Studies on Factors Controlling the Response of Rat Liver to Acute Inflammation

by

Barry M.R.N.J. Woloski

A thesis submitted to
the Faculty of Graduate Studies
of the University of Manitoba
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ABSTRACT

Acute inflammation induces a generalized reaction, the acute phase response, which includes changes in hormone levels, metabolic activity of the liver and hepatic biosynthesis of specific proteins known as acute phase reactants. This thesis examines factors involved in control of hepatic aspects of the acute phase response.

Studies were performed to characterize the acute phase response in the rat. Incorporation of radioactive precursors into the acute phase reactants α_1-acid glycoprotein and α_2-macroglobulin, and the serum glycoprotein α_1-macroglobulin did not identify aspects of hepatic biosynthesis of glycoproteins peculiar to acute phase reactants. Serum levels of corticotropin and cortisol increased at early times after inflammation, insulin increased at later times, and the thyroid hormones decreased. A general increase in serum amino acid levels was found at early times after inflammation, while most serum amino acid levels returned to control levels at later times; hepatic levels were depressed at early times and elevated at later times. Hepatic taurine levels were an exception to this pattern and were elevated from 4-48 h after inflammation.

A rat cytokine preparation was found to elevate serum α_1-acid glycoprotein and cortisol levels and sialyltransferase activities when administered to rats; it also depressed serum albumin and thyroxine levels and hepatic hexosaminidase and galactosidase activities. These effects were less than that observed with inflammation. The cytokine originates from monocytes and is heat- and pronase-labile. Rat cytokine, cortisol and ornithine were also found to have direct effects on RNA

synthesis by liver slices and release of sialyltransferase from liver slices. These effects were seen with single agents or combinations of the agents which suggested a synergistic action of cytokine, cortisol and ornithine.

The drugs indomethacin, sulfinpyrazone, phenylbutazone and salicylate were found to inhibit RNA and glycoprotein biosynthesis by liver slices. RNA and secretable glycoprotein biosynthesis were more sensitive to the effects of the drugs in liver slices from inflamed rats, which exhibit elevated acute phase reactant biosynthesis, than in liver slices from control rats.

The hepatic response to inflammation is complex and regulated at a number of levels; cytokines appear to be critically important to much of this regulation.

List of Abbreviations

A	Adenosine
AA-tRNA	Aminoacyl-transfer ribonucleic acid
Ala	Alanine
AMP	Adenosine 5'-monophosphate
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Asx	Asparagine and aspartic acid
ATP	Adenosine 5'-triphosphate
C	Cytidine
Ci	Curie
CMP	Cytosine 5'-monophosphate
Con A	Concanavalin A
CRF	Corticotropin Releasing Factor
CTP	Cytosine 5'-monophosphate
Cys	Cysteine
d.p.m.	disintegrations per minute
Dol	Dolichol
G	Guanosine
Gal	Galactose
Glc	Glucose
GlcNAc	N-acetylglucosamine
$GlcNH_2$	Glucosamine
Gln	Glutamine

List of Abbreviations - continued

Glu	Glutamic acid
Glx	Glutamine and glutamic acid
Gly	Glycine
GTP	Guanine triphosphate
His	Histidine
hnRNA	heteronuclear ribonucleic acid
HSF	Hepatocyte Simulating Factor
Ile	Isoleucine
I.P.	Intraperitoneal
I.V.	Intravenous
kd	kilodalton
LAF	Lymphocyte Activating Factor
LEM	Leukocytic Endogenous Mediator
Leu	Leucine
LPS	Lipopolysaccaride
Lys	Lysine
Man	Mannose
MCE	Million Cell Equivalents
Met	Methionine
MHC	Major Histocompatability Complex
mRNA	Messenger ribonucleic Acid
mRNP	Messenger ribonucleoprotein
NeuAc	N-acetylneuraminic acid

List of abbreviations - continued

NK	Natural Killer Cells
Orn	Ornithine
PEC	Peritoneal Exudate Cells
Phe	Phenylalanine
PMA	Phorbal Myristic Acetate
PMN	Polymorphonuclear Cells
Pro	Proline
RER	Rough Endoplasmic Reticulum
RNA	Ribonucleic Acid
rRNA	Ribosomal ribonucleic Acid
rT3	Reverse Triiodothyronine
SAA	Serum Amyloid A
SAASF	Serum Amyloid A Stimulating Factor
Ser	Serine
SER	Smooth Endoplasmic Reticulum
SRP	Signal Recognition Particle
T	Thymidine
Tau	Taurine
Thr	Threonine
TRH	Thyrotropin Releasing Hormone
tRNA	Transfer ribonucleic Acid
Trp	Tryptophan
Tyr	Tyrosine

List of Abbreviations - continued

T3	Triiodothyronine
T4	Thyroxine
U	Uridine
UTP	Uridine 5'-triphosphate
Val	Valine

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INTRODUCTION

1. The Acute Phase Response

1.1 Historical Perspective of the Problem

The study of the acute phase response dates back fifty-three years to the discovery of the first acute phase reactant (reviewed by McCarty, 1982). In 1930, William S. Tillet and Thomas Francis discovered that the serum of pneumonia patients agglutinated with the addition of the polysaccharide fraction of the pneumococcal capsule known as the somatic "C" fraction while these researchers were working in O.T. Avery's laboratory at the Hospital of the Rockefeller Institute in New York. They also established that the humoral substance responsible for agglutination was elevated in patients suffering from pneumonia, subacute bacterial endocarditis, acute rheumatoid fever, lung abscess and staphylococcal osteomyletis. The titer of the material returned to normal upon recovery of the patients. In 1933 Rachel Ash at the Children's Hospital in Philidelphia reported that the substance in serum that reacted with pneumococcal "C" fraction was elevated in children suffering from infections with Gram-negative organisms (typhoid fever and *E. coli* pyelitis). By 1941 Avery, Theodore J. Abernathy and Colin MacLeod had established that the substance in serum was a protein, had partially purified this protein and had shown that the C-reactive protein was inactivated at temperatures above 65°C. Since that time a number of investigators have observed elevated levels of the C-reactive protein in a variety of pathological conditions. The site of synthesis of the protein long remained unknown although in 1961 Irving Kushner and Mervin H. Kaplan attempted unsuccessfully to answer this question using

the sensitive technique of immunohistology with fluorescent antibody. In 1964 J.S. Hurliman, J. Thorbecke and G.M. Hockwald established that the liver synthesizes C-reactive protein.

Since that time a great deal of work has been done related to C-reactive protein and a related group of serum proteins (Gordon, 1970; Kushner, 1982). C-reactive protein and those plasma proteins which increase in pathological conditions have come to be known as acute phase reactants. In addition to the alterations in the levels of these proteins, a number of other humoral, metabolic and biochemical alterations occur in response to a variety of pathological conditions and to tissue injury. This generalized response has come to be known as the acute phase response which is a term originally used by Avery to describe the rapidity of elevation of C-reactive protein following the onset of pathological conditions. The acute phase response to stress and tissue injury is a generalized and highly coordinated phenomenon which has received a great deal of attention in recent years from physicians because of its potential clinical value and biochemists, molecular biologists, physiologists and immunologists because of the potential of its use as a model for the understanding of the workings of biological systems.

1.2 Acute Inflammation

Tissue injury and infection perturb physiological homeostasis of organisms and require a response by the organism. The local response to such a challenge is acute inflammation (Kushner, 1982). Acute inflammation involves damage to tissue at the site of inflammation, changes in

vascular flow and permeability, exudation or accumulation of fluid, attraction of leukocytes and pain (Kushner, 1982; Zweifach, 1965; Vinegar, 1982; Grant, 1965; Ross, 1972). These events comprise the immediate response at the site although the generalized response to tissue injury or infection is far more encompassing.

While there are different types of inflammation depending on the causative agents, many aspects of the response to different inflammatory stresses are similar. One model of inflammation that has drawn a great deal of interest because of the ease of analysis in the system is that of carrageenan-induced pleurisy in the rat (Vinegar et al., 1982). It has been possible to study in detail the sequence of events during inflammation in this system and this sequence is presented in Scheme 1. This model will be used in this thesis to illustrate pertinent aspects of the inflammatory response.

As is shown in Scheme 1, damage to cells at the site of inflammation is caused, in part, by the release of lysosomal enzymes (Gleinser, 1979; Baggiolini & Schnyder, 1982; Thomas, 1965; Woessner, 1979). Lysosomes are cell organelles with a semipermeable membrane and which contain more than forty enzymes. Lysosomal enzymes are released from cells by one or more of four mechanisms 1) release of cellular constituents upon cell death 2) selective secretory release in response to appropriate stimuli 3) "regurgitation during feeding" or escape of lysosomal enzymes during phagocytosis and 4) reverse endocytosis or release of lysosomal contents when phagocytes attempt to phagocytose material too large to be taken up into the cell. Most of the lysosomal enzymes are hydrolases and are involved in the degradation of carbohydrates (glycosidases,

Scheme 1 - Events in the Pathway of Carrageenan Pleurisy

First Twenty Minutes

1. Intrapleural injection of carrageenan
2. Adsorption of carrageenan onto pleural surface and into pleura
3. Carrageenan-induced damage to organelles of cells of pleura
4. Release of soluble vasoactive cytoplasmic enzymes from injured cells of pleura
5. Increased permeability of venules and capillaries in pleura
6. Development of mild subpleural edema
7. Plasminogen activator released by injured vessels of pleura
8. Plasmin formed in subpleural edema fluid
9. Emigration of neutrophils and monocytes into subpleural tissue

First and Second Hours

10. Cytoplasmic injury to neutrophils and/or monocytes from phagocytosis of absorbed carrageenan
11. Release of soluble cytoplasmic enzymes from injured cells
12. Selective enzymatic injury to pleura responsible for intrapleural localization of neutrophils but not monocytes
13. Phagocytosis of adsorbed carageenan by intrapleural neutrophils
14. Injury to phagocytic vacuoles by ingested vacuoles
15. Fusion of lysosomes with injured phagocytic vacuoles
16. Degranulation of lysosomes within phagocytic vacuoles

Scheme 1 - continued

17. Lysosomal enzyme injury to membrane of phagocytic vacuoles

18. Intracellular release of lysosomal enzymes

19. Enzymatic injury to plasma membrane of neutrophils

20. Release of lysosomal enzymes of neutrophils into pleural cavity

21. Enzyme induced increase in permeability of venules and capillaries

Third Hour

22. Development of large subpleural edema

23. Neutrophil phospholipase liberates arachidonic acid from cells of pleura

24. Synthesis of vasoactive prostaglandin by cells of pleura

25. Release of vasoactive prostaglandins by cells of pleura

26. Increased permeability of pleura

27. Development of first phase of pleura exudation

Fourth Hour

28. Severe damage to pleura by enzymes released from injured neutrophils

Fifth Hour

29. Initiation of second exudative phase

30. Release of an agent chemotactic for monocytes from injured neutrophils

Scheme 1 - continued

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31. Intrapleural localization of monocytes

Sixth Hour

32. Monocyte-neutrophil interaction

Tenth Hour

33. Reduction of exudate formation

34. Phagocytosis of neutrophils by monocytes

Day 1

35. Cessation of exudate formation

36. Passive decay of exudate volume

Day 2

37. Return of pleural cavity to normal except for large number of intrapleural monocytes (tissue macrophages)

Taken from Vinegar et al. (1982)

polysaccharides), proteins (cathepsins, collagenase, elastase), nucleic acids (ribonucleases) and lipids (lipases). While these enzymes cause some damage at the site of inflammation upon their release, their activity is controlled by the fact that most have acid pH optima while the extracellular space is slightly alkaline. Lysosomal hydrolases are involved primarily with degradation within cells.

During acute inflammation there are vascular alterations leading to the accumulation of exudate fluid. Prostaglandins and other metabolites of arachidonic acid play an integral role in these reactions as well as the generation of pain (Ferriara, 1979; Weissman et al., 1982; Vane, 1972). In addition, vascular changes are also influenced by proteases such as plasmin and kallikrein, polypeptides such as bradykinin and kallidin and biogenic amines such as histamine and serotonin (Wilhem 1965; Busse, 1979; Movat, 1979; Kaplan et al., 1982).

An integral part of inflammation is the emigration of white blood cells to the site of tissue injury. Cells usually migrate in the orderly sequence of neutrophils, monocytes, lymphocytes and fibroblasts (Ross, 1972). While fibroblasts are not leukocytes, they are involved in the fibroplasia which follows exudation (Dumont, 1965; Ross, 1972). Fibroplasia is the accumulation of fibroblasts and the deposition of proteoglycans and collagen fibers at the site of tissue injury or infection. As will be discussed later, cytokines play an integral role in the regulation of emigration of leukocytes; these regulators which are derived from activated leukocytes also are involved in regulating other aspects of the generalized response to tissue injury.

1.3 Systemic, Metabolic, Physiologic and Humoral Alterations during the Acute Phase Response

As previously mentioned, the acute phase response is a generalized reaction to a variety of stresses (Cooper & Stone, 1979; Beisel, 1980; Kushner, 1982). It is induced by infection, surgical and other trauma, chemical inflammatory agents, burns, tissue infarctions and rheumatoid arthritis. Some acute phase phenomena occur during pregnancy, in neonates and in neoplastic states. There is a wide variety of processes affected which range from alterations in thermo-regulation to altered serum concentrations of cations. While the physiological consequences of these alterations are only partly understood, these changes are probably functional as experimentally stressed animals are often more able to resist an induced infection than are non-stressed controls (Suskind, 1977; Hill, 1979).

The temperature of mammals is regulated by complex processes which involve sensing of body temperature by cutaneous, medullar and thalamic thermoreceptors, neural and endocrine responses to altered body temperature and adaptive changes which range from altered heat production and perspiration to the growth of fur (Boulant, 1981; Hensel, 1981; Poulos, 1981; Stitt, 1981). Fever forms a part of the acute phase response to a variety of stresses, and functions to provide sub-optimum growth conditions for invasive microorganisms (Beisel, 1980; Stitt, 1981; Kushner, 1982). During the acute phase response, elevated body temperature results from altered hypothalamic control of thermoregulation and cytokines play an integral role in this process. Fever induces a hypermetabolic state in which there is a stimulation of cellular

metabolic rates, an increased utilization of nutrients and an increased production of glucose to meet the elevated energy requirements of body cells at elevated temperatures. There also are losses of body nutrients *via* perspiration.

The changes mentioned above bring about considerable changes in the nutritional status of animals typified by altered mineral and vitamin metabolisms (Beisel, 1975, 1980; Squibb, 1979; Kushner, 1982). There are altered body losses of magnesium, potassium, phosphate, zinc, sulfate and most vitamins during generalized acute infections. There also is an elevated hepatic sequestration of iron and zinc during the acute phase response due to elevated levels of the iron binding proteins ferritin and hemosiderin and the zinc binding protein metallothionein. Decreased serum levels of the iron binding protein transferrin also contribute to this redistribution of available iron. There also is an elevated hepatic secretion of copper into the serum due to elevated rates of secretion of the copper binding protein ceruloplasmin. While the mechanisms involved have not been delineated, these alterations are believed to play protective roles for the host as altered iron levels are correlated to increased resistance to infection (Hill, 1979).

During the acute phase response, available energy sources change (Beisel, 1975, 1980; Shuttler *et al.*, 1977; Langstaff *et al.*, 1980; Kushner, 1982). There are elevated rates of degradation of amino acids with accelerated ureogenesis and ammoniogenesis, increased gluconeogenesis, increased glycogenolysis, increased catabolism of somatic proteins, reduced ketogenesis and hypertriglyceridemia following stress. There also have been reports of both accelerated hepatic free fatty acid

synthesis and a blockage of inductive synthesis of hepatic fatty acid synthetase. These alterations would bring about considerable changes in the utilization of available protein, carbohydrate and lipid for anabolism.

There are alterations involving leukocytes during the acute phase response in addition to migration to the site of inflammation which was previously mentioned (Kushner, 1982). There is an increase in the blood granulocyte count which results from increased production of granulocytes and release from bone marrow storage pools. Phagocytosis by the reticuloendothelial system is also transiently depressed during the acute phase response.

In addition to the above changes, there are a number of hormones which are altered during the acute phase response. Circulating levels of the glucocorticoids, mineralocorticoids, catecholamines, insulin, growth hormone, glucagon and thyroid hormones are all altered following inflammation (Beisel, 1980; Langstaff et al., 1980).

As previously mentioned, during the acute phase response there are differences in the levels of some of the plasma proteins. The changes of serum levels of many of these proteins are shown in Table 1. Those proteins which increase following stress have been referred to as acute phase reactants (Koj, 1974) and the increases for these proteins range from less than 100% for ceruloplasmin, α_2-plasmin inhibitor and complement C3 to several thousand-fold for human and mouse C-reactive protein. Other proteins fall in concentration following stress and the term "negative acute phase reactant" has been used to describe them (Kushner, 1982). These proteins include albumin, transferrin, and

Table 1 - Plasma Proteins Altered in Concentration following Stress and Tissue Injury

Protein	Normal Levels (mg/ml)	Extreme Levels Following Stress (mg/ml)	References
α_1-Acid Glycoprotein			
Rat	2.4	12	Jamieson et al., 1972b
Rat	0.064	2.5	Ricca et al., 1981
α_2-Macroglobulin			
Rat	3.3	13	Jamieson et al., 1972b
Major Acute Phase α_1 Protein			
Rat	0.35	7	Urban et al., 1979
α_1-Antitrypsin			
Human	2.0	3.9	Matsuda et al., 1980
α_2-Plasmin Inhibitor			
Human	0.058	0.066	Matsuda et al., 1980
α-Fetoprotein			
4 day Rats	6	4	Savu et al., 1983
10 day Rats	2	1.5	Savu et al., 1983
Fibrinogen			
Rat	2	3.5	Kampschmidt & Upchurch, 1974
C-Reactive Protein			
Rat	0.3-0.6	0.9	Baltz et al., 1982
Human	0.0001	0.3	Baltz et al., 1982
Mouse	trace	0.002	Baltz et al., 1982
Haptoglobin			
Human.	1.25	6.5	Putnam, 1975b
Rat	1.4	7.5	Savu et al., 1983
Serum Amyloid A			
Mice	0.0002	0.8	McAdams & Sipe, 1976
Human	0.00001	0.001	Ignaczak et al., 1977

Table 1 - continued

Protein	Normal Levels (mg/ml)	Extreme Levels Following Stress (mg/ml)	References
Serum Amyloid P			
Human	0.04	0.1	Pepys et al., 1982
Syrian Hamster Female Protein			
Males	0.02	0.1	Coe, 1982
Females	2.1	1.0	Coe, 1982
Complement C3			
Human	1.6	2.0	Gordon, 1970
Ceruloplasmin			
Human	0.38	0.47	Gordon, 1970
Transferrin			
Human	2.0	1.6	Gordon, 1970
Thyroxine Binding Prealbumin			
Human	0.40	0.28	Gordon, 1970
Albumin			
Rat	40	33	Jamieson et al., 1972b
Human	42	20	Peters, 1975

thyroxine binding prealbumin. There are also species differences in the response of acute phase reactants to stress. For example, while human, murine and rabbit C-reactive protein are all elevated over one hundred-fold during the acute phase response, the rat protein increases less than three-fold.The sex-limited female protein of Syrian hamsters, which has recently been reported to be an acute phase respondant, shows an interesting response to an inflammatory challenge. The protein is at relatively high serum levels and is a negative acute phase reactant in females of the species, while it is normally present at lower levels and is an acute phase reactant in males (Coe, 1982). The variation in serum levels of acute phase reactants attained is probably related to the physiological regulation of the synthesis and secretion of these proteins.

Although the acute phase reactants are a diverse group of proteins, most of them are also glycoproteins as is shown in Table 2. The carbohydrate composition of those which are glycoproteins varies from 4% for fibrinogen to 40% for α_1-acid glycoprotein. The carbohydrate moieties of the acute phase reactants are usually of the N-linked complex type (Jamieson, 1983), however, there may be alterations in the carbohydrate structures of some of the acute phase reactants following an inflammatory challenge (Koj et al., 1982). Notably, human and rabbit C-reactive protein are not glycoproteins while rat C-reactive protein is a glycoprotein. This difference in carbohydrate content may account for the species differences in normal serum levels of this protein (I.Kushner, personal communication).

Although roles for acute phase reactants are only beginning to be

Table 2 - Physical and Chemical Properties of Some Acute Phase Reactants

Protein	Molecular Weight (kd)		pI	Percent Carbohydrate	References
	Native	Subunit/Polypeptide			
Rat α_1-Acid Glycoprotein	43	43	2.95	34	Jamieson et al., 1972a
Human α_1-Acid Glycoprotein	40	40	2.7	42	Putnam, 1972a Schmid, 1975
Rat α_2-Macroglobulin	700-800	190	4.1-4.6	10-16	Jamieson et al., 1972a Hudig & Sell, 1979 Gauthier & Mouray, 1976 Niewenhuizen et al., 1979
Rat Fibrinogen	340	61,58,51	-	4	Nickerson & Fuller, 1981
Human Fibrinogen	340	-	5.5	4	Doolittle, 1975
Frog Fibrinogen	340	63,59,55	-	-	Wangh et al., 1983
Rat α_1-Major Acute Phase Protein	86	86	4.6	20	Urban et al., 1979
Rat Haptoglobin	90	35, 9.5	4.2	20	Haugen et al., 1981 Putnam, 1975

Table 2 - continued

Protein	Molecular Weight (kd)		pI	Percent Carbohydrate	References
	Native	Subunit/Polypeptide			
Rat C-Reactive Protein	100	20	3.8	11	Baltz et al., 1982
Human C-Reactive Protein	120	20	7.9	0	Baltz et al., 1982 Liu et al., 1982
Rabbit C-Reactive Protein	120	20	-	0	Baltz et al., 1982 Liu et al., 1982
Plaice C-Reactive Protein	120	20	-	5.3	Baltz et al., 1982

understood, it is evident that these proteins are important for non-specific immunity as well as the recovery from tissue injury. A number of acute phase reactants including α_2-macroglobulin, α_1-antitrypsin and α_2-plasmin inhibitor, have antiprotease activity. The activity of these proteins may be important in limiting the extent of tissue damage caused by protease release at the site of inflammation (James, 1980; Stein-Streilein & Hart, 1978; Beisel, 1980). Fibrinogen, as a precursor to fibrin, is involved in localizing infections through clot formation (Beisel, 1980; Lorand, 1983). Fibrinogen peptides also have roles in the modulation of activity of monocytes (Fuller & Ritchie, 1982). Ceruloplasmin may play a role in protecting cells from damage by superoxide anion radicals generated during tissue injury (Goldstein et al., 1979). C-reactive protein may play a role in modulating the immune response of leukocytes (Mortensen et al., 1975; Beisel, 1980; Mold et al., 1982; James et al. 1982). There is evidence that haptoglobin is a natural bacteriostat which binds free hemoglobin and thus removes hemoglobin which would support prolific bacterial growth (Eaton et al., 1982). Human α_1-acid glycoprotein has been implicated as having immunosuppressive roles (Bennet & Schmid, 1980). While a great deal remains to be delineated about the action of the acute phase reactants, these proteins are undoubtably an integral and necessary part of the acute phase response to inflammation and tissue injury.

1.4 Hepatic Events during the Acute Phase Response

The liver is physically, biochemically and physiologically different in animals suffering from stress as compared to control animals. This

is not suprising since it is the primary, if not sole, site of synthesis of the acute phase reactants. As was previously mentioned, it was first established in 1964 that the liver was the site of synthesis of C-reactive protein. Since that time it has been shown that the liver is the site of synthesis of α_1-acid glycoprotein (Miller & John, 1970; Jamieson & Ashton, 1973; Jamieson et al., 1975), α_2-macroglobulin (Sarcione & Bogden, 1966; Sarcione & Bohne, 1969),fibrinogen (Miller & John, 1970; Bouma et al., 1975; Rupp & Fuller, 1980), serum amyloid A (Benson, 1982; McAdam et al., 1982), haptoglobin (Miller & John, 1970), albumin (Miller & John, 1970; Jamieson et al., 1975), C-reactive protein (Hurliman et al., 1966; Kushner & Feldman, 1978; McIntyre et al., 1982) and transferrin (Gordon, 1970); altered rates of hepatic biosynthesis are responsible for the altered plasma levels of the proteins.

During the acute phase response there are a number of structural changes which occur concommitant with the alterations in hepatic biosynthesis of proteins (Turchen et al., 1977; Lombard et al., 1980; Kushner, 1982). There are increased formation of microtubules in the Golgi area of the endoplasmic reticulum, increased amounts of smooth endoplasmic reticulum, proliferation of membranes of the Golgi complex, increased synthesis of hepatocyte plasma membranes and increased cytoplasmic actin synthesis. These changes are consistent with increased synthesis, transport and secretion of plasma proteins.

In addition to the structural alterations mentioned above, there are alterations in activity of many hepatic enzymes as is shown in Table 3. Altered alkaline ribonuclease, nuclear protein kinase and ornithine decarboxylase activities are consistent with increased

Table 3 - Hepatic Enzyme Activities Altered Following Tissue Injury and Inflammation

Enzyme	Function	Comments	References
Sialyltransferase	Transfers sialic acid to carbohydrate moieties of complex type glycoproteins.	Golgi enzyme increased following inflammation and neoplasia. Primarily $\alpha 2 \rightarrow 6$ form increased.	Bernack & Kim, 1977 Kaplan et al., 1983b Lombart et al., 1980
Galactosyltransferase	Transfers galactose to carbohydrate moieties of complex type glycoproteins.	Golgi enzyme increased following inflammation and neoplasia.	Kaplan et al., 1983b Lombart et al., 1980
N-Acetylglucosaminyl-transferase	Transfers GlcNAc to carbohydrate moieties of complex type glycoproteins.	Golgi enzyme increased following inflammation.	Lombart et al., 1980
CTP-Dependent Dolichol Kinase	Increases dolichol phosphate pool available to participate in glycoprotein biosynthesis.	Mostly localized in smooth microsomes, increases following inflammation.	Coolbear & Mookerjea, 1981
CMP-NeuAc Synthase	Synthesizes CMP-NeuAc.	Cytosolic enzyme which increases following inflammation.	Kaplan et al., 1983a
β-Galactosidase	Hydrolyzes Gal from partially degraded glycoconjugates.	Lysosomal enzyme decreased following inflammation.	Kaplan & Jamieson, 1977

Table 3 - continued

Enzyme	Function	Comments	References
N-Acetyl-β-D-glucosaminidase	Hydrolyzes GlcNAc from partially degraded glycoconjugates.	Lysosomal enzyme decreased following inflammation.	Kaplan & Jamieson, 1977
Glucosamine-6-phosphate synthase	Regulatory enzyme in the synthesis of amino sugars.	Cytosolic enzyme increased following inflammation.	Kaplan et al., 1983a Bley et al., 1973
UDP-GlcNAc-2'-epimerase	First enzyme in the pathway to CMP-NeuAc formation.	Cytosolic enzyme increased following inflammation.	Kaplan et al., 1983a
HMG-CoA Reductase	Key enzyme in the synthesis of the lipids cholesterol, retinal and dolichol from hydroxymethylglutyryl CoA.	Increased following tissue injury.	Kushner, 1982
Fatty Acid Synthetase	Synthesizes fatty acids from acetyl-CoA.	Inflammation blocks inductive synthesis of fatty acid synthetase.	Shuttler et al., 1977 Langstaff et al., 1980
Tyrosine Transaminase	Catabolic enzyme providing energy for metabolic activity.	Increased following inflammation.	Kushner, 1982
Tryptophan Oxidase	Catabolic enzyme providing energy for metabolic activity.	Increased following inflammation.	Kushner, 1982

Table 3 - continued

Enzyme	Function	Comments	References
NADH-Cytochrome c-Reductase	Catabolic enzyme providing energy for metabolic activity.	Increased following inflammation.	Kushner, 1982
Phosphoenolpyruvate Carboxykinase	Participates in gluconeogenesis.	Decreased with endotoxemia.	Kushner, 1982
Glucose-6-Phosphatase	Participates in gluconeogenesis.	Decreased with endotoxemia.	Kushner, 1982
5'-Nucleotidase	Splits nucleotides.	Endoplasmic reticulum enzyme decreased with infection & neoplasia.	Kushner, 1982 Cooper & Stone, 1979
Nonspecific Esterase	Involved in drug detoxification.	Endoplasmic reticulum enzyme decreased with infection.	Kushner, 1982
Na,K,ATPase	Cell membrane transport enzyme maintaining Na and K gradients.	Inhibited by endotoxin administration.	Kushner, 1982
Alkaline Ribonuclease	Hydrolyzes RNA.	Decreased following stress.	Liu & Neuhaus, 1968

Table 3 - continued

Enzyme	Function	Comments	References
Ornithine Decarboylase	Key enzyme in the synthesis of polyamines from ornithine.	Increased following laparotomy.	Raina & Janne, 1970
Nuclear Protein Kinase	May phosphorylate nuclear regulatory proteins and modulate genomic information that controls differentiation.	Increased at early times after laparotomy.	Laks et al., 1981

RNA synthesis and accumulation of polyribosomes following inflammation. The enzymes NADH-cytochrome c-reductase, tyrosine transaminase and tryptophan oxygenase may serve to provide fuel for the increased metabolic acitivity manifested by the liver during the acute phase response. Sialyltransferase, galactosyltransferase, N-acetylhexosaminyl-transferase, glucosamine-6-phosphate synthase, UDP-GlcNAc-2'-epimerase, CMP-NeuAc synthase and CTP-dependent dolichol kinase are all involved in the bioysynthesis of glycoproteins. The alterations in the above mentioned enzymes activities are all consistent with one aspect of the acute phase response, namely an increased hepatic capacity for the biosynthesis and secretion of the acute phase reactants. While the liver is a multifunctional organ, available evidence (Beisel, 1975, 1980; Kushner, 1982) suggests that one of the most important roles that this organ plays during the acute phase response is to synthesize and release into serum increased amounts of acute phase reactants.

2. Hepatic Glycoprotein Biosynthesis

Mammals maintain homeostasis by coordinated response of organs and tissue to any disruptive situation or stimulus. The mammalian liver plays a central role in the response to many stimuli because of its multifunctional nature (Hargreaves, 1968; Guyton, 1976). In humans, about 1.5 litres of blood flow through the liver every minute which represents about 30% of total cardiac output. This access to a large fraction of the circulatory system makes the liver cell well suited for central roles in nutrient storage and metabolism, in degradation of toxic substances formed endogenously such as

bilirubin or exogenous material such as drugs, in control of levels of physiologically active agents such as insulin and in the synthesis of most of the plasma proteins. This last role involves the synthesis of RNA, proteins and in the case of glycoproteins, carbohydrate moieties. As will be discussed, these processes are complex in themselves, but they are also regulated at many levels in response to stimuli such as those generated by acute inflammation and tissue injury.

2.1 RNA Synthesis and Metabolism

One level at which gene expression is controlled is by the number of functional transcripts of a gene present in a cell at a given time. These levels are controlled by the rates of synthesis of RNA from DNA, by the processing and transport of RNA, and by post-transcriptional events which affect the ability of a RNA transcript to participate in protein synthesis.

RNA synthesis or transcription is the first step in the expression of gene products (Goodenough, 1974; Goldberger, 1975; Beathnach & Chambon, 1981). RNA polymerases catalyze transcription: RNA polymerase I transcribes ribosomal RNA, RNA polymerase II transcribes messenger RNA and RNA polymerase III transcribes 5 S ribosomal RNA and transfer RNAs. Sequences of DNA adjacent to or within the transcribed region determine the RNA polymerase involved in transciption. For example, a AT-rich region of DNA known as the Goldberg-Hogness bow is involved in the promotion of transcription by RNA polymerase II. Thus effects on the different RNA polymerases would effect transcription of the different classes of RNA.

The metabolism of RNA past the transcriptional step is a complex process as is shown in Figure 1. RNA transcripts which are not transcribed in forms which can be utilized in the translation process. RNA processing is the series of enzymic reactions that convert primary transcription products into mature functional molecules (Breathnach & Chambon, 1981; Perry, 1976; Abelson, 1979; Revel & Groner, 1978). Primary transcriptional products are often larger than the mature forms of RNA. For example, heteronuclear RNA (hnRNA), which is the precursor of mRNA,may be larger than 10^7 daltons while mRNA molecules are generally smaller than 2×10^6 daltons. The processing of hnRNA to mRNA is a multistep process as is shown in Figure 2. After transcription of hnRNA, a stretch of fifty to two hundred adenine residues are added to the 3'-terminus. Then some of the internal adenine residues are methylated and a guanine residue is added at the 5'-terminus. Methylations occur at the 7 position of the 5'-terminus as well as at the 2'-position of the riboses of the two adjacent nucleotides. This structure of methylated nucleotides is referred to as a "cap". The RNA molecule also contains sequences of RNA referred to as introns or intervening sequences which separate the translatable sequences of mRNA or exons. Introns are removed by splicing enzymes after cap formation. The splicing enzymes recognize specific sequences around the splicing points and it has been suggested that small nuclear RNAs hybridize near the splice point and "guide" the splicing enzymes. By these mechanisms, a mature mRNA is formed which has a cap at the 5' end, a non-translatable leader sequence 5' to the translatable mRNA sequence and a polyadenylate tract at the 3' end of the molecule.

Figure 1 - General Scheme of Mammalian RNA Metabolism

Taken from Woloski (1980)

Abbreviations used are:

pre	precursor form of RNA
mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	transfer RNA
A,G,C,U,	nucleotides
mRNP	messenger ribonucleoprotein particle
60S & 40S	large and small ribosomal subunits
AA	aminoacyl unit

Processes involved in this scheme are:

1,2,3	transcription
4,5,6	RNA processing
7,8,10	RNA transport across nuclear membrane
9	mRNA-protein association/dissociation
11	aminoacylation of tRNA
12	initiation of polypeptide synthesis
13	elongation of polypeptides
14	segregation of bound polyribosomes
15	termination of polypeptide synthesis
16	cytoplasmic RNA degradation

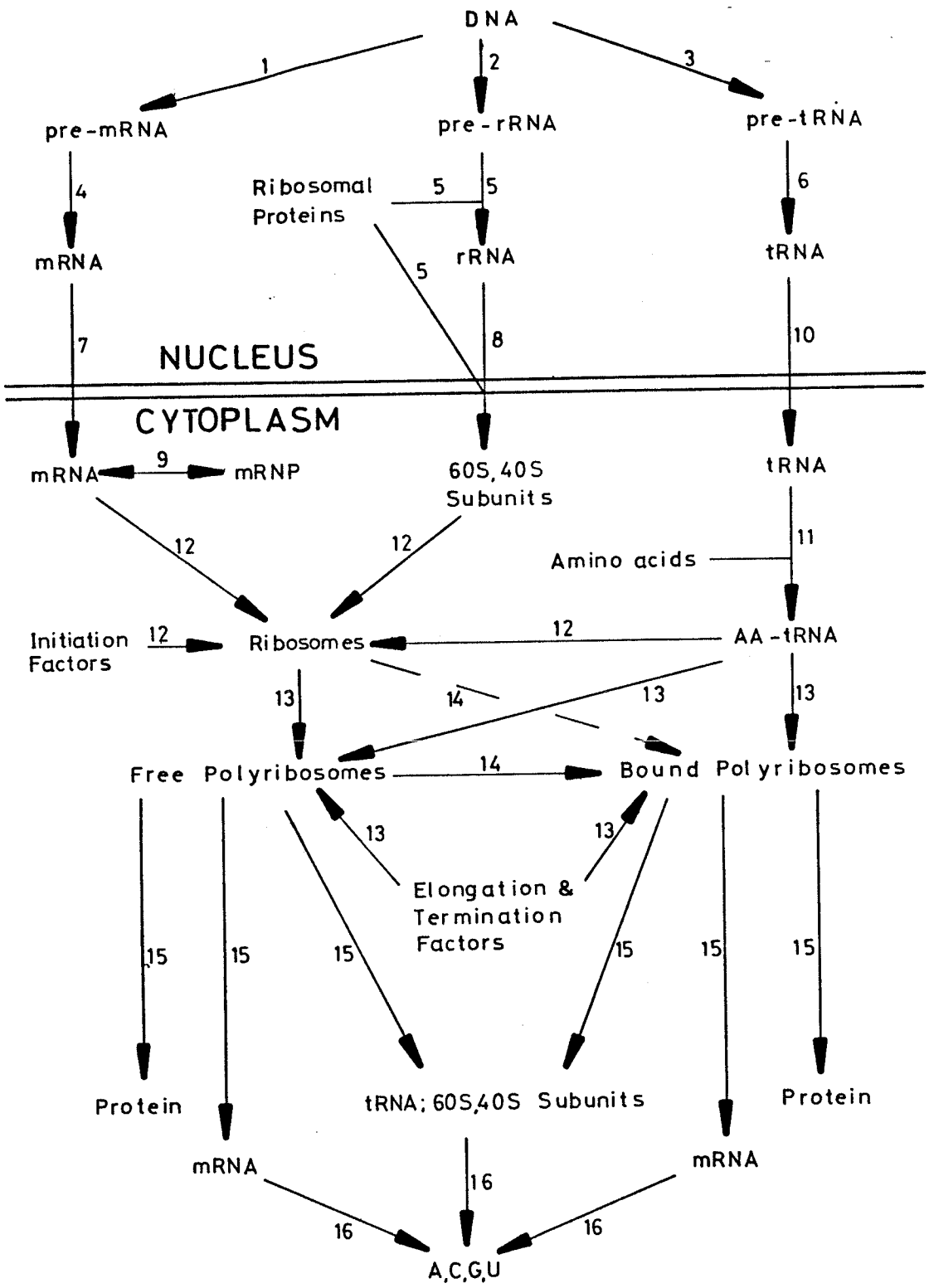

DNA
1
2
3
pre-mRNA
pre-rRNA
pre-tRNA
4
Ribosomal Proteins
5
5
6
mRNA
rRNA
tRNA
5
7
8
10
NUCLEUS
CYTOPLASM
mRNA
9
mRNP
60S, 40S Subunits
tRNA
12
12
Amino acids
11
Initiation Factors
12
Ribosomes
12
AA-tRNA
13
14
13
13
Free Polyribosomes
14
Bound Polyribosomes
13
13
Elongation & Termination Factors
15
15
15
15
15
15
Protein
tRNA; 60S,40S Subunits
Protein
mRNA
mRNA
16
16
16
A,C,G,U

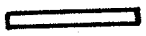
Figure 2 - Maturation of messenger RNA

Taken from Woloski (1980)

Abbreviations used are:

DNA	deoxyribonucleic acid
hnRNA	heteronuclear ribonucleic acid
mRNA	messenger RNA
A,C,G,U	adenine, cytosine, guanine, uracil
N	ribonucleotide
ATP	adenosine triphosphate
PP_i	pyrophosphate
P	phosphate
m	methyl group, nitrogenous base is methylated if written on left side of the base, sugar is methylated if written on right side of base

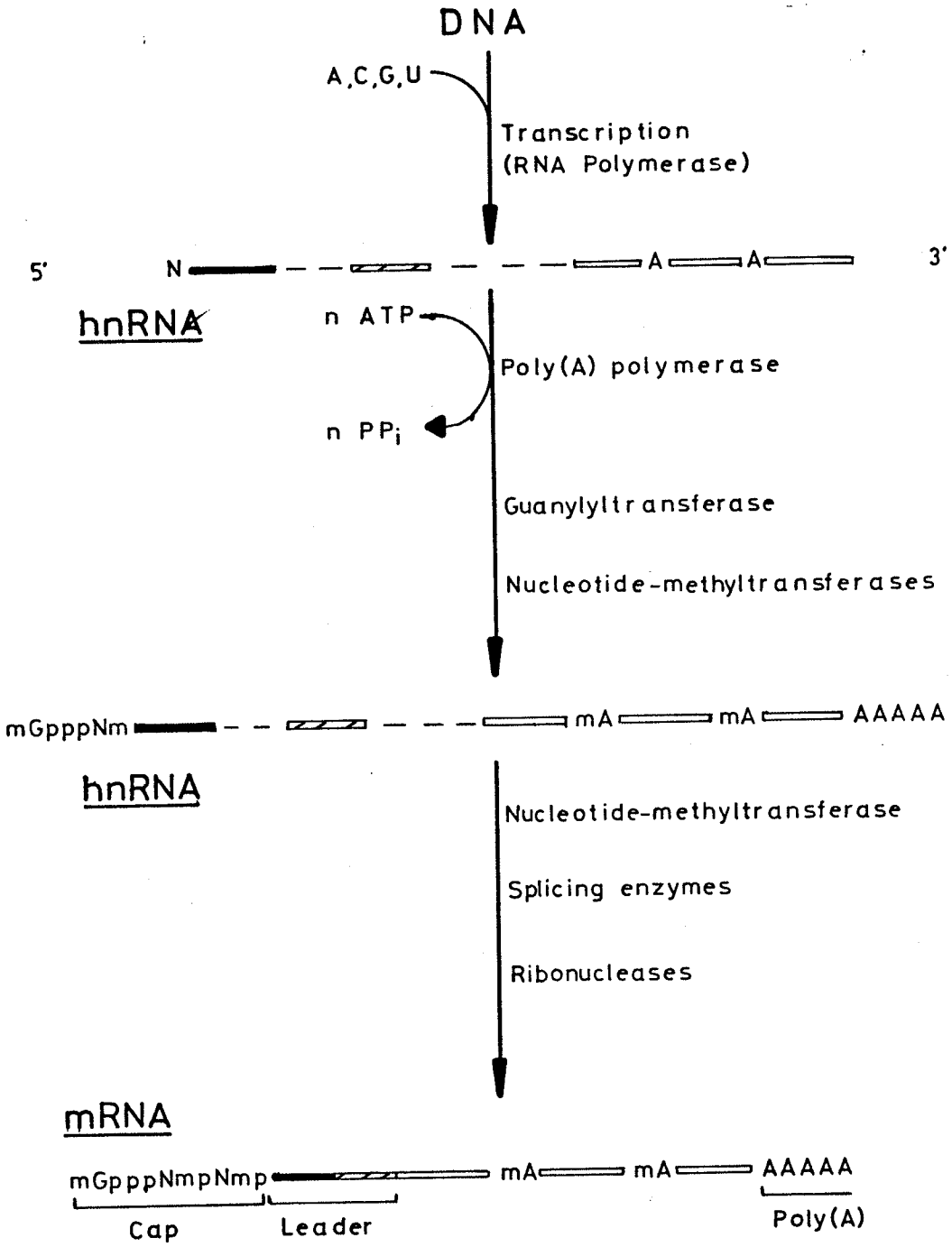
Symbols used are:



translatable transcript

leader sequence

sequences which are cleaved from hnRNA during processing


DNA
A,C,G,U
Transcription
(RNA Polymerase)
5'
N
A
A
3'
hnRNA
n ATP
Poly(A) polymerase
n PPi
Guanylyltransferase
Nucleotide-methyltransferases
mGpppNm
mA
mA
AAAAA
hnRNA
Nucleotide-methyltransferase
Splicing enzymes
Ribonucleases
mRNA
mGpppNmpNmp
mA
mA
AAAAA
Cap
Leader
Poly(A)

By similar mechanisms transfer and ribosomal RNA precursor molecules are processed to mature forms although features such as the cap and poly(A) tract do not appear in these species of RNA.

In addition to the above processing reactions, control is exerted over the transport of mRNA into the cytoplasm (Goldfine, 1982) and, indeed, the majority of hnRNA does not enter the cytoplasm as mRNA transcripts but is degraded in the nucleus (Greenberg, 1975). The nuclear envelope is a bilayered membrane structure separating the nucleoplasm and cytoplasm of eukaryotic cells; in it are dispersed pores filled with a unique structure termed the nuclear pore complex. The nuclear pore complex plays a role in transporting mRNA from the nucleus. The transport of mRNA is an endergonic process requiring the hydrolysis of one high energy phosphate bond to transport one nucleotide of mRNA. There is considerable evidence (Goldfine, 1982) that this energy is provided by nuclear triphosphatase (NTPase) which can utilize ATP, UTP, CTP and GTP with equal efficiency. This process may be important to gene expression. For example, there is evidence (Purello et al., 1983) that insulin-stimulated increases in hepatic mRNA levels result from the stimulation of the NTPase.

Levels of available mRNA transcripts can be controlled by other post-transcriptional processes. It is known (Greenberg, 1975; Shafritz, 1977) that mRNA may bind specific classes of proteins to form messenger ribonucleoprotein particles (mRNP). Functions suggested for these mRNPs include involvement in messenger storage, transport and initiation. Also, it has been suggested that binding of specific proteins to mRNA results in an untranslatable mRNP, whereas dissociation

of the proteins results in the translation of the mRNA transcript (Greenberg, 1975). The synthesis of rat liver ferritin may be controlled post-transcriptionally in this manner (Munro, 1977).

It is by the above processes that RNA appears in the cytoplasm of cells in a form that is required for the synthesis of polypeptide gene products. As RNA transcripts are essential for the synthesis of polypeptide, regulation of the level of RNA transcripts also has effects on the translation process.

2.2 Protein Synthesis

Translation, or protein synthesis, is a multi-step process which involves initiation or formation of mRNA-ribosome complexes, elongation of the polypeptide encoded for by the mRNA transcript, and termination of the process. There are processes, such as localization of some polypeptides within subcellular fractions which are coupled to the translation process. Also, a number of modifications to the polypeptide can occur which alter its mature form. These processes are coordinately controlled such that the product of these processes is a functional protein which is present at levels appropiate to the physiological requirements of the organism.

Essential participants in the translation process are the ribosomal subunits which have been studied in great detail (Brimacombe, et al., 1978; Wool, 1979). Ribosomes are large structures of proteins and ribosomal RNA. Ribosomal RNA is transcribed and processed in the nucleus. Ribosomal proteins, however, are translated in the cytoplasm and transported to the nucleolus where the ribosomal subunits are

assembled. Eukaryotes have more ribosomal proteins than prokaryotes and these extra proteins may participate in functions such as transport of ribosomal subunits from the nucleus to the cytoplasm and in the attachment of ribosomes to membranes. Once ribosomal subunits are in the cytoplasm, they are able to participate in the translation process.

Initiation of protein synthesis is essentially a two step process (Revel, 1977; Revel & Groner, 1978; Ochoa & Haro, 1979) as is shown in Figure 3. First, the initiation region of a mRNA transcript binds to a complex of 40 S ribosomal subunit and initiation factors 1, 2, 3, 4A, 4B and 4C; energy for this process is provided by hydrolysis of ATP. The second step of initiation is the binding of the 60 S ribosomal subunit to the above complex; this step requires initiation factor 5 which has a GTPase activity. The overall result of these reactions is the formation of a 80 S monoribosome which can proceed to chain elongation with an adequate supply of AA-tRNAs and transfer factors. It appears that the initiation process exerts control over selection and discrimination of mRNA transcripts for translation. In a reticulocyte lysate translation system, increasing levels of hemoglobin mRNA decreases the ratio of α- to β-globin translation. This is believed to result from a dependence of initiation of α-globin mRNA on cap recognition and β-globin mRNA on initiation factor 4B binding. This and other examples of apparent mRNA selection illustrate the importance of initiation of translation in the differential expression of gene products.

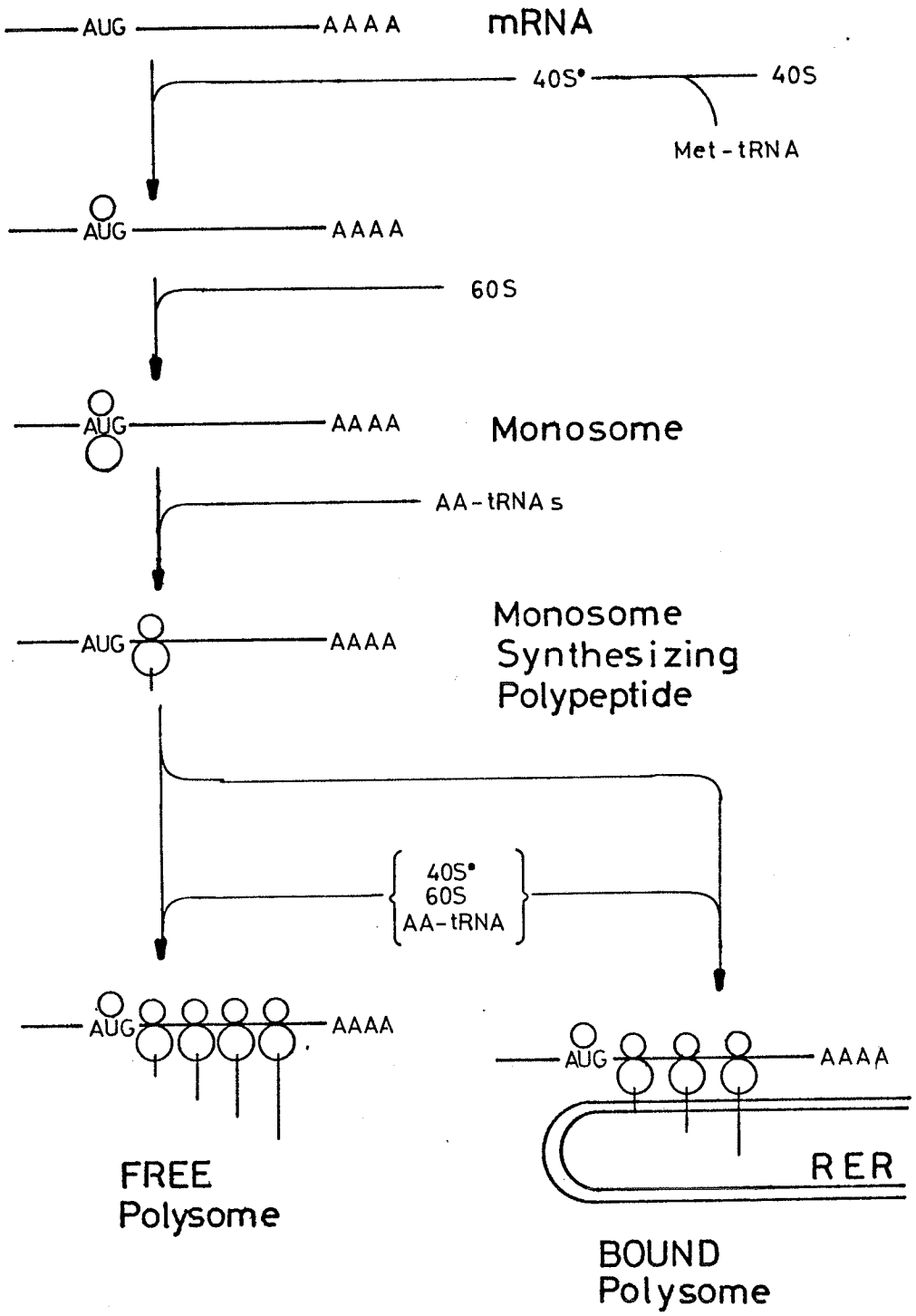
The elongation of nascent polypeptides attached to ribosomes

Figure 3 - Initiation and Translation of Messenger RNA

Taken from Woloski (1980)

Abbreviations used are:

AUG	initiation codon
AAAA	poly(A) tract
40S	small ribosomal subunit
Met-tRNA	methionyl-tRNA
40S•	complex of 40S, Met-tRNA, GTP and initiation factor 2
AA-tRNA	aminoacyl-tRNA
RER	rough endoplasmic reticulum

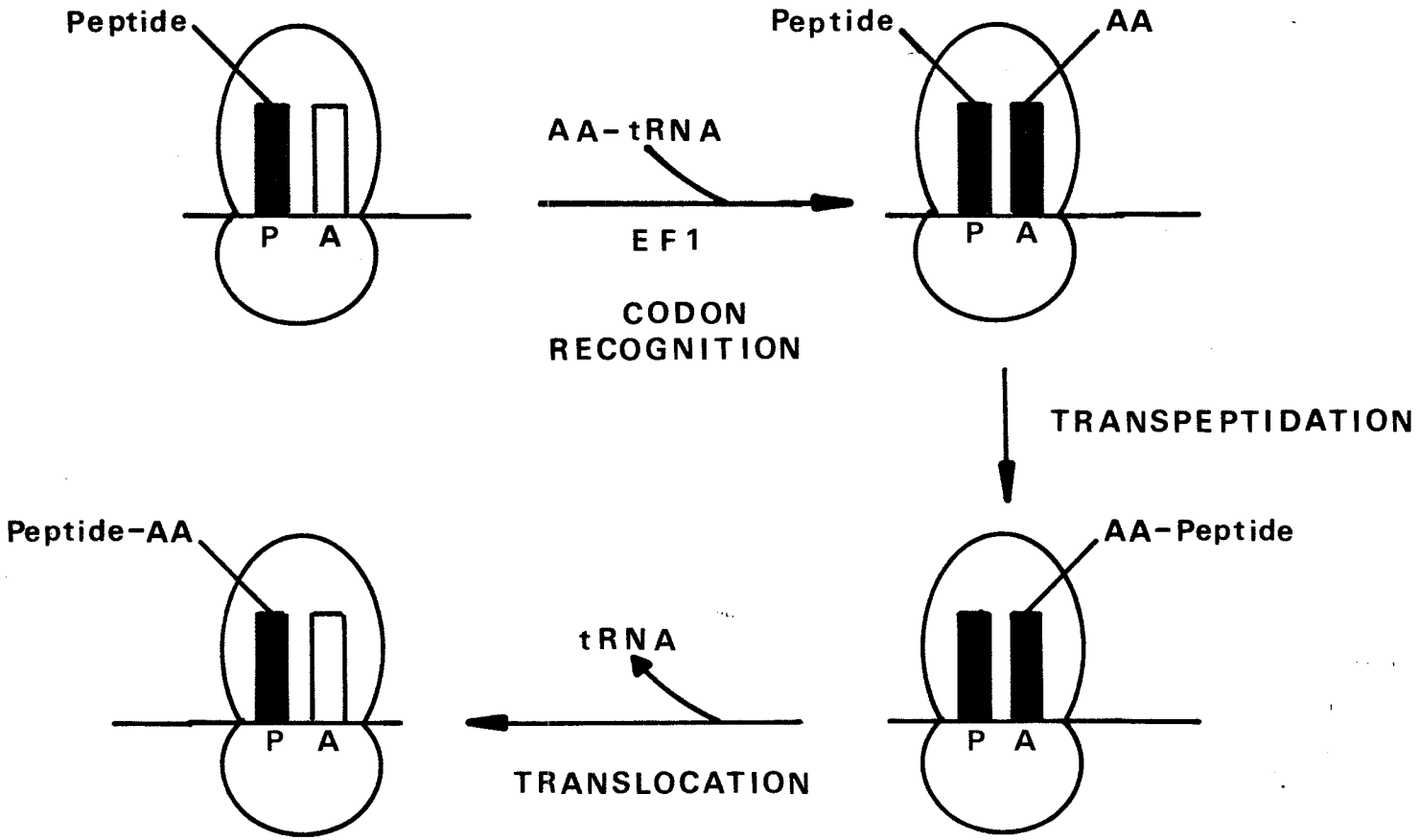

AUG
AAAA
mRNA
40S*
40S
Met-tRNA
AUG
AAAA
60S
AUG
AAAA
Monosome
AA-tRNAs
AUG
AAAA
Monosome
Synthesizing
Polypeptide
40S*
60S
AA-tRNA
AUG
AAAA
FREE
Polysome
AUG
AAAA
RER
BOUND
Polysome

requires AA-tRNAs, elongation or transfer factors and the expenditure of energy as GTP hydrolysis (Brot, 1977; Harris & Pestka, 1977; Miller & Weissbach, 1977; Schimmel & Söll, 1979). This process is summarized in Figure 4. AA-tRNAs required for polypeptide chain elongation are formed by the attachment of amino acids to their cognate tRNAs; these reactions are catalyzed by aminoacyl-tRNA synthetases specific for each amino acid and require energy from the hydrolysis of ATP. Elongation factor 1 transfers an AA-tRNA into the acceptor site of a mRNA-ribosome complex. A component of the large ribosomal subunit catalyzes the formation of a peptide bond between the amino acid on the tRNA in the acceptor site and the nascent polypeptide chain. Elongation factor 2 translocates the ribosome three bases closer to the 3' end of the mRNA and, in doing so, ejects the deacylated tRNA from the peptidyl site. This process is repeated until a termination codon is encountered in the mRNA sequence.

Peptide chain termination results in the release of the completed polypeptide from ribosomes and requires a soluble protein factor (Caskey, 1977). Termination of translation occurs when a termination codon (UAA, UAG or UGA) is encountered in the nucleotide sequence of mRNA transcripts. The releasing factor binds to ribosomes with a termination codon in their acceptor site and this binding is stimulated by GTP. Hydrolysis of the peptidyl-tRNA bond and release of the polypeptide from the ribosome is brought about by the combined action of the releasing factor and the ribosomal enzyme peptidyl transferase. These steps represent the end of polypeptide synthesis; however, a number of post-translational events such as processing of polypeptide and

Figure 4 - Polypeptide Chain Elongation

One cycle of polypeptide chain elongation is shown. Abbreviations used are AA-tRNA -aminoacyl tRNA, EF1- elongation factor 1, AA- amino acid, tRNA- transfer RNA, P - peptidyl site, and A - acceptor site. See text for further explanation.


Peptide
P A
AA-tRNA
EF1
CODON
RECOGNITION
Peptide
AA
P A
TRANSPEPTIDATION
AA-Peptide
P A
tRNA
TRANSLOCATION
Peptide-AA
P A

carbohydrate moieties of proteins and glycoproteins occur before the synthesis of protein is complete.

2.3 Glycosylation of Proteins

While there is a great deal of diversity in the carbohydrate moieties of glycoproteins, there are common features in the structures found in nature (Hussa, 1980; Jamieson, 1983). Carbohydrate may be attached to protein with linkages either to asparagine (N-linked) or serine or threonine (O-linked) residues of the polypeptide chain (Winzler, 1973; Hussa, 1980; Jamieson, 1983). The O-linked carbohydrate moieties tend to be smaller than those of the N-linked oligosaccharide and show more variation in their structure. The N-linked glycoproteins have a common "core oligosaccharide" with the structure $Man_2Man\beta(1\rightarrow4)GlcNAc\beta(1\rightarrow4)GlcNAc$-Asn. The high mannose type of N-linked glycoprotein has 2-6 mannose residues in addition to those found in the core structure. The complex type of N-linked glycoprotein has 2, 3 or 4 triplets of sugars with the sequence NeuAc, Gal, GlcNAc attached to the mannose residues of the core oligosaccharides; fucose may also be attached to GlcNAc residues in the complex type of glycoprotein (Hussa,1980; Jamieson, 1983). Acute phase reactants such as α_1-acid glycoprotein are complex N-linked glycoproteins.

While the synthesis of O-linked glycoproteins is brought about by the sequential addition of sugars to growing oligosaccharides attached to the polypeptide, the synthesis of N-linked glycoproteins is more complex (Winzler, 1973; Hussa, 1980; Hubbard & Ivatt, 1981; Jamieson, 1983). N-linked glycoproteins have a common biosynthetic origin in a

oligosaccharide precursor synthesized while attached to a carrier lipid; the oligosaccharide is then transferred en bloc to acceptor proteins. The lipid involved in this process is dolichol which is a linear polyisoprene of 16-23 repeating units. The synthesis of the precursor oligosaccharide is shown in Figure 5 and has come to be known as the dolichol cycle. Dolichol does not participate in these reactions as the free alcohol, but rather must be phosphorylated. In many tissues, most of the dolichol is present as the free alcohol or is esterified to fatty acids and thus cannot participate in oligosaccharide synthesis. The glycose residues which are transferred to the dolichol phosphate or lipid-linked oligosaccharides are attached either to nucleotides or lipid. The end product of these reactions is a lipid-linked oligosaccharide containing three glucose, nine mannose and two N-acetylglucosamine residues (Figure 5).

The carbohydrate moiety of the lipid-oligosaccharide is transferred en bloc to the polypeptide and this involves the formation of a N-glycosylamine bond between a GlcNAc and an asparagine (Jamieson, 1983). Protein structure is important to the site of attachment and the asparagine must be part of what has come to be known as the "asparagine sequon". The amino acid sequence in the asparagine sequon is Asn-X-Ser(Thr) where X is any amino acid other than proline and the third amino acid on the carboxyl side is serine or threonine. En bloc glycosylation of proteins occurs in the rough endoplasmic reticulum; however, the timing of this event does not appear to be the same for all proteins. While initial glycosylation of many proteins occurs while they are still being translated (Lingappa et al., 1978), there is

Figure 5 - The Dolichol Cycle

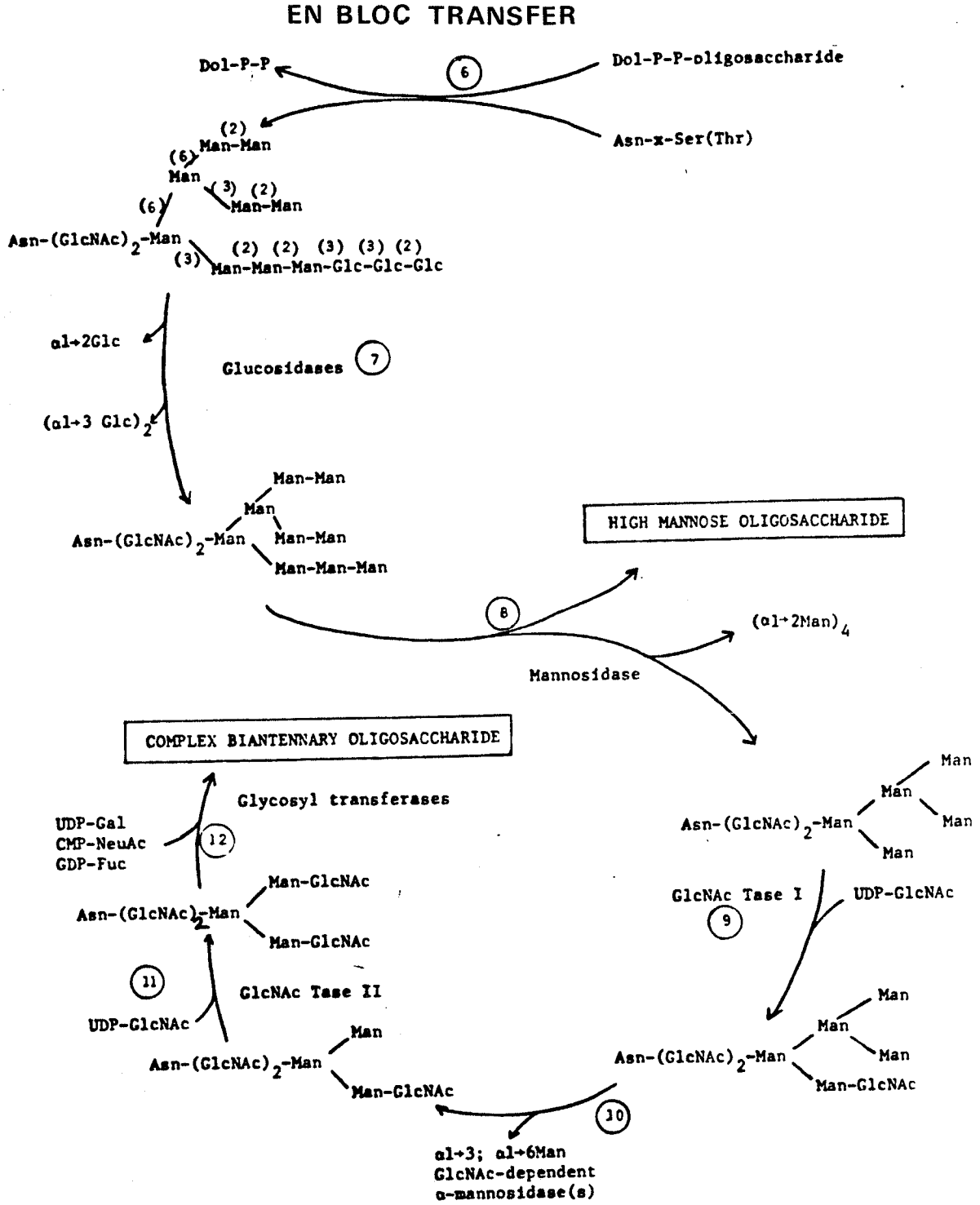
Adapted from Jamieson (1983). Oligosaccharide precursor is synthesized while attached to the lipid dolichol, and then the high mannose-containing oligosaccharide is transfered en bloc to acceptor sites on proteins. Circled numbers indicate discrete steps in this process; the numbers in parentheses indicate the nature of the linkages. See text for further explanation.

evidence that others are glycosylated after completion of translation (Jamieson, 1977; Hubbard & Ivatt, 1981; Nickerson & Fuller, 1981). The functional significance, if any, of these differences in timing of glycosylation is not clear.

After the en bloc transfer of oligosaccharide, some of the sugars are removed from the precursor glycoprotein by what is now known as oligosaccharide processing (Hubbard & Ivatt, 1981; Hussa, 1981; Jamieson, 1983) and this process is summarized in Figure 6. The first sugars to be removed are the glucose residues and this occurs in the rough endoplasmic reticulum immediately after glycosylation. Processing glycosidases responsible for the removal of sugars from the precursor glycoproteins have neutral pH optima in contrast to the lysosomal glycosidases which have acidic pH optima and which are responsible for the degradation of glycoconjugates. Removal of mannose residues occurs in two steps. First the $\alpha 1 \rightarrow 2$ linked mannoses are removed in the Golgi complex. If these mannoses are not removed, then a high mannose type of glycoprotein is produced, whereas removal of these mannoses commits the precursor to becoming a complex-type of glycoprotein. After removal of these mannoses, a GlcNAc is transferred to $Man_5GlcNAc_2$ complex by GlcNAc Transferase 1. The $\alpha 1 \rightarrow 3$ and $\alpha 1 \rightarrow 6$ linked mannoses are then removed by $\alpha 1 \rightarrow 3, \alpha 1 \rightarrow 6$-Man GlcNAc-dependent α-mannosidase(s). Past this point, complex carbohydrates are built up by the sequential action of N-acetylglucosaminyl-, galactosyl-, sialyl- and fucosyl-transferases. (Bretz et al., 1980; Hubbard & Ivatt, 1981; Hussa, 1981; Jamieson, 1983). The end product of these reactions is a glycoprotein which may remain in

Figure 6 - Processing of N-linked Oligosaccharide

Adapted from Jamieson (1983). After en bloc transfer of carbohydrate to protein, sugars are either removed from the carbohydrate moieties of the precursor glycoproteins by the processing glycosidases mannosidase and glucosidase, or added by the glycosyltransferases N-acetylhexosaminyltransferase (GlcNAc Tase I and II), galactosyl-transferase, sialyltransferase or fucosyltransferase. Circled numbers indicate discrete steps in this process; the numbers in parentheses indicate the nature of the linkages. See text for further explanation.


EN BLOC TRANSFER
Dol-P-P
Dol-P-P-oligosaccharide
6
Asn-x-Ser(Thr)
(2) Man-Man
(6) Man
(3) (2) Man-Man
(6)
Asn-(GlcNAc)2-Man
(3)
(2) (2) (3) (3) (2)
Man-Man-Man-Glc-Glc-Glc
α1→2Glc
Glucosidases 7
(α1→3 Glc)2
Man-Man
Man
Asn-(GlcNAc)2-Man
Man-Man
Man-Man-Man
HIGH MANNOSE OLIGOSACCHARIDE
8
(α1→2Man)4
Mannosidase
COMPLEX BIANTENNARY OLIGOSACCHARIDE
Glycosyl transferases
UDP-Gal
CMP-NeuAc
GDP-Fuc
12
Man-GlcNAc
Asn-(GlcNAc)2-Man
Man-GlcNAc
11
GlcNAc Tase II
UDP-GlcNAc
Man
Asn-(GlcNAc)2-Man
Man-GlcNAc
Man
Man
Asn-(GlcNAc)2-Man
Man
GlcNAc Tase I
9
UDP-GlcNAc
Man
Man
Asn-(GlcNAc)2-Man
Man
Man-GlcNAc
10
α1→3; α1→6Man
GlcNAc-dependent
α-mannosidase(s)

the cell as a membrane glycoprotein or a lysosomal enzyme, or may be secreted from the cell.

2.4 Transport, Localization and Secretion of Proteins and Glycoproteins

The intricate process by which proteins and glycoproteins are localized at their sites of action is coupled to the process of synthesis and processing (Jamieson, 1983). The mechanisms by which these processes are coupled are beginning to be understood.

The translation of polypeptide can occur on ribosomes free in the cytoplasm of the cell or attached to the rough endoplasmic reticulum. This distinction appears to be functional as there is a bias for the translation of secretory proteins on membrane bound polyribosomes (Rolleston, 1974). The generally accepted model for this subcellular localization of protein synthesis is the Signal Hypothesis (Blobel & Dobberstein, 1975a, 1975b; Blobel *et al.*, 1979; Walter & Blobel, 1981a, 1981b; Walter *et al.*, 1981; Schreiber & Urban, 1978; Kreil, 1981). The Signal Hypothesis of Blobel & Dobberstein proposes that there exist "signal codons" 3' to the initiation codon and that these codons specify a "signal" polypeptide which will interact with membrane proteins prior to completion of the nascent polypeptide chain. Transcripts of mRNA for secretory proteins contain these signal codons while those for cytoplasmic proteins do not. Initiation of translation of all proteins is thought to occur on ribosomes free in the cytoplasm. Once the signal sequence of secretory proteins is translated and extruded from the ribosome, it is believed to bind to a signal recognition protein (SRP) which arrests the synthesis of the protein and thus prevents

completion in the cytoplasm. This arrested ribosome-mRNA-nascent polypeptide complex binds to "translocation competent sites" on the rough endoplasmic reticulum and this allows the synthesis of the protein to continue concomitant with its translocation across the membrane. A signal peptidase present in the translocation competent sites is thought to remove the signal peptide from the amino terminal of the polypeptide prior to completion of protein synthesis and release of the polypeptide from the ribosome. In this way, it is believed that the synthesis of secretory proteins is localized at the first step of secretion from the cell.

Following segregation of proteins in the rough endoplasmic reticulum, secretory, membrane and lysosomal proteins are transported to the Golgi complex *via* the smooth endoplasmic reticulum (Jamieson,1981; Hand & Oliver, 1981). While with hepatocytes there is evidence that smooth tubular continuities may exist between the rough endoplasmic reticulum and the Golgi, in most other tissues the transfer of protein occurs *via* small transporting vesicles that bud from the ribosome-free portions of the rough endoplasmic reticulum, termed transitional endoplasmic reticulum, and which is adjacent to the cis side of the Golgi apparatus. The "smooth microsomes" of most tissues are believed to consist primarily of these transporting vesicles. The transport of secretory proteins from the RER to the Golgi requires energy in the form of ATP; the energy-requiring step may be at the level of formation of the transporting vesicles and microtubule-mediated movement of these vesicles. The Golgi complex is a complex subcellular component with cis

and trans compartments and is the site of post-translational modifications such as the addition of GlcNAc, Gal and NeuAc to complex glycoproteins (Rothman, 1981; Bretz et al., 1981; Hand & Oliver, 1981; Jamieson, 1983). Usually transporting vesicles fuse with the cis side of the Golgi apparatus, although with some physiological states of the cell the vesicles can fuse at various levels of the Golgi apparatus or even with the forming secretory granules at the trans side,and thus bypass the Golgi altogether (Hand & Oliver, 1981; Jamieson, 1981).

Proteins which will be secreted from cells are packaged into secretory granules at the trans side of the Golgi apparatus (Palade, 1975; Chertow, 1981; Cohn & MacGregor, 1981; Hand & Oliver, 1981; Wilson, 1981). After packaging of proteins into these vesicles, maturation of the vesicles and the contents occurs. This maturation includes proteolytic processing of some precursor proteins. Contents of secretory granules are either stored, degraded or secreted and there is evidence for different classes of secretory granules in both parathyroid gland and liver (Schreiber et al., 1979; Cohn & MacGregor, 1981; Ledford & Davis, 1983). Different secretory granules could explain different transit times of proteins though cells. After the formation of secretory granules, the microtubular-microfilament system contracts and the secretory granule membrane fuses with the cell membrane and ruptures. Proteins associated with the membrane of the secretory granules become membrane proteins and those not associated are secreted from the cell.

In addition to the localization of membranes and secretory protein,

the Golgi complex plays a role in the targetting of glycoproteins to lysosomes (Hand & Oliver, 1981; Sly et al., 1981; Sly & Fisher, 1982; Natowicz et al., 1983). Lysosomes appear to arise in a smooth membrane tubular network that is closely associated with the endoplasmic reticulum and the Golgi. This network has been referred to as the Golgi-Endoplasmic Reticulum-Lysosome (GERL). Enzymes destined for the lysosomes are high mannose forms and covalent modification of the carbohydrate moieties of these glycoproteins results in attachment of a phosphate to mannose residues; this 6-phosphomannose appears to be a recognition marker for both internal transport of enzymes to the lysosomes and the uptake of extracellular enzymes into lysosomes.

While it is evident that there is a coupling of synthesis, transport, post-translational modification and localization of proteins and glycoproteins, alterations in these processes in different physiological states are only beginning to be understood.

3. Regulation by Leukocyte Products

It is evident that tissue not normally considered as part of the endocrine systems produce factors which are involved in the regulation of the activity of cells. Leukocytes are responsible for the production of some of these regulators; an understanding of both the cells responsible for producing these factors and the leukocytes themselves is necessary prior to delineation of their mechanism of action.

3.1 Leukocytes

Blood is a fluid that contains cells and plays numerous roles in the maintanence of homeostasis in higher animals. Blood cells may be classified as either erythrocytes (red blood cells) which function primarily in the transport of oxygen to tissue, or leukocytes (white blood cells) which have roles in host defence. Leukocytes can be classified into two families on the basis of granular or non-granular cytoplasms and also into subgroups of these families. Table 4 gives the relative proportions of the different types of leukocytes present in normal blood.

Blood also contains platelets which are not complete cells but rather fragments of protoplasm from megakaryotes of the bone marrow (Ham & Leeson, 1961). Platelets play integral and complex roles in the response to blood vessel injury (Hoak et al., 1980; Walsh, 1981). Platelets participate in blood coagulation by 1) secretion from α-granules of coagulation proteins including factor V, fibrinogen and an antiheparin protein 2) membrane binding of coagulation enzymes including thrombin and factor Xa 3) surface-mediated activation of coagulation proteins including prothrombin and factors XII, XI and X 4) protection of coagulation enzymes including factors XIa and Xa from inactivation by plasma protease inhibitors. By these and other mechanisms, platelets are intricately involved in blood clotting and thrombosis.

The traffic of granular leukocytes, granulocytes or polymorphonuclear leukocytes is unidirectional in that they are made, stored and

Table 4 - Distribution of Leukocyte Types in Human Blood

Leukocyte Type	Percentage of Blood Leukocytes*
Polymorphonuclear Leukocytes	
Neutrophils	60 - 70
Acidophils	1 - 3
Basophils	0.5
Non-Granular Leukocytes	
Lymphocytes	20 - 35
Monocytes	3 - 8

*Taken from Ham & Leeson (1961) and Rapaport (1971)

discharged from the bone marrow; stay only briefly in the blood; pass into the tissues where they live for only one or two days and do not reenter the blood (Rapaport, 1971; Guyton, 1976; Bainton, 1981). Granulocytes can be subclassified as neutrophils, basophils and acidophils or eosinophils on the basis of the pH of the cytoplasmic granules (Ham & Leeson, 1961). At the site of inflammation, granulocytes are involved in phagocytosing and destroying foreign matter. In addition to this bactericidal role, acidophils participate in aspects of allergic reactions by phagocytosing antibody-antigen complexes, while basophils release the vasoactive substance histamine from storage granules when triggered by immunoglobulin E binding (Weiss, 1973; Sirigian et al., 1982).

There are two types of non-granular leukocytes; lymphocytes and monocytes (Ham & Leeson, 1961). Monocytes are motile cells which migrate into tissues during inflammation and differentiate into highly phagocytic macrophages (Weiss, 1973; Adams, 1982). Activated macrophages also acquire the ability to kill neoplastic cells. Monocytes may remain in tissues for some time and thus become part of the reticuloendothelial system. A good example of this are the Kupffer cells of the liver. Monocytes are also capable of synthesis of immunoglobulin E which suggests a role in allergies (Buckley et al., 1981).

Lymphocytes are divided into two populations: "T" and "B" cells (Guyton, 1976; Cantor, 1982). T-lymphocytes, T-cells or thymocytes are preprocessed by the thymus gland shortly before or after birth, and in general are responsible for cellular immunity. B-lymphocytes or B-cells are preprocessed in the Bursa of Farbricius in birds, but not in mammals, and in general are responsible for humoral immunity or the

production of antibodies of immunoglobulins. B-lymphocytes, upon exposure to an antigen, can differentiate either into plasma cells which are actively involved in the production of antibodies against the stimulating antigen, or into "memory cells" which are dormant B-cells and which respond to a second stimulation by the same antigen with a much more rapid and potent antibody production. The clonal selection theory of antibody production assumes that each immunologic cell is genetically programmed to express a single receptor that binds a unique chemical configuration or antigenic determinant. When the antigenic determinant is bound by the receptor, the cell is stimulated to multiply rapidly and to give rise to a clone of thousands of daughter cells. Some cells within this clone will produce and secrete antibody against that antigen while others will provide the memory of exposure to the antigen.

It is important that the events described above occur when antigens from foreign invaders are present and also that it does not occur in response to chemical determinants of the body's own tissue. Three major types of T-cells can be defined: inducer T-cells which stimulate immunity, suppressor T-cells which suppress immune responses and the killer T-cells (Rosenstreich & Wahl, 1979; Cantor, 1982). Inducer T-cells secrete peptides which activate B-cells to respond to antigens. Inducer T-cell peptides also activate suppressor T-cells to turn off the inducer cells which leads to a transitory stimulation of B-cells. The third type of T-cell subpopulation is the killer T-cell (Ballas & Henney, 1979; Rosenstreich & Wahl, 1979). These killer T-cells are active in the rejection of transplanted tissue and in the regulation of growth of certain neoplasias. The cytotoxic action on these cells is

independent of the antibody and complement systems. Lysis mediated by T-cells is specific in that only target cells bearing antigens shared with those cells used to initiate T-cell production are killed. By these and other more complex mechanisms, non-granular leukocytes are involved in protecting an organism from microbial invasion.

There also is a population of cytotoxic leukocytes which do not require exposure to antigen to manifest activity (Warner & Dennart, 1982). These cells have been termed natural killer cells (NK) and have been defined by their ability to lyse tumor cells *in vitro* as well as by their presence in normal animals. It has been proposed that since NK cells arise spontaneously without priming, they play an important role in the immunosurveillance and control of early neoplastic growth. NK cells are difficult to classify as they lack many of the classical surface markers of T- and B-cells and it is known that they do not require processing by the thymus (Ballas & Henney, 1979; Warner & Dennart, 1982). However, it has been proposed that NK cells are a part of the T-cell subpopulation because they share one surface marker with mature T-cells and since clones of NK cells arise from T-cell cultures (Warner & Dennart, 1982). There is also evidence that the precursors to the blood monocytes (promonocytes) have natural killing activity (Lohmann-Mathes *et al.*, 1980).

Mast cells are leukocytes which are found primarily in loose connective tissue (Bloom, 1965; Laurence, 1979; Winslow & Austen, 1982). While the embryological source of the mast cells is not clear, there is evidence that mast cells and basophils share a common origin in the granulocyte precursor. Mast cells and basophils both produce histamine

and heparin and they are similar to the point that basophils have been termed the blood mast cells. Both basophils and mast cells have cell surface receptors for immunoglobulin E and the binding of this ligand leads to the release of many substances including the biogenic amines histamine and serotonin, acidophil and neutrophil chemotactic factors, the neutral proteases chymase and carboxypeptidase A, glycosidases and products of arachidonic acid metabolism. These factors play an important role in allergic reactions.

In addition to the morphological and functional differences described above, leukocytes, like all cells, can be classified on the basis of their cell surface alloantigens (Eisen, 1974). Allo- or iso-antigens are defined as substances which are immunogenic, or capable of stimulating an immune response, in some other members of the species but not in the donor. Two groups of alloantigens have been defined: the blood group antigens and the histocompatability or transplantation antigens.

The blood group antigens are responsible for determining whether transfused blood cells are recognized by the body's immune system as "self" and accepted or "non-self" and destroyed (Eisen, 1974). In humans these antigens have been classified by the A, B, H, Rh and Le antigens. The differences between these antigens arise from differences in the structures of the carbohydrate moieties of glycoconjugates on the cell surface.

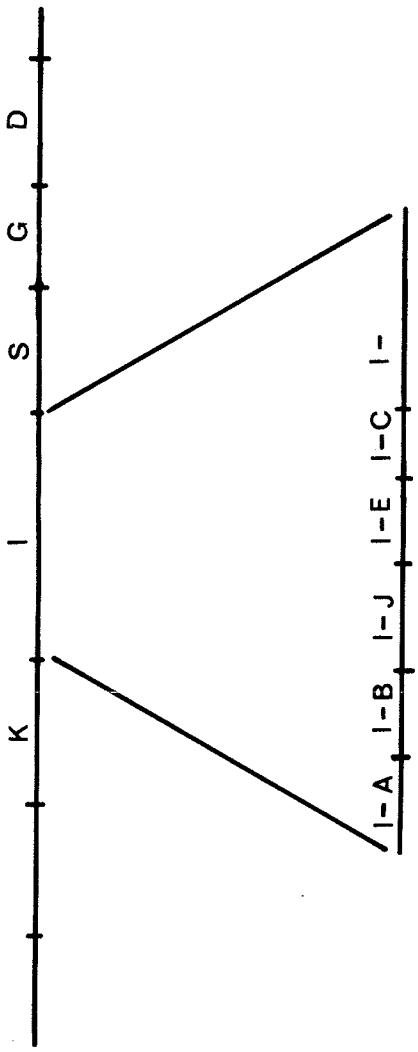
Histocompatability antigens are those portions of the cell surface of tissues which initiate rejection of transplanted tissue (Eisen, 1974; Feldman et al., 1979; Nathenson et al., 1981; Scott et al., 1982).

Histocompatability antigens are cell surface glycoproteins and the specificity with respect to immune tolerance resides in the polypeptide moiety. Histocompatability antigens are specified by the histocompatability or H genes. In the mouse, it has been determined that there are about thirty H loci, one of which, the H2-locus, has about nineteen alleles and is noteworthy because its gene products evoke the most intense of rejection reactions. The H2 region of the major histocompatability complex (MHC) of the mouse is shown in Figure 7. In humans, the major histocompatability locus A (HLA) is quite similar to the mouse H2 locus and has been located on the short arm of chromosome 6 (Farid & Bear, 1981). The HLA-A,B region of the human MHC is analogous to the K region of the mouse H2 system and the HLA-D is equivalent to the I-A region. HLA-A, B and C alloantigens are expressed on the cell surface of all nucleated cells while HLA-D antigens are found on B-lymphocytes, monocytes, sperm and some T-lymphocytes. It is likely that multiple amino acid changes account for the differences in alloantigenicity of the cell surface glycoproteins.

It has become obvious in recent years that alloantigens are important to the interaction of leukocytes. For example, it is accepted that stimulation of T-cells by antigens requires the presence of macrophages (Sprent, 1980). It is believed that T-cells have detectable specificity not for free antigens but only for antigens which are presented in association with MHC gene products. Macrophages seem to "process" antigen and attach small moieties of antigens to cell surface H2 determinants; T-cells recognize the complex and are stimulated. Similarily, the activation by stimulated T-cells of B-cells to produce

Figure 7 - H2 Region of Murine Histocompatability Complex

Adapted from Feldmann et al. (1979). The position of the K, I, S, G and D regions of the seventeenth chromosome of the mouse are shown. Subregions of the I region are also shown and are in a state of flux. See text for further explanation.


D
G
S
I
K
I-
I-C
I-E
I-J
I-B
I-A

antibody appears to require that I determinants of the H-2 region are presented with surface bound antigen by the B-cell to the T-cell (Macrack et al., 1980). While these and other cell-cell interactions of leukocytes are complex by themselves, they are augmented, suppressed or replaced by factors produced by the leukocytes.

3.2 Cytokines

It is evident that lymphocytes produce non-antibody substances which are involved in the mediation of many of the responses to inflammation or infectious agents (Pick et al., 1979; deWeck, 1981). A group of these substances have been termed "lymphokines", a term which is synonymous with the terms lymphocyte mediator, lymphocyte activation product, mediator of cellular immunity and soluble mediator of immunologic regulation. Lymphokines are the protein products of lymphocytes activated in response to the presentation of antigens. Lymphokines are not immunoglobulins or immunoglobulin fragments. Lymphokines do not exhibit specificity for the inducing agent. As their synonyms suggest, lymphokines are involved in mediating activity of cells during the immunological response to infection or inflammation, and they exhibit multiple effects of inflammatory cells as well as proliferative and cytodestructive activities. Lately it has been discovered that nonlymphoid cells can produce mediators with physiological and biological properties similar to or identical with conventional lymphokines. The term cytokine has been used for all soluble substances that are characterized by their ability to cause the same effects on target cells as classical lymphokines (Bigazzi, 1979; Oppenheim, 1982). The term

monokine refers specifically to those cytokines produced by monocytes. Rapid accumulation of knowledge on the production, nature and activity of cytokines has greatly expanded this field of research.

Cytokines are a very diverse group of regulators and can be produced by lymphocytes, monocytes, platelets, fibroblasts and keratinocytes (Oppenheim et al., 1982). These factors are very potent and are physiologically active at concentrations of 10^{-10} to 10^{-15} M. A range of activities can be seen even from the small selection of cytokines shown in Table 5. While many of the activities of cytokines are exerted on leukocytes, these regulators also modulate the activity of osteoclasts, fibroblasts, hepatocytes and hypothalamo cells. Often an individual cytokine is produced by a single cell type but this is not a strict generalization. Production of colony stimulating factor by lymphocytes, macrophages and fibroblasts is a good example of the release of a cytokine activity by various cell types. Individual cytokines do not necessarily have single activities. While the only activity reported to date of osteoclast activating factor is on the degradation of bone, interleukin-1 has a variety of effects on lymphocytes, fibroblasts, hepatocytes and hypothalamo cells. It should be possible in the future to make better generalizations about the properties as more work is done on the isolation, characterization and bioactivities of these substances.

Despite the difficulties inherent in the process, there have been numerous attempts to find a suitable basis for the classification of cytokines. The cell type producing the cytokines has represented one

Table 5 - Activities of Representative Cytokines

Target Cell	Origin	Name	Activity	References
Lymphocytes	Monocytes	Interleukin-1, Lymphocyte Activating Factor (LAF)	Augments mitogen-induced proliferation	Oppenheim et al., 1982
T-Lymphocytes	Lymphoma	Interleukin-1	Augments mitogen-induced proliferation	Palacios et al., 1982
T-Lymphocytes	T-Lymphocytes	Interleukin-2, T-Cell Growth Factor (TCGF)	Augments induction of cytotoxic response, enhances mitogen induced proliferation	Farrar & Fuller-Farrar, 1981; Lachman et al., 1981; Mier & Gallo, 1982
T-Lymphocytes	Lymphocytes	Interleukin-3	Regulates early T-cell differentiation	Ihle et al., 1981, 1982
B-Lymphocytes	T-Lymphocytes	T-Cell Replacing Factor (TCRF)	Induces terminal differentiation of proliferating B-cells into antibody secreting cells	Farrar & Hilfiker, 1982; Schimpl & Wecker, 1979
Polymorphonuclear Leukocytes (PMN)	Lymphocytes	PMN-Migration Inhibitory Factor	Inhibits PMN migration	Pick, 1979
Monocytes	T-Lymphocytes, Macrophages, Fibroblasts	Colony Stimulating Factor (CSF)	Increases RNA and protein synthesis and secretion of prostaglandin E and interferon	Farrar & Hilfiker, 1982; Moore et al., 1981

Table 5 - continued

Target Cell	Origin	Name	Activity	References
Macrophages	Lymphocytes, Monocytes	Macrophage-Migration Inhibitory Factor	Inhibits macrophage migration	Pick, 1979; Cohen, 1980; Weiser et al., 1980
Macrophages	Lymphocytes	Macrophage Activating Factor (MAF)	Enhances macrophage mediated cytotoxicity	McDaniel, 1980; Weiser et al., 1980; Peissens et al., 1981
Natural Killer (NK) Cells	T-Lymphocytes	Interleukin-2, TCGF	Augments NK cell activity	Mier & Gallo, 1982
Natural Killer Cells	Monocytes, Lymphocytes	Interferon	Induces cytotoxic activity	Epstein, 1979; Sakela et al., 1980; Warner & Dennart, 1982
Rheumatoid Synovium Cells	Monocytes	Interleukin-1, Monocyte Cell Factor (MCF)	Modulates collagenase and prostaglandin E2 production	Krane & Dayer, 1980
Osteoclasts	Lymphocytes	Osteoclast Activating Factor (OAF)	Stimulates release of bone mineral and degradation of bone matrix	Mundy, 1981
L-929 Fibroblasts, HeLa Cells	Lymphocytes	Lymphotoxin (LT)	Inhibits growth and induces cell lysis	Granger et al., 1979

Table 5 - continued

Target Cell	Origin	Name	Activity	References
Fibroblasts	Macrophage	Interleukin-1, Fibroblast Activating Factor (FAF)	Stimulates fibroblast proliferation	Wahl et al., 1980; Wahl & Wahl, 1981; Schmidt et al.,1982
Fibroblasts	T-Lymphocyte	Fibroblast Activating Factor	Stimulates fibroblast proliferation	Wahl & Gately, 1983
Fibroblasts	Lymphocytes	Fibroblast Activating Factor	Stimulates collagenase synthesis	Wahl et al., 1979
Hypothalamo Cells	Monocytes	Interleukin-1, Endogenous Pyrogen (EP)	Stimulates fever	Bornstein, 1982; Oppenheim et al., 1982
Hepatocytes	Monocytes	Interleukin-1, Leukocytic Endogenous Mediator (LEM)	Stimulates amino acid uptake, RNA synthesis and acute phase reactant synthesis	Bornstein, 1982; Kampschmidt et al., 1982; Oppenheim et al., 1982
Hepatocytes	Monocytes	Hepatocyte Stimulating Factor (HSF)	Stimulates fibrinogen synthesis and secretion	Fuller & Ritchie, 1982

basis of classification although, as discussed above, cytokines are not always produced by a single cell type. Yoshida and Cohen (1982) have suggested classification on the basis of activity. Many cytokines affect 1) proliferation (eg. interleukins 1 and 2, lymphotoxins); 2) differentiation (eg. interleukin 3, T-cell replacing factor); 3) mobility (eg. migration inhibitory factor); or 4) activation or cytotoxicity (macrophage activating factor, interferon). However, cytokines such as interleukin-1, osteoclast activating factor and colony stimulating factor have effects which do not readily fit into these classifications. It has also been suggested that cytokines be classified on the basis of whether or not they are coded within the genetic region known as the major histocompatability complex (Pick et al., 1979; Waksman, 1979). Of the cytokines that are gene products of the MHC, many have been localized in particular subregions of the immune response region: I-A for the Antigen-Specific Helper Factors, I-C or I-F for the Mixed Leukocyte Reaction Suppressor Factors and I-J for the Antigen-Specific Suppressor Factors. As yet, no coherent theory exists which explains the production and action of the cytokines much less a generally accepted basis for classification and naming of the cytokines. This has complicated the interpretation of experimental results.

The production of cytokines is generally agreed to be one of the manifestations of stimulation or activation of leukocytes (Mastro, 1979; Metcalf, 1979; Pick et al., 1979; Rosenstreich & Wahl, 1979; Levy et al., 1981; Adams, 1982). Stimulation or activation of leukocytes is a complex and poorly understood process. One definition of "activated macrophages" that has been offered is "those macrophages with enhanced antimicrobial

activity as a result of infection of the animal with facultative intracellular parasites" (Soberman & Karnovsky, 1981). Some difficulties with this definition have arisen from the fact that leukocytes prepared from peritoneal exudate, and which are brought into the peritoneum in response to an intraperitoneal injection of agents such as sterile caseinate, glycogen and proteose-peptone, have some biochemical properties similar to those of immunologically activated cells. In this thesis, "resident cells" will be used to describe cells prepared from untreated control animals, "elicited cells" to described cells prepared after treatment with sterile agents such as caseinate, glycogen and proteose-peptone, and "activated cells" to describe cells responding to foreign antigen or cells prepared under conditions that mimic the action of foreign antigen.

There are a number of differences between the above states of leukocytes in the properties and functions of the cellular membrane (David & Remold, 1979; Edelson, 1981; Sharma & Remington, 1981; Soberman & Karnovsky, 1981). A number of cell surface ecto-enzymes, or enzymes external to the cell, are altered. Ecto-5'-nucleotidase and NAD-cleaving enzyme are depressed in elicited and activated cells compared to resident cells, while ecto-p-nitrophenylphosphatase, ecto-aminopeptidase and alkaline phosphatase I are increased in elicited and activated cells. Also, it has been shown that a cell surface antigen (AMØSCA) is present on activated but not resident or elicited cells. In addition to differences in the cell membrane, there are differences in enzyme levels and secretion. Generally, levels of lysosomal hydrolases are increased with activation or elicitation.

Collagenase, elastase and plasminogen activator also increase upon activation or elicitation. There are also differences between the states of cells with respect to oxidative metabolism. Elicited and activated macrophages undergo a significant respiratory burst during phagocytosis along with a generation of active oxygen species such as superoxide and hydrogen peroxide, while resident macrophages do not. Active oxygen species play a role in the cytotoxic properties of macrophages. As previously mentioned, activated leukocytes also exhibit elevated secretion of cytokines.

There are a number of agents which will trigger the activation of leukocytes and these may be specific or non-specific (Rosenstreich & Wahl, 1979; Fauci, 1982). Specific agents or antigens only trigger the small proportion of cells which bear specific receptors for the agents. Non-specific agents are often referred to as mitogens and trigger cells independent of antigenic specificity. Mitogens tend to exhibit cell type specificity. Lectins such as phytohemagglutinin A (PHA) and concanavalin A (Con A) activate T-cells while others such as pokeweed mitogen (PWM) also activate B-cells. Generally though, B-cell mitogens tend to be large, polymeric compounds such as lipopolysaccharide. There is cooperation between cells in the activation process. The activation of B-cells by most protein antigens requires the presence of T-cells; however, the activation by other stimulants such as lipopolysaccharide (LPS) does not require T-cell help. The activation of T-cells by antigens requires the presence of macrophages. This is due to macrophage secretion of factors such as interleukin-1 and the uptake and presentation of antigens to lymphocytes in a stimulatory form. Also, as previously mentioned, T-cell

activation by certain antigens requires histocompatible macrophages. There has been a recent report (Gilman et al., 1983) that monoclonal antibodies to the Ia alloantigen inhibit T-cell activation and the production of cytokines, a fact which demonstrates the importance of the MHC in leukocyte activation. In addition to the agents described above, a number of membrane perturbing agents such as phorbol esters are capable of activating leukocytes (Mastro, 1982). The phorbol ester, phorbol myristic acetate (PMA), also called 12-O-tetradecanoylphorbol-13-acetate, binds to cell membranes, stimulates membrane phospholipid synthesis and release of arachidonic acid and alters the glycoprotein composition of the cell membrane. PMA-treated leukocytes resemble activated leukocytes described earlier with regard to many biochemical parameters including cytokine synthesis and secretion. Thus the process known as activation, which in vivo occurs in response to antigens, stimulators and cell help, is required for cytokine production and can be mimicked in vitro by appropriate treatment.

Despite the fact that cytokine research is still a very new field, some understanding of the mechanisms of synthesis and secretion of these mediators has been achieved (Pick et al., 1979; Rosenstreich & Wahl, 1979; Mizel, 1981; Mizel & Mizel, 1981). Some of this information has been obtained by studying the effects of pharmacological agents on cytokine production as is shown in Table 6. Although there are some exceptions, cytokine production generally requires RNA and protein synthesis but not DNA synthesis. Secretion of cytokines also seems to involve the microfilaments as is shown in Table 6. In a murine macrophage cell line, the production of interleukin-1 can be "superinduced" by the addition of

Table 6 - Effect of Pharmacological Agents on Lymphokine Production

Agents and Effects	Effects on Production of		
	MIF	LIF	LT
DNA Synthesis Blockers			
Mitomycin C	↓,↔	↔	-
Cytosine Arabinoside	↔	-	-
X Irradiation	↓	↔	↔
RNA Synthesis Blockers			
Actinomycin D	↓	↓	-
Protein Synthesis Blockers			
Puromycin	↓	↓	↓
Pactomycin	↓	-	↓
Cycloheximide	-	↓	↓
Blocking of Microfilaments			
Cytochalasin B	↓	↓	↓
Blocking of Microtubules			
Colchicine	↔	↔	↔
Vinblastine	↔	-	↔

Table 6 - continued

Agents and Effects	Effect on Production of		
	MIF	LIF	LT
Elevated cAMP Levels			
cAMP, dibutyryl cAMP	↓	↓	↓
Cholera Toxin	↔	-	↔
Phosphodiesterase Inhibitors	↓	↓	↓
β-Adrenergic Stimulators	↔	-	↓
Prostaglandin E	↓	↓	↓
Elevated cGMP			
Cholinergic Drugs	↑	↓	↓
Miscellaneous			
Glucocorticoids	↓,↔	-	-

Taken from Rocklin et al. (1980). MIF - Macrophage-Migration Inhibitory Factor; LIF - Leukocyte Inhibitory Factor (LIF); LT - Lymphotoxin. (↓) indicates decreased lymphokine production, (↑) indicates increased lymphokine production, (↔) indicates no effect on lymphokine production, (-) indicates that effect of drug was not examined.

actinomycin D two to four hours after the addition of PMA as the primary stimulator of cytokine production (Mizel & Mizel, 1981). These results suggest that the protocol results in blockage of a protein inhibitor of interleukin-1 production. These observations are also consistent with the work of others (Laurence, 1979; Houck & Patt, 1981) which suggests the existence of "chalones" or factors which inhibit leukocyte activation. Clearly though, a great deal remains to be learned about the production of cytokines by activated leukocytes. Undoubtedly much will be learned about cytokines in general in the near future.

4. Introduction to the Present Work

The acute phase response to inflammation represents a very complex yet coordinated series of reactions which must be regulated such that they provide a benefit to the host at an opportune time following inflammation. The problem of regulation of this response is compounded by the fact that tissue injury and inflammation can occur at any site within the organism and often at sites distal to the organs which must respond and to endocrine organs which may be involved in regulatory aspects. Also, the initial site of tissue injury and acute inflammation may be peripheral to the major network of intracellular communication, the cardiovascular system, and yet an acute phase response is still mounted. The conditions complicate theories on the response of a biological system to the disruption of homeostasis by inflammation and tissue injury.

The studies which will be presented in this thesis were concerned with one aspect of this problem, that is the regulation of hepatic events during the acute phase response to inflammation. Many of these studies examine the involvement of cytokine(s) produced by activated leukocytes on parameters which are altered during the acute phase response. These parameters include serum and hepatic levels of the acute phase reactant α_1-acid glycoprotein and the negative acute phase reactant albumin, hepatic and serum sialyltransferase activities and hepatic glycosidase activities. The studies included a partial characterization of the physicochemical properties of the cytokine(s) and an examination of the *in vivo* and *in vitro* effects of the cytokine(s).

Other studies presented in this thesis were concerned with a further characterization of the experimental system in which the effects of the cytokine(s) were tested: in these, hepatic transcription and biosynthesis of plasma glycoproteins were examined. The specific plasma glycoproteins that were studied were the acute phase reactants α_1-acid glycoprotein and α_2-macroglobulin and the protein α_1-macroglobulin. In addition, some studies were concerned with the physiological changes which occurred within the animals during the acute phase response since the humoral environment and the metabolic activity of the liver is important to the hepatic response to inflammation. These studies consisted of assays of serum hormone levels and of hepatic and serum amino acid pool sizes.

The aims of the studies which are presented in this thesis were to identify some of the levels at which hepatic activity is regulated

during the acute phase response and to identify some of the aspects involved in this regulation. These studies also provide a framework within which delineation of the mechanisms of regulation could be pursued in studies that go beyond the scope of this thesis.

EXPERIMENTAL

1. Materials

1.1 Radioactive Compounds

Sodium [^{3}H]-orotate (19 Ci/mmole), L-[^{3}H]-leucine (1 Ci/mmole), D-1-[^{14}C]-glucosamine (55 mCi/mmole), D-1-[^{14}C]-mannose (50 mCi/mmole) were from Amersham Corp., Oakville, Ont.; cytidine-5'-monophosphate-[^{14}C]-N-acetylneuraminic acid was from New England Nuclear Corp., Lachine, P.Q.

1.2 Chemicals and Kits used with Radioisotopes

ACS scintillation cocktail, [^{125}I]-insulin and [^{125}I]-corticotropin radioimmunoassay kits were from Amersham Corp., Oakville, Ont.; GammaCoat [^{125}I]-cortisol, [^{125}I]-thyroxine and [^{125}I]-triiodothyronine radioimmunoassay kits from Clinical Assays, Travenol Laboratories, Cambridge, Mass.

1.3 Proteins

Bovine serum albumin and trypsin were from Sigma Chemical Co., St. Louis, Mo.; pronase from Calbiochem, La Jolla, Ca.

1.4 Chromatographic Media

Sepharose 6-B and Concanavalin A-Sepharose 4B were from Pharmacia, Uppsala, Sweden; Dowex 1-X8 and Affi-Gel Blue from BioRad Laboratories, Richmond, Ca.; CM-Cellulose (coarse) from Sigma Chemical Co., St. Louis, Mo.

1.5 Hormones and Drugs

Prostaglandins E_1, E_2 and $F_{2\alpha}$ were from Upjohn Co., Kalamazoo, Mich., $N^6,O^{2'}$-dibutyryl adenosine-3',5'-cyclic monophosphoric acid (dibutyryl cAMP), insulin (bovine pancrease crystalline), cortisol-21-acetate, thyroxine, triiodothyronine, phenylbutazone and indomethacin from Sigma Chemical Co., St. Louis, Mo.; salicylate from Merck, Sharp and Dohme Research Laboratories and sulfinpyrazone from Ciba-Geigy.

1.6 Chemicals

Trizma base (tris), glycine, Triton X-100, sodium deoxycholate, glutathione, thimerosal, sucrose, Percoll, Wright Stain WS-1, Rinse Solution RS-1, E Toxate assay kit, Slipper limpets type VIII glycogen, phenol extracted E. coli lipopolysaccharide, heparin, streptomycin sulfate, penicillin G, imidazole, p-nitrophenyl-N-acetyl-β-D-glucosamide, p-nitrophenyl-β-D-galactoside, sodium cacodylate, and L-ornithine were all from Sigma Chemical Co., St. Louis, Mo.; Ampholine Carrier Ampholytes from LKB Produkter AB, Stockholm-Bromma 1, Sweden; Dextran T-70 from Pharmacia, Uppsala Sweden; picric acid from BDH, Poole, England; Celite from Chromatographic Specialities Ltd., Rockville, Ont.; Nobel agar and Casein from Difco Laboratories, Detroit, Mich.; turpentine oil (double rectified) from Fisher Scientific Co., Toronto, Ont. Other chemicals were of analytical grade obtained from local suppliers.

2. Methods

2.1 Physical and Chemical Methods

Absorbances in the visible and ultraviolet regions of the spectrum were measured with a Carl Zeiss PMQ 2 spectrophotometer. Radioactivity was determined with an LKB model 1215 BetaRac II liquid scintillation counter. Conversions to disintegrations per minute were performed using an automatic external standard, a preprogrammed calculation routine and quenching data generated by quenching radioactive standards in ACS counting cocktail with chloroform. Determination of pH values was done with a Fisher Accumet model 610A pH meter.

For isoelectric focusing, a 100 ml isoelectric focusing column (LKB 8100-10) and a gradient mixing device obtained from LKB Produkter AB, Stockholm-Bromma 1, Sweden, were used. Temperature was maintained at 2°C by circulating 10% ethanol from a Haake FKN Circulating Bath with a Haake F4391 Line Heater through the cooling jacket. Power was supplied from a Volkam Mk IV Power Supply.

Immunoelectrophoresis was carried out on equipment supplied by Shandon Scientific Co., Ltd., London.

Examination of slides by bright field microscopy utilized a Series 1820 Biostar Biological Microscope from American Optical, Buffalo, N.Y.

Gel chromatography was performed using an LKB Microperspex model 2132 peristaltic pump; fractions were collected with an LKB Ultrorac fraction collector. For some preparations, protein present in effluent was monitored continuously with an LKB Uvicord at 254 nm.

Triply distilled water was used in the isolation of amino acids for quantitation of hepatic and serum pool sizes. This was prepared by fractional distillation of glass distilled water from alkaline potassium permanganate followed by fractional distillation from acidic potassium dichromate. Hydrochloric acid used in the isolation of amino acids was prepared as constant boiling hydrochloric acid and then diluted with triply distilled water as appropiate. In all other experiments, water used for solutions and medium was purified by a Milli-RQ water purifier (Millipore Corp., Bedford, Ma.) which involved removal of contaminants by reverse osmosis, ion exchange adsorption and sterile filtration through a 0.22 μM filter. Sterilization of aqueous solutions injected into animals was done by filtration through a Millipore Millex GV filter immediately prior to injection. Solutions were tested for endotoxin contamination by the Limulus Amoebocyte Lysate Assay using the E Toxate Assay kit.

Total protein was isolated from samples as described by Jamieson et al. (1975). All operations were performed at 4°C unless otherwise noted. Proteins were precipitated from samples by adding an equal volume 10% trichloroacetic acid and the precipitated proteins were collected by centrifugation at 1,000 g_{av} for 10 min. The pellet was washed twice with each of: 5% trichloroacetic acid, a 4:1 mixture of acetone:0.15 M sodium chloride, a 2:2:1 mixture of ethanol:diethyl ether:chloroform and petroleum ether. The precipitated protein was then dissolved in 0.1 M sodium hydroxide and heated at 80°C to remove ether. Aliquots of the protein solution were neutralized with hydrochloric acid and radioactivity was determined using 10 ml ACS counting cocktail.

Protein content of samples was assayed as described by Lowry et al. (1951) but with modified reagents and volumes described by Miller (1959). Bovine serum albumin was used as the standard. Amino acid analyses were performed with an NC-2P Technicon Amino Acid Analyzer System (Technicon Instruments Corp., Tarrytown, New York). External standards were used to determine amino acid contents of samples analyzed.

A modification of the procedure of Munro and Fleck (1966) was used to isolate total cellular RNA from liver slices. Liver was homogenized in 10 ml water containing 5 mM orotic acid with 5 up and down strokes of a Potter-Elvehjem homogenizer rotating at 2,000 r.p.m. Unless otherwise noted, all subsequent operations were performed at 4°C. Proteins and nucleic acids were precipitated by the addition of 0.5 volumes 0.6 M perchloric acid, collected by centrifugation at 8,500 g_{av} for 20 min and washed three times with 0.2 M perchloric acid. The precipitate was then suspended in 4 ml 0.3 M potassium hydroxide and incubated 1 h at 37°C to hydrolyze RNA to mononucleotides. Protein and DNA were precipitated by the addition of 2.5 ml 1.2 M perchloric acid and the pellet was removed by centrifugation as above. Volumes of 1 ml of supernatants were counted in 10 ml ACS counting cocktail to determine radioactivity incorporated into RNA. The RNA content of supernatants was calculated using the absorbance at 260 nm and an extinction of 1.00 as equivalent to 32 µg RNA as suggested by Munro and Fleck (1966).

2.2 Treatment of Experimental Animals

Male Long Evans Hooded rats of 200-250 g body weight were purchased

locally and were maintained under constant light conditions on a diet of Purina Rat Chow and water ad libitum. Rats which received injections were lightly anesthesized by placing the animals in an air-diethyl ether atmosphere for about 2 min. Experimental inflammation was induced by a subcutaneous injection in the dorsolumbar region of 0.5 ml turpentine oil per 100 g body weight (Ashton et al., 1970); rats which served as controls for studies on inflammation received injections of sterile 0.15 M sodium chloride. Saline and cytokine preparations were sterilized by filtration through Millipore Millex GV filters. Intravenous injections were made into the femoral vein. Intraportal injections were made on rats under ether anesthesia after a laparotomy had been performed. Rats were starved for 16 h prior to sacrifice which was between 9 and 11 a.m. to minimize any diurnal or nutritional effects.

For studies on amino acid pool sizes, blood was collected from heavily etherized animals by heart puncture using a plastic syringe and an 18 gauge needle. Livers were perfused in situ via the portal vein with ice-cold 0.15 M sodium chloride, rapidly excised and transferred to ice-cold 0.15 M sodium chloride. Rats used in the preparation of cytokines were sacrificed by etherization and blood was collected after severing the jugular vein. In all other studies, rats were sacrificed by cervical dislocation; blood was collected in a Nalgene plastic beaker after severing the jugular vein and allowed to clot for 10 min at room temperature. Serum was prepared by centrifuging clotted blood at 5,000 g_{av} for 20 min. Livers were perfused as described above and transferred, unless otherwise noted, to ice-cold 0.15 M sodium chloride.

2.3 Separation of Serum and Hepatic Amino Acids

The procedure used to isolate free amino acids from serum and liver samples was based on that described by Stein and Moore (1954). Serum samples were deproteinized by the addition of 5 volumes 1% w/v picric acid, vortexed, and centrifuged for 2 min at 8,000 g_{av}. Liver samples were homogenized in 10 volumes 1% picric acid using 10 strokes of a Potter-Elvehjem homogenizer rotating at 2,000 r.p.m., and were centrifuged for 10 min at 5,000 g_{av} at 4°C. Picric acid and contaminating acid soluble metabolites were removed from the supernatants by ion-exchange chromatography on Dowex 1-X8. Dowex was converted to the H^+ form by washing with 1M hydrochloric acid, washed extensively with triply distilled water and packed in 5 cm x 1.2 cm columns. Volumes of 0.8 ml serum supernatants or 8 ml liver supernatants were applied directly to these columns and the free amino acids were eluted with 40 ml 0.04 M hydrochloric acid. The effluents were reduced to about 1 ml by rotary evaporation, mixed with 100 mg Celite and filtered through acid-washed Whatman No. 2 filter paper with eight 5 ml aliquots of water. The filtrates were again reduced to about 1 ml and then made to a volume of 2 ml with distilled water. Aliquots of these samples were used directly for amino acid analysis while the rest of the sample was hydrolyzed *in vacuo* with 4 M hydrochloric acid at 100°C for 3 h. Samples were then taken to dryness in a vacuum desiccator containing sodium hydroxide, dissolved in 1 ml triply distilled water and analyzed for amino acids. External standards were used to calculate amounts of amino acids isolated. In some trials, [^{14}C]-leucine was added to samples prior to addition of picric acid and the radioactivity was followed through the procedure

to determine recoveries. In these trials, recovery of the tracer was quantitative.

2.4 Isolation of α_1-Macroglobulin and Albumin from Rat Serum

A method similar to that described by Virca et al. (1978) was used as the first step in the isolation of α_1-macroglobulin and albumin from rat serum. Serum was dialyzed overnight at 4°C against 50 mM sodium phosphate, 0.05% sodium azide, pH 8.0. A 2.5 x 25 cm column of Affi-Gel Blue was packed and equilibrated at room temperature with the same buffer. A 10 ml volume of dialyzed serum was centrifuged at 8,000 g_{av} for 3 min to remove precipitated protein (mostly euglobulin) and the supernatant applied to the column. Elution of an α_1-macroglobulin containing fraction was performed by pumping equilibrating buffer through the column at a rate of 20 ml/h. Protein eluted from the column was monitored by following the absorbance at 254 or 280 nm and fractions were collected at 4°C. The material eluting at the void volume of the column was pooled, dialyzed against water and freeze-dried. This material is referred to as fraction A. After washing the column with 2 bed volumes of equilibrating buffer, fraction B was eluted with 5 bed volumes of 50 mM sodium phosphate, 3 M sodium chloride, 0.05% sodium azide, pH 8.0. Albumin was then eluted from the column with 0.5 M sodium thiocyanate and the albumin containing eluent was dialyzed exhaustively against water and freeze-dried. This material is referred to as fraction C. The Affi-Gel Blue column was regenerated by washing with 5 bed volumes water, then 5 bed volumes 50 mM sodium acetate, 0.05% sodium azide, 0.01% thimerosal, pH 5.0, then 5 bed volumes water and

finally 5 bed volumes 50 mM sodium phosphate, 0.1% sodium azide, pH 8.0.

Albumin was purified to homogeneity from fraction C by isoelectric focusing (Jamieson et al., 1972a). A dense electrode solution containing 0.4 ml concentrated sulfuric acid, 24 g sucrose and 24 ml water was added to the anode at the bottom of the column. A linear sucrose gradient containing ampholytes was introduced into the column using the LKB gradient device. The dense gradient solution contained 1.8 ml Ampholine Carrier Ampholytes pH 4-6, 28 g sucrose and 42 ml water; the light gradient solution contained 0.6 ml Ampholine Carrier Ampholytes pH 4-6, 25 mg fraction C and 60 ml water. Light electrode solution containing 0.1 M sodium hydroxide was added to the cathode at the top of the gradient solution. During isoelectic focusing, the potential applied to the column was increased to 600 V while keeping the current below 15 mA. The column was run until the current dropped below 1 mA. Then material absorbing at 280 nm and which focused at pH 4.7 was pooled, dialyzed exhaustively against water and freeze-dried. This material was pure rat serum albumin as determined by double diffusion analysis against anti-whole rat serum and anti-albumin.

The α_1-macroglobulin was further purified from fraction A by affinity chromatography on concanavalin A-Sepharose as described by Lostutoff (1978). A 15 x 1.0 cm column of concanavalin A-Sepharose was packed and equilibrated at room temperature with 0.1 M Tris, 0.1 M sodium chloride, 0.01% sodium azide, pH 7.4. A sample of 400 mg fraction A dissolved in the same buffer was applied to the column which was then washed with the same buffer. The α_1-macroglobulin was then eluted from the column with 0.1 M Tris, 0.1 M sodium chloride, 0.01% sodium azide,

0.1 M α-methyl-D-mannoside, pH 7.4 at 37°C. The above fraction was dialyzed against water, freeze-dried and designated fraction A-2. The concanavalin A-Sepharose column was regenerated by washing with 5 bed volumes 0.1 M Tris, 0.5 M sodium chloride, 0.01% sodium azide, pH 8.5; then 5 bed volumes 0.1 M sodium acetate, 0.5 M sodium chloride, 1 mM magnesium chloride, 1 mM calcium chloride, 0.01% sodium azide, pH 4.5; then with 10 bed volumes 0.1 M sodium acetate, 0.5 M sodium chloride, 1 mM magnesium chloride, 1 mM calcium chloride, 0.01% sodium azide, pH 6.0 after which it was stored at 4°C for future use.

A pure preparation of α_1-macroglobulin was obtained by chromatography of fraction A-2 on Sepharose 6B. A 45 x 5 cm column of Sepharose 6B was packed and equilibrated with 20 mM sodium phosphate, 0.01% sodium azide, pH 7.0 at 4°C. A sample of 100 mg fraction A-2 was dissolved in 2 ml equilibrating buffer, centrifuged at 8,000 g_{av} for 2 min and the supernatant applied to the column which was then eluted at a rate of 25 ml/h with equilibrating buffer. Fractions were analyzed by monitoring the absorbance at 280 nm and by double diffusion analysis against anti-whole rat serum and anti-fraction 5B prepared as described by Jamieson et al. (1972a). Those fractions which gave a single precipitin line against both antisera were pooled, dialyzed and freeze-dried. Determination of the isoelectric point of α_1-macroglobulin was performed as described for the isoelectric focusing of albumin. Amino acid analysis of the α_1-macroglobulin was performed on samples that had been hydrolyzed in vacuo with 6 M hydrochloric acid for 24, 48 and 72 h. Extrapolation to 0 h hydrolysis was performed to account for loss of some amino acids due to hydrolysis.

2.5 Isolation of α_1-Acid Glycoprotein from Rat Serum

A method based on that described by Simkin et al. (1964a) was used to prepare a glycoprotein-rich fraction from rat serum as the first step in the isolation of α_1-acid glycoprotein. Nine volumes 0.15 M sodium chloride were added to rat serum and the mixture was stirred rapidly with a magnetic stirrer at room temperature. The solution was made to 0.6 M perchloric acid by slow addition of 1.8 M perchloric acid, allowed to stand for 10 min and centrifuged at 650 g_{av} for 20 min at 4°C. The supernatant was neutralized to pH 6.5 with 2 M sodium hydroxide, dialyzed exhaustively against water and concentrated using an Amicon model 202 Ultrafiltration Cell fitted with an UM10 Diaflo filter and freeze-dried. This material was designated PCA-soluble fraction.

The PCA-soluble fraction was further purified by chromatography on CM-cellulose as described by Jamieson et al. (1972a). Fines were removed from the CM-cellulose by suspension in water followed by decanting. CM-cellulose was regenerated by washing twice with 0.25 M sodium hydroxide, 0.25 M sodium chloride, with water until neutral and then with 50 mM sodium acetate, pH 4.9. A 45 x 2.5 cm column was packed with CM-cellulose and equilibrated with 50 mM sodium acetate, pH 4.9 at 4°C. A sample of 250 mg PCA-soluble fraction was dissolved in 5 ml equilibrating buffer, the solution centrifuged at 8,000 g_{av} for 2 min and the supernatant applied to the column. The column was eluted at a rate of 40 ml/h with equilibrating buffer. Material that absorbed at 254 nm was pooled, dialyzed against water, freeze-dried and designated fraction CMC-1.

The CMC-1 fraction was further purified by isoelectic focusing on a pH 1-3 gradient as described by Jamieson et al. (1972a). The dense electrode solution contained 0.15 ml concentrated sulfuric acid, 16 ml glycerol and 4 ml distilled water. The dense gradient solution contained 0.1 g monochloracetic acid, 0.1 g orthophosphoric acid, 0.1 g dichloroacetic acid, 35 ml glycerol and 20 ml water. The light gradient solution contained 0.1 g acetic acid, 0.1 g formic acid, 0.1 g citric acid, 0.1 g glutamic acid, 0.05 g aspartic acid, 0.30 ml Ampholine Carrier Ampholytes pH 5-8, 30 mg CMC-1 and 60 ml distilled water. The light electrode solution was 0.1 M sodium hydroxide. Isoelectric focusing was performed as described for albumin and the material which focused at pH 2.95 was pooled, dialyzed exhaustively against water and freeze-dried. This material was pure α_1-acid glycoprotein as determined by double diffusion analysis against anti-whole rat serum and anti-α_1-acid glycoprotein.

2.6 Immunological Methods

Antisera were prepared essentially as described by Simkin et al. (1964b) using male Full Lop Albino rabbits. A mixture of 0.75 mg antigen, 0.25 ml sterile 0.15 M sodium chloride and 0.4 ml Freund's complete adjuvant was emulsified by sonication using 15 sec bursts at 60 percent maximum power setting of an Artec Sonic Dismembrator fitted with a P200-1 probe. This emulsion was injected intramuscularly, one-half of the dose into each thigh of the rabbit. Six days later, 1.25 mg antigen in an emulsion prepared as above was injected. After a further 22 days, a series of intravenous injections of a coprecipitate of antigen with aluminum hydroxide were given. To prepare the coprecipitate, 1 M sodium

bicarbonate was added to neutralize a mixture of 0.4 ml 10% potassium alum and 6 mg antigen in 0.2 ml water. The neutralized solution was allowed to stand 16 h at 2°C, and the precipitate was collected by centrifugation and suspended in 0.4 ml sterile 0.15 M sodium phosphate, 0.01% thimerosal, pH 7.4. Samples of 0.05 ml, 0.10 ml and 0.25 ml of the suspension were injected on alternate days. The rabbits were bled 5 days after the final injection. Blood obtained was allowed to clot at room temperature for 1 h, centrifuged at 3,000 g_{av} for 20 min to remove the clot and the supernatant was taken as antiserum. This was stored at -20°C until required.

Double diffusion analysis of test solutions and antisera was performed as described by Ouchterlony (1953). The medium used consisted of 1.25% Nobel Agar, 0.15 M sodium chloride, 0.05% sodium azide and the plates were allowed to develop at room temperature in a humidity cabinet. Wells were cut using a BioRad Template System and Universal Puncher. Radial immunodiffusion was performed essentially as described by Shuttler et al. (1977). Gels contained 1.5% antiserum, 1% Nobel Agar, 0.15 M sodium chloride, 0.01% sodium azide. Wells of 2.5 mm diameter were cut as described above and diffusion was allowed to proceed for 24-48 h at room temperature in a humidity cabinet. The amount of antigen in a test sample was determined by measuring the diameter of the precipitin ring with a Hyland Precision Viewer fitted with a micrometer scale.

Quantitative immunoprecipitation of serum and liver slice medium samples was performed as described by Jamieson et al. (1972b). Medium

samples were first concentrated by ultrafiltration with concurrent dialysis against 0.15 M sodium chloride. Samples containing antigen were made to a volume of 0.45 ml in 0.15 M sodium chloride, 1 mM sodium azide and 4.7% Dextran T70. A volume of 0.05 ml anti-albumin, anti-α_1-macroglobulin or anti-α_2-macroglobulin or 0.15 ml anti-α_1-acid glycoprotein was added to the samples which were incubated at 37°C for 45 min and allowed to stand at 4°C for 48 h. Precipitates were collected by centrifuging at 2,000 g_{av} for 10 min, washed once with 0.3 ml 0.15 M sodium chloride, 4% Dextran T70, and three times with 0.3 ml 0.15 M sodium chloride and dissolved in 0.1 M sodium hydroxide for protein determinations. Immunoprecipitation of liver subcellular fractions were first concentrated by ultrafiltration with concurrent dialysis against 0.15 M sodium chloride, 1% Lubrol. These samples were cleared of interfering material by heterologous immunoprecipitation. Immunoprecipitation mixtures were prepared as described above with the exception that 0.05 ml anti-whole human plasma proteins was used as the antisera and 50 µg human albumin was added to the mixture; after incubations at 37°C and 4°C, immune complexes were removed by centrifugation and quantitative immunoprecipitation was performed on these cleared samples as described for the serum and medium samples.

Immunoelectrophoresis was performed in 1% agarose gels as described by Ashton et al. (1970) using equipment supplied by Shandon Scientific Co. Ltd. Immunoelectrophoresis buffer was 15.8 mM sodium diethylbarbiturate, 22 mM hydrochloric acid, 18 mM sodium acetate, pH 8.6. A potential of 80 V was applied for 90 min and then the center well was

filled with antiserum and allowed to develop in a humidity cabinet until precipitin lines appeared.

Antiserum to α_2-macroglobulin prepared as described by Jamieson et al. (1972a) was used in some experiments as was antiserum to fraction 5B. These antisera were a kind gift of Dr. J.C.Jamieson.

2.7 Subcellular Fractionations

Rough and smooth endoplasmic reticulum fractions were prepared as described by Jamieson and Ashton (1973). Livers were perfused in situ with 0.15 M sodium choride, excised into 0.25 M sucrose and homogenized in 3 volumes 0.25 M sucrose with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 36,000 g_{av} for 20 min at 4°C to remove nuclei and mitochondria and the supernatant was made to a final concentration of 15 mM cesium chloride using 1 M cesium chloride. The solution was then layered on 10 ml 1.3 M sucrose, 15 mM cesium chloride and centrifuged at 106,000 g_{av} for 150 min in a Ti60 rotor at 4°C. Rough endoplasmic reticulum formed a pellet and smooth endoplasmic reticulum was found at the interface of the sucrose solutions. The smooth endoplasmic reticulum was aspirated with a Pasteur pipette, made to 10 mM magnesium chloride with 1 M magnesium chloride and diluted with an equal volume of 0.25 M sucrose. This solution was centrifuged at 106,000 g_{av} for 150 min to pellet smooth endoplasmic reticulum.

Golgi fractions were obtained as described by Jamieson and Ashton (1973). Livers were perfused in situ with 0.15 M sodium chloride and excised into 0.5 M sucrose in medium A (0.1 M Tris, 10 mM magnesium

chloride, 1% Dextran, pH 7.6). Livers were homogenized in 4 volumes 0.5 M sucrose in medium A with a Potter-Elvehjem homogenizer and 10 ml of the homogenate was layered over a discontinuous density gradient of 8 ml 0.7 M sucrose over 8 ml 1.3 M sucrose over 8 ml 1.7 M sucrose all in medium A. This was centrifuged at 70,000 g_{av} in a SW 27 rotor for 45 min at 4°C; the Golgi fraction was aspirated from the 0.7:1.3 M sucrose interface, diluted four-fold with 0.25 M sucrose and pelleted by centrifuging at 106,000 g_{av} for 150 min.

Lubrol extracts of subcellular fractions were prepared by washing the surface of the pellet with 0.25 M sucrose, suspending the pellet in 1% Lubrol at a concentration of 7-10 mg protein/ml and homogenizing by hand with a Potter-Elvehjem homogenizer.

In experiments in which radioactive compounds were incorporated into nascent polypeptide chains of ribosomes, bound and free polyribosomes were prepared as described by Ikehara and Pitot (1973). Livers were perfused as before and excised into ice-cold 0.44 M sucrose in TKM buffer (50 mM Tris, 25 mM potassium chloride, 10 mM magnesium chloride, pH 7.4). Livers were homogenized in 2 volumes 0.44 M sucrose, TKM buffer as before and centrifuged at 20,000 g_{av} for 10 min in a Sorvall SS-34 rotor. The upper two-thirds of the supernatant was aspirated, mixed with 1.47 volumes 2 M sucrose, TKM buffer and layered on 10 ml 2 M sucrose in TKM buffer. Over this discontinuous gradient was layered 4 ml 0.44 M sucrose in TKM buffer and the preparation was centrifuged at 150,000 g_{av} for 6 h in a Ti60 rotor at 4°C. The pellet formed represented a free polyribosome preparation and the material at the interface between the 1.35 M and 2.0 M sucrose solutions contained the

bound polyribosomes. The bound polyribosomes were aspirated with a Pasteur pipette, made to 0.5% Triton X-100 using 20% Triton X-100, homogenized by hand in a Potter-Elvehjem homogenizer and layered on 10 ml 2.0 M sucrose in TKM buffer. Bound polyribosomes were collected by centrifugation of this preparation at 150,000 g_{av} for 6 h in a Ti60 rotor at 4°C. Bound and free polyribosomes were washed by suspending in 0.5 M ammonium chloride, 0.44 M sucrose, 50 mM Tris, 10 mM magnesium chloride, pH 7.4, homogenizing this suspension by hand and pelleting as before. Puromycin extracts of washed polyribosomes were prepared by suspension and homogenization as above in 2 mM puromycin, 50 mM Tris, 0.5 M potassium chloride, 5 mM magnesium chloride, pH 7.4, incubating at 37°C for 30 min and centrifuging for 6 h as before. The supernatant represented puromycin extracts of polyribosomes and was concentrated by ultrafiltration with concurrent dialysis against 0.15 M sodium chloride.

2.8 Liver Slice Experiments

Liver slice incubations were carried out as described by Jamieson et al. (1975). Rats were starved for 16 h prior to sacrifice which was by cervical dislocation. Livers were extensively perfused with 0.15 M sodium chloride and excised into ice-cold 0.15 M sodium chloride. Slices were cut on a pre-cooled aluminum template with grooves 7 mm wide and 0.36 mm deep. The template was covered during slicing with ice-cold 0.15 M sodium chloride. Slices were washed twice with 0.15 M sodium chloride and then once with medium saturated with oxygen:carbon dioxide. Incubation medium contained 77 mM potassium chloride, 39 mM sodium

chloride, 32.5 mM sodium bicarbonate, 3.1 mM magnesium sulfate, 1.3 mM calcium chloride, 0.6 mM monobasic potassium phosphate, 25 mM glucose, 25 mg/l penicillin G and 25 mg/l streptomycin sulfate. Medium was saturated with gases prior to use by bubbling a 95:5 mixture of oxygen: carbon dioxide through the solution. Incubations contained 0.5 g or 1.0 g liver slices, 5 ml incubation medium, 0.1 ml of a stock amino acid solution and various tracers and supplementation as noted in Results. The stock amino acid mixture is described in Appendix A; the final concentration of amino acids in the flasks represents roughly twice that normally found in blood. Cortisol-21-acetate was prepared as stock solutions in 20% ethanol such that addition of 0.005 ml gave the appropriate final concentration in the incubation medium. All other supplementation was with stock aqueous solutions; volumes of 0.1 ml of these solutions were added to incubation medium.

Liver slices were incubated at 37°C with gentle shaking for 1-6 h under a stream of 95:5 oxygen:carbon dioxide. The gas mixture was kept humid by bubbling through water before being passed over the slices. Carbon dioxide present in the gas mixture after being passed over the slices was removed by bubbling through a saturated solution of barium hydroxide. Liver slice incubations were terminated by stopping the gas flow over the slices, placing the flasks on ice, aspirating the medium with a Pasteur pipette and washing the slices with ice-cold 0.15 M sodium chloride or 0.25 M sucrose.

2.9 Preparation and Fractionation of Peritoneal Exudate Cells; Preparation of Cytokines

Peritoneal exudate cells (PEC) were prepared by modifications of the procedure of Kampschmidt et al. (1980). To prepare activated PEC, rats were injected intraperitoneally 18 h prior to sacrifice with 20 ml 0.2% shellfish glycogen, 10 µg/ml lipopolysaccharide, 0.15 M sodium chloride, 0.5 mg/ml streptomycin sulfate, 100 U/ml penicillin G. In initial experiments, elicited PEC were used and 6% casein neutralized to pH 7.4 with 2 M sodium hydroxide replaced the lipopolysaccharide. Rats were sacrificed by etherization, the abdomens swabbed with 70% ethanol and 20 ml 0.15 M sodium chloride, 0.5 mg/ml streptomycin sulfate, 100 U/ml penicillin G was injected i.p. The peritoneum was massaged gently for 1 min and the peritoneal exudate was collected with a syringe after performing a laparotomy. Exudates containing large numbers of erythrocytes were discarded to prevent sepsis of cytokine preparations. Heparin was added to the peritoneal exudate at a concentration of 10 U/ml to prevent cell aggregation. Cells were collected by centrifugation at 800 g_{av} for 10 min at 4°C and then suspended in 0.168 M ammonium chloride to lyse erythrocytes as described by Watt et al. (1979). The cell suspension was then pelleted and washed three times with 0.15 M sodium chloride.

The procedure of Kampschmidt et al. (1980) was used to prepare cytokines. Washed PEC pellets were suspended in a minimum volume of 0.15 M sodium chloride, cell density was determined with a hemocytometer and the cells were diluted with 0.15 M sodium chloride to a concentration

of about 1 x 10^8 cells/ml. The suspension was incubated at 37°C for 2 h with gentle shaking. Cells were then removed by centrifugation and the resulting supernatant was taken as the cytokine preparation. Some preparations were heat inactivated by incubating at 80°C for 1 h. Pronase and trypsin digestions were with 0.1 mg/ml protease for 3 h at 37°C; digestions were stopped by heating at 100°C for 1 min. Dialysis of cytokine preparations was with Spectropor 1 tubing which was sterilized by heating in a boiling water bath for 2 min. Units of cytokine are defined in terms of million cell equivalents (MCE) as described by Bornstein (1982). One MCE is the amount of cytokine present in 0.01 ml of leukocyte supernatant after incubation under the conditions described above.

In some experiments, leukocytes present in PEC preparations were fractionated into cell types using a self-generating Percoll density gradient according to the procedure of Watt et al. (1979). Diluted isotonic Percoll was prepared by mixing 1 part PBS (20 mM sodium phosphate, 0.149 M sodium chloride, pH 7.3), 9 parts Percoll and 1 part 0.2 M sodium phosphate, 1.49 M sodium chloride, pH 7.3. PEC were prepared as described above, suspended at a concentration of 2-5 x 10^7 cells/ml and mixed with 8 volumes diluted isotonic Percoll. This preparation was then centrifuged at 60,000 g_{av} in a Ti60 rotor for 20 min at 4°C. Monocytes and lymphocytes band together about one-third of the way down the gradient; polymorphonuclear leukocytes band one-half to two-thirds the way down the gradient and erythrocytes band below the PMN cells in the gradient. The leukocyte bands were aspirated with a Pasteur pipette, diluted with 20-50 volumes PBS, collected by

centrifugation at 2,000 g_{av} for 20 min and washed three times with PBS.

In other experiments, leukocytes were fractionated into adherent and non-adherent populations using a procedure described by Opitz et al. (1980). The adherent cells are a monocyte enriched population of leukocytes. PEC were prepared as described above and washed with 0.15 M sodium chloride. Cells were suspended at a concentration of 1 x 10^8 cells/ml, plated on Lux plastic Petri dishes and incubated for 2 h at 37°C in a humid environment with a 95:5 air:carbon dioxide mixture. After this time, non-adherent cells were aspirated with a pipette and the adherent cells were gently washed three times with 0.15 M sodium chloride warmed to 37°C. Adherent cells were then dislodged with a rubber policeman and aspirated from the plate.

2.10 Wright's Staining of Blood Cells

Wright's staining of blood cells was performed by a modification of the procedure described by Humanson (1979) and utilized solutions prepared by Sigma Chemical Co. Smears of cells in 0.15 M sodium chloride were heat-fixed on a hot plate at low setting and covered with 10-15 drops of Sigma Wright Stain WS-10 diluted with an equal volume of Sigma Rinse Solution RS-1. Staining was allowed to proceed for 0.5 to 3 min. Slides were then rinsed 3 times with distilled water, blotted between filter paper, air-dried, dipped in xylene and mounted with cover slips using Permount. Slides were examined by bright field microscopy.

2.11 Enzyme Assays

Sialyltransferase (EC 2.4.99.1) assays were performed by a modification of the procedure of Baxter and Durham (1979) as described by Kaplan et al. (1983b). Rat asialo-α_1-acid glycoprotein was used as the acceptor protein. This was prepared by removal of covalently bound N-acetylneuraminic acid from α_1-acid glycoprotein by hydrolysis in 0.05 M sulfuric acid at 80°C for 1 h. The free N-acetylneuraminic acid was removed from the asialo-α_1-acid glycoprotein by extensive dialysis and the acceptor protein was freeze-dried. Serum sialyltransferase assay mixtures contained 25 µl serum, 250µg acceptor protein, 1.1 nmoles CMP-[^{14}C]-NeuAc (40 nCi) and 7.5 µmoles imidazole pH 7.0 in a total volume of 0.15 ml. Prior to analysis of hepatic sialyltransferase activity, livers were homogenized in 15 volumes 0.25 M sucrose using 10 up and down strokes of a Potter-Elvehjem homogenizer rotating at 2,000 r.p.m. Hepatic sialyltransferase assay mixtures contained 25 µl liver homogenate, 250 µg acceptor protein, 3.1 nmoles CMP-[^{14}C]-NeuAc (30 nCi), 25 µl 2% Triton X-100 and 7.5 µmoles imidazole, pH 7.0 in a total volume of 0.15 ml. Liver slice medium and PEC supernatant assay mixtures were identical to that of the serum sialyltransferase assay mixtures with the exception that serum was replaced with 50 µl liver slice medium or PEC supernatant. Serum, liver slice medium and PEC supernatant assay mixtures were incubated for 40 min and liver mixtures were incubated for 15 min at 37°C. The reaction was stopped by rapidly chilling the assay mixtures to 0°C. The assay mixtures were spotted on Whatman No. 1 paper discs of 2.5 cm diameter (40 µl/disc) and the discs were washed

three times with ice-cold 10% trichloroacetic acid, once with a 2:1 mixture of ethanol:ether and once with diethyl ether and were then air-dried. Radioactivity adsorbed to the paper discs was determined by liquid scintillation counting using 10 ml ACS counting cocktail.

Hepatic β-D-galactosidase (EC 3.2.1.51) and N-acetyl-β-D-glucosaminidase (EC 3.2.1.30) were determined using p-nitrophenyl substrates as described by Kaplan and Jamieson (1977). Assay mixtures contained 1 µmole p-nitrophenylglycoside, 25 µl 2% Triton X-100, 400 µl McIlvane buffer, 25 µl liver homogenate and for β-D-galactosidase assays, 1 mmole potassium chloride, all in a final volume of 0.5 ml. McIlvane buffer was prepared by using 0.2 M dibasic sodium phosphate to adjust 0.1 M citric acid to pH 3.7 for β-D-galactosidase assays and pH 4.2 for N-acetyl-β-D-glucosaminidase assays. For N-acetyl-β-D-glucosaminidase assays, a 1:60 liver homogenate, and for β-galactosidase assays, a 1:15 liver homogenate, prepared as described above, was used. Incubations were at 37°C and reactions were stopped by the addition of 1.5 ml 0.25 M glycine pH 10.2. The mixtures were then centrifuged at 8,000 g_{av} for 2 min to remove any insoluble material, absorbances were determined at 400 nm and the amounts hydrolyzed determined using a molar extinction coefficient of 1.77×10^4 $M^{-1}cm^{-1}$ (li & Li, 1972). Assays of glycosidase activity in PEC supernatants were similar to that for the liver samples with the exception that Triton X-100 was omitted and the PEC supernatant replaced the liver homogenate.

2.12 Hormone Radioimmunoassays

Cortisol, thyroxine and triiodothyronine present in serum samples were assayed using radioimmunoassay kits from Clinical Assays. For cortisol assays, serum samples or standard cortisol solutions were added to Gamma Coat tubes which were coated with antibodies directed against cortisol; a volume of 1 ml [^{125}I]-cortisol in PBS buffer was added to each tube; the tubes were gently vortexed and incubated at 37°C for 45 min. For total thyroxine assays, samples or standards were added to Gamma Coat tubes coated with antibodies against thyroxine; 1 ml [^{125}I]-thyroxine in a buffer containing anilino-1-naphthalenesulfonic acid and salicylate was added to the tubes; incubations were at room temperature for 45 min. For free thyroxine assays, samples or standards were added to the same Gamma Coat tubes described above; 1 ml of incubation buffer was added; tubes were incubated at 37°C for 20 min and then incubation mixtures were discarded. After the first incubation, 1 ml of [^{125}I]-thyroxine solution was added to tubes which were then incubated at 37°C for 1 h. For total triiodothyronine assays, samples or standards were added to Gamma Coat tubes coated with antibodies against triiodothyronine; 1 ml of [^{125}I]-triiodothyronine in assay buffer was added to the tubes; incubations were at 37°C for 1 h. Immediately after incubation periods, 0.8 ml of the solutions in the tubes was aspirated and counted in 10 ml ACS counting cocktail to determine the amount of unbound [^{125}I]-hormone.

Insulin was assayed in serum samples using a radioimmunoassay kit from Amersham. Serum samples or insulin standard solutions were

incubated at 4°C for 45 min with binding reagent, then a [^{125}I]-insulin solution was added to the tubes which were incubated for a further 16 h at 4°C. After this incubation, insoluble material was pelleted by centrifugation at 8,000 g_{av} for 5 min and 0.8 ml of the resulting supernatant was counted as above.

Corticotropin present in serum was assayed using a radioimmunoassay kit from Amersham. Fresh serum samples or corticotropin standards in human plasma were pipetted into extraction tubes containing 0.1 g glass adsorbant, the tubes were vortexed, incubated for 30 min with gentle shaking and centrifuged at 1,000 g_{av} for 2 min. The supernatant was aspirated and discarded.The glass adsorbant was washed with 2 ml water and then 2 ml 1 M hydrochloric acid. Corticotropin was then desorbed from the glass by incubating at room temperature for 20 min with 50% (v/v) acetone. After centrifugation, the glass adsorbant was washed with 50% acetone, the acetone extracts pooled and evaporated to dryness at 55°C under a stream of nitrogen. Buffer solution was then added to the residue of the acetone extracts and the tubes were vortexed and centrifuged at 8,000 g_{av} for 3 min to settle solids. Aliquots of these solutions were then incubated at 4°C for 16-20 h with rabbit anti-corticotropin serum, and [^{125}I]-corticotropin was added to the tubes which were incubated for a further 6-8 h at 4°C. After this second incubation, activated charcoal was added to the tubes, the tubes were vortexed and immediately centrifuged at 8,000 g_{av} for 1.5 min. The supernatant was aspirated and the charcoal residues counted in 10 ml ACS counting cocktail.

Data obtained for radioimmunoassays were analyzed by an iterative curve fitting for competitive binding assays as described by Chang et al. (1975). Details of the calculation and the program used for the calculation are given in Appendix B.

RESULTS

1. Biosynthesis of Acute Phase Reactants

Many of the experiments presented in this thesis are concerned with one aspect of the acute phase response, the hepatic biosynthesis of the acute phase reactants. A great deal of work on the biosynthesis of a specific acute phase reactant, α_1-acid glycoprotein, has been carried out in this laboratory (Jamieson et al., 1972a, 1972b, 1975; Jamieson & Ashton, 1973a, 1973b; Friesen & Jamieson, 1980). These studies indicated several interesting aspects of the biosynthesis of this protein. The protein is synthesized as a precursor in the rough endoplasmic reticulum; the oligosaccharide moiety of the precursor is high in mannose content (Friesen & Jamieson, 1980). Evidence for the existence of a precursor of α_1-acid glycoprotein came from the kinetics of in vivo labelling of anti-α_1-acid glycoprotein reactive material with [^{14}C]-mannose and from the isolation of an intrahepatic form of α_1-acid glycoprotein which differed in its physicochemical properties from the mature form of the protein found in normal serum (Friesen & Jamieson, 1980). It also appears that the en bloc addition of carbohydrate to α_1-acid glycoprotein occurs as a post-ribosomal event; the evidence for post-translational glycosylation of the protein comes from the observation that radiolabelled glucosamine is not readily incorporated into nascent polypeptide chains of ribosomes which are immunoreactive with anti-α_1-acid glycoprotein (Jamieson, 1977). It is not known if aspects of the biosynthesis of α_1-acid glycoprotein are unique to this glycoprotein or are representative of the biosynthesis of serum proteins of hepatic origin or of acute phase reactants. Indeed, the hepatic

biosynthesis of only a few specific glycoproteins has been studied (Jamieson, 1983). For these reasons, experiments were performed to examine the biosynthesis of two serum glycoproteins, specifically α-macroglobulins, which had considerably different physicochemical properties than α_1-acid glycoprotein. While α_2-macroglobulin is an acute phase reactant and increases 4-fold in serum levels during the acute phase response (Jamieson et al., 1972b), serum levels of the closely related α_1-macroglobulin are not altered following inflammation. Comparison of labelling patterns of these specific glycoproteins with radioactive precursors of polypeptide and carbohydrate moieties was used in this thesis as a basis for comparison of the biosynthesis of the acute phase reactants α_1-acid glycoprotein and α_2-macroglobulin and the serum glycoprotein α_1-macroglobulin.

1.1 Isolation and Partial Characterization of α_1-Macroglobulin

Difficulties have arisen in the separation of α_2-macroglobulin (also referred to as acute phase α_2-macroglobulin and α_2-macrofetoprotein) and α_1-macroglobulin (also referred to as normal α-macroglobulin) because of the many similarities in the physicochemical properties of these two proteins (Gauthier & Mouray, 1976; Hudig & Sell, 1979). Chromatography on immobilized Cibacron Blue (Affi-Gel Blue) was used as the first step in the isolation of α_1-macroglobulin from normal rat serum as it proved to be efficient in this separation. A number of other workers have utilized this procedure in the fractionation of plasma proteins including α-macroglobulins (Virca et al., 1978; Leatherbarrow

& Dean, 1980; Gianazza & Arnaud, 1982). The procedure of Virca et al. (1978) for the isolation of α_1-macroglobulin from human serum was used in the fractionation of normal rat serum. Three fractions were obtained as described in Methods; fraction A contained α_1-macroglobulin but little α_2-macroglobulin (see Figure 8), fraction B contained both α-macroglobulins (see Figure 8) and fraction C was highly enriched in albumin. Affinity chromatography on concanavalin A-Sepharose was then used to prepare a glycoprotein-enriched fraction of fraction A as is shown in Figure 9. Concanavalin A-Sepharose chromatography has also been used in other purification schemes of α-macroglobulins from rat plasma (Niewenhuizen et al., 1979) and fetal bovine serum (Loskutoff, 1978). In order to prepare α_1-macroglobulin from the glycoprotein-enriched fraction A-2, gel filtration chromatography on Sepharose 6B was performed as is shown in Figure 10. Fractions from this column were tested by double diffusion analysis against anti-whole rat serum and antiserum against the α-macroglobulin-enriched fraction $5B_1$ described by Jamieson et al. (1972a); those fractions which developed single immunoprecipitin lines against both antisera were pooled, dialyzed, and freeze-dried. This preparation represented purified rat serum α_1-macroglobulin; recovery of the protein by this method was 1.5 mg/ml of control rat serum.

The α_1-macroglobulin was characterized and the physicochemical properties compared to those reported for rat α-macroglobulins. Analytical isoelectric focusing of the purified α_1-macroglobulin is shown in Figure 11; a pI of 4.4 was obtained for the protein which

Figure 8 - Immunological Analysis of Protein Fractions obtained from Chromatography of Rat Serum on Affi-Gel Blue

Protein fractions were analyzed by reactivity against anti-fraction $5B_1$ which contains antibodies against both α-macroglobulins present in the serum of inflamed rats.

Upper figure shows the results of double diffusion analysis of the fractions

1 - fraction A

2 - fraction B

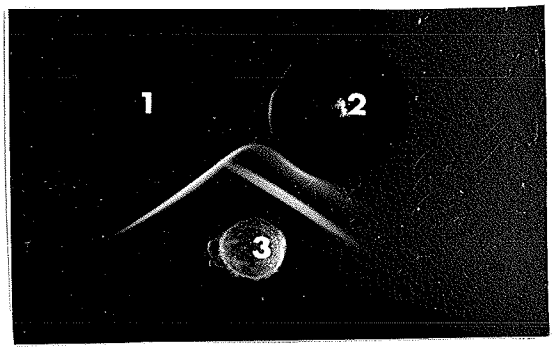
3 - anti-fraction $5B_1$

Lower figure shows the results of immunoelectrophoresis of the fractions

1 - fraction A

2 - fraction B

trough - anti-fraction $5B_1$


1
2
3

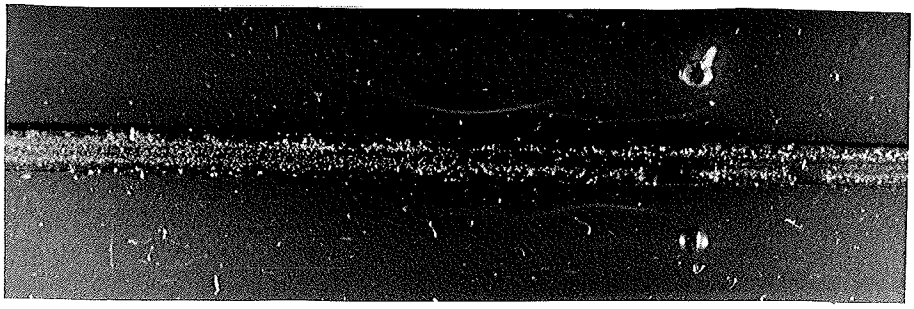

4

Figure 9 - Concanavalin A-Sepharose Chromatography of Fraction A

Fraction A was prepared as described in Methods; a sample of 400 mg of the protein fraction was applied to the concanavalin A-Sepharose column and 100 drop fractions were collected; elution of the column is described in Methods. The arrow indicates where elution of a glycoprotein-enriched fraction with α-methyl mannoside began. Protein present in fractions 56-75 was pooled and designated fraction A-2.

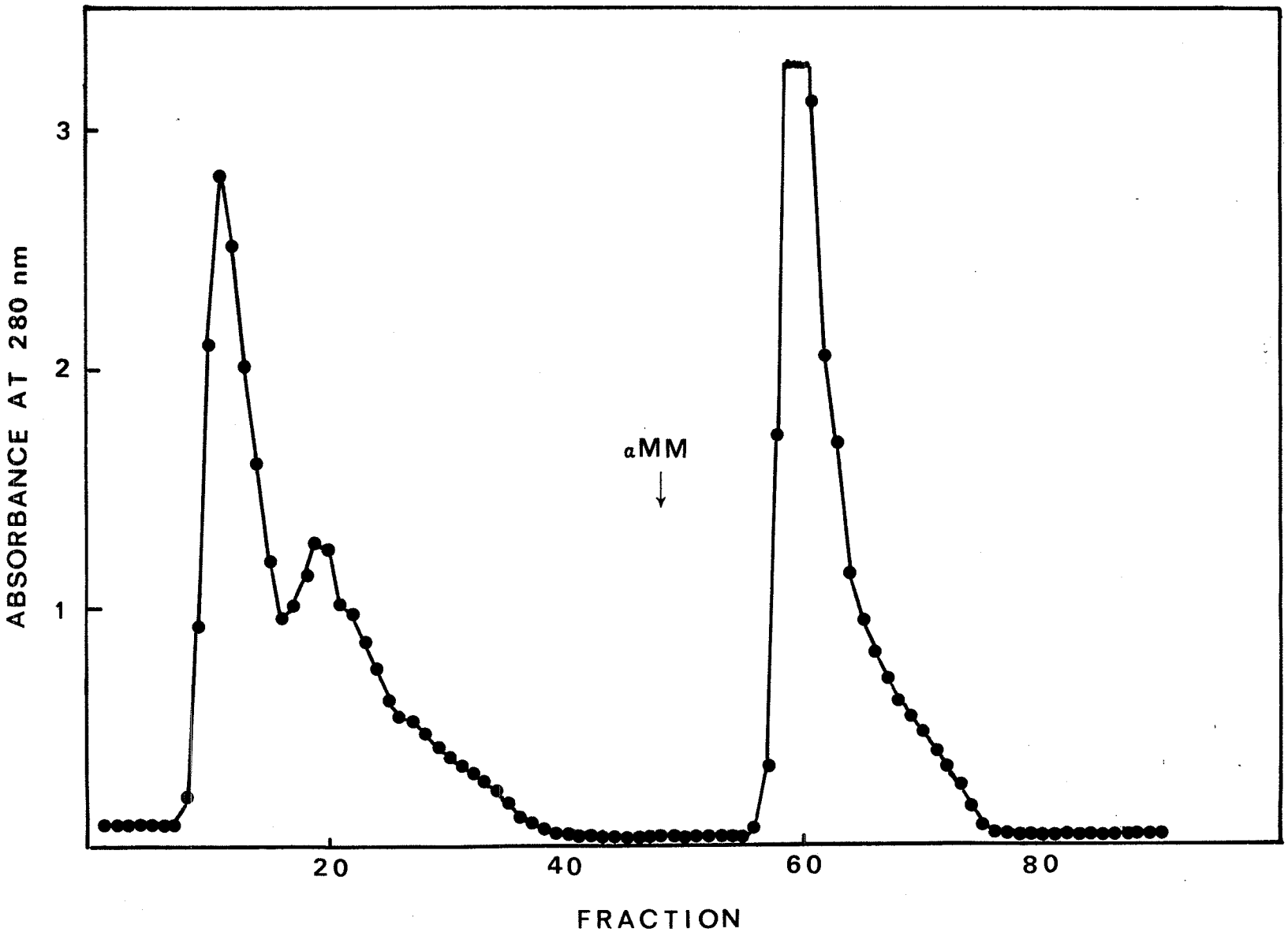

ABSORBANCE AT 280 nm
3
2
1
αMM
20
40
60
80
FRACTION

Figure 10 - Gel Filtration Chromatography of Fraction A-2 on Sepharose 6B

Fraction A-2 was chromatographed on Sepharose 6B as described in Methods; fractions of 200 drops were collected and tested by double diffusion analysis using antisera against fraction $5B_1$ and whole rat serum. The single bars indicate reactivity to anti-fraction $5B_1$ and double bars indicate those fractions which developed more than one precipitin line against anti-whole rat serum. Fractions 34-40 were pooled and designated purified α_1-macroglobulin.
Arrows indicate the position of elution of ferritin (400 kd) and thyroglobulin (670 kd).

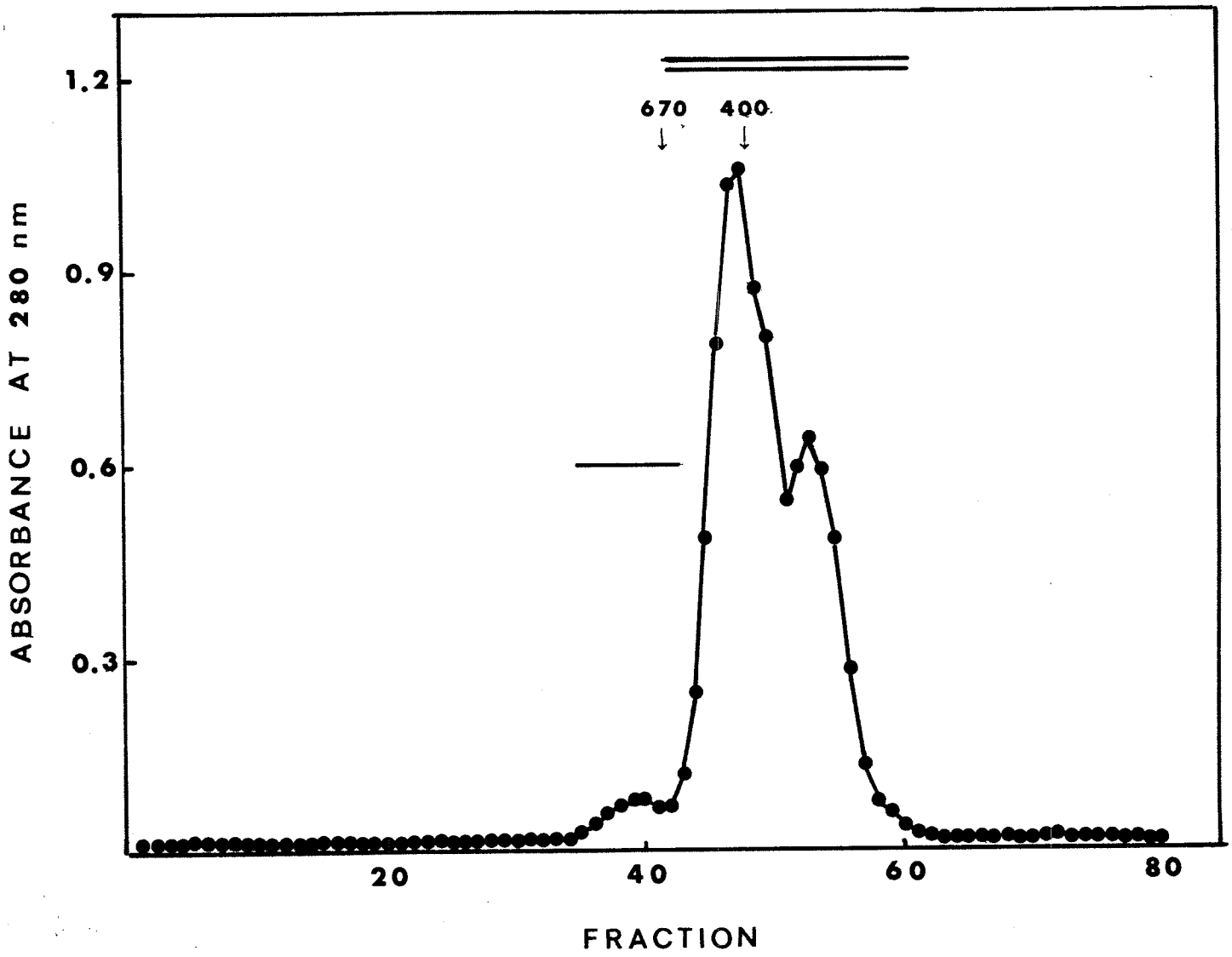
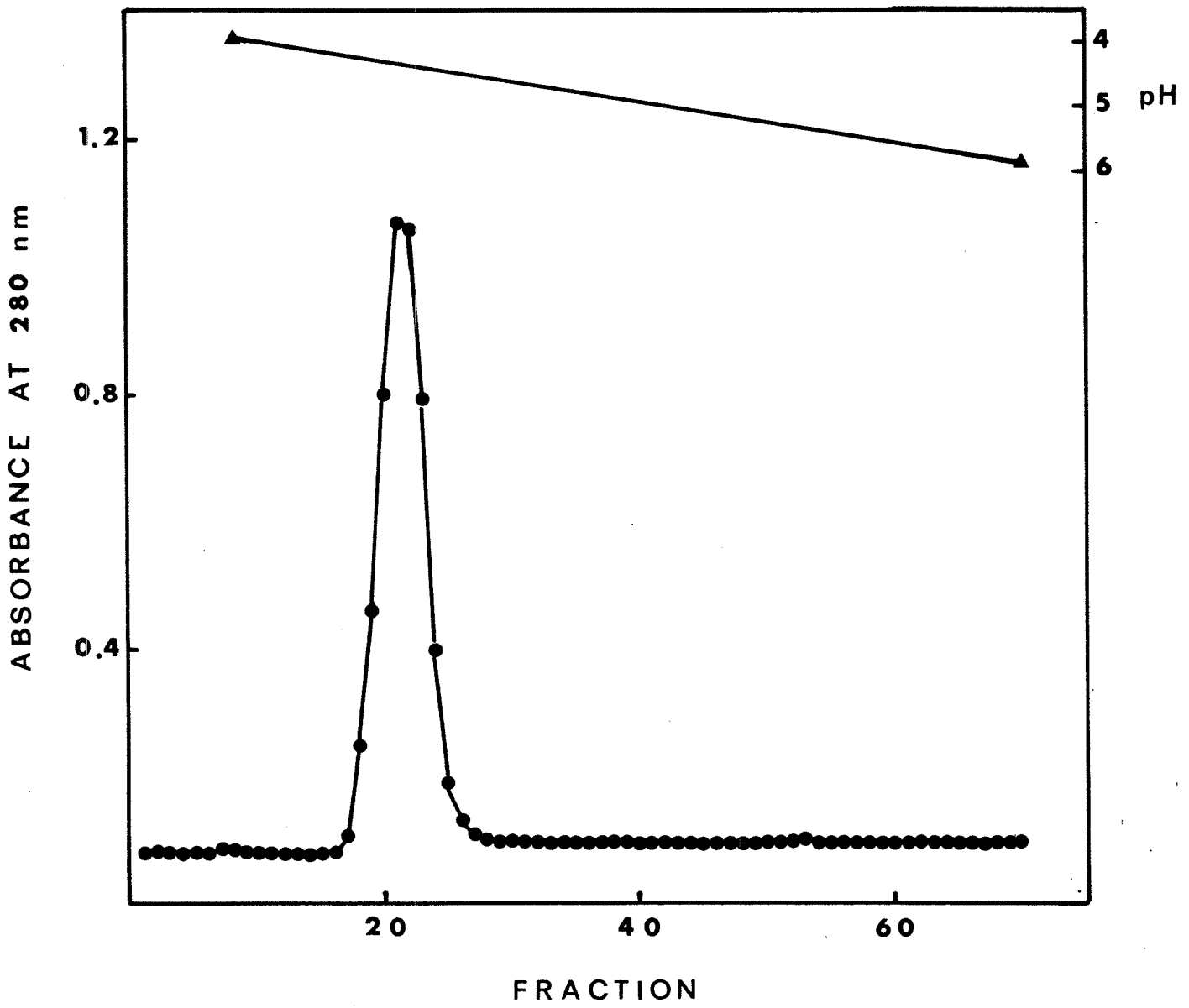

1.2
0.9
0.6
0.3
ABSORBANCE AT 280 nm
670
400
20
40
60
80
FRACTION

Figure 11 - Isoelectric Focusing of α_1-Macroglobulin

An isoelectric focusing column was run on the α_1-macroglobulin prepared as described in Methods. Closed circles indicate the absorbances at 280 nm of the eluted fractions. The line between the triangles indicates the pH of the eluted fractions.


ABSORBANCE AT 280 nm
1.2
0.8
0.4
4
5
6
pH
20
40
60
FRACTION

compares well with pIs of 4.4-4.7 which have been reported for the rat α-macroglobulins (Jamieson et al., 1972a; Gauthier & Mouray, 1976; Hudig & Sell, 1979; Nieuwenhuizen et al., 1979). The results of amino acid analysis of the α_1-macroglobulin isolated in this work are shown in Table 7; these results are compared in Appendix C to the amino acid contents of α_1-macroglobulin and α_2-macroglobulin reported by Gauthier and Mouray (1976). The results of amino acid analysis of the protein isolated in this work and the α_1-macroglobulin of Gauthier and Mouray (1976) are identical considering experimental uncertainty; the protein isolated in this work differed from the α_2-macroglobulin of these authors in Asx, Ser, Gly, Ile, Tyr, His and Arg content. Serum concentrations of α_1-macroglobulin were 2.10 ±0.07 mg/ml as determined by quantitative immunoprecipitation; this level did not change in rats suffering from inflammation for 0-96 h.

1.2 Hepatic Biosynthesis of Plasma Proteins

Previous studies on the biosynthesis of α_1-acid glycoprotein have led to the isolation of an intracellular precursor of this protein which has a high mannose content (Friesen & Jamieson, 1980). The existence of the precursor to α_1-acid glycoprotein was inferred, in part, from the kinetics of labelling with [^{14}C]-mannose of anti-α_1-acid glycoprotein reactive material present in subcellular fractions of the liver (Friesen & Jamieson, 1980). Similar experiments were performed to examine the incorporation of this radiolabel into α_2-macroglobulin as is shown in Figure 12. The kinetics of radiolabelling of α_2-macroglobulin

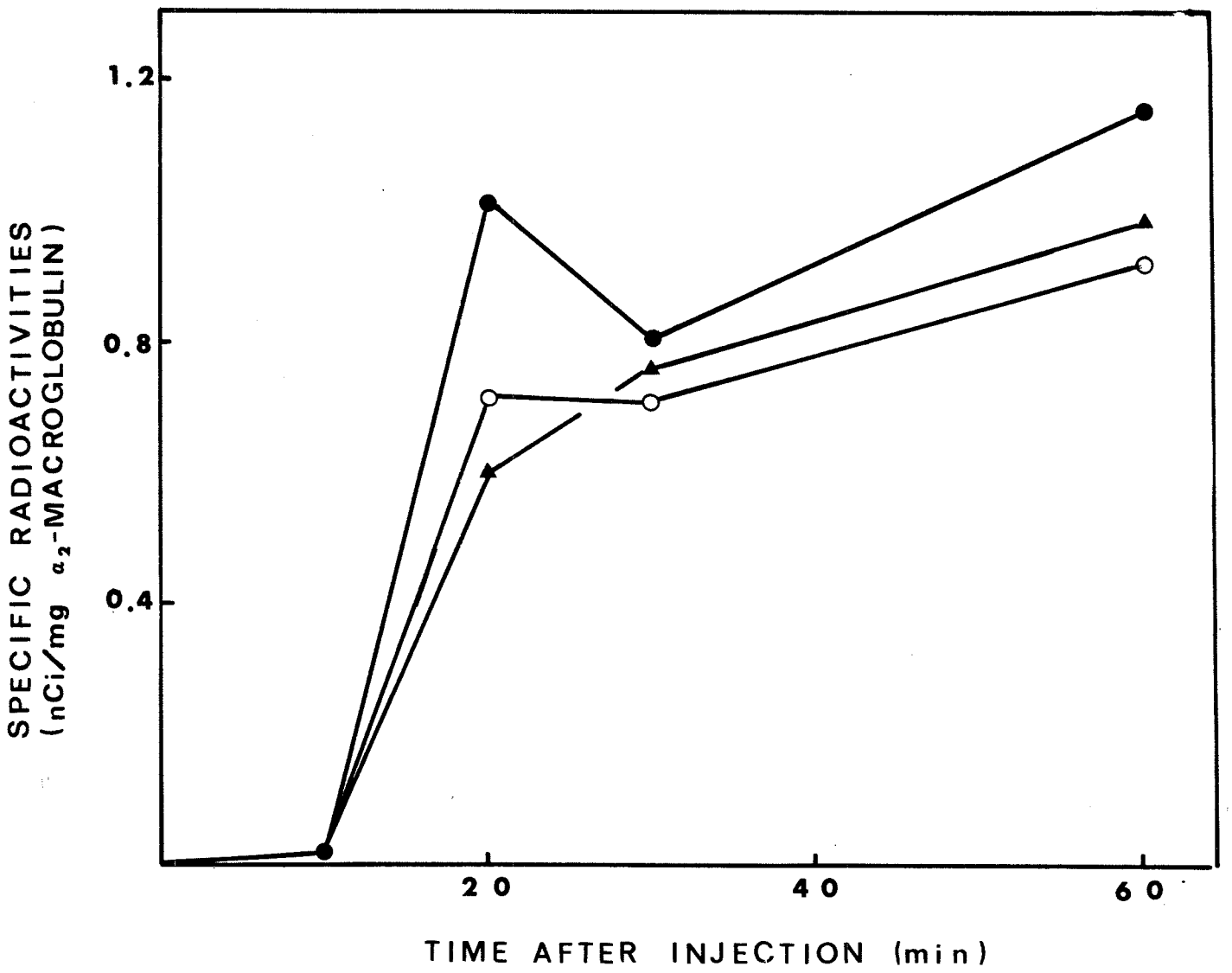
Table 7 - Amino Acid Composition of α_1-Macroglobulin

Amino Acid	moles/100 moles[1]
Asx	9.81 ±0.27
Thr	6.03 ±0.02
Ser	8.18 ±0.02
Glx	12.67 ±0.08
Pro	5.80 ±0.17
Gly	5.74 ±0.14
Ala	6.09 ±0.15
Val	7.45 ±0.21
Met	1.80 ±0.15
Ile	4.99 ±0.13
Leu	10.44 ±0.10
Tyr	4.26 ±0.10
Phe	4.09 ±0.03
His	2.65 ±0.10
Lys	6.05 ±0.08
Arg	2.86 ±0.21
Trp	1.09 ±0.25

[1]Results shown are the means and standard deviations of quadruplicate analyses of samples hydrolyzed for 24, 48 and 72 h. Corrections are made for loss of amino acids due to hydrolysis.Tryptophan was determined from ultraviolet absorbances as described by Edelhoch (1967).

Figure 12 - Time Course of Incorporation of [^{14}C]-Mannose into α_2-Macroglobulin of Subcellular Fractions of Rat Liver

Animals inflamed for 24 h prior to sacrifice received intravenous injections of 10 μCi [^{14}C]-mannose; α_2-macroglobulin was isolated immunologically from Lubrol extracts of subcellular fractions as described in Methods and specific radioactivities were determined. Specific radioactivities of [^{14}C] in α_2-macroglobulin of rough microsomes (●), smooth microsomes (▲) and Golgi (○) are indicated. Each point represents the mean of 4 analyses and the standard deviations of the mean were less than ±8%.


SPECIFIC RADIOACTIVITIES
(nCi/mg α2-MACROGLOBULIN)
1.2
0.8
0.4
20
40
60
TIME AFTER INJECTION (min)

was very similar to the pattern Friesen and Jamieson (1980) obtained for α_1-acid glycoprotein. Little radioactivity was incorporated into the protein in the first 10 min following injection, but by 15 min after injection, α_2-macroglobulin in Golgi and microsome fractions was extensively labelled; protein present in the rough microsome fractions had incorporated more label than protein present in other subcellular fractions. At later times of labelling, specific radioactivity of [^{14}C]-mannose in α_2-macroglobulin from rough microsomes had declined while that in smooth microsomes had increased. The similarities in the kinetics of radiolabelling of α_1-acid glycoprotein and α_2-macroglobulin would suggest that these two proteins share a similar, if not identical, pathway of synthesis.

Further experiments in these studies were concerned with one aspect of the biosynthesis of serum proteins; that is, the timing of glycosylation. In these experiments the immunological approach of Jamieson (1977) was used to examine the biosynthesis of two acute phase reactants, α_1-acid glycoprotein and α_2-macroglobulin, and the serum glycoprotein α_1-macroglobulin. Table 8 shows the labelling patterns of these specific glycoproteins with [^{3}H]-leucine. For all three of these proteins, the highest specific radioactivities were obtained with puromycin extracts of ribosomes; proteins associated with bound polyribosomes had higher specific radioactivities than proteins associated with free polyribosomes. This pattern may reflect the fact that, in the 2 min labelling period, protein associated with the ribosomes would be, for the most part, newly synthesized protein, while

Table 8 - Incorporation of [^{3}H]-Leucine into Protein isolated from Subcellular Fractions of Rat Liver

Fraction	Specific Radioactivities (nCi/mg protein)†			
	Total Protein	α_1-Acid Glycoprotein	α_2-Macroglobulin	α_1-Macroglobulin
Rough Microsomes	39.6 ±3.2	540 ±8	144 ±14	50 ±10
Smooth Microsomes	30.8 ±0.8	324 ±36	62 ±10	14 ±5
Golgi	39.0 ±1.0	560 ±24	560 ±24	124 ±17
Bound Ribosomes	556 ±62	5220 ±920	2880 ±640	3621 ±760
Free Ribosomes	122 ±20	1510 ±360	880 ±220	1110 ±150

†Protein was isolated from Lubrol extracts of microsome and Golgi fractions or puromycin extracts of ribosome fractions either immunologically or by precipitation with trichloroactetic acid as described in Methods. Results shown are the means and standard deviations of 3-6 analyses performed on protein isolated from 6-12 rats inflamed for 24 h. Rats received an intraportal injection of 50 μCi [^{3}H]-leucine 2 min prior to sacrifice.

Golgi and microsome fractions would contain a great deal of protein synthesized prior to tracer administration. Also, the high specific radioactivities of these proteins in puromycin extracts of bound polyribosomes is consistent with the Signal Hypothesis which predicts that the synthesis of secretory proteins will occur primarily on bound polyribosomes (see Introduction). Different patterns of *in vivo* labelling of the three proteins were obtained when radioactive precursors to the carbohydrate moieties of the glycoproteins were used; results obtained following [^{14}C]-glucosamine and [^{14}C]-mannose administration are shown in Tables 9 and 10, respectively. While little or no radiolabel was incorporated into immunoreactive material associated with puromycin extracts of polyribosomes, the radioactive sugars were readily incorporated into immunoreactive material of the microsome and Golgi fractions. As was suggested by Jamieson (1977) from the results of [^{14}C]-glucosamine labelling of α_1-acid glycoprotein, this labelling pattern suggests that initial glycosylation of these proteins occurs as a post-translational event rather than during protein synthesis. While it is possible that incorporation of tracer into immunoreactive material of puromycin extracts is a misleading measure of the addition of carbohydrate moieties of glycoproteins, this is unlikely as puromycin extracts are readily labelled with [^{3}H]-leucine under the same conditions.

The experiments presented in this thesis on hepatic biosynthesis of plasma proteins were performed with the aim of identifying parameters of hepatic biochemistry which are unique to the acute phase response.

Table 9 - Incorporation of [^{14}C]-Glucosamine into Protein isolated from Subcellular Fractions of Rat Liver

Fraction	Specific Radioactivities (nCi/mg protein)			
	Total Protein	α_1-Acid Glycoprotein	α_2-Macroglobulin	α_1-Macroglobulin
Rough Microsomes	0.78 ±0.02	1.44 ±0.08	1.96 ±0.09	0.61 ±0.08
Smooth Microsomes	1.05 ±0.04	2.22 ±0.10	2.70 ±0.09	0.95 ±0.06
Golgi	1.55 ±0.03	2.93 ±0.13	6.06 ±0.18	1.13 ±0.11
Bound Ribosomes	0.51 ±0.10	0.09 ±0.06	-	-
Free Ribosomes	0.20 ±0.12	0.07 ±0.05	0.12 ±0.09	0.09 ±0.07

Experimental protocol was identical to that for the results presented in Table 8 with the exception that tracer was administered as an intraperitoneal injection of 20 µCi [^{14}C]-glucosamine 45 min prior to sacrifice. (-) indicates that no appreciable labelling occured.

Table 10 - Incorporation of [^{14}C]-Mannose into Protein isolated from Subcellular Fractions of Rat Liver

Fraction	Specific Radioactivities (nCi/mg protein)			
	Total Protein	α_1-Acid Glycoprotein	α_2-Macroglobulin	α_1-Macroglobulin
Rough Microsomes	0.25 ±0.01	11.90 ±0.21	2.64 ±0.27	4.93 ±0.08
Smooth Microsomes	0.20 ±0.01	8.79 ±0.34	1.12 ±0.05	1.37 ±0.10
Golgi	0.36 ±0.04	8.19 ±0.74	2.84 ±0.42	3.24 ±0.33
Bound Ribosomes	0.76 ±0.18	0.76 ±0.43	0.36 ±0.20	-
Free Ribosomes	0.09 ±0.07	0.18 ±0.15	0.05 ±0.05	0.11 ±0.10

Experimental protocol was identical to that for the results presented in Table 8 with the exception that tracer was administered as an intravenous injection of 20 μCi [^{14}C]-mannose 20 min prior to sacrifice.

The results did not identify an aspect of biosynthesis unique to acute phase reactants, but, rather, demonstrated similarities in hepatic biosynthesis of serum glycoproteins. For this reason, these studies were not pursued further.

2. Physiologic Alterations induced by Acute Inflammation

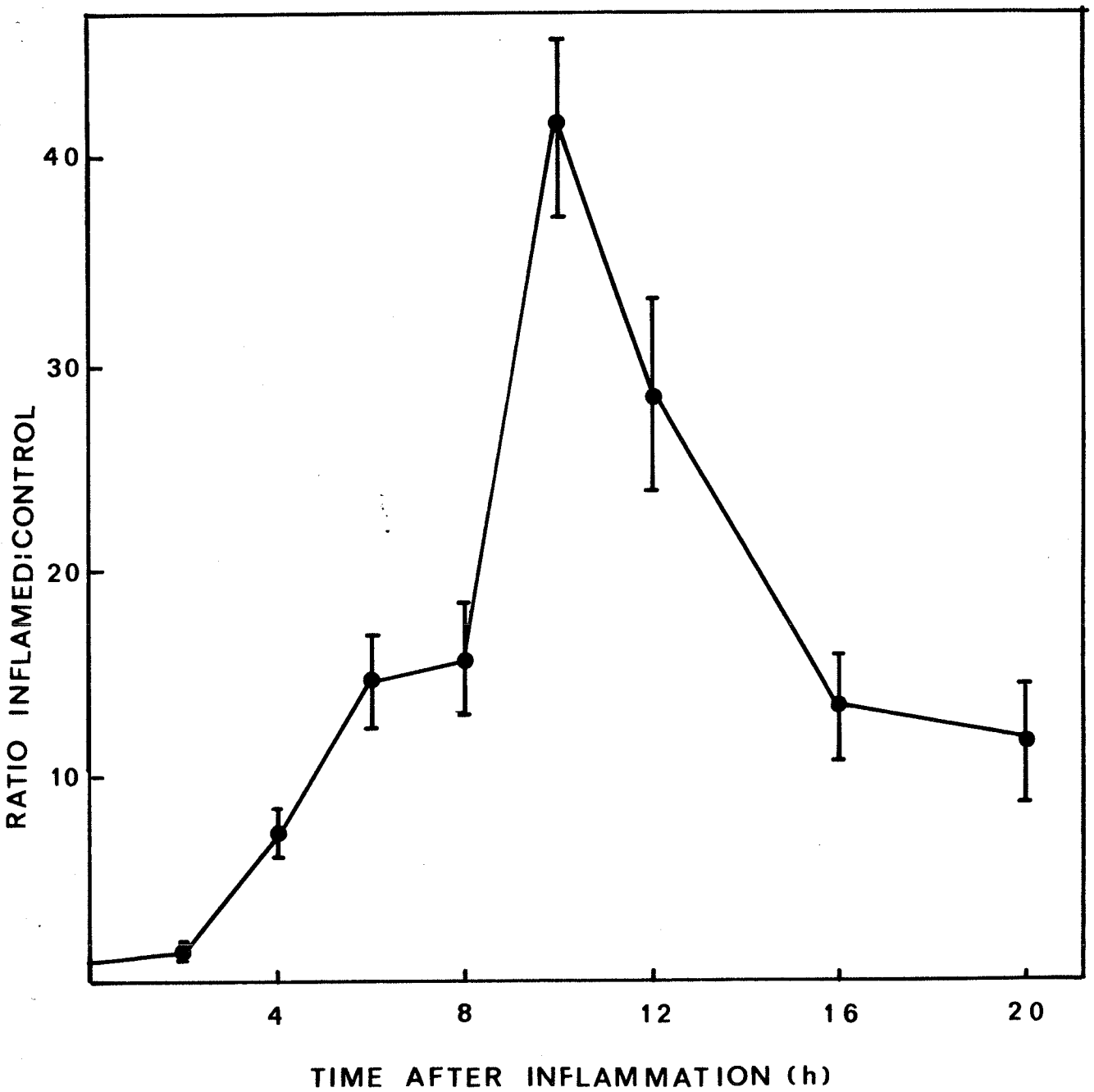
As previously mentioned, tissue injury and acute inflammation result in a variety of physiological and biochemical changes. Many of the changes are related and interdependent. In the work presented here, endocrine alterations were examined at early times after inflammation to provide information which would allow a better understanding, in the rat model, of the mechanisms by which hepatic activity is altered during the acute phase response. Also, the effect of inflammation on serum and hepatic amino acid pools was examined to add to our understanding of metabolic changes during the acute phase response.

2.1 Endocrine Changes following Acute Inflammation

One level at which endocrine changes may occur is in elevations of serum levels of hormones, such as corticotropin, which result from enhanced secretion of hormone from the hypophysis or pituitary gland. Figure 13 shows the effect of experimental inflammation on rat serum corticotropin levels. Control levels of corticotropin varied from 700±90 pg/ml in animals sacrificed immediately after a 2 min etherization and subcutaneous injection of sterile saline, to a mean of 150 ±20 pg/ml

Figure 13 - Effect of Inflammation on Serum Corticotropin Levels

Serum was obtained from rats and corticotropin levels were determined as described in Methods. Means and standard deviations from the means of 4 analyses are indicated. Control levels varied from 150-700 pg/ml. All experimental values other than the 2 h experimental deviated from control values at the 99.9% confidence levels as determined by the Student t test. See text for further explanation.

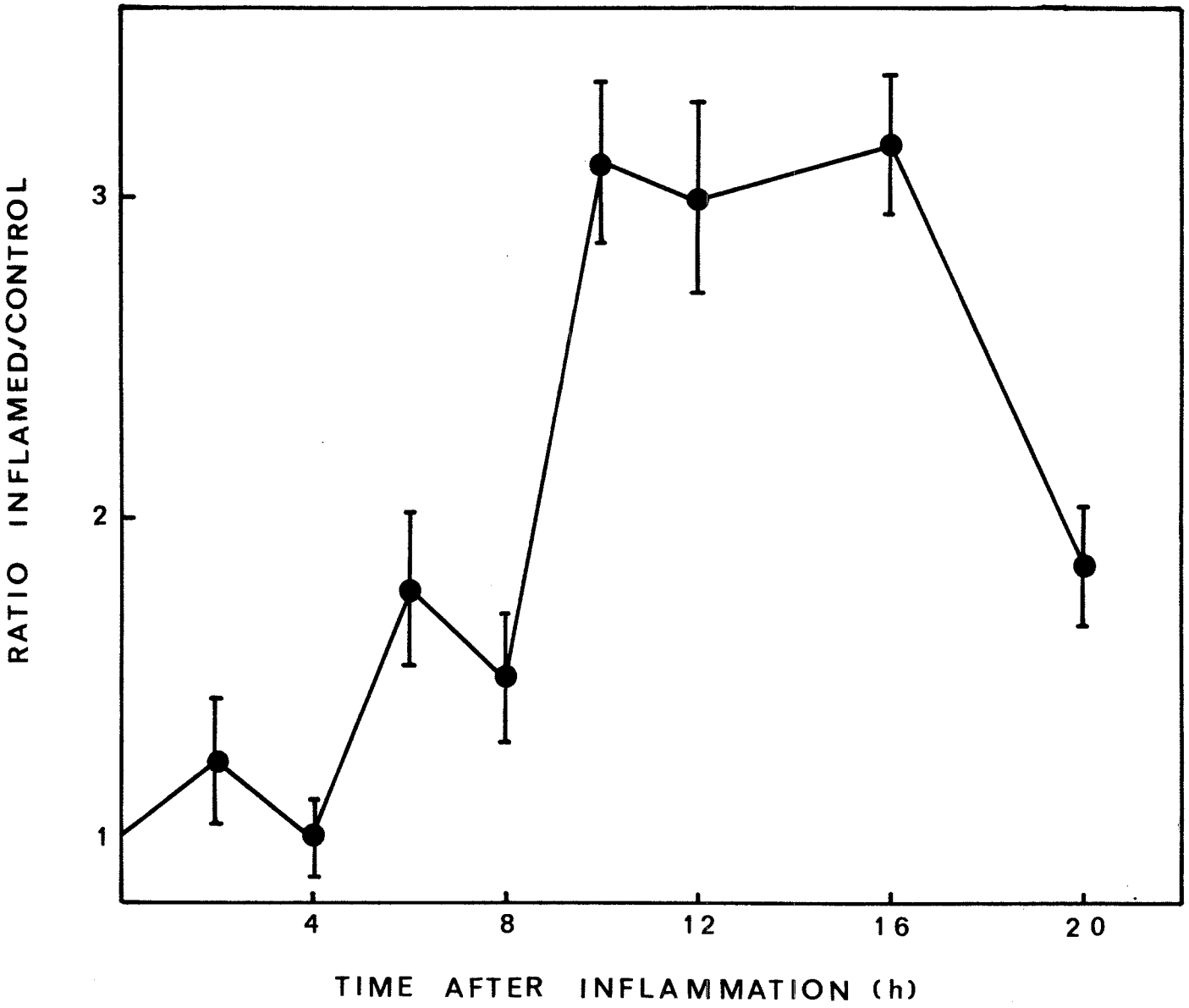

40
30
20
10
RATIO INFLAMED:CONTROL
4
8
12
16
20
TIME AFTER INFLAMMATION (h)

in animals receiving injections of sterile saline 4 h or longer prior to sacrifice. These values are higher than values of 77 ±12 pg/ml obtained by Witek-Janusek and Marotta (1981) two days after laparotomy and of 66 ±14 pg/ml obtained by Matsuyama et al. (1970) for unstressed control rats. The variation in corticotropin levels of control rats in the work presented here is likely due to the stress of etherization and handling involved in injections of saline. De Souza and Van Loon (1982) have reported levels of 400 pg/ml after a 2 min restrain of rats; Matsuyama et al. (1970) have reported a range of 270-3940 pg/ml and a mean of 959 pg/ml for plasma corticotropin levels in female rats immediately following 10 min of ether anesthesia and laparotomy. These levels are comparable to the values obtained for control rats immediately after etherization and saline injection. In contrast to the pattern following saline injection, serum corticotropin levels are elevated following turpentine-induced inflammation; by 4 h after experimental inflammation there is a substantial (7-fold) increase in levels. Maximum levels were obtained 10 h after inflammation at which time serum levels were 6,000 pg/ml. After this time, the levels decreased such that 20 h after inflammation, serum corticotropin is at 2,000 pg/ml. These changes are consistent with other reports that a variety of stresses and tissue injuries result in elevated serum corticotropin levels (Ruhmann-Wennhold & Nelson, 1977).

Corticotropin is the trophic hormone of the adrenal cortex and stimulates synthesis and secretion of glucocorticoids such as cortisol by this gland (Harding, 1977). Figure 14 shows the effect of experimental

Figure 14 - Effect of Inflammation on Serum Cortisol Levels

Serum was obtained from rats and cortisol levels determined by radioimmunoassay as described in Methods. Means and standard deviations of 4 analyses are indicated. Control levels of cortisol were 220 nM. All experimental values other than the 2 and 4 h experimentals deviated from control levels at the 99.9% confidence levels as determined by the Student t test. See text for further explanation.

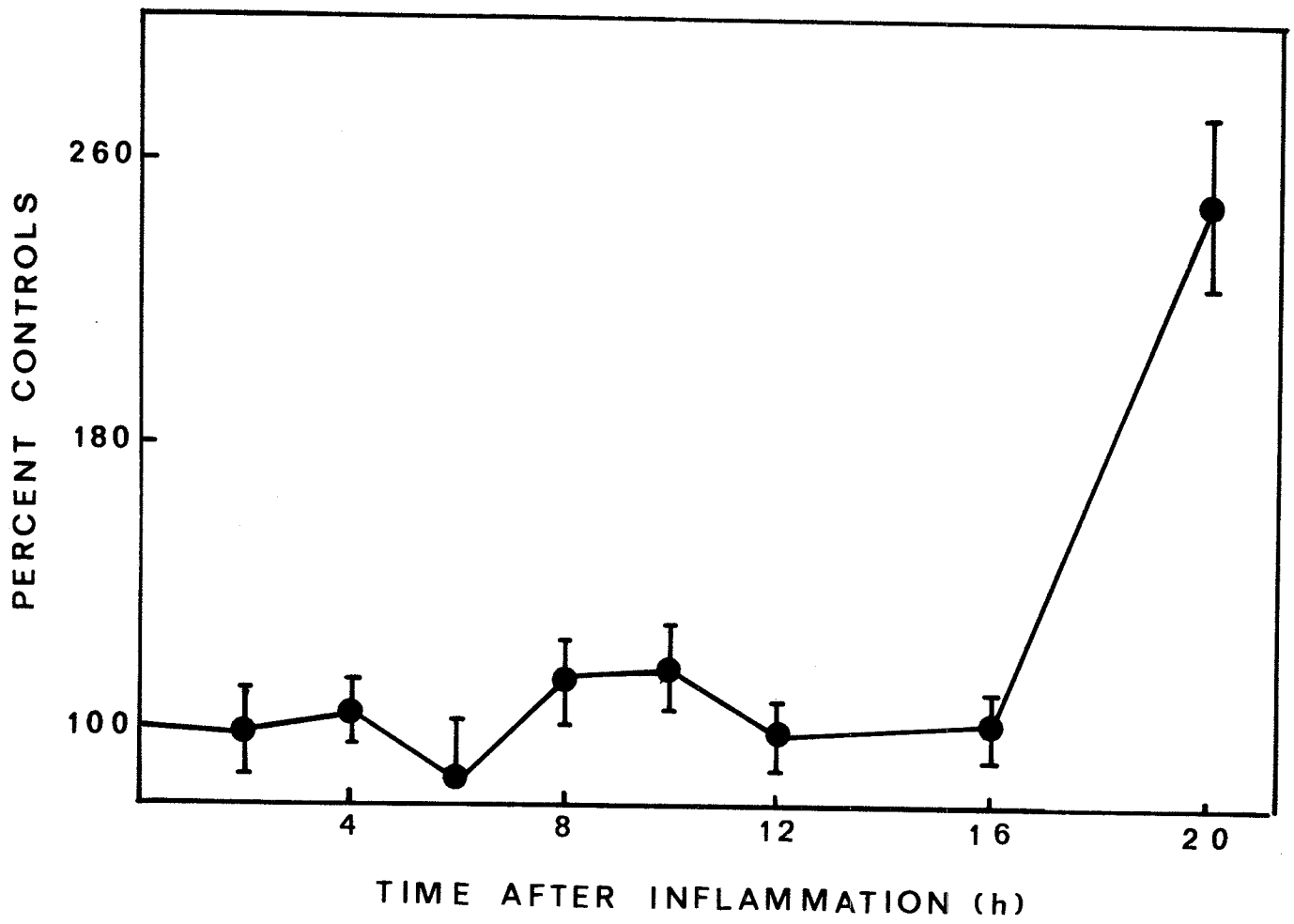

RATIO INFLAMED/CONTROL
3
2
1
4
8
12
16
20
TIME AFTER INFLAMMATION (h)

inflammation on serum cortisol levels. Control levels were constant at 220 ±41 nM. This level is in agreement with that found by Langstaff et al. (1980) for control animals after a 48 h fast. While in the first 4 h following inflammation, cortisol levels did not deviate from control values, by 6 h there was a significant increase observed. Maximum levels were found at 10-16 h following inflammation and these levels represented a 3-fold increase above control values. The increased serum cortisol levels found following inflammation in the work presented here is in agreement with a 3-fold increase reported at 12 h after inflammation by Langstaff et al. (1980). Also, it has been reported that a 3-fold increase in plasma cortisol levels occurs after the stress of surgical laparotomy in the rat, although maximum levels were observed 6 h post-operative (Brodish, 1977). In the work presented here, a biphasic response to experimental inflammation occurs with an 80% increase in levels at 6-8 h after inflammation and a 3-fold increase at 10-16 h after inflammation. A similar biphasic response was observed by Brodish (1977) but at shorter times following the stress of surgical laparotomy.

Alterations in serum insulin levels have been reported by Langstaff et al. (1980) at later times after inflammation but not at short times following stress. Figure 15 shows serum insulin levels at 2-20 h after inflammation. Control levels of insulin were 10.1 ±0.6 μU/ml. This level is lower than the 35 μU/ml obtained by Langstaff et al. (1980) for control animals after a 48 h fast. The reason for this discrepancy

Figure 15 - Effect of Inflammation on Serum Insulin Levels

Serum was obtained from rats and insulin levels were determined by radioimmunoassay as described in Methods. Means and standard deviations of 4 analyses are indicated. Control levels of insulin were 10.1 ±U/ml. Only the 20 h experimental value deviated from control levels at a confidence level higher than 95% as determined by the Student t test. See text for further explanation.

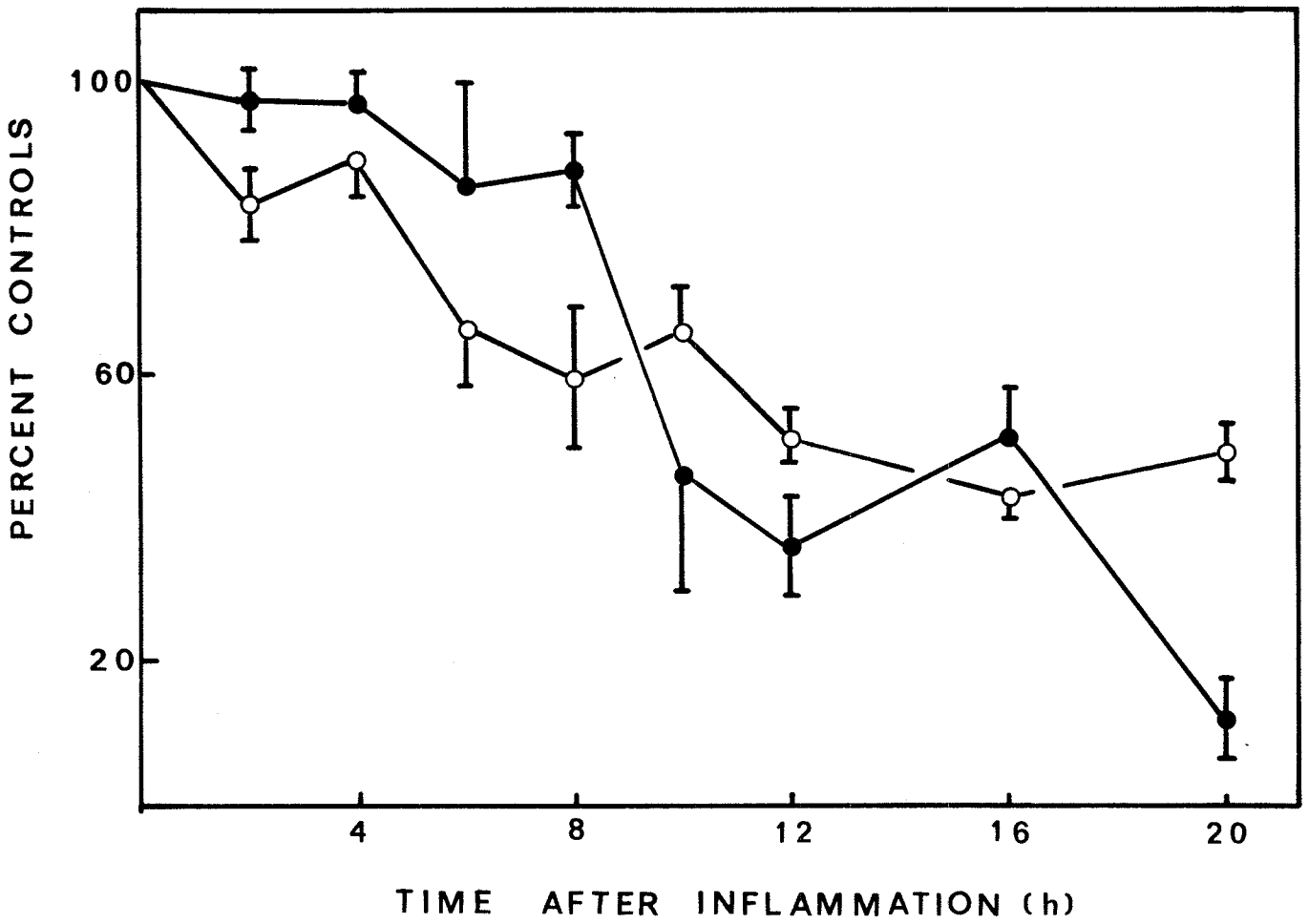

PERCENT CONTROLS
260
180
100
4
8
12
16
20
TIME AFTER INFLAMMATION (h)

is not known, however, Neufeld et al. (1980) have reported that serum insulin levels fall from 21 μU/ml in rats fed ab libitum to 9 μU/ml in rats after a 24 h fast. These values are consistent with the values obtained in the work presented here using rats after a 16 h fast. Serum insulin levels did not deviate from control values in the first 16 h after inflammation although a 2.6-fold increase was observed at 20 h after inflammation. Neufeld et al. (1980) have reported a 3-fold increase in insulin levels in rats infected for 24 h with S. pneumoniae and fasted for 48 h. However, these authors also report a 2-fold increase in 12 h-infected, 36 h-fasted rats, while no differences were observed in the work presented here for 12 h turpentine-inflamed rats fasted for 16 h. Langstaff et al. (1980) reported no difference at 12 h after inflammation and a 2-fold increase at 24 h in rats fasted for 48 h and refed at the time of inflammation. These discrepancies in the effect of stress on serum insulin levels may be related to the nutritional status of the animals.

The effect of inflammation on serum thyroid hormone levels were also examined in these studies. Figure 16 shows these effects on total and free serum thyroxine levels. Control levels of total thyroxine were 61 ng/ml while levels of free thyroxine were 15 pg/ml. Okamura et al. (1981) have reported total thyroxine levels of 42-67 ng/ml for control rats on iodide-containing diets and 3-52 ng/ml for rats on iodide-depleted diets. These values agree with the values obtained in the work presented here for rats feeding on Purina Rat Chow with normal iodide supplementation and starved for 16 h prior to sacrifice.

Figure 16 - Effect of Inflammation on Free and Total Serum Thyroxine Levels

Serum was obtained from rats and total (●) and free (○) thyroxine levels were determined by radioimmunoassay as described in Methods. Means and standard deviations of 4 analyses are indicated. Control levels were 15 pg/ml free thyroxine and 61 ng/ml total thyroxine. Experimental total thyroxine levels other than the 2-6 h values, and all free thyroxine levels, deviated from control levels at the 99.5% confidence levels as determined by the Student t test. See text for further explanation.


PERCENT CONTROLS
100
60
20
4
8
12
16
20
TIME AFTER INFLAMMATION (h)

Okamura et al. (1981) have also reported free thyroxine levels of 21-32 pg/ml for control rats which are slightly higher than the 15 pg/ml reported here. Figure 17 shows the effect of inflammation on serum triiodothyronine levels. Control levels were 490 pg/ml which were similar to the 520-1090 pg/ml reported by Okamura et al. (1981) for control rats. Total thyroxine levels were unaltered for the first 6 h after inflammation after which the levels fell to a minimum of 16% of controls at 20 h after inflammation. Free serum thyroxine levels were depressed at all times following inflammation and were at a minimum of 45% of controls at 16 h following inflammation. At all times other than 2 h after inflammation, serum triiodothyronine levels were depressed and a minumum of 20% of controls was observed at 20 h after inflammation. These decreases are consistent with depressed thyroid hormone levels reported by Beisel (1980) for infected animals.

The results presented in this thesis indicate that a number of endocrine changes occur following inflammation. Some of these changes are rapid and may occur as early as 2 h after an inflammatory stimulus.

2.2 Alterations in Free Amino Acid Pools following Acute Inflammation

While a number of metabolic changes have been identified during the acute phase response (Beisel, 1975, 1980; Shuttler et al., 1977; Langstaff et al. 1980), the effect of inflammation on the pools of free amino acids was unknown and as such was examined in this work. The effect of experimental inflammation on serum and hepatic free amino pools is shown in Tables 11 and 12, respectively. The values obtained

Figure 17 - Effect of Inflammation on Total Serum Triiodothyronine Levels

Serum was obtained from rats and total triiodothyronine levels were determined by radioimmunoassay as described in Methods. Means and standard deviations of 4 analyses are indicated. Control levels were 490 pg/ml. All experimental values other than the 2 h experimental deviated from control levels at the 99.9% confidence level as determined by the Student t test. See text for further explanation.

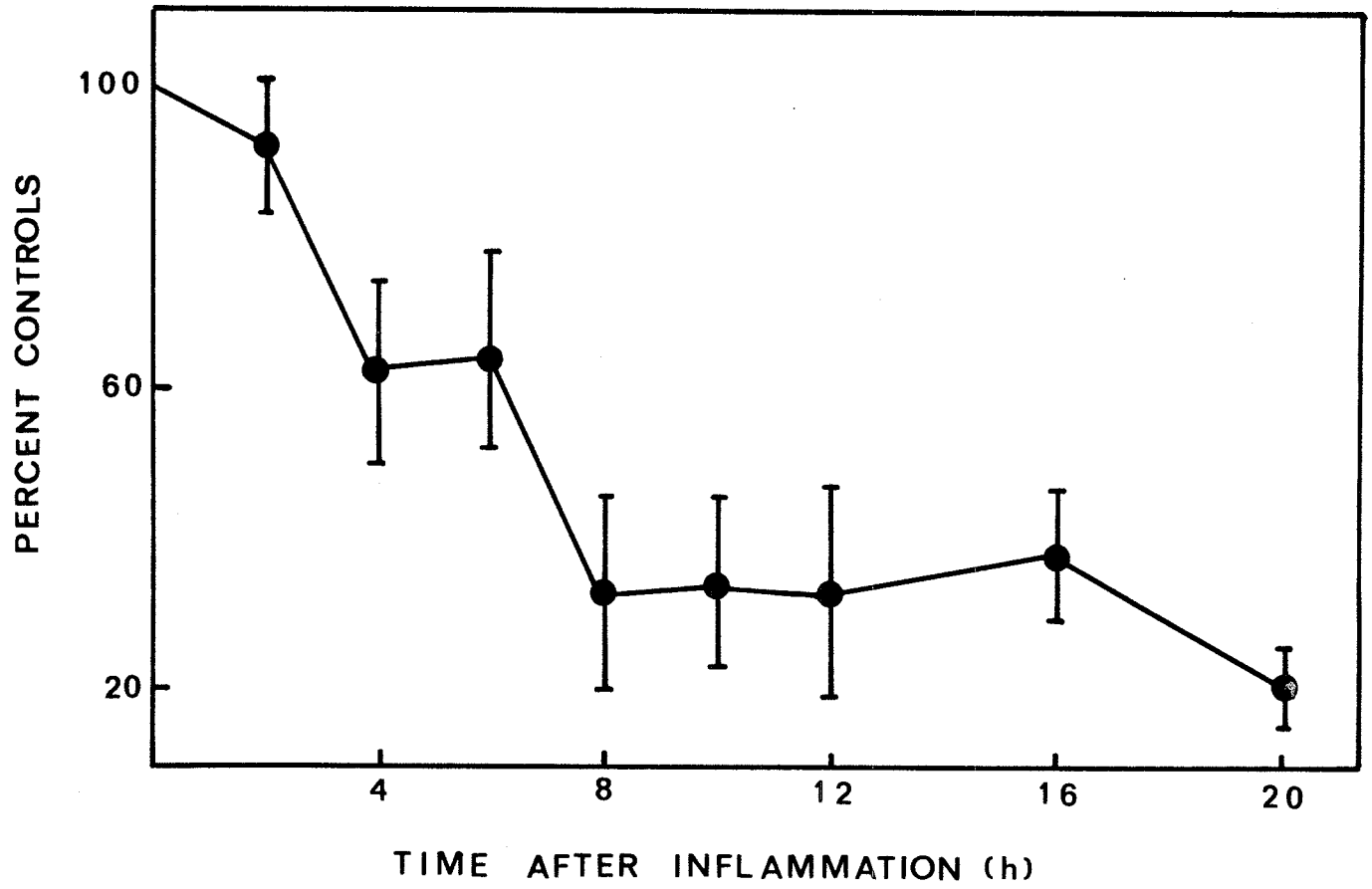

PERCENT CONTROLS
100
60
20
4
8
12
16
20
TIME AFTER INFLAMMATION (h)

Table 11 - Effect of Inflammation on Serum Amino Acid Pools

Amino Acid	Control (26)	Time after Inflammation					
		4 h (12)	8 h (8)	12 h (12)	16 h (6)	24 h (8)	48 h (8)
	nmol/ml	Ratio Inflamed:Control					
Tau	402 ±80	1.42[2]	2.24[1]	1.17	1.13	0.69[2]	0.87
Asx†	194 ±19	1.90[1]	1.31[1]	1.04	1.10	1.81[1]	1.27[2]
Thr†	287 ±14	2.30[1]	0.80[2]	0.95	0.98	1.12	1.00
Ser	337 ±44	1.23	0.92	0.81	0.79	1.27	0.99
Glx†	980 ±49	2.28[1]	1.10	1.04	0.97	1.17	0.83
Pro	195 ±23	1.33	0.42[1]	0.89	0.56[1]	1.46[2]	1.40
Gly	566 ±62	1.62[1]	0.83	0.95	0.89	0.88	0.70[2]
Ala	512 ±66	2.31[1]	1.32	1.11	0.98	1.54[1]	0.96
Val	278 ±34	1.76[1]	1.49[2]	1.03	1.09	0.96	0.93
Ile	143 ±21	1.61[1]	1.21	0.90	0.86	0.81[2]	1.01
Leu	230 ±30	1.70[1]	1.36	1.08	1.11	0.91	1.03
Tyr	106 ±11	1.18	1.30	0.85	0.85	0.97	0.88
Phe	94 ±15	1.68[1]	1.54[1]	1.33	1.28	1.15	0.84
His	94 ± 9	1.86[1]	2.06[1]	1.28[2]	1.12	1.29[2]	1.00
Orn	77 ±18	1.84[1]	1.52[1]	0.59[2]	0.72	0.91	0.96
Lys	600 ±72	1.26[1]	1.47[2]	0.81[2]	0.83[2]	0.98	1.38[2]
Arg	211 ±21	1.37	1.24[2]	0.93	1.22	1.16	1.30[2]

†- amino acid pools calculated from acid hydrolyzed samples. Numbers in parentheses indicate number of analyses performed on group. Statistically significant deviation from controls at the 99.95% ([1]) and 99% ([2]) confidence levels, as determined by the Student t test, are indicated.

Table 12 - Effect of Inflammation on Hepatic Amino Acid Pools

Amino Acid	Control (26)	Time after Inflammation					
		4 h (12)	8 h(8)	12 h (12)	16 h (6)	24 h (8)	48 h (8)
	nmol/g	Ratio Inflamed:Control					
Tau	931 ±242	2.47[1]	2.39[1]	3.57[1]	3.06[1]	1.94[1]	3.04[1]
Asx†	502 ±40	0.64[1]	0.66[1]	1.10	1.73[1]	1.10	1.32
Thr†	192 ±35	0.67[1]	1.18	1.09	1.78[1]	1.08	1.81[1]
Ser	270 ±70	0.65[2]	0.79[2]	1.46	1.51[2]	1.14	2.32[1]
Glx†	3788 ±682	0.76	0.51[1]	0.99	1.73[1]	1.55[1]	1.25
Pro	150 ±54	0.58[2]	0.25[1]	2.38[1]	1.48[2]	0.75	1.53
Gly	1201 ±240	0.63[2]	0.77	2.13[1]	2.49[1]	1.21	1.15
Gly†	3916 ±822	0.70	0.47[1]	1.08	1.86[1]	1.55[1]	1.06
Ala	859 ±94	0.51[1]	0.33[1]	2.54[1]	2.64[1]	1.20	0.84
Val	114 ±18	0.77	1.45[2]	1.31	1.91[1]	0.42[2]	1.17
Ile	56 ±8	0.71	1.40[2]	1.22	1.82[1]	0.81	0.98
Leu	100 ±13	0.78	1.39	1.20	1.58[1]	0.51[1]	1.12
Tyr	41 ±9	0.54[1]	0.63[2]	1.13	1.23	1.04	1.37
Phe	30 ±8	0.80	1.27	1.78[1]	2.05[1]	0.68[2]	1.46[2]
His	206 ±35	0.69[2]	1.06	1.75[1]	1.98[1]	0.63[2]	1.16
Orn	146 ±42	0.64	1.18	1.19	1.23	0.50	1.14
Lys	346 ±62	0.95	1.35	1.71[1]	1.09	1.29	1.54[1]

See footnotes of Table 11 for explanation of symbols.

from control rats generally agree with those found by Stein and Moore (1954) for human plasma and Tallan et al. (1954) for cat plasma and liver. Cysteine, methionine and tryptophan levels in serum and liver samples and arginine levels in liver samples are not given as they were either below the level for reliable quantitation or were not quantitatively recovered in the isolation procedure. Also, asparagine, glutamine and threonine were not sufficiently resolved during amino acid analysis, and so samples were hydrolyzed with acid to convert asparagine to aspartic acid and glutamine to glutamic acid. Acid hydrolysis did not affect the analysis of the other amino acids except for hepatic glycine levels, which were three times higher in acid hydrolyzed samples as compared to untreated controls (see Table 12). Tallan et al. (1954) reported a similar increase in glycine levels with acid hydrolysis of hepatic amino acid samples and attributed the observation to the presence of conjugated ninhydrin-negative forms of glycine which were hydrolyzed to release free glycine.

There was an increase in the pool sizes of all serum amino acids at early times after inflammation as is shown in Table 11. The effects on some amino acids, such as serine and threonine, which deviated less than 30% from controls, may not be significant. Other amino acid pools, such as taurine, aspartic acid and asparagine, threonine, glutamic acid and glutamine, alanine, valine, leucine, phenylalanine, histidine and ornithine, attained levels about twice those of controls in the first 4-8 h following inflammation. After 12-16 h of inflammation, the serum amino acid pools have returned to control levels. Some of the serum amino acids do not follow this simple pattern. The aspartic acid

and asparagine, proline, alanine, histidine and lysine pools were elevated at longer times after inflammation (24-48 h), although the levels attained are smaller than those seen at earlier times. Proline levels were below controls at 8 and 16 h, ornithine and lysine levels were depressed at 12 h, taurine and isoleucine levels were depressed at 24 h and glycine levels were depressed at 48 h after inflammation.

As is shown in Table 12, the response of the hepatic free amino acids to an inflammatory stimulus was considerably different than that of the serum amino acids. At 4 h after inflammation, there was a reduction of the hepatic amino acid pools with the exception of lysine, which was unchanged, and taurine, which was increased substantially. The pools of aspartic acid and asparagine, proline, glycine, alanine, and tyrosine remained depressed at 8 h after inflammation, while at this time, taurine, valine and isoleucine pools were elevated. At 12-16 h after inflammation, the levels of many of the hepatic amino acids were elevated about 2-fold. This elevation of pool size persisted for glutamic acid and glutamine, and glycine at 24 h, and for threonine, serine, phenylalanine and lysine at 48 h after inflammation. Hepatic taurine levels were elevated 2- to 3.5-fold at all times after inflammation which were studied. At 24 h after inflammation, the pools of valine, leucine, phenylalanine, histidine and ornithine were lowered to about one-half of control values.

The alterations in the individual serum and hepatic amino acid pool sizes were examined to ascertain if the behaviour of groups of amino acids were related at all times following an inflammatory stimulus. This statistical approach utilized a one way analysis of

covariance. By this method, only the behaviour of the pool sizes of leucine and isoleucine following the inflammatory stimulus appeared to be interdependent at a confidence level higher than 99%.

3. In Vivo Studies of Cytokines as Mediators of the Acute Phase Response

As mentioned in the Introduction, studies have suggested that factors produced by stimulated leukocytes, or cytokines, are involved in mediating many aspects of the acute phase response. Much of this work had been performed with cytokines from rabbit, human and murine leukocytes; rat cytokines have not been characterized to the same extent. In the work presented here, the *in vivo* effects of cytokines produced by rat leukocytes on parameters of the acute phase response in the rat were examined.

3.1 Characterization of Cytokine Preparations

As a prelude to other studies, initial experiments were performed with the intent of characterizing cytokine preparations with respect to bioactivity, stability and cell origin. Parameters of the acute phase response chosen for these studies were serum sialyltransferase activities, serum α_1-acid glycoprotein and albumin levels, and hepatic sialyltransferase and β-N-acetylglucosaminidase (or hexosaminidase) activities. These parameters were chosen as they represented a spectrum of alterations induced by an inflammatory stimulus. Also, changes in all these parameters could be explained in terms of the action of physiologic regulation of the activity of the liver. Control levels of serum

sialyltransferase activity was found to be 26 pmoles NeuAc transferred/min/ml serum and levels have been reported (Kaplan et al., 1983b) to be elevated 5-fold at 48 h following inflammation. Control levels of serum α_1-acid glycoprotein of 2.3 mg/ml and 37 mg/ml for serum albumin were obtained; a 5-fold elevation of α_1-acid glycoprotein and a 17% reduction of albumin have been reported by Jamieson et al. (1972b). Control levels of hepatic sialyltransferase were 37 pmoles NeuAc transferred/min/mg protein and of hepatic hexosaminidase, 31 nmoles p-nitrophenyl-N-acetyl-β-D-glucosaminide hydrolyzed/min/mg protein. Hepatic sialyltransferase activity was elevated 3-fold (Kaplan et al., 1983b) and hexosaminidase was lowered to 80% of controls (Kaplan & Jamieson, 1977) at 48 h after inflammation.

In preliminary experiments, it was found that PEC, prepared 18 h after infusion of rats with a solution containing casein and glycogen, produced a factor, during a 2 h incubation at 37°C, which elevated serum sialyltransferase activities and lowered serum albumin levels when administered to rats. Further experiments demonstrated that lipopolysaccharide (LPS) could replace the casein in the solution infused into the rats without loss of bioactivity; therefore, a solution of glycogen and LPS in saline was used as described in Methods in all subsequent experiments.

It was of interest to know what handling procedures could be used with the cytokine preparations and so a series of experiments were performed which are summarized in Table 13. It was found that the cytokine was retained by dialysis tubing, which would suggest a molecular weight greater than 8,000 for the bioactive component.

Table 13 - Stability of Cytokine Preparations to Handling Procedures[1]

Treatment	Percent Controls		
	Serum Sialyltransferase	Serum Albumin	Hepatic Hexosaminidase
None	171 ±27[2]	85 ±6[2]	89 ±5[2]
Dialysis (overnight against 0.15 M sodium chloride at 4°C)	165 ±3[2]	82 ±2[2]	71 ±5[2]
Storage at 4°C overnight	159 ±9[2]	91 ±3[2]	86 ±4[2]
Storage at 4°C, 3 days	92 ±6	101 ±2	98 ±1
Storage at 20°C overnight	106 ±10	97 ±3	106 ±4
Storage at -20°C, 1 month	215 ±23[2]	81 ±4[2]	92 ±2[2]
Lyophilized, reconstituted	182 ±11[2]	72 ±5[2]	95 ±2[2]
Amicon filtrate	115 ±5	99 ±3	100 ±7
Amicon-concentrated preparation	96 ±4	101 ±1	102 ±4

[1]PEC were prepared 18 h after infusion of a glycogen, LPS, saline solution. Cells were incubated at a cell density of 0.7-1.3 x 10^8 cells/ml. Rats received intraperitoneal injections of 1-3 ml of cytokine preparations 48 h prior to sacrifice. Results shown are the means and standard deviations of 2-8 analyses represented as percentages of controls receiving injections of heat-inactivated cytokine preparations. See text for further explanation.

[2]Values which differ from controls at the 99.9% confidence level as determined by the Student t test.

Cytokine bioactivity was also stable to freeze-drying and storage overnight at 4°C, or for one month at -20°C. The cytokine lost activity during attempts at ultrafiltration with an Amicon UM-2 membrane, when kept at room temperature overnight or when stored at 4°C for 3 days. Although these observations suggest a reasonably stable factor, in all subsequent in vivo experiments, cytokine preparations were stored at 4°C until administration to animals, which was within hours of preparation.

The chemical nature of the cytokine preparations was further studied as is shown in Table 14. These experiments demonstrated that the bioactivity of the cytokine is lost with incubation with pronase but not with trypsin. This observation suggests a protein nature of the bioactive cytokine. The retention of bioactivity with trypsin digestion of cytokine preparations suggests that there are no trypsin-sensitive sites in the bioactive portion of the cytokine, or perhaps in the molecule(s). Pronase is a broader spectrum protease than trypsin. The results presented in Table 14 also show that bioactivity, with the exception of depression of hepatic hexosaminidase activity, is lost upon heat treatment. The observation that hepatic hexosaminidase activity responded differently to cytokine preparations than other parameters examined was observed in several experiments reported in this thesis. Heat-treated cytokine preparations were partially active towards hepatic hexosaminidase activity, had small (less than 20%) effects on serum sialyltransferase activities and did not affect the other parameters studied. In comparison, pronase treatment of cytokine preparations resulted in a complete loss of all bioactivity towards all the parameters examined. For this reason, pronase-treated cytokine

Table 14 - Stability of Cytokine Preparations[1]

Parameter	Percent Controls			
	No Treatment	Trypsin	Pronase	Heat
Serum Sialyltransferase	183 ±21[2]	160 ±15[2]	95 ±16	92 ±15
Serum Albumin	82 ±2[2]	85 ±3[2]	100 ±2	102 ±2
Serum α_1-Acid Glycoprotein	142 ±3[2]	135 ±5[2]	105 ±3	97 ±2
Liver Sialyltransferase	162 ±11[2]	151 ±5[2]	104 ±2	96 ±5
Liver Hexosaminidase	83 ±4[2]	81 ±5[2]	98 ±4	85 ±3[2]

[1]Cytokine was prepared from PEC at a cell density of 9×10^7 cells/ml as described in Methods. Rats received i.p. injections of 250 MCE cytokine 48 h prior to sacrifice. Treatments of cytokine preparations are described in Methods. Results shown are the means and standard deviations of 4 analyses represented as percentages of untreated controls. See text for further explanation.
[2]Values which differ from controls at the 99.9% confidence level as determined by the Student t test.

preparations were used as controls in all subsequent experiments.

Table 15 shows the effect of cytokine administration, by an intravenous versus an intraperitoneal route, on the response by parameters of the acute phase response. The results demonstrated no statistically significant difference for the two routes of administration. This suggests that it was not necessary for the cytokine to be present in the circulatory system at very short times after administration for elicitation of a response at 48 h after administration.

Table 16 shows the effect of cytokine administration at varying dosages on parameters of the acute phase response. There was no difference observed in the response of sialyltransferase activities and serum albumin to 50-500 MCE of cytokine, however, hepatic hexosaminidase activitity did not respond to less than 200 MCE of cytokine. This observation is consistent with the observation that the factor in cytokine preparations affecting hexosaminidase activity had a different heat stability from those affecting the other parameters examined.

As cell origin represents one basis for the classification of cytokines, and there is the possibility that more than one cytokine was responsible for the bioactivities observed, experiments were performed in which leukocytes were fractionated prior to preparation of cytokine. Table 17 indicates that after fractionation of PEC using a continuous Percoll density gradient, the bioactive cytokine was produced by the fraction enriched in monocytes and lymphocytes, whereas the preparation of PMN cells did not produced detectable cytokine. As this approach did not clearly distinguish the cell type which was responsible for cytokine production, another method of fractionation which separated monocytes

Table 15 - Effect of Route of Administration on Cytokine Bioactivity[1]

Parameter	Percent Controls	
	Intravenous	Intraperitoneal
Serum Sialyltransferase	181 ±21	197 ±15
Serum Albumin	88 ±4	85 ±2
Liver Sialyltransferase	141 ±6	157 ±9
Liver Hexosaminidase	83 ±5	82 ±3

[1]Cytokine was prepared as described in Methods; rats received i.v. or i.p. injections of 120 MCE of cytokine 48 h prior to sacrifice. Results shown are the means and standard deviations of 3 analyses represented as percentages of controls which received injections of heat-inactivated preparations. All values differed from controls at the 99.9% confidence level as determined by the Student t test. Values obtained for the different routes of administration did not show a statistically significant difference.

Table 16 - Effect of Cytokine Dosages on Cytokine Bioactivity[1]

Parameter	Percent Controls			
	Dosage			
	50 MCE	100 MCE	200 MCE	500 MCE
Serum Sialyltransferase	210 ±28[2]	235 ±40[2]	251 ±18[2]	242 ±9[2]
Serum Albumin	85 ±2[2]	79 ±3[2]	81 ±2[2]	83 ±4[2]
Liver Sialyltransferase	141 ±8[2]	169 ±12[2]	136 ±5[2]	147 ±6[2]
Liver Hexosaminidase	103 ±4	93 ±4	85 ±3[2]	87 ±4[2]

[1]Cytokine was prepared as described in Methods; rats received i.p. injections of the cytokine preparations 48 h prior to sacrifice. Volumes of 0.5 ml (50 MCE) to 5 ml (500 MCE) were injected. Results shown are the means and standard deviations of 3 analyses represented as percentages of controls receiving injections of pronase-treated cytokine. See text for further explanation.
[2]Values which differ from controls at the 99.9% confidence level as determined by the Student t test.

Table 17 - Fractionation of Cytokine Producing Cells on a Percoll Gradient

Parameter	Percent Controls[1]		
	PEC	Band 1	Band 2
Serum			
α_1-Acid Glycoprotein	153 ±6[2]	161 ±8[2]	104 ±5
Albumin	81 ±3[2]	83 ±2[2]	94 ±3
Sialyltransferase	297 ±24[2]	225 ±19[2]	116 ±8
Liver			
Sialyltransferase	149 ±5[2]	129 ±5[2]	94 ±4
Hexosaminidase	88 ±2[2]	77 ±4[2]	98 ±3

[1]PEC were prepared and fractionated on a Percoll Gradient as described in Methods. Band 1 contained 57% lymphocytes, 14% monocytes, 28% PMN and 1% mast cells as determined by Wright's staining; Band 2 contained 98% PMN cells, 1% lymphocytes, 0.5% mast cells and 0.3% monocytes. Cells were incubated in 0.15 M sodium chloride at a cell density of 6×10^7 cells/ml for 2 h; 180 MCE of cytokine was injected i.p. into rats 36 h prior to sacrifice. Results shown are the means and standard deviations of 3 analyses represented as percentages of controls receiving injections of pronase-treated cytokine perparations.
[2]Represents values which differ from controls at the 99.9% confidence level as determined by the Student t test.

from other cells on the basis of adherence to surfaces was used. Table 18 shows that the cytokine from the monocyte fraction was more active than that from the original PEC preparation in producing the changes in acute phase parameters. The non-adherent cells in the PEC preparation produced cytokine which did not affect sialyltransferase activities, α_1-acid glycoprotein levels and albumin levels, although hepatic hexosaminidase activity was lowered by cytokines produced by non-adherent cells. These results lead to the conclusion that the cytokine responsible for alterations in sialyltransferase activities, α_1-acid glycoprotein levels and albumin levels is produced by monocytes. The cell origin of the cytokine(s) responsible for depressed hexosaminidase activity is ambiguous. There may be more than one cytokine which will depress hepatic hexosaminidase activity when administered to rats. Non-adherent cells may be producing one of these cytokines. Also, lymphocytes may be producing the cytokine or may be necessary for monocyte production of it.

The results presented in this thesis indicate that administration of a heat- and pronase-sensitive rat monokine results in alterations of a number of parameters of the acute phase response to inflammation in the rat.

3.2 In Vivo Response to Cytokines

A series of experiments were performed to characterize the changes in parameters of the acute phase response of animals to which cytokine had been administered. These experiments provide a basis for comparison of the effects of cytokines to those of an inflammatory stimulus such as turpentine.

Table 18 - Fractionation of Cytokine Producing Cells by Adherence on Plates

Parameter	Percent Controls[1]		
	PEC	Adherent	Non-Adherent
Serum			
α_1-Acid Glycoprotein	141 ±7[2]	168 ±9[2]	109 ±7
Albumin	85 ±2[2]	83 ±3[2]	94 ±4
Sialyltransferase	195 ±2[2]	275 ±12[2]	121 ±17
Liver			
Sialyltransferase	138 ±8[2]	152 ±9[2]	107 ±6
Hexosaminidase	57 ±12[2]	70 ±9[2]	85 ±8[2]

[1]PEC were prepared and fractionated by adherence on plates as described in Methods. PEC were also plated for 1 h after which the cells were scraped from the plates, washed three times with 0.15 M sodium chloride and replated. Cell preparations were incubated on plates at a cell density of 4 x 10^7 cells/ml for 3 h at 37°C in a humid atmosphere of a 95:5 mixture of air:carbon dioxide. Rats received i.p. injections of 120 MCE of cytokine preparations 36 h prior to sacrifice. Adherent cells contained 90% monocytes, 8% PMN cells and 2% lymphocytes; non-adherent cells contained 80% PMN cells, 15% lymphocytes 4% monocytes and 1% mast cells as determined by Wright's staining. Results shown represent the means and standard deviations of 3 analyses represented as percentages of controls receiving injections of pronase-treated cytokine preparations.
[2]Represents values which differ from controls at the 99.9% confidence levels as determined by the Student t test.

Figure 18 shows the effect of cytokine administration on serum albumin and α_1-acid glycoprotein levels. The response of serum albumin was similar to the 80% reduction in levels following an inflammatory stimulus, but the serum α_1-acid glycoprotein response was attenuated compared to that seen following inflammation. Serum α_1-acid glycoprotein levels increased only 1.6-fold following cytokine administration, whereas inflammation elicited a 5-fold elevation (Jamieson et al., 1972b). Figure 19 shows the effect of cytokine administration on hepatic levels of albumin and α_1-acid glycoprotein. While the serum levels of these proteins were altered at all times following administration, hepatic levels did not change significantly for the first 24 h following administration. Liver albumin content was lowered 15% at 36 h after cytokine administration and α_1-acid glycoprotein content was elevated 30% at 36 h and 70% at 48 h. In comparison, liver albumin content was depressed to a minimum of 78% of controls at 24 h and α_1-acid glycoprotein elevated to a maximum of 350% of controls at 12 h after inflammation (Jamieson & Ashton, 1973). Hepatic albumin levels responded similarly, while α_1-acid glycoprotein levels changed less upon cytokine administration than after an inflammatory stimulus.

Figure 20 shows the effect of cytokine administration on serum and hepatic sialyltransferase activities. Enzyme activities were elevated as early as 8 h after administration and both activities peaked at 36 h. Although cytokine caused a hepatic sialyltransferase response similar to that following turpentine injection, the serum sialyltransferase response to cytokine was attenuated compared to an acute phase response (Kaplan et al., 1983b). Also, the response of sialyltransferase activities to cytokine peaked earlier than that for

Figure 18 - Effect of Cytokine Administration on Rat Serum Albumin and α_1-Acid Glycoprotein Levels

Rats received i.p. injections of 300 MCE cytokine and albumin and α_1-acid glycoprotein levels were determined as described in Methods. Control levels of albumin (-● -) were 37 mg/ml and of α_1-acid glycoprotein (-■ -) were 2.3 mg/ml. Results shown are the means and standard deviations of 4-6 analyses represented as percentages of controls. Values in rats receiving pronase-treated cytokine did not deviate appreciably from untreated controls. See text for further explanation.

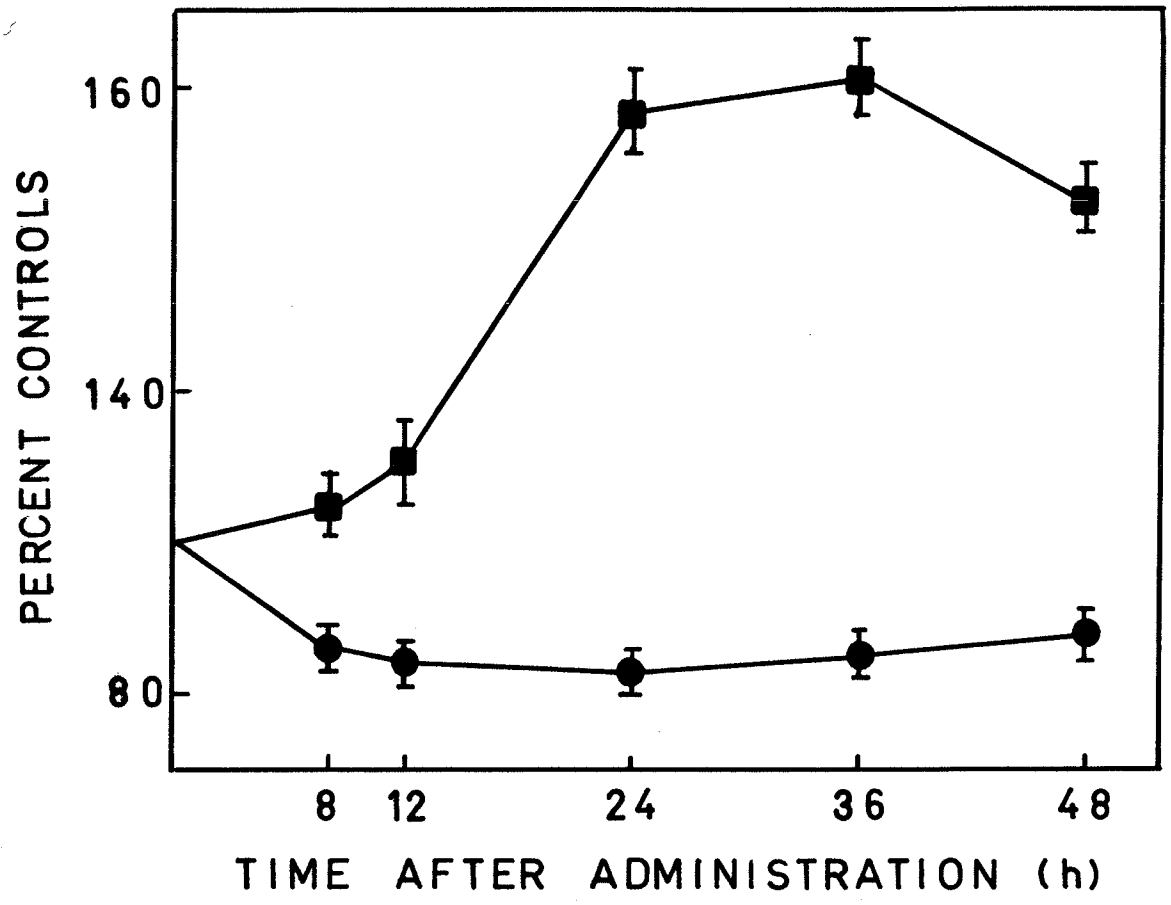

160
140
80
PERCENT CONTROLS
8
12
24
36
48
TIME AFTER ADMINISTRATION (h)

Figure 19 - Effect of Cytokine Administration on Hepatic α_1-Acid Glycoprotein and Albumin Levels

Control levels of albumin (-○-) were 0.44 mg/g wet weight liver and of α_1-acid glycoprotein (-●-), 0.16 mg/g wet weight liver. See text and Figure 18 for further explanation.

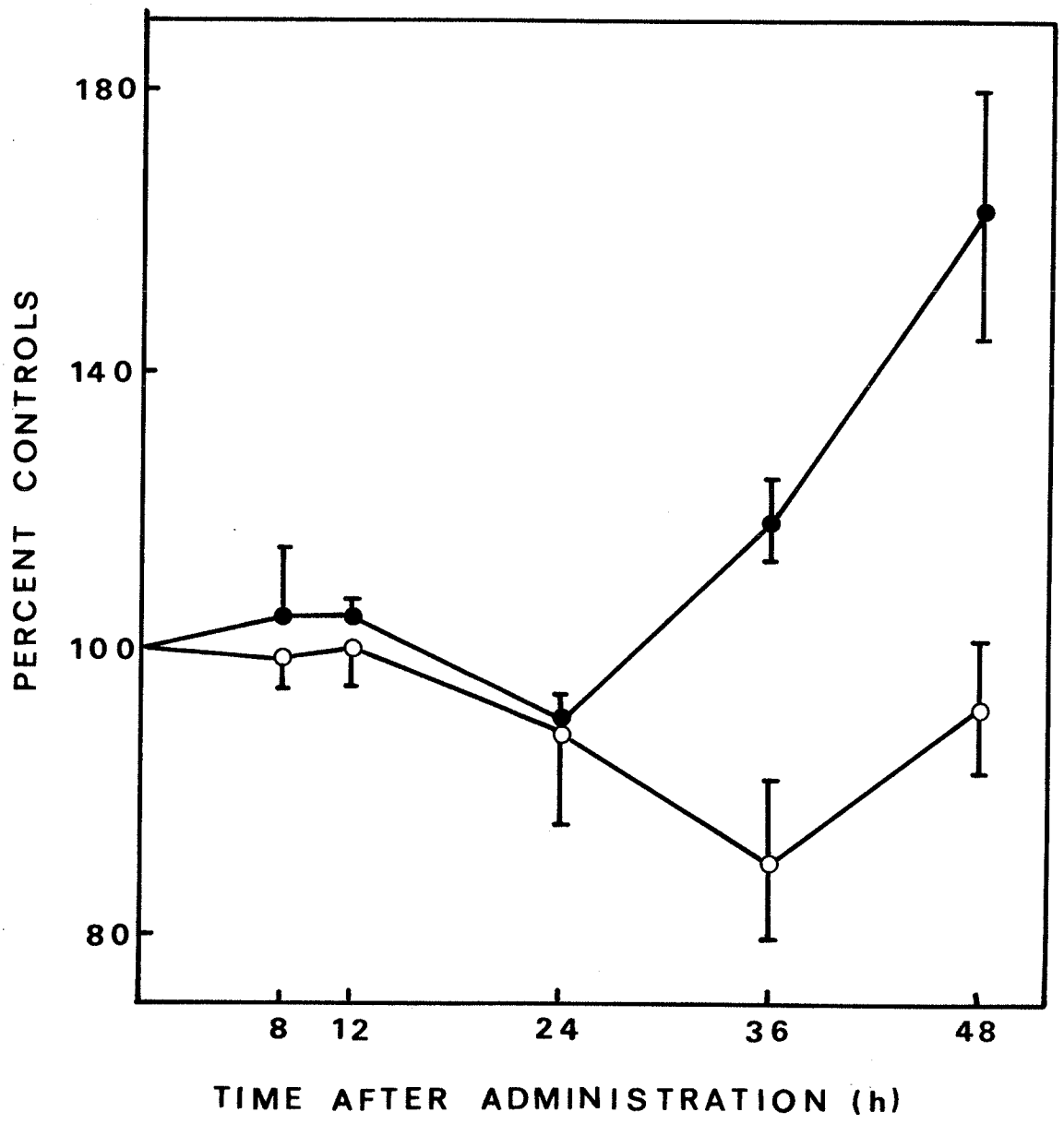
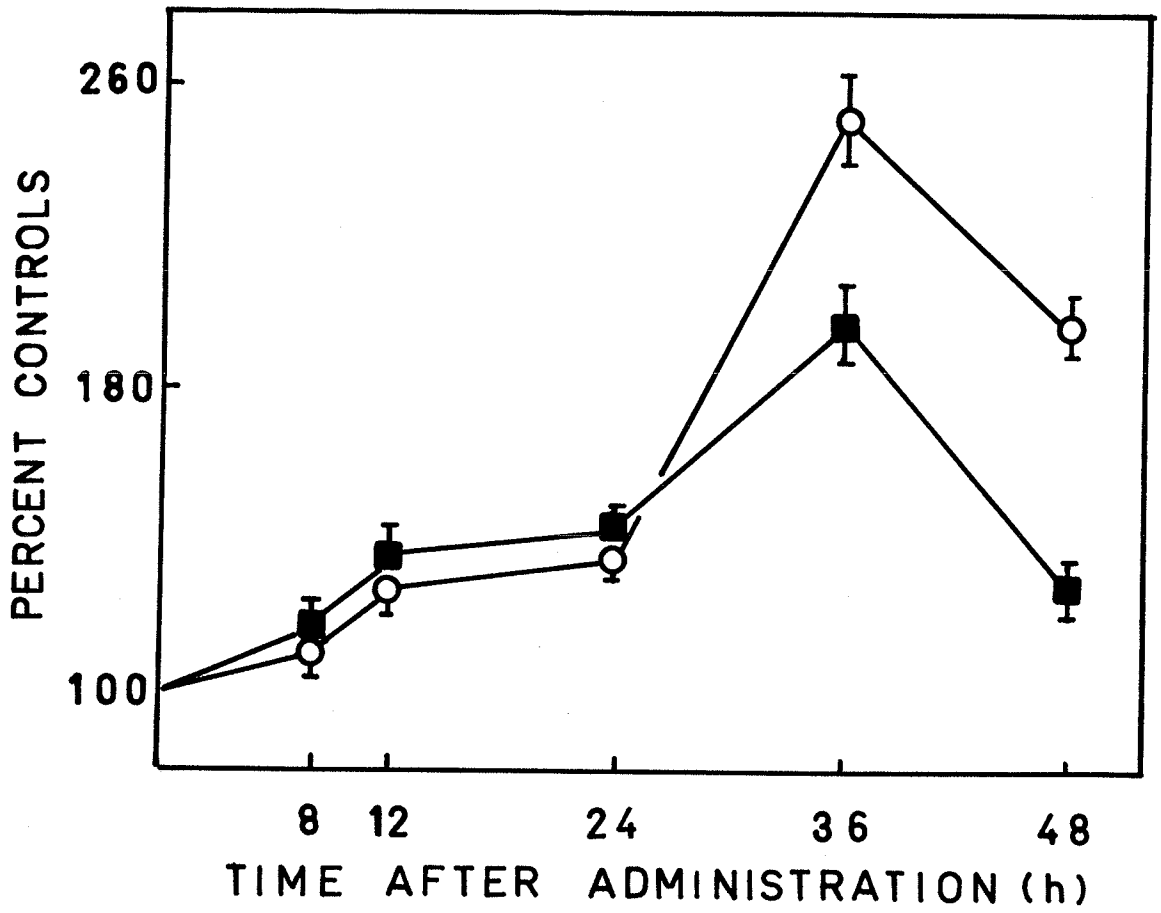

180
140
100
80
PERCENT CONTROLS
8
12
24
36
48
TIME AFTER ADMINISTRATION (h)

Figure 20 - Effect of Cytokine Administration on Serum and Hepatic Sialyltransferase Activities

Control levels of serum sialyltransferase (-■-) were 26 pmoles N-acetylneuraminic acid transferred/min/ml, and of hepatic sialyltransferase (-O-), 37 pmoles N-acetylneuraminic acid transferred/min/mg protein. See text and Figure 18 for further explanation.


260
180
100
PERCENT CONTROLS
8
12
24
36
48
TIME AFTER ADMINISTRATION (h)

an inflammatory stimulus. Figure 21 shows that cytokine administration depressed hepatic β-galactosidase and hexosaminidase activities to a minimum of 80% of controls at 24 h after administration. This response is only one-half that seen following an inflammatory stimulus (Kaplan & Jamieson, 1977).

The effect of cytokine was also examined on two hormones known to change during the acute phase response. Figure 22 shows that while serum cortisol levels were not altered for the first 24 h after cytokine administration, a 2-fold elevation occurred at 36 h after administration. This is lower and much later than the 3-fold elevation observed at 10-16 h after an inflammatory stimulus (see Figure 14). Figure 23 shows that while serum thyroxine levels are depressed to 40% of controls at 8 h after cytokine administration, there is an elevation at 36-48 h. Serum thyroxine levels are consistently lowered following inflammation (see Figure 16), and as such, the response of this parameter to cytokine resembles that following an inflammatory stimulus only at shorter times.

The results presented in this thesis indicate that administration of rat cytokines to rats mimics several aspects of inflammation, however, the response to cytokine administration is the same as that to an inflammatory agent such as turpentine only for serum albumin levels.

4. Liver Slice Studies

A series of experiments was performed utilizing liver slices prepared from control and experimentally inflamed rats. The approach avoids the difficulties inherent in in vivo studies where the liver

Figure 21 - Effect of Cytokine Administration on Hepatic β-Galactosidase and β-N-Acetylhexosaminidase Activities

Control levels of galactosidase (-O-) were 0.51 nmoles p-nitrophenyl-β-D-galactoside hydrolyzed/min/mg protein and of hexosaminidase, (-●-) 31 nmoles p-nitrophenyl-N-acetyl-β-D-glucosaminide hydrolyzed/min/mg protein. See text and Figure 18 for further explanation.

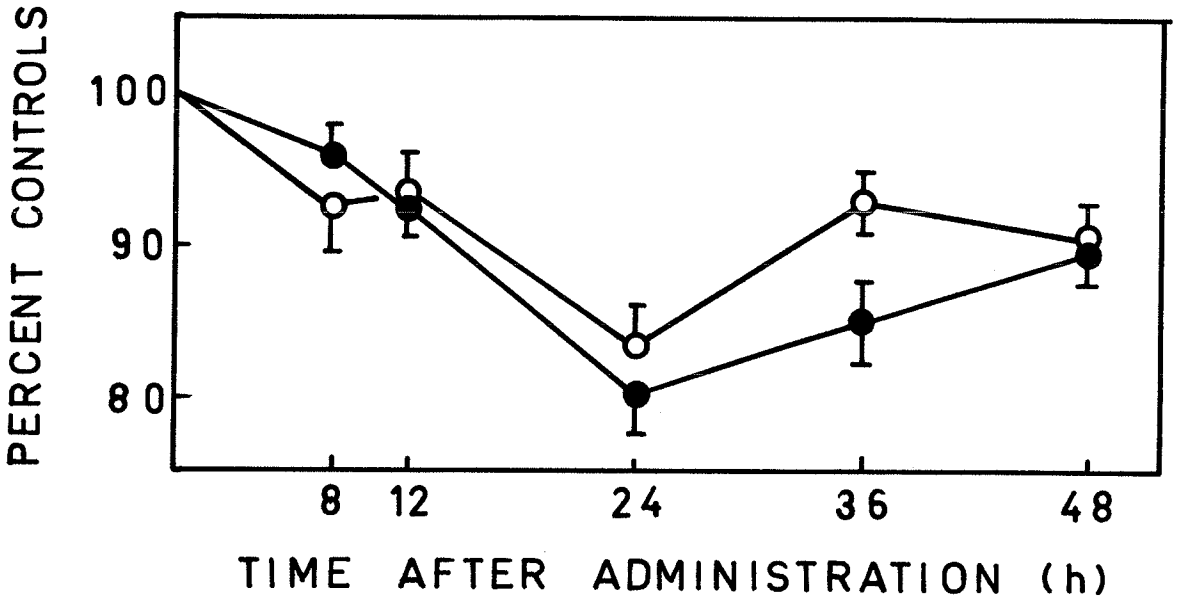

PERCENT CONTROLS
100
90
80
8
12
24
36
48
TIME AFTER ADMINISTRATION (h)

Figure 22 - Effect of Cytokine Administration on Serum Cortisol Levels

Control levels of cortisol were 220 nM. See text and Figure 18 for further details.

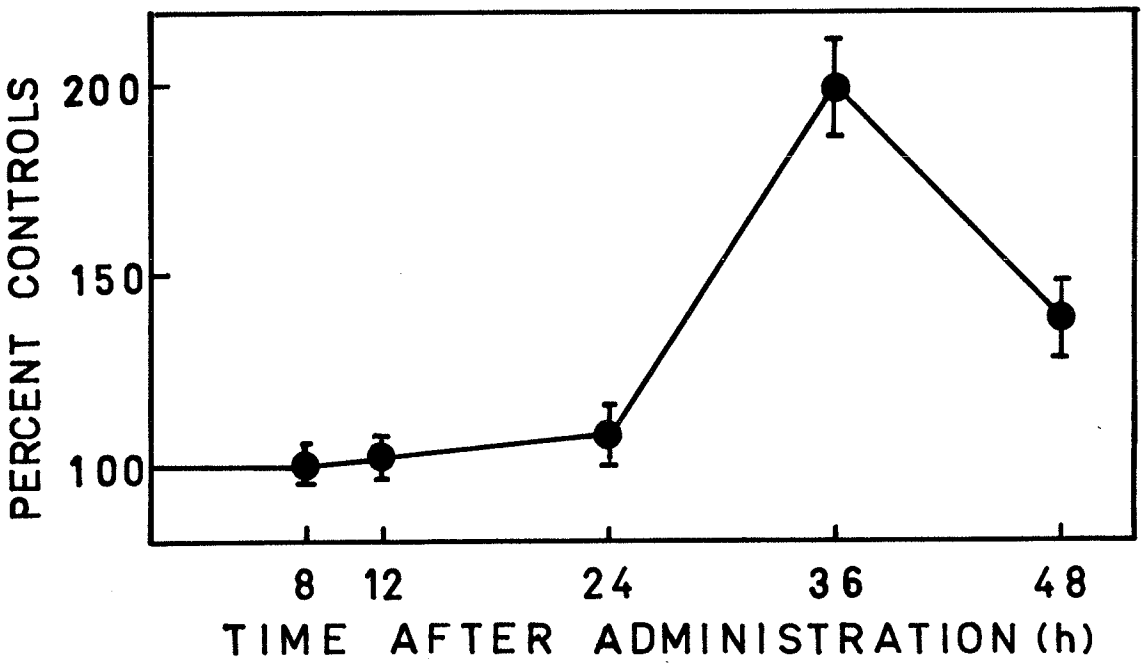
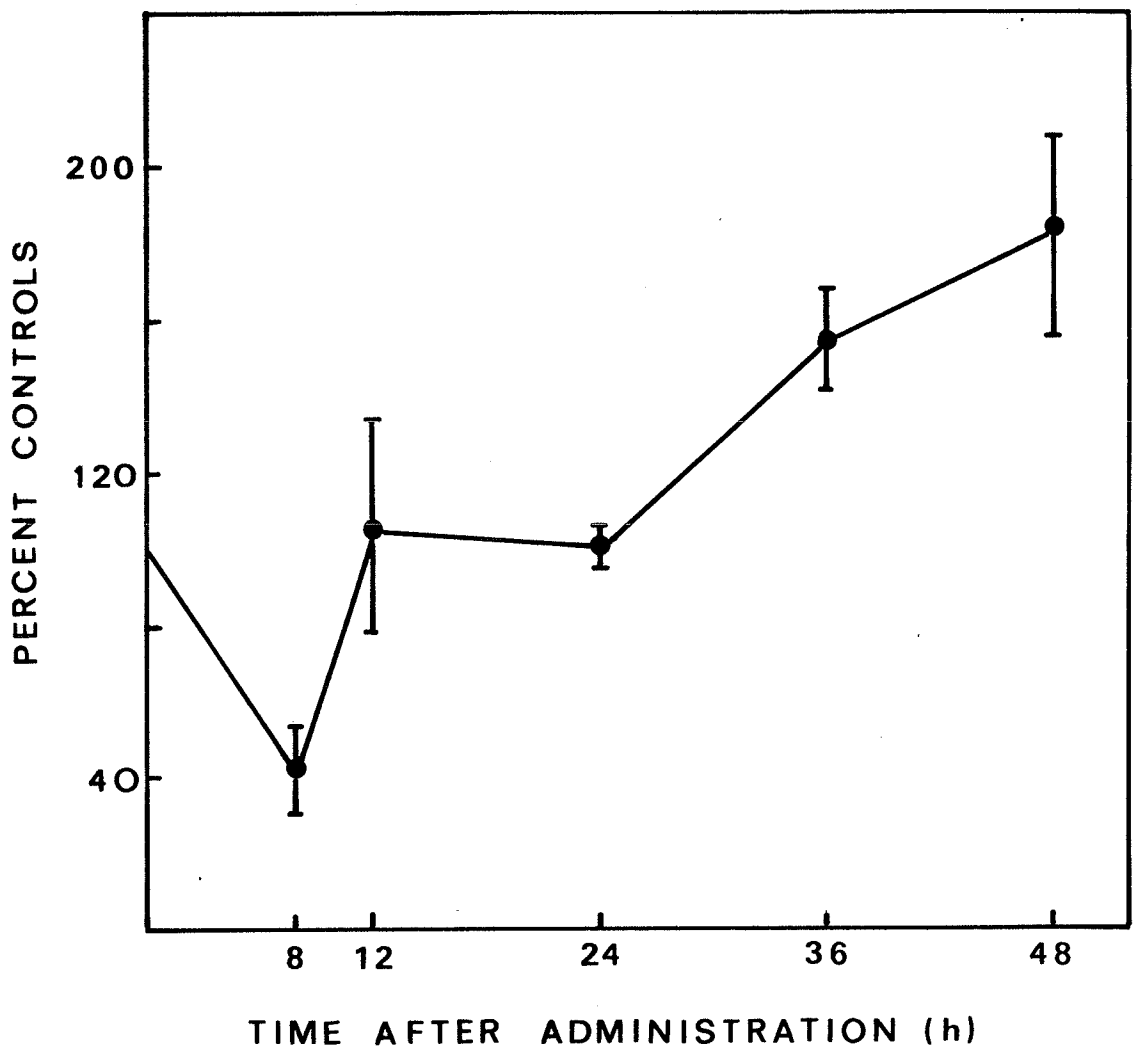

PERCENT CONTROLS
200
150
100
8
12
24
36
48
TIME AFTER ADMINISTRATION (h)

Figure 23 - Effect of Cytokine Administration on Total Serum Thyroxine

Control levels were 58 pg/ml. See text and Figure 18 for further explanation.


200
120
40
PERCENT CONTROLS
8
12
24
36
48
TIME AFTER ADMINISTRATION (h)

is in a constantly changing humoral environment. The use of liver slices also allows the testing of direct effects of physiological or pharmacological agents.

4.1 RNA Synthesis by Liver Slices

The first experiments performed in these studies were aimed at characterizing the synthesis of RNA by the liver slices. Figure 24 shows the time course of incorporation of [^{3}H]-orotate into total RNA isolated from liver slices. Under the conditions utilized, there was a linear increase in the specific radioactivities of [^{3}H] in the RNA of the liver slices over a period of 6 h, at which time about 30% of the tracer had been incorporated into RNA. Clemens and Korner (1970) have shown that liver slices exhibit a linear uptake of orotate into the acid-soluble fraction for at least the first hour of incubation. Also, orotate is a precursor for the biosynthesis of uridine-5'-monophosphate, which serves as one of the precursors for the biosynthesis of RNA (Domagh, 1968). Thus, the linearity of incorporation of [^{3}H] shown in Figure 24 indicates that the processes of orotate transport into hepatocytes, synthesis of nucleotides and transcription remained intact over 6 h of incubation of the liver slices. This also indicates that the rate of synthesis of RNA in liver slices is constant over the incubation times. Orotate incorporation has been used by others as a measure of RNA synthesis (Clemens & Korner, 1970; Mishra & Feltham, 1973; Thompson & Wannemacher, 1973; Ch'ih *et al.*, 1977) and the relative patterns of *de novo* labelling with radiolabelled orotate of hepatic RNA of inflamed rats did not differ when incorporation was expressed as percentages of total radiolabel, specific radioactivities

Figure 24 - Time Course of Incorporation of [^{3}H]-Orotate into Total Cellular RNA of Liver Slices

Samples of 1 g liver slices from control rats were incubated for 1-6 h with 5 μCi [^{3}H]-orotate as described in Methods. Total cellular RNA was then isolated and specific radioactivities were determined. Results shown are the means and standard deviations of 4 analyses. See text for further explanation .

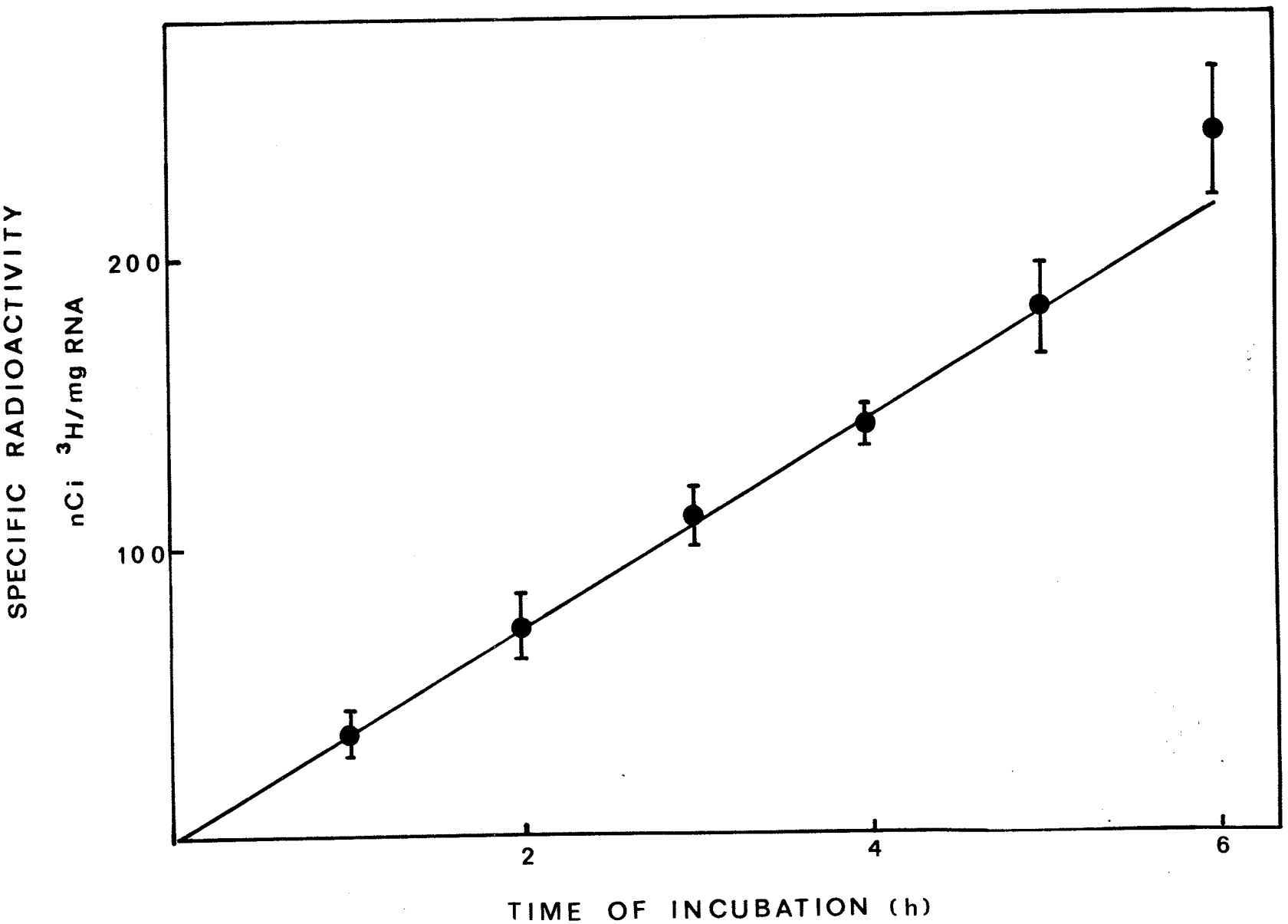
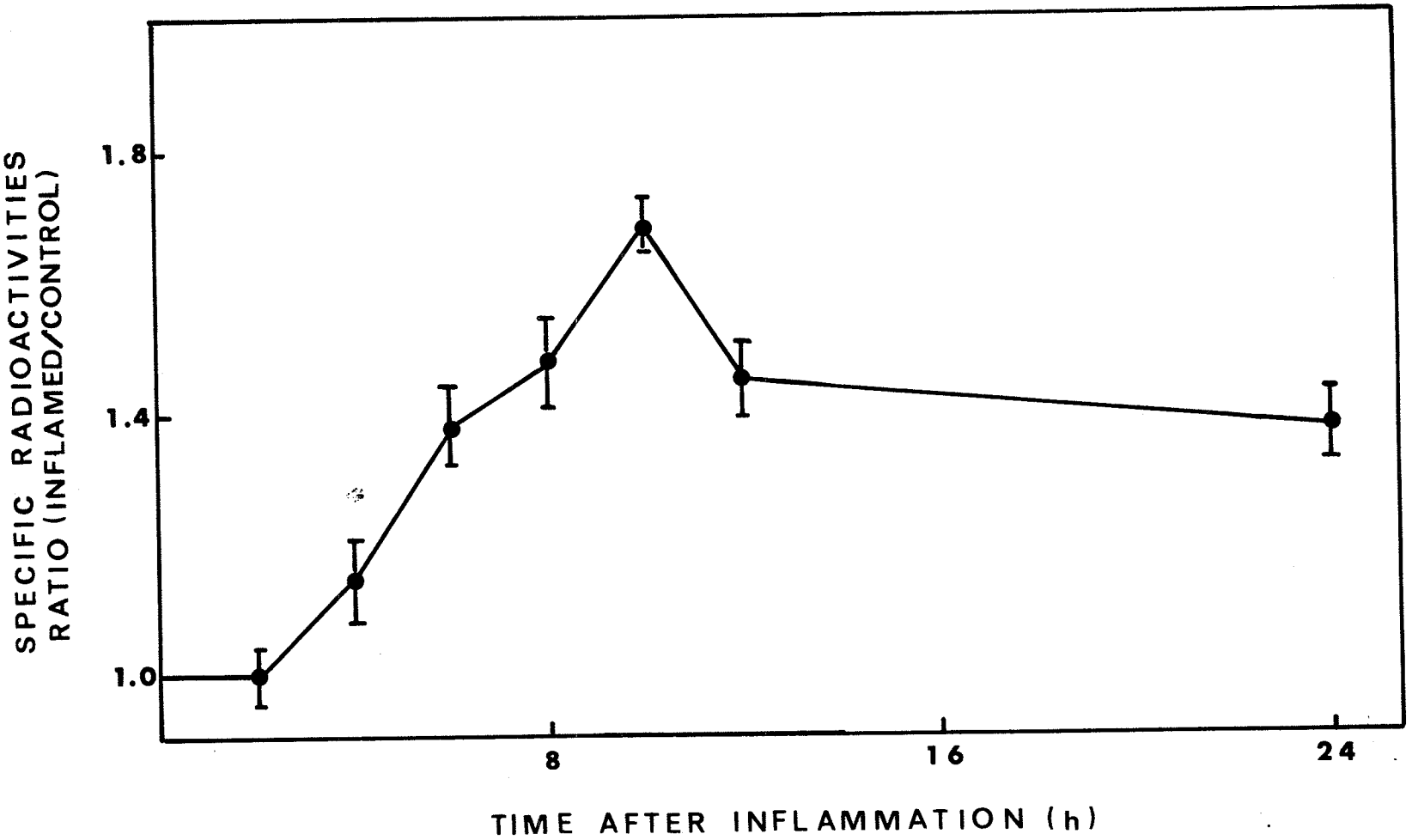

SPECIFIC RADIOACTIVITY
nCi ^{3}H/mg RNA
200
100
2
4
6
TIME OF INCUBATION (h)

Figure 25 - Effect of Inflammation on Incorporation of [^{3}H]-Orotate into Total Cellular RNA of Rat Liver Slices

Samples of 1 g liver slices from control and inflamed rats were incubated with 5 μCi [^{3}H]-orotate for 3 h as described in Methods. Results shown are the means and standard deviations of 4 analyses. See text for further explanation.


SPECIFIC RADIOACTIVITIES
RATIO (INFLAMED/CONTROL)
1.8
1.4
1.0
8
16
24
TIME AFTER INFLAMMATION (h)

or amount of RNA radioactivity per unit of DNA (Thompson & Wannemacher, 1973). These observations lead to the conclusion that orotate labelling of RNA is a suitable measure of transcription rates in liver slices. Others have shown that the processes of synthesis and glycosylation of proteins, the synthesis of lipids and the metabolism of putrescine remain viable in rat liver slices (Clemens & Korner, 1970; Patwardhan & Lanthier, 1974; Jamieson et al., 1975; Lundgren & Hankins, 1978). This would suggest that rat liver slices are suitable for the examination of at least some aspects of the biochemistry and physiology of the liver.

It has been reported that de novo synthesis of RNA is increased in the liver following inflammation and tissue injury (Chandler & Neuhaus, 1968; Thompson & Wannemacher, 1973; Atryek & Fausto, 1979; Haugen et al., 1981; Ricca et al., 1981; Hauer & Little, 1983). This parameter of the acute phase response was examined in liver slices as is shown in Figure 25. Liver slices prepared from rats suffering from inflammation for 4 h or longer had higher rates of RNA synthesis, as determined by [^{3}H]-orotate incorporation; maximum levels of RNA synthesis were seen with liver slices prepared from 10 h inflamed rats. These observations lead to the conclusion that once the liver is stimulated by factors produced or elevated during the acute phase response, humoral or neurological factors are not necessary for maintenance of elevated rates of RNA synthesis.

4.2 Effects of Non-Steroidal Anti-Inflammatory Drugs on Liver Slices' Transcription, Translation and Protein Glycosylation

Administration of non-steroidal anti-inflammatory drugs to

experimental animals and humans results in decreased levels of a variety of serum proteins (Dawkins et al., 1966; Glenn et al., 1968; Akamatsu & Miura, 1972). In experiments using liver slices, two of these drugs, salicylate and phenylbutazone, have been shown to exert direct inhibition on the hepatic biosynthesis of both the polypeptide and carbohydrate moieties of the plasma proteins (Jamieson & Kutryk, 1980). A closer examination of the effects of the anti-inflammatory drugs on liver slices is one approach that could provide some insight into those factors important to the biosynthesis of the plasma proteins, particularly the acute phase reactants.

Figure 26 shows the effects of sulfinpyrazone and indomethacin on α_1-acid glycoprotein and albumin synthesis in rat liver slices as determined immunologically. Both of these drugs substantially decreased the rates of synthesis of the plasma proteins at concentrations of 1 mM and greater. The effects were similar in liver slices from both control and 24 h inflamed rats despite the fact that there was elevated synthesis of α_1-acid glycoprotein and decreased synthesis of albumin in liver slices from inflamed rats. The inhibitory effects of these drugs on biosynthesis were also shown when rates of synthesis were examined by measuring the incorporation of [^{3}H]-leucine into polypeptide and [^{14}C]-glucosamine into carbohydrate moieties of glycoprotein. These measures of hepatic protein synthesis proved to be more sensitive to the effects of the drugs. Indomethacin and sulfinpyrzone inhibited the synthesis of total protein (Figure 27) as well as the specific proteins α_1-acid glycoprotein and albumin (Figures 28 and 29) when rates of synthesis were examined by tracer incorporation. Significant inhibition of translation and glycosylation were always observed with

Figure 26 - The Effect of Indomethacin and Sulfinpyrazone on the Rates of Synthesis of Albumin and α_1-Acid Glycoprotein by Rat Liver Slices

Liver slices were prepared from control and 24 h inflamed rats and incubated with drugs as described in Methods; rates of synthesis of α_1-acid glycoprotein (A and B) and albumin (C and D) were determined by immunoprecipitation of these proteins from liver slices prepared from control (-●-) and inflamed (-O-) rats. Rates of synthesis are given in units of µg albumin or α_1-acid glycoprotein synthesized/g wet weight liver slices/h. The results shown are the means of 3-4 analyses, standard deviations of the mean were within 12%. Values deviated from controls at the 95% confidence levels for 0.5 mM concentrations and at the 99.9% confidence levels at higher concentrations of drugs when statistical significance was analyzed by the Student t test. See text for further details.

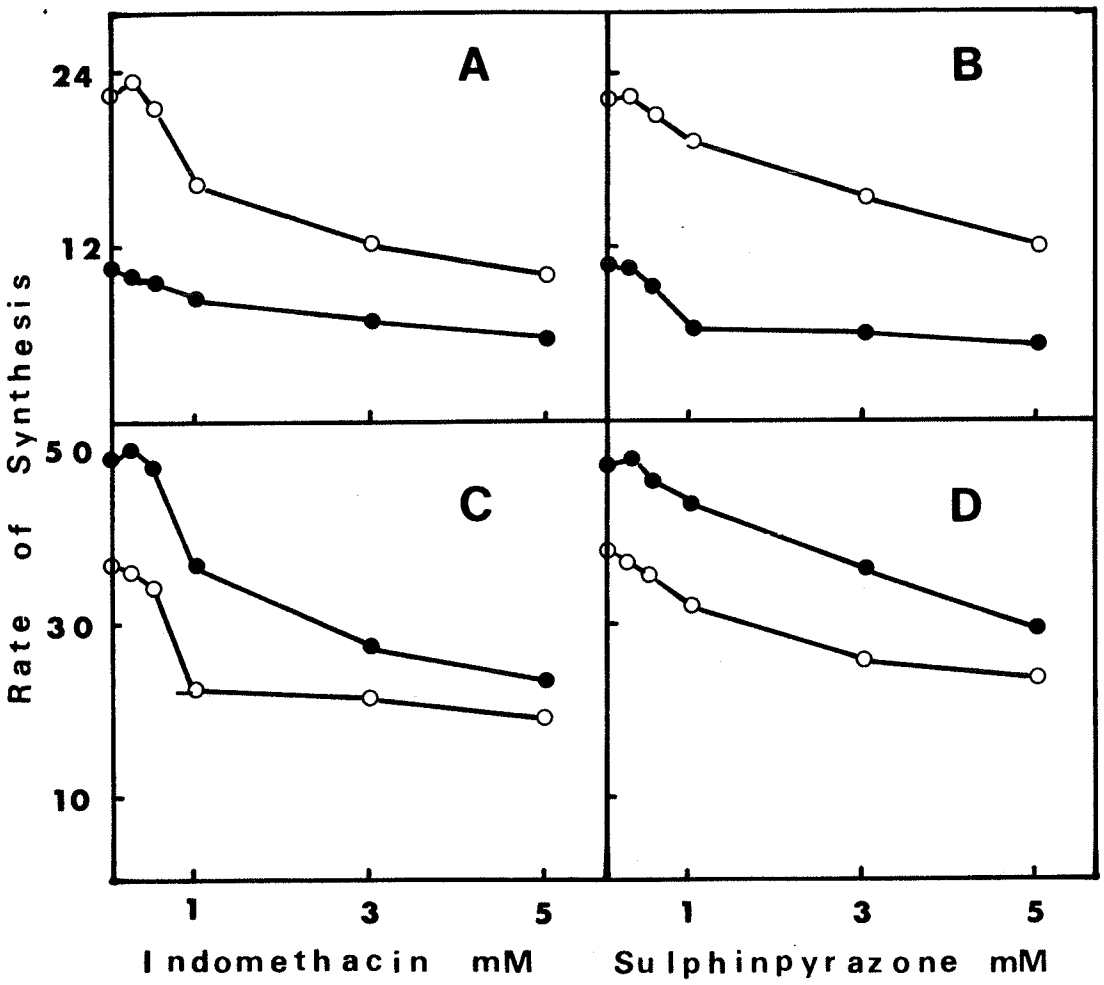

A
B
C
D
24
12
50
30
10
Rate of Synthesis
1
3
5
Indomethacin mM
Sulphinpyrazone mM

Figure 27 - Effect of Indomethacin and Sulfinpyrazone on the Incorporation of [^{3}H]-Leucine and [^{14}C]-Glucosamine into Total Liver and Medium Proteins of Liver Slices

Liver slices were prepared from control and 24 h inflamed rats and incubated with the drugs for 3 h as described in the Methods. After this, total protein present in medium (A and B) and in liver (C and D) were recovered by precipitation with trichloroacetic acid and specific radioactivities were determined. Specific radioactivities of [^{3}H]-leucine in control (-○-) and inflamed (-△-) liver slices and of [^{14}C]-glucosamine in control (-●-) and inflamed (-▲-) liver slices are indicated. Results shown are the means of 3-4 analyses, standard deviations from the means were within ±10%. At concentrations of drugs above 0.5 mM, values differed from the controls at the 99.9% confidence level as determined by the Student t test. See text for further explanation.

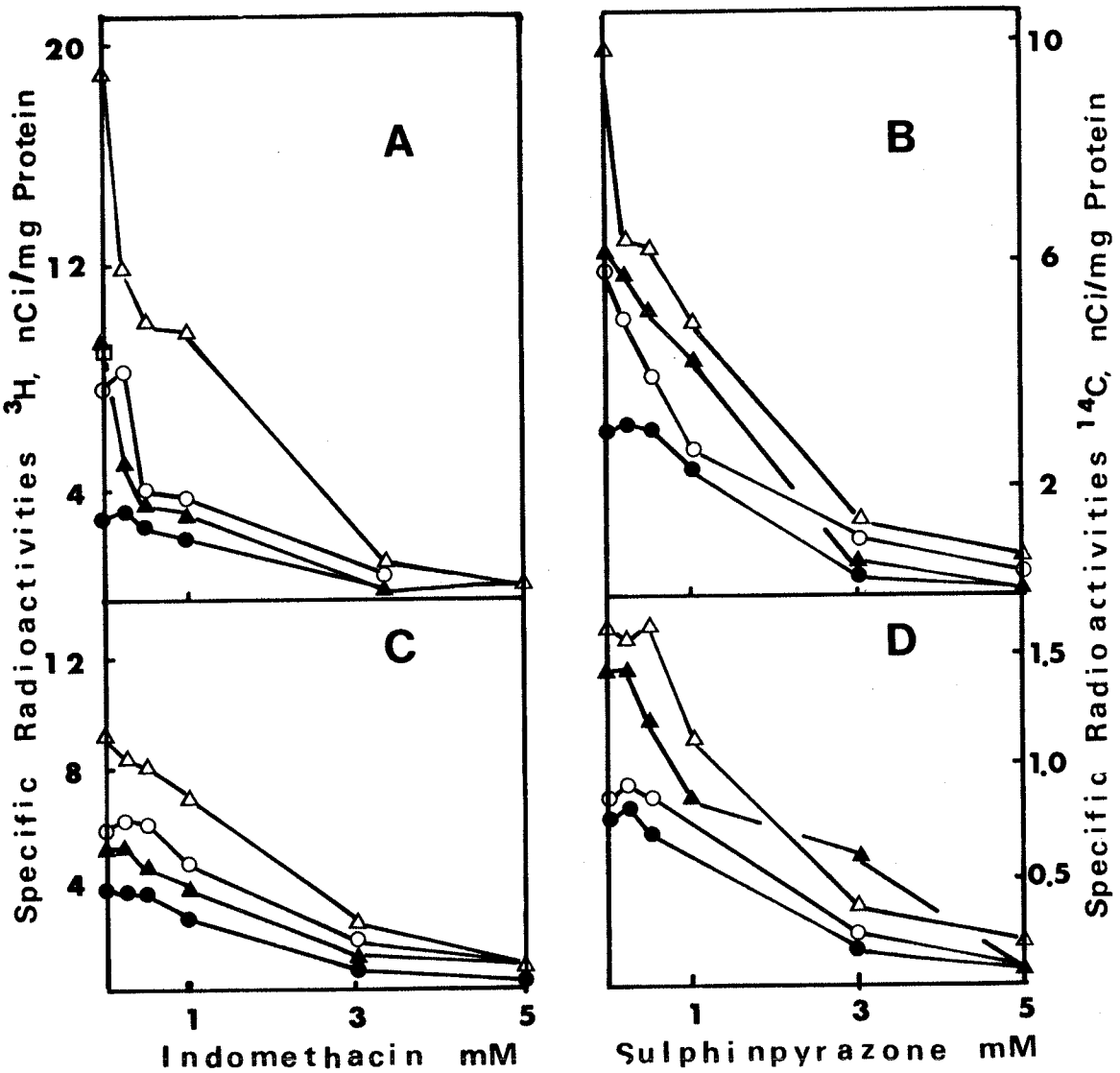

A
B
C
D
Specific Radioactivities ^{3}H, nCi/mg Protein
Specific Radioactivities ^{14}C, nCi/mg Protein
20
12
4
10
6
2
12
8
4
1.5
1.0
0.5
1
3
5
Indomethacin mM
Sulphinpyrazone mM

Figure 28 - Effect of Indomethacin on [^{3}H]-Leucine and [^{14}C]-Glucosamine Incorporation into α_1-Acid Glycoprotein and Albumin of Liver Slices

Liver slices were prepared from control and 24 h inflamed rats and incubated with indomethacin for 3 h as described in the Methods. Specific radioactivities of medium albumin (A) and α_1-acid glycoprotein (B) and of hepatic albumin (C) and α_1-acid glycoprotein (D) were determined after immunoprecipitation of these proteins from medium and Lubrol extracts of liver slices. Specific radioactivities of [^{3}H]-leucine in albumin from control (-○-) and inflamed (-●-) liver slices, [^{3}H]-leucine in α_1-acid glycoprotein from control (-△-) and inflamed (-▲-) liver slices and [^{14}C]-glucosamine in α_1-acid glycoprotein of control (-□-) and inflamed (-■-) liver slices were determined. Results shown are the means of 3-4 analyses, standard deviations of the means were within ±10%. Concentrations of indomethacin above 0.25 mM resulted in an inhibition significant at the 99.9% confidence levels as determined by the Student t test. See text for further explanation.

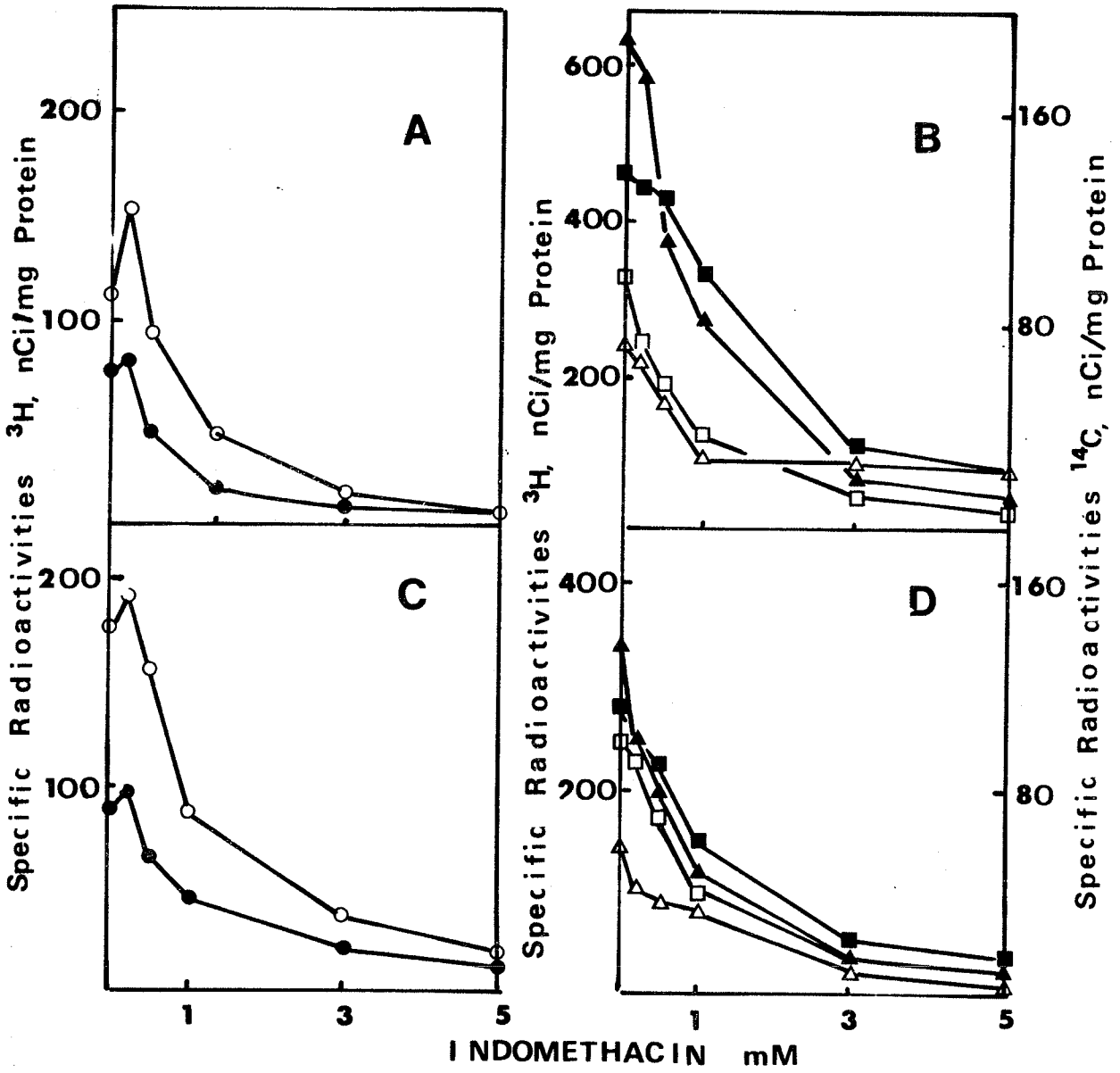
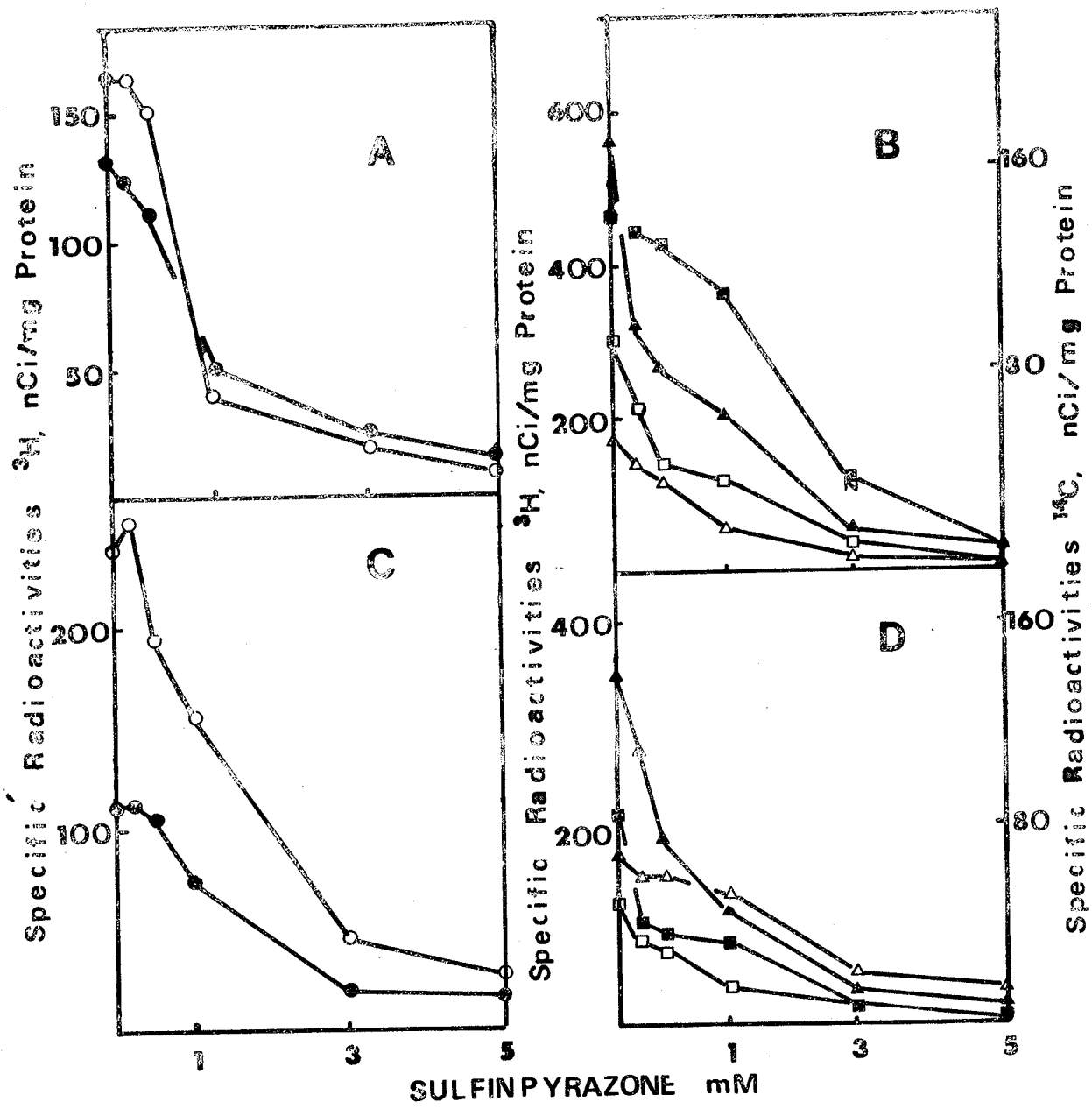

A
B
C
D
Specific Radioactivities ^{3}H, nCi/mg Protein
Specific Radioactivities ^{14}C, nCi/mg Protein
200
100
600
400
160
80
1
3
5
INDOMETHACIN mM

Figure 29 - Effect of Sulfinpyrazone on [^{3}H]-Leucine and [^{14}C]-Glucosamine Incorporation into α_1-Acid Glycoprotein and Albumin of Liver Slices

Liver slices were prepared from control and 24 h inflamed rats and incubated with sulfinpyrazone for 3 h as described in the Methods. Specific radioactivities of medium albumin (A) and α_1-acid glycoprotein (B) and of hepatic albumin (C) and α_1-acid glycoprotein (D) were determined after immunoprecipitation of these proteins from medium and lubrol extracts of liver slices. Specific radioactivities of [^{3}H]-leucine in albumin from control (-○-) and inflamed (-●-) liver slices, [^{3}H]-leucine in α_1-acid glycoprotein from control (-△-) and inflamed (-▲-) liver slices and [^{14}C]-glucosamine in α_1-acid glycoprotein from control (-□-) and inflamed (-■-) liver slices were determined. Results shown are the means of 3-4 analyses, standard deviations of the mean were within ±10%. Concentrations of sulfinpyrazone above 0.25 mM resulted in an inhibition significant at the 99.9% confidence levels as determined by the Student t test. See text for further explanation.

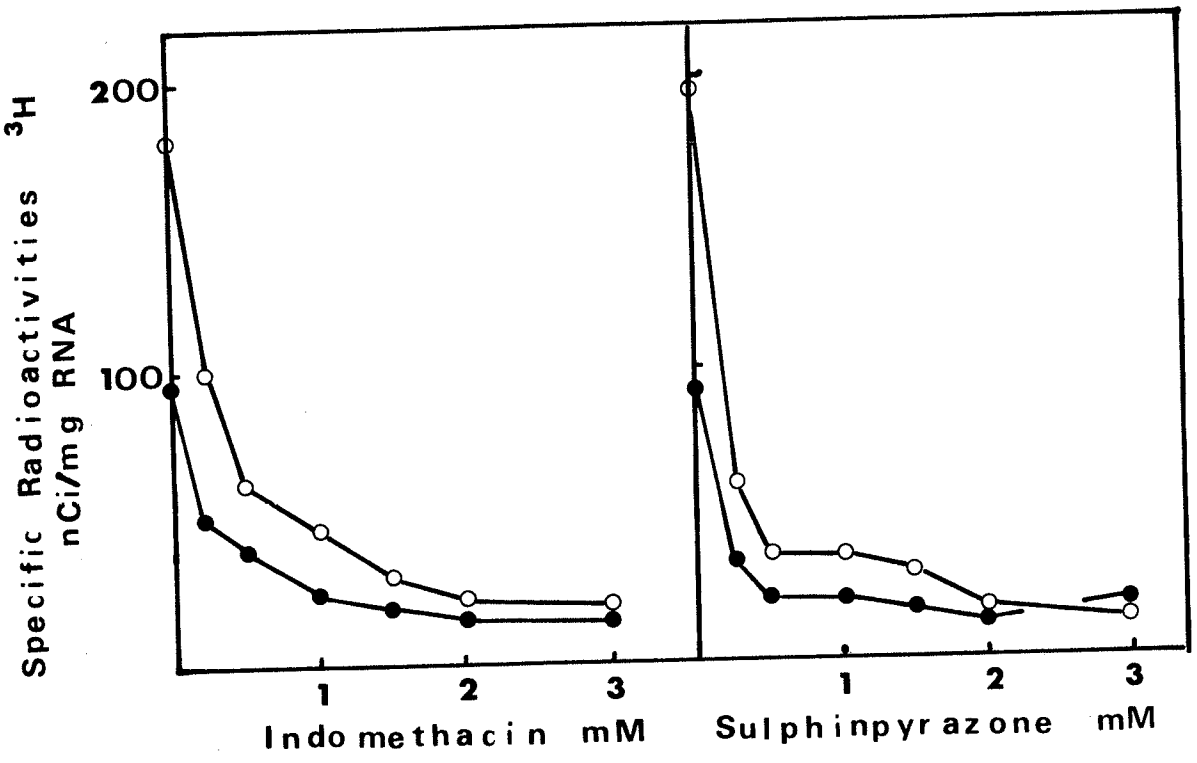

A
B
C
D
Specific Radioactivities ³H, nCi/mg Protein
Specific Radioactivities ¹⁴C, nCi/mg Protein
150
100
50
600
400
200
160
80
SULFINPYRAZONE mM
1
3
5

concentrations of 1 mM or greater of the drugs. While there appeared to be no significant differences in the inhibitory effects of the drugs on [^{3}H]-leucine and [^{14}C]-glucosamine incorporation into liver proteins, differences were observed in the medium proteins. Incorporation of the tracers into medium proteins of liver slices from inflamed rats was more sensitive to the effects of the drugs than into medium proteins of liver slices from control rats. For example, while 0.25 mM indomethacin had little effect on the incorporation of tracers into total medium protein of control liver slices, this concentration resulted in 40-50% inhibition of tracer incorporation into medium proteins of liver slices from inflamed rats (Figure 27). There were little or no differences in the effects of the drugs on [^{3}H]-leucine incorporation into albumin of liver slices from control and inflamed rats (Figure 28 and 29), yet 0.25 mM sulfinpyrazone inhibited [^{3}H]-leucine incorporation into medium α_1-acid glycoprotein about 10% with control liver slices and about 50% with liver slices from inflamed rats (Figure 29). Similarly, 0.25 mM phenylbutazone slightly elevated tracer incorporation into α_1-acid glycoprotein from control liver slices while it inhibited tracer incorporation about 40% in liver slices from inflamed rats (Jamieson & Kutryk, 1980). These differences in sensitivity to the action of the drugs may be related to the elevated rates of biosynthesis of acute phase reactants, such as α_1-acid glycoprotein, in livers from inflamed rats.

As previously mentioned, the synthesis of RNA precedes translation and glycosylation in the biosynthesis of intracellular and secretable proteins. For this reason, the effects of the drugs were examined on RNA synthesis as determined by [^{3}H]-orotate incorporation (Figure 30). Since the studies of Jamieson and Kutryk (1980) demonstrated that

Figure 30 - Effect of Indomethacin and Sulfinpyrazone on the Incorporation of [^{3}H]-Orotate into Rat Liver Slice RNA

Liver slices were prepared from control and 24 h inflamed rats and incubated with indomethacin and sulfinpyrazone for 3 h as described in the Methods. Results shown are the means of 4 analyses, standard deviations of the mean were within ±6%; specific radioactivities of RNA from control (-●-) and inflamed (-○-) liver slices are indicated. Inhibition by the drugs was statistically significant at a confidence level of 99.9%, as determined by the Student t test, at all drug concentrations tested. See text for further explanation.

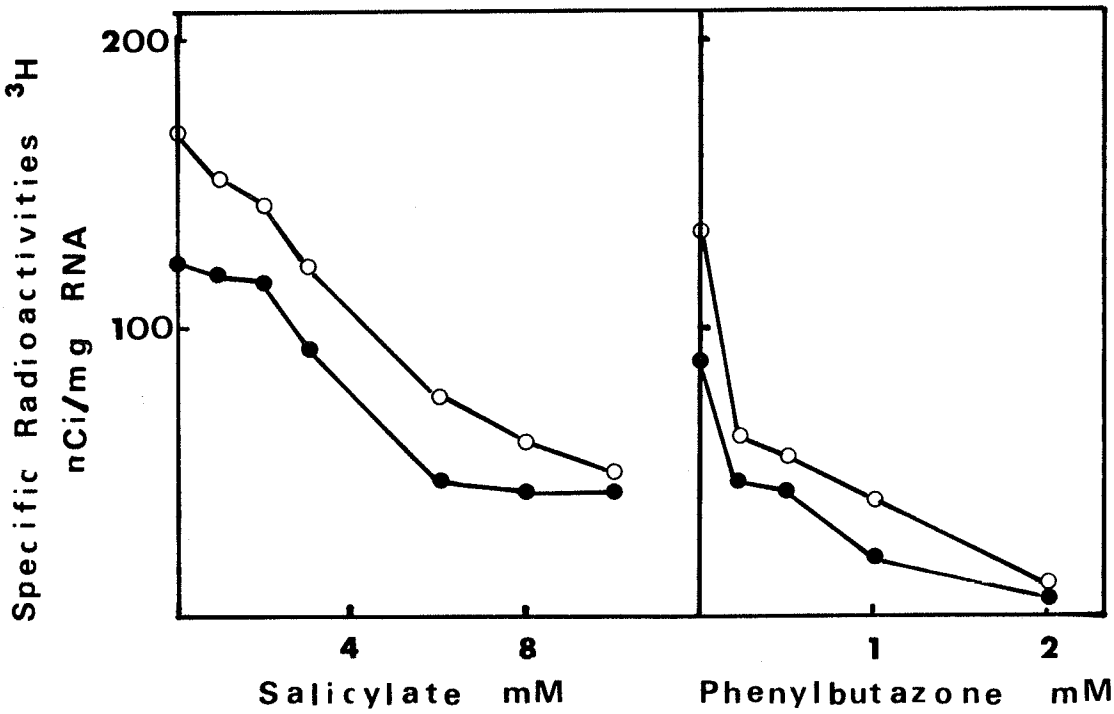

Specific Radioactivities ^{3}H nCi/mg RNA
200
100
1
2
3
Indomethacin mM
1
2
3
Sulphinpyrazone mM

salicylate and phenylbutazone inhibit translation and glycosylation in liver slices, the effects of these drugs on RNA synthesis was also examined (Figure 31). All four of these drugs inhibited the incorporation of [^{3}H]-orotate into rat liver slice RNA. This inhibition was significant at concentrations of 0.25 mM and greater for indomethacin, sulfinpyrazone and phenylbutazone, and at concentrations of 3 mM and greater for salicylate.

These studies show that RNA synthesis in liver slices is generally more sensitive to the effects of the drugs than are the processes of translation and glycosylation. For example, 0.25 mM sulfinpyrazone resulted in about 70% inhibition of [^{3}H]-orotate incorporation into RNA of liver slices of inflamed rats (Figure 30), while this concentration had little or no effect on protein synthesis in control liver slices as determined immunologically (Figure 26), or by tracer incorporation (Figure 27). However, as previously mentioned, tracer incorporation into medium proteins of liver slices from experimentally inflamed rats appeared to be more sensitive to the actions of the drugs. It may be important that, while there is a 70% inhibition of RNA synthesis by 0.25 mM sulfinpyrazone (Figure 30), there also is a 50% inhibition of incorporation of [^{3}H]-leucine into medium α_1-acid glycoprotein from liver slices of inflamed rats (Figure 29). This observation would suggest that biosynthesis of acute phase reactants, such as α_1-acid glycoprotein, is more dependent upon an intact transcriptional process in livers from inflamed rats than in livers from control rats.

Figure 31 - Effect of Salicylate and Phenylbutazone on the Incorporation of [^{3}H]-Orotate into Rat Liver Slice RNA

Liver slices were prepared from control and 24 h inflamed rats and incubated with salicylate and phenylbutazone for 3 h as described in the Methods. Results shown are the means of 4 analyses, standard deviations of the means were within ±6%; specific radioactivities of RNA from control (-●-) and inflamed (-○-) liver slices are indicated. Inhibition by the drugs was statistically significant at a confidence level of 99.9%, as determined by the Student t test, at all phenylbutazone concentrations, and at concentrations above 2 mM salicylate. See text for further explanation.


Specific Radioactivities ^{3}H
nCi/mg RNA
200
100
4
8
Salicylate mM
1
2
Phenylbutazone mM

4.3 Effects of Physiologically Active Agents on Liver Slices

As previously mentioned, it is difficult to determine the mode of action of physiologically active agents on target tissue using in vivo techniques. For this reason, a series of experiments were performed using liver slices as the experimental tissue. These experiments were aimed at determining the direct effects on liver cells of factors which may be involved in regulating hepatocyte activity during the acute phase response.

The results presented in Section 2.2 of Results indicated that there were changes in serum and hepatic amino acid pools following inflammation; these changes included the pools of the non-translatable amino acids taurine and ornithine. As these two amino acids are not normally constituents of the medium used for liver slice studies, and yet are present in serum, experiments were performed to determine their direct effects on liver slice activity. Table 19 shows the effect of taurine supplementation on the incorporation of [^{3}H]-leucine and [^{14}C]-glucosamine into total protein present in liver slices and medium. Taurine supplementation was at a concentration of 800 nmol/ml, which is that present in control rat serum, and at 1600 nmol/ml, which is the maximum level present in serum of inflamed rats. Taurine supplementation, at these concentrations, had no effect on transcription or glycosylation in liver slices from control rats and rats suffering from inflammation for 24 h. In contrast, ornithine supplementation did exert effects on liver slice translation and glycosylation as is shown in Table 20. Ornithine supplementation, at physiological concentrations, inhibited the incorporation of [^{3}H]-leucine and [^{14}C]-glucosamine into glycoprotein when livers from control rats were used.

Table 19 - Effect of Taurine Supplementation on [^{3}H]-Leucine and [^{14}C]-Glucosamine Incorporation Into Medium and Liver Proteins of Liver Slices

Taurine Concentration (nmol/ml)	Specific Radioactivities (nCi/mg protein)			
	Liver		Medium	
	[^{3}H]-Leu	[^{14}C]-GlcNH$_2$	[^{3}H]-Leu	[^{14}C]-GlcNH$_2$
Control Liver Slices				
0	7.34 ±0.10	2.38 ±0.05	7.15 ±0.80	6.65 ±0.63
800	6.72 ±0.28	2.14 ±0.07	6.41 ±0.48	5.98 ±0.58
1600	6.87 ±0.45	2.13 ±0.07	8.13 ±1.93	7.12 ±1.67
24 h Inflamed Liver Slices				
0	7.56 ±0.41	1.90 ±0.26	12.24 ±2.07	8.50 ±1.17
800	7.16 ±0.29	1.67 ±0.12	15.06 ±5.07	9.95 ±3.48
1600	7.66 ±0.09	1.98 ±0.05	12.55 ±0.40	8.89 ±0.43

Experiments were performed with 1 g liver slices, 5 µCi [^{3}H]-leucine and 0.5 µCi [^{14}C]-glucosamine per flask as described in Methods; incubations were for 4 h. Results shown are the means and standard deviations of 3 analyses. See text for further explanation.

Table 20 - Effect of Non-Translatable Amino Acids on [^{3}H]-Leucine and [^{14}C]-Glucosamine Incorporation into Medium and Liver Proteins of Liver Slices

Supplementation	Specific Radioactivities (nCi/mg protein)			
	Liver		Medium	
	[^{3}H]-Leu	[^{14}C]-$GlcNH_2$	[^{3}H]-Leu	[^{14}C]-$GlcNH_2$
Control Liver Slices				
None	7.44 ±0.62	1.17 ±0.09	5.02 ±0.68	2.83 ±0.42
150 nmol/ml Orn	6.76 ±0.50	1.02 ±0.10	3.69 ±0.41	2.04 ±0.32
150 nmol Orn, 1600 nmol/ml Tau	6.74 ±0.51	1.02 ±0.11	3.74 ±0.99	2.16 ±0.49
24 h Inflamed Liver Slices				
None	7.69 ±0.89	0.77 ±0.08	9.39 ±1.68	3.59 ±0.64
150 nmol/ml Orn	9.34 ±1.42	0.94 ±0.15	14.19 ±4.47	4.73 ±1.37
150 nmol/ml Orn, 1600 nmol/ml Tau	10.07 ±0.73	1.02 ±0.05	13.78 ±0.48	4.69 ±0.06

Experiments were performed with 1 g liver slices, 5 µCi [^{3}H]-leucine and 0.5 µCi [^{14}C]-glucosamine per flask as described in Methods; incubations were for 3 h. Results shown are the means and standard deviations of 3 analyses. See text for further explanation.

Table 21 - Effect of Ornithine Supplementation on [^{3}H]-Orotate Incorporation into Liver Slice RNA

Ornithine Concentration (mmol/ml)	Specific Radioactivity (nCi/mg RNA)
Control Liver Slices	
0	93 ±2
50	91 ±1
200	91 ±1
12 h Inflamed Liver Slices	
0	144 ±3
50	161 ±2
200	179 ±4

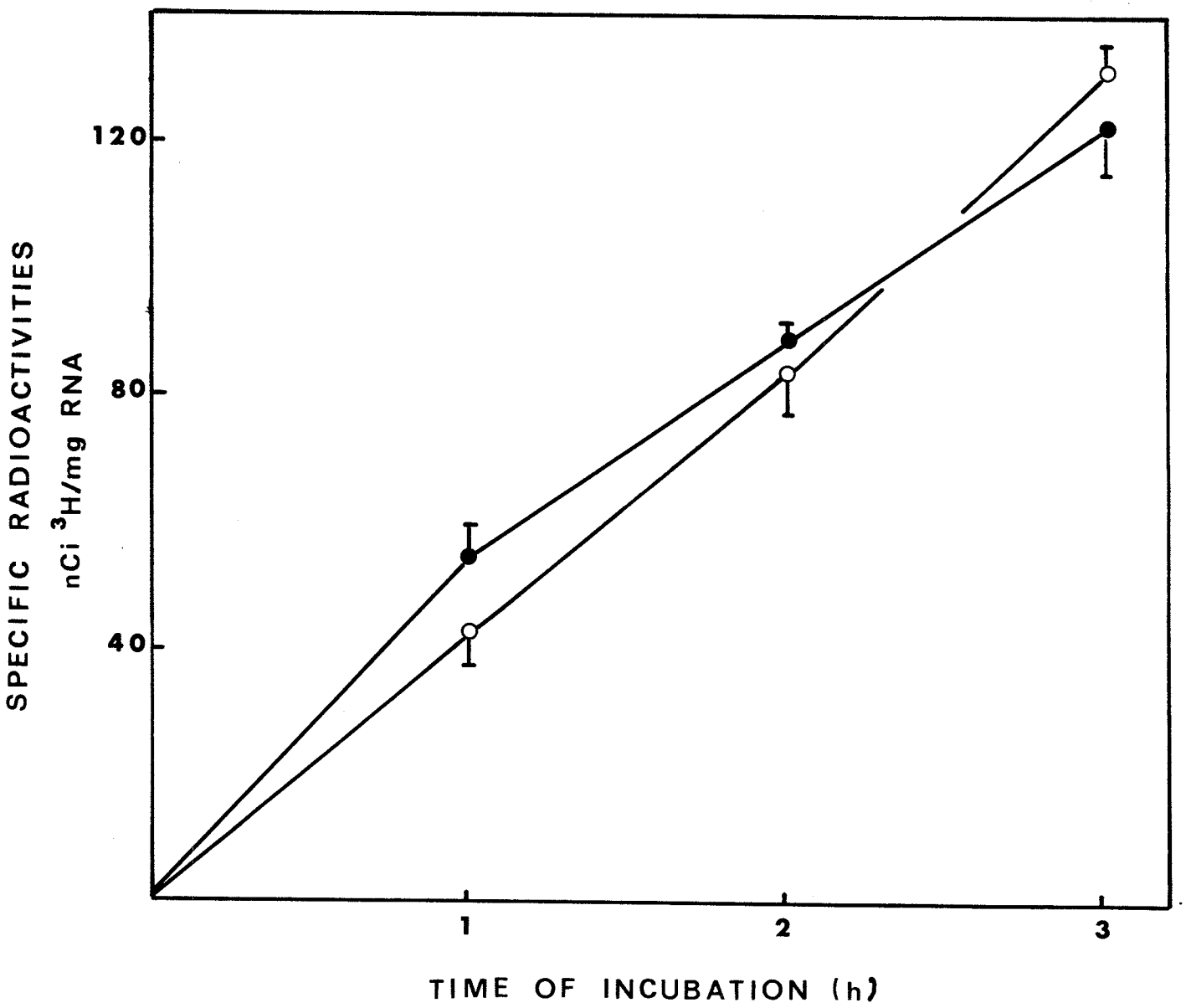
Experiments were performed with 0.5 g liver slices and 3 μCi [^{3}H]-orotate per flask as described in Methods; incubations were for 4 h. Results shown are the means and standard deviations of 3 analyses. See text for further explanation.

Suprisingly, ornithine stimulated incorporation of these tracers into protein when livers from 24 h inflamed rats were used. Taurine supplementation had no significant influence on these effects of ornithine. The effects of ornithine were more pronounced on specific radioactivities of medium proteins than on liver proteins. These observations would suggest that the effects exerted by ornithine on liver slices were influencing specifically the biosynthesis of secretory proteins. Also, while ornithine supplementation had little effect on [^{3}H]-orotate incorporation into control liver slice RNA, it enhances the incorporation into RNA of liver slices from 12 h inflamed rats (Table 21). In fact, the ratio of specific radioactivities of inflamed to control liver slice RNA increased from 1.55 without supplementation, to 1.97 at a concentration of 200 nmol/ml ornithine. These observations suggest a modulatory role for ornithine in glycoprotein biosynthesis by livers of experimentally inflamed rats.

Many physiologically active agents exert their effects *via* cyclic AMP-dependent protein kinase catalyzed phosphorylation of proteins. Also, it has been shown (Langstaff *et al.*, 1980) that there are elevated levels of cyclic AMP in the liver following inflammation. For these reasons, the effects of dibutyryl cyclic AMP, an analogue of cyclic AMP which readily traverses the cell membrane, were examined on RNA synthesis. As is shown in Figure 32, this cyclic nucleotide had no significant effect on [^{3}H]-orotate incorporation into RNA of liver slices of control rats between 1 and 3 h of incubation. This observation suggests that hormones which exert their effects *via* the second messenger cyclic AMP are not important regulators of RNA synthesis by liver slices.

Figure 32 - Effect of Dibutyryl-Cyclic AMP on [^{3}H]-Orotate Incorporation into Rat Liver Slice RNA

Experiments were performed with 1 g liver slices and 5 μCi [^{3}H]-orotate as described in Methods. Liver slices were from control rats and were incubated with (-●-) or without (-○-) supplementation of 20 μM dibutyryl cyclic AMP. Results shown are the means and standard deviations of 3 analyses.


SPECIFIC RADIOACTIVITIES
nCi ^{3}H/mg RNA
120
80
40
1
2
3
TIME OF INCUBATION (h)

As previously mentioned, one of the alterations in activities of leukocytes during the acute phase response is an elevated biosynthesis and release of metabolites of arachidonic acid such as the prostaglandins. Table 22 shows the effect of prostaglandins of the E and F series on the incorporation of [^{3}H]-orotate into liver slice RNA. Prostaglandins E_1, E_2 and F_{2_α} all inhibited tracer incorporation into RNA about 30% during a 6 h incubation of liver slices from control rats. These prostaglandins have also been reported (Molasky, 1976) to inhibit [^{3}H]-leucine and [^{14}C]-glucosamine incorporation into medium proteins of liver slices by 60-80%. This inhibition of liver slice activity is similar to that seen with physiological concentrations of ornithine (see Tables 20 and 21), and may reflect the fact that liver slices are not maintained in a truly physiological environment during the experiments.

Table 23 shows the effects of hormones, which are known to change during the acute phase response to inflammation, on RNA synthesis by liver slices. Thyroxine levels are decreased , while insulin and cortisol increase following inflammation (see Section 2.2 of Results). Individually, thyroxine, insulin and cortisol all inhibited [^{3}H]-orotate incorporation into RNA about 20%; together, physiological concentrations of these hormones had little effect on RNA synthesis. Obviously, the action of these hormones is not simple in nature.

Parameters, other than RNA and glycoprotein synthesis, which are known to be altered during the acute phase response, have been examined in liver slices. For example, the enzyme sialyltransferase is elevated

Table 22 - Effect of Prostaglandins on [^{3}H]-Orotate Incorporation into Liver Slice RNA

Supplementation	Specific Radioactivities (nCi/mg RNA)
None	144 ±13
Prostaglandin E_1	100 ±8
Prostaglandin E_2	102 ±14
Prostaglandin F_{2_α}	101 ±14

Experiments were performed with 0.5 g liver slices and 3 μCi [^{3}H]-orotate per flask as described in Methods; incubations were for 6 h. Results shown are the means and standard deviations of 4 analyses. See text for further explanation. Prostaglandins were added to flasks at concentrations of 40 μM.

Table 23 - Effect of Thyroxine, Insulin and Cortisol on [^{3}H]-Orotate Incorporation into Liver Slice RNA

Supplementation	Specific Radioactivities (nCi/mg RNA)
None	85 ±7
10 ng/ml Thyroxine	70 ±6
60 ng/ml Thyroxine	75 ±7
8 μU/ml Insulin	70 ±7
300 nM Cortisol-21-Acetate	68 ±8
60 ng/ml Thyroxine & 8 μU/ml Insulin & 300 nM Cortisol-21-Acetate	85 ±6

Experiments were performed with 0.5 g liver slices and 3 μCi [^{3}H]-orotate per flask as described in Methods; incubations were for 4 h. Results shown represent the means and standard deviations of 3 analyses. See text for further explanation.

in serum and liver following inflammation; liver slices from experimentally inflamed rats release more of this enzyme activity than slices from control rats (Kaplan et al., 1983b). Table 24 shows the effect of cortisol supplementation on the sialyltransferase activity secreted into medium from control liver slices during a 6 h incubation. At concentrations of 280 nM and greater, cortisol-21-acetate inhibited the release of the enzyme activity from the liver slices. Figure 33 shows the effect of cortisol on both medium sialyltransferase and [^{3}H]-orotate incorporation into control liver slice RNA over a 4 h incubation period. A concentration of 320 nM cortisol-21-acetate inhibited both enzyme release and tracer incorporation into RNA. While lower concentrations of cortisol had little or no effect on enzyme release, there was a 20-30% increase in tracer incorporation into RNA. These results may reflect a dependence of sialyltransferase release on the synthesis of RNA.

Results presented in Section 3 of this thesis indicated that administration of cytokine preparations resulted in alterations in a number of parameters of the acute phase response. The effects of cytokine preparations on these parameters was examined in liver slices in attempts to more clearly understand the mechanism(s) of action of cytokine(s) on the liver. Table 25 shows the effect of cytokine supplementation on incorporation of [^{3}H]-glucosamine and [^{14}C]-leucine into medium proteins, and on medium sialyltransferase activity. Cytokine supplementation at a level of 5 MCE inhibited these parameters by 20-30%, however, the heat-treated cytokine preparations also inhibited medium sialyltransferase by 40%. Supplementation with 10 MCE

Table 24 - Effect of Cortisol on Medium Sialyltransferase Activity of Liver Slices

Cortisol-21-Acetate (nM)	Medium Sialyltransferase Activity (pmol NeuAc/min/ml)
0	9.8 ±0.3
28	10.4 ±0.5
56	9.5 ±0.4
280	8.9 ±0.3
560	6.9 ±0.4

Experiments were performed with 1 g liver slices per flask as described in Methods; incubations were for 6 h. Results shown are the means and standard deviations of 3 analyses. See text for further explanation.

Figure 33 - Concentration Dependent Effects of Cortisol on [^{3}H]-Orotate Incorporation into Liver Slice RNA and Medium Sialyltransferase

Samples of 0.5 g liver slices from control rats were incubated with 3 µCi [^{3}H]-orotate for 4 h as described in Methods. Specific radioactivities of liver slice RNA (-●-) and medium sialyltransferase activities (-O-) are indicated. Results shown are the means and standard deviations of 4 analyses. See text for further explanation.

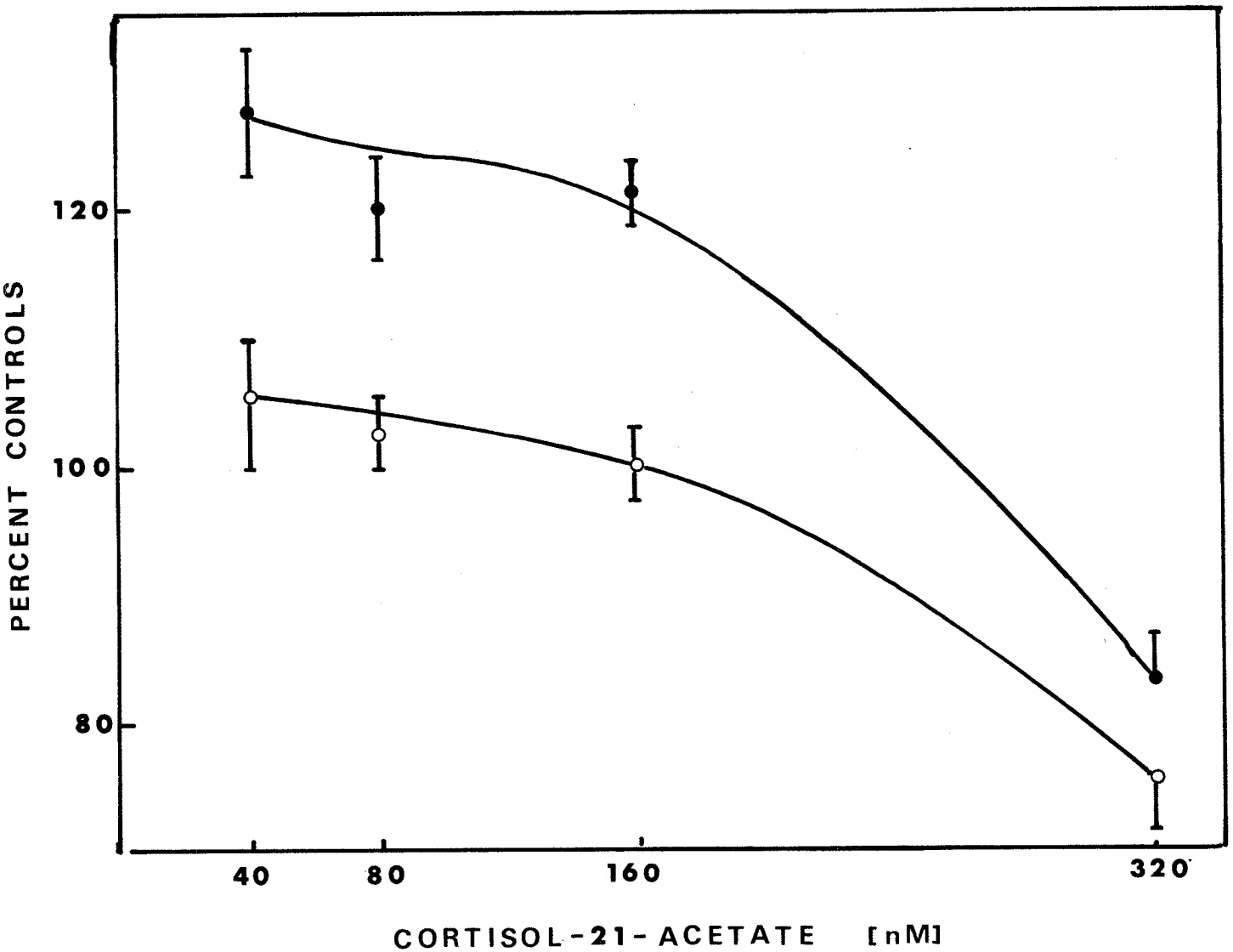

PERCENT CONTROLS
120
100
80
40
80
160
320
CORTISOL-21-ACETATE [nM]

Table 25 - Effect of Cytokine on Medium Sialyltransferase Activity and Incorporation of [^{3}H]-Glucosamine and [^{14}C]-Leucine into Medium Proteins of Liver Slices

Supplementation	Sialyltransferase Activity (pmoles NeuAc/min/ml)	Specific Radioactivities (nCi/mg protein)	
		[^{3}H]-Glucosamine	[^{14}C]-Leucine
None	5.63 ±0.10	4.48 ±0.27	0.28 ±0.03
5 MCE Cytokine	4.15 ±0.15	3.48 ±0.10	0.23 ±0.04
5 MCE Heat-Treated Cytokine	3.35 ±0.10	4.73 ±0.11	0.28 ±0.04
10 MCE Cytokine	4.21 ±0.11	4.41 ±0.30	0.31 ±0.02
10 MCE Heat-Treated Cytokine	2.35 ±0.13	4.96 ±0.08	0.38 ±0.04
50 MCE Cytokine	4.39 ±0.09	4.18 ±0.06	0.26 ±0.01
50 MCE Heat-Treated Cytokine	4.06 ±0.12	3.97 ±0.08	0.27 ±0.02

Experiments were performed with 1 g liver slices, 1 µCi [^{14}C]-leucine and 5 µCi [^{3}H]-glucosamine per flask as described in Methods; incubations were for 3 h. Cytokine was prepared as described in Methods; the solution for incubation of PEC contained 10 µg/ml LPS, 0.15 M sodium chloride, 0.5 mg/ml streptomycin sulfate and 100 U/ml penicillin G. Results shown are the means and standard deviations of 3 analyses. See text for further explanation.

cytokine did not appreciably affect tracer incorporation into medium proteins, but inhibited medium sialyltransferase; heat-treated material elevated tracer incorporation into medium proteins and inhibited sialyltransferase activity by 60%. Supplementation with 50 MCE cytokine did not appreciably affect tracer incorporation, whereas, both untreated and heat-treated cytokine inhibited medium sialyltransferase activity. These are not simple effects and interpretation of the results is complicated by the effects of the heat-treated material. As mentioned in Section 3.1 of Results, the cytokine preparations are heterogenous and there appeared to be heat-stable factors which are physiologically active. Also, the level of antibiotics present in the cytokine preparations was considerably higher than that normally present in the liver slice medium (see Methods). Table 26 shows the effects of antibiotic supplementation of liver slice medium on the activity of enzymes in the medium and the liver slices. While supplementation with lipopolysaccharide, saline solution had no effect on enzyme activities, supplementation with lipopolysaccharide, streptomycin sulfate, penicillin, saline solution resulted in an inhibition of medium sialyltransferase and hepatic hexosaminidase and galactosidase; these inhibitions were dependent on antibiotic concentrations. For this reason, antibiotics were excluded in subsequent experiments from saline, lipopolysaccharide solutions in which PEC were incubated for the preparation of cytokine. Also, cytokine preparations without antibiotics had no detectable sialyltransferase activity and did not affect enzyme assays for serum or medium sialyltransferase.

Table 26 - Effect of Antibiotics on Enzyme Activities in Liver Slices

Supplementation	Sialyltransferase	Hexosaminidase	Galactosidase
None	7.28 ±0.30	38 ±3	0.25 ±0.02
0.1 ml LPS, Saline	7.32 ±0.35	38 ±2	0.27 ±0.02
0.5 ml LPS, Saline	7.06 ±0.20	40 ±5	0.24 ±0.02
0.1 ml LPS, Streptomycin, Penicillin, Saline	5.44 ±0.12	29 ±1	0.24 ±0.01
0.5 ml LPS, Streptomycin, Penicillin, Saline	4.02 ±0.36	28 ±1	0.20 ±0.01

Experiments were performed with 1 g liver slices per flask, incubation time was 6 h. Enzymes were assayed as described in Methods. Solutions containing 10 µg/ml lipopolysaccharide in 0.15 M sodium chloride, with or without 0.5 mg/ml streptomycin sulfate and 100 U/ml penicillin G, were added to the flasks as indicated. Medium sialyltransferase and hepatic hexosaminidase and galactosidase were assayed as described in the Methods. Units of enzyme activities are pmoles NeuAc transferred/min/ml medium for sialyltransferase, nmoles p-nitrophenyl-N-acetyl-β-D-glucosaminide hydrolyzed/min/mg protein for hexosaminidase and nmoles p-nitrophenyl-β-D-galactoside hydrolyzed/min/mg protein for galactosidase. The results shown are the means and standard deviations of 3 analyses. See text for further explanation.

Table 27 shows the effect of cytokine supplementation on [^{3}H]-orotate incorporation into RNA of control liver slices. While 10 MCE of cytokine had no influence on tracer incorporation into RNA; 10 MCE of heat-treated cytokine inhibited tracer incorporation; this inhibition was greater after 4 h than after 2 h of incubation. Cytokine supplementation with 30 MCE enhanced tracer incorporation slightly at 2 h and inhibited at 4 h of incubation; heat-treated cytokine at this level inhibited tracer incorporation about 45%. As mentioned above, these cytokine preparations did not contain antibiotics. Also, subsequent experiments demonstrated that this inhibitory effect of heat-treated cytokine preparations was not present with pronase-digested cytokine preparations. This suggests that a heat-stable protein factor may be responsible for the inhibitory effects of the heat-treated cytokine preparations. If a single factor in the cytokine preparations is responsible for the differences between 30 MCE untreated and heat-treated cytokine, then this factor must also be responsible for a 100% increase in tracer incorporation into RNA at 2 h and a 55% increase at 4 h of incubation. A heat-labile cytokine in the preparations may elevate liver slice RNA synthesis at relatively short times of exposure, although, as previously mentioned, heterogeneity of cytokine preparations complicates this interpretation.

Liver slices can respond to mixtures of physiologically active agents in quite different ways than to single agents. Table 28 shows the synergistic effects of cytokine and cortisol on medium sialyl-transferase activity and on [^{3}H]-orotate incorporation into RNA.

Table 27 - Effect of Cytokine Supplementation on [^{3}H]-Orotate Incorporation into Liver Slice RNA

Supplementation	Specific Radioactivities (nCi/mg RNA)	
	2 h	4 h
None	79 ±4	147 ±6
10 MCE Cytokine	75 ±3	143 ±5
10 MCE Heat-Treated Cytokine	72 ±5	119 ±6
30 MCE Cytokine	91 ±5	126 ±7
30 MCE Heat-Treated Cytokine	45 ±7	82 ±8

Experiments were performed with 1 g liver slices and 5 µCi [^{3}H]-orotate per flask as described in Methods; incubations were for 2 h or 4 h as indicated. PEC were prepared as described in Methods and cytokine was prepared by incubation of these cells in a solution containing 10 µg/ml LPS and 0.15 M sodium chloride. Results shown are the means and standard deviations of 3 analyses. See text for further explanation.

Table 28 - Synergism of Cytokine and Cortisol on Medium Sialyltransferase Activity and [^{3}H]-Orotate Incorporation into Liver Slice RNA

Supplementation	Sialyltransferase Activity (pmol NeuAc/min/ml)	Specific Radioactivity (nCi/mg RNA)
None or Pronase-Treated Cytokine	2.16 ±0.09	42 ±1
10 MCE Cytokine	1.70 ±0.05	40 ±2
280 nM Cortisol-21-Acetate	1.93 ±0.02	37 ±1
10 MCE Cytokine & 280 nM Cortisol-21-Acetate	1.65 ±0.08	48 ±3

Experiments were performed with 0.5 g control liver slices and 3 µCi [^{3}H]-orotate per flask as described in Methods; incubations were for 2 h. Cytokine was prepared as described in Table 28. Results shown are the means and standard deviations of 3-5 analyses. See text for further explanation.

Supplementation with 10 MCE cytokine and 280 nM cortisol inhibited medium sialyltransferase; together, these agents stimulated RNA synthesis, whereas separately they inhibited this parameter. Table 29 shows the synergistic effects of cytokine, ornithine and cortisol on medium sialyltransferase activity and [^{3}H]-orotate incorporation into RNA. Supplementation with 80 nM cortisol, 150 nmol/ml ornithine and 30 MCE cytokine together resulted in the highest specific radioactivity of RNA; a lower level of cytokine, cytokine alone or ornithine alone depressed tracer incorporation. Similar effects were exerted by these agents on medium sialyltransferase activities. Table 30 shows that the effects of 10 MCE cytokine, 150 nmol/ml ornithine and 60 nM cortisol on release of sialyltransferase did not differ when liver slices were prepared from control or 36 h inflamed rats. Table 31 shows the synergistic effects of cortisol and cytokine on liver slice medium sialyltransferase and hepatic hexosaminidase activities. Addition of cytokine depressed sialyltransferase activities in the presence of 28 nM cortisol and elevated these enzyme activities in the presence of 280 nM cortisol. Addition of several doses of cytokine throughout the incubations did not produce dramatically different results, although 50 MCE cytokine in multiple doses did result in higher medium sialyltransferase activity in the presence of 28 nM cortisol than the addition of 10 MCE or 50 MCE cytokine. Addition of cytokine depressed hepatic hexosaminidase activity in the presence of 28 nM cortisol; effects of cytokine on this enzyme activity were less pronounced in the presence of 280 nM cortisol. These results indicate that cytokine(s) exert direct effects on liver slice activity, however,

Table 29 - Synergisms of Cytokine, Ornithine and Cortisol on Medium Sialyltransferase and [^{3}H]-Orotate Incorporation Into Liver Slice RNA

Supplementation	Specific Radioactivity (nCi/mg RNA)	Sialyltransferase Activity (pmol NeuAc/min/ml)
None or Pronase-Treated Cytokine	24.2 ±0.8	3.21 ±0.03
30 MCE Cytokine	19.1 ±0.6	2.35 ±0.27
150 nmol/ml Ornithine	20.2 ±1.3	2.52 ±0.05
80 nM Cortisol-21-Acetate	28.6 ±0.6	3.43 ±0.11
30 MCE Cytokine & 150 nmol/ml Ornithine	22.3 ±0.3	2.88 ±0.09
30 MCE Cytokine & 150 nmol/ml Ornithine & 80 nM Cortisol-21-Acetate	29.2 ±0.7	3.18 ±0.36
10 MCE Cytokine & 150 nmol/ml Ornithine & 80 nM Cortisol-21-Acetate	22.3 ±0.1	2.50 ±0.21

Experiments were performed with 0.5 g liver slices and 3 µCi [^{3}H]-orotate per flask as described in Methods; incubations were for 3 h. See footnotes of Table 28 for details of cytokine preparation. The results shown are the means and standard deviations of 3 analyses. See text for further details.

Table 30 - Synergism of Cytokine, Ornithine and Cortisol on Liver Slice Medium Sialyltransferase

Supplementation	Sialyltransferase Activity (pmol NeuAc/min/ml)	
	Control	36 h Inflamed
None or Pronase-Treated Cytokine	2.78 ±0.12	7.91 ±0.48
10 MCE Cytokine	2.57 ±0.06	5.73 ±0.49
10 MCE Ctyokine & 150 nmol/ml Ornithine	2.21 ±0.09	-
10 MCE Cytokine & 60 nM Cortisol-21-Acetate	2.20 ±0.10	-
10 MCE Cytokine & 150 nmol/ml Ornithine & 60 nM Cortisol-21-Acetate	2.56 ±0.32	6.20 ±0.25

Experiments were performed with 0.5 g liver slices as described in Methods; incubations were for 4 h. See footnotes of Table 28 for details of preparation of cytokine. Results shown are the means and standard deviations of 3 analyses. See text for further explanation.

Table 31 - Synergism of Cytokine and Cortisol on Liver Slice Enzyme Activity

Supplementation	Sialyltransferase Activity (pmol/min/ml)	Hexosaminidase Activity (nmol/min/mg protein)
None or Pronase-Treated Cytokine	8.2 ±0.2	34 ±1
28 nM Cortisol-21-Acetate	10.4 ±0.3	36 ±2
& 10 MCE Cytokine	8.7 ±0.2	30 ±1
& 50 MCE Cytokine	6.1 ±0.4	28 ±1
& 50 MCE Cytokine*	9.3 ±0.2	27 ±2
280 nM Cortisol-21-Acetate	6.2 ±0.4	29 ±1
& 10 MCE Cytokine	9.6 ±0.3	28 ±1
& 50 MCE Cytokine	7.8 ±0.2	33 ±2
& 50 MCE Cytokine*	7.6 ±0.2	28 ±1

Experiments were performed with 1 g liver slices as described in Methods; incubations were for 6 h. Unless otherwise noted, supplementations were at the beginning of the incubations. (*)- indicates addition of 10 MCE cytokine at hour 0, 20 MCE at hour 2 and 20 MCE at hour 4 of the incubations. See footnotes of Table 28 for details of cytokine preparation. Results shown are the means and standard deviations of 3 analyses. Medium sialyltransferase and hepatic hexosaminidase were assayed as described in Methods. See text for further explanation.

the effects of cytokines are influenced by the presence of agents normally present in the blood of animals.

It is evident that supplementation of liver slices with physiologically active agents results in a number of effects on glycoprotein and RNA synthesis and on intracellular hepatic enzymes and enzymes released from the liver slices. Cytokines exert direct effects on liver slices, but these effects are not the same as the *in vivo* effects of cytokines. The synergistic effects of cytokine and cortisol on a number of activities of liver slices suggests that hormones may play a role in the *in vivo* response of the liver to cytokines.

DISCUSSION

1. Hepatic Biosynthesis of Plasma Glycoproteins

The biosynthesis of three plasma glycoproteins, α_1-acid glycoprotein, α_1-macroglobulin and α_2-macroglobulin, were examined in this thesis. While α_1-acid glycoprotein and α_2-macroglobulin are acute phase reactants (Jamieson et al., 1972b; Gauthier & Mouray, 1976), α_1-macroglobulin levels do not change following inflammation (Gauthier & Mouray, 1976; this thesis). In vivo labelling of these proteins was studied in this thesis to gain insight into the pathway of biosynthesis of acute phase reactants, as compared to that of other plasma glycoproteins.

While the two α-macroglobulins that were studied were very similar in their physicochemical properties, these properties differed considerably from that of α_1-acid glycoprotein. Jamieson et al. (1972a) have reported a molecular weight of 800 kd, and Gauthier and Mouray (1976) a molecular weight of 770 kd, for α_2-macroglobulin. The α_1-macroglobulin isolated in this work has a molecular weight greater than 670 kd (see Figure 10); Gauthier and Mouray (1976) have reported a molecular weight of 760 kd for α_1-macroglobulin. These proteins are considerably larger than α_1-acid glycoprotein which has been reported to have a molecular weight of 43 kd (Jamieson et al., 1972a). While α_1-acid glycoprotein is a monomer with a polypeptide moiety of 23 kd (Ricca et al., 1981), α-macroglobulins are tetramers of identical subunits of molecular weights of about 200 kd (Jones et al., 1972; Barret et al., 1979; Burden, 1980; Howard et al., 1980). The carbohydrate contents of these proteins have been reported to be 34% for α_1-acid glycoprotein (Jamieson et al., 1972a), 10-16% for

α_2-macroglobulin (Jamieson et al., 1972a; Gauthier & Mouray, 1976) and 9% for α_1-macroglobulin (Gauthier & Mouray, 1976). Considering these differences in properties of the plasma glycoproteins, if patterns of in vivo labelling of glycoproteins with radioactive precursors of polypeptide and carbohydrate moieties are related to structural features of the glycoproteins, then the in vivo radiolabelling of α_1-acid glycoprotein and α-macroglobulins should have been considerably different.

Differences that were observed in the in vivo radiolabelling patterns of the specific proteins were subtle; the kinetics of incorporation of [^{14}C]-mannose into α_2-macroglobulin were remarkably similar to those for incorporation of this tracer into α_1-acid glycoprotein (Friesen & Jamieson, 1980). Differences did exist though, and these could be explained in terms of the properties of the proteins. Lower specific radioactivities of tracers in α_1-macroglobulin, compared to the two acute phase reactants, probably reflects a lower carbohydrate composition and a lower rate of synthesis in inflamed rats. The α-macroglobulins present in Golgi fractions had higher specific radioactivities of [^{3}H]-leucine and [^{14}C]-glucosamine than α_1-acid glycoprotein had; two explanations could be offered for this observation. The transit times from the rough endoplasmic reticulum to the Golgi may be shorter for the α-macroglobulins than for α_1-acid glycoprotein. Also, while en bloc transfer of high mannose-containing oligosaccharide precursors could be the same for the proteins, later addition of terminal triplets of sugars by Golgi glycosyltransferases, such as N-acetylglucosaminyltransferase (see

Introduction) may differ for α_1-acid glycoprotein and α-macroglobulins. Evidence suggesting elevated levels of a high-mannose containing α_1-acid glycoprotein in the serum of inflamed rats (Koj et al., 1982) supports this second suggestion. Further studies would be necessary to answer questions posed by the above observations; these studies were beyond the scope of this thesis.

Radiolabelling of the three proteins studied with ^{3}H-leucine was highest in the proteins associated with bound polyribosomes; the specific radioactivities of radioactive mannose and glucosamine in α_1-acid glycoprotein and α-macroglobulins associated with ribosomes was much lower than those of the proteins associated with the proteins in microsome and Golgi fractions. These observations lead to two conclusions: translation of the three serum glycoproteins occurs primarily on bound polyribosomes and en bloc transfer of carbohydrate to these proteins occurs primarily after completion of polypeptide synthesis. While the synthesis of secretory proteins on bound polyribosomes is consistent with the Signal Hypothesis of Blobel and Dobberstein, many proteins are thought to be glycosylated as a cotranslational event (see Introduction). However, there are precedents for post-translational glycosylation. Nickerson and Fuller (1981) have demonstrated that hepatic en bloc glycosylation of the Bβ chain of fibrinogen is a post-translational event. Bielinska et al. (1978) have shown that glycosylation of nascent chains of secretory proteins is not obligatory for the proteolytic processing of pre-proteins, or for the translocation of secretory proteins across microsomal membranes. Also, Hanover and Lennarz (1980) have reported

that *in vitro* glycosylated proteins do not copurify with polyribosomes, and that release of nascent chains from ribosomes with puromycin did not affect *in vitro* glycosylation of proteins. Although the initial glycosylation of proteins may often be a cotranslational event, the above observations suggest a loose coupling of translation and glycosylation, and that the initial attachment of carbohydrate to α_1-acid glycoprotein, α_1-macroglobulin and α_2-macroglobulin is primarily a post-translational event in the livers of inflamed rats.

The similarities in the *in vivo* labelling of the acute phase reactants α_1-acid glycoprotein and α_2-macroglobulin with the serum glycoprotein α_1-macroglobulin raise the question of the role of glycosylation in enhanced biosynthesis of acute phase reactants following inflammation. Glycosylation and translation of acute phase reactants do not appear to be tightly coupled (Jamieson, 1977; Nickerson & Fuller, 1981a; this thesis). Also, the drug tunicamycin, which inhibits glycosylation of proteins, does not affect secretion of transferrin or fibrinogen (Schreiber *et al.*, 1979; Nickerson & Fuller, 1981b); both these proteins are glycoproteins and acute phase reactants. Thus, it appears that glycosylation is not essential to the secretion of the acute phase reactants. However, it is known that following inflammation, there are elvated rates of biosynthesis of carbohydrate moieties of glycoproteins (Jamieson *et al.*, 1975, 1983; Kushner, 1982); levels of available dolichol phosphate becomes rate limiting to glycosylation during the acute phase response (Coolbear & Mookerjea, 1981). Also, during the acute phase response, there are elevated synthesis and levels of nucleotide sugar precursors of

glycoproteins, and elevated glycosyltransferase activities responsible for attachment of carbohydrate to glycoprotein precursors (Bley et al., 1973; Turchen et al., 1977; Lombart et al., 1980; Jamieson et al., 1983; Kaplan et al., 1983a, 1983b); these changes are consistent with elevated rates of synthesis of carbohydrate moieties of glycoproteins. While glycosylation may not be an essential component of the enhanced biosynthesis of acute phase reactants following inflammation, the biosynthesis of the carbohydrate moieties of acute phase reactants may be important for the properties of glycoproteins secreted from the liver during the acute phase response, as is suggested by the work of Koj et al. (1982). The importance of glycosylation of acute phase reactants during the acute phase response remains to be fully delineated; undoubtedly, this will be the subject of many future studies.

2. Physiologic Response to Inflammation

2.1 Hormone Secretion and Levels

The work presented in this thesis, as well as that by others, indicates that stress and inflammation result in significant changes in hormone secretion. Some of these changes can be understood in terms of changes in the status of the hypophysis or pituitary gland. Corticotropin in synthesized, stored and released from cells, known as corticotrophs, which are located in the anterior and intermediate lobes of the hypophysis (Moriarty, 1977; Nakane et al., 1977). Elevated serum levels of corticotropin following inflammation would result from increased release of this hormone from corticotrophs.

The primary action of corticotropin is to stimulate synthesis and secretion of glucocorticoids by the adrenal cortex (Harding, 1977), and thus, elevated corticotropin levels would account for elevated serum cortisol levels following inflammation. The appearance of corticotropin following inflammation would also suggest other endocrine changes. It has been shown that, in response to all physiological stimuli affecting corticotropin secretion, there is a coordinate release of the products of processing of the precursor proteins of this hormone (Eipper & Mains, 1980). This would mean that, following inflammation, there should be equimolar secretion of corticotropin and β-endorphin-related molecules (β-endorphin and β-lipotropin), and that circulating levels of β-endorphin-related molecules should be elevated at 10 h after inflammation.

More than one factor may be responsible for stimulating corticotropin release following acute inflammation. Corticotrophs of the adenohypophysis, or anterior lobe of the pituitary, are stimulated to release hormone by blood-born corticotropin releasing factor (CRF), and hormone secretion is inhibited by elevated levels of circulating glucocorticoids (Eipper & Mains, 1980). In contrast, release of hormone from intermediate lobe corticotrophs is controlled only by neurological factors. CRF originates primarily in the hypothalamus, which is a portion of the central nervous system located adjacent to the hypophysis; CRF is delivered from the hypothalamus to the adenohypophysis *via* the hypophysial portal veins (Hiroshige *et al.*, 1977). Also, there is evidence of a CRF which does not originate in the hypothalamus. Rats show a biphasic release of CRF peaking at 1 h and 6 h

following surgical laparotomy; rats with hypothalamic lesions do not release CRF at 1 h after laparotomy, but still have an elevation in CRF activity at 6 h after laparotomy; this later CRF activity has been referred to as tissue-CRF (Lymangrover & Brodish, 1973; Brodish, 1977). Tissue-CRF, which cannot originate in the hypophalamus, is present in the blood of injured rats, is extremely potent and presumably originates at the site of tissue injury (Brodish, 1977). Also, there is a biphasic response of corticotropin in response to turpentine-induced inflammation (this thesis), which suggests that both hypothalamic- and tissue-CRFs may be responsible for the elevated levels observed. It is tempting to speculate that the tissue-CRF may be a cytokine. Interleukin-1 is known to act on the hypothalamus to stimulate fever (Oppenheim et al., 1982; Bornstein, 1982). Also, administration of cytokine results in an elevation of serum cortisol levels (this thesis), and presumably corticotropin. Intracerebroventricular injection of cytokine is more effective in eliciting acute phase-like like response, including fever, than intravenous injections (Bornstein, 1982). Together these observations suggest action of the cytokines on the hypothalamo-hypophysial axis.

Inflammation and disease also affect the release of thyrotropin from the adenohypophysis (Larsen et al., 1981; Morley, 1981). Hypothalamic thyrotropin releasing hormone (TRH) stimulates the release of thyrotropin from thyrothrophs; there is evidence for extrahypothalamic TRH originating in tissues as diverse as the pineal gland, the spinal cord, the pancreas and the testes. A number of substances, including glucocorticoids and endogenous opiates, are involved in the

inhibition of thyrotropin secretion and may lower thyrotropin levels following inflammation. Thyrotropin stimulates secretion of thyroid hormones from the thyroid gland, and thus lower levels of thyrotropin may contribute to the depressed levels of thyroxine and triiodothyronine following inflammation.

Uptake and deiodination of thyroid hormones by tissues also contributes to the depression of thyroxine and triiodothyronine following acute inflammation (Beisel, 1975, 1980; Larsen et al., 1981). Thyroxine or T4 is a hormone which contains four covalently attached iodine atoms. Deiodination from a position distal to the amino function of T4 results in the formation of triiodothyronine or T3; deiodination from an inner position of T4 results in the formation of reverse triiodothyronine or rT3, which is physiologically inactive; removal of two or more iodines yields physiologically inactive compounds. Following active infections, there is an accelerated deiodination of thyroid hormones and an accumulation of rT3 in the liver, which contributes to the depression of serum thyroid hormones (Beisel, 1980).

Serum glucose levels are elevated following inflammation, and these levels would stimulate release from the pancreas, and thus elevate serum insulin levels (Langstaff et al., 1980). Catecholamines act on α-adrenergic receptors of the pancreas to inhibit secretion of insulin (Young & Landsberg, 1979). In view of the well known effects of stress on catecholamine release from sympathetic nerves and the adrenal medulla (Young & Landsberg, 1979), it seems likely that the basal levels of insulin at early times following inflammation, which are times that serum glucose levels are elevated, are due to

catecholamine inhibition of insulin release in response to elevated serum glucose levels.

The acute phase response includes complex, yet orderly, endocrine changes in experimental animals. In view of the physiological activities of the hormones which are altered, these changes undoubtably contribute to other complex, yet orderly, responses which include those involved in the hepatic biosynthesis of acute phase reactants.

2.2 Amino Acid Levels

The results presented in this thesis indicate that there is a general increase in serum amino acid pools at early times after inflammation, and a return to control levels at later times. Hepatic amino acid pools are depressed at early times and increase at later times after inflammation. Hepatic taurine levels, which are consistently elevated after inflammation, are an exception to this pattern. This redistribution of amino acids may be related to humoral changes which are known to occur in response to inflammation.

It is known that following inflammation, there is an increased catabolism of protein in most tissue, particularly muscle, and an increased flux of amino acids into the plasma (Beisel, 1975, 1980). This increased protein catabolism and amino acid flux may account for the lower hepatic levels of amino acids, and for the elevated serum amino acid pools, at early times after inflammation. Elevated cortisol stimulates catabolism of protein, promotes release of amino acids from muscle, reduces amino acid uptake into muscle and enhances hepatic gluconeogenesis (Goldberg et al., 1980). Thus, the elevated cortisol

levels observed at 6 h, and later times, after inflammation may account, in part, for the altered amino acid pools. However, cortisol is at basal levels at 4 h after inflammation, and cannot account for the alterations in amino acid pools observed this early following the inflammatory stimulus. The rapidity of the response, though, suggests neuro-humoral involvement in modulating amino acid pools at this early time.

Lombardini (1980) has studied the effect of the β-adrenergic agonist isoproterenol, and the α-adrenergic agonist methoxamine, on amino acid contents of rat tissues. Stress induces the rapid release of catecholamines such as norepinephrine and epinephrine from sympathetic nerves and from the adrenal medulla; different aspects of catecholamine action are stimulated via α- and β-adrenergic receptors on the cell surfaces of target tissues (Young & Landsberg, 1979). Isoproterenol administration reduced cardiac taurine content, increased the serum taurine pools from 3-16 h after administration and increased the hepatic taurine pool from 4-14 h after administration. Isoproterenol also depressed serum taurine, arginine, valine, isoleucine, leucine and ornithine levels at 7 h, and elevated serum lysine levels at 24 h after administration. Methoxamine administration resulted in a reduction of cardiac taurine levels from 2-60 h, and an increase in serum taurine levels from 4-16 h after administration; hepatic taurine levels doubled at 4 h, reached a maximum at 16 h and returned to control levels at 48 h after administration. Lombardini (1980) also noted that while loss of taurine from cardiac tissue could quantitatively account for elevated serum taurine pools, hepatic taurine pools

cannot be accounted for by transfer from the cardiac and serum pools; catecholamines either stimulate de novo taurine biosynthesis in the liver, or sequester taurine from other tissues. This last possibility is unlikely as the fourth major pool of taurine in the body is skeletal muscle, and pools in this tissue were unchanged by catecholamine agonist administration (Lombardini, 1980). There are some striking similarities between the effects of inflammation and catecholamines; these similarities suggest that stress-induced catecholamine release can account for the alterations in taurine pools following inflammation. Some exceptions to the general pattern of the amino acids, such as the increased lysine content of the liver at late times following inflammation, may also be accounted for by the action of the catecholamines.

Altered hormone levels at later times after inflammation may modulate the changes in the amino acid pools. Depression of thyroid hormones and elevations of insulin levels (Figures 14-16) would decrease protein catabolism, and promote uptake into muscle (Goldberg et al., 1980). The net effect of this should be to lower serum amino acid pools to near basal levels. It is known that cytokines released by leukocytes following inflammation increase hepatic amino acid uptake (Wannemacher et al., 1975); catecholamines also stimulate amino acid uptake into the liver (Young & Landsberg, 1979). The action of these agents may account, in part, for the elevated hepatic amino acid pools seen at later times after inflammation.

Several alterations in metabolism lead to an increased utilization

of amino acids following inflammation. There is an increased synthesis of proteins during the acute phase response (Beisel, 1980; Kushner, 1982; Jamieson et al., 1983). Increased gluconeogenesis from amino acids during the acute phase response (Beisel, 1980) results in an increased production of glucose which is released into the blood (Langstaff et al., 1980), or contributes to an increased hepatic production of other sugars and nucleotide sugars (Kaplan et al., 1983a). A part of this increased degradation of amino acids is the increased production of urea; it may be important that ornithine has been shown to have stimulatory effects on hepatic ureagenesis (Stewart & Walser, 1980). This suggests that fluctuations in ornithine pools may modulate amino acid degradation during the acute phase response.

It is evident that the alterations in amino acid pools following inflammation are complex and likely to be regulated at a number of levels. These changes are important to the understanding of overall metabolic alterations during the acute phase response to acute inflammation. Those metabolic changes occurring in the liver may be particularly important because of the synthesis of the acute phase reactants by this organ.

3. In Vivo Response to Cytokine

3.1 Comparison of Cytokine Action and Inflammation

Cytokines produced by stimulated leukocytes are responsible for mediating some aspects of the acute phase response to inflammation. As such, a comparison of the effects of cytokine administration and

turpentine-induced inflammation should provide some insight into the extent and mechanisms of involvement of cytokine in controlling the acute phase response.

One feature of the response to cytokine administration seen in these studies is that while changes in albumin levels were of a similar magnitude as that elicited by inflammation, the alterations in sialyltransferase, hepatic glycosidase activities and α_1-acid glycoprotein levels were attenuated in comparison to that seen with turpentine-induced inflammation. Dependence on cytokine concentration is an unlikely explanation for this effect since there was no apparent difference in the response of sialyltransferase activities and serum albumin levels over a 10-fold range of dosage of cytokine administered. This would suggest that the maximum response to a single administration of cytokine alone may be a doubling of serum sialyltransferase and a 20% reduction of serum albumin. The serum sialyltransferase response is significantly lower than the 5-fold increase obtained following turpentine-induced inflammation (Kaplan et al., 1983a). Also, while serum α_1-acid glycoprotein levels are elevated 5-fold following turpentine-induced inflammation (Jamieson et al., 1972b), it may not be possible to attain these levels by administration of cytokine alone. The response of serum albumin levels to cytokine administration, though, was very similar to that found following inflammation (Jamieson et al., 1972b).

While a number of possible explanations exist for the diminished response to cytokine of some parameters of the acute phase response, one of the more obvious is a lack of synchrony which might optimize

the in vivo response to cytokine. Serum cortisol levels were at basal levels for the first 24 h following cytokine administration; this hormone was elevated from 6-24 h after turpentine administration. Serum thyroxine levels were lowered at short times after cytokine administration, but were at basal levels, or were elevated, at 12-48 h after cytokine administration. These hormones are known to have various effects on liver, and may be necessary at specific times after treatment for the full expression of the acute phase response. At 36 h after cytokine administration, sialyltransferase activities and serum α_1-acid glycoprotein levels were maximum and hepatic α_1-acid glycoprotein levels were increased; serum cortisol levels also were maximum at this time. This suggests that cortisol may play a role in elevation of α_1-acid glycoprotein levels and sialyltransferase activities. This hypothesis is supported by the observation that cortisol administration elevated hepatic, but not serum, sialyltransferase activities in the adrenalectomized rat (Ip, 1979). Also, Baumann et al. (1983) have reported that glucocorticoids elevate rat liver α_1-acid glycoprotein mRNA levels, but these authors also noted that acute phase reactants can be induced in the absence of glucocorticoids. These observations suggest that during inflammation a synergism may exist involving the effects of cytokines and glucocorticoids such that α_1-acid glycoprotein and sialyltransferase are elevated maximally. Alterations in other hormone levels, such as thyroxine, following inflammation may serve to modulate other parameters of the acute phase response.

Another explanation for the diminished response of parameters of

of the acute phase response to cytokines comes from the heterogeneity of the cytokine preparations, and a number of experiments support this hypothesis. Effects of cytokine preparations on sialyltransferase activities, and serum α_1-acid glycoprotein and albumin levels, were lost upon heat treatment; both heat-treated and untreated cytokine preparations depressed hepatic hexosaminidase activity. Hepatic hexosaminidase activities did not respond to dosages of cytokine less than 200 MCE; sialyltransferase activities were elevated, and serum albumin levels were lowered, by administration to rats of 50 MCE cytokine. Preparations of non-adherent cells produced cytokine which depressed hepatic hexosaminidase activities, but had no effect on other parameters of the acute phase response. These observations suggest that hepatic hexosaminidase activity is controlled by a cytokine distinct from those responsible for elevations of sialyl-transferase activities and α_1-acid glycoprotein levels, and the depression of albumin levels. This cytokine, which may be controlling hepatic hexosaminidase activities, also may conteract the effects of other cytokines on parameters of the acute phase response.

A third possible explanation for the diminished response to cytokine administration is related to the stability of the bioactive material. A rabbit cytokine preparation, which has effects on hepatic activity, has a half-life of less than 10 min when administered to rats (Kampschmidt et al., 1982). Also, a number of parameters which include amino acid flux to the liver, haptoglobin and C-reactive protein levels and α_2-macrofetoprotein synthesis exhibited larger responses at lower dosages when administration of a rabbit cytokine was via an intracerebroventricular route, as opposed to an

intraperitoneal route (Bailery et al., 1976; Bornstein, 1982). These observations suggest that the loss of bioactivity between the time of cytokine administration and the action of cytokine on target tissue may be important to the magnitude of the response elicited. However, there was no difference in the response elicited when rat cytokine was administered to rats by intraperitoneal or intravenous routes. This would suggest that the transfer of rat cytokine from the peritoneum to the circulatory system does not represent an important step in the response of the liver to cytokine administration. Nevertheless, this does not discount the possibility that the fate of the cytokine following administration plays a role in the magnitude of the response elicited.

It is evident that while the response to cytokine administration resembles the acute phase response to inflammation in many ways, these responses differ. These differences undoubtedly reflect faults with the simple hypotheses that all of the changes elicited during the acute phase response are controlled by cytokine released by activated leukocytes at the site of inflammation, and that the acute phase response can be duplicated by administration of in vitro produced cytokine. An understanding of these differences in response, though, will undoubtedly be greatly beneficial to an understanding of the complex problem of the control of the acute phase response.

3.2 Leukocyte Endogenous Mediation of the Acute Phase Response

It is evident that leukocytes produce factors which modulate or control many aspects of the acute phase response to inflammation.

Indeed, it has been suggested that cytokine(s) are the central mediator(s) of the acute phase response (Bornstein, 1982; Kampschmidt et al., 1982; Fuller & Ritchie, 1982; Oppenheim et al., 1982). What is suprising is the range of effects that cytokines exhibit, and the possibility that a single protein, or a closely related family of proteins, may be responsible for the very wide ranging physiological and biochemical response to inflammation.

Cytokines have effects on many tissues of the body, and thus upon many of the physiological responses characteristic to the acute phase reaction. A cytokine activity, which has been referred to as Leukocytic Pyrogen (LP) and Leukocytic Endogenous Mediator (LEM), has been shown to stimulate cells of the preoptic nuclei of the anterior hypophalamus to produce fever (Rafter et al., 1966; Merriman et al., 1977; Atkins & Bodel, 1979; Dinarello, 1979; Bornstein, 1982). LEM is involved in increasing blood granulocyte numbers by stimulating release of granulocytes from bone marrow (Kampschmidt et al., 1973; Kampschmidt & Upchurch, 1977); this cytokine also stimulates blood granulocytes such that they exhibit an increased oxidative metabolism and exocytosis of granules (Bornstein, 1982). LEM elevates both plasma glucagon and insulin levels at 5 h after administration (George et al., 1977); a cytokine from rat leukocytes elevates serum cortisol levels and depresses thyroxine levels (this thesis). Reductions of plasma zinc and iron levels are induced by LEM administration (Pekarek & Beisel, 1971; Kampschmidt et al., 1973; Pekarek et al., 1974; Mapes & Sobocinski, 1977; Bornstein, 1982). Cytokine administration has been shown to elevate serum levels of the acute phase reactants α_1-acid

glycoprotein, fibrinogen, C-reactive protein, haptoglobin, α_1-macrofetoprotein, α_2-acute phase globulin, ceruloplasmin and serum amyloid A, and to depress serum albumin and transferrin levels (Eddington et al., 1972; Pekarek et al., 1974; Wannemacher et al., 1975; Bornstein, 1982; this thesis). Rat cytokines elevate sialyltransferase activity in this species (this thesis). Cytokines have been shown to be mitogenic to fibroblasts (Wahl et al., 1980; Wahl & Wahl, 1981; Oppenheim et al., 1982; Wahl & Gately, 1983). The above examples illustrate the depth of involvement of cytokines in mediating aspects of the acute phase response, in addition to the well documented effects on leukocytes (see Introduction).

As was previously mentioned, alterations in the profile of plasma proteins during the acute phase response results from altered hepatic activity following inflammation. Cytokines also have a number of effects on the livers of treated animals. Administration of cytokine stimulated hepatic amino acid transport (Beisel, 1975; Wannemacher et al., 1975; George et al., 1977; Mapes & Sobocinski, 1977; Kampschmidt et al., 1982), stimulated orotate incorporation into bound ribosomal RNA (Wannemacher et al., 1975), depressed hepatic hexosaminidase and galactosidase activities (this thesis) and increased hepatic cAMP content (George et al., 1977). In addition to these effects, there were elevated hepatic synthesis of the acute phase reactant fibrinogen (Kampschmidt & Upchurch, 1974), elevated hepatic content of α_1-acid glycoprotein and depressed hepatic content of albumin (this thesis) following cytokine administration. All these observations are consistent with the proposed role of cytokine(s)

as the central mediator(s) of the acute phase response to inflammation.

It appears that the primary mediator(s) of the acute phase response is of monocytic origin. Experiments presented in this thesis indicated that monocytes produced the factor(s) responsible for elevations in sialyltransferase activities, α_1-acid glycoprotein levels and for depression of albumin levels. Monocytes and lymphocytes may both produce the cytokine(s) which depress hepatic hexosaminidase activities (this thesis). Administration of cytokines produced by cultured macrophages resulted in fever in treated animals, and in the induction of serum amyloid A production by liver cells (Simon & Willoughby, 1982). Aveolar or peritoneal macrophages and Kupffer cells or macrophages from peripheral blood cells will produce leukocytic pyrogen and LEM activities (Bornstein, 1982; Kampschmidt et al., 1982). Also, experiments examining the effect of supplementation of hepatocyte cultures on acute phase reactant synthesis indicate that monocytes are the source of the mediator(s) of the acute phase response (Fuller & Ritchie, 1982; Ritchie & Fuller, 1983; Tatsuda et al., 1983). However, there also has been a report that cytokine activity believed to be similar to this mediator can be produced by a human lymphoma cell line (Palacios et al., 1982). Generally, though, it is accepted that endogenous mediators of the acute phase response are monokines (Bornstein, 1982; Kampschmidt et al., 1982; Kushner, 1982; Fuller & Ritchie, 1982).

Most of the work on monokine regulation of the acute phase response performed to date has utilized unfractionated cytokines from mixtures of leukocyte cell types, or monocyte-enriched preparations.

This has resulted from experimental problems associated with obtaining large amounts of cytokines, and with the loss of bioactivity during purification (Bornstein & Walsh, 1978; Mizel & Mizel, 1981; Borstein, 1982; Simon & Willoughby, 1982). Despite these difficulties, studies with fractionated cytokines have illustrated two points: 1) there is heterogeneity in the monokine responsible for mediating the acute phase response, and, 2) individual molecular species of monokine have multiple biological activities, and a single protein could be responsible for mediation of most aspects of the acute phase response. LP, LEM, Lymphocyte Activating Factor (LAF) and Serum Amyloid A Stimulating Factor (SAASF) cannot be separated from one another; the activities copurify during a variety of sequential purifications (Mizel & Mizel, 1981; Bornstein, 1982; McAdam et al., 1982; Oppenheim, 1982; Simon & Willoughby, 1982). For this reason, the use of the term interleukin-1 has been proposed to describe all these cytokine activities (Bornstein, 1982; Oppenheim et al., 1982; Simon & Willoughby, 1982). While these activities are not distinguishable from one another, there are differences in the molecular forms of interleukin-1. Two forms of rabbit interleukin-1 exist with molecular weights 12 kd and 14 kd; the smaller monokine has a pI of 7.4, while the larger monokine has a pI of 4.6 (Simon & Willoughby, 1982). Two forms of murine interleukin-1 have been reported, one with a molecular weight of 12-16 kd and a pI of 5.0-5.4, and a second with a molecular weight of 18 kd and pI of 4.8 (Simon & Willoughby, 1982). Human interleukin-1 is a 12-15 kd polypeptide which has forms with pIs of 5.2, 6.0 and 6.9 (Oppenheim, et al., 1982; Simon & Willoughby, 1982). Higher molecular weight

(eg. 50-85 kd) forms of interleukin-1 have been identified, but these are believed to result from binding of the monokine to proteins as these forms dissociate into low molecular weight interleukin-1 under appropriate conditions (Simon & Willoughby, 1982). These differences in physicochemical characteristics are not understood at this time, but it may be important that the pI 4.6 form of rabbit interleukin-1 has a greater biological stability than that of the pI 7.4 form (Simon & Willoughby, 1982). Also, when rabbit peritoneal exudate cells, which contain large numbers of granulocytes, are used as a cell source, predominantly the pI 7 form is produced, whereas predominantly the pI 5 form is obtained using rabbit monocytes as the cell source (Bornstein, 1982). For the human interleukin-1, it has been suggested that the different forms result from differences in post-translational glycosylation (Oppenheim et al., 1982), although the differences are not due to differences in sialic acid content (Bornstein, 1982). Undoubtedly, improved methods for the production of large quantities of cytokines, and for the handling and purification of cytokines, will answer many questions on the structural basis of interleukin-1 function by providing large quantities of the purified cytokines.

The simple view that a single monokine mediates the acute phase response contrasts with the results presented in this thesis which suggests the involvement of several cytokines. Also, a monokine which has been referred to as hepatocyte stimulating factor (HSF), and which is responsible for stimulation of fibrinogen synthesis by hepatocyte cultures, has recently been demonstrated to be distinct from interleukin-1 (Ritchie & Fuller, 1983). While many parameters examined in this thesis

may be controlled by the rat counterpart to interleukin-1 of other species, it is likely that more than one active cytokine is present in the preparations used for the studies presented here.

In addition to the heterogeneity which is exhibited in the physicochemical properties of interleukin-1, it appears that there are species differences in this monokine. While both the pI 5 and 7 forms of rabbit interleukin-1 elicit the acute phase response in rabbits, only the pI 7 form of the rabbit interleukin-1 is active when administered to rats (Kampschmidt et al., 1982). Also, rabbit interleukin-1 is pyrogenic in the rat, while the rat cytokine is not (Kampschmidt & Upchurch, 1969). Rabbit interleukin-1 is sensitive to trypsin digestion and is unstable at 40°C, while human interleukin-1 is stable at 40°C and resistant to trypsin digestion (Simon & Willoughby, 1982); murine interleukin-1 also is resistant to trypsin digestion (Mizel, 1980) and pyrogenic activity is lost by incubation at 56°C for 1 h (Bodel & Miller, 1976). The rat cytokine activity responsible for alterations in sialyltransferase activities, and in albumin and α_1-acid glycoprotein levels, is lost with heating at 80°C for 1 h, but is stable to trypsin digestion; the cytokine activity that depresses hepatic hexosaminidase activity is stable to heat treatment and trypsin digestion (this thesis). The rat cytokine activity also is lost during attempts at ultafiltration using Amicon filters (this thesis), and the human interleukin-1 binds irreversibly to ultrafiltration membranes (Lachman et al., 1981); murine interleukin-1 retains its bioactivity during ultrafiltration on Amicon UM filters (Mizel & Mizel, 1981). These observations suggest that the cytokine(s) from rat leukocytes resembles human interleukin-1 more so than cytokines

from other species.

The potency of the rat cytokine in the rat was examined in this thesis. A dosage of 50 MCE cytokine will elicit a full response of sialyltransferase activity, or albumin levels, while over 100 MCE cytokine is necessary to lower hepatic hexosaminidase activity in the rat. The minimum dosage of rabbit cytokine that must be administered to elicit a pyrogenic response in the rabbit is 17.5 MCE; neutrophil-releasing activity is stimulated maximally at dosages of 175 MCE, or more, of rabbit interleukin-1 (Bornstein, 1982). Dosages of 100 MCE of rabbit LEM elevate acute phase reactant levels in the rat (Pekarek et al., 1974), and stimulate hepatic amino acid transport (Wannemacher et al., 1975). Approximately 20 MCE of rabbit cytokine is the minimum dosage required to elicit a pyrogenic response in mice (Kampschmidt et al., 1980). While 5 MCE of human cytokine is pyrogenic in the rabbit, maximum responses are elicited by dosages of 60 MCE, or greater; these dosages are smaller if purified monocytes are used as the cell source (Dinarello et al., 1974). Dosages of 4 MCE of mouse cytokine elicit a pyrogenic response in mice (Bodel & Miller, 1976). Considering the differences in the size in experimental animals tested, and the differences in experimental procedures, the rat cytokine described in this thesis is as potent as that from other species.

As discussed above, a consideration of biological activity, cell sources, physicochemical properties and potencies leads to the conclusion that the preparation of rat cytokines that has been studied in this thesis contains a cytokine which is very similar to, or identical with the interleukin-1 described in rabbits, mice and humans.

4. Liver Slice Studies

4.1 Comparison of Liver Slices and Hepatocyte Cultures

Regulatory mechanisms are best studied with isolated cells, or tissues, as testing of agents may be carried out without interference from homeostatic control by the organism. Two *in vitro* systems have been used to test the direct effects on livers of physiologically active agents and drugs: liver slices and liver cell culture. As differences exist between these two systems which have been used in attempts to answer the same basic questions, an understanding of these differences would be beneficial to interpretations of results obtained from both systems.

The liver contains many cell types which make up its two major compartments, the portal tracts and lobular parenchyma (Grisham, 1980). The portal tracts contain epithelial cells of arteries, veins, capillaries, and lymphatic vessels, smooth muscle cells of arteries and veins, fibroblasts, and nerve cells. Hepatocytes comprise most of the volume of the lobular parenchyma, but there are also numerous macrophages (Kupffer cells), sinusoidal endothelial cells, so-called pit cells and fat-storing (Ito) cells. Liver slices would contain all of these cells in proportions equal to that normally found in the liver. Cultures of liver cells would contain different proportions of the cells, and these proportions depend on the method of preparation, and the time spent in culture. Tissue digestion of liver with trypsin, collagenase or Dispase I causes severe damage to hepatocytes, and thus, primary hepatocytes established using these enzymes are enriched in

epithelial cells and fibroblasts (Marceau et al., 1980). The extracellular matrix is composed of collagen, glycosaminoglycans and glycoproteins, it is produced and secreted by cells, and separates cells in situ (Gospadarowicz & Tauber, 1980). While liver slices retain much of the extracellular matrix, preparation of cultures of liver cells results in loss of much of the extracellular matrix (Gospadarowicz & Tauber, 1980; Reid et al., 1980). The extracellular matrix plays an important role in the regulation of growth of cells, and for this reason, loss of this component results in morphological and biochemical alterations of cells (Gospadarowicz & Tauber, 1980; Reid et al., 1980).

Another important difference between liver slices and liver cell culture is the length of time which the cells remain viable. Liver slices remain viable for 6 h of incubation to judge from rates of synthesis of protein (Jamieson et al., 1975) and RNA (this thesis) and from the release of sialyltransferase activity into medium (Kaplan et al., 1983b), and for 5 h when the metabolism of putrescine is considered (Lundgren & Hankins, 1978). This may be related to the fact that in the process of liver regeneration, events critical to the promotion of growth, such as altered membrane potentials, intracellular pH, amino acid transport, and RNA and protein metabolism, occur within the first 6 h after hepatectomy (Koch & Leffect, 1980; Swierenga et al., 1980). Conditions used in liver slice studies may not allow liver cells to maintain hepatocyte functions past this time. It may be relevant that Liberti et al. (1971) were able to maintain protein synthesis in liver slices for 12 h of incubation, however, their protocol used unperfused livers and serum supplementation; RNA synthesis

was maintained for 2 h of incubation in their system. Liver cell cultures are viable for considerably longer times than liver slices, and the time that liver cell will remain alive depends on the conditions of culture. Primary cultures of hepatocytes can be prepared from normal liver, and are established within 4-6 h of plating if medium is supplemented with serum, vitamins, insulin and corticosterone; serum-free medium can be used providing that ornithine and insulin are added (Bissell & Guzelian, 1980). Maintenance of specific functions of liver cells may require more complex media. Primary cultures of hepatocytes can be maintained in this way for days, but there is a loss of protein and DNA with each change of culture medium. This loss is one of the phenotypic changes which represent an ordered metabolic response in which hepatocytes lose many of the characteristic features of the intact liver, and adapt to conditions of culture (Bissell & Guzelian, 1980).

Liver slices and hepatocyte cultures differ from intact liver, and yet, they maintain, under suitable conditions, some of the biochemical properties of the intact liver. Liver slices synthesize and secrete plasma proteins, and liver slices from inflamed rats exhibit elevated rates of synthesis of acute phase reactants, such as α_1-acid glycoprotein, as does the intact liver (Jamieson et al., 1975). Primary cultures of hepatocytes also synthesize and secrete plasma proteins, including the acute phase reactants (Jeejeebhoy et al., 1980; Seglon et al., 1980; Ritchie & Fuller, 1981; McInteyre et al., 1982; McAdam et al., 1982; Tatsuda et al., 1983; Wangh et al., 1983; Weiner & Cousins, 1983). However, rates of synthesis of proteins by liver

cells are not always directly comparable. For example, while albumin is synthesized by intact liver at a rate of 290 µg/g liver/h, and by hepatocyte cultures at a rate of 320 µg/g hepatocytes/h (Jeejeebhoy et al., 1980), liver slices synthesize this protein at a rate of 33-66 µg/g liver/h (Jamieson et al., 1975). This difference may be related to the fact that only those cells on the outer surface of the liver slices are in intimate contact with medium, and no fluid perfuses the inner cells of the liver slices as blood would perfuse an intact liver. However, the rate of synthesis of α_1-acid glycoprotein in liver slices is 9-20 µg/g liver/h (Jamieson et al., 1975), which is comparable to the rate of 20 µg/g hepatocytes/h found for this protein in hepatocyte culture (Jeejeebhoy et al., 1980). Also, Miller and Griffin (1975) have estimated that the rates of net synthesis of these proteins in isolated, perfused, whole liver were 150 µg/g liver/h for α_1-acid glycoprotein, and 75-200 µg/g liver/h for albumin. Thus, the rates of synthesis of specific proteins by liver cells vary considerably with the experimental conditions. In addition to protein synthesis, other activities of liver are maintained in these in vitro systems. RNA synthesis is maintained in both liver slices (Clemens & Korner, 1970; this thesis), and hepatocyte cutlures (Chen & Feigelson, 1980; Roy, 1983; Roy et al., 1983). Also, liver slices release the enzyme sialyltransferase into incubation medium (Kaplan et al., 1983b), as does the hepatoma cell line SK-H-MA (Waxman et al., 1980); hepatic release of this enzyme is mainly responsible for elevated serum levels of the enzyme activity during the acute phase response (Kaplan et al., 1983b). These similarities between liver cell activities in vivo and

in in vitro systems are used as the basis for use of the results of experiments with liver slices and hepatocyte cultures in explaining the biochemistry and physiology of the intact liver.

While interpretation of the results of experiments with liver slices or hepatocyte cultures must take a critical view of the experimental conditions if an extrapolation is to be made to the intact liver, the use of these in vitro systems simplifies experiments examining the direct effects of agents on this organ.

4.2 RNA Synthesis by Liver Slices

Alterations in hepatic RNA synthesis are an important part of the acute phase response to inflammation, and are essential for altered biosynthesis of acute phase reactants (see Introduction; Woloski, 1980). Rates of incorporation of radiolabelled orotate into RNA were examined in this thesis, and were taken as suitable measures of RNA synthesis (see Section 4.1 of Results).

Liver slices from inflamed rats have higher rates of synthesis of RNA than liver slices from control rats (this thesis). This observation is consistent with a number of reports of altered RNA metabolism during the acute phase response (Chandler & Neuhaus, 1968; Thompson & Wannemacher, 1973; Atryek & Fausto, 1979; Woloski, 1980; Haugen et al., 1981; Ricca et al., 1981). The results from liver slices, though, indicate that the maximum elevation to 170% of control levels of RNA synthesis occurred with slices prepared from 10 h inflamed rats (this thesis); Thompson and Wannemacher (1973) reported de novo labelling of hepatic RNA peaked at 20 h after inflammation, and maximum

levels were 300% of controls. While the reason for the diminished response in orotate labelling of liver slice RNA is not immediately obvious, some insight can be obtained by examination of results presented in this thesis.

As previously mentioned, results of liver slice experiments should be interpreted with a consideration of experimental conditions. Unless otherwise noted, liver slice medium contained only inorganic salts, glucose, translatable amino acids and antibiotics. However, when medium was supplemented with physiological concentrations of prostaglandins, thyroid hormones, insulin and cortisol, orotate incorporation into control liver slice RNA was diminished (this thesis). While ornithine supplementation had little effect on orotate incorporation into control liver slice RNA, it resulted in an enhancement of tracer incorporation into 12 h inflamed liver slice RNA; thus 200 nmol/ml ornithine increased the ratio of specific radioactivities of RNA in 12 h inflamed to control liver slices from 1.5 without supplementation to 2.0. Also, orotate labelling of RNA by liver slices is dependent upon amino acid concentrations, and increases with amino acid supplementation 1-6 times that normally present in serum (Clemens & Korner, 1970). These observations suggest that differences between de novo RNA labelling patterns and that obtained with liver slices are due, at least in part, to the conditions of incubation of liver slices. However, these observations do not invalidate the observation that even in a very simple medium, liver slices from inflamed rats exhibited elevated tracer incorporation into RNA in a similar way that inflamed rats exhibit elevated de novo RNA

synthesis.

From the results of radiolabelling of RNA in liver slices, it is not possible to distiguish which classes of RNA were labelled, however, patterns of de novo radiolabelling of hepatic RNA with [^{3}H]-orotate are known. While rRNA accounts for about 80% of hepatic RNA, and poly(A)-RNA (a mRNA enriched fraction) about 2%, differences in rates of synthesis of classes of RNA are such that, in a 3 h period, about 10% of the [^{3}H]-orotate incorporated into hepatic RNA appeared in the poly(A)-RNA fraction (Woloski, 1980). This would suggest that while much of the [^{3}H]-orotate incorporated into liver slice RNA may appear in the rRNA fraction, a reasonable fraction of mRNA is also radiolabelled. While there is an increase in the synthesis of rRNA (Chandler & Neuhaus, 1968; Thompson & Wannemacher, 1973), others have reported a more substantial elevation in the synthesis of mRNA transcripts of acute phase reactants, such as α_1-acid glycoprotein, (Haugen et al., 1973; Ricca et al., 1981; McAdam et al., 1982; Stearman et al., 1982) during the acute phase response; mRNA transcripts for α_1-acid glycoprotein become the most abundant hepatic mRNA species following tissue injury (Ricca et al., 1981). Low concentrations of drugs inhibit tracer incorporation into RNA and into α_1-acid glycoprotein secreted from inflamed, more so than control, liver slices (this thesis). Also, it may be important that the mRNA transcripts for α_1-acid glycoprotein are relatively short-lived and exhibit half-lives of 2-4 h (Miller & Griffin, 1975). Taken together, these observations lead to the conclusion that the increased labelling of liver slice RNA with [^{3}H]-orotate in slices prepared from inflamed,

as compared to control, rats reflects, at least in part, increased synthesis of mRNA transcripts for acute phase reactants.

4.3 Effects of Drugs on Liver Slices

Studies presented in this thesis and by Jamieson & Kutryk (1980) demonstrate that the drugs indomethacin, sulfinpyrazone, phenylbutazone and salicylate inhibited protein synthesis and RNA synthesis in liver slices from control and experimentally inflamed rats. The transcription process, and the translation of the acute phase reactant α_1-acid glycoprotein in liver slices from inflamed rats, was more sensitive to the effects of the drugs than the synthesis of proteins in liver slices from control rats. The reasons for these differential effects of the drugs are not obvious from the studies presented here, although some insight can be obtained from studies reported by others.

Two possible explanations have been offered for the effects of these drugs on liver slices: a direct effect on hepatic biosynthesis of glycoproteins, and an indirect effect on the energy-generating processes within the cell (Jamieson & Kutryk, 1980). Dawkins et al. (1966) have shown that concentrations of 0.1-20 mM of salicylate inhibits incorporation of radiolabelled leucine into protein by rat liver cell-free systems; these results indicate a direct effect on the translation process of the liver. Also, phenylbutazone has been shown to have direct effects on protein synthesis in rabbit reticulocytes (Mainzer, 1968). The exact mechanisms of inhibition by these drugs was not identified in the above studies. In addition, concentrations above 2 mM salicylate uncouple oxidative phosphorylation

reactions in respiring mitochondria (Brody, 1956); these results indicate a direct effect on the energy-generating process of the cell. Results presented in this thesis show that the drugs also inhibited RNA synthesis in rat liver slices. While these studies did not further delineate the mechanisms of action of the drugs, it is likely that the inhibitory effects are at a number of levels: transcription, translation, glycosylation of proteins and the generation of energy via oxidative phosphorylation. The actual mechanism could vary with the drugs studied and with different concentrations of a drug.

The biosynthesis of the acute phase reactant α_1-acid glycoprotein by liver slices appears to be dependent upon the synthesis of RNA, this may be the result of high levels and short lifetimes of mRNA transcripts for this protein (see preceding Section). This is an important observation for the interpretation of results presented in this thesis. Liver slices were used as an experimental system in this work to test the effects of physiological regulators on the liver; alterations in RNA synthesis occur earlier after an inflammatory stimulus than other changes, such as those seen in acute phase reactant biosynthesis, and as such, RNA synthesis may be more sensitive than other parameters to the effects of physiological regulators in the short-lived liver slices (see preceding Section); de novo synthesis of acute phase reactants, such as α_1-acid glycoprotein, at elevated rates following inflammation is dependent upon elevated rates of transcription (Thompson & Wannemacher, 1973; Sipe, 1978; Ricca et al., 1981; McAdam et al., 1982).

4.4 Physiologic Regulation of Liver Slice Activity

A number of effects of various agents on the activity of liver slices have been identified in this thesis. Some of these effects may be the result of the conditions of the liver slice experiments, while others may reflect *in vivo* regulation of activity.

While the medium used in the liver slice experiments is a relatively simple defined medium, it does contain a mixture of nineteen of the translatable amino acids at concentrations twice that normally found in serum. Clemens and Korner (1970) have reported that the incorporation of radiolabelled leucine into liver slice protein was stimulated by amino acid concentrations 1-6 times that normally present in serum, while orotate incorporation into RNA was reduced at concentrations 2-12 times that normally present. Miller and Griffin (1975) have found that the biosynthesis of specific proteins by isolated perfused liver responds differently to hormones depending on the availability of amino acids. Seglon *et al*. (1980) have investigated in some detail the stimulation of protein labelling in cultured hepatocytes by amino acid concentrations 1-12 times that normally present in serum. They have found that hepatocyte protein synthesis is not affect by the amino acids cit, glu, asp, met, ile and leu; it is stimulated by the amino acids ala, ser, gly, pro and thr; and it is inhibited by the amino acids cys, arg, orn, trp and lys. The amino acids gln, asn, his, phe and tyr exhibited biphasic effects; they were stimulatory at moderate concentrations (generally below 15 nmol/ml), but were inhibitory at higher concentrations. The stimulatory effects of amino acids by themselves probably reflect their roles as energy

substrates, as the stimulatory effects were not seen with optimal concentrations of known energy substrates such as glucose (20 mM) or pyruvate. Complete amino acid mixtures stimulate protein synthesis even in the presence of optimum concentrations of energy substrates, which suggests that these effects may be due to the function of amino acids as precursors of proteins. This suggestion is strengthened by the observation that propylamine or leupeptin, both of which inhibit the endogenous production of free amino acids by lysosomal proteolysis, reduced protein synthesis and enhanced the stimulatory effects of exogenous complete amino acid mixtures in the presence of optimum concentrations of energy substrates. Seglon et al. (1980) did not offer an explanation of the inhibitory effects of some of the amino acids, such as ornithine, on protein biosynthesis. While the results of these experiments with hepatocyte cultures may not be compared directly to results of experiments with liver slices, they do illustrate several points common to both systems. Isolated liver cells use exogenous amino acids as a source both of energy and of precursors for translation. Exogenous amino acids also exert effects on isolated liver cells which cannot be explained on the basis of these two functions.

In the experiments using liver slices which are presented in this thesis, the non-translatable amino acid taurine had no effect on glycoprotein biosynthesis, while ornithine supplementation inhibited glycoprotein biosynthesis in liver slices from control rats, and was stimulatory with liver slices from inflamed rats. Ornithine also stimulated RNA synthesis in liver slices from inflamed, but not control, rats. While the inhibition of protein synthesis in control liver slices

is similar to the effects of ornithine on protein synthesis in liver cell culture (Seglon et al., 1980), the stimulatory effects of ornithine are difficult to explain. It is unlikely that ornithine is acting as an energy source since the liver slice medium contains an adequate supply of glucose and amino acids to meet that need. Also, ornithine is not capable of being incorporated into protein during the translation process. Ornithine is an intermediate in urea biosynthesis, and also is a precursor for the polyamines putrescine, spermidine and spermine (Pegg et al., 1982). A key step in the pathway of biosynthesis of polyamines is the formation of putrescine from ornithine by the action of the enzyme ornithine decarboxylase (Raina & Janne, 1970; Kuehn & Atmur, 1982; Pegg et al., 1982; Tabor et al., 1982). During the acute phase response there are elevated hepatic levels of ornithine decarboxylase and polyamines (Raina & Janne, 1970). The polyamines are essential growth factors for certain microorganisms, they have effects on various steps of DNA, RNA and protein synthesis and they are present at elevated levels in proliferating and differentiating cells (Raina & Janne, 1970; Oka et al., 1982; Tabor et al., 1982). At least some of these effects are controlled through the action of a polyamine-dependent protein kinase (Kuehn & Atmur, 1982; Morishita et al., 1983a, 1983b). The effects of ornithine reported in this thesis may be reflecting aspects of polyamine metabolism. Rat liver slices are able to metabolize putrescine for at least 5 h of incubation (Lundgren & Hankins, 1978). A polyamine-dependent Chinese hamster ovary cell line, which is deficient in the arginase catalyzed synthesis of ornithine, can be maintained in serum-free medium containing the polyamines putrescine, spermidine

and spermine, or ornithine itself (Holta & Pohyanelto, 1982). These observations suggest that liver slices from inflamed rats undergo an accelerated synthesis of polyamines due, at least in part, to elevated ornithine decarboxylase activity. Endogenous pools of ornithine in liver slices may be depleted by the action of this enzyme. This assumption is strengthened by the observation that serum and liver from inflamed rats both show depleted pools of ornithine at later times after inflammation. Thus, the effects of ornithine on liver slice activity may be reflecting a dependence of liver slices on an exogenous supply of ornithine to meet the needs for an accelerated biosynthesis of the polyamines.

Cortisol supplementation of liver slice medium had two effects: low concentrations (40-170 nM) stimulated RNA synthesis and had little or no effect on the release of sialyltransferase activity into the medium, while higher concentrations (280-330 nM) resulted in an inhibition of both RNA synthesis and sialyltransferase release from liver slices. Others have reported effects of cortisol on liver slice activity, although the studies may not be comparable to the work presented here. Molasky (1976) reported a slight increase in medium protein synthesis with cortisol supplementation, although the concentration used (27,000 nM) was not within the physiological range. Liberti et al. (1971) reported that supplementation of unperfused liver slices with 100 nM cortisol had no effect on protein and RNA synthesis, although serum supplementation and the loss of RNA synthesis after 2 h may mean that these studies are not comparable to the ones presented in this thesis. The effects reported here are similar, though,

to those of Ritchie and Fuller (1981), who reported that adult rat hepatocytes cultured in serum-free medium exhibited elevated rates of fibrinogen synthesis and secretion with concentrations of 1-10 nM of the glucocorticoid dexamethasone, and depressed rates of fibrinogen synthesis and secretion with concentrations of 100 nM dexamethasone. Tsukada et al. (1979) have reported that concentrations of 5-500 nM dexamethasone stimulated secretion of α-fetoprotein from hepatoma cells cultured in serum-free medium without having effects on albumin secretion. Also, Baumann et al. (1983) have reported that concentrations of 11 nM, or greater, of dexamethasone elevated mRNA transcripts for α_1-acid glycoprotein in hepatoma cells cultured in serum-supplemented medium. While the presence of serum may complicate interpretations of direct effects of agents, such as the glucocorticoids, the results presented in this thesis, as well as by others, indicate that glucocorticoids have direct effects on liver cells at physiological concentrations (200-700 nM), such that parameters characteristic of the acute phase response are altered.

A major target for the glucocorticoids is the hepatocyte, and these hormones have a number of physiological effects on these cells (Litwack & Singer, 1972; Cake & Litwack, 1975; Szego & Pietras, 1981; Roy, 1983; Roy et al., 1983). One effect is the rapid stimulation of polyamine synthesis from ornithine due to activation of the enzyme ornithine decarboxylase (Cake & Litwack, 1975). This observation would offer an explanation for the differences observed in this thesis between the responses to ornithine supplementation of liver slices from control and inflamed rats. Elevated levels of hepatic ornithine

decarboxylase activity during the acute phase response (Raina & Janne, 1970) could result from elevated serum cortisol levels (this thesis). Ornithine supplementation may be necessary to maintain polyamine synthesis in liver slices from inflamed rats which have cortisol-stimulated ornithine decarboxylase activities. Cortisol action on hepatic ornithine decarboxylase activity may also explain some of the synergistic effects of cortisol and ornithine on control liver slices which were observed in this thesis. Also, supplementation with arginine, a precursor to ornithine, or ornithine is necessary in the culture medium which best maintains hormone responsiveness of protein synthesis in isolated rat hepatocytes (Seglon et al., 1980). Ritchie and Fuller used ornithine in culture medium in which the effects of dexamethasone on rat hepatocyte fibrinogen synthesis were examined. Some of the effects of glucocorticoids on hepatic parameters of the acute phase response may be mediated via effects on polyamine metabolism.

While cytokine supplementation had little or no effect on protein synthesis and glycosylation in liver slices, it did depress the levels of sialyltransferase activities released into medium. Kampschmidt and Upchurch (1974) have reported that addition of 100 MCE of rabbit cytokine to medium had no effect of the synthesis of fibrinogen by rat liver slices during a 2 h incubation. This choice of conditions may be important as the results presented in this thesis indicate that following 2 h of exposure to 30 MCE of rat cytokine, liver slices from control rats exhibit slightly elevated rates of RNA synthesis, while 4 h of incubation with 30 MCE of rat cytokine depresses RNA synthesis. The effects of cytokine on liver slice activity may

depend on time of incubation. While addition of cytokine to medium of control liver slices did not cause the liver slices to mimic the acute phase response, cytokines have been shown to stimulate acute phase reactant biosynthesis in hepatocyte cultures (Rupp & Fuller, 1979; Fuller & Ritchie, 1982; McAdam et al., 1982; Ritchie & Fuller, 1983; Tatsuda et al., 1983). While it is not obvious why liver slices do not respond in the same manner, these differences may be related to time of exposure of liver cells to cytokine or to the synergistic effects of medium components on the action of cytokine (Kampschmidt et al., 1982).

In vivo studies indicated that, in addition to a heat-labile factor that may well be analogous to the interleukin-1 of other species, there appeared to be a heat-stable factor which depressed hepatic hexosaminidase activity. The inhibition of liver slice RNA synthesis provides further evidence for a heat-stable cytokine in these preparations which exerts effects on liver cell activity. As previously mentioned, Ritchie and Fuller (1983) have isolated a monokine which stimulates fibrinogen production by hepatocytes, and which is distinct from interleukin-1. Also, Tatsuda et al. (1983) have demonstrated that while purified murine interleukin-1 stimulates synthesis of the acute phase reactant serum amyloid A by cultured hepatocytes, it does not affect the synthesis of the acute phase reactant serum amyloid P; macrophage culture supernatants, which contain a heterologous mixture of monokines, stimulated hepatic synthesis of both serum amyloid A and serum amyloid P. These observations indicated that there are more than one molecular species of cytokine that can affect acute phase

reactant synthesis and other parameters of the acute phase response.

There appeared to be synergistic effects of the action of cytokine and cortisol on the synthesis of RNA by liver slices. Cytokine alone inhibits liver slice RNA synthesis, whereas addition of cytokine along with cortisol enhances RNA synthesis in liver slices, particularly at concentrations of cortisol which inhibit RNA synthesis. These results are similar to the effects reported by Thompson et al. (1976) on isolated perfused rat livers; while rabbit cytokine had no direct effect by itself on hepatic RNA synthesis, and cortisol inhibited the synthesis of nuclear RNA, cytokine and cortisol together stimulated the synthesis of bound ribosomal RNA. Also, cortisol is a requirement in serum-free medium for cultured hepatocytes which respond to monokines with elevated fibrinogen synthesis (Ritchie & Fuller, 1981, 1983; Fuller & Ritchie, 1982), and serum, which would contain cortisol, is a culture medium requirement for cultured hepatocytes to respond to monokines with serum amyloid A and serum amyloid P synthesis (McAdam et al., 1982; Tatsuda et al., 1983). A synergism also exists in the effect of cytokine and cortisol on sialyltransferase activity released from liver slices; cytokine inhibits medium sialyltransferase at low concentrations of cortisol and elevates medium sialyltransferase at high cortisol concentrations. The above effects are obviously not simple, and an understanding of the effects of cortisol, and other normal serum components, on the biochemistry and physiology of liver cells is necessary for the understanding of the role of cytokines in regulating hepatic aspects of the acute phase response.

The studies presented in this thesis have indicated that a number

of agents have direct effects on liver cells to alter parameters, such as release of sialyltransferase and enhanced RNA synthesis, which are characteristic of the acute phase response. The effects are not simple and no single agent examined in the work presented in this thesis will elicit a full acute phase-like behaviour from liver slices of control rats. Clearly, an in-depth understanding of the metabolism of isolated liver cells is necessary for a full understanding of the regulation of hepatic aspects of the acute phase response to inflammation.

5. Regulation of Hepatic Aspects of the Acute Phase Response

At early times following onset of the acute phase response, the liver is quite distal to activity which is confined primarily to the site of tissue injury and inflammation; at later times of the acute phase response, many changes have occurred such that body temperature is elevated, and the humoral environment, the nutritional status of the organism, and metabolic activity of the liver are all altered (Beisel, 1975, 1980; Cooper & Stone, 1979; Kushner, 1982). An understanding of the processes by which these changes occur must begin with an understanding of the early events which occur at the site of tissue injury.

Many alterations occur at the site of tissue injury at very early times of inflammation (see Introduction); the one most pertinent to this discussion is the production of cytokine(s) there. Following activation of leukocytes at the site of injury (see Introduction), cytokines are produced which play a role in mediation of the acute

phase response. It is not clear whether cytokine activity is produced exclusively at the site of inflammation, since monokines produced by Kupffer cells are also capable of stimulating acute phase reactant synthesis (Fuller & Ritchie, 1982). Regardless of Kupffer cell production of monokines, cytokines produced at the site of tissue injury must enter the circulatory system to exert the generalized effects they have. It would appear that lymphatic drainage of the site of inflammation is important to transmission of the "message" from the site (Poulter & Pandolph, 1982; Issekutz et al., 1982) and presumably this is the mechanism by which cytokines are delivered to the cardiovascular system. It would seem important that cytokines actually enter the blood stream in view of the observation by Keenan et al. (1982) that critically ill patients with low serum levels of LEM often do not survive (71% of patients studied died) while most (88%) critically ill patients with high serum levels of LEM do survive. This would suggest that the production of cytokine and its delivery to the blood stream represents an important aspect of the host's response to stress.

Once cytokines produced in response to tissue injury have entered the blood stream, they are involved in many aspects of the generalized response to inflammation. These include fever induced via action of cytokine on the hypothalamus (Rafter et al., 1966; Merriman et al., 1977; Atkins & Bodel, 1979; Dinarello, 1979; Bornstein, 1982), secretion of glucagon and insulin by the pancreas (George et al., 1977) and alteration of serum glucocorticoid and thyroid hormone levels (this thesis). While experimental evidence has led to the suggestion

that cytokine may also act on neurohumoral pathways (Bailey et al., 1976; Kushner & Feldman, 1978; Turchik & Bornstein, 1980; Kampschmidt et al., 1982; this thesis), this aspect of cytokine action remains to be uniquivocally demonstrated. Still, the action of cytokine to produce a generalized response to inflammation in many ways changes the environment in which the liver functions.

The hepatic response to tissue injury is constantly changing throughout the period following an inflammatory challenge. Among the earlier changes to be observed are alterations in pools sizes of amino acids (this thesis) and nucleotide sugars (Kaplan et al., 1983a). These changes reflect altered metabolic activity (Beisel, 1975, 1980; Goldberg, 1980; Shuttler et al., 1980; Kushner, 1982; Kaplan et al., 1983a) and hepatic transport of amino acids (Beisel, 1975, 1980; Wannemacher et al., 1975; Kushner, 1982), and serve, in part, to meet elevated requirements for glucose necessitated by fever (Beisel, 1980). Cytokines mediate hepatic transport of amino acids (Wannemacher et al., 1975) and may be involved in other aspects of this metabolic response. Glucocorticoids, insulin, catecholamines and thyroid hormones are also involved in this part of the hepatic response to acute inflammation (see Section 2.2 of this Discussion).

Hepatic transcriptional activity is altered at early times of the acute phase response (this thesis). This may be initiated by alterations in nuclear protein kinase (Laks et al., 1981). These changes effect rRNA levels (Chandler & Neuhaus, 1968; Thompson & Wannemacher, 1973; Woloski, 1980), and mRNA content of acute phase reactants (Sipe, 1978; Haugen et al., 1981; Ricca et al., 1981; McAdam et al., 1982). Cytokines, glucocorticoids and polyamines are

involved in regulating hepatic transcription (Raina & Janne, 1970; Wannemacher et al., 1975; this thesis). These changes are particularly important as the altered rates of translation of plasma proteins during the acute phase response are probably controlled at the level of the mRNA transcripts for the proteins (Sipe, 1978; Haugen et al., 1981; Ricca et al., 1981; McAdam et al., 1982). Many studies are now underway to investigate this aspect of the acute phase response and cytokine mediation of the process (A.M. Chandler, G.M. Fuller, I.Kushner, J.D.Sipe, J.M. Taylor, personal communications).

Hepatic translation of acute phase reactants is elevated while lower levels of negative acute phase reactants are translated following inflammation (see Introduction). Elevated hepatic levels of amino acids and available energy may contribute to this enhanced biosynthetic capacity (Beisel, 1980; Kushner, 1982; this thesis). Monokines have been shown to be involved in elevated acute phase reactant biosynthesis and depressed synthesis of negative acute phase reactants (Eddington et al., 1972; Kampschmidt & Upchurch, 1974; Pekarek et al., 1974; Wannemacher et al., 1975; Bornstein, 1982; Simon & Willoughby, 1982) via direct effects on hepatocytes (Rupp & Fuller, 1979; Fuller & Ritchie, 1982; McAdam et al., 1982; Ritchie & Fuller, 1983; Tatsuda et al., 1983), although it is not clear whether this results from effects on levels of mRNA transcripts, or actual effects on the translation apparatus. Cortisol appears to be involved with cytokines in a synergistic action on hepatic acute phase reactant synthesis (Ritchie & Fuller, 1981, 1983; Fuller & Ritchie, 1982;

Kampschmidt et al., 1982; McAdam et al., 1982; Tatsuda et al., 1983). However, cytokine(s) may not be involved in the induction of synthesis of the hepatic protein metallothionein during the acute phase response by direct effects on the liver; rather glucocorticoids and glucagon induce the synthesis of this protein (Sobocinski & Canterbury, 1982). Cytokine effects on hepatic metallothionein synthesis may be via alterations in glucagon levels (George et al., 1977) and cortisol levels (this thesis). A great deal of work remains to be done to elucidate fully the mechanisms by which cytokines induce acute phase reactant synthesis.

As many acute phase reactants are glycoproteins (see Introduction), elevated glycosylation of proteins represents one aspect of enhanced acute phase reactant biosynthesis (Bley et al., 1973; Lombart et al., 1980; Coolbear & Mookerjea, 1981; Kushner, 1982; Jamieson et al., 1983; Kaplan et al., 1983a, 1983b). Control of alterations in glycosylation following inflammation has not been studied in as much detail as other aspects of altered liver function during the acute phase response. Glycosylation and translation of acute phase reactants do not seem to be tightly coupled as addition of carbohydrate to α_1-acid glycoprotein, α_2-macroglobulin and fibrinogen apparently occurs as post-ribosomal events (Jamieson, 1977; Nickerson & Fuller, 1981a; this thesis). It is known that the rates of glycosylation during the acute phase response are controlled at the level of available dolichol phosphate, as the dolichol cycle becomes rate limiting to glycosylation during inflammation (Coolbear & Mookerjea, 1981). Therefore, regulation of the enzyme CTP-dependent dolichol kinase must

be important to the rate of glycosylation during the acute phase response, although, as yet, factors important in the control of this enzyme have not been studied. As previously mentioned, synthesis and levels of the nucleotide sugars, which serve as precursors for the carbohydrate moieties of glycoproteins, are part of the metabolic alterations which are controlled by cytokines and hormones such as cortisol, insulin, catecholamines and thyroid hormones. Also, cytokines and cortisol appear to regulate the activity of the enzyme sialyl-transferase (this thesis), which may reflect a more general control of later aspects of glycoprotein synthesis. The importance of glycosylation and the control of these reactions during the acute phase response remain to be fully delineated, and undoubtedly will be the subject of many future studies.

The last stage of biosynthesis of plasma proteins is the secretion of these proteins from hepatocytes (see Introduction). Several ultrastructural changes of hepatocytes such as increased synthesis and formation of microtubules, increased dilation and vesiculation of the endoplasmic reticulum, increased amounts of smooth endoplasmic reticulum and increased synthesis of plasma membranes have been reported during the acute phase response (Turchen et al., 1977; Lombart et al., 1980; Kushner, 1982). These changes are consistent with elevated levels of secretion of acute phase reactants (Kushner, 1982). Corticotropin and glucocorticoids regulate alterations of the endoplasmic reticulum of liver cells following stress (Lando et al., 1980); it remains to be seen if cytokines are also involved in this aspect of the acute phase response.

The hepatic response to stress is complex and regulated at a number of levels by numerous physiologically active agents. The mechanisms by which this regulation occurs largely remain to be delineated. Of those agents involved in regulation of the acute phase response, cytokines produced by activated monocytes appear to be critically important.

6. Suggestions for Future Studies

The work presented here suggests possible studies, related to this work, that went beyond the scope of this thesis.

A number of experiments examining in vivo effects of cytokine administration could be performed. Experimental manipulation of animals can produce alterations in nutritional and endocrine status of animals; examination of the effects of cytokine in these animals may provide insight into the mechanisms of cytokine action.

As previously mentioned, interpretation of the results of experiments on the effects of cytokines are complicated by the heterogenous nature of the preparations. The use of purified cytokine preparations would solve some of these problems, however, the protocol used in this thesis for preparation of cytokine would be unsuitable for the preparation of large amounts of cytokine which would be needed for these purifications. The use of cultured cells as a continuous source of cytokine would avoid problems assocated with the use of peritoneal exudate cells as a source of cytokine. Thus, the establishment of a permanent leukocyte cell line in culture conditions optimum for cytokine production would be beneficial to studies further characterizing

the physicochemical properties of the cytokine(s), and examining the effects of purified cytokine(s).

The results of experiments examining the effects of cytokine(s) on liver slices led to the conclusion that cytokine(s) have direct effects on liver slices, however, these results did not clearly identify the *in vivo* mechanism(s) of cytokine action. Studies aimed at identifying the optimum conditions of liver slice incubations for *in vitro* effects of cytokine(s) may facilitate this extrapolation. Also, different liver slice incubation conditions may result in a greater sensitivity of the liver slices to cytokine supplementation; this would be beneficial to studies aimed at purifying the active components of cytokine preparations by providing a more sensitive bioassay of the cytokine.

Undoubtedly, some or all of these experiments will be attempted in the future, and will add to our understanding of the control of hepatic aspects of the acute phase response.

APPENDICES

Appendix A - Composition of Stock Amino Acid Mixture

Amino Acid	Concentration (mM)
Arg	22
Asp	3.8
Thr	29
Ser	29
Glu	19
Gly	43
Ala	48
Val	20
Met	7.0
Ile	9.0
Leu	17
Tyr	9.0
Phe	8.0
Pro	24
Cys	3.8
Gln	19
Trp	6.9
His	9.0
Lys	48

Appendix B - Radioimmunoassay Calculation

The program which will be described is based on the log-logit model of Chang et al. (1975) for fitting competitive binding assay data.

In this model, the response variable Y is defined by

$$Y = \frac{B_o - \bar{N}}{\bar{B}_o - \bar{N}} \qquad (1)$$

where B_o is the number of bound d.p.m. in the presence of sample or standard ligand, $\bar{B}_o$ is the mean number of bound d.p.m. in the absence of unlabelled ligand, $\bar{N}$ is the number of non-specific d.p.m. in the absence of antibody or binding protein.

I is defined as the logit of the response variable

$$I = \text{logit}(Y) - \ln \frac{Y}{1 - Y} \qquad (2)$$

and z is defined as the logarithm of the concentration (c) of unlabelled ligand

$$z = \ln (c) \qquad (3)$$

The method uses an iterative weighted least squares procedure to fit a dose-response regression line to the standard data; the line is of the form

$$I = a + bz \qquad (4)$$

The response variable and the function I are determined from the radioactivity bound by samples, and the concentration of unlabelled ligand determined from the equation

$$c = e^{(I-a)/b} \qquad (5)$$

Values for a and b are determined from standard data by an iterative weighted least squares procedure as follows

$$\bar{N} = \frac{\Sigma N}{n} \tag{6}$$

where N is the background d.p.m. in the absence of antibody or binding protein, n is the number of samples, and $\bar{N}$ is the mean background d.p.m.

$$\sigma(\bar{N}) = \frac{\Sigma N^2 - (\Sigma N)^2/n}{n - 1} \tag{7}$$

where $\sigma(\bar{N})$ is the variance in $\bar{N}$

$$\bar{B}_o = \frac{\Sigma B_o}{n} \tag{8}$$

$$\sigma(\bar{B}_o) = \frac{\Sigma B_o^2 - (\Sigma B_o)^2/n}{n - 1} \tag{9}$$

where B_o is the d.p.m. bound in the absence of unlabelled ligand, $\bar{B}_o$ is the mean d.p.m. in the absence of unlabelled ligand, and $\sigma(\bar{B}_o)$ is the variance in $\bar{B}_o$.

The values described above are used to determine the variance in the response variable ($\sigma(Y)$) by the following approximations:

$$\sigma(Y) = a_0 + a_1 Y \tag{10}$$

where

$$a_0 = \frac{\sigma(\bar{N})}{(\bar{B}_o - \bar{N})^2} \tag{11}$$

and

$$a_1 = \frac{\sigma(\bar{B}_o)}{(\bar{B}_o - \bar{N})^2} - a_0 \tag{12}$$

The variance in the logit function of the response variable ($\sigma(I)$) is calculated from

$$\sigma(I) = \frac{(Y)}{Y^2(1 - Y^2)} \tag{13}$$

The variance in the I function for each concentration of unlabelled ligand is used to determine the weight which will be assigned to each point

$$w = 1/\sigma(I) \tag{14}$$

Using these weights for each point, the weighted means of z and I are determined from

$$\bar{z} = \Sigma wz/\Sigma w \tag{15}$$

$$\bar{I} = \Sigma wI/\Sigma I \tag{16}$$

Values for a and b are determined from the equations

$$b = \frac{\Sigma w(z - \bar{z})I}{\Sigma w(z - \bar{z})^2} \tag{17}$$

and $$a = \bar{I} - b\bar{z} \tag{18}$$

The variances in these parameters are determined from the equations

$$\sigma(\bar{I}) = 1/r\Sigma w \tag{19}$$

$$\sigma(b) = 1/[r\Sigma w(z - \bar{z})^2] \tag{20}$$

$$\sigma(a) = \sigma(I) + \bar{z}^2\sigma(b) \tag{21}$$

where r is the number of replicates at each concentration of unlabelled ligand. Standard deviations of $\bar{I}$, b and a are given by taking the square roots of the variances given in equations 19-21.

Use of the Program

The program listing which follows is for use with a Hewlett-Packard 41-C Progammable Calculator. This program utilized 86 registers for program memory and 21 registers for data storage. Memory must be allocated by executing SIZE 021 on the calculator. The program is entered into the calculator either from magnetic cards using a card reader (HP 82104A), or is keyed in manually. If a card reader is used, step 1 of the program use is not necessary as program execution begins automatically. Do not attempt to enter data while program is being executed; PGRM is visible in lower right hand corner of the display while program is being executed.

Notes on program use:

1. Press RTN, then R/S to begin program execution."BG,A=DONE" is shown in the display, and then "BG1".
2. Key in the first value for N (radioactivity in the absence of antibody or binding protein) and press R/S. Continue entering values when cued by "BG X" (where X is the replicate number) until all N data are entered, then press A. "BLANK,B=DONE" is shown in the display, and then "BLANK 1".
3. Key in the first value for B_o (radioactivity in the absence of unlabelled ligand) and press R/S. Continue entering data when cued until all B_o data are entered, and then press B. "STD DATA" is shown in the display, then "C=DONE", and then "DPMAV 1".
4. Key in the first value of B (average radioactivity bound in the presence of unlabelled ligand) for the standard data, press R/S

and "CONC 1" is shown in the display. Enter the appropriate concentration of unlabelled ligand and press R/S. Continue entering data for B and concentration as cued. When all standard data are entered, press C, "D=DONE" will be shown in the display, then "DPMAV 1".

5. Reenter standard data as cued above. When all data are entered, press D. "REP NUM?" will be shown in the display.
6. Key in the number of replicates for the standard points and press R/S.
7. "B=" will be shown in the display with a value for the constant b described earlier. Press R/S.
8. "SDEV B=" will be shown in the display with a value for the standard deviation in the value for the constant b. Press R/S.
9. "SDEV I=" will be shown in the display with a value for the standard deviation in the function $\overline{I}$ described earlier. Press R/S.
10. "A=" will be shown in the display with a value for the constant a described earlier. Press R/S.
11. "SDEV A=" will be shown in the display with a value for the standard deviation in the constant a. Press R/S.
12. "SAMP DPM" will be shown in the display. Key in the value for B (radioactivity bound) for a sample of unknown concentration. Press R/S.
13. "CONC=" will be shown in the display with a value for the concentration of unlabelled ligand in the sample. Press R/S.
14. Repeat steps 12 and 13 for data from another sample.

15. Repeat steps 1-14 if another set of standard data is to be used.

16. Once standard data are entered (steps 1-6), calculator can be shut off. When calculator is turned on again, press GTO SAMP and then R/S. Perform steps 12 and 13. Calculator will remember data from curve fitting if no values are entered into memory registers 6-11 or 15-16.

Program Listing

Below is a listing of the program steps for the program. Expressions which are underlined are entered in the alpha mode of the calculator.

	STEP		STEP		STEP		STEP
001	LBL RIA	031	1	061	1	091	1
002	CLRG	032	-	062	-	092	ST+ 00
003	ΣREG 00	033	/	063	/	093	RCL 00
004	SF 27	034	STO 07	064	STO 09	094	DPMAV
005	FIX 0	035	CLΣ	065	CLΣ	095	ARCL X
006	BG,A=DONE	036	BLANK,B=DONE	066	STD DATA	096	AVIEW
007	AVIEW	037	AVIEW	067	AVIEW	097	STOP
008	PSE	038	PSE	068	PSE	098	XEQ I
009	PSE	039	PSE	069	PSE	099	ST+ 01
010	LBL 00	040	LBL 01	070	C=DONE	100	STO 10
011	RCL 05	041	RCL 05	071	AVIEW	101	RCL 11
012	1	042	1	072	PSE	102	*
013	+	043	+	073	PSE	103	ST+ 02
014	BG	044	BLANK	074	RCL 07	104	CLA
015	ARCL X	045	ARCL X	075	RCL 08	105	RCL 00
016	AVIEW	046	AVIEW	076	RCL 06	106	CONC
017	STOP	047	STOP	077	-	107	ARCL X
018	Σ+	048	Σ+	078	x^2	108	AVIEW
019	CLA	049	CLA	079	/	109	STOP
020	GTO 00	050	GTO 01	080	STO 07	110	LN
021	LBL A	051	LBL B	081	CHS	111	RCL 10
022	MEAN	052	MEAN	082	RCL 07	112	*
023	STO 06	053	STO 08	083	RCL 08	113	ST+ 03
024	RCL 01	054	RCL 01	084	RCL 06	114	GTO 02
025	RCL 00	055	RCL 00	085	-	115	LBL C
026	x^2	056	x^2	086	x^2	116	RCL 02
027	RCL 05	057	RCL 05	087	/	117	RCL 01
028	/	058	/	088	+	118	/
029	-	059	-	089	STO 09	119	STO 12
030	RCL 05	060	RCL 05	090	LBL 02	120	RCL 03

	STEP		STEP		STEP		STEP
121	RCL 01	171	RCL 02	221	SDEV A=	271	RTN
122	/	172	/	222	ARCL X		
123	STO 13	173	STO 15	223	AVIEW		
124	RCO 01	174	CLA	224	STOP		
125	STO 14	175	B=	225	LBL SAMP		
126	CLΣ	176	ARCL X	226	SAMP DPM		
127	CLA	177	AVIEW	227	PROMPT		
128	D=DONE	178	STOP	228	XEQ I		
129	AVIEW	179	CLA	229	RCL 11		
130	PSE	180	RCL 00	230	RCL 16		
131	PSE	181	RCL 02	231	-		
132	CLΣ	182	*	232	RCL 15		
133	LBL 03	183	1/x	233	$/_x$		
134	1	184	STO 18	234	e^x		
135	ST+ 00	185	SQRT	235	CLA		
136	RCL 00	186	SDEV B=	236	CONC=		
137	DPMAV	187	ARCL X	237	ARCL X		
138	ARCL X	188	AVIEW	238	AVIEW		
139	AVIEW	189	STOP	239	STOP		
140	STOP	190	CLA	240	GTO SAMP		
141	CLA	191	RCL 00	241	LBL I		
142	XEQ I	192	RCL 00	242	RCL 06		
143	STO 03	193	RCL 14	243	-		
144	RCL 00	194	1/x	244	RCL 08		
145	CLA	195	STO 17	245	RCL 06		
146	CONC	196	SQRT	246	-		
147	ARCL X	197	SDEV I=	247	/		
148	AVIEW	198	ARCL X	248	STO 10		
149	STOP	199	AVIEW	249	ENTER↑		
150	LN	200	STOP	250	ENTER↑		
151	RCL 13	201	CLA	251	1		
152	-	202	RCL 12	252	-		
153	STO 04	203	RCL 15	253	CHS		
154	x^2	204	RCL 13	254	1		
155	RCL 03	205	*	255	LN		
156	*	206	-	256	STO 11		
157	ST+ 02	207	STO 16	257	RCL 10		
158	RCL 04	208	A=	258	RCL 09		
159	RCL 11	209	ARCL X	259	*		
160	*	210	AVIEW	260	RCL 07		
161	RCL 03	211	STOP	261	+		
162	*	212	CLA	262	RCL 10		
163	ST+ 01	213	RCL 17	263	x^2		
164	GTO 03	214	RCL 13	264	/		
165	LBL D	215	x^2	265	1		
166	CLA	216	RCL 18	266	RCL 10		
167	REP NUM?	217	*	267	-		
168	PROMPT	218	+	268	x^2		
169	STO 00	219	SQRT	269	/		
170	RCL 01	220	CLA	270	1/x		

Appendix C - Comparison of Amino Acid Composition of α_1-Macroglobulin isolated in this Thesis and α-Macroglobulins of Gauthier and Mouray (1976)

Amino Acid	moles/100 moles		
	This Thesis	α_1-Macroglobulin	α_2-Macroglobulin
Asx	9.81	9.63	8.67
Thr	6.03	6.72	6.97
Ser	8.18	7.55	7.28
Glx	12.67	12.32	12.32
Pro	5.80	5.16	5.02
Gly	5.74	6.10	6.46
Ala	6.09	6.83	7.12
Val	7.45	8.29	8.67
Met	1.80	1.92	2.06
Ile	4.99	5.18	4.33
Leu	10.44	9.11	11.05
Tyr	4.26	3.85	3.40
Phe	4.09	4.13	4.46
His	2.65	2.14	1.58
Lys	6.05	6.22	5.69
Arg	2.86	3.09	3.63
Trp	1.09	-	-

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