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THE EFFECT OF FEEDING AND STARVATION ON PROTEIN TURNOVER IN THE LIVER AND THE BREAST MUSCLE OF JAPANESE QUAIL

by

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ABSTRACT

The half-lives of the soluble and insoluble proteins from liver and breast muscle and total plasma protein were measured in laying female and 52-wk-old male quail and compared to the same proteins from mature 16-wk-old male birds. The female birds showed faster turnover of liver and plasma proteins and negligible turnover of muscle proteins. This was thought to be the response of the female birds to the high metabolic demands for egg laying. There were no significant differences ($P<0.01$) in protein turnover between tissues from old and young male birds.

Proteins from the livers of fed and starved birds were purified and the half-lives were calculated using a single pulse injection of ^{14}C-leucine. The fed birds had the shortest half-life for aldolase at 2.2 days followed by the extract, breakthrough peak and 3 M salt peak at 3 days and fructose-1,6-diphosphatase at 4.1 days. The starved birds had the shortest half-life associated with the salt peak at 3 days, followed by aldolase at 4 days and fructose-1,6-diphosphatase, extract and breakthrough peak at 5.7 days. Glyceraldehyde-3-phosphate dehydrogenase appeared to turnover very slowly so that the rate was not readily measurable. The half-lives of the muscle proteins, extract, aldolase and glyceraldehyde-3-phosphate dehydrogenase were measured, however, the turnover was slow enough that it could only be demonstrated graphically. All measurements showed that muscle proteins had considerably longer half-lives than did liver proteins. This may be a reflection of the relative metabolic activity of muscle

and liver.

Starvation was studied in tissues from young male birds using pulse and dual labelling experiments. Starvation resulted in longer half-lives for purified liver proteins, soluble and insoluble liver proteins and plasma proteins, while muscle proteins showed shorter half-lives in the starved birds. Shorter half-lives in breast muscles were thought to occur so that the muscle can produce most of the nutrients necessary to sustain the animals over the period of stress. It was hypothesized that liver proteins had longer half-lives in order to maintain the integrity of the liver as a regulatory organ even though it has been shown to absorb the initial shock to the system when an animal is starved.

The relative turnover of proteins of varying molecular weight was measured using dual labelling and protein separation on Sephadex G-200. There was some relationship between decreasing protein turnover rate and increasing molecular weight for large proteins (>160,000) but this relationship did not exist for proteins which were smaller. The calculated turnover of the pure proteins suggested that there was not a relationship between half-life and molecular size. Other possible relationships and recycling are also discussed.

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ABBREVIATIONS

FDP	Fructose-1,6-diphosphate
EDTA	ethylenediaminetetraacetic acid
TCA	trichloroacetic acid
Tris	2 amino-2(hydroxymethyl)-1,3 propandiol
DPN	diphosphopyridine nucleotide
DPNH	diphosphopyridine nucleotide (reduced)
TPN	triphosphopyridine nucleotide
PPO	2,5-diphenyloxazole
POPOP	1,4 bis-[2-(5-phenyloxazoyl)] benzene
Aldolase	fructose 1,6-diphosphate : D-glyceraldehyde-3-phosphate lyase
FDPase	D-fructose-1,6-diphosphate : 1-phosphohydrolase
TDH	D-glyceraldehyde-3-phosphate : NAD oxidoreductase
GDH	L-glycerol-3-phosphate : NAD oxidoreductase
DEAE-cellulose	Diethylaminoethylcellulose

INTRODUCTION

All proteins within the body are in a continual process of synthesis and degradation. Until the advent of isotopic labelling, whereby a compound could be monitored in the presence of other compounds, the process of dynamic equilibrium had only been theorized from nitrogen balance studies(44). Radioactive isotopes allowed for the direct observation of incorporation and elimination of labelled amino acids in proteins.

The amount of amino acid which is utilized for daily protein synthesis is generally much more than that provided by the diet. Individual tissues from mature rats may derive from 30 to 50% of the amino acid required for protein synthesis from endogenous sources rather than the diet(30). Thus, it is apparent that the animal synthesizes much more protein than is required.

The flux of amino acids as the result of synthesis and degradation is the key component of turnover. External stimuli to the animal may cause an alteration in the degradative or synthetic rate, so that a rapid adjustment may be made and the corresponding condition may reflect the new steady state for the animal. Such an adjustment may occur very rapidly, if it is accomplished within the constraints of protein turnover. Control of synthesis or degradation may be the result of different stimuli, however, together the stimuli may produce a multiple response(56).

The objectives of this study were to compare protein turnover rates in the breast muscle and the liver of starved and control quail. The specific proteins studied included total soluble and insoluble

protein, aldolase and glyceraldehyde-3-phosphate dehydrogenase prepared from both liver and breast muscle, as well as, fructose-1,6-diphosphatase prepared from liver. The preparation of the enzymes required the development of the isolation techniques described in the text. A second series of experiments was conducted to determine the relationship, if any, between the molecular weight of liver and breast muscle proteins and the relative turnover as determined by dual labelling.

These experiments represented new findings, in that, this is one of the first major studies on the turnover of avian proteins. It is also unique because quantitative preparations of a number of enzymes were made from the same tissue to determine the relative rates of turnover. Finally, proteins were separated on the basis of molecular size using a molecular seiving gel, which was sufficient to separate over the range of 40,000 to 400,000 molecular weight.

LITERATURE REVIEW

I. The necessity of protein turnover

Protein turnover refers to the amount of a protein or group of proteins which is replaced by an equal quantity of the same proteins which have been newly synthesized from the available metabolic precursor within the cell during some specified period of time(47). While total turnover is defined in terms of flux, the individual reactions have been designated as the rate of synthesis, which is a linear or zero order reaction defined by a rate constant (ks), and the rate of degradation, which is an exponential or first order reaction defined by a rate constant (kd) and the specific protein concentration (E) within the cell(3).

The amino nitrogen derived from food intake has been found to represent one-half to one-third of the total amino nitrogen involved in daily protein flux(71). Further, it has been shown that there is a daily balance of nitrogen within the body such that under steady state, the intake of nitrogen equals the excretion of nitrogen(44). Therefore, the body reserves of protein must undergo extensive synthesis and degradation to maintain the daily equilibrium.

Siekevitz(60) has proposed three possible reasons for the existence of protein turnover. A system involving continual synthesis and degradation could respond to changes in environment, which require a change in enzyme patterns, most efficiently by altering the synthesis or degradation. Another possible function was thought to be for removal of useless proteins whether these proteins are the results of mistakes in protein synthesis or of denaturation. The final possible function was to increase the efficiency of energy utilization. He contends that the energy cost to synthesize the protein is far less than that necessary to synthesize the

mRNA, tRNA and ribosomes, therefore it is more efficient to produce excess proteins per unit of synthetic machinery, than to produce the machinery as required.

Understanding the process of protein turnover may result in some practical benefits. In establishing the protein requirements for man and animals, it was felt that an understanding of the mechanism by which the animal adapts to different levels of dietary protein would result in more accurate estimates(70). Waterlow's work was designed to establish minimum maintenance protein requirements in an attempt to eliminate protein malnutrition in developing countries. Most of the protein synthesized by animals was shown to be broken down rather than added to total body weight(70,64). The proper control of protein degradation might be utilized to grow an animal faster and larger if the conditions necessary for such control were more adequately understood(64).

II. Measurement of protein turnover

Many isotopic methods have been described to measure the turnover of specific proteins and groups of proteins on specific tissues. The original introduction of labelled amino acid into protein occured when Schoenheimer et al.(70) demonstrated that ^{15}N-tyrosine was accumulated in rat liver proteins. The method employed for any turnover determination must be adapted to the specific situation(20) because each tissue involves different problems.

Many methods have been developed to meet the requirements of individual experiments. These methods may be separated into those which measure degradation rates and those which measure synthetic rates.

A. Measurement of protein degradation

The most direct method of studying protein degradation has been by

the injection of a single pulse dose of a labelled amino acid. The loss of label from protein is then followed over a period of days(54,16). The degradation rate is linear when the log of the radioactivity is plotted against the time after the initial injection. This method, although widely used, has been critized because it does not account for the amount of amino acid which is released during catabolism and is reincorporated into protein before it is metabolized or excreted(33,51). This effect tends to produce overestimates of the true half-life.

Koch(33) showed mathematically that an amino acid which was incorporated into protein at a faster rate than it was metabolized, was reutilized to a greater extent than an amino acid which was more readily metabolized than used in protein synthesis. Poole(51) reported that proteins having half-lives of 0.1 to 2 days would have apparent half-lives of 3 to 4 days if [4,5-^{3}H] leucine was used to measure the rate of degradation. This increase was due to reutilization of the label which could not be accurately corrected. It was estimated that protein catabolism was responsible for approximately 50% of the liver and 30% of the muscle intracellular free amino acid pool when considering lysine and tyrosine(18). These two amino acids must therefore be recycled to a considerable extent.

Swick(63) reported that estimates of turnover in rat liver proteins were most accurate using [6-^{14}C] arginine because this amino acid was minimally recycled. When arginine was liberated from liver proteins, it immediately entered the urea cycle where the guanidine carbon (C-6) was lost as urea and was replaced with non-radioactive carbon from the bicarbonate pool(70). McFarlane(39) showed that there was some recycling of [6-^{14}C] arginine, but in comparison with other amino acids

this reutilization was very small. The tendency of guanidino-^{14}C-arginine to lose its label through metabolism has been shown to result in very low levels of label being incorporated into protein.

The use of arginine is not useful for determining turnover in birds because this amino acid is subject to extensive reutilization. Tamir and Ratner(66) found that chicken liver contained no urea cycle enzymes except small amounts of arginase. It was shown that the amount of arginase in chicken liver was approximately 0.2% as great as in rat liver(61). Sykes(65) suggested that arginase was present in chicken liver and kidney to produce ornithine for detoxification reactions of the kidney. Since there is very little arginase in avian liver, arginine is more likely to be involved in recycling.

Incorporation of $Ca^{14}CO_3$ into rat diets was shown to produce substantial labelling of the guanidine moiety of arginine in liver proteins(63). Labelling of arginine occured in mammalian liver because this tissue was the only one containing the urea cycle(39). Continuous administration of $^{14}CO_2$ pool precursors was advantageous because the guanidino arginine label was localized in liver proteins and was not substantially recycled. Swick(63) calculated the turnover rate constants by measuring the specific activities of the CO_2 pool, arginine bound to protein and urea excreted, as well as the rate of incorporation of arginine into protein, in animals which were approaching a steady state with respect to $^{14}CO_2$ specific radioactivity.

Another method called dual labelling was developed to circumvent many of the problems posed by recycling of leucine and the low level of incorporation of [^{14}C-guanidino] arginine(3,22). Animals were injected with 25 μCi of [U-^{14}] leucine followed three days later with 75 μCi of

[4,5-^{3}H] leucine. Four hours after the second injection, the animals were killed. Proteins to be studied were isolated and the $^{3}H:^{14}C$ ratios were determined. Some of the protein half-lives were determined using the guanidino-arginine technique. A standard curve was constructed by comparing the measured half-life and the log of the corresponding $^{3}H:^{14}C$ ratio. This work(22) showed that there was a good linear relationship between the log of the ratio and half-life determined with arginine. The half-lives of other proteins may be determined by measuring the $^{3}H:^{14}C$ ratios of these proteins from the same tissues used to prepare the standard curve; after which the half-life is read directly from that standard curve.

B. Measurement of protein synthesis

One of the most simple methods of measuring protein synthesis rates involves a single intravenous injection of labelled amino acid(27). At specific short time intervals after injection, animals are killed, specific radioactivity is determined and the rate of accumulation of label is calculated. This method has been critized because the single injection results in a precursor pool of variable specific radioactivity and the incorporation rate is not linear(19).

A second method involves the continuous administration of labelled amino acid. This allows the specific activity of the amino acid pool to rapidly reach that of steady state(19). It was concluded that the measurements made after a definite period of time reflected the entire time course of the synthetic process. Continuous infusion may be accomplished by feeding diets containing ^{14}C-lysine or $Ca^{14}CO_3$(63) and intravenous infusion of labelled amino acid(72).

Austin et al.(5) repeatedly injected [U-^{14}C] glucose into rats and measured the incorporation of labelled aspartate and glutamate into brain proteins. They showed that conversion of ^{14}C-glucose into aspartate and glutamate produced pools which remained at a relatively constant specific activity for up to 4 hours after the start the experiment.

III. Effects of changes in physiological parameters

The turnover of protein has been measured under many nutritional and hormonal states. One of the objectives of this work has been to determine the mechanisms by which the animal adapts to variations in environment when compared to a basal state.

A. Effects of amino acids on turnover

Tryptophan injection produced a linear increase in the amount of tryptophan pyrrolase in rat liver(56). The control animals showed a typical exponential decay of prelabelled enzyme, however in the presence of tryptophan, the label remained at a constant level. It was concluded that tryptophan protected tryptophan pyrrolase from degradation by converting it to a form which was less subject to enzymatic attack.

An amino acid mixture lacking in valine, tyrosine, leucine, isoleucine, glutamine and asparagine was shown to decrease the release of ^{14}C-valine from perfused prelabelled rat livers(73). It was also showed that methionine, phenylalanine, proline and tryptophan each caused a slight reduction in the rat of release of ^{14}C-valine, however the combination of all four amino acids produced an inhibition of the same magnitude as the original mixture. It was concluded that the amino acids produced an inhibition of protein degradation, but no mechanism was suggested. Glycine, ornithine, proline, alanine and arginine

decreased the rate of inactivation of arginase by purified lysosomes from 1.5 to 10 times while the rate for alanine aminotransferase was decreased by less than a factor of two(26). This showed the preferential protection of one enzyme as compared to another *in vitro*.

Livers from rats fed tryptophan-free diets were shown to contain a greater number of polysomes containing 3 or less ribosomes per unit(74). These polysomes had a decreased capacity to incorporate ^{14}C-leucine and ^{14}C-tryptophan into polysomes and post-polysomal pellets. Perfusion of rat liver *in situ* with amino acid mixtures deficient in one of arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan or valine also increased the number of polysomes containing 3 or less ribosomes(31). The rate of incorporation of ^{14}C-amino acids into proteins synthesized *in vitro* by isolated polysomes was increased in the presence of arginine, histidine, cysteine, lysine, methionine, phenylalanine, threonine, tryptophan and valine(6). It was concluded that certain amino acids may be necessary to produce the maximal aggregation of polysomes.

B. Effects of dietary protein on turnover

The level of dietary protein appeared to influence the turnover of proteins from many tissues. As compared with that found for the control diet, the synthetic rate of liver proteins was slower in rats fed low protein and protein-free diets(72,27) and slightly faster in rats fed a high protein diet(27). The turnover of mixed serum proteins was more rapid in rats fed low protein and protein-free diets(72). The synthetic rate of plasma albumin was increased in rats fed low protein diets and decreased in rats fed high protein and protein-free diets(27). Low protein and protein-free diets decreased the rate of protein synthesis

in rat muscle(70). Schimke(55) reported that the quantity of rat liver arginase decreased when the diet was changed from 70% casein to 8% casein. Initially, liver adapted to the change by increasing degradation and decreasing synthesis and following 9 days of adjustment, the synthetic and degradation rates had established a new lower steady state level.

C. Effects of age on turnover

The synthetic rate in liver of rats weighing 100 g was 12% greater than it was in rats weighing 300 g(41). Skeletal muscle was synthesized at a rate which was 67% greater in the young rats than in the older rats. They concluded that much of the synthesized muscle protein was necessary for growth and that after growth ceased, the net synthesis of protein was decreased. Yousef and Johnson(75) showed that total body protein turnover was less in old rats (572-605 days old) than it was in young rats (47-49 days). This may also reflect the necessity of synthesis to produce the necessary protein for growth.

D. Effects of starvation on turnover

Restriction of food intake was shown to decrease total turnover in young growing rats(75), however the same restrictions placed on older mature rats did not affect the total turnover. In other studies, it was demonstrated that starvation for 48 hours resulted in no change in the rate of synthesis or degradation of rat liver protein(70,21). Both reports showed that starvation decreased the the rate of protein synthesis in rat muscle. Three days of starvation caused a 20% decrease in rat liver protein synthesis(40) while the decrease in muscle synthesis was similar to that shown for 48 hours. In addition, starvation produced a small decrease in the rate of serum protein

synthesis(72).

The effects of starvation have been demonstrated for specific enzymes. Rechcigl(52) showed that moderate starvation had no effect on synthesis or degradation of hepatic catalase but severe starvation resulted in a 50% decrease in the rate of synthesis with no change in degradation. Starvation for 20 hours resulted in the amount of phosphoenolpyruvate carboxylase in rat liver and adipose tissue being 3% of the total synthesized protein(30). Subsequent refeeding produced levels of 0.2% in the liver and 1% in the adipose tissue. This adaptation was considered to be the result of changes in synthesis with relatively little change in degradation.

After starving rats for periods varying from one to seven days, [U-^{14}C] lysine was infused intravenously for 10 hours and the ratios of liver lysine to plasma lysine or muscle lysine to plasma lysine specific activity (dpm/mg lysine) were compared(18). The authors concluded that the first two or three days of fasting induced mobilization of the labile liver proteins accompanied by a decrease in liver weight and an increase in protein degradation while after three days the degradative rate decreased and liver weight was essentially stabilized. The relative specific activity of lysine in muscle progressively decreased for seven days, which indicated that the muscle was being involved to a greater extent in maintenance of homeostasis during starvation.

E. Effects of hormones on turnover

The control of synthesis and degradation exhibited by such factors as starvation, amino acid balance and dietary composition may be

mediated by the influence of hormones. The effect of the original stimulus or the net effect of a hormone may be inseparable in establishing the true cause for alterations in turnover.

Insulin decreased the rate of release of valine from prelabelled perfused rat livers(43,73). Shrago et al.(59) used livers from rats treated with insulin and showed that there was an increased quantity of malic enzyme and no change in phophoenolpyruvate carboxykinase content, which is associated with gluconeogenesis. Glucagon and alloxan induced diabetes reduced malic enzyme activity but increased the carboxykinase activity. Waterlow and Stephen(72) showed that insulin produced a significant increase in the turnover time for rat liver proteins.

Rat serum and muscle proteins did not show a change in the turnover rate in the presence of insulin(72). Insulin stimulated the incorporation of labelled amino acid into rat muscle tissue in vitro(25). Inclusion and deletion of metabolites involved in cellular transport showed that insulin was involved in stimulation of amino acid uptake by muscle tissue but there was no evidence of any direct influence on protein synthesis.

Hormones from the adrenal medulla also appear to perform a regulatory function in connection with protein turnover. Adrenalectomy did not change the amount of total body protein turnover in old or young rats(75). The amount of tryptophan pyrrolase in rat liver increased after injection of hydrocortisone(56). It was shown that hydrocortisone induced tyrosine transaminase synthesis in hepatoma cell culture and that cycloheximide and puromycin blocked the

turnover of this enzyme(7). Cortisone was shown to produce a 30% increase in nucleic acid and protein moieties of the 30S and 50S ribosome subunits from rat liver(28). They also showed that cortisone induced a rapidly turning over RNA, which was smaller than 10S. They concluded that cortisone leads to increased synthesis of a rapidly turning over RNA such as mRNA which would be responsible for the enhanced biosynthetic rate of hormonally inducible enzymes such as tryptophan pyrrolase(56). Goldberg(24)reported that cortisone produced atrophy of those muscles studied by decreasing protein synthesis and increasing protein degradation. This appeared to involve a mechanism which was different from that operating in liver.

Growth hormone appeared to increase the amount of protein synthesis while having no effect on degradation(23). Injection of the glucocorticoid, triamcinolone, into adrenalectomized animals produced an apparent half-life for tyrosine aminotransferase which was twice as long as that found under basal dietary conditions(9). Thyroidectomy of rats decreased body weight and total body protein turnover(75). Thyroidectomy decreased the activity of phosphoenolpyruvate carboxylase in rat liver(46). Treatment with thyroxine enhanced the carboxylase activity in normal and throidectomized animals. Thyroxine was thought to stimulate the synthesis of protein because cycloheximide blocked the activation.

Although the actual stimulus to alterations in protein turnover has not been clearly established, it may be seen that hormones appear to have the greatest demonstrable direct action. It may also be the case that the effects of other stimuli such as protein and amino acid supply or starvation may be mediated by hormones,

however, hormones cannot be looked upon as the only source of direct control. Schimke et al.(56) showed that tryptophan and hydrocortisone had a stimulatory effect on the amount of tryptophan pyrrolase but together the effect was equal to the product of the individual stimuli, thus demonstrating quite different mechanisms for each.

IV. Alternative factors regulating turnover

Boctor and Grossman(9) stated '...it is conceivable that protein turnover may range from a few seconds to several weeks. Even if this time gap is narrowed, what kind of apparatus would be required to bestow a characteristic rate of decay upon several hundred different types of protein?' In the light of what is known of hormonal, genetic and metabolic control of turnover of specific proteins there are other relationships which have been suggested as possible regulators of total turnover.

A. Relationship of protein size and turnover

Dehlinger and Schimke(12) labelled soluble rat liver proteins with ^{3}H and ^{14}C and separated these on Sephadex G-200 in the presence and absence of sodium dodecyl sulfate (SDS). They found a direct correlation between proteins of decreasing molecular weight and decreasing $^{3}H:^{14}C$ ratio. There was a far better correlation between the decreasing size of protein subunits which had been dissociated with SDS and decreasing $^{3}H:^{14}C$ ratio(12,22). Subsequent studies on soluble proteins(15) from rat liver, kidney, muscle, testes and brain also showed the correlation between subunit size and turnover rate. The correlation with subunit size has also been demonstrated for individual proteins in ribosomal protein(14) dissociated with SDS and membrane proteins(13) dissociated with sodium lauryl sulfate (SLS).

It was shown that proteins of specific molecular weights as separated

on Sephadex G-100 were subject to degradation by pronase in direct proportion to the fraction molecular weight(15). Bond(10) studied the rate of inactivation of specific enzymes, with known molecular weights, by proteolytic enzymes. There appeared to be no correlation between the rate of inactivation and the half-lives reported elsewhere in the literature.

B. Lysosomes, protein instability and turnover

It was suggested that protein denaturation was a prerequisite for the digestion by lysosomal enzymes(11). Rat liver alanine aminotransferase was shown to have a half-life of thermal inactivation of 400 days(58). As this was many times greater than the true half-life, they concluded that the effect of thermal inactivation was not significant in determining the rate of protein degradation.

Segal et al.(58) proposed a degradative system with a flux of cellular material through the lysosome. The minimum cellular half-life was defined by the rate of incorporation into the lysosomes while longer half-lives occured because some of the protein returned to the cytoplasm before being degraded. Based on the most rapid turnover rate reported in the literature ($t_{1/2}$ = 2 hr) and the quantity of lysosomal protein per unit of cellular protein(3%), Haider and Segal(26) calculated that the lysosome must handle 8 volumes of cytoplasmic material per hour. They also showed that certain amino acids and Mn^{2+} decreased the susceptability of arginase to be inactivated by purified lysosomes in vitro, whereas the same conditions did not decrease the inactivation of alanine aminotransferase. It was concluded that cellular components may stabilize proteins to varying degrees which would tend to account for the variable turnover of cellular components.

MATERIALS AND METHODS

I. Materials

Young Japanese quail (Coturnix japonica) were obtained from a flock maintained by the Department of Entomology at the University of Manitoba. Rabbit muscle aldolase, glycerophosphate dehydrogenase, triose phosphate, glucosephosphate isomerase and glucose-6-phosphate dehydrogenase were purchased from Boehringer-Mannheim (GmbH). Cellulose phosphate, Tris, FDP, EDTA, DPN+, DPNH, TPN+, cytochrome c and bovine serum albumin were purchased from Sigma. Toluene(scintillation grade), PPO, POPOP, and Triton x-100 were purchased from Packard. NCS, L-[1-^{14}C]-leucine and L-[4,5-^{3}H]-leucine were purchased from Amersham/Searle. Other chemicals purchased were Cellex-D from Bio.Rad Laboratories, heparin from BDH, ferritin from Pentex biochemicals, dextran blue from Pharmacia, Uppsala and phenol reagent from Harleco. Chick starter containing 21 % protein was obtained from Feedrite Ltd.

II. Methods

A. Experimental design

1. Experiment 1

One hundred and twenty seven Japanese quail were divided into four experimental groups. Groups I and II each contained 34, sixteen-wk-old males while group III contained twenty five, 52-wk-old males and group IV contained 34, sixteen-wk-old laying females. Group II was starved for the duration of the experiment and allowed only water. The remaining groups were allowed feed and water ad lib, throughout.

The feed was removed from all birds for 4 hr, after which each bird received 1.56 µCi of L-[1-^{14}C] leucine per 100 g of body weight. Four hr after the injection, feed was returned to all of the birds except those in group II. Eight birds from each group (6 from group III) were killed

by decapitation at 1, 3, 5 and 7 days after injection. Blood was collected from the neck vein into 5 USP units of heparin. The blood samples were centrifuged at 1500 x g for 10 min and the plasma was frozen until it could be used. The breast muscle and liver was removed from each animal frozen in liquid nitrogen and stored at -70 C until it was analyzed.

2. Experiment 2

Fourteen 17-wk-old quail were allotted to three groups and their respective controls. Group I contained 4 birds fed ad lib as in experiment 1, while group II contained 4 birds that were starved for three days before they were killed and group III contained 3 birds that were starved for 10 days before they were killed and 7 days prior to the initial injection. There was one bird used as a control for each group.

All birds were starved for at least 4 hr. Each bird was injected with 6.2 µCi of L-[1-^{14}C] leucine per 100 g of body weight. The control birds were injected with 6.2 µCi of L-[1-^{14}C] leucine and 18.5 µCi of 1-[4,5-^{3}H] leucine per 100 g. The birds from group I with the control were refed after 4 hr. After three days the test birds were injected with 18.5 µCi of L-[4,5-^{3}H] leucine per 100 g of body weight. The control birds did not receive a second injection. The birds were killed 4 hr after the administration of the ^{3}H. Blood plasma, breast muscle and liver were collected and stored as in Experiment 1.

B. Preparation of soluble and insoluble protein

Two groups of four, 0.2 g samples of liver or breast muscle, were weighed from eight different tissue samples. Each composite sample was suspended in 7.2 ml of 50 mM Tris; 5 mM EDTA; 5 mM 2-

mercaptoethanol (pH 7.8) and homogenized at maximum speed for 1 min at O C in the Polytron homogenizer (Kinematica, GmbH). The homogenate was separated into supernatant and precipitate fractions by centrifugation at 50,000 x g for 15 min.

1. Precipitate (insoluble protein)

The precipitate was resuspended in 5.0 ml of homogenization buffer, mixed thoroughly and recentrifuged at 50,000 x g for 15 min. This washing was repeated once. The precipitate was suspended in 5.0 ml of a 1:1 mixture of petroleum ether and ethanol, allowed to extract for 30 min and centrifuged at 50,000 x g for 10 min to remove the lipid components. The precipitate was suspended in 5.0 ml of petroleum ether and centrifuged at 50,000 x g for 10 min. This precipitate was suspended in 4.0 ml of the water and blended with the Polytron homogenizer. Two, 0.2 ml aliquots were taken from each preparation for solublization in NCS solublizer and were counted. Duplicate 0.4 ml aliquots were diluted to 2 ml with water and were frozen until Lowry protein analysis could be performed.

2. Supernatant (soluble protein)

The initial supernatant was recentrifuged at 200,000 x g for 60 min. This supernatant was mixed with an equal volume of 10% trichloroacetic acid (TCA). The precipitate was collected by centrifugation of 50,000 x g for 10 min. The precipitate was washed as before, in 5% TCA, petroleum ether-ethanol and petroleum ether. The washed precipitate was suspended in 2.0 ml of water and blended with the Polytron homogenizer. The same aliquots were taken for counting and Lowry protein determination as for the insoluble proteins.

Lowry protein determination (Section VI) and radioactivity

(Section V) were measured in soluble and insoluble proteins.

C. Isolation of the liver and muscle enzymes

Two groups of four, 0.3 g samples of liver or breast muscle were weighed from eight different tissue samples. Each composite sample was homogenized at maximum speed in the Polytron homogenizer at 0 C with 10.8 ml of 50 mM NaCl: 5 mM EDTA; 5 mM 2-mercaptoethanol (pH 7.0 at 23 C). The homogenates were centrifuged at 50,000 x g for 15 min and resulting liver supernatants were centrifuged at 200,000 x g for 35 min. Eight ml of each final supernatant was diluted with an equal volume of water and placed on a cellulose phosphate column (1.4 cm x 14 cm) previously equilibrated with 25 mM Tris; 1 mM EDTA; 5 mM 2-mercaptoethanol, pH 7.7 at 23 C (buffer I). Each column was washed overnight with 1.5 to 2 l of buffer I plus 50 mM NaCl.

A column containing DEAE (1.5 mm x 4.0 mm) was placed in line after the cellulose phosphate. Muscle aldolase was eluted with 150 ml of buffer I, plus 50 mM NaCl and 1 mM fructose-1,6-diphosphate (FDP). The DEAE was disconnected and glyceraldehyde-3-phosphate dehydrogenase was eluted with 250 ml of buffer I plus 150 mM NaCl. Liver aldolase was eluted with 150 ml of buffer I plus 50 mM NaCl and 0.2 FDP and the dehydrogenase was removed with 250 ml of buffer I plus 150 mM NaCl. The column was washed with buffer I plus 100 mM NaCl and fructose-1,6-diphosphatase was removed with buffer I plus 100 mM NaCl and 3 mM FDP. The remaining protein was eluted with 150 ml of 3 M NaCl. The eluate was continuously collected in 20 ml fractions. Those fractions containing the maximum enzyme activity were determined by assaying for enzymes using the subsequently described assays. Each pooled fraction was dialyzed for two hr against distilled water. The elution

Fig. 1. Chromatography of quail liver soluble proteins on phosphocellulose. The protein peaks for the breakthrough (BT), aldolase (ALD), glyceraldehyde-3-phosphate dehydrogenase (TDH), fructose-1,6-diphosphatase (FDPase) and the 3M NaCl peak were separated as described in the Methods. The absorbance at 280 nm (x) and the enzyme activity (o) for the specific enzymes and the additions to the basic elution buffer are indicated.

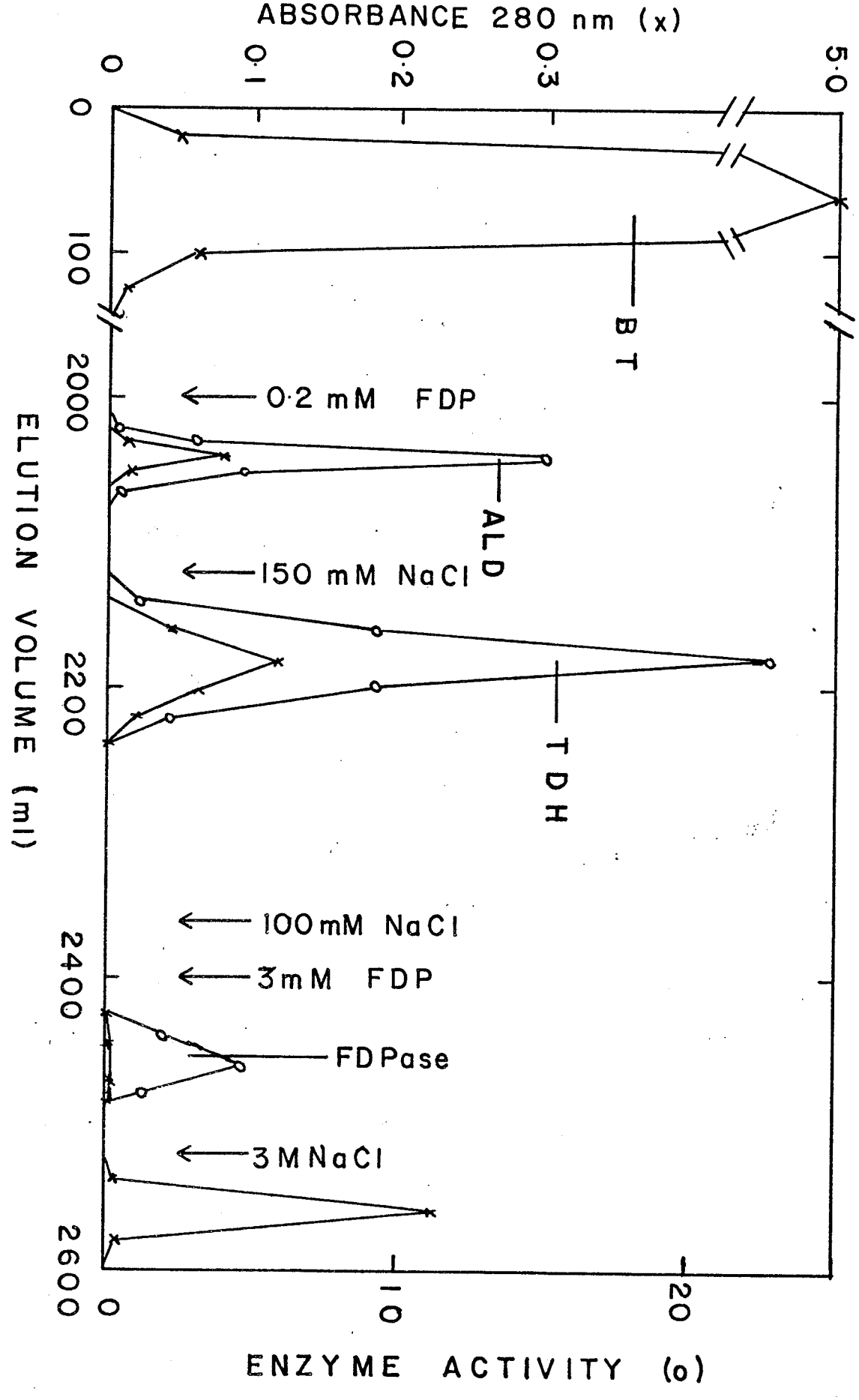
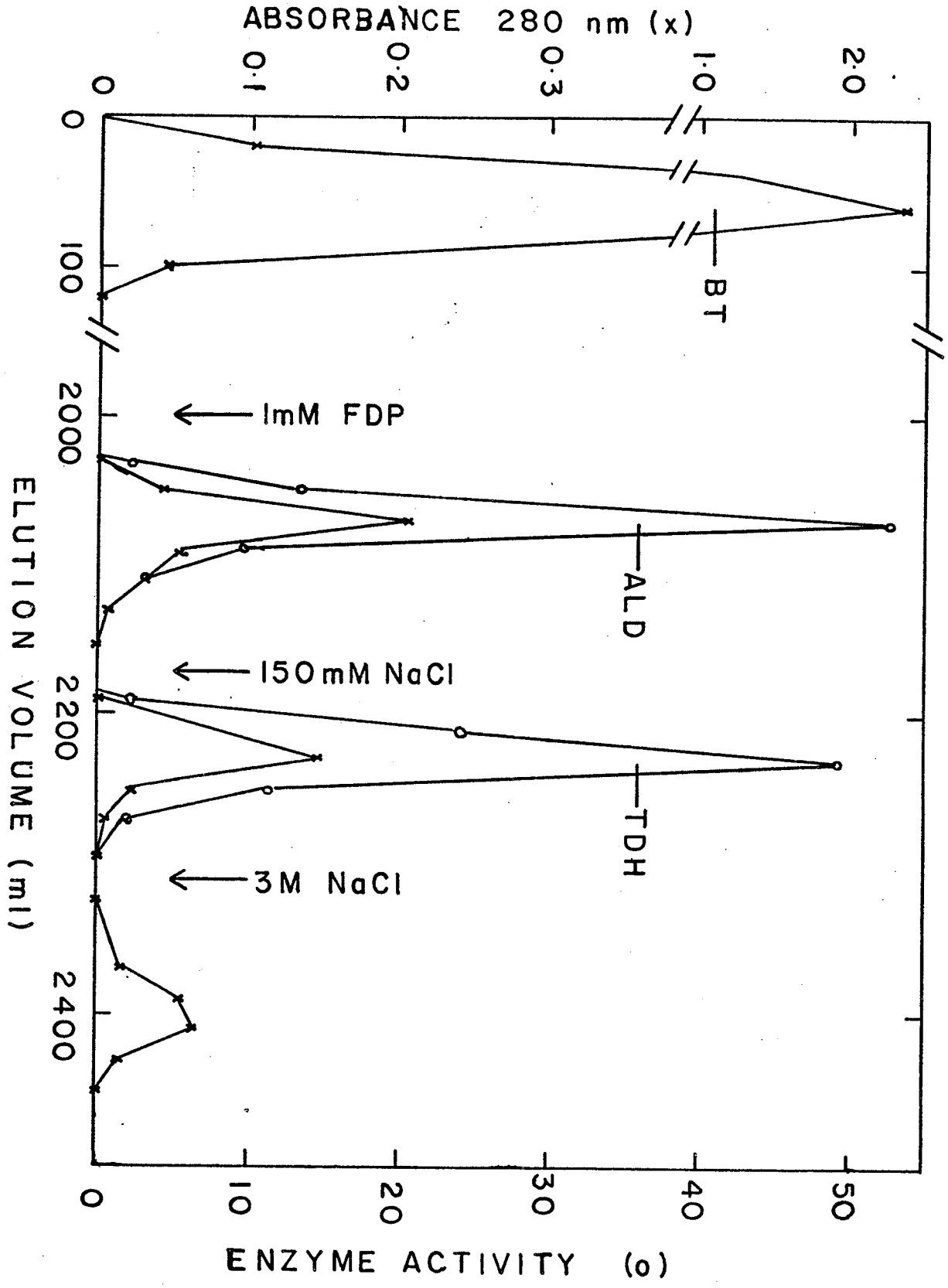

ABSORBANCE 280 nm (x)
0
0·1
0·2
0·3
5·0
0
100
2000
2200
2400
2600
ELUTION VOLUME (ml)
BT
0·2 mM FDP
ALD
150 mM NaCl
TDH
100mM NaCl
3mM FDP
FDPase
3M NaCl
0
10
20
ENZYME ACTIVITY (o)

Fig. 2. Chromatography of quail breast muscle soluble proteins on phosphocellulose. The protein peaks for the breakthrough (BT), aldolase (ALD), glyceraldehyde-3-phosphate dehydrogenase (TDH) and the salt peak were separated as described in the Methods. The absorbance as 280 nm (x) and the enzyme activity (o) for the specific enzymes and the additions to the basic elution buffer are indicated.


ABSORBANCE 280 nm (x)
0
0·1
0·2
0·3
1·0
2·0
0
100
2000
2200
2400
BT
1mM FDP
ALD
150mM NaCl
TDH
3M NaCl
ELUTION VOLUME (ml)
0
10
20
30
40
50
ENZYME ACTIVITY (o)

pattern of liver and muscle proteins are shown in Figs. 1 and 2.

Glyceraldehyde-3-phosphate dehydrogenase was purified by first adding dithiothreitol to a final concentration of 1 mM and crystalline ammonium sulfate to a concentration of 2.77 M. The precipitate was removed by centrifugation at 50,000 x g for 10 min and the supernatant was adjusted to a final ammonium sulfate concentration of 3.5 M. The enzyme was collected by centrifugation at 50,000 x g for 10 min, suspended in 1 ml distilled water and dialyzed against distilled water. A 0.2 ml aliquot was taken for Lowry protein and the remainder was divided for duplicate counting.

Duplicate samples of the other proteins, equaling 40% of the peak volume to a maximum of 15 ml, were collected into scintillation vials and were dried in vacuo at 58 C. The proteins were then solublized and counted. One 2 ml fraction was taken from the same peak volume for Lowry protein analysis.

D. Enzyme Assays

1. Aldolase (EC.4.1.2.13)

The aldolase assay was that reported by Marquardt(37). Each cuvette contained 1.45 ml of the reaction cocktail which consisted of 50 mM Tris, 2 mM FDP, 5mM EDTA (pH 7.5 at 30 C), 0.18 mM DPNH, 15 µg rabbit muscle α-glycerophosphate dehydrogenase, and 15 µg triose phosphate isomerase (EC.5.3.1.1). The reaction was initiated by addition of 0.05 ml of the appropriate dilution of the enzyme.

2. Fructose-1,6-diphosphatase (EC.3.1.3.11)

This assay was that reported by Olson and Marquardt(49). Each cuvette contained 1.45 ml of 50 mM Tris, 1.0 mM EDTA (pH 7.5 at 30 C),

15 mM $MgCl_2$, 0.1 mM FDP, 0.2 mM TPN^+, 8 µg glucosephosphate isomerase (EC.5.3.1.9), 8 µg glucose-6-phosphate dehydrogenase (EC.1.1.1.49), and 16 µg bovine serum albumin. The reaction was initiated with 0.05 ml of the appropriate dilution of the enzyme.

3. Glyceraldehyde-3-phosphate dehydrogenase (EC.1.2.1.12)

This assay was a modification of that reported by Velick(67). Each cuvette contained 1.45 ml of 6.7 mM EDTA, 26.7 mM sodium pyrophosphate, 13.4 mM sodium arsenate, 3mM 2-mercaptoethanol (pH 8.4 at 30 C), 0.33 mM DPN^+, 2 mM FDP, 15 µg aldolase and 15 µg triose phosphate dehydrogenase. The reaction was initiated by addition of 0.05 ml of the appropriate dilution of the enzyme.

4. Glycerol-3-phosphate dehydrogenase (EC.1.1.1.18)

This assay was a modification of that reported by Beisenherz et al.(8). Each cuvette contained 1.45 ml of the aldolase cocktail with 15 µg of aldolase replacing the α-glycerophosphate dehydrogenase. The reaction was initiated with 0.05 ml of the appropriate dilution of the enzyme.

All assays were monitored with a Gilford Model 2000 Recording Spectrophotometer. The molar extinction coefficients for the enzyme reactions were 12.44×10^3 cm^{-1} for aldolase and 6.22×10^3 cm^{-1} for the remaining three enzymes. Each reaction was followed at 340 nm for 10 min and the linear portion of the recording was taken as the rate of reaction.

E. Radioisotope Counting

All proteins, which required solublization, were suspended in 0.2 ml of water and 1.0 ml of NCS. Samples were incubated overnight at 60 C. Each sample was neutralized with 0.04 ml of glacial acetic

acid, suspended in 10 ml of scintillation solvent and refrigerated at 0 C for 24 hr. Samples of soluble proteins were made up to a total aqueous volume of 0.8 to 1.5 ml and were dissolved in 10 ml of scintillation solvent. Duplicate 0.05 ml samples of plasma were dissolved in 10 ml of scintillation solvent. Samples were counted in a Nuclear Chicago Mark II Liquid Scintillation Counter using external standardization.

The scintillation solvent contained 16.5 g of 2,5-diphenyloxazole (PPO) and 0.30 g of 1,4-bis [2-(5-phenyloxazolyl)] benzene (POPOP) dissolved in 2000 ml of toluene and 1000 ml of Triton X-100.

F. Protein determination

Protein was determined by the Lowry method(36). Protein samples were mixed with an equal volume of 1N NaOH and diluted to a concentration between 20 and 200 µg/ml with 0.5N NaOH. One ml of the dilute protein solution was mixed with 5 ml of copper reagent (2% Na_2CO_3, 0.01% $CuSO_4.5H_2O$ and 0.02% Na.K tartrate; prepared fresh daily). Tubes were mixed and allowed to stand for 15 min. To each tube, 0.5 ml of 1N phenol reagent was added and mixed thoroughly. After 30 min the absorbance at 660 nm was measured and compared to the standard.

The standards consisted of 1 ml samples of bovine serum albumin (20 to 200 µg/ml) in 0.5 N NaOH. These were treated in the same manner as the test proteins.

G. Sephadex chromatography

From each experimental group a composite sample weighing 1.2 g was homogenized at 0 C in 4.8 ml of 50 mM Tris; 100 mM KCl; 5 mM EDTA; 2 mM 2-mercaptoethanol, pH 7.6. The homogenate was centrifuged at 50,000 x g for 15 min and recentrifuged at 200,000 x g for 30 min. Four ml of the supernatant was added to the bottom of a Sephadex G-200 column (1.8 cm x 66 cm) and separated using an ascending buffer system.

The elution buffer was identical to the homogenization buffer plus pentachlorophenol. The eluent was collected in fractions of 4 to 5 ml and each eluted protein was compared to the void volume obtained with dextran or the void peak obtained from the nucleic acids and high molecular weight proteins. The liver and muscle extracts were standardized by measuring the peaks for aldolase and α-glycerophosphate dehydrogenase using the assays in section IV. The extreme points on the standard curve were determined by separating a mixture of ferritin and cytochrome c by measuring the peaks using the absorbance at 280 nm. A 1.0 ml aliquot was counted for ^{3}H and ^{14}C in those fractions that had an absorbancy at 280 nm of greater than 0.05 units.

The molecular weights used were ferritin, 480,000(29); liver aldolase, 160,000(38); muscle aldolase, 158,000(37); glycerophosphate dehydrogenase, 66,000(17); cytochrome c, 12,400(2); fructose-1,6-diphosphatase, 143,3000(48); and glyceraldehyde-3-phosphate dehydrogenase, 138,000(4).

H. Zone electrophoresis

Cellulose acetate electrophoresis was performed for 90 min at 2 C on 2.5 x 17 cm Sepraphore III strips in a Gelman electrophoresis chamber using a pH 8.8 running buffer (Gelman barbital-Tris salt mixture, 17.8 g/l; 1 mM EDTA; 1 mM 2-mercaptoethanol). The strips were stained with Ponceau S in 7% acetic acid.

TREATMENT OF THE DATA

I. Calculations

Simultaneous analysis of ^{3}H and ^{14}C involved counting the samples in two preset channels (A and B), where the A channel contained 99% of the ^{3}H and some of the ^{14}C and the B channel contained 70% of the ^{14}C and less than 1% of the ^{3}H. The true efficiency of counting each isotope in each channel was calculated from the efficiency of counting the machine external standard. These efficiencies are designated as Ha, Hb, Ca and Cb while the measured counts are designated Na and Nb. The true counts (dpm) are calculated from the simultaneous eqns 1 and 2.

$$^{3}H\ (dpm) = \frac{(Cb \times Na) - (Ca \times Nb)}{(Cb \times Ha) - (Ca \times Hb)} \qquad (1)$$

$$^{14}C\ (dpm) = \frac{(Ha \times Nb) - (Hb \times Na)}{(Cb \times Ha) - (Ca \times Hb)} \qquad (2)$$

Turnover coefficients were calculated from the log of the specific radioactivity (dpm/mg of protein) change over the four time points used in experiment 1, by the least squares analysis (62) which produced the regression coefficient (eqn 3). The half-life was

$$b = \frac{\Sigma xy}{\Sigma x^2} = k_d \qquad (3)$$

calculated using eqn 4 which is analogous to that shown by Arias *et al.* (3).

$$t_{\frac{1}{2}} = \frac{\log 2}{b} = \frac{0.301}{b} = \frac{0.301}{k_d} \qquad (4)$$

Enzyme units were calculated using eqn 5. The molar extinction

$$\text{Enzyme units} = \frac{\text{Change in absorbance 340 nm}}{\text{Extinction coefficient}} \times \text{dilution factors} \qquad (5)$$

coefficients for NADP, NAD and NADH were all $6.22 \times 10^{3}\ cm^{-1}$.

II. Statistics

Statistics were performed according to methods reported by Steele and Torrie(62). Standard error was calculated using eqn 6.

$$S.\ E.\ = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n(n-1)}} = \sqrt{\frac{\Sigma x^2}{n(n-1)}} \qquad (6)$$

The slopes of two regression lines were compared by the t test in eqn 7 where S^2p is described by eqn 8.

$$t = \frac{b_1 - b_2}{[S^2p(1/\Sigma x_1^2 + 1/\Sigma x_2^2)]^{\frac{1}{2}}} \qquad (7)$$

$$S^2p = \frac{\Sigma y_1^2 - \frac{(\Sigma x_1 y_1)^2}{\Sigma x_1^2} + \Sigma y_2^2 - \frac{(\Sigma x_2 y_2)^2}{\Sigma x_2^2}}{n_1 + n_2 - 4} \qquad (8)$$

The standard error of the slope was calculated using eqn 9.

$$S.\ E.\ (b) = \sqrt{\frac{\Sigma y^2 - \Sigma xy/\Sigma x^2}{\Sigma x^2 (n-1)}} \qquad (9)$$

The standard error of the half-life was calculated as the same percentage of the half-life as the standard error of the slope was of the slope from which the half-life was calculated.

RESULTS

I. Enzyme purification

The effectiveness of an enzyme purification procedure may be assessed directly by measurement of specific contaminating proteins during electrophoresis or by enzyme assay or indirectly by comparison of the specific activity and fold purification of the enzyme to similar isolations from the literature. The purification of liver aldolase, fructose-1,6-diphosphatase and glyceraldehyde-3-phosphate dehydrogenase was measured (Table 1,Fig.3). The aldolase fraction contained 0.4% of the protein as fructose-1,6-diphosphatase, however there were no extra visible bands upon electrophoresis. The specific activity and fold purification were almost identical to that reported for chicken muscle aldolase(38). The fructose-1,6-diphosphatase fraction contained 3% of the protein as aldolase and 0.07% of the protein as glyceraldehyde-3-phosphate dehydrogenase and the electrophoresis showed no visible contaminating proteins. The fold purification was almost identical to that reported in chicken liver(49). Glyceraldehyde-3-phosphate dehydrogenase fraction contained 0.02% of the protein as fructose-1,6-diphosphatase and 0.7% of the protein as aldolase and the electrophoresis showed no visible contaminating proteins.

The purity of muscle aldolase and glyceraldehyde-3-phosphate dehydrogenase were measured (Table 2,Fig.4). The aldolase fraction contained 0.02% of the protein as glyceraldehyde-3-phosphate dehydrogenase but no visible contaminating proteins were found during electrophoresis. The specific activity of aldolase was roughly equal to that reported for chicken muscle aldolase(37). Glyceraldehyde-3-phosphate dehydrogenase fraction contained 0.14% of the protein as aldolase but

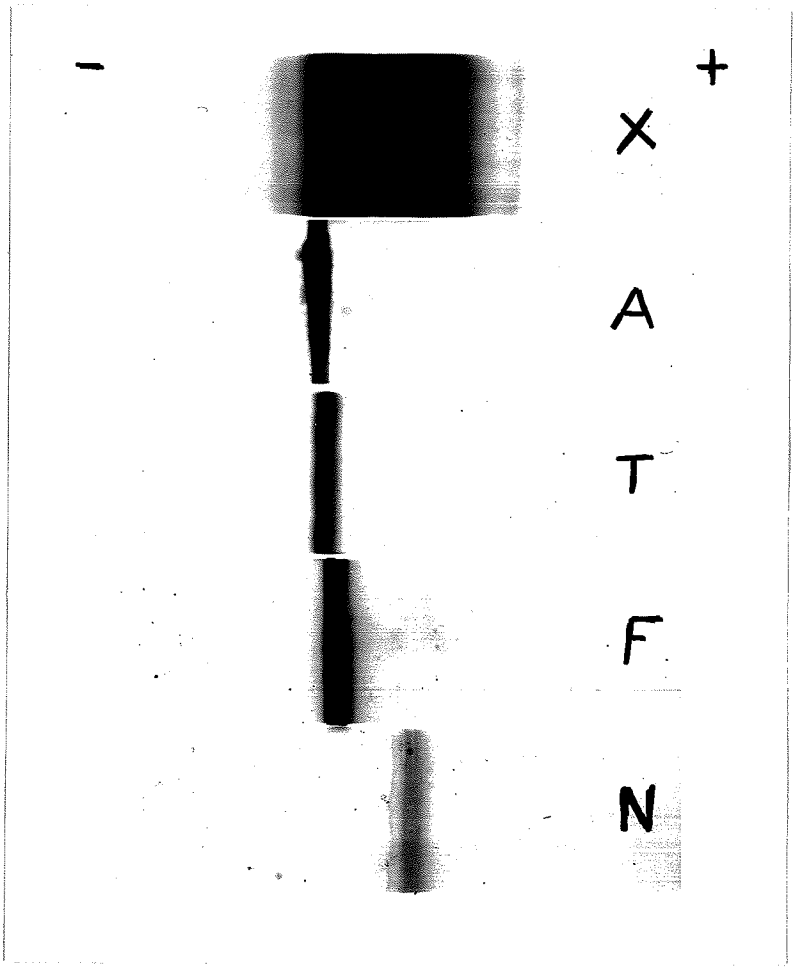


Fig. 3. Electrophoresis of quail liver purified proteins on cellulose acetate strips. The specific proteins shown are extract (X), aldolase (A), glyceraldehyde-3-phosphate dehydrogenase (T), fructose-1,6-diphosphatase (F) and the salt peak (N).

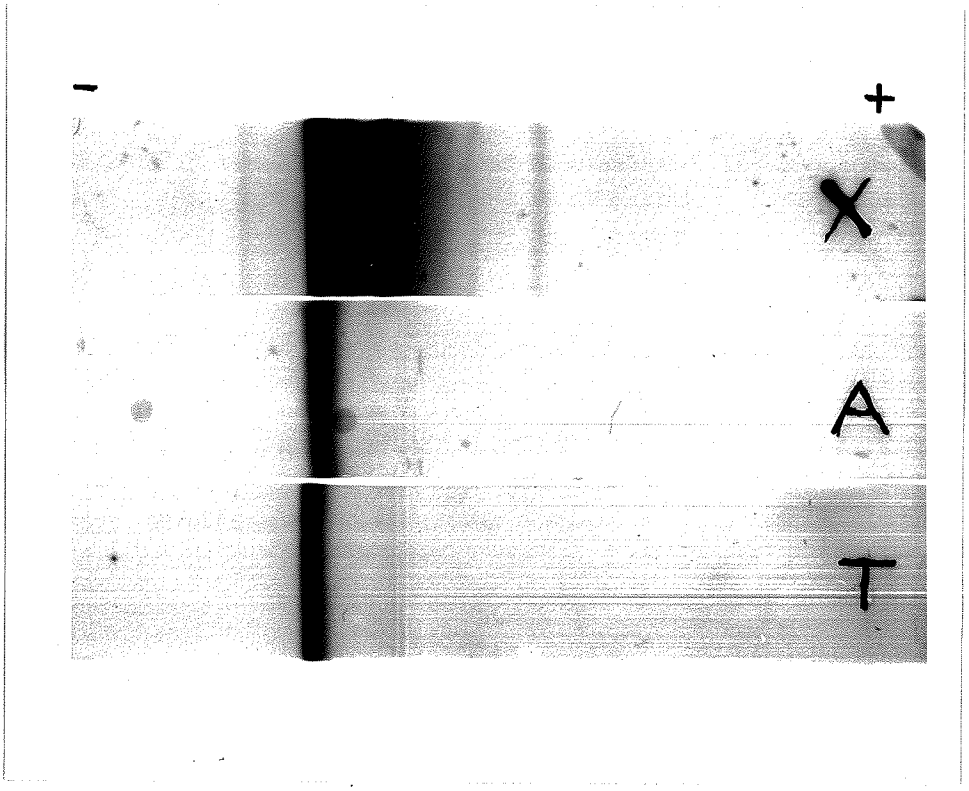


Fig. 4. Electrophoresis of quail breast muscle purified proteins on cellulose acetate strips. The specific proteins shown are extract (X), aldolase (A) and glyceraldehyde-3-phosphate dehydrogenase (T).

Table 1. Purification of aldolase, fructose-1,6-diphosphatase and glyceraldehyde-3-phosphate dehydrogenase from quail liver.

Fraction	Specific activity (enzyme units / mg protein)			Fold Purification
	Ald[1]	TDH[1]	FDPase[1]	
Extract	0.041	0.845	0.051	
Ald	1.38	0.0	0.012(0.40)[2]	34
TDH	0.010(0.70)	34.6	0.006(0.02)	41
FDPase	0.043(3.10)	0.024(0.07)	3.10	61

[1] Ald, aldolase; TDH, glyceraldehyde-3-phosphate dehydrogenase; FDPase, fructose-1,6-diphosphatase.

[2] Values in brackets are estimated % contaminating protein as determined by enzyme assay and specific activity values of the pure enzyme.

Table 2. Purification of aldolase and glyceraldehyde-3-phosphate dehydrogenase from quail breast muscle.

Fraction	Specific Activity (enzyme units / mg protein)		Fold Purification
	Ald[1]	TDH[1]	
Extract	1.23	7.69	
Ald	19.12	0.01(0.02)[2]	15.5
TDH	0.03(0.14)	34.36	4.5

[1] Ald, aldolase; TDH, glyceraldehyde-3-phosphate dehydrogenase

[2] Values in brackets are estimated % contaminating protein as determined by enzyme assay and specific activity values of the pure enzyme.

there was no visible contaminating protein found during electrophoresis. The muscle enzyme had an identical specific activity to that measured for the liver enzyme.

II. Effects of starvation, sex and age on the physical condition of birds

The body weights (Table 3) and physical condition of each group of animals were compared. The body weight of the old and young male birds (groups I and III) were not significantly different ($P<0.01$). This suggested that the young birds were physically mature by sixteen weeks. There were no physical differences between these two groups. The female birds (group IV) were consistently heavier than the male birds however, the difference was not significant. The female birds appeared to have large fat reserves and many of the plasma samples were milky white with fat. Most of the birds had been laying eggs since they were eight weeks old. The increased body weight may be accounted for by the body fat reserves and the unlaid eggs. The starved birds (group II) showed a progressive loss of weight with the time of starvation. This loss of weight may be accounted by atrophy of the tissues. During the first seven days of starvation the body fat reserves around the abdomen were much depleted and the apparent size of the liver was markedly decreased. By ten days (Experiment 2) it was observed that the amount of breast muscle and the size of the heart, testes and liver were decreased and the fat associated with the heart and kidneys was virtually absent. The amount of plasma collected was much less in the starved birds.

III. Turnover of plasma, liver and muscle proteins

A. Plasma proteins

The half-lives of mixed plasma proteins are reported in Table 3. The female birds had a plasma half-life of 2 days. The old and young

Table 3. The body weights(g) and plasma half-lives from four groups of quail from experiment I.

Treatment	Days after injection[1]				Plasma[2] half-life (days)
	1	3	5	7	
I[3]	111 ± 6	120 ± 8	117 ± 9	120 ± 5	2.55 ± 0.13[a]
II[3]	98 ± 8	93 ± 16	85 ± 10	83 ± 8	4.77 ± 0.50[b]
III[4]	106 ± 10	110 ± 10	113 ± 7	107 ± 11	2.27 ± 0.15[ac]
IV[3]	116 ± 6	125 ± 11	131 ± 18	127 ± 12	2.00 ± 0.12[c]

1 See Materials and Methods for treatments.
2 Half-lives with different superscripts are significantly different ($P<0.01$)
3 Mean of eight observations ± S.E.
4 Mean of six observations ± S.E.

males had plasma half-lives which were 0.27 and 0.55 days longer, respectively. The starved male birds had a plasma half-life which was approximately double that of the other groups. The plasma $^{3}H:^{14}C$ ratio (Table 4) for the fed birds (group I) was significantly ($P<0.01$) larger than that for birds starved for either 3 or 10 days (groups II and III, respectively). The average ratio and the standard error of the mean for the control birds was much smaller than that from any of the treatment.

B. Liver proteins

The turnover of the soluble and insoluble liver proteins was measured in each treatment group (Fig.5). The old males (5c) and young females (5d) had turnover rates of approximately 2.8 days for both soluble and insoluble protein. The young male birds (5a) had half-lives which were almost double those of the previous two groups. The starved birds (5b) had half-lives which were much longer for the soluble fraction and slightly longer for the insoluble fraction. All fractions were initially labelled at 200 to 300 dpm/mg of protein except the starved insoluble proteins which were labelled at approximately five times that level. The turnover of soluble proteins was not significantly different ($P<0.01$) from that of insoluble proteins within each group (Table 5).

Half-lives of isolated liver proteins from fed and starved birds were compared (Table 6). The fed birds had the shortest half-life for aldolase at 2.2 days followed by the extract, breakthrough peak and salt peak protein at 3 days and fructose-1,6-diphosphatase at 4.1 days. The starved birds had the shortest half-life associated with the salt peak at 3 days, followed by aldolase at 4 days and fructose-1,6-diphosphatase, extract and the breakthrough peak at 5.7 days. Starvation, except for the salt peak, increased the corresponding protein half-lives by 1.6 to

Table 4. The effects of feeding and starvation on blood plasma $^{3}H:^{14}C$ ratios in Experiment 2.

Treatment	Plasma $^{3}H:^{14}C$ ratio[1] [2]
I	14.06 ± 1.12^{a} (4)[3]
II	9.76 ± 1.72^{b} (4)
III	6.97 ± 2.20^{b} (3)
Mean control	3.57 ± 0.32 (3)

1. All ratios are mean ± S.E.
2. Means with different superscripts are significantly different ($P<0.01$).
3. Values in brackets are the number of birds used to obtain mean.

Fig. 5. The rate of loss of label from soluble and insoluble proteins from the livers of young male (a), young starved male(b), old male (c), and young female (d) birds, which were injected with ^{3}H-leucine and measured at 2 day intervals up to 7 days.

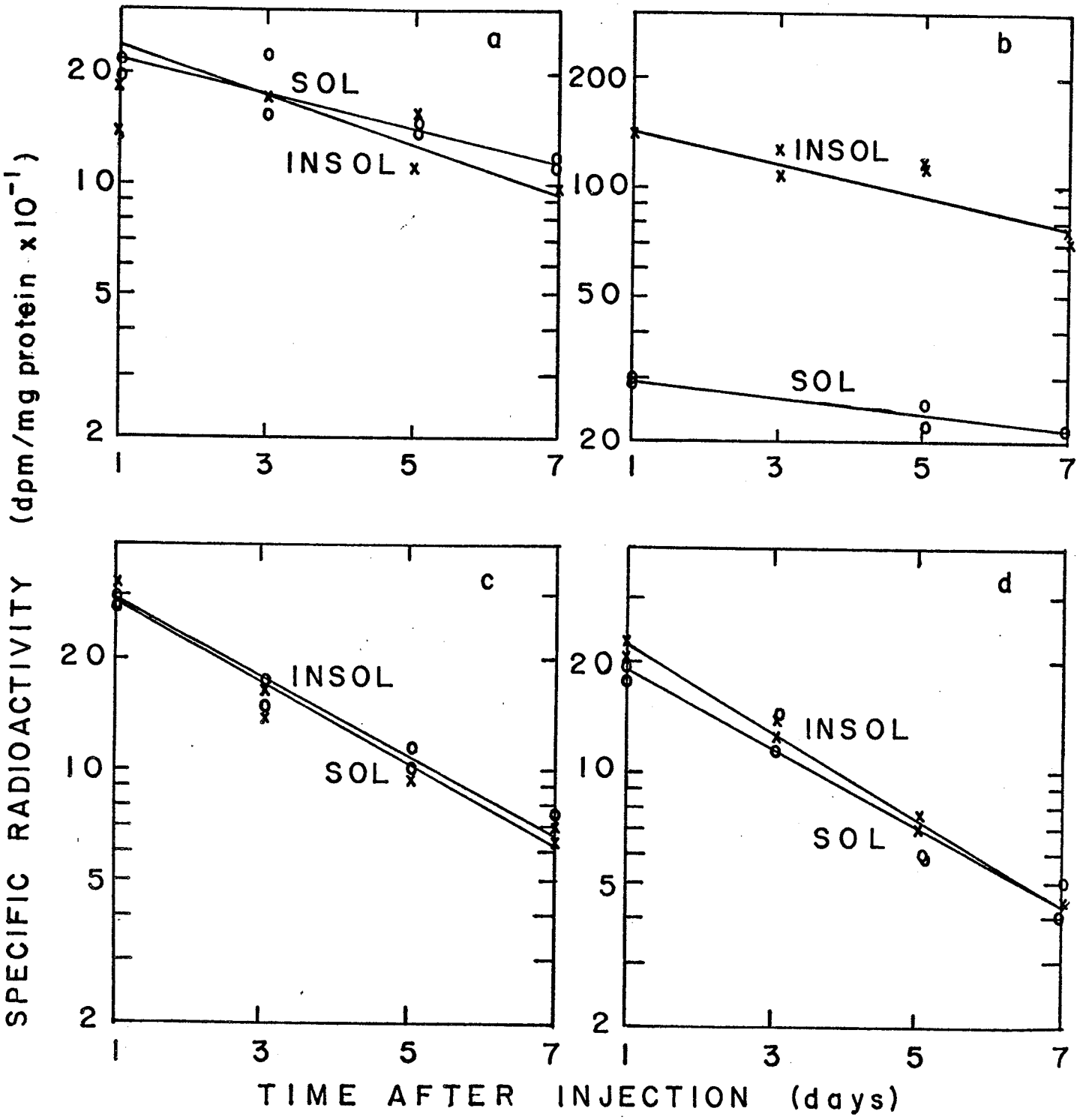

a
SOL
INSOL
b
INSOL
SOL
c
INSOL
SOL
d
INSOL
SOL
SPECIFIC RADIOACTIVITY (dpm/mg protein x10⁻¹)
TIME AFTER INJECTION (days)
2
5
10
20
50
100
200
1
3
5
7

Table 5. The half-life of the soluble and insoluble liver protein from quail exposed to four treatments.

Treatment	Half-lives (days)[1] [2]	
	Insoluble protein	Soluble protein
I	4.79 ± 1.14^{ab}	6.60 ± 1.52^{a}
II	6.80 ± 1.57^{ac}	13.56 ± 4.87^{c}
III	2.91 ± 0.28^{bd}	2.73 ± 0.28^{d}
IV	2.62 ± 0.09^{d}	2.80 ± 0.33^{d}

[1] All values are mean ± S.E.

[2] Means with different superscripts are significantly different ($P<0.01$).

Table 6. The half-life of liver soluble extracts and pure protein fractions from those extracts.

Fraction	Half-lives (days)[1] [2]	
	Group I	Group II
Extract	2.99 ± 0.25^{abcd}	5.73 ± 0.65^{de}
Breakthrough	3.10 ± 0.22^{ac}	5.94 ± 1.16^{e}
Aldolase	2.18 ± 0.16^{b}	4.03 ± 0.79^{cde}
FDPase[3]	4.12 ± 0.47^{ade}	5.69 ± 1.18^{de}
TDH[3]	> 75	> 150
Salt[3]	2.98 ± 0.18^{c}	2.96 ± 0.57^{bcde}

[1] All values except TDH are mean ± S. E.

[2] Means with different superscripts are significantly different ($P<0.01$).

[3] FDPase, fructose-1,6-diphosphatase; TDH, glyceraldehyde-3-phosphate dehydrogenase; salt, 3 M NaCl.

2.8 days. Glyceraldehyde-3-phosphate dehydrogenase appeared to turnover very slowly so that the rate was not readily measurable .

The $^{3}H:^{14}C$ ratio for each of the proteins was compared with the measured half-life (Fig.6). The starved birds (group II) showed a linear relationship between the log $^{3}H:^{14}C$ ratio and half-life of aldolase, salt peak and fructose-1,6-diphosphatase. The relationship was less linear for the fed birds (group I). As the slopes of the two lines were almost identical, the middle value (salt peak) may have a turnover rate which is more rapid than that which was measured in the fed birds. This would also allow for an increase as a result of starvation.

C. Muscle proteins

The turnover of the muscle soluble and insoluble proteins is shown in Fig.7. The old (7c) and young (7a) birds had almost the same turnover of the insoluble proteins while that of the soluble proteins was 5 days faster in the old birds. These half-life values were not considered exact because the period of measurement was much shorter than the half-life however, some trends were established in muscle. The female birds (7d) showed virtually no measurable turnover. The large dispersion of points about the lines (7d) may be the result of the level of incorporation of the labelled amino acid which were somewhat lower in both fractions of the female muscle. The starved birds (7b) showed a continual incorporation of label up to seven days. Therefore, there was no net net degradation associated with the muscle during the initial seven days of starvation.

The net degradation of aldolase, glyceraldehyde-3-phosphate dehydrogenase and extract from muscle are shown in Fig.8. The extract and aldolase showed similar rates of degradation however, there was no apparent turnover of the glyceraldehyde-3-phosphate dehydrogenase. The

Fig. 6. The relationship between the turnover coefficients (kd) for the salt peak, aldolase and fructose-1,6-diphosphatase from liver and the corresponding $^{3}H:^{14}C$ ratios. The livers used were from the fed (x) and starved (o) groups.

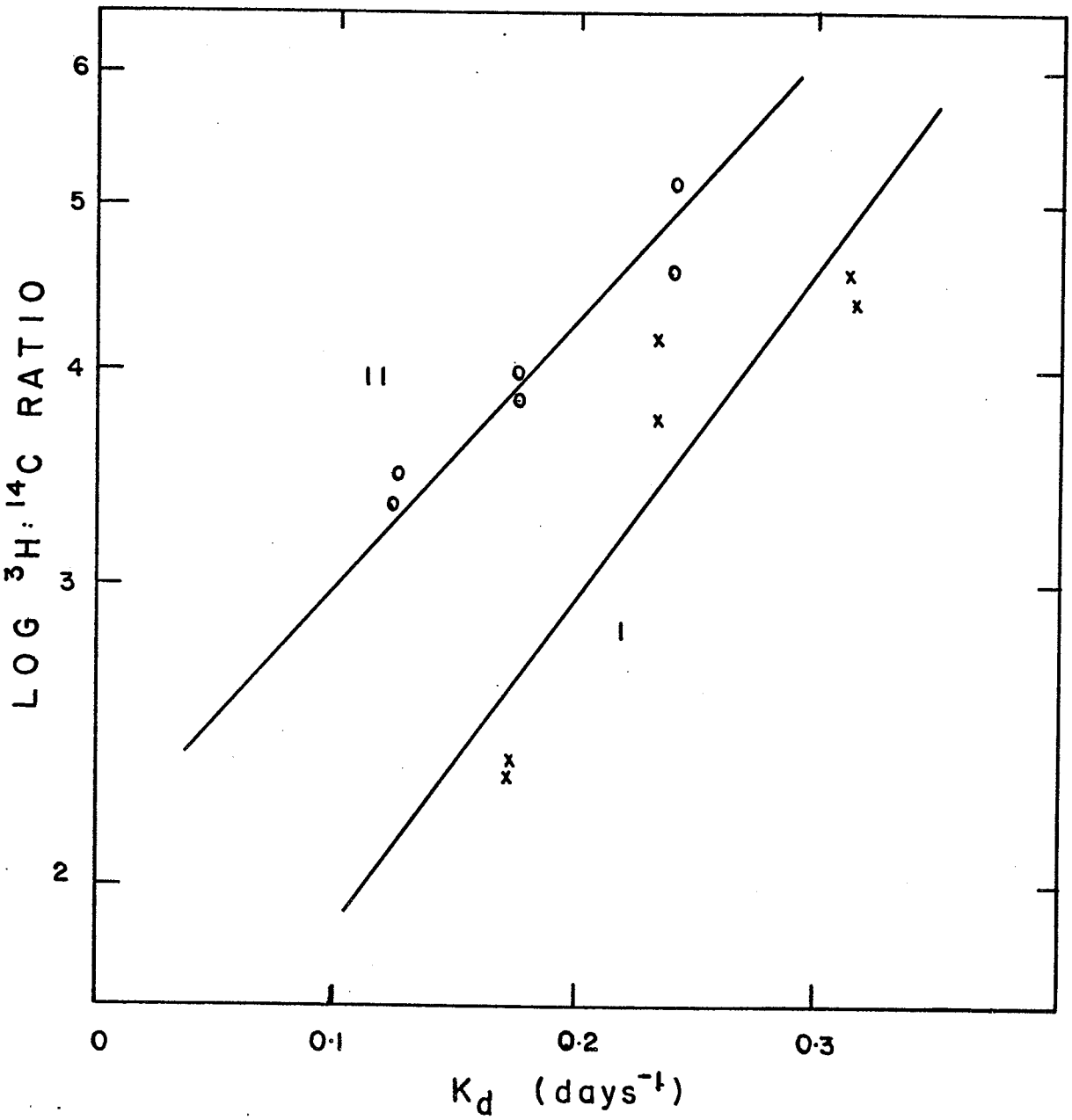

LOG $^{3}H:^{14}C$ RATIO
6
5
4
3
2
0
0·1
0·2
0·3
K_d (days^{-1})
II
I

Fig. 7. The rate of loss of label from soluble and insoluble proteins from the breast muscle of young male (a), young starved male (b), old male (c) and young female (d) birds, which were injected with ^{3}H-leucine and measured at 2 day intervals up to 7 days.

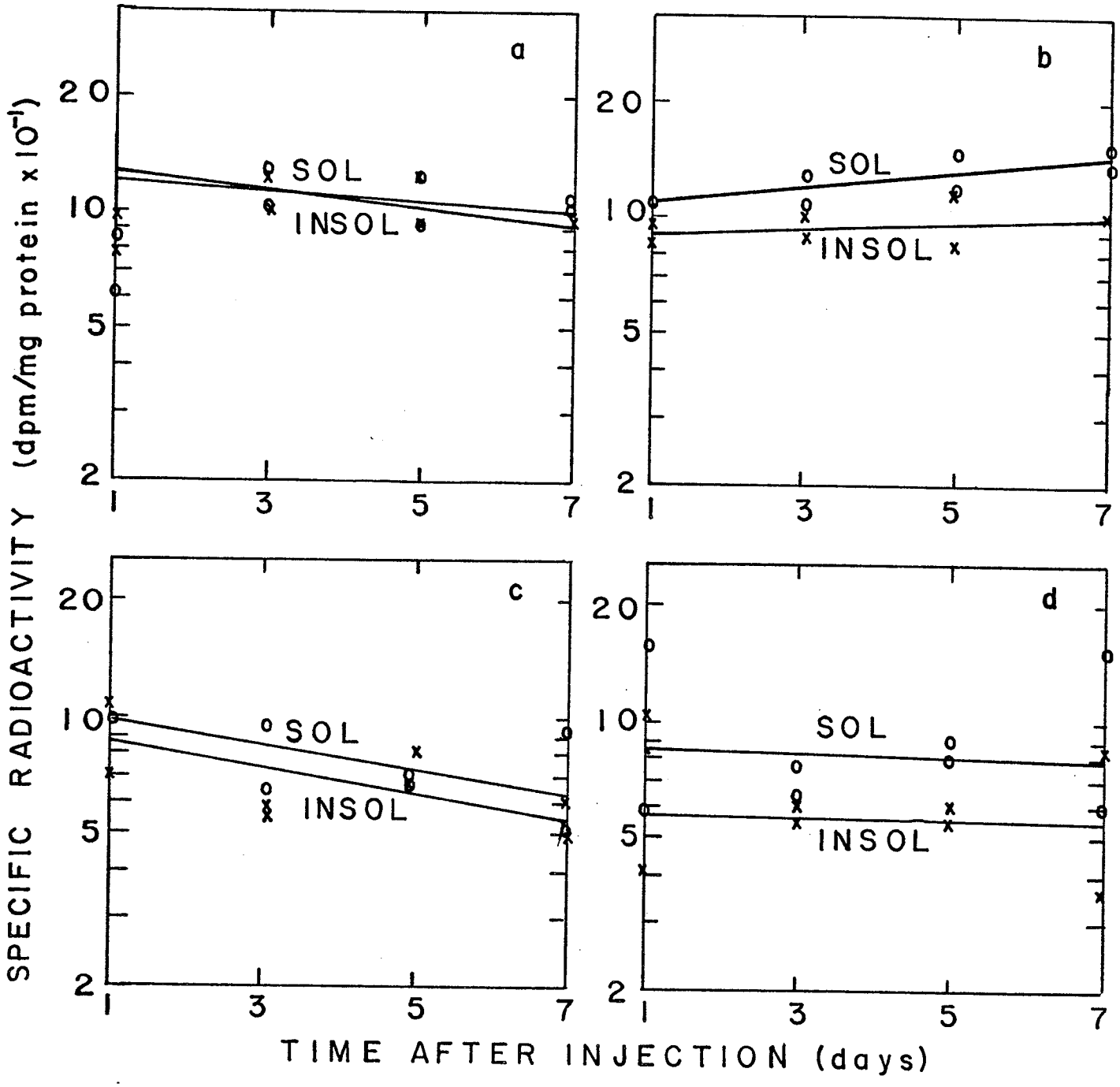
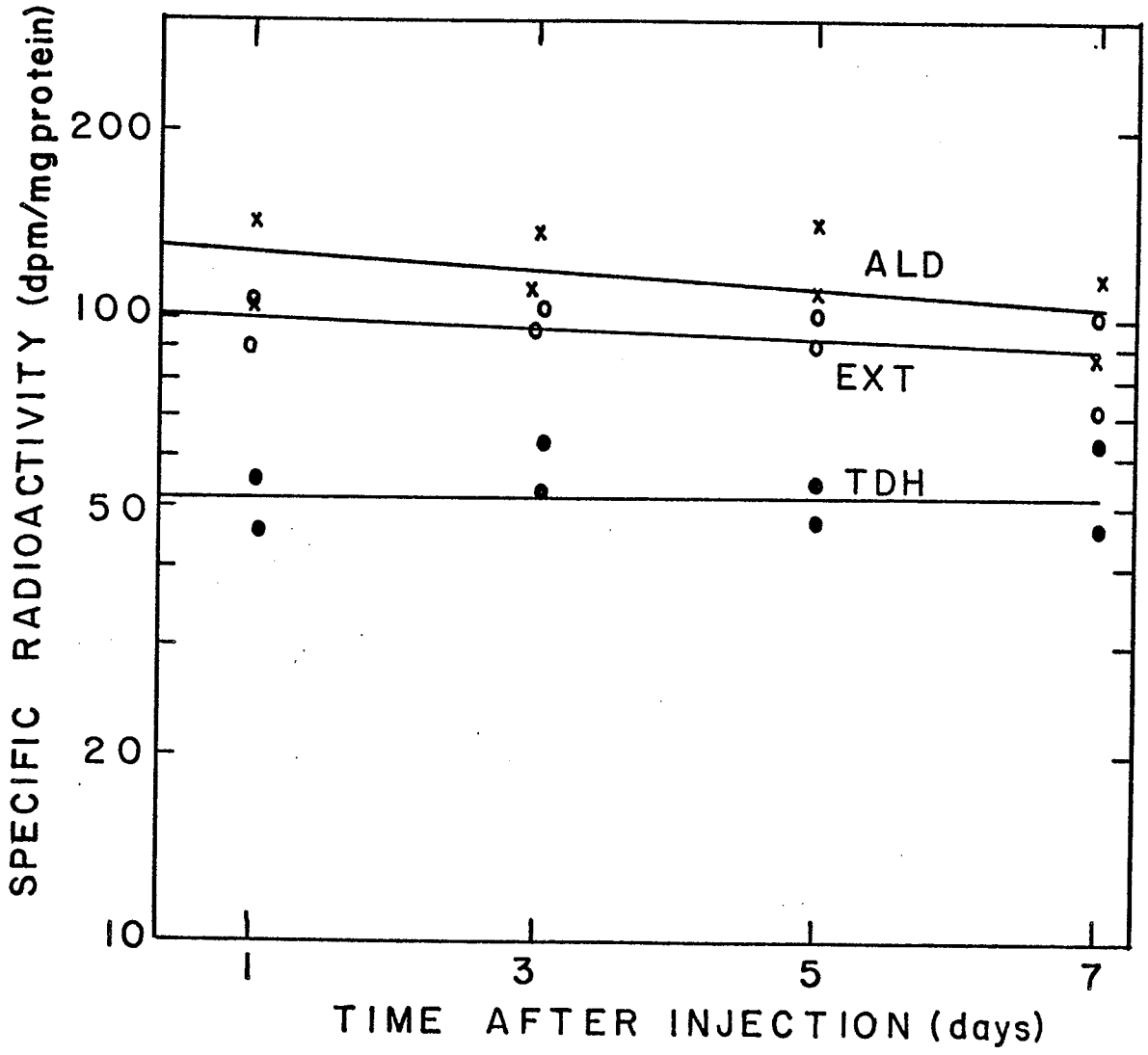

a
b
c
d
SOL
INSOL
20
10
5
2
1
3
5
7
SPECIFIC RADIOACTIVITY (dpm/mg protein x 10⁻¹)
TIME AFTER INJECTION (days)

Fig. 8. The rate of loss of label from the breast muscle proteins, extract (EXT, o), aldolase (ALD, x) and glyceraldehyde-3-phosphate dehydrogenase (TDH, ●) as measured by following the level of ^{3}H-leucine at 2 day intervals up to 7 days after injection.


SPECIFIC RADIOACTIVITY (dpm/mg protein)
200
100
50
20
10
ALD
EXT
TDH
1
3
5
7
TIME AFTER INJECTION (days)

initial level of incorporation of label (dpm/mg protein) was greatest in those proteins with measurable turnover.

IV. Metabolism of leucine

The rate of dialysis of soluble muscle proteins was measured over a six-hour period (Fig.9). Unbound ^{3}H-leucine in a muscle extract of a bird which had been injected 4 hours prior to killing, was almost completely removed after 80 minutes of dialysis. Therefore, dialysis for 90 minutes against two changes of buffer was considered adequate to remove non-protein-bound label.

A comparison of ^{3}H:^{14}C ratios (Tables 7 and 8) before and after dialysis and the percent dialyzable ^{3}H and ^{14}C in soluble liver and muscle extracts that were injected with each label 3 days prior to sacrifice would suggest that the two isotopes were being metabolized differently. This may be explained on the basis that the second step in the metabolism of leucine involves the decarboxylation of the carboxyl carbon(34). Since the two forms of leucine injected simultaneously into the control birds were [1-^{14}C] and [4,5-^{3}H]-leucine, partial metabolism of leucine labelled with ^{14}C should result in the loss of that label whereas, metabolism of ^{3}H-leucine should not remove the label.

A comparison of the amount of dialyzable ^{14}C-leucine from all birds showed that in the liver, 5% of the ^{14}C was unbound (Table 7) while in the corresponding muscle samples, there was almost no free ^{14}C. Since the ^{14}C-leucine had been injected 3 days prior to killing it may be hypothesized that the dialyzable ^{14}C represents the leucine resulting from recycling. These results would indicate that there is a higher relative level of amino acid recycling in liver than in muscle. There was much more dialyzable ^{3}H in muscle extracts from the test birds (Table 8) than

Fig. 9. The effect of dialysis of muscle soluble proteins prepared from animals injected with ^{3}H and ^{14}C-leucine at four hours and three days prior to killing, respectively.

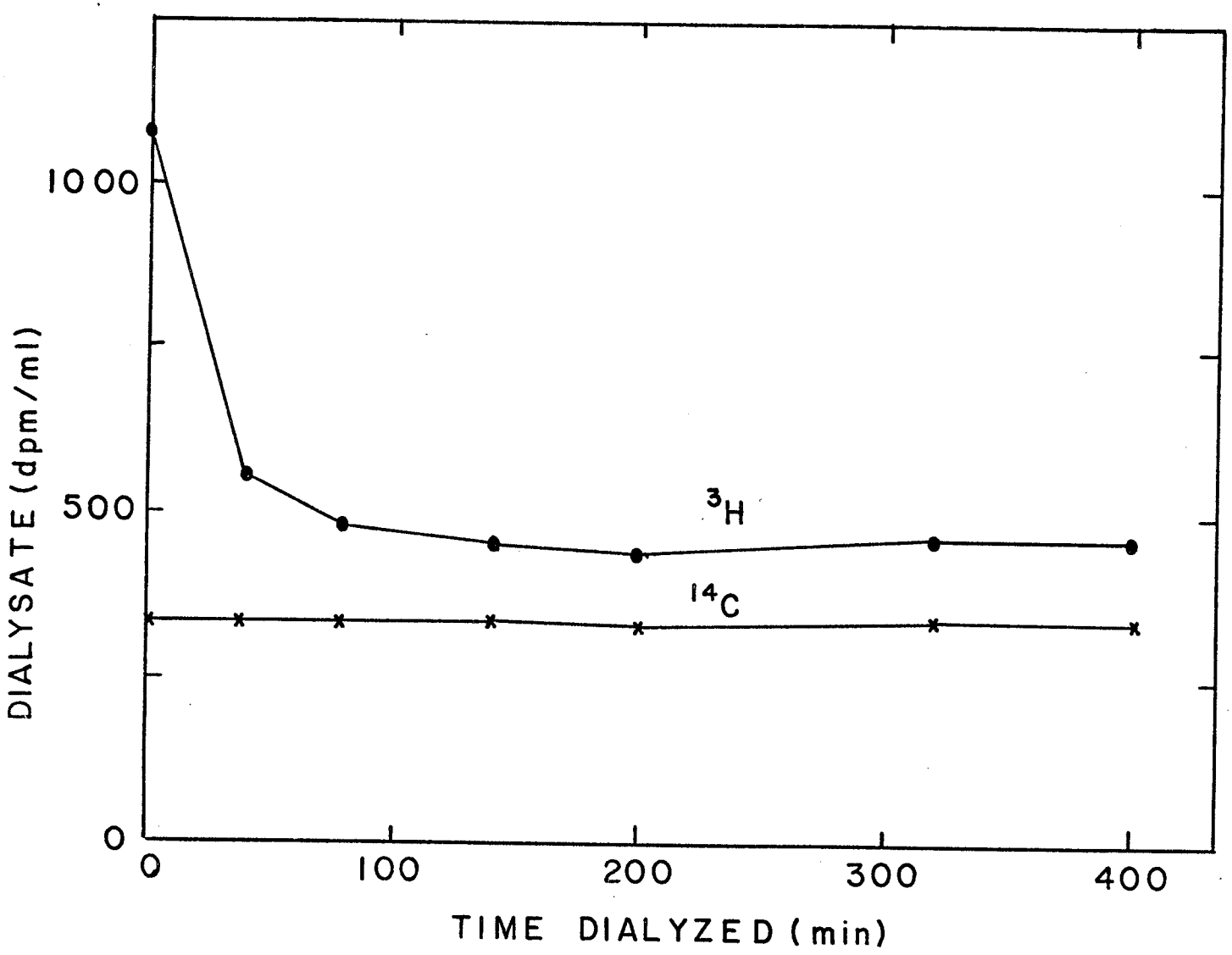

1000
500
0
DIALYSATE (dpm/ml)
^{3}H
^{14}C
0
100
200
300
400
TIME DIALYZED (min)

Table 7. The effect of dialysis on the $^{3}H:^{14}C$ ratio in liver soluble protein preparations.

Treatment	$^{3}H:^{14}C$ ratio		Dialyzable label (%)[1]	
	Pre-dialysis	Post-dialysis	^{3}H	^{14}C
I	6.61	5.00	26.4	2.6
II	4.44	3.67	23.8	8.0
III	3.72	3.18	18.6	4.9
I control	1.88	1.71	14.4	5.8
II control	1.89	1.62	18.1	4.2
III control	2.38	2.06	18.3	5.5

[1] Dialyzable values calculated allowing 4% for the sample swelling during dialysis.

Table 8. The effect of dialysis on the $^{3}H:^{14}C$ ratio in breast muscle soluble protein preparations.

Treatment	$^{3}H:^{14}C$ ratio		Dialyzable label (%)[1]	
	Pre-dialysis	Post-dialysis	^{3}H	^{14}C
I	2.79	0.85	70.3	2.1
II	5.36	1.71	68.1	0.0
III	3.70	1.51	60.7	3.7
I control	2.91	1.84	36.8	0.0
II control	3.17	1.81	36.4	0.0
III control	5.33	2.97	44.3	0.0

[1] Dialyzable values calculated allowing 4% for the sample swelling during dialysis.

there was in the liver extracts from the same birds (Table 7). Since the ^{3}H-leucine was injected into these birds 4 hours prior to killing, these differences between these tissues should reflect the relative incorporation of amino acid into tissue protein. Thus liver incorporates the amino acid more extensively than does muscle.

V. Relationship between protein size and turnover

The incorporation of ^{14}C and ^{3}H into the spectrum of liver proteins as separated by Sephadex G-200 is shown in Fig. 10. The level of radioactivity was measured as dpm per unit of absorbance at 280 nm. The incorporation of ^{3}H and ^{14}C leucine into protein was greatest in starved birds (II and III) and lowest in fed birds (I). The level of ^{3}H, in the liver proteins of birds subjected to the different treatments was more than 3 times greater than that of ^{14}C which would suggest that the turnover of liver proteins was quite high. The level of incorporation of both isotopes decreased with decreasing protein size.

Sephadex G-200 separated liver (Fig.11) and muscle (Fig.13) proteins in direct proportion to their molecular weight. The proteins were separated into two distinct peaks. The initial peak was eluted with the same volume of buffer as was blue dextran, therefore, it was used as an internal standard for the void volume (Vo). Peaks of aldolase and glycerophosphate dehydrogenase were measured in each sample. Ferritin and cytochrome c were also used to calibrate the column. The log of each molecular weight was plotted against the corresponding relative elution volume (V/Vo) and the relationship was found to be linear for both tissues.

The size of the $^{3}H:^{14}C$ ratio has been shown to be inversely proportional to the half-life of the protein being measured(3,22). The apparent turnover of liver proteins as estimated from the $^{3}H:^{14}C$ ratios

Fig. 10. The amount of ^{3}H and ^{14}C (dpm / mg protein) in the spectrum of proteins separated by Sephadex G-200 from livers of fed (I), starved for 3 days (II) and starved for 10 days (III) groups of birds as plotted against the relative elution volume (V/Vo).

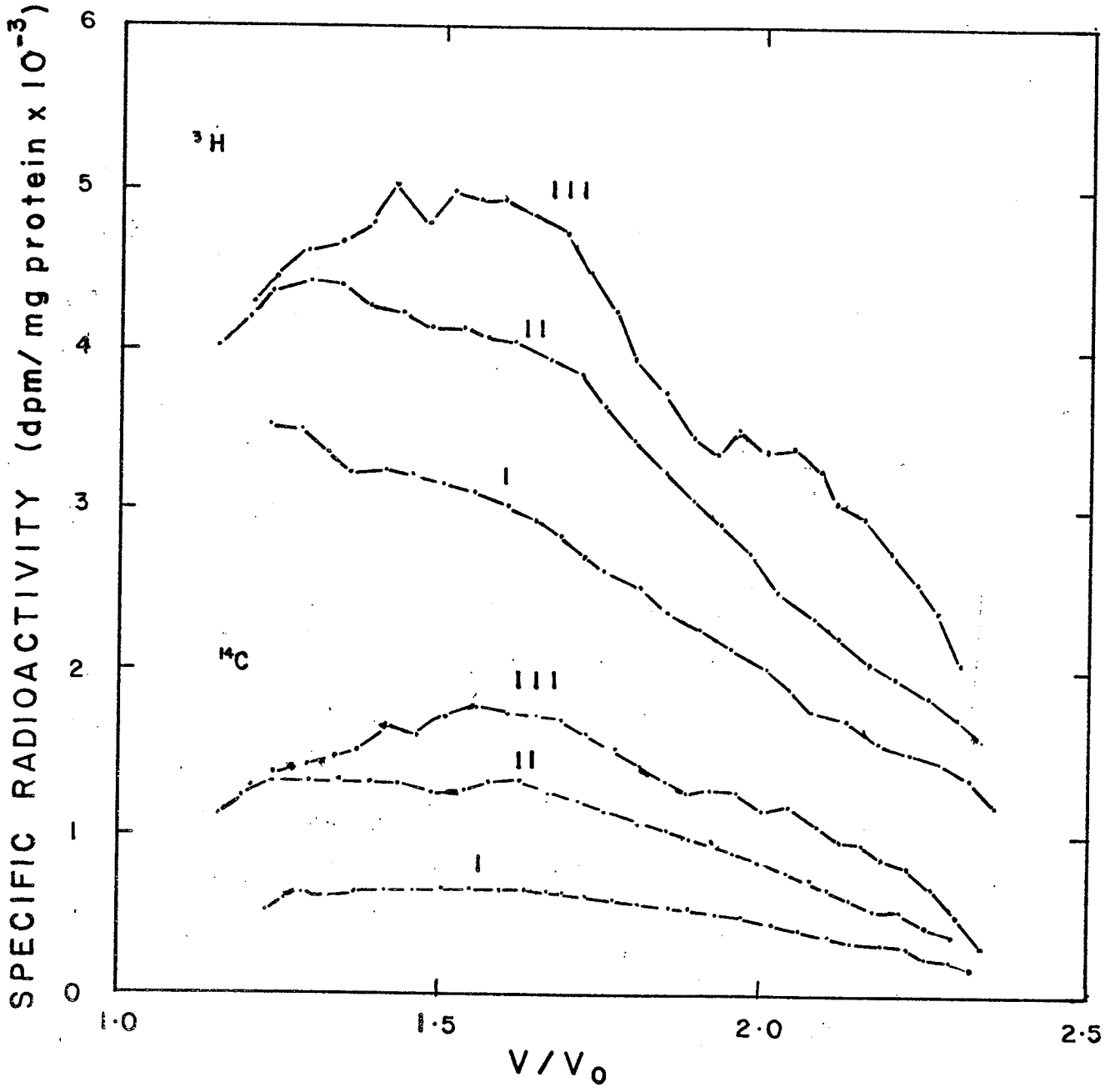

SPECIFIC RADIOACTIVITY (dpm/ mg protein x 10^{-3})
^{3}H
^{14}C
III
II
I
III
II
I
0
1
2
3
4
5
6
1·0
1·5
2·0
2·5
V / V_0

Fig. 11. Separation of liver proteins using Sephadex G-200. The protein profile and the $^{3}H:^{14}C$ ratios from the fed and starved groups (I,II and III) are shown with the typical molecular weight standard curve constructed using ferritin (a), aldolase (b), glycerophosphate dehydrogenase (c) and cytochrome c(d). Also shown are the $^{3}H:^{14}C$ ratios for the control birds.

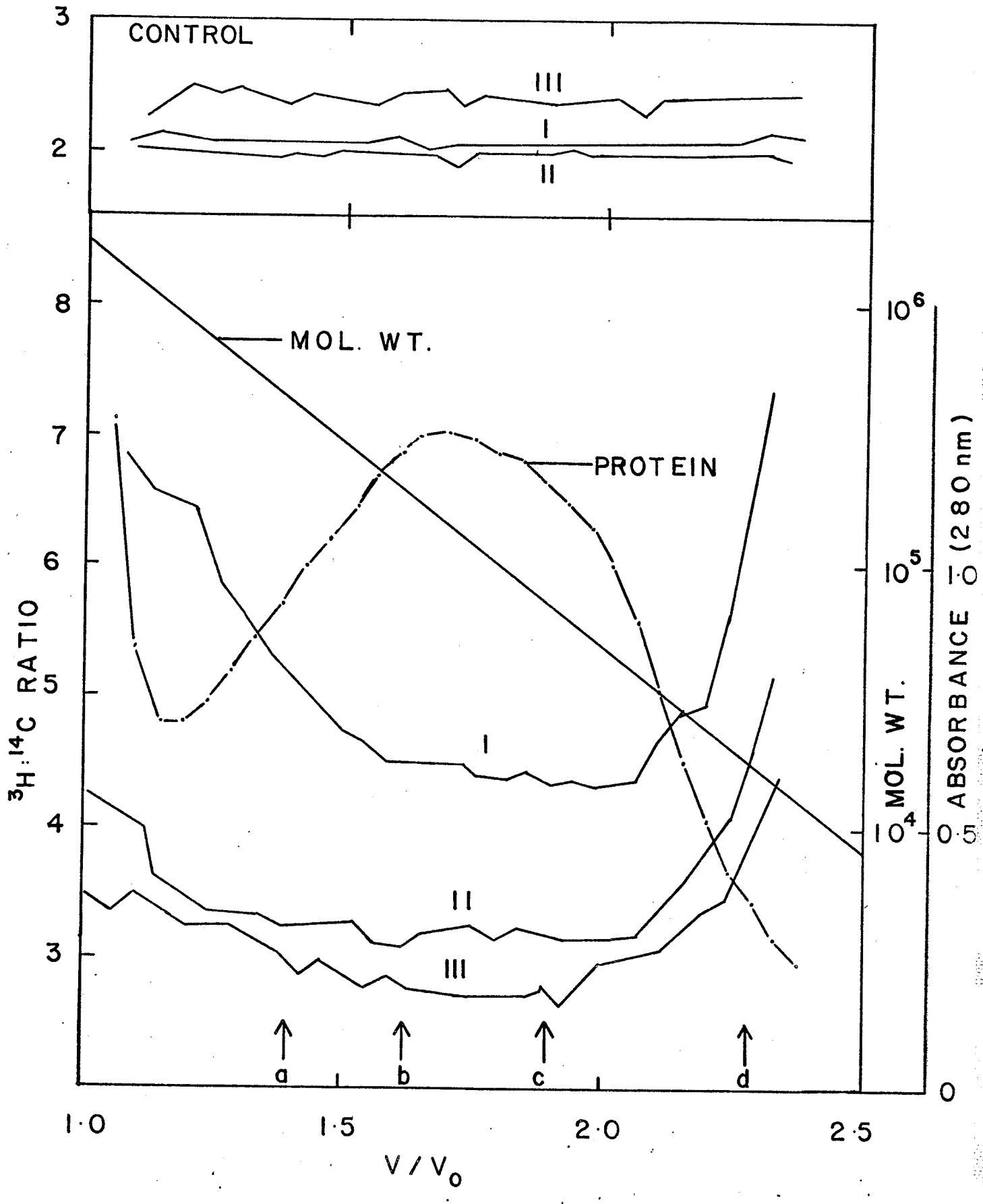

CONTROL
III
I
II
MOL. WT.
PROTEIN
$^3H:^{14}C$ RATIO
MOL. WT.
ABSORBANCE (280 nm)
V / V$_o$
a
b
c
d

Fig. 12. The amount of ^{3}H and ^{14}C (dpm / mg protein) in the spectrum of proteins separated by Sephadex G-200 from the breast muscle of fed and starved groups of birds (I, II and III).

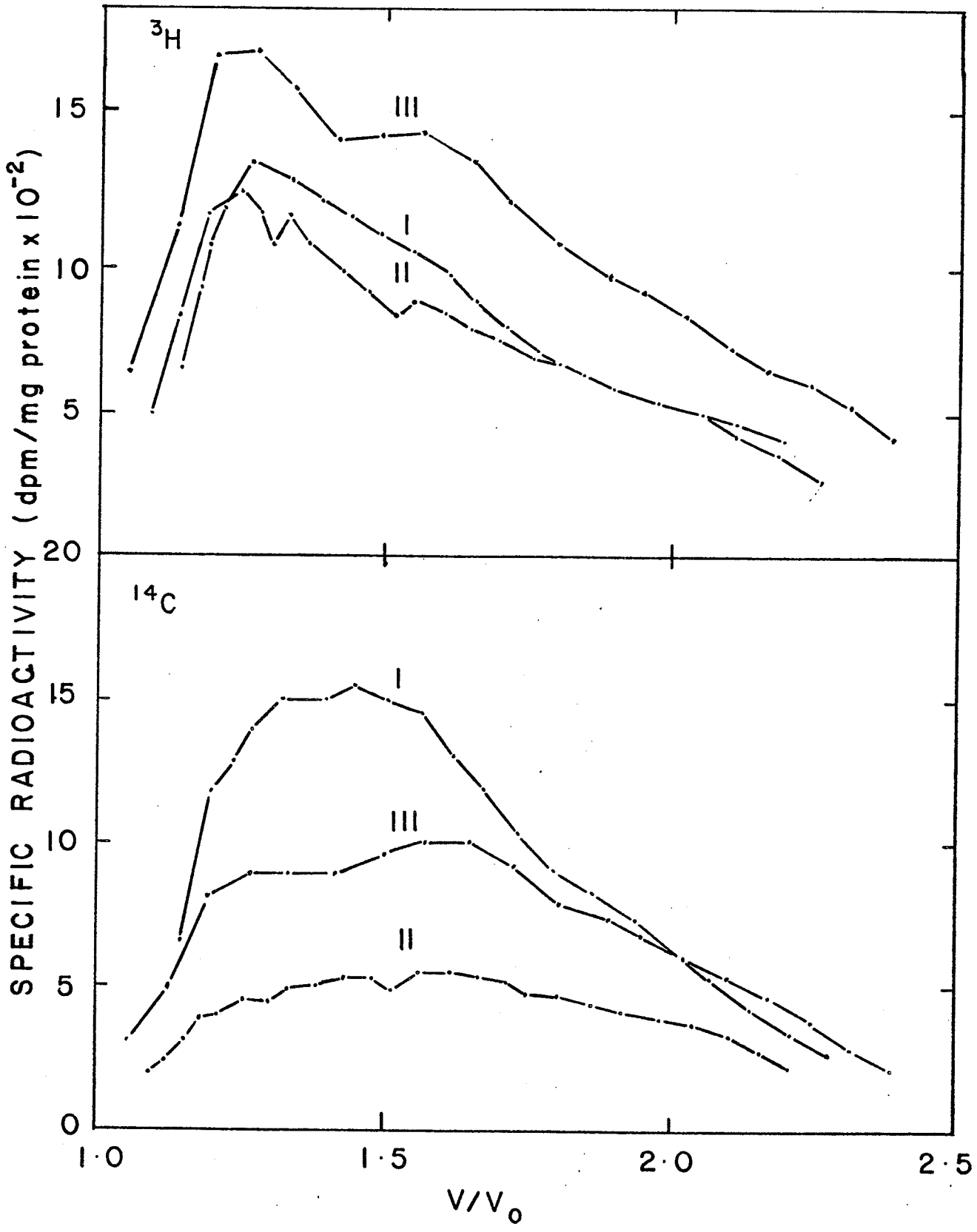
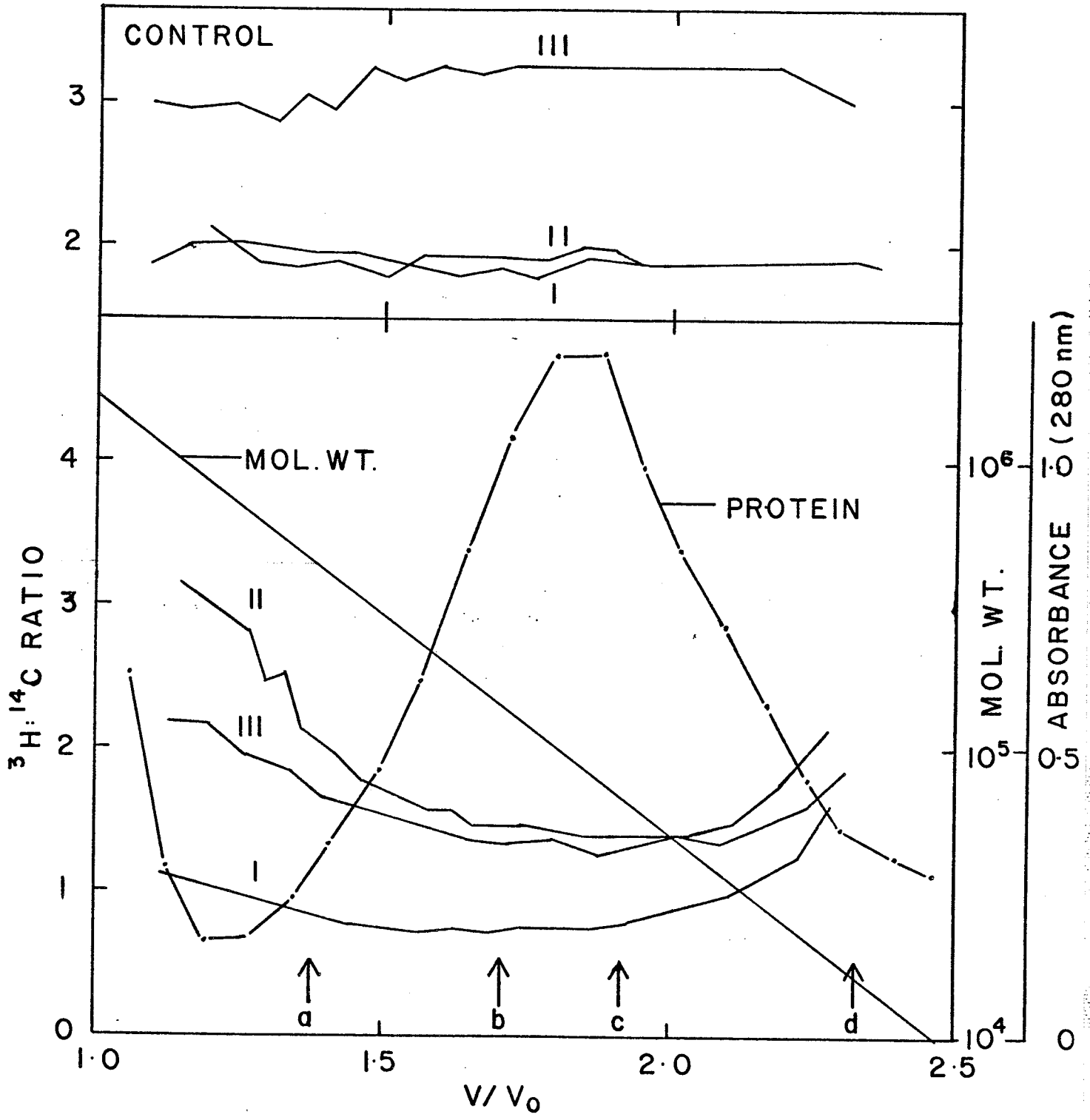

^3H
III
I
II
15
10
5
20
^{14}C
I
III
II
15
10
5
0
SPECIFIC RADIOACTIVITY (dpm/mg protein x 10^{-2})
1·0
1·5
2·0
2·5
V/V_o

Fig. 13. Separation of breast muscle proteins using Sephadex G-200. The protein profile and the $^{3}H:^{14}C$ ratios from the fed and starved groups (I,II and III) are shown with the typical molecular weight standard curve constructed using ferritin (a), aldolase (b), glycerophosphate dehydrogenase (c) and cytochrome c (d). Also shown are the $^{3}H:^{14}C$ ratios for the control birds.


CONTROL
III
II
I
3
2
MOL. WT.
PROTEIN
$^3H:^{14}C$ RATIO
MOL. WT.
ABSORBANCE (280 nm)
10^6
10^5
10^4
0·5
0
4
3
2
1
0
a
b
c
d
1·0
1·5
2·0
2·5
V/ V_0

was much more rapid in the fed birds than in either group of starved birds (Fig.11). The proteins from all groups appeared to be divisible into three size categories. The large molecular weight proteins (>160,000) showed increasing $^{3}H:^{14}C$ ratios and therefore, increasing turnover rates with increasing molecular size while the small proteins (<40,000) showed increasing ratios with decreasing molecular size. The intermediate group showed a roughly constant ratio over the entire range. The control birds showed a constant ratio over the entire spectrum which was used as evidence for uniform handling of both radioactive forms of the amino acid.

The incorporation of label into the breast muscle proteins was also measured (Fig.12). There was more ^{14}C in proteins from the fed birds (I) than from either starved group. The protein-^{3}H was almost identical in the fed and starved group (II) however, the extensively starved group (III) had somewhat higher incorporation. As with liver, the muscle tissue showed a decreased level of label as the molecular size decreased. The $^{3}H:^{14}C$ ratios for the spectrum of muscle proteins separated by Sephadex G-200 were greater in both groups of starved birds than in the fed group. There also appeared to be three categories of protein which produced a pattern similar to that found in the liver, however, the magnitude of the increased ratios was not as great as that in liver.

DISCUSSION

I. Female protein turnover

Female rats were shown to have shorter half-lives for serum and liver proteins and a longer half-life for muscle proteins when compared with male rats(72). The synthetic rate of liver catalase was slower in female rat liver than in male rat liver however, there was no difference in the degradative rate(53). The data presented for plasma (Table 3), liver (Fig. 5) and muscle (Fig. 7) proteins agreed with the trends reported for rats. There was no metabolic stress applied to the female rats of the preceeding experiments however, the female quail were actively laying eggs. The differences between male and female birds were much greater than those of rats. The level of label in the female muscle proteins was much lower than that in the male muscle proteins (Fig. 7). This was thought to occur because much of energy and protein components were being channelled into egg production, hence, there will be less labelled amino acid available for muscle metabolism. The rechanneling of nutrients may make the increased muscle half-lives a necessity in female birds or it may be a reflection of reduced recycling of labelled amino acid.

II. Effect of age on protein turnover

The turnover of liver and serum proteins was shown to be slower in older rats when compared to younger, lighter rats on the same dietary treatment(72). The muscle tissue from older rats had a slower rate of protein synthesis than did muscle from young growing rats(72,41). Total body protein turnover was also slower in older rats(75). Each of these articles compared young growing rats to older mature rats and established differences, however, these differences were more likely

related to the requirements for growth than a response to old age. Moog(42) cited many instances of changes in specific proteins as a result of aging and concluded that the most likely regulating factor was a decline in the supply of hormones and other factors in the blood. In the present study, plasma (Table 3), liver (Fig. 5) and muscle (Fig. 7) proteins all had shorter half-lives in the old birds than they did in the young birds. Since the body weights were not significantly different (Table 3), the comparison must have been made between groups of mature birds. The differences between plasma, muscle and insoluble liver proteins were not significant and therefore, may have reflected natural variation. The differences also may have been due to lower recycling in the old birds which in turn could be the result of factors such as hormones, feed intake and basal metabolic rate.

III. Protein turnover and recycling

Specific glycolytic enzymes have had their half-lives measured by following the rate at which the enzyme loses radioactivity after a single pulse administration of labelled amino acid. Muscle aldolase was found to have a half-life of 20 days in the rat(54). This was of the same order of magnitude although slightly longer than that determined for quail muscle aldolase (Fig. 8). Rabbit muscle glyceraldehyde-3-phosphate dehydrogenase had a reported half-life of 100 days(53), a value not inconsistent with the line in Fig. 8. Rat liver aldolase and glyceraldehyde-3-phosphate dehydrogenase had half-lives of 4.9 and 5.4 days, respectively(35). The value calculated for quail liver aldolase was 2.2 days (Table 6) whereas glyceraldehyde-3-phosphate dehydrogenase had an unmeasurably slow rate of isotope loss.

The differences between the half-lives observed in this study

and similar values in the literature may be the result of amino acid reutilization. It was shown that approximately 50% of the liver and 30% of the muscle amino acid pools in rats were derived from protein catabolism and was available for recycling(18). Poole(51) has shown that ^{3}H-leucine will extend true protein half-lives of 0.1 to 2 days to apparent half-lives of 3 to 4 days. This extension was due to reutilization of the amino acid. Half-lives of rat liver subcellular particles and enzymes were found to be substantially greater using ^{3}H-leucine than they were using [^{14}C-guanidino] arginine(22).

Swick(63) suggested that [^{14}C-guanidino] arginine was ideal for measuring mammalian liver protein turnover because the level of hepatic arginase was sufficient to metabolize most of the arginine before it is reutilized. The level of chicken liver arginase was about 0.2% of that found in rat liver(61). The purpose of avian arginase is for the production of ornithine rather than urea(65). Therefore, arginine is not a satisfactory label to overcome the effects of recycling in species where the primary excretory form of amino nitrogen is uric acid.

The ^{3}H:^{14}C ratio method has been shown to separate proteins in direct proportion to the corresponding turnover rates(3,22). One precaution that must be taken in working with ^{3}H:^{14}C ratios is to be certain that individual proteins are pure. Although the relative turnover of proteins can be determined using ^{3}H:^{14}C ratios, absolute turnover rates can not be calculated for birds, because there are no methods which have been developed to produce absolute standards. Poole(51) has also warned that trace contamination by a protein with a very rapid turnover may make two proteins having different half-lives to appear the same or

conversely, two proteins having identical half-lives appear to be different. The results of the present study demonstrated that the degree of purity of aldolase and fructose-1,6-diphosphatase was good while that of glyceraldehyde-3-phosphate dehydrogenase did not agree with data presented in the literature(50,1) and was therefore, suspect. This would suggest that the turnover rates of the pure proteins would not be greatly modified by impurities having somewhat different turnover rates.

IV. Starvation and protein turnover

The response of the body to any form of external stress is to alter the overall metabolism of the body to counteract the harmful effects of that stress. In the case of starvation, all inputs of protein and energy cease dramatically and the body must alter its metabolism to restrict the rate at which components are lost by being less extravagant with the available energy. Siekevitz(60) showed that the energy expenditure for maintaining protein turnover may actually be the most economic method for the body to conserve energy and still respond to alterations in tissue metabolic requirements. During starvation, an expenditure of energy equivalent to that of the normal steady state condition may be considered extravagant.

Starvation causes the animal to begin mobilization of the labile protein reserves described by Munro(45). It has been shown that liver protein is much more labile(68) than muscle protein(69). Gan and Jeffay(18) showed that initially rat liver protein reserves were mobilized. For the first three days of starvation the amount of plasma lysine which contributed to liver metabolism was decreased from 50% to 10% but after this time the value again approached 50%. Also, over this 3-day period the liver lost 35% of its weight and remained relatively constant

thereafter. The muscle obtained progressively less plasma lysine until by day seven it had fallen from 60% to 30%. This demonstrated that liver was able to absorb the initial shock to the system while muscle became involved only when the stress became somewhat more lasting.

Severe starvation decreased the rate of synthesis of rat liver catalase but it did not affect the rate of degradation(52). Two days of starvation did not appear to decrease protein synthesis in rat liver, however three days of starvation brought about a general 20% reduction(40). It was also shown that total muscle synthesis decreased more than total liver synthesis, whereas total muscle catabolism decreased less than total liver catabolism. Based on the relative sizes of rat liver and muscle it may be seen that during the initial stages of starvation, a unit weight of muscle may produce less effect than the same amount of liver(18), but the total body requirements are met by the muscle tissue because it is so much greater in mass(40).

The condition of the well fed quail at the beginning of starvation may be quite different from that of the rats used elsewhere. The quail had considerable reserves of body fat around the organs and lining the abdominal cavity. It required seven days of starvation to remove most of the fat and show a definite atrophy of the organs (liver, testes, breast muscle). It appeared that the half-life of liver proteins was substantially increased during starvation (Fig.5, Table 6). If protein is not required for energy production, then a decrease in turnover could be a mechanism to conserve the amino acids before they are required. The level of incorporation of labelled amino acid into liver proteins tends to show net conservation (Fig. 10). The relative amounts of ^{14}C in protein from fed (group I) and starved (group II) birds shows that either degradation of the liver protein has been

decreased or the amino acids from the degraded proteins are all being reutilized when the liver is decreasing its size during the first 3 days of starvation. The latter case would result in a constant level of specific radioactivity because unlabelled amino acid would be sacrificed at the same rate as labelled amino acid. Gan and Jeffay(18) reported that liver size decreased 35% over three days while Millward(40) showed only 20% reduction in the rate of synthesis. If these conditions prevailed in the quail then the same dose of ^{3}H-leucine into a starved or non-starved bird should result in somewhat higher level of incorporation into protein of the starved birds on the basis of dose per unit of tissue size. This was the case in Fig. 10.

Muscle tissue (Fig. 7) appeared to accumulate label as a result of starvation. This may be the result of a recycling effect being offset by a decrease in tissue size. The ^{3}H:^{14}C ratios (Fig. 13) from muscle proteins indicate that the turnover of protein in starved animals is faster than in non-starved animals. The relative levels of incorporation of ^{3}H and ^{14}C for groups I and II would suggest that the rate of muscle synthesis is not changed whereas the rate of muscle degradation is substantially increased during starvation.

One major consideration in turnover studies should be the measurement of synthesis and degradation under steady state conditions. Jones and Mayer(32) showed that the level of intestinal enzymes began adjusting to a new steady state almost immediately after the onset of starvation. Arias et al.(3) described the equation for adjustment to another steady state. The process of starvation becomes progressively more severe with time so that a new steady state condition can not be established even though over a long enough period, turnover may approach steady state.

The $^{3}H:^{14}C$ ratios for groups II and III (Figs.11 and 13) show nearly the same values suggesting that the birds under ten days of starvation were approaching a new steady state.

V. Molecular size and protein turnover

It was demonstrated that there was a correlation between increased protein half-life measured as decreased $^{3}H:^{14}C$ ratio and decreasing protein molecular weight(12). Further work revealed that this correlation was better when half-life and protein subunit size were compared (13-15). Separation of proteins from liver (Fig.11) and muscle (Fig. 13) soluble protein extracts using Sephadex G-200 showed partial agreement with the literature. Very large proteins (>160,000) showed a decrease in $^{3}H:^{14}C$ ratio which was directly proportional to the decrease in molecular size. Intermediate and small proteins (<160,000) did not demonstrate this phenomenon. It is of interest that one isolation of non dissociated rat liver proteins(15) on Sephadex G-100 produced a pattern very similar to that in Fig. 11 however, the molecular weight of the standards were considerably smaller than those used in the present study. Thus it would appear that molecular size may be a factor in determining the turnover rates for large proteins.

Dice et al.(15) showed that protein fractions of size ranges from 20000 to 100,000 were progressively more subject to degradation by pronase as the molecular weight increased. Comparison of the protein elution pattern obtain for rat liver on Sephadex G-100 and that of quail liver on Sephadex G-200 (Fig. 11) shows that a large group of high molecular weight compounds (>100,000) were not adequately resolved by the G-100 material. Bond(10) showed that five pure proteins of known

molecular weight and known half-life did not show a correlation between these parameters nor did they correlate to the rate of degradation by four proteolytic enzymes. The molecular weight of chicken aldolases (37,38) fructose-1,6-phosphatase(48) and glyceraldehyde-3-phosphatase dehydrogenase(4) are within 20,000 of one another however, the rates of degradation (Table 6, Fig. 8) and $^{3}H:^{14}C$ ratios are definitely not equal for these proteins. Thus, it would appear that proteins of intermediate and low molecular weight do not show a direct relationship between molecular size and protein degradation rate.

Those reports (13-15) which showed a correlation between half-life and subunit size had the protein separation conducted on acrylamide of varying concentration using bromphenol blue as a tracking dye. This tracking dye moves through the gel quite rapidly and may be completed before the gradient in acrylamide concentration has been able to complete the molecular seiving process. Electrophoretic mobility at any given pH is related to the molecular charge at that pH and to a very small extent, to the molecular weight. Therefore, it may be the case that molecular charge as well as molecular weight is responsible for the differences in half-life.

It was shown that arginase was stabilized against lysosomal proleolytic attack by Mn^{2+} and certain amino acids to a far greater extent than was alanine amino transferase(26). It was also shown that deficiency of certain amino acids influenced the aggregation of polysomes(31) as well as the rate of protein synthesis(6) and degradation(73). Therefore, it may be concluded that the entire intracellular environment must play an important role in regulation of the rates synthesis and degradation of protein.

CONCLUSIONS

1. The turnover of female muscle proteins was much less than male muscle proteins while female liver proteins were substantially faster. This was thought to be the result of higher metabolic activity associated with the laying of eggs.

2. The turnover of proteins from both tissues in older birds were faster than in younger birds however, the differences were not significant and no reasons were established to account for the differences.

3. The purified proteins, liver aldolase, muscle aldolase and muscle glyceradehyde-3-phosphate dehydrogenase had measured half-lives which were similar to values reported elsewhere in the literature while the dehydrogenase from liver was considerably different. Except for the liver dehydrogenase, the other enzymes including fructose-1,6-diphosphatase were considered to be adequately pure to allow direct comparison of the calculated turnover rates.

4. Starvation increased the half-life of liver proteins and decreased the half-life of muscle proteins. The primary response to starvation appeared to involve progressive degradation of the tissues to provide nutrients for maintenance over the time when there was a severe stress placed on the system. The turnover of liver protein was decreased to maintain the integrity of the tissue whereas the turnover rate of muscle proteins was increased to provide amino acids for the rest of the body.

5. There was some correlation between decreasing molecular weight of very large proteins (>160,000) and increasing half-life as determined by $^{3}H:^{14}C$ ratio. The intermediate and low molecular weight proteins do not show this relationship, suggesting that there may be a correlation between molecular size and turnover rate but there must be also other controlling factors involved.

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