EVALUATION OF THE NUTRITIVE QUALITY OF RAPESEED PROTEIN FOR THE RAT AND HUMAN

by

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Two experiments with growing rats and a human metabolic study were conducted to evaluate the nutritional quality of rapeseed protein.

Utilization of a low-thioglucoside-content rapeseed meal (LoRSM) by growing rats was compared with that of a high-thioglucoside-content rapeseed meal (HiRSM) and casein (Cas). Each protein source served as the sole source of dietary nitrogen (2.4%). In Experiment 1, 3 males and 3 females were allotted to each diet. Apparent protein digestibility was lower for the rapeseed meals (80 and 82%) than for casein (94%). However, 4-day nitrogen retention was significantly lower ($P<0.05$) only for rats fed HiRSM (169, 440 and 526 mg for HiRSM, LoRSM and Cas resp.). In Experiment 2, 48 rats were allotted to the same 3 diets for an 8-week growth study. Weight gains by rats fed Cas or LoRSM was nearly 3 times that of animals fed HiRSM. Thyroid size (mg/g body weight) was similar for the LoRSM and Cas groups whereas a pronounced thyroid enlargement as well as increased size of liver, adrenals, kidneys and testes were observed in rats fed HiRSM. Histochemical examination of hepatic tissue revealed marked changes in activity and localization for acid and alkaline phosphatases and adenosine triphosphatase of animals fed HiRSM but only slight changes in the patterns of rats

fed LoRSM. Males fed LoRSM and both sexes fed HiRSM exhibited lipid infiltration in the liver. The results suggest that thioglucosides are responsible for the poor performance reported for animals fed rapeseed meal.

The human metabolic study was undertaken to evaluate the potential of rapeseed flour (RSF) as a protein supplement in an all-vegetable diet for the human. Four subjects were randomly allotted to the RSF diet and 4 subjects to a vitamin-free casein (Cas) diet in a cross-over experiment. Four subjects, two at each period, also were assigned to a wheat gluten (WG) diet. Each protein source, rapeseed flour, casein and wheat gluten was incorporated into baking powder biscuits at a level to provide 20 g of protein per day (40% of daily intake) during the two 7-day periods. Four-day metabolic studies showed that apparent protein digestibility was significantly ($P<0.01$) lower for the RSF diet than the Cas diet (81% and 86%, respectively). However, N retention data showed that all subjects, except one, were either in N equilibrium or positive N balance regardless of diet consumed. Since amino acid analyses revealed each diet to provide adequate quantities of essential amino acids, except methionine and valine, caution must be exercised in interpreting these results.

TABLE OF CONTENTS

Table of Contents (cont'd)

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LIST OF TABLES

LIST OF FIGURES

I. GENERAL INTRODUCTION

Rapeseed meal is a valuable by-product of the rapeseed oil industry. Rapeseed is Canada's largest and most important oil crop, occupying 1.62 million acres in Western Canada in 1969 (Bell et al. 1967; Canada Year Book 1969). The meal is potentially an excellent protein supplement for farm livestock and poultry since its high quality protein is comparable to soybean. Unrestricted use of rapeseed meal as livestock feed has been hampered, however, by the poor performance observed when high levels of rapeseed meal are fed.

Nutritional problems have been encountered in the development of nearly every oil seed and rapeseed is no exception. Soybean possesses a trypsin inhibitor, cottonseed has gossypol and flax a cyanogen factor (Liener 1966). The toxic principles present in rapeseed mainly arise from the thioglucosides, which on hydrolysis by the enzyme myrosinase, yield isothiocyanates, oxazolidine-thione and nitriles. These hydrolytic products, of which oxazolidine-thione is believed to be responsible for the thyroid enlargement in non-ruminants (Jackson 1969), have also been reported to adversely affect growth and reproduction when high dietary levels of rapeseed are consumed by animals (Bowland et al. 1965).

The potential for rapeseed production, processing and use by food and feed manufacturers is great. During the last two decades, RSM has evolved from use mainly as a fertilizer to an important protein supplement for livestock and poultry. Although much has

been done to improve rapeseed, much still remains to be accomplished. Expanding research in breeding new rapeseed varieties, changes in processing methods and increased knowledge of the nutritional properties of rapeseed meal (RSM)* suggests that if success is achieved, rapeseed could mean as much to Canadian agriculture as the soybean has meant to the United States.

Two exciting developments during the past two years, the discovery of thioglucoside-free varieties and the development of a process for removing thioglucosides has made possible the complete elimination of these compounds from rapeseed protein supplements. The Food Research Institute of the Canada Department of Agriculture has reported a water extraction process for the production of thioglucoside-free rapeseed flour (RSF)**. The availability of tailor-made thioglucoside-free rapeseed varieties, and edible, nutritious rapeseed flours, concentrates and/or isolates may truly transform rapeseed into a "Cinderella crop".

The work presented in this thesis attempted to evaluate a low-thioglucoside-content rapeseed meal and rapeseed flour. Experiments with rats were prompted by the availability of a low-thioglucoside-content rapeseed meal and the possibility that thioglucosides may be directly responsible for the deleterious effects observed with the feeding of rapeseed meal. Since the nutritional value of rapeseed flour was essentially unknown, a human

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* Subsequently, the abbreviation RSM will refer to rapeseed meal.

** Subsequently, the abbreviation RSF will refer to rapeseed flour.

metabolic study attempted to evaluate its potential as a protein supplement in an "all-vegetable" diet for the human.

II. OBJECT OF RESEARCH

The presence of free hydrolyzed thioglucosides have been reported to be responsible for the deleterious effects observed with the feeding of rapeseed meal to farm animals. Recently a genetically low-thioglucoside rapeseed, which originated in Poland, has been grown on an experimental basis in Saskatchewan. Part of the research reported in this thesis was prompted by the availability of this low-thioglucoside rapeseed meal, Brassica napus L. var. Bronowski and was undertaken to assess the nutritional value of this product for growing rats.

Even more recently, the development of a water-extracted thioglucoside-free rapeseed flour in Ottawa, prompted the investigation in our laboratory of its supplemental protein value for human use.

Two rat experiments and a human metabolic study were conducted to evaluate the nutritional value of the rapeseed protein sources described above. The rat experiments were devoted to a study of protein metabolism, 8-week weight changes, organ weight changes and histochemical examination of liver slices in growing animals. Response to the low-thioglucoside product was compared to high-thioglucoside-content rapeseed meal and vitamin-free casein. The human metabolic study assessed the supplemental protein quality of rapeseed flour in an all-vegetable diet and was compared to vitamin-free casein and wheat gluten.

III. REVIEW OF LITERATURE

A. INTRODUCTION

During the last two decades research on the nutritive value of rapeseed has resulted in a substantial increase in the use of rapeseed meal in livestock and poultry rations. Deleterious effects on growth and efficiency of feed utilization have been observed, however, when high levels of rapeseed were included in the diet (Hussar and Bowland 1959a; Manns and Bowland 1963a). Poor performance in response to rapeseed meal has been attributed to the presence of giotrogens (Bell and Belzile 1965) although tannins have also been implicated (Clandinin and Heard 1968).

Thyroid enlargement has been the only consistent tissue change observed in animals fed rapeseed meal although slight changes also have been reported in other organs and tissues (Bowland et al. 1963; Manns et al. 1963c). Thyroid hypertrophy, however, has been reported to reach a maximum between 6 and 9 weeks in rats following which thyroid-to-body weight tended to stabilize (Bowland and Standish 1966).

The discovery of a genetic low-thioglucoside-content rapeseed (Downey 1969) and the development of a water extracted thioglucoside-free rapeseed flour (Eapen et al. 1969) has opened a whole new area of research. Since the thioglucosides in rapeseed are water soluble, these toxic principles can be completely eliminated by aqueous extraction. One of the exciting qualities about this product is its high protein content (60%). Considerable attention,

therefore, has been focused on the potential of rapeseed flour as a possible future supplemental protein source for the human.

B. SUPPLEMENTAL VALUE OF RAPESEED PROTEIN

B.1. Potential Use for the Human

The rapidly growing world protein requirement has directed major attention to the use of oilseed proteins since animals are inefficient protein producers. Oilseed proteins have in the past been used almost exclusively for fertilizers. However, refinements in the oil processing methods have not only increased the quantity and quality of the oil, but also improved the quality of the meals and by-products derived from it. The developments which have led to the use of oilseed protein concentrates, particularly soybean and cottonseed, as human food have been based largely on the results of extensive animal feeding trials (Altschul 1966).

The use of RSM, the by-product of the oil industry, as a potential protein supplemental in human food is limited. The presence of thioglucosides, high fibre content, black hulls and the possibility of recontamination with myrosinase are the main factors restricting the use of RSM to animal feeds (Eapen et al. 1968; Eapen et al. 1969).

A light-colored, bland, thioglucoside-free flour containing 60% protein (RSF) recently has been produced from rapeseed at the Food Research Institute, Canada Department of Agriculture, Ottawa (Eapen et al. 1969). An aqueous extraction procedure for the removal of thioglucosides has proved to be practicial as well as

economical since rapeseed thioglucosides are completely soluble in water while the protein and carbohydrate of rapeseed is only sparely soluble. In order to fully realize the potential of this process research now must be directed toward the reduction of processing costs, evaluation of the nutritional properties of RSF and its by-product, a 30% protein, thioglucoside-free meal and determination of the functional properties of rapeseed protein.

Owen et al. (1970) also have devised a successful method for the isolation and detoxification of the proteins of rapeseed press-cake meal. They were able to reduce the oxazolidinethione and isothiocyanate content from original levels of 7.5 and 4.9 mg per g meal, respectively, to less than 0.2 and 0 mg per g protein respectively. Protein efficiency ratios in rats was 1.86 and when mixed with an equal quantity of skim milk powder, the PER increased to 2.5. In addition, selected organ weights and histological examination of livers and thyroids showed these organs to be normal in rats fed the diets for 2 months. It is difficult to assess these results as neither the level of protein nor the content of rapeseed isolate in the diet is given. Nevertheless, results such as these and those of Shaikh et al. (1968) and Eapen et al. (1969) offer promise that rapeseed protein concentrates and flour may have tremendous potential as a human food source, especially in alleviating protein shortages in developing countries.

B.2. Present Use of Livestock and Poultry

Excellent reviews have been published regarding safe and economical levels of RSM for use in livestock and poultry diets

(Bowland et al. 1965; Bell et al. 1969a). These recommendations are necessary because it is difficult for the average livestock and poultry feeder to assess the overall performance of animals on diets containing RSM. Many of the studies that have been conducted were of short duration and the results were frequently confounded by the fact that several sources of protein, including RSM, were used. In addition, commercial RSM purchased from any one of the four major rapeseed processing plants in Western Canada will vary from year to year and from plant to plant depending upon species and variety of rapeseed and processing method used. Even more confusing to the livestock producer are the contradictory results reported by the same research workers (Manns and Bowland 1963a; Bowland 1965; Schuld and Bowland 1968; Bowland and Schuld 1968).

Differences exist between species of animals in their response to the inclusion of RSM in the diet. Little or no deleterious effect has been observed with ruminants (Whiting 1965), while slight effects have been reported with growing swine and poultry (Bowland 1965; Clandinin 1965). By contrast, impairment of reproductive ability has been reported with breeding sows, especially gilts fed diets containing RSM during rearing, gestation and lactation. Schuld and Bowland (1968) concluded that a level of 8% solvent-extracted RSM in diets did not influence the performance of mature sows or their litters during the first or second reproductive cycles. However, these same levels (8%) should not be fed to gilts to be used for reproductive purposes as it resulted

in a decreased number of pigs weaned per litter with a corresponding decrease in litter weaning weight in the first reproductive cycle. These results were not surprising as Manns and Bowland (1963a) had suggested that solvent-extracted RSM should not be used at levels above 3% of the total diet for breeding females during pregestation, gestation and lactation. On the other hand, Bowland and Schuld (1968) found that 8% RSM in a sow's diet had no effect on the future performance of their litters after weaning.

An animal's age and sex also affects its response to dietary RSM. A review of these factors in relation to overall weight gain performance will be discussed in Section C.2.a.

Experiments with poultry stress the need for adjusting the energy levels of RSM diets in order to compensate for its low metabolizable energy content as compared to soybean meal (Clandinin 1969). Lodhi et al. (1969a; 1969b) found that the average metabolizable energy values were approximately one-half the values reported for soybean (Hill and Renner 1960). This same group (Lodhi et al. 1969b) also showed that the level of available carbohydrate in RSM is lower than in soybean meal, which may help to explain the low metabolizable energy value of RSM. By contrast, the energy of RSM appears to be much more available to swine than poultry. According to one recent study with pigs (Bell 1969a), RSM contains 12% less metabolizable energy than soybean meal (3,100 versus 3,600 kcal per kg).

Surprisingly, in many of the experiments conducted, RSM was substituted on an isonitrogenous basis only for soybean meal

(Bell 1957b; Hussar and Bowland 1959a; Bowland et al. 1963; Schuld and Bowland 1968). Studies (Sibbald et al. 1956) have demonstrated that digestible energy consumption influence N retention in the weanling rat. In the chick, energy content of the diet is a major factor controlling feed intake (Hill and Dansky 1954). One exception to this general oversight in diet formulation was the study by Drouliscos and Bowland (1969) who formulated isocaloric and isonitrogenous RSM diets for feeding weanling and mature rats. They concluded that the higher indices (N digestibility, NPU, BV, PER and growth) with mature rats as compared to those for weanling rats could be explained on the basis of the nature and availability of the carbohydrate fraction of the diets. Much of the variability in growth with animals fed RSM may be due in part to a failure to maintain diets, within any one experiment, isocarloric as well as isonitrogenous.

It had been literally impossible in the past to assess the nutritive value of a thioglucoside-free RSM, but today it has become a reality with the discovery of Brassica napus var. Bronowski, a genetically low-thioglucoside rapeseed which originated in Poland. Evaluation of this rapeseed is currently being carried out. Bell (1969b) suggests that such thioglucoside-free or thioglucoside-low meals would be more palatable, free of growth depressing factors and likely higher in metabolizable energy values. The performance of turkeys fed "Bronowski rapeseed" (10 and 20% of the diet) was essentially the same as soybean-fed control birds, suggesting that thioglucoside-free varieties have the potential to substantially

improve the nutritive value of RSM (Salmon 1970).

Although Bell (1969b) only generalizes on their findings with RSM essentially free of thioglucosides (Bronowski rapeseed), he reported that growth response was equal to soybean meal while digestibility of protein and energy were slightly better than for soybean meal. One would expect a favorable growth response since the RSM is essentially thioglucoside-free, but the improved protein digestibility, equal to the 90.4% reported for soybean meal (Drouliscos and Bowland 1969), is more difficult to explain. In fact, results in our laboratory (Oliver et al. 1970) have shown that the growth response of rats fed low-thioglucoside RSM was essentially the same as casein-fed control rats, but protein digestibility for the low-thioglucoside RSM group was similar to those fed a high-thioglucoside content RSM (81 versus 79%).

Brassica napus, var. Bronowski has several undesirable features such as: low yield, long growing season and low oil content (Stefannson 1970). Results to date suggest that complete removal of all the thioglucosides from rapeseed species is possible. Attempts to develop a rapeseed with the following characteristics: thioglucoside-free, eruric acid-free, low fibre, high protein and high oil content continue to challenge the plant geneticist. Equally as challenging to plant breeders is the development of a rapeseed variety possessing, in addition to these desirable nutritive qualities, a high acreage yield and high oil yield coupled with early maturity.

C. EVALUATION OF RAPESEED PROTEIN QUALITY

C.1. Protein Metabolism in the Rat and Pig

The laboratory rat has been used extensively as an experimental animal to study the effect of rapeseed meal consumption on growth, metabolism and physiology, not only because of its economic advantage over productive species, but also, because its response appears to be similar to that of the pig. With the increasing importance of rapeseed production in Western Canada (Canada Year Book 1969), newer varieties of rapeseed (Downey 1966; Downey et al. 1969) and better methods of processing (Youngs 1965), extensive research has occurred with this oilseed.

Since the amino acid composition of rapeseed is comparable with that of soybean, the animal's quantitative requirement for protein and qualitative requirements for amino acids are adequately met (Wetter 1965; Goering et al. 1960). In a review, Robblee (Bowland et al. 1965) noted that the protein level of RSM ranges from 32 to 44%. Similarly, Josefsson (1970) reported that rapeseed contained 40 to 45% protein in the dry matter with a well balanced amino acid composition. Clandinin and Bayly (1963) found that the protein and amino acid composition of rapeseed is affected by variety and growing conditions. They found the lysine content of the protein of the Polish variety, Brassica campestris, was significantly higher than that of the Argentine variety, Brassica napus. Recent studies with plants grown on S-deficient soil showed that, besides species and varietal differences, the amounts of protein deposited in the rapeseed and the structures of complex proteins were affected by

the amounts of nutrients in the soil (Finlayson et al. 1969). Josefsson and Applequist 1968; Josefsson 1970 also reported that thioglucoside content and amino acid composition of rapeseed (Brassica napus) is affected by the presence of fertilizer containing sulfur and nitrogen. They found that a lack of sulfur in the growth medium of soil-free culture resulted in seed low in thioglucosides, protein content and methionine content.

Relatively low protein digestibility coefficients have been reported for both ruminants and non-ruminants (Bell 1955; Bell and Linton 1961). These authors attribute most of the lowered digestibility to the nitrogen-free extract fraction and some to the crude fibre. An average of 13.2 to 14.7% crude fibre has been reported to be present in rapeseed meal (Ballester et al. 1970a), which is higher than that for most other vegetable meals. True protein digestibility for solvent and prepressed-solvent processed rapeseed meals has been reported to be significantly lower ($P<0.01$) than that of casein or soybean, 78.9 and 77.9 versus 96 and 90.4% respectively (Drouliscos and Bowland 1969). Ballester et al. (1970a) concluded that low protein digestibility values could be explained on the basis of the presence of toxic factors and the high level of crude fibre, although they concluded that rapeseed meal has an adequate level of available lysine. Youngs (1965) attributes the higher available lysine content of solvent-extracted RSM to the reduced heat damage that occurs by this method of processing. However, Jackson (1969) reported that the availability of lysine from RSM (71%) is lower than that of soybean meal (90%).

Hussar and Bowland (1959b) reported that the addition of expeller-extracted Argentine-type RSM, as 10% of the total diet for 7, 28 and 62 kg pigs and 4- and 6-week-old rats depressed apparent digestibilities of protein, energy and dry matter although the results were significant with rats only. Nitrogen retention was unaltered. Rapeseed when incorporated at the 2% level in the diet had no effect on these digestibility coefficients.

Manns and Bowland (1963b) suggested that protein digestibility and energy and dry matter utilization values were similar for solvent-extracted Polish RSM and expeller-extracted Argentine RSM with both rats and pigs. In this study, solvent-extracted RSM represented up to 15% of the total diet when substituted on an equivalent protein basis for soybean meal. Apparent protein, energy and dry matter digestibility coefficients were reduced when RSM replaced 50 or 100% of dietary soybean meal for growing rats. Similarly nitrogen retention either on the basis of digested N or gross N was significantly lower for rats when RSM replaced 50 or 100% of dietary soybean meal. However during gestation N digestibility was lower when RSM replaced as little as 25% of the dietary soybean meal. Less consistent results however were obtained with pigs and boars were more efficient than gilts in digesting energy and dry matter.

Bowland and Standish (1966) reported that protein digestibility of rapeseed meal was unaffected by length of time rats were fed the diet. Protein digestibility after 2 weeks on the diets

was 83.1% whereas after 10-11 weeks it was 83.7%. No significant sex differences were observed in digestibility or N retention.

C.2. Growth Performance of Rats and Pigs

C.2.a. Weight Changes

The goitrogenic properties of rapeseed and in turn the nutritive value of this product are influenced by numerous factors, including seed variety, environmental growing conditions, processing methods and level in the diet. In addition, physiological factors such as age, sex and species of the animal have an appreciable effect on the growth response obtained. The results of numerous experiments have been excellently reviewed and summarized in several publications (Bell 1955; Bowland et al. 1965; Bell et al. 1969a).

Goering et al. (1960) compared expeller- and solvent-extracted RSM following enzymatic treatment designed to remove the toxic factors and found that the solvent-extracted RSM promoted more rapid gain and higher efficiency of food utilization in rats. Clandinin and co-workers (1959; 1961) concluded that the improved temperature control in the oil extraction process resulted in proteins of higher biological value. Increased lysine content and altered myrosinase activity were reported to be responsible for the superior performance when solvent-extracted rapeseed meal was fed to poultry. Youngs (1965) attributed the lower protein quality of expeller processed RSM to a decrease in lysine content.

Argentine RSM has been found to be more detrimental to growth

than the Polish RSM (Renner et al. 1955; Clandinin et al. 1959). However, in two separate rat experiments researchers concluded that solvent-extracted Polish RSM depressed rate of gain and efficiency of food utilization in rats as much as expeller-extracted Argentine RSM (Hussar and Bowland 1959a; Manns and Bowland 1963a). Manns and Bowland (1963a) found that rats fed solvent-extracted Polish RSM at 15.6% of the diet gained 24% more slowly and 19% less efficiently than those receiving 0% RSM, whereas no demonstrable effects were observed with rats fed 2% expeller-extracted RSM (Hussar and Bowland 1959a). It should be observed that the levels of the two rapeseed species used, Polish and Argentine were not identical in these two studies and the authors reported an average oxazolidinethione and isothiocyanate content of 5.65 mg and 4.20 mg. per gram of sample, respectively, for the solvent-extracted Polish RSM, which is considerably higher than the 2 mg oxzaolidinethione per gram of sample reported for the Polish species (Bell et al. 1967).

Food intake has not been found to be significantly influenced by the addition of high levels of RSM (up to 15.6% of the diet), although there was a trend toward decreasing food intake as levels of RSM in the diet increased (Hussar and Bowland 1959a; Manns and Bowland 1963a; Bowland and Standish 1966).

Rats fed 15% solvent-extracted RSM for 20 weeks had a reduced rate of gain for only 8 to 10 weeks (Bowland and Standish 1966). These results coincide with an earlier report where differences in rate of gain and efficiency of food utilization of rats fed RSM at 5-8 weeks of age was less marked than at 3-5 weeks of age, suggesting that a tolerance to RSM appears to develop in the

older animal (Manns and Bowland 1963a). Similarly, older pigs (above 110 pounds liveweight) seem to be more tolerant to high levels of rapeseed as body weight gains and feed efficiency were equal to soybean-fed animals whereas addition of solvent-extracted RSM as 15.6% of the diet to group-fed or individually-fed pigs weighing 20 to 50 pounds caused a severe depression in rate of gain and efficiency of feed utilization. These results with rats and pigs are in agreement with those obtained with poultry. Lodhi et al. (1969a) showed that the ability of the chicken to utilize RSM improved with age. By contrast Hussar and Bowland (1959a) observed a significant reduction in weight gain for 9, 28 and 62 kg pigs fed 10% RSM as compared to pigs receiving 0 or 2% dietary rapeseed. Bell (1965) reported that the inclusion of 5 or 10% Polish RSM in the grower rations of pigs (23 or 45 kg) significantly depressed rate of gain and daily feed intake without affecting efficiency of feed utilization.

The variable response to RSM may be partly the result of the availability of goitrogens to the animal. Addition of myrosinase to swine grower rations containing RSM depressed weight gain in pigs to one-third that of animals fed RSM-containing diets alone (Bell 1965). Results such as these confirm previous in vitro findings and studies with mice, in which myrosinase had been implicated in the toxicity of RSM (Belzile et al. 1963; Belzile and Bell 1966).

Evidence of sex differences has been observed for a number

of species and will be reviewed only briefly (Bowland et al. 1965; Bell et al. 1969a). Female rats appear to be more severely affected than male rats whereas with mice, males are more susceptible than females (Bell 1955; Manns and Bowland 1963a). The latter authors also reported that the relative growth rate of boars from 16 to 210 pounds liveweight was superior to the weight gain performance of gilts of an identical age.

Various dietary supplements have been incorporated into rapeseed diets in numerous studies conducted to determine if the goitrogenic properties of rapeseed could be counteracted (Bell and Williams 1953; Bell and Baker 1957; Bell 1957a; Bell 1957b). Manns and Bowland (1963a) found that addition of 0.2% L-lysine to a diet containing 15.6% RSM had no influence on gain, efficiency of food utilization or reproductive performance of rats or pigs. The inclusion of DL-lysine at 0.5% of the diet produced no beneficial effect for mice (Bell 1957b) suggesting that lysine was not a limiting amino acid in diets containing RSM. It likewise has been shown that supplementation with potassium iodide, iodinated casein, antibiotics, linseed oilmeal, skim milk powder, fishmeal and thyroxine failed to counteract the growth depressant effects which occurred when mice were fed diets containing 17 to 50% Argentine or Polish RSM (Bell and Williams 1953; Bell and Baker 1957; Bell 1957a; Bell 1957b). Thus, no practical methods of directly counteracting the action of the growth depressant have been found.

C.2.b. Organ Weight Changes

Studies on organ weight changes occurring in monogastric animals consuming RSM are limited. They thyroid gland has been the organ most intensively studied, perhaps, because of the well known thyrotoxic effect observed in mice, rats, swine and poultry (Bowland et al. 1965; Bell et al. 1969a).

Normal adrenal and gonad weights have been reported (Manns et al. 1963c) for rats and pigs fed diets containing 7.8 to 15.6% RSM for 9 to 12 weeks despite poorer reproductive performance reported for female rats and gilts fed similar diets during gestation (Bowland and Standish 1966; Schuld and Bowland 1968). Significant increases in the relative weights of spleen, kidneys and liver of rats fed a 55% RSM diet were observed by Ballester et al. (1967).

The liver is known to undergo variable degrees of compensatory hypertrophy to overcome nutritional stress (Barber et al. 1961). Although a significant degree of liver hypertrophy was observed only is sows fed RSM (12% of diet); a non-significant trend towards increase liver size and likewise observed in this same study with market pigs was rats (Bowland et al. 1963). Signs of liver necrosis and regeneration observed in the study by Ballester et al. (1967) further suggest that severe hepatic change may occur when high levels of RSM are ingested. Evidence of definite hepatic toxicity has recently be published by Jackson (1969). They observed fatty livers in laying hens fed a diet containing up to 20% RSM for 252 days. The main cause of

death was haemorrhage in the liver. Further investigation by these workers revealed that many of these RSM-fed birds when sacrificed showed evidence of liver haemorrhage which had not proved fatal. They suggest that, "the fatty livers did render the birds more susceptible to the toxic effect of the rapeseed meal, the main cause of death being haemorrhage of the liver."

C.2.c. Mode of Action on Thyroid Hypertrophy

Goitrogenesis as a consequence of thyroid malfunction is the primary disorder frequently observed with rapeseed toxicity in non-ruminants (Bell and Belzile 1965). It is well established that rapeseed contains a glucoside, which on enzymatic hydrolysis by myrosinase, yields a goitrogen, (-)-5-vinyl-2-oxazolidinethione (Astwood et al. 1949a; Astwood et al. 1949b; Ettlinger 1950). Isothiocyanates (3-bytenyl and 4-pentenyl) and occasionally nitriles are also released on enzymatic hydrolysis. Greer et al. (1964) state that certain isothiocyanates which will cyclize to form oxazolidinethione derivatives, have been found to form compounds possessing significant antithyriod activity. Pitt-Rivers (1950) had postulated that 3-butenyl isothiocyanate could cyclize and was therefore, a possible biological precursor of oxazolidinethione.

Two characteristics are evident for Brassicae, (1) the predominance of allyl isothiocyanate and (2) the appearance of a mixture of 2 or 3 different isothiocyanates. In general the latter is the rule rather than the exception (Jensen et al. 1953). Numerous isothiocyanates in RSM have been identified by Kjaer and co-workers (1953a; 1953b, 1953c) using paper chromatographic

techniques. Recently, Youngs and Wetter (1967) have identified the last of the major isothiocyanates as 4-methylthio-butyl and 5-methylthio-pentyl isothiocyanates by GLC.

Myrosinase, the enzyme responsible for the hydrolysis of the mustard oil thioglucosides, is a 2-component enzyme (Gaines and Goering 1962). Both components, the sulfatase factor and the thioglucosidase factor must be present for total hydrolysis to occur. In addition, thioglucoside hydrolysis is favored above 10% moisture and rapidly increases with increasing temperature until inactivation occurs (80-90°C). Hence, regardless of processing method, myrosinase must be inactivated as quickly as possible by a rapid rise in temperature in order to minimize thioglucoside hydrolysis (Bell 1965). However, even though the enzyme is inactivated during processing of RSM there is always danger of reintroducing myrosinase in other feedstuffs (Downey et al. 1969). Excellent reviews on the enzymatic mechanism and chemical reactions involved in thioglucoside hydrolysis have been published by Bell (1955) and Greer et al. (1964)

Clandinin (1967) has indicated that rapeseed produced from 1965 to 1967 was lower in goitrogenic factors than earlier produced crops. This may be due in part to the greater use of the Polish rapeseed species, Brassica campestris, which contains lower levels of thioglucosides than does the Argentine rape, Brassica napus. Argentine rape has an oxazolidinethione content of 0.5 to 1.0% on an oil-free basis compared to 0.2% found in the Polish variety

(Bell et al. 1967; Clandinin et al. 1959). Complete removal of these toxic components may be possible through plant breeding or a combination of processing and plant breeding programs (Ballester et al. 1970b; Downey et al. 1969). Success to date has been partially attained by the discovery of Bronowski rapeseed. Furthermore, varieties even lower in thioglucoside content have been achieved through plant breeding research currently being carried out at the University of Manitoba (Steffansson 1970).

The literature is well documented with reports (Bell and Belzile 1965) of thyroid hypertrophy and hyperplasia occurring in non-ruminants as a consequence of ingesting moderate to high levels of RSM (10% or more). Clandinin and Bayly (1960) have described the histology of the thyroid glands of growing chickens and laying hens fed RSM. They observed that the increase in thyroid size was due to not only an increase in the number of follicles, but also to an increase in the size of the epithelial cells in the glands. Clandinin and Robblee (1966) cite a report by New Zealand workers which showed that thyroid changes in the rat are at a maximum after 3 weeks on diets containing RSM. Apparently the feeding of RSM of rats interferes with the ability of the thyroid to synthesize thyroxine. In turn, the resultant fall in the level of circulating thyroxine induces the pituitary to secrete excessive amounts of thyrotropin which acts on the thyroid causing hypertrophy and hyperplasia.

The effects of the goitrogen, (-)-5-vinyl-2-oxazolidinethione, on the thyroid activity of chicks have been studied (Clandinin et al. 1966; Matsumoto et al. 1968). They suggest that early effects of feeding this goitrogen to chicks include the suppression of iodine uptake by the thyroid glands and reduced secretion of thyroxine from the glands. After feeding a RSM diet for 3-4 weeks compensatory changes occur in the thyroid glands which permit a return to normal rates of thyroxine secretion. Hence, poultrymen need have no fear of adverse effects from goitrogen since compensatory adjustments are made in the thyroid glands to counteract the absorbed goitrogen.

D. DEVELOPMENT OF RAPESEED FLOUR

The recent development of RSF at the Food Research Institute, Ottawa has attracted considerable attention but in contrast to the well documented literature available on the development and improvement of RSM, little literature is available at this time on RSF. Preliminary studies have shown that it is possible to produce a bland, light-colored, thioglucoside-free, 60% protein flour from rapeseed (RSF) (Eapen et al. 1969), although extensive research and development are still needed to improve its palatability, flavour, color and baking qualities.

During commercial processing crushed rapeseed is cooked for 30 minutes without water at 80-90°C to inactivate myrosinase (Youngs 1965). The thioglucosides are still intact in the meal and can be subsequently liberated by the enzyme produced in the

gastro-intestinal tract by certain bacteria, especially E. coli and A. Aerogenes (Greer et al. 1964; Eapen et al. 1968). Hence, toxicity might arise if thioglucoside containing rapeseed products are to be used for food purposes.

Preliminary investigations have indicated that myrosinase inactivation and oil color were markedly affected by either wet- or dry-heat processing treatments. Observations have showed the importance of a short, controlled heat-treatment for the production of a light-colored oil and enzyme inactivated meal; both qualities are equally important for rapeseed processors. Furthermore, it was observed that wet-heat treatment resulted in the formation of a higher quality oil and meal. Another advantage of this processing method was that fibrous hulls were easily removed (Eapen et al. 1969).

Since thioglucosides are completely water soluble, these toxic principles can be completely eliminated by aqueous extraction. However, thioglucoside removal by this method (2 min in 100°C water) must precede grinding to prevent increased loss of protein and fine solids in the extract. Eapen et al. (1969) reported that nearly 44% of the raw material (moisture- and oil-free basis) can be separated as a light-colored, bland "powder" with an exceptionally high protein content. The by-product, a thioglucoside-free seed coat fraction is grey in color and contains approximately 33% protein. Hence, it could be used for animal purposes although the high fibre content of this product reduces its nutritive quality.

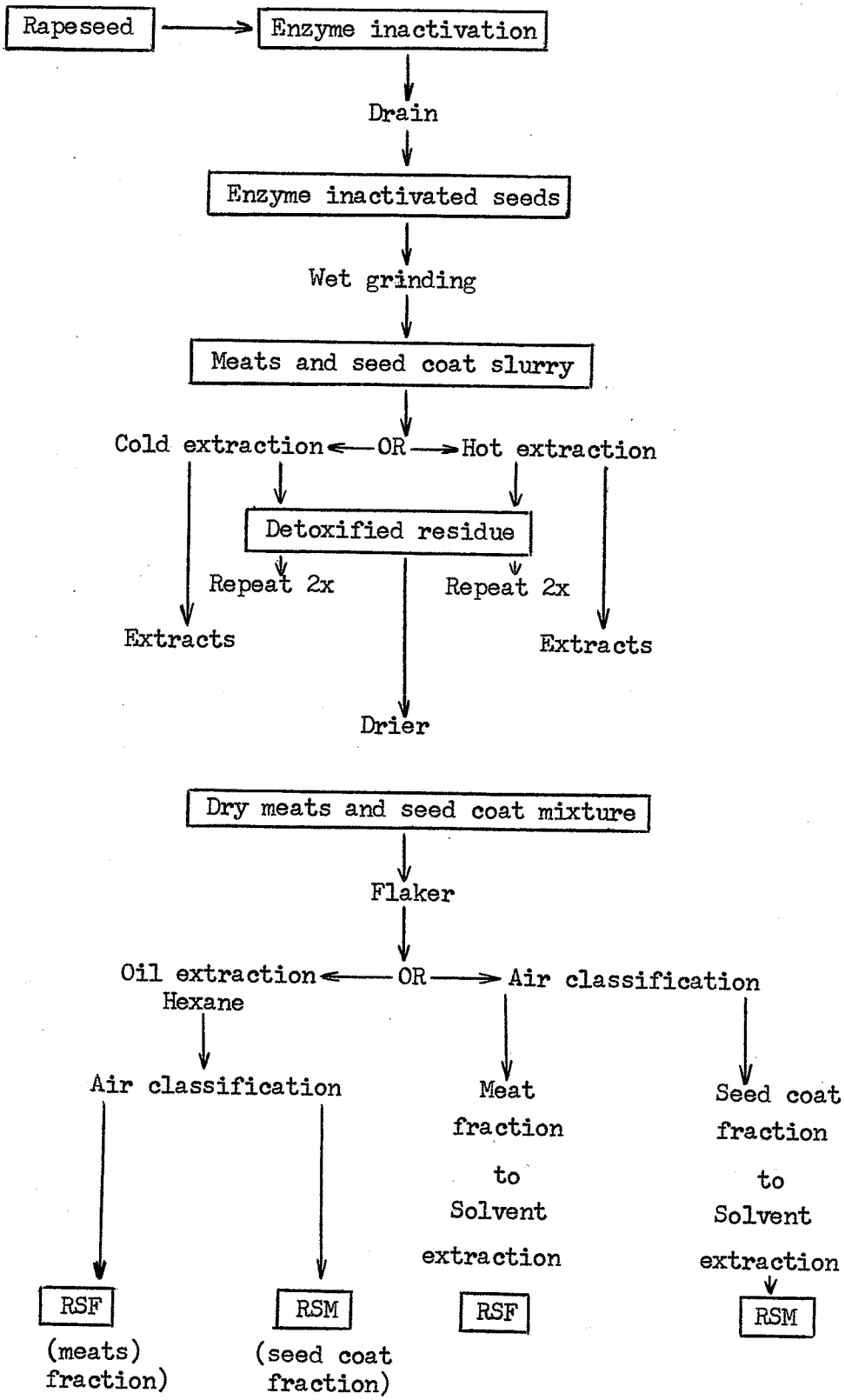


Fig. 1. A simplified diagram outlining preparation of RSF and RSM.

A simplified diagram outlining the production of RSF for human nutrition is presented in Figure 1. Tape et al. (1970) have reported that the protein in RSF was equal to, or better than casein in promoting growth in rats. Further research is necessary however to determine whether RSF production, utilization and consumption by man is feasible.

E. NITROGEN BALANCE IN HUMANS

E.1. Assessment of Protein Requirement

Most protein requirement studies for adult man have been concerned with the measurement of nitrogen (N) retention. Available evidence at present favors the view that protein requirement for adults should be based upon the minimum protein intake which will maintain N equilibrium. Leverton et al. (1956) have defined N equilibrium as "the zone in which the excretion of N is within 95-105% of the intake, rather than the point at which excretion and intake are numerically equal".

An adult subject can be maintained in a state of N equilibrium over a wide range of N intakes and the problem, therefore, is to find the minimum dietary protein level. This can be achieved in two ways, by long-term N balance studies or by separate evaluation of the magnitude of each of the components of N loss from the body. The latter procedure is described as the "factorial method" (Hegsted 1968; Miller and Payne 1969).

Even though the nitrogen balance method is costly, time consuming, and difficult to measure accurately, it will continue

to be used until a superior method is developed (Allison and Bird 1964; Fisher et al. 1967; Miller and Payne 1969). Ideally, nitrogen balance studies should be of relatively long term duration and provide evidence of continued maintenance of health for the individual. However, it is impossible to specify a minimum physiological level of proteinthat will give a person protection against disease or resistance to various stresses (Miller and Payne 1969).

The "factorial method" of estimating minimum protein requirements considers separate evaluation of the major losses of N from the body: urine, feces, skin and lungs.

The major loss of N is through the urine. Urinary N excretion decreases rapidly when a person is fed a protein-free diet and approaches a constant excretion ("endogenous N excretion") within 7 to 14 days. Endogenous urinary N excretion has been calculated at approximately 2 mg N per basal calorie per day. The real problem, however, is that the longer the N-free diet is consumed, the more depleted the subject becomes and as pointed out by Hegsted (1968) there is no way to distinguish between a normal subject in N equilibrium and a slightly depleted one.

Fecal losses of nitrogen are composed of unabsorbed residues from ingested food and "metabolic fecal nitrogen". The latter is composed of gastrointestinal secretions, mucosal cells and intestinal flora. Fecal N losses constitute the second major loss of N from the body and have been calculated to be 10-20% of dietary

intake. However the situation is complicated by the fact that there is no satisfactory way to distinguish between food residues and metabolic fecal nitrogen with possible exception of the use of isotopic markers (Hegsted 1968), although even the use of isotopes is limited by rapid re-cycling of label.

Adequate evidence is not available at present to state the actual losses of N via the skin and lungs, although these losses are considered to be minimal (Munro 1968; Hegsted 1968). Miller and Payne (1969) cite a report by Costa et al. (1968) which suggested that losses of nitrogen from the lungs may provide an explanation for long-continued apparent positive N balances in men and animals unaccompanied by changes in body weight. Costa et al. (1968) apparently based this conclusion on the observation that in mice, N losses from the lungs was equivalent to 5-10% of the dietary N intake.

Using the factorial method of estimating minimum protein requirements, Hegsted (1968) suggested that minimal adult protein requirement is between 20 and 30 g of high quality protein per day. This figure is obtained by adding together the minimal N excretions, assuming there is a loss of 2 mg N (equivalent to 12.5 mg protein) per basal calorie per day in the urine, 20% additional in the feces and 10% more in losses from the skin and lungs:

Minimum protein requirement = (Basal calories x 12.5 mg protein) +
(20% x 12.5 mg protein x basal calories) +
(10% x 12.5 mg protein x basal calories).
= 1.3 (12.5 mg protein x basal calories)

Normal protein requirements should not be set too low as long-term studies on low N intakes have not been conducted. Current studies seem to indicate that minimum protein intakes for uremic patients (20-30 g daily) are probably adequate, provided the caloric intake can be maintained and only proteins of highest quality are fed (Tsaltas 1969; Berlyne 1969). Whether the long-term effects of such a regimen are beneficial or not remains to be seen. Furthermore, whether uremic patients handle protein in the same fashion as healthy people is another question that must be answered.

E.2. National versus International Dietary Protein Allowances

The principle objective of dietary allowances is to ensure, from a nutritional standpoint, the maintenance of health for the majority of people. However, considerable differences do exist among national and international dietary protein recommendations as indicated below, although these differences are gradually narrowing as more knowledge accumulates and dietary allowances are periodically revised (Patwardhan 1970).

Origin of Allowances	Date	Typical Body Weight, Kg	Recommended Protein Allowance, g/day
Canada	1964	70	55*
United States	1968	65	65
United Kingdom	1969	65	68
FAO/WHO	1965	65	68

* Canada's Standard revised in 1968. Recommended protein allowance decreased to 48 g.

The recommended daily protein allowance for the adult Canadian was based on the "factorial" approach, which in turn, was based on N balance data (Sabry 1970). The formula used to calculate the Canadian standard was:

Protein allowance = (U + F + S) x 1.3 x 1.43

where U, F and S represented nitrogen loss in the urine, feces and skin respectively. A factor of 1.3 allowed for individual variation and a factor of 1.43 adjusts for the quality of protein in a typical Canadian diet i.e. NPU = 70.

E.3. Factors Affecting Nitrogen Retention in Humans

Numerous factors affecting N retention in humans have been investigated and included such items as: amount and source of total N, prior protein intake, length of time on diet and frequency of eating.

There is lack of agreement among researchers with respect to the influence of N intake on N retention. Swendseid et al. (1960) reported that changes in N retention of young men and women were neglible when dietary N was increased from 6.5 to 10 g by addition of non-essential nitrogen in the form of glycine and diammonium citrate to a diet containing whole egg. On the other hand, the N balance of women consuming either purified amino acids or corn shifted from slightly positive to distinctly negative when N was decreased from 10 to 6 g (Linkswiler et al. 1960). Clark et al. (1963) reported that a daily intake of 9 g N resulted in significantly higher N retention than an intake of 6 g N,

but no further improvement in N retention was obtained by increasing the N intake to 12 g per day.

Nitrogen balance has also been reported to be slightly better when the source of N was in the form of an intact protein food rather than crystalline amino acids (Kirk et al. 1962; Leverton and Steel 1962; Swenseid et al. 1961; Swendseid et al. 1962; Oldham and Dickinson 1965; Kolski et al. 1969). More recently, though, studies by Morse et al. (1969) seem to indicate that a crystalline amino acid control diet (FAO pattern) was better utilized than an amino acid supplemented wheat gluten diet formulated to contain the same levels of amino acids as the control diet.

The influence of variable quantities of amino acids on N retention of adults has been studied for a number of practical reasons. Supplementation of maize with lysine and tryptophan, for example, improved N retention in men, but neither amino acid gave any benefit when administered alone (Truswell and Brock 1961). In addition, studies on establishment of amino acid requirements, interrelationships among amino acids and essential amino acid balances has contributed information on N retention. Fisher et al. (1969) studied lysine and tryptophan requirements in young women. They found that with a daily N intake of approximately 5 g, the requirements for both essential amino acids were in the range of 50 mg per day. Since the requirement levels are considerably lower than those reported in earlier studies (Jones et al. 1956; Clark et al. 1963), Fisher et al. (1969) suggested that the difference

may be explained by the lower level of the other essential amino acids in their study.

Another group of investigators, Kolski et al. (1969) studied the interaction between branched-chain amino acids and found a wide zone of tolerance existed for leucine and valine when adequate amounts of other essential amino acids were consumed. Furthermore, data by Clark et al. (1966) indicates that an intricate balance must exist among the essential amino acids if they are to fulfill their nutritive roles.

Prior protein intake has been reported to influence later nitrogen balance trial results (Fisher et al. 1965; 1967). Another group of workers (Watts et al. 1964) noted that when a metabolic study was conducted in two parts, subjects were in negative N balance during the first few days of the second adjustment period. Watts et al. (1964) suggested that the amount of protein eaten before the beginning of the second part of the study was responsible for the negative N balances. Fisher et al. (1964; 1967) also questioned the importance of protein reserves on N retention. They concluded that the total quantity of N retained could not be accounted for in terms of body-weight changes or body-composition measurements based on K^{40} whole body counting and suggested that a major part of the retained N must be present as non-protein N.

Calories also have been reported to affect N retention in humans. Linkswiler et al. (1958) observed increased caloric need when pure amino acids rather than natural foods were used as a source of N. They attributed this, however, to the source of N,

rather than the total amount in the diet. These data coincide with those of Doyle et al. (1965) who reported that average weight loss for male subjects consuming vegetable diets, which provided 0.5 g protein per kg body weight, was 2.6% during the course of the entire study even though their caloric intake was increased by 23.7%. However, when the protein level was increased to 0.75 g per kg body weight the average weight loss was only 0.8%, in spite of the fact that caloric intake increased only 9.2% in the second part of their study.

Several other factors affecting N retention have also been investigated and will only be briefly summarized. Nitrogen retention has been reported to improve with time (Clark et al. 1962; Doyle et al. 1965), but was not influenced by frequency of eating when diets providing 5.3 to 9 g nitrogen were consumed (Shortridge and Linkswiler 1963). In addition, no correlation was found between body weight of male and female subjects and total N retention in experiments conducted by Swendseid et al. (1961 ; 1962) and Leverton et al. (1962). The times of menstrual bleeding and ovulation should be considered when N blanace studies are conducted with women. Swindells et al. (1968) reported that urinary N excretion tended to vary with different phases of the menstrual cycle. Four of their six subjects showed an increase in urinary N excretion during the postovulatory phase and a decrease during the premenstrual phase of the menstrual cycle. Since the number of subjects used in this study was small, further investigation

of this factor on N retention seems imminent.

When one considers the variability in N retention values reported in these studies which have been briefly reviewed, only then does one realize that many factors are responsible for the inconsistent results frequently obtained. Emphasis on amino acid balances, interrelationships and requirements, for example, have only within the last decade been considered to be of real importance to researchers in their evaluation of N retention data. Different laboratory procedures have also contributed to the variability of N retention results, but at the same time, it has opened new avenues of research.

F. LIVER HISTOCHEMISTRY

Rapid development of enzyme histochemistry during the last two decades has uncovered an abundance of stainable enzymes in the liver and has made this technique a versatile and powerful tool in the localization and identification of many cytoplasmic organelles (Novikoff and Essner 1960; Wachstein 1963; Tanikawa 1968). The correct localization of various enzymes identified by enzyme histochemistry have been confirmed by differential centrifugation studies and electron microscopy. Alkaline phosphatase, for example, has been shown to be a specific marker of cell membranes whereas certain hydrolytic enzymes, particularly acid phosphatase, are localized in the lysosomes (Wachstein et al. 1962; Tanikawa 1968). Two other hepatic enzymes, thiamin pyrophosphatase and adenosine triphosphatase have been extensively used as

specific markers, for the Golgi regions in the case of the former and bile canaliculi in the case of the latter (Novikoff and Essner 1960; Novikoff and Goldfischer 1961).

Marked changes in the distribution and localization of histochemically demonstrable enzymes are considered to reflect intracellular disorganization and have been shown to coincide with damage in the liver. In general, the degree of change observed in enzyme patterns reflect the degree of damage induced by the epxerimental treatment regardless of the means used to induce experimental hepatic injury (Wachstein et al. 1962; Shrader and Zeman 1969; Madi et al. 1970). Adenosine triphosphatase activity, for example, exhibits immediate changes following administration of agents which induce acute necrosis, following the administration of carcinogenic diets, during infections or after experimental bile obstruction. Thus, a decrease in adenosine triphosphatase activity is an early and reliable indication of hepatocellular damage and usually will be followed by diffusion and reduction of acid phosphatase activity. On the other hand, alkaline phosphatase activity appears to increase in response to necrosis and inflammatory infections (Wachstein 1963).

Difficulties in methodology have been repeatedly encountered by enzyme histochemists (Gomori 1952; Novikoff and Essner 1960; Burstone 1962; Wachstein 1963). Gomori (1952) pointed out that inactivation of the enzyme in the course of sectioning, embedding and storage may result in either uniform or patchy staining depending on the degree of enzyme inactivation. In addition,

varying degrees of cytoplasmic staining and the presence of artifacts, such as impregnation of nerve axons with dye, may result in conflicting interpretations of results. Wachstein (1963) stressed that optimal procedures have not been clearly established for enzyme histochemistry and the results obtained depend not only on the techniques involved in cutting and fixing the tissue slices, but also the type of substrate, composition of the incubation mixture and staining method used to visualize the enzyme. Another important factor in achieving optimal results is the most favorable pH for the enzyme itself. Alkaline phosphatase in the living organism, for example, exists at a pH close to neutrality (7.2) whereas pH 10 has usually been used for optimal staining in vitro (Burstone 1962). Perhaps a more accurate picture of cellular activity would be obtained if physiological conditions were used. Considerable variation in the apparent activity of various enzymes has been observed among different species of animal (Wachstein 1963). Acid phosphatase, for example, has not been demonstrated in the liver cells of the mouse in spite of its abundance in the rat. On the other hand, some enzymatic reactions are universal in all species, such as the presence of adenosine triphosphatase in bile canaliculi. Another point that must be kept in mind is that only potential sites of enzyme activity, not quantity of enzyme, can be visualized in histochemical staining reactions.

In spite of many difficulties in methodology, considerable progress has been made in enzyme histochemistry. Nevertheless the

technique is still in its infancy and continues to be used almost exclusively by researchers to gain an understanding of the functioning of normal and abnormal cells as well as delineation of cell structures. Ultimately the technique may have application in the early diagnosis of certain diseases or assessment of the degree of damage produced by the accidental or unwitting intake of harmful substances.

IV. EXPERIMENTAL

A. NUTRITIONAL EVALUATION OF A LOW-THIOGLUCOSIDE RAPESEED, Brassica napus L. var. Bronowski

A.1. Object

The present study was prompted by the availability of a low-thioglucoside-content rapeseed meal and the possibility that thioglucosides may be directly responsible for the deleterious effects observed with the feeding of rapeseed.

A.2. Methods and Materials

Composition of the diets, formulated to be isocaloric and isonitrogenous, is given in Table 1. Each protein source, low-thioglucoside rapeseed meal (LoRSM), high-thioglucoside rapeseed meal (HiRSM) and vitamin-free casein (Cas) provided the sole source of nitrogen. The rapeseed meal samples were prepared by commercial oil seed processors. LoRSM was prepared from seeds of Brassica napus L. var. Bronowski by an all-solvent process while HiRSM was prepared from seed composed primarily of B. napus L. by a prepress-solvent process. Chromic oxide (200 mg/100 g of diet) was added for apparent protein digestibility determinations. The diets were pelletted in a Templewood pellet mill equipped with a 3.2 mm die.

A.2.a. Experiment 1

Three male and 3 female rats of the Holtzmann strain were randomly allotted to each diet. The rats were housed in individual metabolism cages and fed ad libitum for 2 weeks. Feces were

Table 1. Composition of diets

Ingredients	Amount of Ingred. (g/100 diet)		
	HiRSM[1]	Cas[1]	LoRSM[1]
Vitamin-free casein[2]	---	18.75	---
Rapeseed meal	43.73	---	36.29
Corn oil (Mazola)	4.00	4.00	4.00
Cerelose (glucose)	47.67	49.55	54.11
Mineral Mix[3]	4.00	4.00	4.00
Vitamin Mix[2]	.60	.60	.60
Alphacel[2]	---	23.10	1.00
Chemical Analysis			
Crude Protein, %	16.1	17.8	15.9
Thioglucosides, mg/100g			
Oxazolidinethione	262.8	---	2.5
Isothiocyanate	157.4	---	5.8
Tannins, %	2.65	---	2.55

1 HiRSM - high-thioglucoside-content rapeseed meal, Cas - vitamin-free casein, and LoRSM - low-thioglucoside-content Bronowski rapeseed meal.

2 Vitamin-free casein, vitamin mix (Vitamin Diet Fortification Mixture) and alphacel were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

3 Mineral Mix as described by Mameesh and Johnson, 1958.

collected on the last 4 days of each week and protein digestibility determined by the chromic oxide method. The third week was devoted to a study of protein digestibility and nitrogen retention. On day 15 the animals were restricted to a maximum of 15 g of diet per rat per day. Daily procedure for collection of urine from the 18th to the 21st day included: rinsing the cages and collecting funnels with distilled water, adding the washings to the urine and addition of 1 ml of 10 N H_2SO_4 to each collection bottle.

Nitrogen of feed, feces and urine was determined by the macro Kjeldahl method (A.O.A.C. 1965) using HgO plus K_2SO_4 as catalyst. Chromic oxide was determined in feed and feces using a Perkin Elmer Model 303 Atomic Absorption Spectrophotometer (Williams et al. 1962).

Microdetermination of the thioglucosides in the rapeseed meal was carried out according to the method of Youngs and Wetter (1967). Method of tannin analysis was that described for cloves and all-spice by the A.O.A.C. (1965).

A.2.b.Experiment 2

Forty-eight animals were randomly allotted in unequal replicates to the same dietary treatments as described in Experiment 1. The animals were fed ad libitum for 10 weeks. Weight changes were recorded during the first 8 weeks and the animals killed by decapitation over the following 2-week period. Thyroid, liver, kidneys, spleen, adrenals and testes were excised, blotted on paper towels, placed in tared glass-stoppered

bottles and weighed immediately. A representative sample of liver from 3 males and 3 females from each treatment group was placed in ice-cold Baker's fluid for 24 hours.

Following fixation, each liver specimen was cut with a cryostat microtome at -20°C into 10 micro sections. Histochemical enzyme assays for acid and alkaline phosphatases, adenosine triphosphatase and thiamin pyrophosphatase were conducted on the free floating sections.

Alkaline phosphatase (Alk P'ase) was determined by the azo-dye method with sodium alpha-naphthyl phosphate as substrate and Fast Red Violet LB or Fast Blue RR diazonium salts, pH 10 (Burstone 1962). Acid phosphatase (Acid P'ase) was determined by the Gomori lead salt method with sodium beta-glycerophosphate as substrate, pH 5.2 (Gomori 1952) or the azo-dye method, according to Sigma Technical Bulletin No. 85, using sodium naphthol AS-MX phosphate and Fast Blue RR diazonium salt, pH 5.2. Adenosine triphosphatase (ATPase) was determined with the Wachstein and Meisel modification of Gomori's metal salt procedure using the disodium salt of ATP as substrate, pH 7.2 (Burstone 1962). Thiamin pyrophosphatase (TPPase) was determined by the metal salt method with thiamin pyrophosphate chloride as substrate, pH 7.2 (Novikoff and Goldfisher 1961). Fatty infiltration of the liver was determined using Fast Red 7B (1% in 60% ethyl alcohol) or Oil Red 0 (1% in 60% triethyl phosphate) staining methods.

A.2.c. Statistical Analyses of Data

The data were subjected to analysis of variance (Snedecor and Cochran 1967) and differences among means were tested using Duncan's Multiple Range (Duncan 1955).

A.3. Results and Discussion

The results of Experiment I are presented in Table 2. Apparent protein digestibility of the two rapeseed meals was significantly ($P<0.05$) lower than that of casein. No differences in digestibility of the individual protein sources were observed due to sex of the animals, length of time on experiment or quantity of food consumed. During the first two weeks of Experiment 1, food was provided ad libitum whereas during the third week food was restricted to a maximum of 15 g per rat per day. Manns and Bowland (1963b) and Drouliscos and Bowland (1969) also reported lower apparent protein digestibility values for rapeseed meal than for casein or soybean meal. Similarly, Bowland and Standish (1966) found no differences in protein digestibility whether rats had been fed a diet containing 15% rapeseed meal for 2, 6, or 10-11 weeks.

Nitrogen retention, determined during the third week, was significantly ($P<0.05$) lower for rats fed HiRSM than those fed Cas or LoRSM. Higher average weekly food consumption by rats fed the Cas and LoRSM diets probably contributed to improved N retention by these groups, although covariance analysis showed a significant ($P<0.05$) difference in N retention among groups even after adjusting for differences in food intake.

Table 2. Protein utilization by growing rats fed high- and low-thioglucoside content rapeseed meals - Experiment 1.

	Dietary group[1]					
	HiRSM		Cas		LoRSM	
	Females	Males	Females	Males	Females	Males
No. of Rats	3	3	3	3	3	3
Av Initial Wt, g	119.7± 1.6[2]	136.1± 1.0	122.3± 1.1	133.1± 0.9	117.1± 1.1	138.0± 1.3
Protein Digestibility, %						
Week 1	78.1± 0.8[a]	76.4± 1.1[a]	91.5± 0.4[b]	91.7± 0.7[b]	80.6± 0.7[a]	80.5± 0.6[a]
Week 2	78.2± 0.8[a]	76.8± 0.5[a]	89.5± 0.7[b]	90.5± 0.4[b]	80.6± 0.2[a]	80.6± 0.2[a]
Week 3	79.3± 1.7[a]	79.6± 1.2[a]	93.8± 0.4[b]	93.7± 0.6[b]	80.1± 0.7[a]	81.9± 0.9[a]
Av Weekly Food Consumption, g						
Weeks 1, 2 and 3	57.2± 11.1[c]	56.1± 5.2[x]	112.0± 14.5[d]	106.1± 7.3[y]	99.3± 10.0[d]	130.5± 20.3[y]
Week 3 only	70.0	61.8	95.3	98.4	87.8	87.3
Nitrogen Retention, mg	212.2± 39.2[c]	124.7± 86.7[x]	550.8± 82.1[d]	492.3± 17.0[y]	339.6± 4.1[d]	480.9± 95.1[y]

[1] See footnote 1, Table 1.

[2] Means ± S.D. $\bar{x}$.

a-b Means with the same letter did not differ significantly, $P<0.05$.

c-d, x-y Means within the same sex with the same letter did not differ significantly, $P<0.05$.

Eight-week weight gains (Table 3, Experiment 2) followed a similar pattern to N retention. Rats fed Cas or LoRSM gained significantly ($P<0.05$) more weight than those fed HiRSM. Growth was particularly poor for females fed the HiRSM, which accounts for the significant ($P<0.05$) sex x treatment interaction for weight gain. Several workers (Bell 1957b; Bowland and Schuld 1968; Drouliscos and Bowland 1969) have observed substantial reductions in liveweight gains of mice, rats and pigs, regardless of sex, when high levels of thioglucoside-containing rapeseed was consumed. In addition, Hussar and Bowland (1959a) and Manns and Bowland (1963a) found that high dietary levels of rapeseed meal had a more pronounced effect on the growth rate of female than male rats. Sex differences in response to rapeseed meal, however, tend to vary with different species; female rats and pigs appear more susceptible (Manns and Bowland 1963a) than female mice (Bell and Baker 1957).

Thyroid enlargement in rats fed the HiRSM diet was the most pronounced gross tissue change observed in these studies although there were also significant increases in weights, when expressed as a ratio of body weight, of the liver, adrenals, kidneys and testes for these animals. The thyroid was the only tissue, however, which was also significantly ($P<0.05$) heavier in animals fed the HiRSM diet when compared on an actual weight basis. Thyroid hypertrophy and accompanying histological changes have been reported for a variety of species fed thioglucoside-containing rapeseed meals (Bell and Belzile 1965) whereas relatively little

Table 3. Weight gain and organ weights of rats fed high- and low-thioglucoside content rapeseed meals - Experiment 2.

	Dietary group[1]					
	HiRSM		Cas		LoRSM	
	Females	Males	Females	Males	Females	Males
No. or rats	7	11	5	5	8	11
Av Initial Wt, g	152.3± 5.3[2]	183.7± 2.3	149.7± 5.4	174.6± 5.4	148.8± 11.9	176.6± 8.1
Total 8 Wk Wt Gain, g[2]	29.3± 9.6^{a}	66.0± 7.7^{x}	101.7± 11.4^{b}	219.5± 11.4^{y}	94.4± 9.0^{b}	209.4± 7.7^{y}
Organ Weights/100 g Body Wt						
Thyroid, mg	14.0± 0.7^{a}	10.3± 0.6^{x}	4.0± 0.9^{b}	5.4± 0.9^{y}	5.5± 0.7^{b}	3.9± 0.6^{y}
Liver, g	4.0± 0.2^{a}	4.6± 0.1^{x}	3.0± 0.2^{b}	2.9± 0.2^{y}	3.3± 0.2^{b}	3.4± 0.1^{y}
Adrenals, mg	31.7± 1.5^{a}	20.6± 1.2^{x}	24.4± 1.7^{b}	11.1± 1.2^{y}	22.7± 1.4^{b}	11.3± 1.2^{y}
Kidneys, mg	858.3± 36.0^{a}	992.1± 28.7^{x}	688.9± 42.5^{b}	651.6± 42.5^{y}	715.8± 33.6^{b}	678.5± 28.7^{y}
Spleen, mg	248.9± 19.4	259.1± 15.5	226.9± 22.9	209.2± 22.9	285.4± 18.1	207.7± 15.5
Testes, mg	---	1113.4± 46.0^{x}	---	850.4± 68.3^{y}	---	889.7± 46.0^{y}

1 See footnote 1, Table 1.

2 Means ± S.D. $\bar{x}$.

a-b, x-y Means within the same sex with the same letter did not differ significantly, $P \leq 0.05$.

information has been published concerning the effect of rapeseed meal on other body organs. Hepatomegaly has been reported in sows fed rapeseed meal during gestation and lactation and a trend towards an increase in liver/body weight ratio was observed in growing rats and market pigs (Bowland et al. 1963). Adrenal and gonad weights of rats and pigs have been reported to be unaffected by consumption of rapeseed meal (Manns et al. 1963c).

Histochemical examination of liver slices revealed striking changes in enzyme patterns in rats fed HiRSM compared to those fed Cas or LoRSM. Since structural integrity of the tissue is maintained by histochemical techniques, changes in activity and localization of these hepatic enzymes has been taken to reflect varying degrees of intracellular disorganization. The following enzymes, Alk P'ase, Acid P'ase, ATPase and TPPase were employed as markers to visualize respectively, cell membranes, lysosomes, bile canaliculi and Golgi regions of the hepatic cell (Novikoff and Essner 1962; Wachstein 1963; Novikoff et al. 1964; Tanikawa 1968).

Wachstein (1963) states that relatively little Alk P'ase is demonstrable in the normal liver, with activity being limited to the bile canaliculi and the odd capillary in the periportal regions, whereas increased activity is seen in the peripheral portions of lobules as a result of dietary necrosis, Salmonella infection of following the administration of carcinogenic agents. A normal distribution of Alk P'ase activity was observed in livers

of the Cas and LoRSM groups (Figs. 2 and 3) while an increased Alk P'ase reaction was evident on the periphery of liver lobules of the HiRSM group, especially among females (Fig. 4). In addition, an intense enzymatic activity was seen in many hepatocellular membranes (Fig. 5).

The livers of the Cas and LoRSM rats exhibited a normal, positive Acid P'ase activity (Novikoff and Essner 1960; Wachstein 1963) with the lysosomes being located primarily along the bile canaliculi and in the Browicz-Kupffer cells (Fig. 6). However, a slight decrease in Acid P'ase activity in the centrilobular areas of liver lobules and changes in lysosomes localization were sometimes visible in the LoRSM-fed rats (Fig. 7). Decreased Acid P'ase activity also was observed in the centrilobular areas of livers of rats fed HiRSM. In addition, formation of cytolysomes, enlarged acid-rich organelles, dislocalization of lysosomes and an increase in the number of positive Browicz-Kuppfer cells were visible (Figs. 8 and 9). Decreased overall staining for Acid P'ase has been associated with general hepatocellular damage (Wachstein et al. 1962).

A strong, positive ATPase reaction, characteristic of normal rat liver (Wachstein 1963) was observed in livers of rats fed Cas or LoRSM (Fig. 10). By contrast, livers from rats fed HiRSM showed decreased ATPase activity in the central part of the

lobule (Fig. 11). In addition, the bile canaliculi were wider and more convoluted. A reduction in bile canaliculi also has been reported following experimental hepatic damage (Wachstein 1963).

Distribution of hepatic TPPase activity was identical for all groups, irrespective of dietary regimen, to that described by Novikoff and Essner (1962) for the normal liver. The Golgi apparatus was visible in only a few cells and in general the lightly stained Golgi lamellae were located near the bile canaliculi. Changes in the Golgi complex has been reported to be affected by dietary factors. A high level of rapeseed oil in the diet of rats has been recently shown to alter the Golgi complex and to disrupt its topographical location in hepatic cells (Ziemlański et al. 1970).

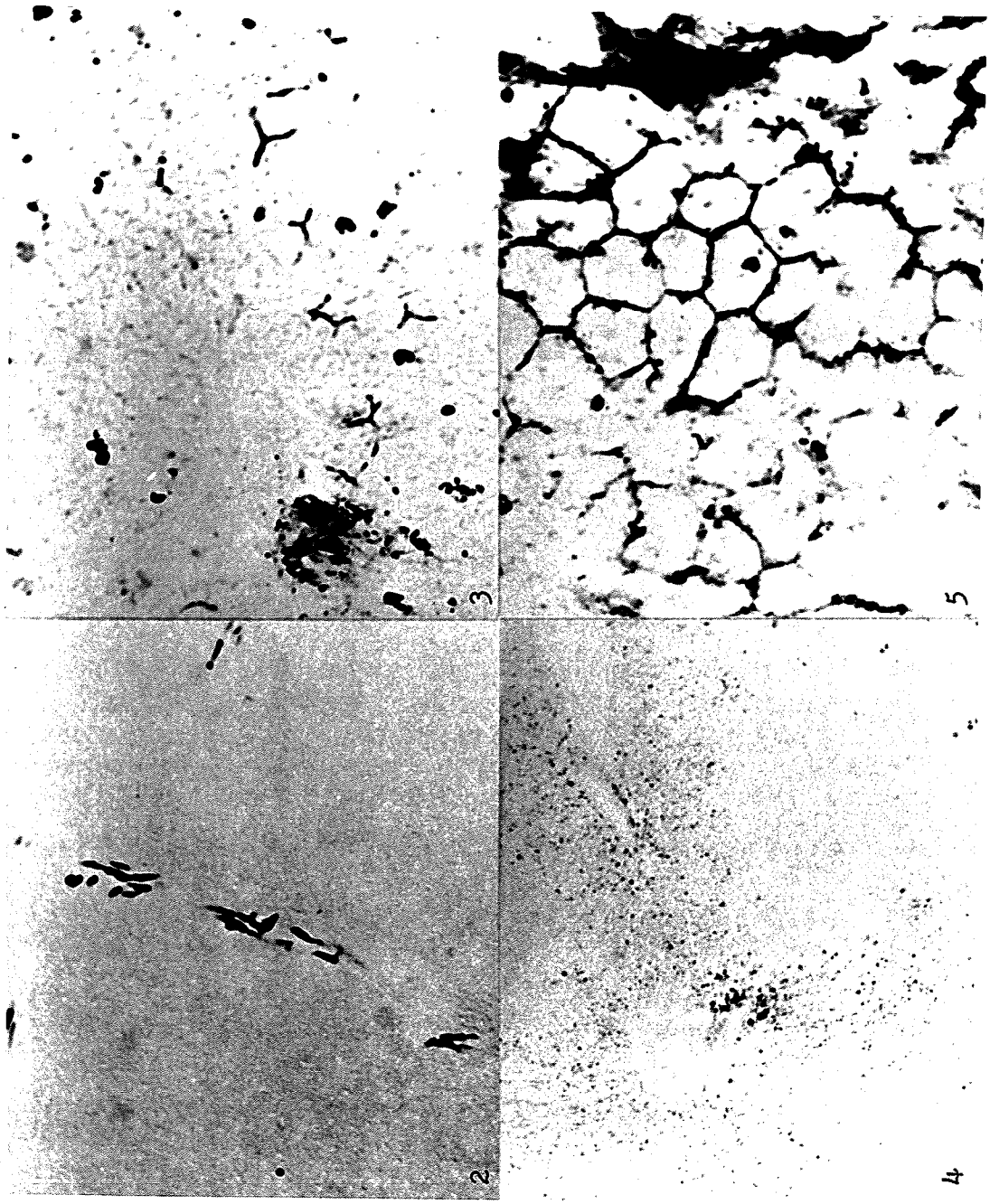
No lipid infiltration was observed in livers of the Cas-fed rats or in females fed LoRSM (Fig. 12). Slight to moderate liver lipid infiltration was observed, however, in males fed LoRSM (Fig. 13). By contrast, lipid infiltration was observed in livers of both sexes fed HiRSM (Fig. 14).

B. EVALUATION OF THE NUTRITIVE VALUE OF RAPESEED FLOUR AS A POTENTIAL PROTEIN SOURCE FOR HUMANS

B.1. Object

The object of the human metabolic study was to determine the supplemental value of rapeseed flour as a protein source in an all-vegetable diet. The rapeseed flour which was prepared by the Food Research Institute, Canada Department of Agriculture

List of Figures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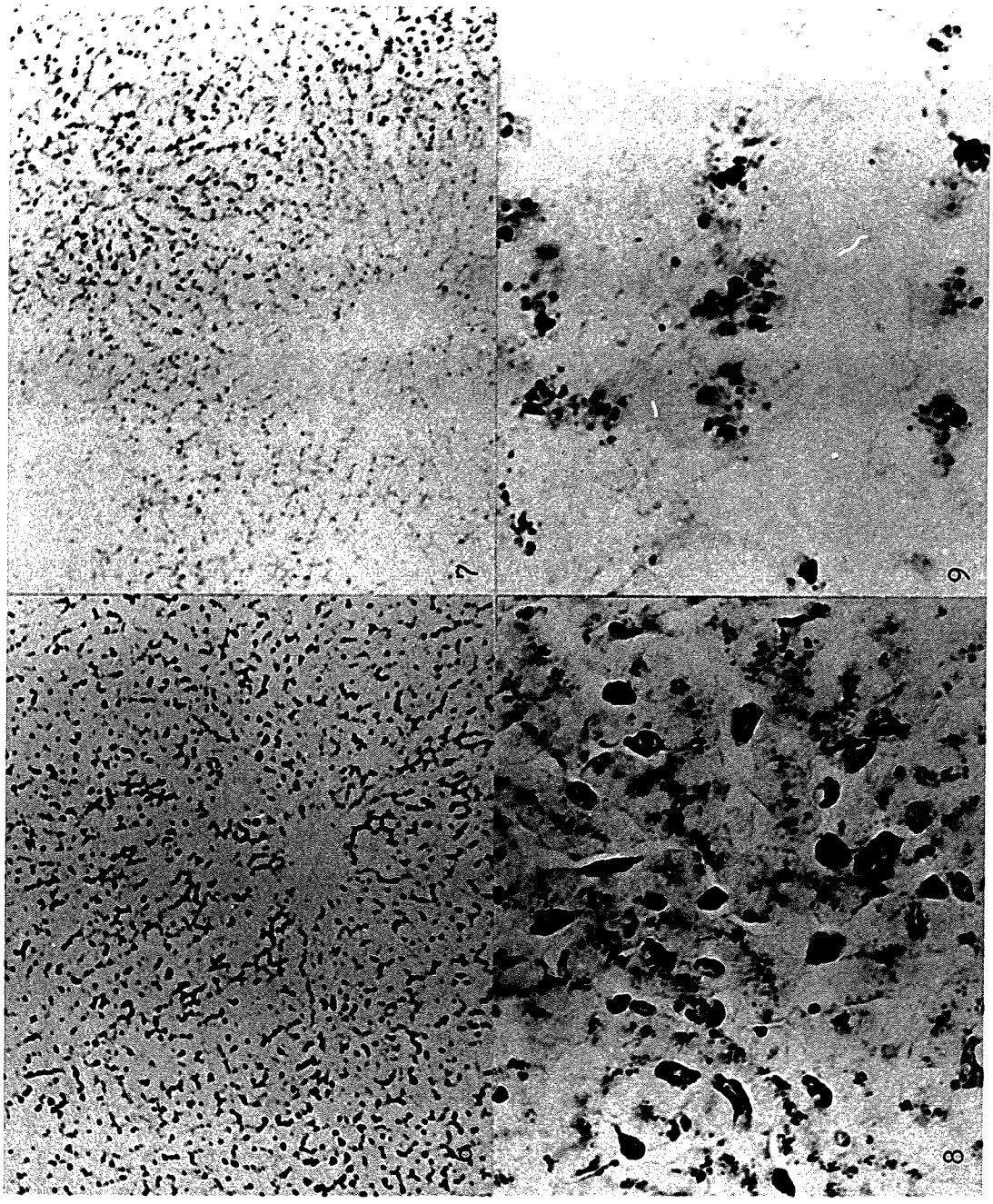
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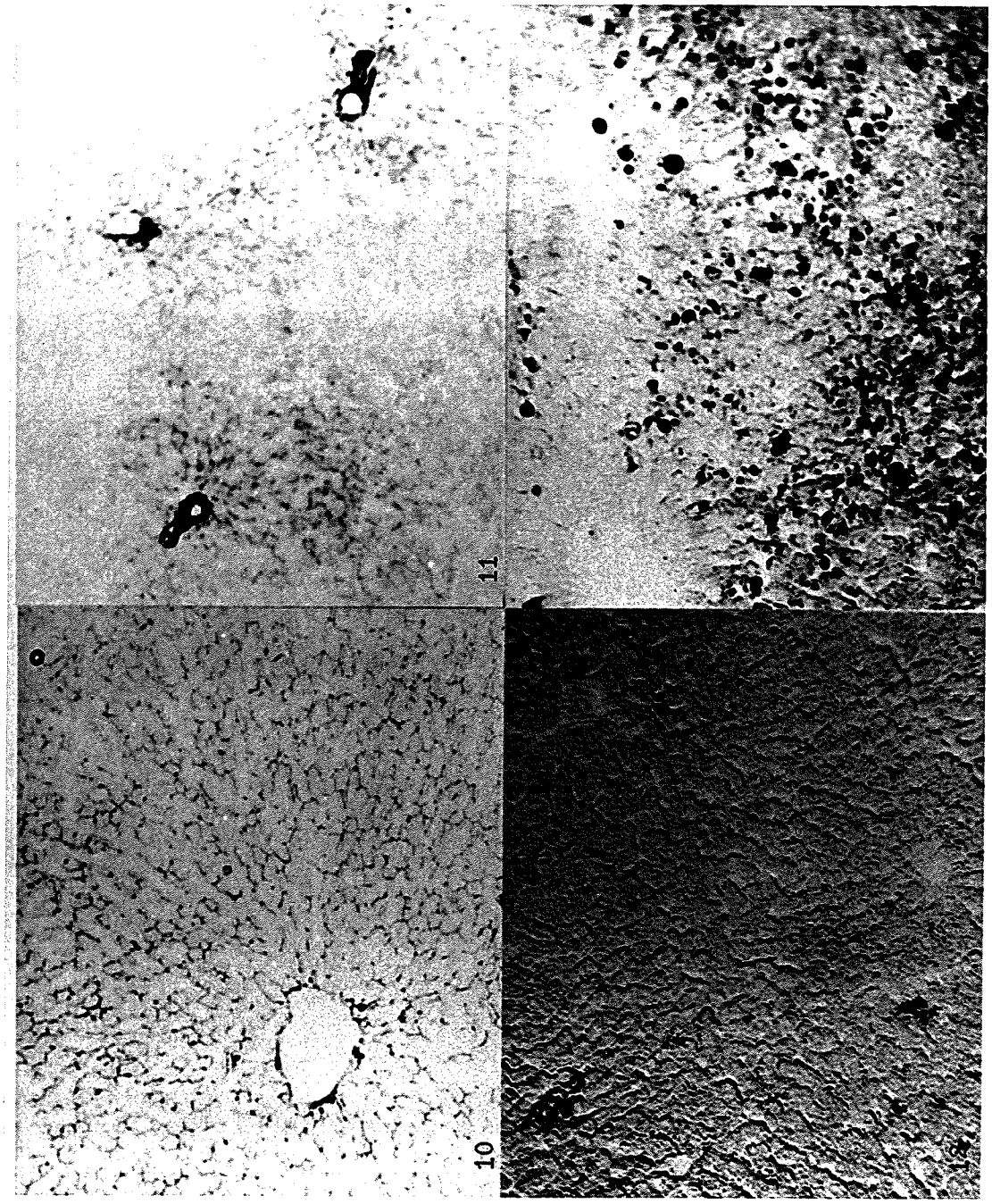
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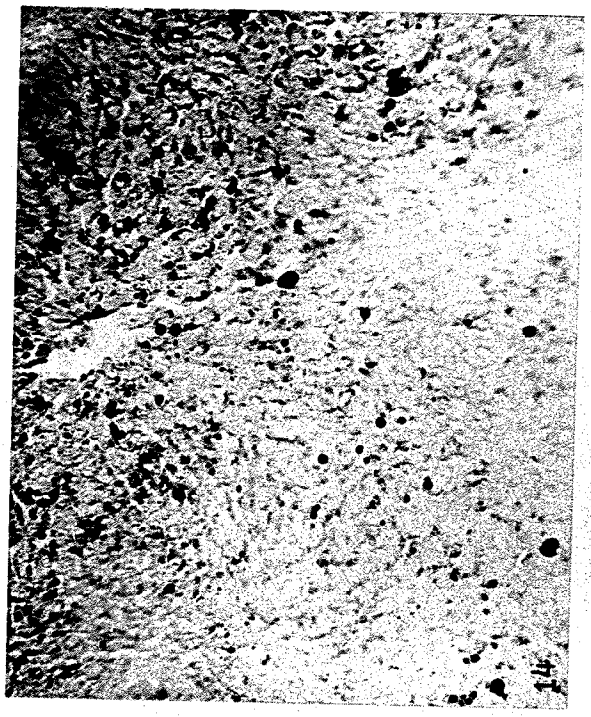

was incorporated into the diet at a level equivalent to 40% of the total daily protein intake (8.0 g).

B.2. Methods and Materials

B.2.a. Diets and Experimental Design

The subjects were 12 female senior and graduate students in Home Economics at the University of Manitoba. They ranged in age from 20 to 27 years, were of average height and weight, 161.3 cm and 53.3 kg, respectively, and had no recent history of illness or poor health. During the conduct of the metabolic study the subjects resided in the Home Management apartments (Home Economics Building, University of Manitoba). They were allowed to maintain their normal activities, in fact normal routines were encouraged (Appendix Tables 1 and 5). The subjects weighed themselves daily before breakfast and were asked to attempt to maintain their weight by adjusting their caloric intake. Each subject was given a notebook in which she recorded her daily weight, caloric intake and hours of sleep. This same book also was used to record the volume of each urine voiding and the size of aliquot preserved for analysis (Appendix Table 6).

Nitrogen intake of the experimental subjects was standardized during an initial 3-day pre-experimental period in which subjects consumed a controlled diet of ordinary foods. The diet provided approximately 60 g protein per day while calories were voluntarily adjusted to need. Extra calories were provided by sugar cubes, butter, jelly (apple, grape, red currant),

Coffee Rich*, carbonated drinks and candies (peppermints, raspberry drops, gumdrops). Coffee and tea were allowed ad libitum during the pre-experimental and experimental periods.

After the 3-day pre-experimental period, subjects were randomly allotted to 3 experimental diets in a modified cross-over design. The diets were designated rapeseed flour (RSF), casein (Cas) and wheat gluten (WG) on the basis of the major source of supplemental protein in each**. The 4 subjects randomized to the RSF diet in Period A were switched to the Cas diet in Period B. Similarly, the 4 subjects on the Cas diet in Period A were switched to the RSF diet in Period B. Since only 2 subjects could be assigned to the wheat gluten diet in each period, 2 new subjects were enlisted for this diet during Period B.

The supplemental protein sources, rapeseed flour, vitamin-free casein and wheat gluten, were each incorporated into isonitrogenous (3.2 g), isocaloric (930.4 calories) "baking powder biscuits". Each subject consumed one and one-third biscuits (1/3 of their daily allottment at each meal). The daily allottment of biscuits of each subject was preweighed prior to baking. Following baking the biscuits were stored at -15°C until 12 hours prior to being served. Since the metabolizable energy content of RSF is

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* Coffee Rich - Trade name of an artificial protein-free beverage cream. Rich Products of Canada Limited, Fort Erie, Canada.

** Subsequently, the abbreviations RSF, Cas and WG will refer to the chief supplemental sources of protein in the 7-day experimental diet, rapeseed flour, vitamin-free casein and wheat gluten, respectively.

unkown, calculations were based on the assumption that all the carbohydrate (approximately 42%) is available.

Canned and frozen foods were purchased in case lots to ensure reasonable uniformity of composition. Fresh vegetables and fruits were purchased as required. All vegetables, except potatoes, were weighed after cooking during both the pre-experimental and experimental periods. Potatoes, which were wrapped in aluminum foil and baked, were weighed prior to baking. Each allotted serving of the diet was weighed to the nearest gram on a Sartorius top-loading balance.

Ingredient composition of the basal portion of the experimental diet, which was common to all subjects, the pre-experimental diets and the biscuits is shown in Appendix Tables 2, 3 and 4. All calculated values were based on Agricultural Handbook No. 8 (1963). Each supplemental protein source provided 40% of the total protein in the all-vegetable experimental diet.

An extra serving of the entire pre-experimental diet was weighed each day. Similarly, an extra serving of the entire basal portion of the experimental diet was weighed on 3 different days. A subject's daily allottment of RSF, Cas and WG biscuits also was taken on 2 separate days. Subsequently, all diets and biscuits were frozen at -15°C, lyophilized and later analyzed for crude protein and amino acids.

B.2.b. Urine and Fecal Collection Procedures

Styrofoam commodes, 30.5 cm polyethylene funnels, one

litre glass collection jars, 10 ml pipettes and polyethylene storage bottles were used for urine collections. A diagram depicting the design of the apparatus is shown in Figure 2. Mimeographed instructions summarizing the procedures to be followed in measuring the volume of urine voided and in obtaining aliquots for analyses was given to each subject (Appendix Table 6). A representative sample (5%) of the total urine voided during each 24-hour period was collected for each subject. A 10% aliquot of each voiding also was taken on 2 consecutive days (7th and 8th) of each period. These samples were stored in individual polyethylene bottles which were identified by subject and the precise time of voiding. The latter samples were collected for another study under the direction of Dr. B. E. McDonald, Department of Foods and Nutrition. All urine samples were preserved with 0.1 ml toluene and 1 ml 10 N H_2SO_4 and kept frozen (-14°C) until analyzed for crude protein.

Scale: 1 cm = 10 cm

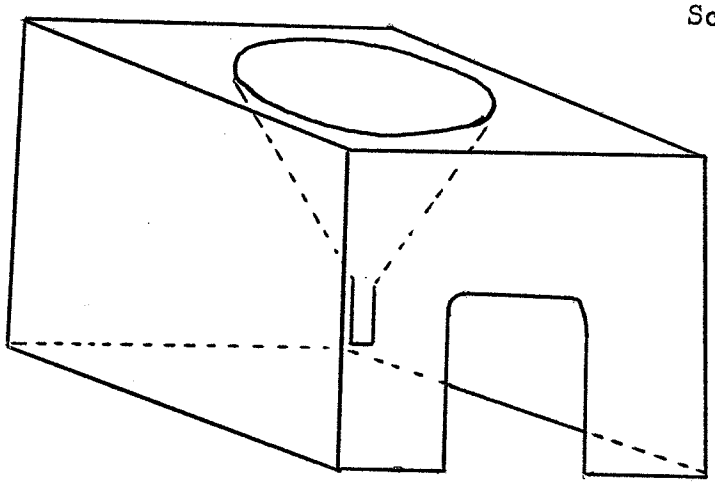

Figure 15. Styrofoam commode.

Feces samples were collected on 4 consecutive days (7th to 10th inclusive) during experimental periods A and B. They were collected on Saran wrap*, wrapped in brown paper, placed on dry ice and stored immediately at -14°C. Four-day composite feces samples were later homogenized in a Model CB-4 Waring Blender**, the final volume adjusted to 2 litres with distilled water and three 10-ml aliquots taken with a slurry pipette for nitrogen determinations.

B.2.c. Chemical Analyses

Diets and biscuits were lyophilized in a Model 10-MR-TR Virtis freeze drier*** and ground in a Wiley Mill Model No. 2+ using a 1 mm mesh screen prior to analyses. Diets, biscuits, 24-total urine samples and feces were analyzed for total nitrogen content by the macro Kjeldahl method as previously described (Section IV. A.2.a.).

A representative sample of each diet and biscuits was hydrolyzed according to the method of Bragg et al. (1966). These hydrolyzed samples were then analyzed for amino acids on a Beckman Model 116 Amino Acid Analyzer++ according to the method of Benson and Patterson (1965).

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* Saran wrap - Trade name of a clear plastic film material. Dow Chemical of Canada, Sarnia, Ontario.

** Waring Products Corporation, Winsted, Conn.

*** Virtis Co., Inc., Gardiner, New York.

\+ Arthur Thomas and Co., Philadelphia, Penn.

++ Beckman Instruments, Inc., Palo Alto, California.

B.2.d. Statistical Analyses of Data

The N retention data was subjected to cross-over analysis of variance (Cochran and Cox 1957), whereas a t-test for paired variance was used in analysis of the protein digestibility data.

B.3. Results and Discussion

Protein digestibility and nitrogen retention data from the Human Metabolic Study are presented in Tables 4, 5 and 6. Apparent protein digestibility was significantly lower ($P<0.01$) for the RSF diet than for the Cas diet (81% and 86%, respectively). These results with the human coincide with those observed for the rat (Section IV A.3.) in that a lower apparent protein digestibility was observed for rapeseed protein than for casein. Mean apparent protein digestibility also was higher for the WG diet than for the RSF diet although the data for wheat gluten was not included in the statistical analysis.

If one considers nitrogen equilibrium as the range within which total N excretion is equal to 95 - 105% of the total dietary N intake (Leverton et al. 1956), then all subjects in the present study, except Subject 4, were in N equilibrium or positive N balance during both periods irrespective of the source of supplemental protein. Subject 4 was in negative N balance during both periods of the study. Subject 5 also approached negative N balance in Period B. Since Subject 4 was on the RSF diet during Period A and the Cas diet during Period B, dietary treatment does not appear to be a major factor contributing to these

Table 4. Summary of nitrogen intake, nitrogen excretion and nitrogen balance of subjects fed experimental diets during Periods A and B - Human Metabolic Study

Diet	No. of subjects	N intake, g	Urinary N, g	Fecal N, g	App. prot. digestibility,[1] %	N absorption, g	N retention g
RSF	8	8.0 ± 0.1	6.7 ± 0.5	1.5 ± 0.3	81.1 ± 4.5[a]	6.4 ± 0.4	-0.3 ± 0.8
Cas	8	8.3 ± 0.1	7.3 ± 0.2	1.2 ± 0.3	85.6 ± 3.8[b]	7.2 ± 0.3	-0.1 ± 0.5
WG	4	8.4 ± 0.3	6.6 ± 0.9	1.0 ± 0.1	87.4 ± 1.2	7.4 ± 0.3	+1.0 ± 0.7

[1] Means with a different letter differ significantly, $P<0.01$.

Table 5. Mean daily nitrogen intake, nitrogen excretion and nitrogen balance for individual subjects during Period A - Human Metabolic Study

Diet[1]	Subject	N intake, g	Urinary N, g	Fecal N, g	App. pro. digestibility, %	N absorption, g	N retention, g
RSF	1	7.9	6.3	1.5	81.6	6.4	+0.1
	2		6.4	1.5	81.0	6.4	+0.0
	3		6.6	1.2	84.9	6.7	+0.1
	4		7.4	2.2	71.6	5.6	-1.7
Group mean ± S.D.			6.7 ± 0.5	1.6 ± 0.5	79.8 ± 5.7	6.3 ± 0.5	-0.4 ± 0.9
Cas	5	8.2	7.0	1.2	85.6	7.1	+0.1
	6		7.3	1.3	84.8	7.0	-0.3
	7		7.2	1.2	85.7	7.1	-0.1
	8		7.2	1.0	87.7	7.2	+0.0
Group mean ± S.D.			7.2 ± 0.1	1.2 ± 0.1	86.0 ± 1.2	7.1 ± 0.1	-0.1 ± 0.2
WG	9	8.2	5.9	1.0	87.0	7.1	+1.2
	10		7.0	1.1	86.5	7.1	+0.1
Group mean ± S.D.			6.4 ± 0.8	1.1 ± 0.1	86.8 ± 0.4	7.1 ± 0.0	+0.7 ± 0.8

[1] RSF, Cas and WG identify the supplemental protein sources which were: rapeseed flour, vitamin-free casein and vital wheat gluten, respectively.

Table 6. Mean daily nitrogen intake, nitrogen excretion and nitrogen balance for individual subjects during Period B - Human Metabolic Study

Diet[1]	Subject	N intake, g	Urinary N, g	Fecal N, g	App. pro. digesti-bility, %	N absorption, g	N retention, g
RSF	5	8.0	7.4	1.7	78.9	6.3	-1.1
	6		5.9	1.3	83.6	6.7	+0.8
	7		6.5	1.1	85.9	6.9	+0.4
	8		6.9	1.5	81.4	6.5	-0.4
Group mean ± S.D.			6.7 ± 0.6	1.4 ± 0.3	82.5 ± 3.0	6.6 ± 0.3	-0.1 ± 0.9
Cas	1	8.4	7.1	1.5	82.2	6.9	-0.2
	2		7.3	0.8	90.4	7.6	+0.4
	3		7.1	0.9	89.6	7.6	+0.4
	4		7.8	1.8	78.9	6.7	-1.2
Group mean ± S.D.			7.3 ± 0.3	1.2 ± 0.5	85.3 ± 5.6	7.2 ± 0.5	-0.1 ± 0.7
WG	11	8.6	7.6	0.9	89.2	7.7	+0.2
	12		5.8	1.1	87.0	7.5	+1.8
Group mean ± S.D.			6.7 ± 1.3	1.0 ± 0.1	88.1 ± 1.6	7.6 ± 0.1	+1.0 ± 1.1

[1] See footnote 1, Table 5.

Table 7. Mean urinary N excretion of subjects during experimental periods A and B - Human Metabolic Study

Diet	Subject	N intakes, g	Experimental Period	
			Days 1 - 3	Days 4 - 7
Period A				
RSF	1	7.9	6.9 ± 0.3	6.3 ± 0.6
	2		---[1]	6.4 ± 0.6
	3		7.5 ± 1.2	6.6 ± 0.4
	4		7.6 ± 0.9	7.4 ± 0.2
Group mean ± S.D.			7.3 ± 0.4	6.7 ± 0.5
Cas	5	8.2	7.6 ± 0.7	7.0 ± 0.4
	6		5.7 ± 0.3	7.3 ± 1.4
	7		6.6 ± 0.2	7.2 ± 0.5
	8		---	7.2 ± 0.1
Group mean ± S.D.			6.6 ± 1.0	7.2 ± 0.1
WG	9	8.2	7.8 ± 0.2	5.9 ± 0.9
	10		7.5 ± 1.0	7.0 ± 0.7
Group mean ± S.D.			7.7 ± 0.2	6.4 ± 0.8
Period B				
RSF	5	8.0	8.2 ± 0.6	7.4 ± 0.4
	6		5.3 ± 0.8	5.9 ± 1.2
	7		6.4 ± 0.5	6.5 ± 0.5
	8		6.7 ± 0.0	6.9 ± 0.5
Group mean ± S.D.			6.6 ± 1.2	6.7 ± 0.6
Cas	1	8.4	7.3 ± 0.6	7.1 ± 0.6
	2		---	7.3 ± 1.1
	3		7.5 ± 0.6	7.1 ± 0.9
	4		8.4 ± 0.5	7.8 ± 0.3
Group mean ± S.D.			7.7 ± 0.6	7.3 ± 0.3

- Continued -

Table 7 (cont'd)

Diet	Subject	N intakes, g	Experimental Period	
			Days 1 - 3	Days 4 - 7
WG	11	8.6	7.8 ± 0.4	7.6 ± 0.3
	12		7.0 ± 0.5	5.75 ± 0.8
Group mean ± S.D.			7.4 ± 0.5	6.7 ± 1.3

[1] Urine not collected for subject during this period.

results. Examination of the data indicates that low protein digestibility and high urinary N excretion, especially the latter, contributed to the negative N balance for these subjects (Tables 5, 6 and 7).

Various investigators (Clark et al. 1962; Clark et al. 1966; Kolski et al. 1969) have observed that N balance of individual subjects within a treatment group varied even when experimental conditional were identical for all. The individual variation observed in the present study coincides with those of Clark et al. (1962; 1966) and Kolski et al. (1969).

One subject fed the WG diet was in positive N balance in each period (Subject 9, Table 5 and Subject 12, Table 6). Examination of the data revealed that nitrogen losses, unlike those of Subjects 4 and 5, were lower than average. Urinary N excretion for Subjects 9 and 12 were low only during the 4-day metabolic study in Period A and B, respectively (Table 7). In fact, N excretion was especially low for two days of the metabolic study (Appendix Table 8).

All subjects maintained fairly constant body weights during both periods (Table 8). The subjects appeared to be successful in adjusting caloric intakes since the extra calories consumed by each subject were adequate to maintain a constant body weight. The original assumption that the carbohydrate in RSF was available to the human appears to be substantiated in this study since there were no changes in body weight whether the subjects were on the RSF or Cas diet and the subjects

Table 8. Mean body weight and extra calories consumed by subjects during experimental periods A and B - Human Metabolic Study.

Diet	Subject	Mean daily body weight, kg	Body weight range, kg	Extra calories consumed (mean daily)
Period A				
RSF	1	63.9 ± 0.2	63.5 - 64.2	232 ± 48
	2	51.8 ± 0.0	51.8 - 51.8	105 ± 77
	3	45.6 ± 0.1	45.5 - 45.9	20 ± 27
	4	56.3 ± 0.2	56.0 - 56.4	339 ± 58
Group mean ± S.D.		54.4 ± 7.7		174 ± 140
Cas	5	60.5 ± 0.3	60.1 - 61.0	288 ± 158
	6	49.6 ± 0.1	49.5 - 50.0	227 ± 78
	7	41.9 ± 0.2	41.7 - 42.3	319 ± 71
	8	50.8 ± 0.1	50.7 - 50.9	269 ± 141
Group mean ± S.D.		50.7 ± 7.7		275 ± 38
WG	9	54.3 ± 0.1	54.2 - 54.5	344 ± 99
	10	53.8 ± 0.2	53.5 - 54.1	519 ± 165
Group mean ± S.D.		54.1 ± 0.4		432 ± 124
Period B				
RSF	5	60.8 ± 0.1	60.6 - 60.9	208 ± 100
	6	50.0 ± 0.2	50.0 - 50.5	260 ± 73
	7	41.7 ± 0.3	41.0 - 42.3	252 ± 46
	8	50.5 ± 0.2	50.1 - 50.7	247 ± 101
Group mean ± S.D.		50.8 ± 7.8		241 ± 23
Cas	1	63.2 ± 0.2	63.1 - 63.5	206 ± 46
	2	51.8 ± 0.0	51.8 - 51.8	148 ± 242
	3	45.9 ± 0.2	45.5 - 46.1	8 ± 20
	4	55.9 ± 0.2	55.8 - 56.3	250 ± 37
Group mean ± S.D.		54.2 ± 7.3		153 ± 106

- Continued -

Table 8 (cont'd).

Diet	Subject	Mean daily body weight, kg	Body weight range, kg	Extra calories consumed (mean daily)
WG	11	51.6 ± 0.1	51.5 - 51.8	136 ± 64
	12	48.6 ± 0.2	48.2 - 48.6	230 ± 155
Group mean ± S.D.		50.1 ± 2.2		183 ± 66

tended to consume the same number of extra calories with both diets.

The pre-experimental diet, basal portion of the experimental diet and biscuits were formulated to supply 60, 13 and 39 g protein per day. Crude protein analyses revealed that the analyzed values differed considerably from the calculated values in some instances (Table 9). The fact that the actual protein intake on Day 2 of the pre-experimental period was 77 g whereas the intake on Day 3 was 54 g illustrates the problem which confronts nutritionists when formulating diets using composition tables such as Agricultural Handbook No. 8 (1963).

The biscuits were formulated to contain 20 g protein from each protein source. Since the actual protein content of the biscuits varied from the calculated values, possible sources of error were investigated. A mistake was discovered in the formulation of the WG biscuits whereby the amount of wheat gluten incorporated in the biscuits resulted in a calculated protein content of 40.24 g instead of 38.67 g. It was impossible to check the analyses for the biscuits because the samples were inadvertently discarded. However, the protein content of rapeseed flour was checked, this time by another person, and similar results were obtained (50.9% protein by the original analysis and 50.1% protein by the re-run). Errors may have occurred in the actual preparation of the RSF biscuits which analyzed only 36.75 g protein, since two people, the experimenter and a

Table 9. Chemical analyses of diets and biscuits - Human Metabolic Study

Item	Calculated calories	Calculated protein g	Analyzed protein (N x 6.25)
Pre-Experimental Diets			
Day 1	1862.26	59.90	65.42
Day 2	1740.06	60.41	76.86
Day 3	1617.26	60.09	54.11
Experimental Diet			
Basal Diet	764.40	12.96	13.17
Biscuits[1]			
Rapeseed flour[2]	930.36	38.67	36.75
Vitamin-free casein[3]	930.36	38.67	38.68
Wheat gluten[4]	930.36	40.24[5]	39.58

1 Each supplemental protein source provided 20 g protein.

2 Since calorie content of RSF is unknown, calculations were based on the assumption that all carbohydrate present is available (approximately 42%).

3 Vitamin-free casein obtained from Nutrition Biochemicals Corp., Cleveland, Ohio.

4 Wheat gluten obtained from Ogilvie Flour Mills Co. Ltd., Winnipeg, Manitoba.

5 An error was made in the original formulation of the WG biscuits.

technician, made the biscuits and mixing facilities and oven space were adequate for only 16 - 20 biscuits at one time. Unfortunately the actual factor(s) responsbile for the lower analyzed protein content of the RSF biscuits could not be determined.

Amino acid analyses of the basal portion of the experimental diet and biscuits (Appendix Tables 9 and 10) revealed that all 3 experimental diets were adequate in all amino acids. Daily lysine intake of the RSF, Cas and WG diets was 1.96, 2.66 and 1.18 g respectively (Table 10). This is considerably higher than the recommended lysine requirements (0.55 g/day) cited by Hegsted (1968). In fact, the WG diet provided twenty times the lysine requirement recently reported by Fisher et al. (1969) for young women. The diets also provided substantially greater quantities than those cited by Hegsted (1968) for the other essential amino acids (Table 10). In fact, except for methionine and valine, the essential amino acid requirements of the subjects were met by the basal portion of the experimental diet in the present study (Appendix Table 10).

It is interesting to note that the amino acid composition of rapeseed flour is reasonably well balanced and is comparable to a high quality protein such as casein. Apparent protein digestibility of rapeseed flour, however, was significantly ($P \leq 0.01$) lower than casein, which is in agreement with earlier results obtained with rats in our laboratory (Section IV a.3.). The protein digestibility of wheat gluten, which was similar to

Table 10. Total amino acid consumption per subject per day - Human Metabolic Study

	g Amino acids			
	Diets			Amino acid requirement[1]
	RSF	Cas	WG	
Essential				
Lysine	1.96	2.66	1.18	0.55
Threonine	1.71	1.73	1.36	0.38
Valine	2.11	2.52	1.98	0.62
Leucine	3.45	4.09	3.32	0.73
Isoleucine	1.70	1.89	1.58	0.55
Methionine	0.84	0.70	0.71	0.19
Phenylalanine	2.02	2.21	2.37	0.26
Non essential				
Serine	2.30	1.97	2.19	
Histidine	1.11	1.22	1.10	
Aspartic acid	3.94	4.23	3.19	
Glutamic acid	11.44	12.27	16.13	
Glycine	2.02	1.37	1.29	
Alanine	2.17	1.81	1.15	
Proline	4.4	4.83	5.90	
Arginine	3.46	1.88	2.19	
Tyrosine	1.32	1.93	1.45	

[1] Hegsted (1968)

casein, also was higher than for rapeseed protein. The N retention data of the present study suggests that rapeseed flour is as well utilized by the human adult as a high quality protein such as casein (Table 4). However, caution must be exercised in drawing conclusions from the present study because the basal portion of the experimental diet supplied adequate quantities of the essential amino acids for the adult human.

More experimental research is required at this time to improve the color, palatability, texture and baking qualities of rapeseed flour, before any further human experimentation is attempted. In addition, since the amino acid requirements of the adult human are low, caution must be exercised in the formulation of the experimental diets in future metabolic studies when protein quality is being evaluated.

V. SUMMARY

Two experiments with growing rats and a human metabolic study with college women were conducted to evaluate the nutritional quality of rapeseed protein. The rat studies were designed to compare the performance of rats fed a high-thioglucoside content and a low-thioglucoside content rapeseed meal with casein. The criteria used to evaluate these rapeseed meals included growth rate, organ weights, liver histochemistry and nitrogen metabolism. The Human Metabolic Study was designed to assess the supplemental value of protein from a water-extracted thioglucoside-free rapeseed flour (RSF) recently developed by the Food Research Institute, Canada Department of Agriculture. The parameters used in evaluating the protein quality of RSF included apparent protein digestibility and N balance.

The results of the rat experiments indicated that the thioglucoside-content of rapeseed meal had an appreciable effect on the performance of rats fed rapeseed meal as the sole source of protein. Protein digestibility of rapeseed meal by growing rats was lower than that of casein, irrespective of thioglucoside content, whereas 4-day N retention and 8-week weight gains were significantly lower for rats fed the high-thioglucoside-content meal (HiRSM), than those fed the low-thioglucoside-content meal (LoRSM) or casein (Cas). Thyroid hypertrophy in theHiRSM-fed rats was the most pronounced change observed in organ weights, which is in agreement with the observations of numerous investigators. However, there

were also increases in the weights of liver, kidneys, adrenals and testes of the HiRSM animals when expressed as a ratio of body weight. The apparent hepatomegaly in the HiRSM-fed rats was accompanied by an appreciable derangement of hepatic cells as evidenced by marked changes in activity and localization of histoenzymatic patterns and severe lipid infiltration. Consumption of LoRSM resulted in a slight decrease in hepatic acid phosphatase activity and slight to moderate lipid infiltration in males when compared to casein-fed control rats, but in general, the histochemical picture was similar to that of Cas-fed animals. These results suggest that the presence of thioglucosides is the primary factor responsible for the poor performance reported for animals fed commercially available rapeseed.

The results of the Human Metabolic Study indicated that the apparent protein digestibility of rapeseed flour was significantly ($P<0.01$) lower than that of casein. This observation was in agreement with the rat experiments where digestibility of rapeseed meal also was significantly lower than casein. Four-day N retention studies with humans showed all subjects, except one, to be in N equilibrium or positive N balance whether they were fed the rapeseed flour, casein or wheat gluten diets. However, caution must be used in interpreting these results because all experimental diets contained adequate amounts of essential amino acids for the young adult human. In fact, the basal portion of the experimental diet contained adequate amounts of essential amino acids with the

exception of methionine and valine. Since all subjects were able to maintain their body weight during both periods of the metabolic study, it suggests that the carbohydrate in rapeseed flour is available to the human.

Appendix Table 1. General Instructions - Human Metabolic Study

(Sept. 8 - 18th and Oct. 7 - 17th, 1969)

1. In a metabolic study since food intake is being carefully controlled by exact weighing it is important that all food on the preliminary and experimental diets be consumed. In other words, it means literally "scraping and/or licking your plate clean". Extra calories are provided by free foods which are allowed (see "constant diet" sheets).
2. Coffee Rich and sugar cubes will be provided where coffee and/or tea is being served. Coffee Rich is a milk substitute with a 0 protein content. For ease of caloric calculation sugar cubes are being used.
3. Meal times are as follows: breakfast----7:45 a.m.
   lunch-------12:30 p.m.
   supper-------5:45 p.m.
   If you are unable to eat at these times please arrange a convenient time for yourself.
4. It is desirable to provide a constant environment in a metabolic study. You will maintain your own normal activities. Please refrain from participating in any strenous, seldom-occurring activities. It is requested that you be back in the apartment by 11:00 - 11:30 p.m.
5. Keep a daily record of your caloric intake, weight (before breakfast), and hours of sleep per night in the notebook provided. It is desirable for you to maintain your weight i.e.

- Continued -

Appendix Table 1 (cont'd)

your average weight during the 3-day pre-experimental period.

6. If you are given keys to the apartment, it is your responsibility that they are not lost.
7. Be sure that all garbage be placed in paper bags or newspaper, and tightly wrapped. Place in the hall container for removal.
8. It is your responsibility to keep neat and tidy all areas which are being used. This is especially important when leaving the apartments in the morning.
9. In order that there will be no shortage of hot water for dishwashing, please stagger the times you will be requiring hot water throughout the day and evening. Bedding will be laundered at the end of each 10 day experimental session. Laundry facilities and detergent are available for your use.
10. Sulfuric acid (10 N) and toluene is being added to each aliquot urine bottle. Be careful when taking your aliquot and handle these bottles with due precaution. Otherwise your clothes may suffer the consequences.

Appendix Table 2. Three-Day Pre-Experimental 60 g Protein Diets - Human Metabolic Study

Day 1 - Pre-Experimental Diet

Item	Amount	Calculated Calories	Calculated Protein, g
Breakfast:			
Bran flakes	20 g	60.6	2.04
Milk, homo	180 g	114.75	6.23
Grapefruit guice, uns.	120 g	49.2	.6
Raisins	30 g	86.7	.75
Toast, white	1 slice	80.0	2.5
Egg, soft-boiled	1	72.0	5.7
	Sub-total	463.25	17.82
Lunch:			
Tomato juice	120 g	22.8	1.08
Grilled cheese sandwich			
Bread, white	2 slices	160.0	5.0
Cheese	1 slice	105.0	6.6
Butter pat	6.0 g	43.06	---
Dill pickle	½	---	---
Coleslaw	100 g	20.0	1.0
Gingerbread	76 g	202.28	.77
Ice cream	78 g	163.0	3.0
	Sub-total	716.14	17.45
Afternoon Snack:			
Orange	½ large	51.0	1.2
Super:			
Tossed salad		---	---
Spaghetti, cooked	100 g	111.0	3.4
Meatballs and	86.5 g	340.87	16.98
Sauce	120 g	---	---
Cake, angel food	50 g	129.5	2.85
Blueberries, canned	50 g	50.5	.2
	Sub-Total	631.87	23.43
	Total Day 1	1862.26	59.90

- Continued -

Appendix Table 2 (cont'd)

Day 2 - Pre-Experimental Diet

Item	Amount	Calculated Calories	Calculated Protein, g
Breakfast:			
Orange juice, sw.	120 g	62.4	.84
Egg, boiled	1	72.0	5.7
Toast, white	2 slices	160.0	5.0
Peanut butter	14 g	81.0	3.7
Bran muffin	1	86.0	2.5
	Sub-total	461.40	17.74
Lunch:			
Apple juice	120 g	60.0	.1
Hot dog			
Bun, wiener	1	160.0	5.0
Wiener	40 g	103.0	5.7
Butter pat	6.0 g	43.06	---
Mustard, as desired		---	---
Pie, lemon meringue	100 g	255.0	3.7
	Sub-total	621.06	14.50
Afternoon Snack:			
Banana	100 g	89.0	1.2
Supper:			
Tomato juice	120 g	22.8	1.08
Sirloin steak, E.P.	100 g	254.0	17.3
Potato, baked	100 g	96.0	2.6
Sour cream	14.5 g	44.5	.35
Asparagus	85 g	20.0	2.0
Roll	30 g	93.0	2.7
Jello	10 g (powder)	38.3	.94
	Sub-total	568.60	26.97
	Total Day 2	1740.06	60.41

- Continued -

Appendix Table 2 (cont'd)

Day 3 - Pre-Experimental Diet

Item	Amount	Calculated Calories	Calculated Protein, g
Breakfast:			
Apple juice	120 g	60.0	.1
Toast, white	2 slices	160.0	5.0
Milk, homo	120 g	114.75	6.23
Egg, fried	1	72.0	5.7
	Sub-total	406.75	17.03
Lunch:			
Macaroni and cheese	75 g	254.25	11.78
Salami	30 g	130.0	7.15
Apple	120 g	62.4	.36
Cookie, fig bar	1	71.6	.78
	Sub-total	518.25	20.07
Afternoon Snack:			
Cookie, fig bar	1	71.6	.78
Supper:			
Ham, cooked, med. fat	75 g	280.5	17.25
Pineapple slice	50 g	29.0	.20
Rice, cooked	100 g	106.0	2.10
Beans, green	90 g	21.6	1.26
Pears, canned	115 g	70.55	.23
Cream, whipping	30 g	92.01	.77
Arrowroot	1	21.00	.40
	Sub-total	620.66	22.21
	Total Day 3	1617.26	60.09

Appendix Table 3. Experimental Diet (Basal Diet + Biscuits) - Human Metabolic Study

Item	Amount	Calculated Calories	Calculated Protein, g
Breakfast:			
Biscuits	1 1/3	310.32	12.89
Sugar cube	1	14.51	---
Applesauce	100 g	91.00	.20
Orange juice, sw.	120 g	62.40	.84
	Sub-total	478.03	13.93
Lunch:			
Biscuits	1 1/3	310.12	12.89
Fruit salad			
Lettuce leaf		---	---
Peaches, canned	51 g	29.58	.20
Grapes, Malaga	50 g	33.50	.30
Oranges	30 g	14.70	.33
Strawberries, frozen, unsw.	25 g	23.00	.10
Banana	50 g	42.50	.55
Glorified rice	110 g	146.16	1.72
	Sub-total	599.56	16.09
Supper:			
Biscuits	1 1/3	310.12	12.89
Potato, baked	100 g	93.0	2.60
Corn, kernel, canned, vacuum pack	75 g	62.25	1.88
Peas, frozen	50 g	34.00	2.55
Carrots, raw	40 g	16.80	.44
Tomato, raw	50 g	11.00	.55
Strawberries, frozen	75 g	69.00	.30
Arrowroot	*1	21.00	.40
(Tossed salad if desired)		---	---
	Sub-total	617.17	21.61
	Day's Total	1694.76	51.63

* May be taken as an afternoon snack if so desired.

NOTE: The biscuits: rapeseed flour, casein and wheat gluten are calculated to be iso-caloric, and iso-nitrogenous (i.e. same amount of protein, and the same number of calories per biscuit regardless of protein source used)

- Continued -

Appendix Table 3 (cont'd)

Glorified Rice (10 servings):

Item	Amount	Calculated Calories	Calculated Protein, g
Minute Rice	187.5 g	701.25	14.06
Salt	1/2-3/4 tsp	---	---
Marshmallows, mini	100 g	319.00	2.00
Maraschino cherries	25 g	29.00	.50
Crushed pineapple, drained, syrup pack	212.5 g	125.38	.64
Spoon n Serve whipped topping	179.6 g	166.63	---
Total for 10:		1461.57	17.20
Per serving:	110 g	146.16	1.72

Caloric content of free foods allowed:

	Calculated Calories
*Apple, 120 g	69.6
Butter, 1 pat (6.0 g)	43.06
Candy:	
Dinner Mint, 1 (1.2 g each)	4.22
English Mint, 1 (2.6 g each)	9.46
Gum drop, 1 (13.2 g each)	26.21
Mint Mix, 1 (3.0 g each)	10.92
Raspberry Drop, 1 (3.5 g each)	12.63
Scotch Mint, 1 (3.0 g each)	10.92
Jelly (21.3 g each) (apple, grape, red currant)	58.04
Sugar, white granulated, 1 (3.8 g)	14.51
Coffee Rich (4 g = 1 tsp)	6.98

* Only 1-120 g apple allowed/day.

Appendix Table 4. Recipe for Biscuits - Human Metabolic Study

Ingredients	Amount of Ingred. (g.) Rapeseed flour	Casein	Wheat gluten
Vitamin-free casein	---	23.5	---
Rapeseed flour	40.0	---	---
Wheat gluten	---	---	26.6
All purpose flour	122.4	122.4	122.4
Shortening	35.5	44.7	44.7
Salt	3.0	3.0	3.0
Baking powder	5.3	5.3	5.3
Water, ml	approx 400.0	105.0	105.0
Sucaryl, ml	5.0	---	---

Method:

1. Sift dry ingredients
2. Cut in shortening with pastry blender
3. Add liquid ingredients
4. Knead 10 - 20 times
5. Leave 10 minutes
6. Roll out, cut with biscuit cutter
7. Bake 425°F for 15 - 20 minutes
8. Yield one subject's daily allottment of biscuits

Appendix Table 5. Schedule for Human Metabolic Study

Period A

Monday evening	Sept. 8, 1969	Move into Home Management apartments for 10 day period
Tuesday	Sept. 9, 1969	Preliminary 60 g protein, 1700 calorie diet
Wednesday	Sept. 10, 1969	Preliminary 60 g protein, 1700 calorie diet
Thursday	Sept. 11, 1969	Preliminary 60 g protein, 1740 calorie diet
Friday	Sept. 12, 1969	Experimental 50 g protein, 1700 (+ 200-400 calories) calorie diet begins: rapeseed casein wheat gluten
Saturday	Sept. 13, 1969	First voiding at exactly the same time as previous day's will be considered part of yesterday's total collection, i.e. completion of first 24 hour collection.
Sunday	Sept. 14, 1969	Second 24 hour urine collection
Monday	Sept. 15, 1969	Third 24 hour urine collection Collection of stools begins
Tuesday	Sept. 16, 1969	First 24 hours metabolism
Wednesday	Sept. 17, 1969	Second 24 hours metabolism
Thursday	Sept. 18, 1969	Third 24 hour metabolism
Friday	Sept. 19, 1969	Fourth 24 hours metabolism
		Resume a normal breakfast!

Period B

Daily schedule repeated from Monday evening, October 6th, 1969 until Friday morning, October 17th, 1969.

Appendix Table 6. Instructions for Urine and Feces Collections - Human Metabolic Study

1. Discard the first voiding on the day the experimental diet begins as this reflects the previous day's metabolism. In order to complete a 24 hour total urine collection, it is necessary for you to include the first voiding (between 7 - 8 A.M.) of each day with the previous day's sample aliquot bottle.
2. Styroform commodes, 12" polyethylene funnels, and 1 litre glass collection jars are provided for urine collection. Be sure when using the commode that the funnel and glass collection jar meet.

   Otherwise an aliquot will be lost!
3. After the first voiding in the morning, get clean items from Room 314: 10 ml pipette, funnel, and urine collection and sample bottles. Be sure the sample bottle has acid (10N H_2SO_4) and toluene added to it. This is indicated by a piece of masking tape on top of the bottle marked with an "A" and "T". After the first voiding in the morning, please place your dirty equipment in boxes in the hallway so they can be removed and cleaned for next day's use.
4. Check which kind of pipette you are using - whether it is volumetric or graduated. Do this every time. Don't take for granted.
5. After voiding, place funnel in graduated cylinder. Take initial volume. Rinse funnel with distilled water. Rinse

- Continued -

Appendix Table 6 (cont'd)

collection bottle with distilled water. Pour into funnel. Bring the volume to the nearest 100th ml - <u>with distilled water</u>.

i.e. after rinsing, volume is 230 ml. Bring it up to 300 ml with distilled water.

Leave funnel in graduated cylinder when bringing the volume to the nearest 100th ml. That is, rinse the funnel by directing the stream from the water bottle around the top edge of the funnel. Remove funnel last of all.

6. Be careful not to pass this 100th ml mark with distilled water.
7. Check your pipette again to make sure you take the correct aliquot (5%).

<u>If final volume is:</u>	<u>take aliquot of:</u>
100 ml	5 ml
200 ml	10 ml
300 ml	15 ml
400 ml	20 ml
500 ml	25 ml
600 ml	30 ml
700 ml	35 ml
800 ml	40 ml
900 ml	45 ml

- Continued -

Appendix Table 6 (cont'd)

8. Record in your book:

- initial urine volume
- final urine volume
- aliquot taken
- time (state whether it is a.m. or p.m.)
- date

9. On the sample bottle marked "total urine aliquot" (i.e. 24 hour aliquot) please record the following on the bottles:

- your number i.e. IAR
- date October 13th
- total urine volume i.e. 3000 ml
- total aliquot taken (5% of total) i.e. 150 ml

Be sure to use the correct colored pen.

- red - casein
- black - rapeseed
- blue - wheat gluten

10. On two consecutive days, September 15 - 16th and October 13 - 14th, 1969, individual time aliquots (10%) will be taken at each voiding. For these individual times aliquot samples, the same procedure as mentioned above should be followed except a 10% aliquot is placed in sample bottles marked "individual sample bottle".

- Continued -

Appendix Table 6 (cont'd)

If final volume is:	take aliquot of:
100 ml	10 ml
200 ml	20 ml
300 ml	30 ml
400 ml	40 ml
500 ml	50 ml
600 ml	60 ml
700 ml	70 ml
800 ml	80 ml
900 ml	90 ml

**Do not forget to add a 5% aliquot to your total urine aliquot bottle on these 2 days when timed individual aliquots are taken.

**Be sure to write the exact time on individual timed aliquots - i.e. whether it is 10:00 A.M. or P.M.

11. During the last four days of each experimental period, stool collections will be made i.e. between 8:00 A.M. October 13th and 8:00 A.M. October 16th.

Saran wrap is provided for this purpose. Place an ample length of Saran in the funnel. After defecating, wrap the stools in the Saran wrap and then in brown paper. Use masking tape to seal the sample. On the sample write:

- your number i.e. IAR
- date - October 14th, 1969

- Continued -

Appendix Table 6 (cont'd)

- time - 10:00 A.M.
- Be sure to use the correct colored marking pen.

Place the stool sample on dry ice in the styroform container placed in hallway.

12. If you get your menstrual cycle during the 7 day experimental period do not take urine collections.

13. If any mistakes at any time are made in taking aliquots, be sure to contact the experimenter - and also, write it in your book.

Appendix Table 7. Amino Acid Composition of Protein Sources[1] - Human Metabolic Study

	Amino Acid Content (g Amino Acid/16 g N)		
	RSF	Cas	WG
Essential			
Lysine	4.67	8.76	1.53
Threonine	4.36	4.38	3.65
Valine	4.91	6.74	4.43
Leucine	7.59	10.07	8.77
Isoleucine	3.05	5.24	3.91
Methionine	1.69	3.10	1.68
Phenylalanine	3.85	5.28	6.79
Cystine	neg	neg	3.48
Non-essential			
Serine	4.40	5.88	6.27
Histidine	2.48	3.47	1.75
Aspartic acid	7.79	7.77	3.09
Glutamic acid	18.31	23.77	12.80
Glycine	5.39	1.98	4.11
Alanine	3.44	3.20	3.73
Proline	4.98	12.24	19.01
Arginine	5.68	4.04	3.09
Tyrosine	2.41	5.65	3.94
Recovery, %	83.1	98.0	97.4
Protein Content, %	50.93	85.52	81.61

[1] RSF, Cas and WG refers to the protein sources, vitamin-free casein, rapeseed flour and wheat gluten respectively.

Appendix Table 8. Individual urinary N excretion of subjects during experimental periods A and B - Human Metabolic Study

Diet	Subject	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Period A								
RSF	1	7.2	6.5	7.0	5.7	7.1	6.4	6.2
	2	---[1]	---	---	6.2	6.1	5.9	7.3
	3	6.2	8.2	8.1	6.7	5.9	7.0	6.7
	4	8.4	7.8	6.6	7.1	7.3	7.6	7.6
Group mean ± S.D.				7.3±0.4				6.7±0.5
Cas	5	7.9	8.0	6.8	7.1	6.4	7.4	7.0
	6	6.0	5.8	5.3	7.0	5.5	8.0	8.7
	7	6.4	6.8	6.7	6.5	7.2	7.5	7.5
	8	---	---	---	7.3	7.1	7.1	7.3
Group mean ± S.D.				6.6±1.0				7.2±0.1
WG	9	7.8	7.9	7.6	6.5	4.7	6.6	5.7
	10	6.4	8.3	7.7	7.5	6.9	7.5	6.0
Group mean ± S.D.				7.6±0.2				6.4±0.8
Period B								
RSF	5	---	8.6	7.8	7.2	7.9	7.6	6.9
	6	5.0	6.1	4.7	7.5	5.2	6.2	4.8
	7	6.6	6.8	5.9	6.4	5.8	6.9	6.8
	8	---	---	6.7	7.6	7.0	6.9	6.3
Group mean ± S.D.				6.6±1.2				6.7±0.6
Cas	1	7.6	7.6	6.5	8.0	7.2	6.6	6.8
	2	---	---	---	8.2	7.4	5.7	7.7
	3	7.9	7.8	6.8	5.9	7.0	8.0	7.6
	4	---	8.8	8.0	7.7	8.3	7.7	7.6
Group mean ± S.D.				7.7±0.6				7.3±0.3
WG	11	8.2	7.7	7.5	8.0	7.3	7.3	7.6
	12	7.4	6.5	7.2	6.6	5.7	4.7	6.0
Group mean ± S.D.				7.4±0.5				6.7±1.3

[1] Urine not collected for subject during this period.

Appendix Table 9. Amino acid composition of biscuits, pre-experimental diets and basal portion of experimental diet - Human Metabolic Study

	Amino acid content (g Amino acid/16 g N)[1]				
	Diets		Biscuits[4]		
	Pre-experimental[2]	Basal portion[3] of experimental diet	RSF	Cas	WG
Essential					
Lysine	4.2 ± 0.1	4.4 ± 0.3	3.8	5.4	1.5
Threonine	2.8 ± 0.3	3.0 ± 0.3	3.6	3.5	2.5
Valine	3.7 ± 0.9	4.2 ± 0.1	4.3	5.1	3.6
Leucine	5.4 ± 0.8	6.0 ± 0.3	7.2	8.3	6.4
Isoleucine	2.6 ± 0.1	2.8 ± 0.2	3.6	3.9	3.1
Methionine	1.2 ± 0.1	0.8 ± 0.3	2.0	1.5	1.5
Phenylalanine	2.9 ± 0.4	3.1 ± 0.1	4.4	4.7	5.0
Non essential					
Serine	3.7 ± 0.7	4.2 ± 0.1	4.8	3.7	4.1
Histidine	1.8 ± 0.0	2.3 ± 0.1	2.2	2.4	2.0
Aspartic acid	8.8 ± 4.2	14.1 ± 1.7	5.7	6.1	3.4
Glutamic acid	14.4 ± 2.4	16.0 ± 0.7	25.4	26.3	35.4
Glycine	3.2 ± 0.5	3.5 ± 0.1	4.3	2.4	2.1
Alanine	4.1 ± 1.3	5.2 ± 0.5	4.0	2.9	1.2
Proline	4.8 ± 0.3	5.0 ± 0.0	10.1	10.8	13.3
Arginine	5.5 ± 2.1	7.3 ± 0.1	6.8	2.4	3.1
Tyrosine	2.4 ± 0.3	2.4 ± 0.1	2.7	4.2	2.9
Recovery, %	85.4	87.2	96.2	86.9	96.5
Protein content, %	18.7	7.1	18.8	19.2	20.0

1 Mean and S.D. for 2 separate days.

2 Pre-experimental diets analyzed for days 1 and 2.

3 Experimental diets analyzed for days 4 and 9.

4 Biscuits analyzed for Period A only.

Appendix Table 10. Contribution of basal portion of experimental diet and biscuits to the daily amino acid intake of subjects - Human Metabolic Study

	g Amino acids			
	Basal portion of experimental diet	Biscuits		
		RSF	Cas	WG
Essential				
Lysine	0.59	1.38	2.08	0.59
Threonine	0.39	1.32	1.34	0.97
Valine	0.55	1.57	1.97	1.43
Leucine	0.80	2.65	3.21	2.53
Isoleucine	0.37	1.33	1.52	1.22
Methionine	0.11	0.73	0.59	0.61
Phenylalanine	0.41	1.61	1.80	1.96
Non essential				
Serine	0.55	1.75	1.42	1.63
Histidine	0.30	0.81	0.92	0.80
Aspartic acid	1.86	2.08	2.37	1.33
Glutamic acid	2.11	0.93	1.02	1.40
Glycine	0.46	1.57	0.92	0.84
Alanine	0.69	1.48	1.12	0.46
Proline	0.65	3.72	4.18	5.25
Arginine	0.96	2.50	0.92	1.22
Tyrosine	0.31	1.00	1.62	1.14
Protein content	13.17	36.75	38.68	39.58

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