NUCLEIC ACIDS AND PROTEIN STORES IN THE MERINO SHEEP

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Summary

The storage of protein in the tissues of Merino sheep has been investigated, and the quantitative distribution of these stores delineated by complementary methods. Muscle and liver are evinced as the main storage tissues, with the protein of the pancreas, heart, and spleen also labile to a considerable extent. Kidney and lung protein is well preserved under the stress of chronic dietary deprivation.

In addition, the nucleic acid concentrations in ovine tissues have been determined, along with the ratios of ribonucleic acid to deoxyribonucleic acid, and protein nitrogen to nucleic acid. Comparative studies have been made of the effect of age, sex, and nutritional status on these values.

In general, the concentrations of nucleic acids in sheep tissues are similar to those of the common laboratory animals. Exceptions are the ovine pancreas and kidney, which exhibit low RNAP/DNAP ratios. Little modificatory effect towards the nucleic acid concentrations seems attributable to the effects of sex and age.

I. Introduction

Previous papers by Masters and Horgan (1962*a*, 1962*b*) directed attention to the fact that sheep in tropical and subtropical areas are often subjected to long periods of low nitrogen intake under natural grazing conditions. As a consequence of this treatment, the protein stores of the grazing animals are depleted; but there is little quantitative information on this process in the literature.

Although extensive investigation of protein storage in monogastric animals has led to the accumulation of considerable experimental data (Addis *et al.* 1940; Whipple 1948), it was considered unwise to assume a valid extrapolation of all this evidence to the case of the ruminant. One objection is that the nitrogen metabolism of the ruminant differs considerably from that in the monogastric animal (Annison and Lewis 1959). Secondly, information was required particularly on chronic aspects of protein depletion, and most of the experimental evidence available relates to short-term effects, which are markedly different (Addis *et al.* 1940; Allison 1955).

As an aid to further understanding of the biochemical processes involved in the protein depletion of Merino sheep, therefore, the loss of tissue protein by these animals on a diet characteristic of drought conditions has been investigated. These protein reserves have been estimated both by direct measurement of total tissue protein in normal and depleted animals; and also by the use of deoxyribonucleic acid as a reference substance, in terms of which the chemical composition of a tissue may be expressed (Thomson *et al.* 1953). Utilization of these different methods enables a comparison and confirmation of results to be made, and also allows of measurements in living objects.

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Forming an integral part of this project was the determination of tissue nucleic acid levels in the sheep, and in supplementation of this aspect, the effects of age, sex, and nutritional status on nucleic acid concentrations and ratios have been studied.

II. Methods

The eight animals used in the protein depletion experiments were well-matched, adult Merino ewes, 3–4 years of age. After being fed for 3 weeks on lucerne chaff *ad libitum*, with free access to water, the animals were changed over to a ration of 3 kg/week of chaffed wheat straw (digestible crude protein 0·5%), with water *ad libitum*, for a period of 8 weeks. This experimental diet was chosen to resemble that under drought conditions; and though approximating an average *ad libitum* intake of this feed, it is a low protein, low energy ration. The duration was shown by preliminary experiments to approach closely the maximum consistent with survival.

At the completion of this dietary treatment, the experimental animals were slaughtered. Organs and tissues required for analysis were obtained by excision, weighed rapidly, and representative samples of approximately 10 g taken from the same areas of tissue in each animal. Further tissue samples were obtained in a parallel manner from well-nourished Merinos: adult females, adult males, and young animals of both sexes.

Tissue samples were weighed, extracted with ethanol–ether (3 : 1, 10 vols.), and dried in a vacuum desiccator over phosphorus pentoxide. After re-weighing, the dried, fat-free tissues were finely ground, and a portion (about 10 mg) accurately measured into a tared glass microhomogenizer. This sample of tissue was homogenized with ice-cold 5% trichloroacetic acid (0·3 ml), centrifuged, and the precipitates washed twice with further 0·3-ml quantities of ice-cold 5% trichloroacetic acid (TCA) in order to remove acid-soluble nucleotides. The residue was extracted with approximately 1 ml of 5% TCA at 90°C for 15 min and after cooling the exact volume of the extract was determined by weighing.

Total nucleic acids in the diluted extract (1 in 10) were measured spectrophotometrically by their absorption at 268·5 mμ (Logan, Maxwell, and Rossiter 1952). On the same solutions, deoxyribonucleic acid (DNA) was measured by the indole reaction of Ceriotti (1952). From these values, the contributions of DNA to the total absorption at 268·5 mμ were calculated, and, by difference, the RNA contributions.

Control solutions for the Ceriotti determinations were prepared from a highly polymerized, white DNA.* An aliquot of this product was dissolved in 5% trichloroacetic acid and heated at 90°C for 15 min. Prepared in this manner, the standard solutions had an atomic extinction coefficient with respect to phosphorus of 9950 at 268·5 mμ. For the purposes of calculation, it was assumed that RNA had the same extinction coefficient under these conditions (Logan, Maxwell, and Rossiter 1952).

Protein nitrogen was measured in the residue remaining after extraction of the nucleic acids, by microKjeldahl digestion and subsequent nesslerization (Hawk, Oser, and Summerson 1954).

* Sigma Chemical Co., St. Louis 18, Missouri, U.S.A.

TABLE 1

NUCLEIC ACID CONCENTRATIONS AND NUCLEIC ACID–PROTEIN RATIOS IN MERINO SHEEP TISSUES

Concentrations of nucleic acids in tissues are expressed as milligrams nucleic acid phosphorus per 100 g fresh tissue. Results are given as mean values ± standard errors of the mean. Abbreviations in the tissue column refer to young male (YM), young female (YF), adult male (AM), adult female (AF), and protein-depleted adult female (D). Significant differences ($P<0\cdot05$) between the latter two tissues are indicated by asterisks. The young animals were approximately 6 months old, and adults 3–4 years of age

Tissue		No. in Group	Dry Fat-free Weight (% fresh tissue)	RNAP Concn. (mg/100 g fresh tissue)	DNAP Concn. (mg/100 g fresh tissue)	Ratio RNAP/ DNAP (mg/mg)	Ratio Protein N to DNAP (mg/mg)	Ratio RNAP to Protein N (μg/mg)
Heart	YM	3	18·3±0·6	7·2±1·5	7·0± 2·3	1·12±0·09	357 ±19	0·031±0·006
	YF	3	18·2±0·4	7·5±0·4	7·2± 1·5	1·03±0·08	340 ±35	0·030±0·006
	AM	6	17·9±0·5	6·5±0·9	6·0± 1·6	1·08±0·09	351 ±40	0·031±0·007
	AF	16	18·3±0·3	8·3±1·0	6·8± 2·0	1·12±0·11	363 ±33	0·034±0·005
	D	6	17·0±0·5	7·0±1·1	8·4± 1·9	0·83±0·09*	268 ±24*	0·032±0·008
Liver	YM	3	25·8±1·2	44·5±2·8	23·6± 1·7	1·89±0·13	110 ±10	0·170±0·009
	YF	3	25·5±1·1	41·4±4·2	25·2± 2·3	1·64±0·07	96 ± 8	0·170±0·029
	AM	6	23·1±0·8	45·5±4·4	23·8± 2·8	1·92±0·18	110 ±14	0·174±0·021
	AF	20	22·0±0·8	41·0±3·5	24·4± 2·4	1·81±0·15	102 ±12	0·180±0·018
	D	6	19·7±1·0	25·0±2·9*	36·4± 3·4*	0·68±0·09*	55 ± 5*	0·130±0·021*
Skeletal muscle	YM	3	20·3±0·3	5·7±2·1	4·1± 1·8	1·39±0·09	519 ±51	0·027±0·006
	YF	3	21·0±1·1	3·9±1·8	3·8± 1·7	1·01±0·16	470 ±52	0·021±0·004
	AM	6	19·8±0·9	5·0±1·1	4·2± 0·9	1·19±0·12	494 ±38	0·024±0·004
	AF	16	20·3±1·0	5·2±0·9	3·9± 0·8	1·34±0·13	508 ±51	0·026±0·005
	D	6	17·6±1·3	4·4±0·8	6·9± 1·1*	0·64±0·10*	218 ±27*	0·029±0·006
Kidney	YM	3	21·6±1·2	10·1±2·4	50·2± 6·3	0·20±0·02	47·3± 5·3	0·042±0·005
	YF	3	20·5±0·4	10·5±1·0	50·2± 5·7	0·21±0·01	48·1± 4·7	0·044±0·009
	AM	6	20·2±0·7	8·7±1·3	48·6± 5·4	0·18±0·02	46·7± 6·1	0·039±0·010
	AF	18	18·7±0·6	8·0±1·1	49·1± 2·6	0·17±0·01	45·2± 4·2	0·040±0·008
	D	6	16·8±0·9	15·4±3·2*	47·8± 8·9	0·32±0·02*	49·4± 5·2	0·090±0·021*
Spleen	YM	3	19·0±0·6	35·2±5·0	76·1±10·2	0·47±0·08	28·3± 3·0	0·161±0·035
	YF	3	19·5±0·5	38·1±4·7	68·6± 8·3	0·55±0·08	30·7± 0·9	0·172±0·019
	AM	6	19·1±0·8	36·7±5·3	70·7±11·4	0·51±0·06	28·6± 2·4	0·184±0·023
	AF	18	20·4±1·3	35·5±5·0	75·3±10·1	0·51±0·07	29·8±·3·1	0·181±0·037
	D	4	17·4±1·4	40·7±3·9	74·4± 8·3	0·54±0·05	31·9± 3·6	0·175±0·041
Pancreas	YM	3	22·1±1·2	57·6±4·3	36·3± 0·3	1·59±0·09	45·8± 4·4	0·366±0·009
	YF	3	18·2±0·3	55·5±3·8	38·1± 3·3	1·52±0·13	46·6± 5·3	0·332±0·041
	AM	6	19·6±0·9	47·9±4·0	37·4± 2·8	1·30±0·11	44·2± 4·0	0·307±0·053
	AF	18	18·2±1·0	42·1±4·1	37·1± 2·5	1·14±0·12	40·3± 3·9	0·284±0·050
	D	2	15·9±1·1	41·0±3·7	50·2± 3·6*	0·81±0·10*	27·1± 5·1*	0·301±0·056
Lung	YM	3	22·2±1·4	15·4±3·0	46·3± 4·2	0·34±0·02	51·8± 3·4	0·067±0·014
	YF	3	19·2±0·3	10·9±3·4	37·7± 4·1	0·29±0·02	48·5± 4·7	0·059±0·007
	AM	6	19·2±0·7	13·3±2·9	43·8± 5·3	0·31±0·02	50·4± 4·1	0·062±0·012
	AF	16	21·1±0·7	17·0±3·1	45·3± 7·1	0·33±0·02	52·4± 5·3	0·060±0·009
	D	6	17·8±0·8	16·1±2·8	43·8± 6·4	0·38±0·03	60·5± 7·1	0·063±0·009
Brain	YM	3	13·5±1·1	11·1±0·9	9·6± 0·7	1·16±0·11	152 ±14	0·077±0·015
	YF	3	12·9±0·7	10·5±1·1	9·3± 0·8	1·14±0·10	148 ± 8	0·077±0·011
	AM	6	13·4±0·9	11·4±1·1	9·5± 0·7	1·20±0·09	163 ±14	0·073±0·019
	AF	18	14·6±1·1	10·1±0·9	9·5± 0·2	1·12±0·14	143 ±10	0·080±0·012
	D	2	12·7±1·2	8·7±0·4	10·1± 0·3	0·86±0·16	131 ±12	0·066±0·014

Reagents used throughout were the highest grade commercially available, and duplicate determinations were made in all instances. For spectrophotometric measurements in the ultraviolet range, a Beckmann model DU spectrophotometer was used, and for all other photometric measurements, a Beckmann model B spectrophotometer.

III. Results

The data presented in Table 1 indicate the concentrations on a wet weight basis of nucleic acid phosphorus (NAP) in the various ovine tissues investigated. In addition, various relative concentrations have been shown: protein nitrogen/DNAP, RNAP/DNAP, RNAP/protein nitrogen. This information provides a basis for assessing normal ranges, for comparing the effects of age and sex, and for assessing the relative status of nucleic acid and protein.

Table 2

EFFECT OF PROTEIN DEPLETION ON THE DNA CONTENT OF SHEEP TISSUES

The same adult female Merino sheep have been used for this comparison as listed in Table 1 (AF and D). Results are expressed as mean values ± standard errors of the mean, and the number of observations is given in parenthesis. There are no significant differences ($P < 0{\cdot}05$) attributable to this treatment

Tissue	DNA Content (mg/organ)	
	Normal Tissue	Depleted Tissue
Heart	12·3 ± 2·9 (16)	12·0 ± 2·1 (6)
Liver	129 ±16 (20)	125 ±11 (6)
Kidney	17·0 ± 1·6 (18)	17·0 ± 1·9 (6)
Spleen	45·8 ± 8·9 (18)	49·1 ± 4·9 (4)
Pancreas	12·9 ± 2·2 (18)	12·1 ± 1·6 (2)
Lung	204 ±28 (16)	214 ±23 (6)
Brain	10·4 ± 1·1 (18)	9·6 ± 0·5 (2)

On the basis of wet weight, nucleic acids occur in highest concentration in the spleen, pancreas, and liver. Of the tissues studied, skeletal muscle has the lowest concentration. In general terms, this distribution is common to both nucleic acids, but the ratios of RNAP/DNAP are lower than average in the case of the lungs, kidney, and spleen. Relative concentrations of protein nitrogen to nucleic acid vary considerably in the different tissues. A considerable overlapping of the standard deviations is apparent in the results for individual tissues, and no statistically significant trend is revealed in differences due to age and sex.

However, significant differences can be attributed to protein depletion. These are indicated in Table 1 by asterisks. The general response is a decrease of protein and RNA relative to the DNA content; usually the protein shift is the more emphatic.

Under the experimental conditions, skeletal muscle, liver, pancreas, and cardiac muscle exhibit significant changes.

Table 2 lists the total DNA content of tissues in normal and protein-depleted animals, the same ewes listed in Table 1 being utilized. The purpose of this comparison is to demonstrate the constancy of tissue DNA under the protracted conditions of the experiment, and hence to verify the validity of using DNA as a reference substance for comparative studies of protein depletion.

TABLE 3

EFFECT OF PROTEIN DEPLETION ON TISSUE PROTEIN AND NUCLEIC ACID–PROTEIN RATIOS IN THE SHEEP

The sheep used for this comparison are those listed in Table 1 (AF and D). Results have been obtained from the values in Table 1, by calculating the percentage alteration of mean values caused by protein depletion

Tissue	Protein Nitrogen	Protein N/ DNAP Ratio	RNAP/DNAP Ratio	RNAP/Protein N Ratio
Heart	−25%	−26%	−26%	− 6%
Liver	−47%	−46%	−62%	−28%
Skeletal muscle	—	−57%	−52%	+11%
Kidney	+12%	+ 9%	+88%	+125%
Spleen	+ 3%	+ 7%	+ 6%	− 3%
Pancreas	−31%	−33%	−29%	+ 6%
Lung	+ 5%	+15%	+15%	+ 5%
Brain	− 5%	− 8%	−23%	−17%

In Table 3, the loss of protein from tissues during depletion is compared with the alterations of the following ratios under identical conditions: protein nitrogen/DNAP, RNAP/DNAP, RNAP/protein nitrogen. For the purposes of this comparison, mean values have been utilized, and there is a good agreement on this basis between protein loss/whole tissue, and protein loss/unit of DNA. Changes of similar magnitude are evident in the RNAP/DNAP ratios.

IV. DISCUSSION

Although the literature on tissue nucleic acids is voluminous (Schneider 1946; Davidson 1947*a*, 1947*b*; Euler and Hahn 1948; Leslie 1955), relatively few studies appear to have been directed to this aspect of biochemistry in the sheep. In 1944, Davidson and Waymouth estimated the total nucleic acids in embryo and adult sheep tissues, and made an approximate assessment of the ratios of RNAP/DNAP. In the following year, Schmidt and Thannhauser (1945) and Schneider (1945) published the first convenient and reliable methods for determining tissue nucleic acids; but it does not appear from a search of the available literature that these procedures have been previously applied to a detailed study of sheep tissues. It is of interest, then, to examine the values obtained for nucleic acid concentrations and to compare them with the extensively documented values for the rat.

The tissue concentrations of DNAP found in the adult female Merino sheep (Table 1) closely parallel the values of Schneider and Klug (1946) for rat tissues. In general, mean values for sheep are lower than the corresponding means in the rat; but in the case of renal tissue, the range of values for the sheep is contiguous with the upper limit of the rat normals.

This tendency towards lower nucleic acid concentrations in the sheep is accentuated in the case of RNAP values (Table 1). The kidney and pancreas of the sheep, in particular, present appreciably lower values than the figures of Schneider and Klug (1946) for the rat.

Comparisons on the basis of tissue concentrations, however, are clouded by the wide variations contained within the normal ranges. Disparities between species are more clearly manifest when the ratios of RNAP/DNAP in the respective tissues are considered. Several points arise in connection with such a comparison of nucleic acid ratios.

Firstly, the Schmidt and Thannhauser extraction yields high values for RNAP/DNAP ratios, because the RNAP fraction separated by this method contains concomitant phosphorus compounds. Consequently, it is necessary to ensure that comparison is made on the basis of similar extraction techniques. Collating with the values of Schneider and Klug (1946), then, a general similarity is apparent for the tissues of the sheep and the rat; the main points of difference are the pancreas and kidney, where the ovine RNAP/DNAP ratios are considerably lower.

With the pancreas, the striking contrast is understandable on the basis of differences in function in the two situations. In the monogastric animal, the pancreas secretes considerable quantities of protein as hormones and enzymes. In the ruminant, pancreatic function is usurped to a degree by the rumen microorganisms. Rumen fermentation ensures that very little carbohydrate is absorbed as monosaccharide from the gut; consequently, there is a typical lack of post-prandial hyperglycaemia in the ruminant, and it would seem, a reduction in the necessity for insulin secretion. Further, the action of the rumen microorganisms greatly reduces the quantities of triglyceride and starch admitted to the gut, and hence minimizes the requirement for the digestive action of pancreatic lipase and amylase (Sammonds, Frazer, and Thompson 1956; Keller, Cohen, and Neurath 1958). Overall, then, there would seem to be a greatly diminished need for protein synthesis by the ruminant pancreas, and this might be expected to be reflected in a considerably lowered RNA concentration (Davidson 1960).

Reasons for the different RNAP/DNAP ratios between species are not so apparent in the case of renal tissue. Perhaps it is related to differences in physiological function; possibly, it is merely an idiosyncrasy of the method. Leslie (1955) reports a distinct lack of agreement among the results of various authors for nucleic acid concentrations in kidney.

With regard to the protein nitrogen/DNAP ratios, sheep liver presents another example of the remarkable similarity of values for this tissue in different species (Leslie 1955). Although there is a paucity of comparative information in the literature for other tissues, a greater intergeneric variability is evident. Values for the ratio

RNAP/protein nitrogen, on the other hand, are comparable with those in the rat for most tissues. Whereas the sex and age of animals are quoted in the literature as being modificatory towards tissue nucleic acid concentrations and the ratios discussed above (Leslie 1955), no significant trend attributable to these variables is evident in the sheep (Table 1).

The validity of using DNA as a standard of reference for measuring the chemical composition of liver has been discussed in detail by Davidson and his colleagues (Thomson *et al.* 1953). They confirmed and extended the earlier observations of Campbell and Kosterlitz (1950) that in the adult rat the amount of DNA in the liver is constant over a wide range of dietary conditions. In addition, Mandel and his collaborators (Mandel, Jacob, and Mandel 1950; Jacob, Mandel, and Mandel 1951) studied the effect of prolonged protein depletion on various rat tissues, and observed that the DNA content of the tissues was not diminished in general. The spleen, however, was an exception, losing half its DNA under these conditions.

In spite of this available evidence on the rat, it was considered necessary to confirm the situation in the sheep under the chronic dietary conditions of the experiment. As the rumen microorganisms contribute to the absorption of purine and pyrimidine bases in the sheep, and as this process is considerably affected by energy-deficient diets (Blaxter 1961), it was deemed prudent to confirm the constancy of tissue DNA in the sheep under the conditions of the experiment (Table 2).

Playing a central part in any description of the effects of protein depletion is the concept of protein stores (Waterlow, Cravioto, and Stephen 1960). Whereas these stores do not appear to have a separate anatomical existence, there does seem to be a reserve protein supply which can be drawn upon to furnish the fundamental nitrogen requirements of the animal when the protein intake is inadequate. This reserve protein appears to be drawn from the tissues themselves, particularly from the cytoplasm, and to possess an important buffer action against nutritional and pathogenetic stresses (Allison 1955).

In measuring these protein reserves in the Merino sheep, two lines of approach have been utilized. Firstly, the total protein contents of tissues from well-nourished sheep have been compared with those in the chronically depleted sheep. Secondly, use has been made of DNA as a reference substance for measuring changes in chemical composition. The first approach necessitated the slaughter of the experimental animals; the second allows for the possibility of measurement in living animals, and in addition provides a useful corroboration of the first method.

Direct measurement of storage protein (Table 3) indicates that the liver has lost nearly half its normal protein content, with the pancreas, heart, and spleen also highly labile; kidney protein and lung protein are well preserved. Good agreement with these results was obtained by measurement of the protein nitrogen/DNAP ratio, and to a lesser extent by the RNAP/DNAP ratios (Table 3). An exact correlation was not expected in the case of the RNAP/DNAP ratios, because it was known that RNA and protein nitrogen can vary independently to a certain extent, especially where energy considerations are involved (Leslie 1955). Nevertheless, the similarities in the alteration of both nitrogen/DNAP and RNAP/DNAP ratios with protein depletion affords a useful confirmation in several instances.

With skeletal muscle, a loss during depletion of more than one-half of the tissue protein is indicated by the protein nitrogen/DNAP ratios. Neither total protein content nor total DNA were measured in this instance because of the impracticability of establishing total tissue mass. Nevertheless this depletion value would seem to be a realistic one (Waterlow and Mendes 1957; Waterlow, Cravioto, and Stephen 1960), and represents muscle as the major protein store of the body, bearing the brunt of the protein depletion, and probably buffering the effect of protein loss in the more essential organs.

The overall picture of protein loss in chronic protein deprivation, then, agrees in general with the results for monogastric animals. The main difference is the conservation of renal protein by the sheep under these conditions; a situation which is in contrast to published results for the rat (Addis, Poo, and Lew 1936).

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