ON THE AMINO ACIDS ESSENTIAL FOR THE TISSUES OF THE SHEEP

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Summary

A lactating ewe was injected intravenously with sodium [1-^{14}C]acetate and samples of the wool and milk produced subsequently were collected. Some of the casein from the milk produced 1–3 hr after the injection was hydrolysed and the distribution of ^{14}C among most of the amino acids determined. The results showed that only the following amino acids became labelled: glutamic acid, aspartic acid, proline, alanine, arginine, and serine. By analogy with earlier work on cows by Black *et al.* (1957) it was concluded that these amino acids are also non-essential for the tissues of the sheep.

The casein isolated from the milk produced during the first few hours had a much higher specific activity than the maximum observed in the wool. However, the results were consistent with the hypothesis that neither the wool keratin nor the casein were formed from the plasma proteins.

I. Introduction

Many living systems cannot synthesize certain of the amino acids required for growth or maintenance, at least in amounts adequate for the demands of anabolism. Rose (1938), in his studies of the nutritional requirements of certain mammals, called such amino acids which have to be supplied in the diet "essential". By the use of diets containing mixtures of highly purified amino acids in place of proteins he developed relatively simple and reliable methods for determining the nutritive significance of the individual amino acids. For example, by the "deletion" technique, in which single amino acids are successively removed from the complete diet, Rose, Oesterling, and Womack (1948) were able to show that for the growing rat 10 amino acids are essential dietary components—valine, leucine, isoleucine, threonine, methionine, phenylalanine, tryptophan, lysine, histidine, and arginine. However, it has been found that the results for the rat are not necessarily transferable to other species. Man, for example, requires the first eight only of the above amino acids for maintenance (Rose *et al.* 1955).

As an alternative to the deletion technique an indirect approach has been used. Steele (1952) showed that there was a striking absence of ^{14}C in the essential amino acids isolated from the mixed proteins of the carcass (with the entire gastro-intestinal tract removed) of a mouse which had received a dose of [^{14}C]sucrose, even though the experimental conditions were favourable for the detection of possible minor metabolic routes to the essential amino acids through intermediates with low turnover rates. Recently Kasting and McGinnis (1958) used the indirect method, with [^{14}C]glucose, to determine the amino acids essential for an insect.

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This indirect approach has been particularly useful in the study of ruminants because nutritionally they do not require the essential amino acids to be supplied in their food. This is due to the presence of large numbers of microorganisms in the rumen which can synthesize enough of the essential amino acids to meet the needs of the animal (Loosli *et al.* 1949). There nevertheless remains the possibility that the tissue metabolism of ruminants differs from that of monogastric animals. To obtain a comparison between the two types of animals Kleiber and his co-workers have used the indirect method (Black, Kleiber, and Smith 1952; Black and Kleiber 1958). They gave intravenous injections of a number of ^{14}C-labelled compounds such as glucose and sodium bicarbonate, acetate, propionate, and butyrate to lactating cows and measured the amount of ^{14}C incorporated into the various amino acids of the casein. The amino acids examined could be divided into two groups—those with low levels of ^{14}C, which corresponded well with the essential amino acids, and those with much higher levels, which were in general the same as the non-essential amino acids. By injecting the compounds intravenously the rumen was largely by-passed. Thus the tissue metabolism of the cow was shown to be similar to that of the rat, dog and, except for histidine, man (Black, Kleiber, and Smith 1952).

There is apparently no published information on the amino acids essential for the sheep. However, some evidence has now been obtained by applying the indirect method outlined above. In this paper a comparison is made between some results obtained in the sheep and those published for the cow after an injection of sodium [1-^{14}C]acetate (Black *et al.* 1957).

II. Experimental

A lactating ewe (32 kg) was injected intravenously with sodium [1-^{14}C] acetate (50 mg; 555 μc). Just before the injection, and at intervals afterwards, the animal was milked and the wool clipped from a large area on each side. Just before the completion of each milking a dose of purified posterior pituitary extract (5 i.u. oxytocic; supplied by the Commonwealth Serum Laboratories, Melbourne), was injected and the animal milked dry.

(i) *Casein Samples.*—The casein was isolated from each sample of milk and purified as described by Askonas, Campbell, and Work (1954).

A portion (1 g) of the second casein sample was hydrolysed with HCl (200 ml, 6N) at 140°C for 20 hr. The phenylalanine and tyrosine were isolated from the hydrolysate as described by Partridge (1949) and the other amino acids eluted from a column (2·2 by 150 cm) of "Amberlite IR120" with increasing concentrations of HCl (Hirs, Moore, and Stein 1954).

Pure samples of all the amino acids were thus obtained except aspartic acid, serine, and the leucines. The aspartic acid and serine were separated on a column (0·8 by 40 cm) of "Deacidite FF" by elution with acetic acid after the method described by Hirs, Moore, and Stein (1954). No attempt was made to separate the leucines, since they contained practically no ^{14}C. The basic amino acids were kept as the hydrochlorides. The other amino acids were obtained as such by adding their

hydrochlorides to small columns of "Deacidite FF" and eluting with acetic acid (0·5N). The acetic acid solutions were evaporated to give the solid amino acids. The purity of each sample was checked by paper chromatography in *n*-butanol–acetic acid–water and in phenol–ammonia.

The casein and amino acid samples were either counted directly on 1-cm^2 polythene disks under an end-window G.M. counter, correcting the results to the infinite thickness value if necessary, or were combusted to CO_2 and counted as the gas as described by Bradley, Holloway, and McFarlane (1954).

(ii) *Wool Samples.*—The wool was washed with ether, ethanol, and water and dried in an oven at 110°C. Samples were counted directly as described by Downes (1961).

TABLE 1

SPECIFIC ACTIVITY OF SAMPLES OF CASEIN AND WOOL FROM A SHEEP AFTER AN INTRAVENOUS DOSE OF SODIUM [1-^{14}C]ACETATE

	Sample No.	Time of Collection Relative to Injection	Specific Activity (mμc/g)
Casein	1	−52 min–68 min	527
	2	68 min–4 hr	219
	3	4–7 hr	83·8
	4	7–10·5 hr	36·6
	5	10·5–22·5 hr	15·9
	6	22·5–30 hr	9·1
Wool, left side	1	−1–4 days	1·6
	2	4–7 days	8·5
	3	7–11 days	12·4
	4	11–15 days	5·1
	5	15–19 days	2·1
Wool, right side	1	1–5 days	1·5
	2	5–8 days	15·0
	3	8–12 days	11·0
	4	12–15 days	6·7
	5	15–19 days	2·1

III. RESULTS

The specific activities of the samples of casein and wool are shown in Table 1. The first sample of casein had the highest specific activity, 0·527 μc per g. Since this was obtained from the first milking, 68 min after the injection, the maximum specific activity must have been reached in the first few minutes. The later samples showed a rapid fall in specific activity. By comparison, much smaller amounts of ^{14}C appeared in the wool, the maximum specific activity observed being 0·015 μc per g in the wool grown from the fifth to the eighth day after the injection.

The specific activities of the individual amino acids from the second casein sample are shown in Table 2, together with the corresponding results obtained by

Black *et al.* (1957) for the amino acids from casein produced by cows during the first 3 hr after an injection of sodium [1-^{14}C]acetate. For this comparison both sets of results are expressed as μc per g-atom of carbon divided by the number of microcuries injected per kg of body weight.

TABLE 2

SPECIFIC ACTIVITY OF THE AMINO ACIDS ISOLATED FROM THE SECOND CASEIN SAMPLE (SEE TABLE 1)

The amino acids whose counting rates were above background (8 counts/min) when counted at infinite thickness on 1-cm^2 polythene disks are compared in the last two columns with the corresponding mean results published by Black *et al.* (1957) for the casein from the milk of three cows

Amino Acid	Specific Activity (counts/min)	Specific Activity (μc/g-atom C/μc injected/kg body wt.)	
	Sheep	Sheep	Cow
Isoleucine*†	3		
Leucine*†			
Lysine*†	3		
Methionine*†	3		
Phenylalanine*†	1		
Threonine*†	7		
Tryptophan*†	—		
Valine*†	0		
Histidine†	0		
Arginine†	112	0·37	0·51
Glycine	75	0·30	0·42
Alanine	145	0·42	0·62
Serine	110	0·36	0·56
Cystine	—		
Tyrosine	2		
Aspartic acid	160	0·52	1·77
Glutamic acid	380	1·09	3·17
Proline	185	0·43	0·46

* Essential for maintenance of nitrogen equilibrium in normal adult man.

† Essential for optimum growth of young rats.

IV. DISCUSSION

The results obtained here for the sheep were substantially the same as those of Black *et al.* (1957) for the cow. In both cases the amino acids could be divided into the same two groups; firstly, tyrosine, phenylalanine, leucine, isoleucine, valine, methionine, threonine, lysine, and histidine, all of which had negligible radioactivity; and secondly, those containing ^{14}C—glutamic acid, proline, aspartic acid, alanine, arginine, serine. Cystine and tryptophan were not isolated. Although there are individual variations, as would be expected in such a comparison, the specific activities were of the same order of magnitude as those published by Black *et al.*

(1957) for casein from cow's milk collected about 3 hr after a similar injection. These authors pointed out that only the non-essential amino acids became labelled significantly. Similarly, the present results suggest that at least 8 of the 10 amino acids essential for the rat are also essential for the tissues of the sheep. Arginine is evidently not essential for the sheep. No statement can be made about cystine or tryptophan since they were not isolated. Tyrosine is not classified as an essential amino acid from the nutritional experiments of Rose. However, since it is synthesized *in vivo* by the hydroxylation of phenylalanine, which is essential, no ^{14}C would be expected in the tyrosine unless the phenylalanine was radioactive also.

Casein is largely synthesized in rabbit and goat mammary glands from the free amino acids in the plasma rather than from large peptides or proteins (Barry 1958). If this also applies in the sheep, and the rapid fall in the specific activity of the casein in the present experiment suggests that it does, then a comparison of the specific activities of the amino acids in the casein and the wool from a sheep injected with labelled amino acids should throw some light on the mechanism of wool biosynthesis. Thus, if wool keratin is also derived from the plasma-free amino acids, the specific activity of any of its constituent amino acids should be the same as that of the same amino acid in the casein synthesized during the same time; if, on the other hand, wool keratin is formed from the plasma proteins, or from peptides derived from these proteins, the specific activity should be much lower in the wool than in the casein.

In the present experiment only a rough comparison of the wool and casein themselves, instead of individual amino acids, could be made. However, although these two proteins have different compositions, the total content of non-essential amino acids is about the same for each (Block and Bolling 1951), and it was calculated that the specific activity of wool derived from amino acids with the specific activities shown in Table 2 would be about the same as that of the casein.

From the specific activities of the casein samples given in Table 1 and the amounts of milk obtained (25 g/hr) it was calculated that the average specific activity of the casein produced during the first day after the injection was about 100 mμc/g. Since the specific activity had fallen to almost negligible proportions after the first day, the average value over the first 3 days must have been about 35 mμc/g, assuming a constant rate of milk production. The wool synthesized during the same period could not be clipped immediately because newly synthesized wool takes about 6 days to appear above the skin surface (Downes 1961). A maximum specific activity of 15 mμc/g was observed in the 3 days' growth clipped 8 days after the injection. The true maximum attained in the wool must have been higher because, as pointed out previously (Downes 1961), the radioactive portions of the fibres cannot all be clipped simultaneously. The specific activity of the wool must therefore have approached that of the casein synthesized during the same time. This fact and the relatively rapid fall in the specific activity of the wool after the maximum was reached suggest that the plasma proteins are not intermediates in the synthesis of wool keratin. It must be emphasized that this tentative conclusion will have to be confirmed by detailed studies of the fate of labelled amino acids and plasma proteins in the sheep.

The results show that the tissue metabolism of the sheep is much the same as in other species. The accumulation of results of experiments similar to those reported here seems to be one of the few ways of comparing the nutritional needs of the tissues of the sheep with those of monogastric animals.

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