The Lysine Requirements of the Adult Rat

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Osborne & Mendel (1915), who found that the presence of an adequate amount of lysine in the diet is necessary for growth, also investigated the question of whether or not lysine is needed for maintenance of weight in the adult rat. They observed that a diet in which all the nitrogen was supplied by zein to which tryptophan had been added, and also by 'protein-free' milk, maintained the body weights of their rats for as long as 182 days. These animals showed wasting of muscles, but remained vigorous and began to grow again when lysine was supplied. The conclusion which Osborne & Mendel (1915, 1916) had reached, that lysine was most probably not required for maintenance, has been questioned by later authors. Thus, it is very doubtful, as Osborne & Mendel (1916) themselves acknowledge, whether 'protein-free' milk is really free from lysine. The term 'maintenance' needs to be more clearly defined. As pointed out by Hart, Nelson & Pitz (1918), maintenance in this connexion means that the nitrogen or protein content of the body should be constant over fairly long periods, and constancy of body weight is in itself not sufficient evidence that this has been achieved. Nitrogen balance experiments in rats (Wolf & Corley, 1939) and in dogs (Rose & Rice, 1939) appeared to show that lysine was indispensable for maintenance. On the other hand, Burroughs, Burroughs & Mitchell (1940), using mixtures of essential amino-acids, found that the lack of lysine in the diet of adult rats led to a small or negligible N balance; these authors concluded that lysine is probably not essential for maintenance of N equilibrium in the rat. From experiments in which deaminized casein was fed to volunteers (Albanese, Holt, Brumback, Hayes, Kajdi & Wangerin, 1941) it was deduced that lysine was essential for the maintenance of N equilibrium in humans; these experiments are, however, not

conclusive, since deaminized casein is toxic and its deleterious effects are specifically relieved by lysine (Hogan, Powell & Guerant, 1941). In view of the conflict of opinion it was decided to reinvestigate the problem. In earlier experiments (Harris, Neuberger & Sanger, 1943) it was found that young rats kept on diets containing zein and a liver concentrate as the only sources of amino-acid-N did not lose weight, though they did not grow, results which are in agreement with the findings of Osborne & Mendel (1915, 1916) and other workers. According to the most careful analyses (Vickery, 1938; Zittle & Eldred, 1944) zein is free from lysine, but the liver extract supplied about 5.5 mg. N of unknown chemical nature per day. It thus appeared that, if lysine is essential for maintenance, the requirements must be small. It was therefore decided to leave out any supplement of unknown composition and to compare diets, made up of zein, tryptophan and the known crystalline B-vitamins, with a similar diet supplemented with lysine, in their effects on the maintenance of weight in adult rats over long periods.

EXPERIMENTAL

Fully grown male black-hooded and albino rats from our stock were used. Weights were measured twice weekly and food consumption was recorded. The basal diet was similar to the one described (Gillespie, Neuberger & Webster, 1945); it differed in only two respects; the percentage of zein was 15, the starch content of the diet was correspondingly increased; secondly, the amounts of the vitamin B supplements, choline and inositol, were halved. When lysine was added, it was incorporated in the diet; food was given *ad libitum*.

RESULTS

The weight changes recorded in Table 1 for adult rats and also the results reported for adolescent animals (Gillespie *et al.* 1945) on a similar diet show

Table 1. *Changes of body weight of rats receiving basal diets with and without added lysine*

Rat no.	Days on diet	Diet	Body weight (g.)		Average change per week	Daily food consumption (g.)
			Initial	Final		
1	98	Basal	287	218	-4.9	11.5
2	91	"	290	225	-5.0	11.4
3	56	"	277	233	-5.5	12.0
4	56	"	309	273	-4.2	12.2
5	56	"	237	208	-3.5	11.1
6	50	"	344	305	-5.6	10.4
7	98	+1% lysine HCl	290	295	—	13.1
8	119	"	287	289	—	14.2

clearly that a loss of body weight invariably occurs if lysine is absent from the diet. The loss is continuous, as shown in Fig. 1, and the amount of body

Fig. 1. Weight changes of adult rat on zein diets supplemented with tryptophan with and without added lysine.

substance lost appears to be related to the initial weight. Thus in this series the average weekly loss of weight varied between 1.4 and 1.9% of the initial body weight with an average figure of 1.75%, whilst with the adolescent animals of initial weight between 110 and 140 g. an average figure of 1.5% was found (Gillespie *et al.* 1945). If lysine is added to the diet, no loss of weight is observed; in some cases slow growth, even in adult animals, has been found. It must be concluded, therefore, that, at least with the diet used in these experiments, the presence of lysine in the food is essential for the maintenance of body weight in adolescent and adult rats.

The lysine-deficient animals appeared to be quite healthy, even after having been on this diet for nearly 100 days. Their fur was quite smooth and

there was no marked wasting of muscles, but the subcutaneous fat was scanty compared with that of the control animals. They also showed marked hypoproteinaemia. It should be noted that these observations were made before those on adolescent rats (Gillespie *et al.* 1945) and as the occurrence of anaemia was not then under consideration no haemoglobin estimations were made. Histological examination of liver, kidney, spleen, testis and bone-marrow, kindly carried out by Dr Glynn, showed no pathological changes. It has been claimed (Albanese, Holt, Frankston, Kajdi, Brumback & Wangerin, 1943) that lysine deficiency in humans caused increased excretion of non-ketonic organic acids in the urine. We have been unable to detect such an increase in lysine-deficient adult rats; this may be due to a species difference. It is also possible that the increase of urinary organic acids observed by the American authors was not due to the lack of lysine in the diet, but was caused by some factor present in the deaminized casein. It may be concluded, therefore, that the absence of lysine in the diet of the adult rat causes no specific lesions.

In the next experiment increasing amounts of lysine were added to the diet and the effects on body weight observed. It can be seen (Table 2) that an intake of up to 10 mg. of lysine hydrochloride per day causes no significant change in the loss of weight. A dose of about 14 mg. reduces the loss of body weight by about one-half, whilst an intake of about 20 mg. of the hydrochloride ensures on the average a maintenance of weight. Higher doses of lysine hydrochloride, i.e. 25 mg. or more, led to an increase of weight even in these rather adult rats. 20 mg. of lysine hydrochloride, or 16 mg. of lysine itself, may be considered the minimal dose for maintenance in a rat of about 300–350 g. body weight.

DISCUSSION

The finding that the presence of lysine in the diet is essential for maintenance of the body weight of the adult rat is in accordance with results obtained in

Table 2. Changes of body weight of adult rats receiving basal diets with varying amounts of lysine

Rat no.	Days on diet	Daily lysine HCl (mg.)		Body weight (g.)			Daily food consumption (g.)
		Added	Eaten	Initial	Final	Average change per week	
18	26	15.0	13.3	240	228	-3.0	12.8
19	26	15.0	13.6	253	245	-2.0	13.1
20	26	15.0	13.8	252	241	-2.7	13.3
25	27	20.0	17.1	305	296	-2.2	13.4
26	27	20.0	18.2	380	380	0	14.2
27	27	20.0	18.8	324	321	-0.7	14.7
30	26	25.0	20.6	310	305	-1.0	14.4
31	26	25.0	24.0	358	380	+5.5	17.4
32	26	25.0	22.6	307	324	+4.2	15.8

other species. The contrary findings of Osborne & Mendel (1915, 1916) may be ascribed to the presence of small amounts of lysine in their 'protein-free' milk. The loss of body weight in lysine deficiency is slow and the over-all loss of N is small. This may explain why Burroughs *et al.* (1940) were unable to establish with certainty a negative N balance in lysine-deficient rats. We have obtained similar equivocal results with the balance method, which is not sufficiently sensitive to detect small differences between intake and excretion. Another point has also to be considered. In the experiments reported here diets were used that did not contain biotin, folic acid and other less well-defined growth factors. If bacterial synthesis of amino-acids in the intestine is of any importance to the lysine supply of the rat, it is possible that the omission of these growth factors from the diet may affect adversely the microbiological synthesis in the gut, and may thus modify the dietary requirements for lysine. This point must, however, await further investigation, when all these factors have become available in a pure form.

There is a considerable disparity between the maintenance requirements of the adult rat for lysine and the requirements of the young animal for optimal growth. Thus the minimal value for maintenance obtained from the present work is about 16 mg. a day, which is equivalent to about 0.1% of the total food. Rose (1938) states that the young animal requires about 1% of lysine in its food for optimal growth. This estimate, which agrees with our (unpublished) observations, means that a young rat needs about 100 mg. of lysine a day for optimal growth; the ratio between growth requirement of the young animal and the maintenance requirement of the adult would therefore be, in absolute amounts, about 6:1. This ratio would become considerably

greater—about 20 or 25:1—if the comparison were made on the basis of body surface. Whatever the correct basis for this calculation, it is clear that the maintenance requirement for lysine is small compared with the growth requirement. There are no comparable figures for other amino-acids, but the results of Burroughs *et al.* (1940) indicate that dietary deficiencies of threonine, isoleucine and methionine produce very abrupt and marked nitrogen losses from the body, whilst the omission of other essential amino-acids like lysine and histidine causes no such negative balance as could be established with certainty. Rubner has called the loss of nitrogen which occurs through irreversible oxidation of amino-acids the 'wear and tear' quota, a quota which has to be replaced if the body protein is to be maintained. It appears now that this 'wear and tear' quota is a factor characteristic not so much for protein as a whole, but for its constituent amino-acids and will differ in value from one amino-acid to another. A low 'wear and tear' quota for lysine such as is postulated here, is in accord with the relative metabolic inertia shown by lysine towards enzymes and tissue slices (Neuberger & Sanger, 1944).

SUMMARY

1. It has been shown that the presence of lysine in the diet is necessary for the maintenance of body weight in the adult rat.

2. The amount of lysine required for maintenance has been determined and found to be very much smaller than the amount needed for optimal growth.

3. It is suggested that the relative proportions of essential amino-acids required for growth and for maintenance respectively may vary considerably for different amino-acids.

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