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Tryptophan Deficiency and Requirements in the Adult Rat

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Many investigations have been made in recent years on the nutritive value of protein hydrolysates and amino-acid mixtures. Nevertheless, it is doubtful whether these are complete substitutes for whole protein. The result of the first experiment reported here suggested that acid-hydrolysed casein supplemented with tryptophan was inadequate both for the maintenance of the adult female rat and for promoting recovery of animals suffering from severe tryptophan deficiency. However, the results of later experiments indicated that the supplemented hydrolysate was only slightly inferior in nutritive value to whole casein for adult, growing and tryptophan-deficient rats. In these experiments it was found possible to maintain adult rats at a constant, if not maximal, weight level on the acid-hydrolysed casein when it was supplemented with tryptophan, and an attempt was made to determine the maintenance requirement of the adult rat for tryptophan for comparison with values reported for the growing rat. At the same time the effects of prolonged tryptophan deficiency were studied, since it was thought that by using only adult animals it would be possible to distinguish between any specific effects that might be caused by this deficiency and those that were secondary to the general stunting.

EXPERIMENTAL AND RESULTS

General

Management of rats. The experiments were performed on hooded rats of the Lister strain. Litter-mates were used and divided into groups, as far as possible similar with regard to age, sex and weight. They were kept in individual cages with mesh bottoms placed in a tray containing sawdust (Exp. 1), or lined with blotting paper to absorb the urine (Exps. 2–4). This made it possible to collect the food that had been scattered and to determine the food intake. For the most part the faeces fell through the mesh; no extra precautions were taken against coprophagy.

Diets. In all the experiments the animals were fed *ad lib.* The percentage composition of the basal diets was: casein (whole or hydrolysed) 14·7, cystine 0·3, starch 62, hardened arachis oil 15, lard 3 and salts 5. The salt mixture had the following composition: NaCl 51·9, $MgSO_4.7H_2O$ 164, NaH_2PO_4 104·1, K_2HPO_4 286·2, $Ca(H_2PO_4)_2.H_2O$ 162,

Ca lactate 390, ferric citrate 35·4 parts. The dry constituents of the diet were well mixed, an equal weight of water was incorporated, and the mixture then steamed until the starch was thoroughly cooked. The diet, when cool, set to a cake of cheese-like consistency.

The acid-hydrolysed casein was prepared by the method of Jackson (1927). The vitamin supplements, which differed slightly, are recorded under the description of the individual experiments. One batch of the food-yeast extract contained in μg./g. extract: riboflavin 300, nicotinic acid 2000, pantothenic acid 400, biotin 0·4 and pyridoxin 100. It had a low amino-acid content and gave a negative glyoxylic acid test for tryptophan.

Exp. 1. *Effect on body-weight of casein and its hydrolysates*

Arrangement of experiment

Twelve young adult female rats from four litters between 129 and 194 days old were divided into three groups. As the protein fraction, the diets contained whole (unhydrolysed) casein (U.C.), acid-hydrolysed casein (A.H.C.), acid-hydrolysed casein with 0·2 g. L-tryptophan/100 g. diet (A.H.C.T.) or a papain and pancreatin digest of casein (E.H.C.). They were given to the three groups according to the details given in Table 1. Vitamins were supplied to each rat as follows: 1 ml. yeast extract (equivalent to 500 mg. yeast) and 15 μg. aneurin hydrochloride daily, three drops cod-liver oil five times a week and three drops of α-tocopherol solution twice a week (equivalent to 8 mg./week).

Table 1. *Average change in body-weight when groups of four adult rats received diets containing whole casein (U.C.), tryptophan-free acid-hydrolysed casein (A.H.C.), A.H.C. supplemented with* 0·2% L-*tryptophan (A.H.C.T.) and an enzymic digest of casein (E.H.C.)*

	Group A (Average initial weight 250 g.)			Group B (Average initial weight 235 g.)			Group C (Average initial weight 235 g.)		
Period no.	Length of period (days)	Diet given	Average change in weight (g.)	Length of period (days)	Diet given	Average change in weight (g.)	Length of period (days)	Diet given	Average change in weight (g.)
1	43	U.C.	−22	43	A.H.C.T.	−41	63	A.H.C.	−120
2	43	A.H.C.T.	−47	64	U.C.	+21·5	23	A.H.C.T.	+ 3
3	47	A.H.C.T. with tryptophan increased to 0·4 %	+ 5	46	E.H.C.	−12	10	A.H.C.T. with tryptophan increased to 0·4 %	+ 6
4	20	U.C.	+19·5	—	—	—	57	U.C.	+ 65

Results

The animals of group A on transference from the stock diet to diet U.C. showed a weight loss that was least with the two smaller animals. After 3–4 weeks the weights of all the animals were similar (220, 228, 227 and 236 g.), regardless of their initial weights (225, 279, 236 and 261 g.). Little change occurred during the last 2 weeks of the period, but when the animals were transferred to diet A.H.C.T. there was

a further loss of weight and inhibition of oestrous cycles. Doubling the tryptophan content of the diet prevented further loss, but in only one of the four animals did it cause an increase (15 g.) of more than 5 g. in 47 days. However, during period 4 when the rats were returned to diet U.C. (Table 1, Fig. 1) a slow increase in weight occurred in all the animals, and normal oestrous cycles were restored. The weight curve of one of the rats is shown in Fig. 1.

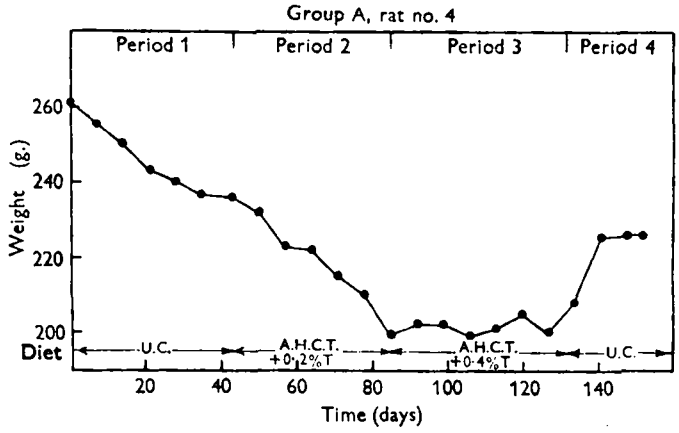

Fig. 1. Exp. 1. Failure of diet A.H.C.T. to maintain adult female rats at their original weight level. For details of diets see p. 307.

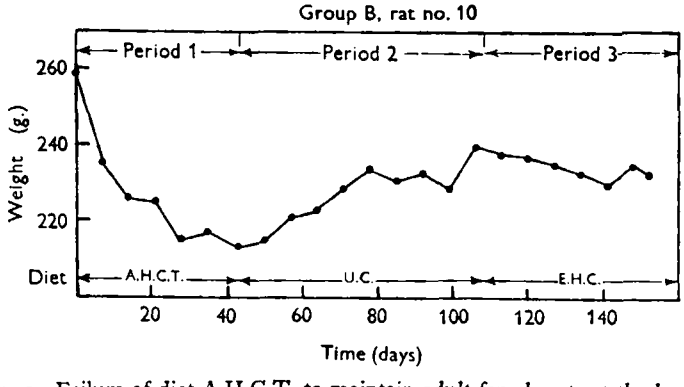

Fig. 2. Exp. 1. Failure of diet A.H.C.T. to maintain adult female rats at the level achieved with diet U.C. For details of diets see p. 307.

Similar results were obtained in group B. During period 1, when the rats of this group were given diet A.H.C.T., considerable weight loss occurred which again was greater in the heavier animals. During period 2 when they received diet U.C., they all made slow increases in weight, although a further loss of weight occurred slowly during period 3 when diet E.H.C. was given. The weight curve of one of these rats is shown in Fig. 2.

The animals of group C received the tryptophan-deficient diet (A.H.C.) for 63 days, during which they lost 46·7, 56·0, 49·6 and 44·0 % of their initial body-weights respectively. During the last few days of this period the rate of loss increased and the

condition of the animals became critical. In an attempt to restore them, 0·2 % tryptophan was added to the diet, but the overall weight changes of 0, 15, 8 and −1 g. in 23 days were slight, and there were considerable day-to-day fluctuations. For the second rat the weight curve suggested that slow recovery was initiated by the inclusion of 0·2 % tryptophan in the diet, but during the following 10 days when the amount was increased to 0·4 %, there was a further gain in weight of only 2 g. The weight changes for the other three animals after increasing the tryptophan were

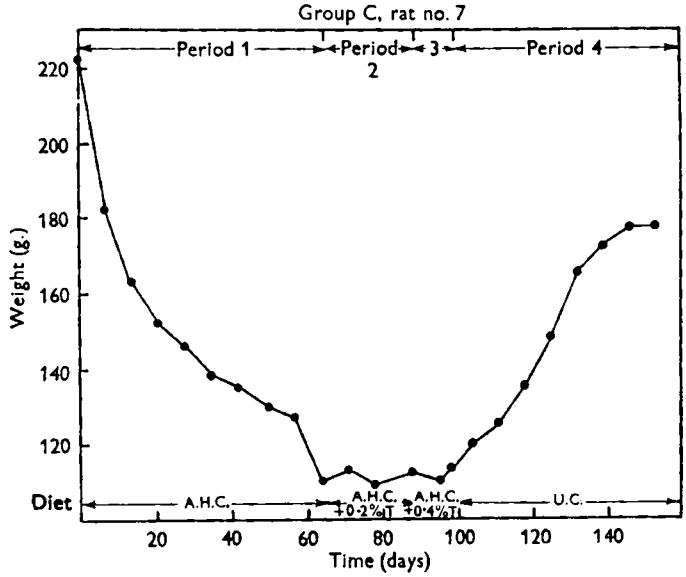

Fig. 3. Exp. 1. Failure of adult tryptophan-deficient rats to recover on the addition of tryptophan to the diet. For details of diets see p. 307.

+7, +1 and +5 g. giving total weight gains of 7, 17, 9 and 4 g. for the 33 days during which tryptophan was given. However, when the rats were transferred to diet U.C. they made immediate and progressive weight gains, and oestrous cycles were restored in from 25 to 40 days. The weight curve of one of these rats is shown in Fig. 3.

The addition of tryptophan to the diet of these animals stopped the steep decline in weight, and was accompanied by an increase in food consumption. This could not be measured accurately owing to some scattering of the food among the sawdust but was about 50 g./rat during the last week of the deficiency period (29 Cal./100 g. body-weight/day) and 77 g. for the 1st week after the addition of tryptophan (44 Cal./100 g. body-weight/day). Later, when the tryptophan content of the food was increased, the food intake appeared to be slightly reduced and the feeding of whole casein did not seem to increase the intake above that seen with diet A.H.C.T. until after the animals had made an appreciable weight gain.

Exp. 2. Effect on body-weight of young rats of a diet containing acid-hydrolysed casein supplemented with tryptophan

The failure of diet A.H.C.T. to maintain adult rats and to enable those suffering from tryptophan deficiency to recover was particularly surprising in view of the known adequacy of such diets for growth in young animals (Jackson, 1927; Berg & Rose, 1929). In the circumstances it was decided to investigate the ability of diet A.H.C.T. to support the growth of young rats of the same strain.

Arrangement of experiment

Groups of four young rats aged 30–33 days and weighing between 62 and 95 g. were used. The composition of the diets was similar to that in the previous experiment except that, in addition to the vitamin supplements given in Exp. 1, each animal was supplied daily with pyridoxin 30 μg., riboflavin 50 μg., calcium pantothenate 100 μg., nicotinic acid 1 mg., inositol 1 mg. and choline chloride 6 mg.

The food residues were collected on blotting paper which lined the trays and absorbed the urine. They were separated from the faeces and weighed daily.

Table 2. *The calorie intake and change in body-weight of young rats given diets A.H.C.T.* and U.C.**

	Diet A.H.C.T.					Diet U.C.				
Rat no. ...	21	22	23	24	Average	25	26	27	28	Average
Initial weight (g.)	93	62	70	72	74	85	73	79	82	80
Final weight after 8 weeks (g.)	169	128	178	182	164	198	174	199	253	206
Weight increase: (g.)	76	66	108	110	90	113	101	120	171	126
(%)	77	106	154	153	122	138	138	151	208	159
Total calorie intake (Cal.)	3485	2633	3516	3241	3219	3673	3424	3738	4329	3791

* See Table 1.

Results

The results are shown in Table 2. Satisfactory growth occurred on both diets U.C. and A.H.C.T., and statistical analysis of the results showed that there was no significant difference in the rates of growth of the two groups. The average total gain in weight with its standard error of an animal in 8 weeks on diet U.C. was 126 ± 16 g. and on diet A.H.C.T. it was 90 ± 11 g., with $t = 1{\cdot}49$; the probability that the difference was significant was $P > 0{\cdot}1$. In each group there was a correlation between the weight increase and the calorific value of the food intake (correlation coefficient $r = +0{\cdot}77$).

The failure of the rats of Exp. 1 to recover when fed diet A.H.C.T. remained unexplained.

Exp. 3. Further investigations of observations made in Exp. 1

Arrangement of experiment

In an attempt to investigate more fully the observations made in Exp. 1, twelve adult female rats were made tryptophan-deficient by diet A.H.C. The vitamin supplements were similar to those of Exp. 2 except that the choline chloride was increased

from 6 to 12 mg./rat/day. After 49 days when the animals had lost an average of 35·9 % of their initial weight, they were divided into groups of four which were then given diets U.C., A.H.C.T. and E.H.C. respectively.

Results

The results are shown in Fig. 4. Recovery occurred in all three groups of animals regardless of the nature of the diet. Five days after changing from the deficient diet, when all the animals were rapidly gaining in weight, two from each group were returned to the deficient diet and their subsequent treatment is described under Exp. 4. The other two in each group were allowed to continue on their respective recovery diets for 3 weeks or more to ensure that their recovery was continuous.

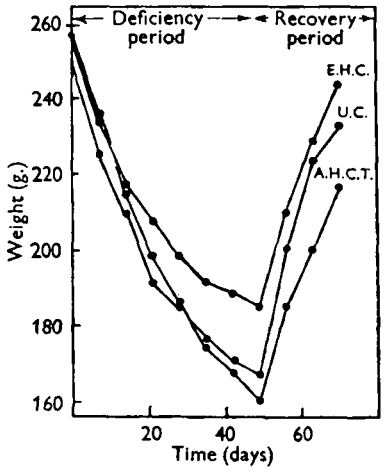

Fig. 4. Exp. 3. Recovery of weight in adult tryptophan-deficient rats when fed diets U.C., E.H.C. and A.H.C.T. For details of diets see p. 307.

Exp. 4. Determination of the tryptophan requirement of the adult rat

Arrangement of experiment

At the conclusion of Exp. 3 the animals, together with a further group of four rats which had been treated similarly, were used to determine the tryptophan requirement of the adult rat. In addition a further four rats (group 1) received the tryptophan-deficient diet until they were moribund. The others were divided into groups of four, two of which had almost completely recovered from the deficiency, the other two having been depleted for the second time. These groups (2–5) all received diet A.H.C. supplemented with 0·01, 0·05, 0·1 and 0·2 % tryptophan respectively.

One of the rats of group 1 died from a respiratory infection after 41 days on the deficient diet and was not included in recording the results. The effects of total deprivation of tryptophan were studied in the three remaining rats and also, so far as gross results were concerned, in the four deficient rats (group C) of Exp. 1. Red-corpuscle counts and haemoglobin values were determined and the results are recorded in Table 3. Included for comparison are the results obtained for two animals which,

Table 3. *Haemoglobin estimations and red-corpuscle counts on groups of adult rats given diets U.C.*, A.H.C.*, and A.H.C.* supplemented with different amounts of tryptophan*

Group no.	Rat no.	Diet	Haemoglobin (g./100 ml.)	Red-corpuscle count (millions/cu.mm.)
Control	C3	Stock	14·6	9·14
	C4		14·6	9·50
	C7		15·6	8·87
	C9		14·1	8·56
	Average		14·7	9·02
6	118	U.C.	14·3	9·53
	119		14·1	8·35
	Average		14·2	8·99
5	122	A.H.C. with 0·2 % tryptophan	13·4	8·12
	135		14·1	8·86
	Average		13·8	8·49
4	120	A.H.C. with 0·1 % tryptophan	13·8	8·96
	127†		13·2	8·74
	Average		13·5	8·85
3	128	A.H.C. with 0·05 % tryptophan	13·7	8·61
	136		14·7	10·11
	Average		14·2	9·36
2	123†	A.H.C. with 0·01 % tryptophan	6·6	3·96
	126†		11·5	7·29
	129		12·3	8·46
	132		13·1	8·65
	Average (of 129 and 132 only)		12·7	8·55
1	113	A.H.C. unsupplemented	7·7	4·61
	115		10·4	—
	116		10·0	4·57
	Average		9·4	4·59

* See Table 1.
† Small blood clots present.

immediately after the deficiency period, increased in weight on diet U.C. Oestrous cycles were studied by the vaginal smear method.

In the latter part of Exp. 4 normal supplies of the acid-hydrolysed casein became inadequate, and for a short period it was necessary to mix the ordinary preparation with equal quantities of one containing some calcium sulphate, or to use the latter preparation alone. Changes in the hydrolysate caused minor weight changes but did not interfere with the main result.

Results

The effects of tryptophan deficiency. The four rats of Exp. 1 received the deficient diet for 63 days and lost between 44 and 56 % of their initial body-weight. Their condition was then critical. The three rats of group 1 (Exp. 4) were killed after 106–112 days on the deficient diet, when they were on the point of death and had lost between 51·6 and 58·1 % of their original weight.

In addition to the usual features associated with nutritional deficiency, e.g. emaciation, staring coat, anorexia and permanent dioestrus, there was necrosis of the tail and staining of the wrists, paws and nose with reddish material. Although oestrus was inhibited 10–25 days after the period of tryptophan-deficiency began, the four animals of Exp. 1 showed normal cycles after recovery on a diet containing whole casein, and two of them subsequently bore young, which, however, were either born dead or died shortly after birth.

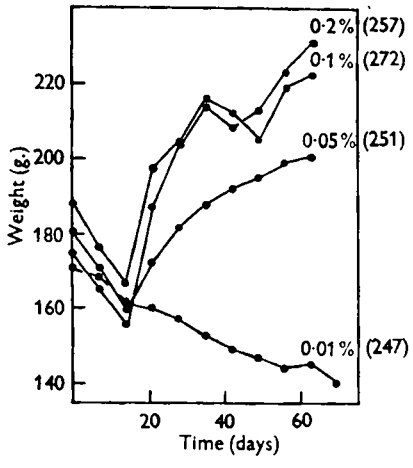

Fig. 5

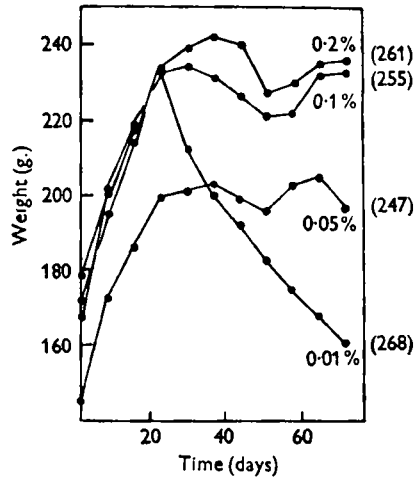

Fig. 6

Fig. 5. Exp. 4. Effect of inclusion of tryptophan at various levels in the diet of tryptophan-deficient rats. The figures in parentheses show the average initial weights of the rats in g.

Fig. 6. Exp. 4. Effect of feeding tryptophan at various levels to rats that had partly recovered from a period of tryptophan deficiency. The figures in parentheses show the average initial weights of the rats in g.

Group 2, *given* 0·01 % *tryptophan.* The animals of this group showed a progressive loss of weight which was more rapid in the two animals that had recovered from the initial deficiency. Not only did 0·01 % tryptophan fail to maintain the animals at any weight level, but in three of the four animals the rate of loss was not appreciably less than when tryptophan was altogether lacking. All the animals of this group showed complete inhibition of oestrus, and symptoms of deficiency similar to those described in the rats totally deprived of tryptophan.

Group 3, *given* 0·05 % *tryptophan.* The rats that had nearly recovered from the deficiency before being given this diet did not complete their recovery, but maintained themselves at a weight level considerably below their initial values for 7 and 11 weeks, respectively, until they were killed. The two deficient animals showed a slow weight increase on this level of tryptophan, followed by maintenance at a level of 190–210 g., similar to that of the other two animals of the group. This level seemed to be independent of the initial weight of the animals, which varied between 231 and 271 g. All were in fairly good condition but noticeably thin. Oestrous cycles returned but there was a tendency to periods of continuous cornification of the vaginal mucosa, or prolonged intervals between the cycles. Some of the results are shown in Figs. 5 and 6.

Group 4, *given* 0·1 % *tryptophan*. The animals of this group that had already recovered much of their lost weight, maintained themselves for the remaining 7–11 weeks on this level of tryptophan. The smaller animal slightly surpassed its initial weight (234 g.) and remained at this level, whereas the larger did not fully regain its initial weight (277 g.).

The rats that were transferred direct from the deficient to the 0·1 % tryptophan diet showed a steady increase in weight except for a short period during which the hydrolysate containing calcium sulphate was given. Here again the smaller animal nearly reached its initial weight but the heavier did not do so within the experimental period, although its weight was still increasing slowly when it was killed. All the animals appeared to be in good health, and oestrous cycles, inhibited during the course of the deficiency, were restored.

Group 5, *given* 0·2 % *tryptophan*. The results for this group did not differ materially from those of the 0·1 % tryptophan group. The smaller animals regained the whole of their lost weight, but recovery of the larger ones was incomplete. The health of all the animals appeared good and oestrous cycles were normal.

Blood analyses. The results of the blood analyses are shown in Table 3. There was pronounced anaemia in the three rats given the totally deficient diet. The haemoglobin level fell to 63·8 % of the value recorded for rats given the stock diet. Owing to clumping of the corpuscles in the blood of one of the rats on dilution it was impossible to make a red-corpuscle count. In the other two, however, the counts were 4·61 and 4·57 million/cu.mm. respectively, as compared with an average of 9·02 for the controls. The values for the two animals given diet U.C. during recovery from the deficiency were only slightly lower than those of the controls given the stock diet, and those of the animals that had recovered after supplementation of the deficient diet with 0·2 % tryptophan were comparable with them. Values as high as those seen in the latter group were found in the animals given diet A.H.C. supplemented with 0·1 and 0·05 % tryptophan.

A sample of the reddish material found on the nose, paws and feet of the deficient rats when washed off with water gave a faint-brown cloudy solution which did not give a positive benzidine test. Spectroscopic examination showed an intensification of the *D* line and a faint band in the green region suggesting that the pigment might be a porphyrin.

DISCUSSION

General effects of tryptophan deficiency

The general condition of the tryptophan-deficient rats was similar to that seen in other forms of protein deficiency except for the caudal necrosis which had not previously been reported in protein or amino-acid deficiencies. The weight loss, averaging 8·9 % in the 1st week and 2·0 % in the 15th week, was much greater than that reported by Neuberger & Webster (1945) in adult male rats suffering from lysine deficiency, but nevertheless the animals survived for periods up to 112 days.

The inhibition of oestrus caused by tryptophan deficiency cannot be considered specific, since deficiency of almost any essential nutrient has this effect. Albanese,

Randall & Holt (1943) found that foetal resorption occurred in female rats given a tryptophan-deficient diet immediately after mating, and Keller (1946) reported that young rats of both sexes when given a tryptophan-deficient diet for from 3 to 18 days became sterile and showed no signs of sexual activity for the remainder of the observation period of 90–150 days. His findings, however, are not supported by the work of Berg & Rohse (1947) nor by the results of Exp. 1, where recovery from extreme tryptophan deficiency led to return of oestrous cycles, two of the rats concerned subsequently bearing young.

The anaemia seen in the rats of Exp. 4 was more pronounced than that previously reported in tryptophan deficiency (Alcock, 1933; Hamada, 1936; Albanese, Holt, Kajdi & Frankston, 1943), but, since adult rats were used in the present work, comparison with results mostly obtained on immature animals is difficult. Tryptophan, however, is not alone among the amino-acids in being essential for the maintenance of normal haemoglobin values, and anaemia has also been reported in deficiencies of lysine, methionine, histidine, phenylalanine and isoleucine. It is extremely doubtful therefore whether tryptophan plays a specific role in blood formation, although suggestions of a direct stimulant action (Matsuoka & Nakao, 1931) have not been finally disproved.

The results of the analyses performed on rats that had recovered from the effects of tryptophan deficiency of 49 days' duration suggest that there was no permanent damage to the haemopoietic system. The rats that received 0·05 % tryptophan had haemoglobin values comparable with those of the rats that received 0·1 and 0·2 % tryptophan, although the 0·05 % group was being maintained at a lower weight level. The haemoglobin values and red-corpuscle counts of the rats receiving 0·01 % tryptophan were higher than those of the totally deficient rats, although the loss of body-weight was comparable, averaging 51·6 and 53·8 % respectively. This finding, which is in agreement with the observation of Hamada (1936) that in young rats given tryptophan at a level of 0·025 % the haemoglobin rose although the weight fell, suggests that small amounts of tryptophan may be used preferentially in the maintenance of haemoglobin levels.

The exact nature of the reddish brown material deposited on the nose and paws of the deficient rats was not determined. Krehl, Sarma, Teply & Elvehjem (1946) found a similar accumulation of porphyrin-like material in young rats fed a diet low in nicotinic acid and containing maize. This is of particular interest in view of the relationship now known to exist between tryptophan and nicotinic-acid metabolism (Krehl, Teply, Sarma & Elvehjem, 1945). Porphyrin deposition has also been reported in pantothenic-acid deficiency (Chick, Macrae & Worden, 1940; McElroy, Salomon, Figge & Cowgill, 1941).

The adequacy of supplemented acid-hydrolysed casein as a substitute for whole casein

In Exp. 1 the tryptophan-deficient rats failed to recover when tryptophan was added to the diet. Similar anomalies have occasionally been reported. Thus Roche, Roche, Drouineau & Passelaigue (1938) found that rats given a protein-free diet until they lost a quarter of their body nitrogen and then transferred to a diet supplemented with

lysine and containing gliadin and known to support growth in young rats failed to recover. Again, Albanese, Holt, Kajdi and Frankston (1943) found that adult rats given a diet containing acid-hydrolysed casein with 0·225 % L-tryptophan lost weight, and also that three adult rats that had lost weight during a prolonged period of tryptophan deficiency regained weight only slowly and incompletely when tryptophan was added to the diet, although the haemoglobin and plasma-protein levels were fully restored.

Roche & Gueit (1945) showed that the lysine requirement for recovery of adult protein-deficient rats is greater than that of young rats for growth. However, since casein contains nearly three times as much lysine as edestin which was used in Roche & Gueit's experiments, it seems unlikely that a lysine deficiency can have been responsible for the failure of recovery in Exp. 1, nor can a relative tryptophan deficiency have been a major factor in the present work, since doubling the amount in the diet caused no appreciable increase in weight.

According to Morgulis (1923), Avrorov believed that only the terminal stages of inanition are accompanied by pathological changes, and it seems possible that such changes had occurred in the animals of Exp. 1 that had lost an average of 49·1 % of their body-weight, but were absent from those of Exp. 3 that had lost an average of only 35·9 % of their body-weight, and appeared to be in much better condition. Histological examination of the gastro-intestinal tract of the two tryptophan-deficient rats that were, at the time of killing, in a state comparable with that of the deficient rats of Exp. 1 before tryptophan was added to the diet, showed sloughing and necrosis of the villi in the ileum (unpublished data) suggesting that absorption might have been impaired. Sun (1926), however, found that repair of the intestinal villi in starving mice occurred very rapidly when an adequate diet was supplied and that the epithelium had regenerated 10 hr. after refeeding. Though the possibility remains that the amount of food absorbed by the rats of Exp. 1 was inadequate to do more than maintain the animals, it seems unlikely that repair of the intestinal epithelium took 33 days, and that it occurred suddenly just at the time when whole casein replaced the supplemented hydrolysate. Thus, though defective absorption may have been one of the factors involved in the failure of the rats to recover, it seems unlikely that it was the only one.

It seemed possible that an absolute or relative deficiency of some factor such as strepogenin (Sprince & Woolley, 1945) or the 'animal protein factor' (Cary, Hartman Dryden & Likely, 1946) might have been involved, since destruction of these factors would have occurred during the acid hydrolysis of the casein. It is unlikely, however, that this was so since a similar hydrolysate was used in Exps. 3 and 4, in which rapid recovery from tryptophan deficiency and maintenance of adult rats at a satisfactory weight level occurred respectively.

The chief difference between the conditions existing in Exp. 1 and in Exps. 3 and 4 was that in the latter experiments various pure vitamins of the B complex were supplied in addition to the yeast extract, and it seemed possible that the anomalous results might be explicable in terms of the tryptophan-nicotinic acid relationship. The yeast extract given in all experiments supplied about 100 μg. nicotinic acid/rat/day,

whereas in Exps. 2–4 an extra 1 mg. nicotinic acid/rat/day was given. Under normal conditions the rat does not require dietary nicotinic acid, presumably because it can synthesize sufficient for its own requirements, and recent work suggests that the source may be tryptophan (Rosen, Huff & Perlzweig, 1946). According to Krehl, Henderson, de la Huerga & Elvehjem (1946) nicotinic acid must be supplied at a level of 1·0–1·5 mg./100 g. diet to growing animals receiving an unbalanced mixture of amino-acids, and in Exp. 1 of the present series it may have been that the nicotinic acid supplied by the yeast extract was insufficient for the needs of the animals receiving the deficient diet but, since the animals were losing weight, it is difficult to assess the effect of the amino-acid imbalance on the requirement. However, the greater ability of the rats of Exp. 4 to withstand the effects of tryptophan deficiency as judged by their survival time, more than 100 days as compared with about 63 days in Exp. 1, supports the suggestion of an increased nicotinic-acid requirement even in the absence of growth. If the animals of Exp. 1 were deficient both in nicotinic acid and in tryptophan this might explain the delay in recovery, time being required for the restoration of nicotinic-acid synthesis. However, if such were the case it is remarkable that the re-establishment of the synthetic process coincided with the replacement of diet A.H.C.T. by diet U.C., particularly in view of the fact that tryptophan as such is said to be more effective in promoting nicotinic-acid synthesis than when given in the form of protein (Singal, Briggs, Sydenstricker & Littlejohn, 1946; Bell, Scheer & Deuel, 1948). It is, however, possible that some nicotinic acid may have been supplied by the casein, since the importance of using a vitamin-free preparation was not realized at the time. However, the loss of weight of the rats fed the tryptophan-supplemented diet (A.H.C.T.) in Exp. 1, and the fact that increasing the tryptophan content of the diet from 0·2 to 0·4 % prevented further weight loss still remain unexplained.

The tryptophan requirement of the adult rat

The tryptophan requirement of the young growing rat is reported to be between 0·1 and 0·2 % of the diet, varying slightly according to the other constituents (Berg & Potgieter, 1931–2; Krehl, Sarma & Elvehjem, 1946), whereas between 0·025 and 0·05 % suffices for its maintenance (Jackson, 1929; Berg, Rose & Marvel, 1929–30; Berg & Potgieter, 1931–2). These levels are equivalent to intakes of about 10 and 2·5 mg./100 g. rat/day for growth and maintenance respectively.

Although the number of animals used was small, the results of Exp. 4 suggest that maintenance of adult female rats after recovery from tryptophan deficiency may be achieved over a fairly wide range of tryptophan intake, but the weight at which the animals are maintained may vary with the level of tryptophan in the diet. Thus there was a direct correlation between the percentage of the initial weight recovered and the logarithm of the percentage of tryptophan in the diet up to the 0·1 % level as shown in Fig. 7. Above this the rate of recovery decreased, suggesting that a level of between 0·1 and 0·2 % is sufficient for maintenance at a maximal weight level on the supplemented A.H.C. diet and that the percentage dietary requirement for tryptophan of the adult female rat after an initial period of tryptophan deficiency is similar to that of the young growing rat. During the later weeks of the experiment when a steady weight

level had been reached the actual amounts of tryptophan consumed by the rats at the 0·2, 0·1 and 0·05 % levels averaged 12·2, 5·8 and 3·3 mg./rat/day respectively. The group given tryptophan at the 0·01 % level lost weight continuously.

This finding does not agree with that of Albanese, Holt, Irby, Snyderman & Lein (1947) that the daily tryptophan requirement of the infant is about 30 mg./kg. body-weight, as compared with a previously determined adult human requirement of 6 mg./kg. (Holt, Albanese, Frankston & Irby, 1944). It is also in marked contrast with the findings of Neuberger & Webster (1945) for lysine that the ratio of the growth requirement of the young rat to the maintenance requirement of the adult was of the order of 6:1, or even higher if the comparison was made on the basis of body surface

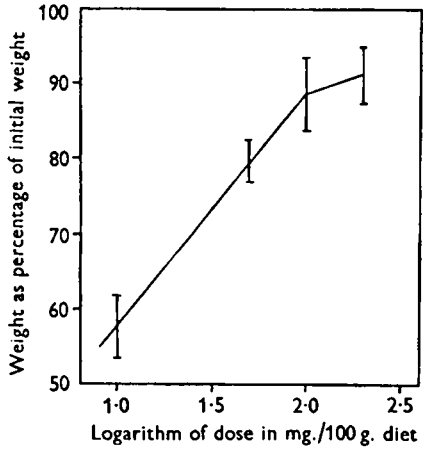

Fig. 7. Exp. 4. Relationship of the weight, as a percentage of the initial weight, of the rats to the logarithm of the dose of tryptophan in mg./100 g. diet.

instead of body-weight. This may indicate that, whereas lysine is required by the adult only for the replacement of endogenous losses, tryptophan performs some additional function, or alternatively that the rate of destruction of tryptophan is very rapid in both the young and adult rat. This latter possibility is suggested by the fact that the amount of ingested tryptophan retained as such in the tissue proteins and body fluids (Fürth & Lieben, 1922) and excreted (Schweigert, Sauberlich, Elvehjem & Baumann, 1946) is low and that it disappears rapidly from the blood after ingestion (Buck & Berg, 1945).

However, since three out of four rats lost weight when tryptophan was given at the low level of 0·01 % of the diet as rapidly as when no tryptophan was supplied, it seems possible that the available tryptophan was used in the performance of special functions, leaving no residue to supply the tissues generally. If this were the case, some tissue or function should have been better maintained in those rats than in the completely deficient ones. The degree of anaemia in the rats given 0·01 % tryptophan was less severe than in those given no tryptophan, and it is hoped that histological examination of tissues may show further differences between the two groups.

SUMMARY

1. The gross symptoms of tryptophan deficiency in the adult female rat were found to be similar to those of protein deficiency. In addition, there was necrosis of the tail and accumulation of porphyrin-like material on the nose and paws. The animals lost about 50 % of their body-weight and survived for periods up to 112 days. Oestrus was inhibited during the deficiency, but even when this was severe, permanent sterility was not induced. Marked anaemia was observed in the deficient rats.

2. The ability of acid-hydrolysed casein supplemented with tryptophan to maintain adult female rats, to promote growth in young rats and to afford recovery from tryptophan deficiency was studied. In one experiment with tryptophan included at a level of 0·2 %, the diet failed to maintain groups of adult rats, and in a further group it failed to support recovery from severe tryptophan deficiency. In a later experiment a similar diet allowed recovery from a moderate degree of deficiency, and thereafter animals were maintained at varying weight levels when tryptophan was fed at levels of 0·05, 0·1 and 0·2 % of the diet. The possible reasons for this discrepancy are discussed.

3. The optimal level of tryptophan in the diet of the adult female rat was found to be between 0·1 and 0·2 %, which is similar to that previously reported for the young growing rat.

We wish to record our thanks to Glaxo Laboratories Ltd. for their gift of acid-hydrolysed casein, to Mr S. Thurlow for preparing much of the tryptophan used in these experiments and to Dr M. Ginsburg who made the statistical analyses.

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Induced Cobalt Deficiency in Lambs

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The object of the experiments described below was to induce cobalt deficiency in lambs by giving a diet composed of foodstuffs sufficiently remote from pasture to avoid the criticism that some unknown pasture factor other than cobalt might be involved in the causation of the marasmus, now called 'cobalt deficiency', and to prove conclusively that a lack in the diet of cobalt *per se* is responsible for 'pining' in lambs.

EXPERIMENTAL

First experiment, 1946

Basic ration. The basic daily ration used was 1100 g. flaked maize, 250 g. hay and 60 g. of a mineral mixture composed of ground limestone, steamed bone-flour and crude rock salt. The introduction of hay was necessary to ensure that the rumen processes would be normal, as it was not considered satisfactory to use wood pulp or a similar source of cellulose, since it might cause upsets in rumination, regarding which little is known. The hay was kept to a minimum (250 g./day) and was obtained from a field of which the soil was deficient in cobalt.

The diet was adequate in starch and protein equivalent for a pregnant or lactating ewe. According to Woodman (1948), a 120 lb. ewe requires 10 lb. starch equivalent (S.E.)/week for maintenance and 4 lb. starch equivalent (S.E.)/week/gal. milk; and 0·46 lb. protein equivalent (P.E.)/week for maintenance and 1 lb. protein equivalent (P.E.)/week/gal. milk. The starch equivalents of the flaked maize and hay were 84 and 30 respectively, and the protein equivalents were about 10 and 3. Thus the diet outlined above supplied the energy requirements for maintenance and for at least 1 gal. weekly of milk, the average weight of the ewes being 115 lb.

The cobalt content of the ration was assayed spectrographically with the following result:

Flaked maize	0·025 p.p.m.
Hay	0·10 p.p.m.
Mineral mixture	0·27 p.p.m.