8. Another intermediate compound, having a transient existence, gives the Rothera reaction. This substance has not been characterized, but evidence is presented which indicates that it may be a keto-acid, although not identical with acetoacetic acid.

9. Formic acid has been isolated from a bacterial culture in which the phenol or benzoic acid had disappeared.

10. It is shown that in the action of a bacterial suspension of vibrio 01 on the two amino-acids, tyrosine and 3:4-dihydroxyphenylalanine, two oxidative mechanisms may operate: deamination

leading to oxidation of the side chain, or nuclear oxidation leading to fission of the ring.

11. Evidence is presented for the occurrence of a 'catechol dehydrogenase system' in cell suspensions of these organisms.

12. Our knowledge of the oxidation of the benzene ring within living organisms and the possible role of these micro-organisms in the prevention of aromatic compounds from accumulating in toxic amounts in the soil is discussed.

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Amino-Acids and Proteins in Haemoglobin Formation

I. TRYPTOPHAN*

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Knowledge of protein and amino-acid metabolism is confined almost entirely to those aspects concerned with degradation and excretion. Of the synthetic processes in which amino-acids are utilized little is known. One of the most important of such syntheses is that of haemoglobin, the new formation of which in the adult human being is estimated at about 25 g./day. Red blood cell destruction followed by the irreversible decomposition of the haemoglobin and excretion of part of the resulting products in the form of urinary and faecal pigments takes place continuously in the normal animal and the nitro-

genous material thus lost has to be replaced by dietary protein. We have at present, however, very little knowledge of the amino-acids essential for the performance of this function.

Tryptophan, which has an obvious chemical relationship to blood porphyrin, is the only amino-acid which has been extensively investigated from this point of view. The efficacy of tryptophan in the treatment of experimental anaemia was first noted by Hirasawa (1922) and similar results were reported by Okagawa & Tatsui (1931). Fontés & Thivolle (1930) claim to have produced anaemia in rats kept on a tryptophan deficient diet for a period of 10 weeks, and further, to have rendered normal

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rabbits and dogs hyperaemic by injections of tryptophan or a mixture of tryptophan and histidine. A mixture of these two amino-acids was claimed by these authors to have also a curative effect in certain types of human anaemia. Alcock (1933), however, was unable to produce anaemia in animals by depriving them of tryptophan, nor could he demonstrate any beneficial effect on administering tryptophan to rats suffering from milk anaemia. Tochowicz (1936) also suggests that tryptophan plays no important role in blood regeneration either in haemorrhagic or pernicious anaemia. Other authors (Cuthbertson, Fleming & Stevenson, 1931; Dominici & Penati, 1931) have confirmed the negative results in pernicious anaemia. But in experimental animals Hamada (1936) reproduced the observations of Fontés & Thivolle, and succeeded in making young rats and mice anaemic by keeping them on a tryptophan deficient diet. Similar results were obtained by Albanese, Holt, Kajdi & Frankston (1943) who found that a diet deficient in tryptophan brings about a reduction of plasma proteins and haemoglobin in the rat.

While the position with regard to tryptophan is thus still controversial, about the role of other amino-acids we have even less information. Whipple & Robschey-Robbins (1937) have shown that in dogs, made anaemic by bleeding, haemoglobin production is usually, though not invariably, increased by addition of histidine or phenylalanine to a basal bread ration of unspecified amino-acid composition. The findings of Harris, Neuberger & Sanger (1943) that lysine deficiency in rats produces a mild anaemia have been recently confirmed by Gillespie; Neuberger & Webster (1945). On the other hand, in experiments in which rats were made anaemic by the ingestion of a low protein diet, Orten & Orten (1945) found no increase in haemoglobin on supplementation with any one of the ten essential amino-acids or with glycine, cystine, glutamic acid, proline or tyrosine. From experiments on rats kept on human or beef globin as the sole source of protein with and without supplements of isoleucine Orten, Bourque & Orten (1945) conclude that isoleucine is essential for haemoglobin formation in the rat. This surprising result implies either that rat globin, unlike human or beef globin, contains isoleucine or that this amino-acid is required for the synthesis of a protein of which it is not a constituent.

These isolated data suggested the need for further research and a systematic investigation was begun in this laboratory of the haemopoietic action of amino-acids and the nutritional value of proteins as assessed from their efficacy to promote haemoglobin regeneration. It was found quite early in our experiments that normal animals were of little use for this purpose as they showed no marked response

in their blood picture either to amino-acid additions or deficiencies. On deficient diets the animals usually lost weight but the percentage of red cells and haemoglobin was not usually altered, although it is probable that there was a decrease in blood volume and therefore a fall in the total haemoglobin in the animal as a whole. It therefore became necessary to make the animals anaemic and to study the rate of recovery from the anaemia on administration of the dietary factor under test. Whipple employed dogs made anaemic by bleeding. The time and expense involved in carrying out large numbers of feeding experiments with amino-acids on dogs made it impossible to use this method. Animals fed on milk proteins and suffering from anaemia on account of a mineral deficiency (Pearson, Elvehjem & Hart, 1937) could also not be considered suitable experimental material for the present purpose. A very convenient means of producing anaemia in experimental animals was found in the use of phenylhydrazine. A single intraperitoneal injection of phenylhydrazine administered at a dose of 2 mg./100 g. body weight induces in rats a consistently reproducible type of anaemia, the red blood cells being lowered to about 50% and haemoglobin to about 60% of the normal values in the course of 3-4 days after injection (see Yeshoda, 1942). The drug is known to act specifically by destroying mature red cells and to have no other toxic effects in the dosages required (Long, 1926*a, b*; Stealy & Sumerlin, 1944). In particular, it has no direct effect on the bone marrow or the spleen, and this anaemia is thought to be due entirely to the fact that phenylhydrazine renders red cells susceptible to those processes which normally destroy old erythrocytes (see Sollmann, 1937). The effect therefore is the same as in haemorrhagic anaemia with the possible difference that some of the disintegration products derived from the haemoglobin might be retained in the system and reutilized for new haemoglobin formation. The animals show no other adverse effects due to phenylhydrazine and on a complete diet rapidly recover from the anaemia. The present paper describes experiments on the effect of tryptophan deficiency on the rate of recovery of animals suffering from phenylhydrazine anaemia.

EXPERIMENTAL

The tryptophan-free diet was made up as follows:

	%
Hydrolyzed casein	18
Starch (tapioca)	40
Sugar	20
Butter fat (melted)	17
Salt mixture	5

Each animal also received 2 drops of cod-liver oil and 200 mg. of yeast daily.

The casein was hydrolyzed with sulphuric acid in the usual way and the hydrolysate after removal of acid by means of baryta was evaporated to a thick syrup. This was mixed with the required quantity of starch and dried at a low temperature in a current of air. To the powdery material the remaining constituents of the diet were added and thoroughly mixed. The food was supplied to the animals *ad libitum*. The animals on the tryptophan diet received daily, in addition to the above, 20 mg. of *l*-tryptophan which, mixed with a little melted butter, was given separately. Records of food intake show that consumption ranged from 6-8 g./day, depending upon the body weight of the animal. Rats in the two groups showed no differences

in the intake in the first 3 weeks of the experimental period; after that there was a gradual falling off in the food consumption of the deficient group.

The animals were made anaemic by means of a single intraperitoneal injection of 2 mg. phenylhydrazine (1 ml. of 0.2% (w/v) aqueous solution) and put on the tryptophan-free basal diet on the day of injection. From the 4th day 20 mg. of tryptophan/rat/day were given to one group of animals while the control group remained throughout the experimental period on the tryptophan-free diet. Red cell count and haemoglobin determinations were carried out every 3rd or 4th day. Blood was obtained by snipping the tail; the acid haematin method was used for haemoglobin determination.

Table 1. *Recovery of rats from phenylhydrazine anaemia; basal diet without tryptophan*

(R.B.C. values are in millions/mm.³; Hb values in g./100 ml. blood.)

Only four representative experiments are given in this table.

Rat no.		Days after injection								
		0	4	8	12	15	20	25	30	35
33	R.B.C.	6.68	2.80	3.45	4.51	5.07	5.10	5.23	6.18	6.29
	Hb	14.7	8.6	9.3	10.9	11.4	12.3	12.4	13.0	14.0
34	R.B.C.	6.91	2.82	3.60	4.26	4.76	5.31	5.91	6.45	6.52
	Hb	14.7	8.5	8.9	11.1	11.9	12.0	12.5	13.0	14.0
35	R.B.C.	6.99	3.27	3.69	3.99	5.01	4.96	6.12	6.13	6.20
	Hb	14.9	8.3	9.0	11.0	11.5	11.5	13.0	12.5	13.9
36	R.B.C.	6.33	3.19	3.67	4.20	4.91	5.20	5.81	5.89	5.94
	Hb	13.5	8.3	9.0	10.5	10.7	11.3	11.9	12.1	12.6

Table 2. *Recovery of rats from phenylhydrazine anaemia; basal diet + 20 mg. oral tryptophan/day*

(R.B.C. values are in millions/mm.³; Hb values in g./100 ml. blood.)

Only four out of eight experiments are reported in this table.

Rat no.		Days after injection								
		0	4	8	12	15	20	25	30	35
41	R.B.C.	6.61	2.65	4.70	5.97	6.71	6.91	6.89	7.02	7.15
	Hb	14.5	8.6	10.6	12.5	13.9	13.9	15.1	15.0	14.8
42	R.B.C.	6.80	3.02	4.98	5.61	6.52	6.60	7.09	7.14	7.20
	Hb	14.9	8.4	11.0	12.4	14.2	14.7	14.9	14.7	14.9
43	R.B.C.	7.02	2.94	4.54	6.06	6.96	6.99	7.09	6.95	7.08
	Hb	15.1	8.5	10.5	12.0	14.1	14.4	14.2	14.5	14.8
44	R.B.C.	6.42	3.16	4.61	5.61	6.26	6.28	6.71	6.81	6.89
	Hb	13.7	8.3	10.3	11.7	13.0	13.5	13.6	13.4	13.6

Table 3. *Body weights of rats mentioned in Tables 1 and 2*

(Body weight is given in g.)

The data are representative of a large number of experiments carried out.

Rat no.	Tryptophan (mg./day)	Days											
		0	7	14	21	28	35	42	49	56	60	64	70
33	20	113	118	120	120	123	128	130	—	—	—	—	—
41	—	118	117	98	94	88	80	75	70	57	54	50	*
34	20	122	126	128	130	131	136	138	—	—	—	—	—
42	—	126	122	118	107	104	101	92	86	80	78	76	75
35	20	126	130	132	134	136	138	138	—	—	—	—	—
43	—	126	124	118	99	98	92	85	79	70	63	60	60
36	20	107	108	110	114	119	125	130	—	—	—	—	—
44	—	107	100	94	88	86	83	72	67	52	*	—	—

* Animal died.

RESULTS

Tables 1 and 2 show clearly the difference in the rates of recovery from anaemia of the groups of animals with and without tryptophan in the diet and are typical of the results of many sets of experiments. The accelerated formation of haemoglobin and red cells in the animals receiving tryptophan is particularly marked in the first 12 days after injection. The weights of the animals are given in Table 3. Throughout the experimental period the animals receiving

are not necessarily in conflict with those of Du Vigneaud, Sealock & Van Elten (1935) who found that tryptophan administered subcutaneously was utilized for growth, as the above authors carried out their experiments on normal animals while the present observations as well as those of Hamada were made on anaemic animals.

Experiments with normal animals

To ascertain the effect of tryptophan deficiency on normal animals three groups of rats were used,

Table 4. *Recovery of rats from phenylhydrazine anaemia; basal tryptophan-free diet + injected tryptophan*

(R.B.C. values are in millions/mm.³; Hb values in g./100 ml. blood.)

Rat no.	Tryptophan injected (mg./day)		Days after injection					
			0	4	7	10	13	16
101	0	R.B.C.	7.51	3.27	4.17	5.02	5.55	5.91
		Hb	14.5	8.3	10.1	10.7	12.3	12.5
102	0	R.B.C.	7.29	3.50	3.56	4.71	5.31	6.01
		Hb	14.3	8.1	9.1	11.9	12.0	13.0
103	0	R.B.C.	7.01	3.91	4.13	4.94	5.05	5.74
		Hb	13.8	8.0	10.1	11.4	12.0	12.2
104	0	R.B.C.	7.53	3.18	4.05	5.00	5.52	5.70
		Hb	15.1	8.1	9.1	12.0	12.3	12.5
105	10	R.B.C.	7.41	3.90	5.15	5.57	6.71	7.22
		Hb	14.2	8.5	11.8	12.3	13.6	14.0
106	10	R.B.C.	7.31	3.80	4.56	5.59	6.91	7.12
		Hb	14.9	8.5	11.0	12.5	13.7	14.2
107	10	R.B.C.	7.20	3.11	5.56	5.86	6.40	7.06
		Hb	13.8	8.3	12.0	13.0	13.5	14.1
108	10	R.B.C.	7.50	3.22	4.81	5.66	6.40	7.35
		Hb	15.2	8.1	11.3	13.0	13.8	14.5
109	20	R.B.C.	7.37	3.47	5.46	6.17	7.19	7.41
		Hb	14.6	8.3	11.8	14.0	14.1	14.8
110	20	R.B.C.	7.29	3.69	5.63	5.90	7.21	7.38
		Hb	14.5	8.5	12.0	13.0	14.5	14.8
111	20	R.B.C.	7.01	8.43	4.91	5.91	6.98	7.15
		Hb	14.0	8.3	12.0	13.1	14.0	14.3
112	20	R.B.C.	7.37	3.44	4.88	5.71	6.69	7.15
		Hb	14.4	8.1	11.7	12.0	13.6	14.3

tryptophan steadily increased in weight while the reverse was the case with the rats on the tryptophan deficient diet.

Injection experiments with tryptophan

Hamada (1936) noted that the effect of tryptophan administered by injection is not the same as when it is given by mouth. Experiments were also carried out therefore in which the animals were given subcutaneous injections of 10 or 20 mg. of tryptophan daily. The R.B.C. and haemoglobin values in comparison with the controls are given in Table 4 and the weights in Table 5. It is obvious that injected tryptophan has the same haemopoietic action as when it is supplied in the diet; but given parenterally the amino-acid has no effect on weight. These results

Table 5. *Body weights of rats mentioned in Table 4*

(Body weight is given in g.)

Rat no.	Tryptophan injected (mg./day)	Days					
		0	3	6	9	12	15
101	0	120	117	114	110	104	98
102	0	125	123	118	110	100	96
103	0	117	114	110	106	98	90
104	0	102	98	90	84	80	76
105	10	120	118	114	110	105	100
106	10	122	118	114	110	108	103
107	10	115	115	111	106	103	102
108	10	107	100	94	86	80	77
109	20	119	116	112	106	105	103
110	20	122	118	114	110	106	108
111	20	115	114	112	108	104	100
112	20	98	96	92	90	88	86

one receiving hydrolyzed casein, the other hydrolyzed casein plus tryptophan and the third group casein. Only R.B.C. determinations were made in this experiment. The values in Table 6 show that although the erythrocyte count is slightly in favour of animals receiving tryptophan (either as a supplement to hydrolyzed casein or as whole casein) the differences are not sufficient to make them statistically significant.

DISCUSSION

The experiments recorded make it clear that tryptophan accelerates the recovery of animals suffering from phenylhydrazine anaemia. It will be seen from Table 1 that animals suffering from phenylhydrazine anaemia on a tryptophan-deficient diet take about 30 days to recover from the anaemia. When the diet is supplemented with tryptophan the

Table 6. *Body weights and erythrocyte counts of normal rats on different diets*(R.B.C. values are in millions/mm.³; body weight in g.)

Rat no.	Days	Casein		Hydrolyzed casein + tryptophan		Hydrolyzed casein	
		0	30	0	30	0	30
113	R.B.C.	7.00	8.10	7.06	8.05	6.81	7.40
	Weight	61	94	60	86	69	51
114	R.B.C.	7.06	8.05	7.47	8.10	7.46	7.35
	Weight	62	80	64	90	66	63
115	R.B.C.	7.09	8.10	7.29	8.09	7.07	6.60
	Weight	50	95	53	68	53	40
116	R.B.C.	7.08	7.98	6.61	8.01	6.73	7.60
	Weight	45	100	44	68	44	30

In another experiment six animals were maintained on a tryptophan-free diet for a prolonged period until, in fact, most of them died. In this experiment also no significant decrease was observed either in haemoglobin or in the number of erythrocytes, but the animals lost weight heavily (Table 7).

red cells are restored to normal in about 12 days, and haemoglobin in less than 15 days (Table 2). The statistical examination of the results (Table 8) shows that differences between the groups of animals on the tryptophan-containing and tryptophan-deficient diet are highly significant. With tryptophan administration, the average increases in 12 days of red

Table 7. *Body weights and blood picture of rats fed a tryptophan-free diet for a prolonged period*(R.B.C. values are in millions/mm.³; Hb in g./100 ml. blood; body weight in g.)

Rat no.		Days									
		0	14	28	35	42	49	56	58	64	75
201	R.B.C.	6.99	6.80	6.94	6.88	6.98	6.91	6.81	—	—	—
	Hb	14.0	13.7	13.9	13.8	13.9	13.8	13.8	—	—	—
	Weight	84	70	64	58	50	42	39	39*	—	—
202	R.B.C.	7.20	7.10	6.99	7.02	6.99	6.98	6.99	—	6.91	—
	Hb	14.2	14.1	14.0	14.1	13.9	13.9	13.9	—	14.0	—
	Weight	80	70	61	60	56	52	48	44	43	40*
203	R.B.C.	7.02	7.01	6.98	6.98	6.85	6.86	6.75	—	—	—
	Hb	14.2	14.0	14.1	14.0	13.9	13.8	13.8	—	—	—
	Weight	90	78	70	60	54	51	48	44	40*	—
204	R.B.C.	6.75	6.65	6.80	6.75	6.71	6.68	6.65	—	6.68	—
	Hb	13.9	13.9	14.0	13.9	13.8	13.8	13.8	—	13.8	—
	Weight	102	87	82	70	66	62	58	54	52	50
205	R.B.C.	6.85	6.96	6.88	6.98	6.86	6.91	6.99	—	—	—
	Hb	14.2	14.2	14.0	14.1	13.9	13.9	14.0	—	—	—
	Weight	100	90	84	70	59	50	46	45	43*	—
206	R.B.C.	7.22	7.18	6.98	7.01	7.00	6.99	7.01	—	6.96	—
	Hb	14.3	14.2	14.2	14.2	14.0	13.9	14.0	—	14.0	—
	Weight	108	94	83	73	66	64	53	48	45	40

* Animal died.

Table 8. *Average increase in R.B.C. and haemoglobin of rats in first 12 days after phenylhydrazine—tryptophan by mouth. (Statistical analysis of data from Tables 1 and 2 and of those from other experiments)*

No. of animals ...	Hydrolyzed casein + tryptophan 8		Hydrolyzed casein 8		$\frac{x_1 - x_2}{\sqrt{(\epsilon_1^2 + \epsilon_2^2)}}$
	Mean increase x_1	Standard error of mean ϵ_1	Mean increase x_2	Standard error of mean ϵ_2	
R.B.C.	3.41	0.1525	1.72	0.1052	9.12
Hb	5.17	0.1608	2.96	0.1056	10.64

Table 9. *Average increase in R.B.C. and haemoglobin of rats in first 12 days after phenylhydrazine—tryptophan by injection. (Statistical analysis of data from Table 4 and of those from other experiments)*

No. of animals ...	Hydrolyzed casein + 20 mg. tryptophan 4		Hydrolyzed casein + 10 mg. tryptophan 4		Hydrolyzed casein 4		$\frac{x_1 - x_2}{\sqrt{(\epsilon_1^2 + \epsilon_2^2)}}$	$\frac{x_2 - x_3}{\sqrt{(\epsilon_2^2 + \epsilon_3^2)}}$
	Mean increase x_1	Standard error of mean ϵ_1	Mean increase x_2	Standard error of mean ϵ_2	Mean increase x_3	Standard error of mean ϵ_3		
R.B.C.	3.76	0.0586	3.68	0.2103	2.37	0.1841	7.24	4.17
Hb	6.23	0.0983	5.83	0.2201	4.43	0.1476	10.19	5.28

cells and haemoglobin exceed by 100 and 74% respectively the corresponding increases on a tryptophan-deficient diet.

Subcutaneous injection of the amino-acid is as effective as admixture with the diet for the purpose of blood regeneration, the R.B.C. and haemoglobin increasing at about the same rate in the two cases (Tables 4 and 9). In one important respect, however, subcutaneously injected tryptophan differs from oral tryptophan. With the former the animal loses as much weight as on a tryptophan-deficient diet. These findings agree generally with the observations of Hamada (1936). But while Hamada found that injected tryptophan in doses larger than about 75–100 mg./kg. body weight retarded red cell regeneration, no such harmful effect was noted in the present experiments in which tryptophan was injected at two levels of about 90 and 180 mg./kg. body weight.

While these experiments establish the beneficial effect of tryptophan in phenylhydrazine anaemia, its mode of action is not yet clear. The possibility that it neutralizes the toxic effects of phenylhydrazine need not be seriously considered as in these experiments tryptophan administration was started 3 days after injection of phenylhydrazine by which time the anaemia had already reached its peak. Further, in experiments on animals maintained on a diet containing whole casein the intensity of the anaemia was unaffected by the presence of tryptophan at the time when phenylhydrazine was injected (Yeshoda, 1942). It is therefore certain that tryptophan exerts its action not by detoxicating phenylhydrazine and preventing the destruction of erythrocytes but by accelerating the formation of new red cells. This it might achieve

either by supplying an essential structural unit for haemoglobin synthesis, or by stimulating the haemopoietic organs. The second explanation was favoured by Alcock (1933), who based this view on two findings: (1) that animals suffering from milk anaemia recover from the anaemia independently of the presence of tryptophan in their diet and (2) that animals on a tryptophan-deficient diet do not develop anaemia. The first observation, which is in contrast to our findings, is probably to be attributed to the unsuitability of the method used for inducing anaemia. In view of the demonstration by Robschey-Robbins and Whipple (1935) that on a favourable diet large reserve stores of haemoglobin-producing material can accumulate in the body, it is not surprising that Alcock's animals, fed on milk proteins and made anaemic by lack of iron and copper, should show no positive response to the amino-acid precursors required for haemoglobin synthesis.

Alcock's second observation that normal animals on a tryptophan-deficient diet do not develop a severe anaemia agrees with our own results. This cannot, however, be considered an argument against the view that it is an essential precursor of haemoglobin, as animals lost weight on the deficient diet and the tryptophan necessary for haemoglobin formation could have arisen from tissue degradation. It is to be remembered that in an animal which is losing weight there is bound to be a proportionate decrease in blood volume and hence of the haemoglobin content, although the relative composition of the blood elements may show no appreciable alteration. Thus in spite of a more or less constant red cell count and haemoglobin percentage there would be a progressive fall in the total haemoglobin

in the body with a consequent decrease in the demand for the structural elements required for haemoglobin regeneration.

Another fact quoted by Alcock in favour of his hypothesis, that the haemopoietic activity of tryptophan is due to its stimulating action on the blood-forming organs, was the observation made by Matsuoka & Nakao (1931) on rats and guinea-pigs suffering from phenylhydrazine anaemia and later confirmed by Chin Kyu-Sui (1938) on rabbits, that injected methyltryptophan accelerates R.B.C. and haemoglobin formation in the same manner as tryptophan. There is no reason, however, to consider that the presence of the 2-methyl group would prevent the utilization of the pyrrole nucleus in tryptophan for porphyrin synthesis. Either demethylation could take place giving rise to tryptophan or the methyl group might be utilized in the formation of the methine bridge present in porphyrins.

The difference in the effects produced by dietary and injected tryptophan still remains to be explained. It is probably to be attributed to possible differences in the metabolic fate of the amino-acid depending upon its mode of entry into the body. It is known that tryptophan metabolism in the animal follows more than one pathway, the amino-acid being converted into indolepyruvic acid, kynurenine acid and urochrome. It does not appear improbable that subcutaneously injected tryptophan undergoes only those transformations that lead to the formation of blood pigments and for this reason is not utilized for the maintenance of weight or growth. It has also to be pointed out that in the injection experiments that have been carried out with

tryptophan as well as with methyltryptophan the quantities used have been of the same order as that derived from the diet. It would be reasonable to assume that much smaller quantities of these substances would be sufficient if this action consisted of a stimulation of the haemopoietic apparatus. But no evidence has been produced to show that such small quantities are in fact effective. In any event Alcock's theory of haemoglobin formation without tryptophan which was later expanded to a theory of protein synthesis without amino-acids (Alcock, 1936) can no longer be taken seriously in the light of recent experiments using amino-acids marked with N^{15} (Schoenheimer, Ratner, Rittenberg & Heidelberger, 1942).

SUMMARY

1. It has been shown that in animals suffering from anaemia produced by phenylhydrazine, regeneration of haemoglobin and red cells is markedly accelerated by dietary tryptophan.

2. Tryptophan given by subcutaneous injection also exercises the same haemopoietic effect, but the amino-acid when administered in this manner is not utilized for maintenance of body weight. The results obtained are highly significant.

3. The mode of action of tryptophan and the contradictory findings of previous authors are discussed in the light of these observations. There is no justification for the view that the beneficial effect of tryptophan in blood regeneration does not depend upon its action as a precursor of structural material for haemoglobin building, but upon its stimulatory action on the haemopoietic organs.

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