Anaemia of Nutritional Origin in the Rat

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Many workers during the past ten years have reported the development in laboratory animals of anaemia resulting from deficiency in the diet of one or more components of the vitamin B complex. Rhoads & Miller (1933) reported an anaemia, macrocytic in type; in a small proportion of dogs maintained on a modified Goldberger diet, and suffering from recurrent attacks of black tongue. Cures were obtained following oral administration of meat, liver, or yeast. Wills & Stewart (1935) observed a macrocytic anaemia in monkeys fed on a diet low in its content of protein and the B vitamins. The anaemia responded to treatment with autolyzed yeast or a yeast filtrate fraction. Birch, Chick & Martin (1937) found that pigs fed on a modified Goldberger diet developed severe enteritis and a progressive anaemia. In later work Chick, Macrae, Martin & Martin (1938 *a, b*) found that the pellagra-like condition was rapidly cured by daily injections of nicotinic acid, though they did not state whether the blood picture responded to this treatment. More recently, Handler & Featherstone (1943) have reported that dogs developed a macrocytic anaemia with hyperplastic marrow about 60 days after remission of symptoms of black tongue following saline therapy. Following administration of nicotinic acid, these workers observed a progressive recovery from the anaemia, even though the food intake was restricted during the therapeutic period to a level similar to that of the period which preceded it. Apart from deficiency of nicotinic acid, many workers have provided evidence for the appearance of anaemias of different types and resulting from absence of other B vitamins in the diet. Chick *et al.* (1938 *c*) clearly differentiated the effects of deficiency in the pig of yeast eluate and yeast filtrate fractions. On a synthetic ration supplemented by aneurin, riboflavin, nicotinic acid, and yeast filtrate fraction, their animals developed epileptiform convulsions and a microcytic anaemia. When the eluate fraction replaced the yeast filtrate in the diet, the clinical picture was of a flaccid hind limb paralysis together with a moderate normocytic anaemia. Wintrobe, Samter & Lisco (1939), Wintrobe, Miller, Follis, Stein, Mushatt & Humphreys (1942) and Wintrobe, Follis, Miller, Stein, Alcayaga, Humphreys, Suksta & Cartwright (1943) extended these observations

on the pig. Using a synthetic ration in which brewer's yeast was subsequently replaced by supplements of aneurin, riboflavin, choline, nicotinic acid, and in some cases pantothenic acid, they reported the development of ataxia, sensory neurone degeneration, epileptiform fits and a microcytic anaemia. The last was characterized by polychromasia, hyperplasia of bone marrow, haemosiderosis in liver, spleen and marrow, and rise of serum iron level. Administration of pyridoxin was followed by reticulocytosis and blood regeneration. The existence of a microcytic hypochromic anaemia resulting from pyridoxin deficiency and cured by its administration has been similarly reported for the dog (Fouts, Helmer, Lepkovsky & Jukes, 1938; Fouts, Helmer & Lepkovsky, 1939, 1940; McKibbin, Madden, Black & Elvehjem, 1939-40; McKibbin, Scharfer, Frost & Elvehjem, 1942; Borson & Mettier, 1940). In the pyridoxin-deficient rat, Fouts & Lepkovsky (1942) observed a fall in haemoglobin less profound than in the dog. There have been suggestions that additional dietary factors may influence normal erythropoiesis. György, Goldblatt, Miller & Fulton (1937) claimed that a condition of panmyelophthisis with aplastic macrocytic anaemia developed in the rat after symptoms of acrodynia had been relieved by pyridoxin. György (1938) at first believed that inclusion of nicotinic acid in the diet prevented the onset of panmyelophthisis, but later (György & Eckhardt, 1940) this claim was withdrawn and was not confirmed by Chick, Macrae & Worden (1940). Borson & Mettier (1940) noted that after amelioration of microcytic anaemia in the dog with pyridoxin, there remained a residual mild normochromic anaemia which responded to large doses of an unknown factor present in a liver filtrate fraction.

A chick anti-anaemia factor, vitamin B₉, has been reported by Hogan & Parrott (1940), which has been identified with folic acid (O'Dell & Hogan, 1943). Campbell, Brown & Emmett (1944) confirmed this claim, and observed that presence of this factor in the diet gave protection, not only against fall of red cells, but also of thrombocytes and leucocytes. In the rat leucopenia and granulocytopenia induced by feeding sulphaguanidine and other sulphonamides are prevented by folic acid

concentrates (Nielsen & Elvehjem, 1942; Axelrod, Gross, Bosse & Swingle, 1943), and by crystalline folic acid (Daft & Sebrell, 1943). Langston, Darby, Shukers & Day (1940) have reported a nutritional anaemia and agranulocytosis which developed in rhesus monkeys fed on a modified Goldberger diet, and which failed to respond to nicotinic acid. The factor responsible for protection against this cytopenia in the monkey has been designated by these workers vitamin M. Wilson, Doan, Saslaw & Schwab (1942) found that intramuscular injections of a folic acid concentrate effected re-establishment of normal white cell equilibrium in the monkey. On the other hand, Totter, Shukers, Kolson, Mims & Day (1944) have failed to find any correlation between the folic acid content of the diet and its potency in protecting monkeys against nutritional cytopenia. They observed that xanthopterin, a fish anti-anaemic factor (Simmons & Norris, 1941), possessed vitamin M activity, although some of their animals appeared to show relapse during the course of treatment. The relationship between folic acid, xanthopterin and vitamin M remains uncertain. It is evident that many factors are involved in normal erythropoiesis.

The present work has for its object a further investigation of this problem in the rat. We have obtained evidence, which is presented in more detail below, that on diets which include an adequate intake of pyridoxin as well as of other factors needed by this species, a severe microcytic anaemia develops.

METHODS

Sixty-six rats of the Wistar strain, weighing 40–50 g., were placed on the experimental diet shortly after weaning. Two simplified diets, which will be referred to as diet R and diet S, have been employed. Diet R had the following composition: rice starch 70%, caseinogen 20%, salt mixture 5%,* cod-liver oil 4%, agar 1%. Diet S was similar in composition to diet R, except that rice starch was replaced by sucrose. In control diets T and U, 15% dried brewer's yeast (Distillers Co. Ltd.) replaced equivalent amounts of starch or sucrose respectively. The starch and casein used in these experiments were twice extracted with 50% ethanol and twice with 96% ethanol over a period of a week, with frequent stirring. These diets were fed *ad libitum*, and each rat received daily supplements† of aneurin 20 µg., riboflavin 40 µg., pyridoxin 40 µg., choline chloride 500 µg., and in certain cases calcium-*D*-pantothenate 240 µg. Blood samples were obtained by tail bleeding, and haemo-

globin estimations were performed by the Haldane method. Red and white cell counts were made at intervals during the course of the experiment.

RESULTS

Signs of nutritional deficiency

Figs. 1–4 illustrate the growth curves in a typical experiment, in which 15 rats, shortly after weaning, were distributed as follows: Group M1 (5 rats, nos. 71, 72, 78, 79, 85) received diet S, together with daily supplements of aneurin 20 µg., riboflavin 40 µg., pyridoxin 40 µg., choline chloride 500 µg., and calcium pantothenate 240 µg. Group M2 (5 rats, nos. 73, 74, 80, 81, 86) received diet S and similar supplements, except for the omission of pantothenate. Group M3 (5 rats, nos. 75, 76, 82, 83, 87) received diet U containing 15% yeast, together with the supplements as for group M1. There seems to be no marked difference in the growth response of groups M1 and M3, though the maximum plateau was in each case lower than that reached by similar rats (group M4, nos. 77, 84, 88) on a stock laboratory diet of commercial rat cake and milk. The growth response of the animals in group M2, deficient in pantothenic acid, was much inferior, and weight became stabilized at about 100 g. In other experiments a terminal fall of weight followed by death was frequently observed unless early treatment with pantothenic acid was begun. The most conspicuous external evidences of deficiency were those which have been attributed to lack of pantothenic acid. These features, which have also been noted by other workers (Unna & Sampson, 1940; Daft, Sebrell, Babcock & Jukes, 1940; Figge & Salomon, 1941–2; Chick *et al.* 1940) and which usually appeared in our rats within 6–8 weeks, included an incrustation of the whiskers and hair of the eyes, nose, and paws with a porphyrin which fluoresced a vivid red in ultra-violet light. The abdominal hair was matted and stained brown, possibly owing to an inability of the animals to clean themselves effectively. In some cases alopecia was a prominent feature. This may be related to absence of inositol in the diet (Woolley, 1941). The only source of fatty acids in the diet was cod-liver oil. Other fat was deliberately excluded in order to avoid possible introduction of traces of B vitamins, and this may have been responsible for the scalliness and patchy dermatitis of the tail which frequently occurred. No symptoms of motor ataxia were observed.

Anaemia

In the assessment of the incidence of anaemia in the rat, it is important to determine the changes in the blood picture which occur during the early period of growth of the animal. The normal blood picture has been established in a series of 12 male

* The salt mixture had the following composition: sodium chloride 4.69%, magnesium sulphate 7.22%, disodium hydrogen phosphate 9.21%, potassium dihydrogen phosphate 25.82%, calcium phosphate 14.62%, calcium lactate 35.22%, ferric ammonium citrate 3.15%, cupric sulphate 0.05%, potassium iodide 0.01%.

† Pyridoxin and riboflavin from Glaxo Laboratories Ltd., choline chloride from British Drug Houses Ltd., calcium-*D*-pantothenate from Roche Products Ltd.

Table 1. *Development of anaemia in rats on various diets*

(Data are given for one rat from each group.)

Group	No. rats in group	Rat-identifying numbers	Diet	Days on diet	Red blood cells (10 ⁶ /cu.mm.)	Haemoglobin index	Colour index	Reticulo-cytes (no./100 red blood cells)
M1	5	85	Diet S + all vitamin supplements*	0	5.05	80	0.78	19.2
				52	8.49	108	0.64	2.4
				101	7.98	98	0.61	1.4
				112	6.44	84	0.65	1.4
				167	4.21	60	0.62	6.8
				188	4.98	64	0.64	1.2
M2	5	74	Diet S + vitamin supplements except pantothenate*	0	5.56	84	0.75	11.2
				52	8.04	100	0.62	4.2
				101	6.60	78	0.60	1.6
				112	6.40	74	0.60	4.3
				146	2.51	15	0.30	64.0
M3	5	83	Diet U + all vitamin supplements*	0	5.96	82	0.68	9.4
				54	9.05	112	0.61	3.4
				102	8.54	104	0.61	2.2
				139	8.10	100	0.59	0
M4	3	77	Rat cake + milk	0	6.70	90	0.67	6.8
				54	8.51	108	0.63	1.8
				102	8.08	98	0.58	3.2
				139	8.10	96	0.60	0.8

* Vitamin supplements: aneurin 20 μ g., riboflavin 40 μ g., pyridoxin 40 μ g., choline chloride 0.5 mg., calcium pantothenate 240 μ g.

and 7 female animals placed after weaning on the control diets T and U, or the stock laboratory diet. Blood counts and haemoglobin estimations were performed at intervals over a period of 6–8 months. At 40–50 g. weight the red cell count and haemoglobin index average 5.5 million/cu.mm. and 80% respectively, and thereafter rise linearly to reach an adult mean of 8.60 ± 1.90 million/cu.mm. and 107 ± 17.5 . This mean is the same for males and females. It is reached at a weight range of 140–150 g. for males and 110–120 g. for females, at about 3 months of age. In the case of animals on a deficient diet the onset of anaemia is a late feature, and in all cases in which it was observed there was an initial rise both of red cell count and of haemoglobin index from the level characteristic of the young animal to the mean seen in the normal at 110–150 g. For our present purpose anaemia is defined as a fall in the erythrocyte count and haemoglobin index below the level of the mean minus 3σ observed in normal animals of the same age group.

Table 1 and Figs. 1–4 record the blood findings for each group in the typical experiment referred to above. None of the control animals in group M3 on a diet containing 15% yeast, together with all vitamin supplements, nor those in group M4 on a stock laboratory diet, developed anaemia during the period in which they were under observation.

Three animals in group M1 on diet S, together with vitamin supplements including pantothenic acid, developed a severe anaemia after 188 days. The remaining two animals of this group showed a

slight fall of haemoglobin index at the 146th and 181st day respectively, when they were sacrificed. The changes in the morphology of the blood cells were much less marked than those associated with pantothenic acid deficiency described below. In severe cases there was polychromasia, but little anisocytosis, and no evidence of granulopenia. In less severe cases the morphology seemed normal. The bone marrow was hyperplastic but orthoplastic, and showed no evidence of failure of maturation. The significance of this anaemia is discussed later.

Table 2. *Incidence of anaemia in rats receiving, and deficient in, pantothenate*

	No. of rats
Animals deficient in pantothenic acid:	
(a) Surviving less than 7 weeks	
Anaemic	0
Not anaemic	7
(b) Surviving longer than 7 weeks	
Anaemic	24
Not anaemic	11
Animals receiving pantothenic acid:	
(a) Surviving less than 7 weeks	0
(b) Surviving longer than 7 weeks	
Anaemic	5
Not anaemic	0
Controls on normal diets:	
Anaemic	0
Not-anaemic	19

Three rats in group M2, deficient in pantothenic acid, developed a severe anaemia in 58, 101 and 112 days respectively. The remaining two animals

Fig. 1. Changes in body weight and haemoglobin index on diet S together with all vitamin supplements. The lower curve gives the average body weight of the group. The number identifying each rat is given in brackets.

Fig. 2. Changes in body weight and haemoglobin index on diet S together with all vitamin supplements except pantothenic acid. The lower curve gives the average body weight of the group. The number identifying each rat is given in brackets. One rat of this group, no. 81, whose haemoglobin curve is not given, died and had a haemoglobin index of 82%.

Fig. 3. Changes in body weight and haemoglobin index on diet U together with all vitamin supplements. The lower curve gives the average body weight of the group. The number identifying each rat is given in brackets.

Fig. 4. Changes in body weight and haemoglobin index on diet of rat cake and milk. The lower curve gives the average body weight of the group. The number identifying each rat is given in brackets.

of this group died after 25 and 57 days respectively, without anaemia. In all we have had 42 rats on pantothenic acid-deficient diets R and S (Table 2). Of these animals, seven survived for less than 7 weeks, and none became anaemic. Thirty-five rats survived for a period longer than 7 weeks; of these 66% developed anaemia. The first change in the blood morphology associated with deficiency of pantothenic acid appears to be an increase of polychromasia as compared with control animals of the same age. This was followed by anisocytosis as the anaemia developed. The final stage was characterized by extreme anisocytosis, polychromasia and poikilocytosis, together with the presence of target cells, polychromatic or even basophilic normoblasts, and by polymorphonuclear leucopenia verging on agranulocytosis. The bone marrow, details of which are described in detail below, showed extreme hyperplasia and evidence of arrested maturation of both erythropoietic and leucopoietic cells (Pl. 4).

Curative tests with pantothenic acid. In the prophylactic experiment described above, the onset of anaemia appears to be delayed in those animals receiving pantothenic acid as compared with animals deficient in this factor. Thus the average time of onset of anaemia in animals of group M1 receiving daily supplements of pantothenic acid was 172 days, whereas the average time for pantothenic acid-deficient animals was 74 days. Too much emphasis must not be placed on this difference at present in view of the small number of animals investigated. Nevertheless it seemed of interest to study the curative effect of pantothenic acid in anaemic animals deficient in this factor. Twelve rats, which had developed anaemia of varying severity under conditions previously described, were given daily supplements of 240 μ g. calcium pantothenate for periods up to 80 days (Table 3). Certain other animals which had become severely anaemic and which had died within 3 days of the commencement of treatment are not included in this series. Three rats, nos. 6, 55 and 65, survived, and a normal blood picture was restored in two cases within 3 weeks, but in no. 6 only after some 11 weeks. No. 50 showed gradual but complete blood regeneration after 10 weeks but subsequently died, while no. 48 showed partial recovery but died before this was complete. Seven rats died without any evidence of recovery from anaemia, but in four of these death occurred within 8 days from the commencement of pantothenate administration. Our present experience is that it is necessary to begin treatment at an earlier stage in the development of anaemia if it is to be successful.

Morbid anatomy and histology

The morphology of the organs was studied in eighteen animals of the present series. Of this group

ten died or were killed at the end of the experiment as a consequence of an outbreak of salmonella infection in another group of rats which spread to our animals. No conclusion as to the significance of the morphological changes seen in the infected animals can be drawn, and they will not be considered further. The remaining eight rats included three animals (nos. 16, 36 and 45) which were normal controls, and one anaemic animal, no. 71, on the synthetic diet with pantothenic acid. Nos. 58, 73, 74 and 86 were on a pantothenic acid-deficient diet and were all anaemic.

A. Pantothenic acid-deficient animals

All were subnormal in weight and showed reddish brown staining of the hair of the abdominal wall and nares with scaliness of legs and tail. There was marked pediculosis. Marked absence of omental and depot fat.

Skin. Marked hyperkeratosis and moderate parakeratosis. No acanthosis, but proliferation of basal cells and irregularity of rete pegs. There were collections of neutrophils in the hyperkeratotic plaques, and areas of ulceration and small subepithelial abscesses.

Skeletal muscle. Degenerative changes with focal loss of striation and proliferation of sarcolemmal nuclei. These features were also seen in animals receiving pantothenic acid, and may be attributed to general undernutrition rather than to any specific deficiency.

Liver. No abnormality.

Spleen. Marked myeloid transformation, both leucopoietic and erythropoietic cells being present. Many of the myeloid and erythroid cells showed the failure of nuclear maturation seen in the marrow. Moderate siderosis. No fibrosis. The myeloid transformation was not observed in the control animals.

Bone marrow. In three animals (nos. 73, 74 and 86) there was extreme hyperplasia in the marrow of femur and ribs, with almost complete absence of fat spaces, and failure of maturation in both leuco- and erythropoietic cells. Polychromatic and orthochromatic erythroblasts with primitive leptochromatic nucleus, and many cells of the pro-erythroblast series were present. In rat 58, which was severely anaemic, there was marked hypoplasia of marrow, with fatty replacement. There were a few islands of basophil erythroblasts, isolated primitive mesenchymal cells, and very few granular cells, though mast cells were prominent. The vertebral marrow of the tail showed no hyperplasia in any of these animals (Pl. 4).

Gonads. Spermatogenesis absent in contrast to the controls and to those pantothenic acid-deficient animals which survived less than 7 weeks. The ovaries were hypoplastic, but there appeared to be normal ripening of the follicles.

(a)

(b)

- (a) Bone marrow of normal rat on diet of rat cake and milk. Section shows normal degree of cellularity in which fat spaces are present. A high proportion of the cells are mature in type—myelocytes, granulocytes, and orthochromatic normoblasts. Magnification, $\times 520$. Barrett's stain. (b) Bone marrow of anaemic rat on pantothenic acid-deficient diet. Section shows considerable hyperplasia with absence of fat spaces, and the cells show failure of maturation both of the erythroid and myeloid series. The increase in cell size and immature type of nucleus can be clearly seen. Magnification, $\times 520$. Barrett's stain.

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Table 3. *Effect of administration of pantothenic acid on anaemia*

Rat no.	Days on pantothenate-deficient diet	Days on pantothenate treatment	Red blood cells (10 ⁶ /cu.mm.)	Haemoglobin index	Colour index	Remarks
6	195	—	7.41	100	0.67	
	223	—	7.48	86	0.57	
	278	—	3.90	50	0.64	
	—	18	6.41	48	0.37	
	—	28	7.66	58	0.37	
	—	44	7.79	68	0.43	
	—	80	8.03	94	0.58	Cured. Survived
55	62	—	8.30	116	0.75	
	76	—	7.63	104	0.68	
	85	—	5.35	72	0.66	
	—	6	4.90	74	0.75	
	—	22	8.30	104	0.63	Cured. Survived
65	14	—	8.34	104	0.61	
	57	—	6.28	78	0.61	
	—	22	8.15	100	0.61	Cured. Survived
50	40	—	7.98	78	0.49	
	—	14	6.11	68	0.47	
	—	25	6.05	66	0.55	
	—	33	7.59	78	0.51	
	—	73	8.18	112	0.67	
	—	107	—	—	—	Cured. Subsequently died
48	57	—	8.23	102	0.65	
	68	—	7.14	86	0.60	
	76	—	6.07	69	0.57	
	—	38	7.38	72	0.49	
	—	48	—	—	—	Improved. Subsequently died
3	136	—	8.79	98	0.56	
	154	—	3.72	42	—	
4	—	8	—	—	—	No improvement. Died
	81	—	8.89	110	0.62	
	154	—	5.73	52	0.45	
49	—	7	—	—	—	No improvement. Died
	40	—	8.22	78	0.47	
	—	14	5.73	48	0.45	
51	—	21	—	—	—	No improvement. Died
	63	—	8.26	110	0.66	
	91	—	8.07	98	0.60	
	—	10	7.10	86	0.60	
	—	26	2.69	24	0.44	
54	—	29	—	—	—	No improvement. Died
	63	—	8.10	106	0.65	
	91	—	6.98	86	0.61	
	—	26	3.70	38	0.51	
67	—	29	—	—	—	No improvement. Died
	59	—	6.25	76	0.61	
	—	5	—	—	—	No improvement. Died
70	37	—	7.70	110	0.68	
	55	—	5.89	84	0.70	
	—	6	—	—	—	No improvement. Died

Adrenals. No evidence of necrosis or haemorrhage.

B. *Animals receiving pantothenic acid*

Well nourished, with ample depot fat. Coat in good condition. No splenomegaly, nor myeloid transformation. The marrow was hyperplastic and orthoplasmic, but did not show the marked failure of maturation seen in the pantothenic acid-deficient animals.

DISCUSSION

The evidence presented above indicates the development in the rat, under the conditions stated, of two types of nutritional anaemia differing in their blood morphology and in their biochemical origin. The first of these anaemias, about which we have at present most evidence, is associated with deficiency of pantothenic acid. It occurs, in conjunction with other manifestations of its deficiency, in about 60%

of cases. It develops within 8–16 weeks, and in a certain proportion of cases is cured by administration of pantothenic acid. It is characterized by a rapid fall of haemoglobin and of erythrocytes and polymorphonuclear leucocytes. In the final stage there is extreme polychromasia, anisocytosis and poikilocytosis. Post-mortem the most striking changes are splenomegaly with myeloid transformation, and, in three of the four cases examined, hyperplasia in the marrow of ribs and femur with failure of maturation in both erythropoietic and leucopoietic cells. Administration of pantothenic acid resulted in restoration of a normal blood picture in only 25% of the animals treated, but our present experience suggests that failure to improve the condition was, in some cases, due to the fact that treatment was begun too late. The morphology of the red cells seen in our animals deficient in pantothenic acid appears to be very similar to that described by Wintrobe *et al.* (1943) in pyridoxin-deficient pigs. On the other hand, the polymorphonuclear leucopenia, verging on agranulocytosis in the final stage, is a feature reminiscent of folic acid deficiency seen in rats treated with sulphonamides. Our animals received no preparation of folic acid, and the question of its lack as a contributory factor in the development of this anaemia is therefore raised. On the other hand, the curative effect of pantothenic acid in certain cases is definite. The extreme degree of reticulocytosis would, in human subjects, be suggestive of a haemolytic origin. However, these features are also seen in pyridoxin deficiency in the pig, in which Wintrobe appears to have excluded haemolysis. It may be noted that we have observed no evidence of bilirubinaemia. The significance of reticulocytosis in the rat is not yet clear, but there seems no doubt that pantothenic acid deficiency leads to a progressive interference with erythropoiesis and leucopoiesis.

In contradistinction to the picture described above is that of rats which received pantothenic acid throughout the experimental period and which also became anaemic. Changes in the morphology of the blood cells were confined to polychromasia

and there was little anisocytosis and no evidence of granulocytopenia. The marrow showed moderate hyperplasia, but no indication of maturation arrest. In view of the small number of animals receiving pantothenic acid throughout the experiment, and also of the fact that as the present work was nearing completion an outbreak of infection in a group of rats unconnected with our experiment spread to some of our animals and led to the temporary suspension of the investigation, it would be inexpedient to draw definite conclusions as to the significance of this anaemia. Further work which is confirmatory of these findings is still in progress.

SUMMARY

1. Rats suffering from deficiency of pantothenic acid develop a severe hypochromic anaemia in about 60% of cases.
2. The anaemia is characterized by fall of haemoglobin, erythrocytes and polymorphonuclear leucocytes. The erythrocytes show marked polychromasia, anisocytosis and poikilocytosis.
3. Post-mortem there is splenomegaly with myeloid transformation. The bone marrow usually shows hyperplasia, and evidence of failure in maturation of both erythropoietic and leucopoietic cells. No pathological changes in the adrenal cortex were observed.
4. Administration of pantothenic acid results in a restoration of a normal blood picture in a proportion of cases, but it is important that treatment should begin at an early stage in the development of anaemia if it is to be successful.
5. An anaemia, differing in morphology from that described above, develops at a later stage in animals receiving pantothenic acid throughout the experimental period. The significance of this anaemia is at present uncertain.

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REFERENCES

- Axelrod, A. E., Gross, P., Bosse, M. D. & Swingle, K. F. (1943). *J. biol. Chem.* **148**, 721.
- Birch, T. W., Chick, H. & Martin, C. J. (1937). *Biochem. J.* **31**, 2065.
- Borson, H. J. & Mettier, S. R. (1940). *Proc. Soc. exp. Biol., N.Y.*, **43**, 429.
- Campbell, C. J., Brown, R. A. & Emmett, A. D. (1944). *J. biol. Chem.* **152**, 483.
- Chick, H., Macrae, T. F., Martin, A. J. P. & Martin, C. J. (1938a). *Biochem. J.* **32**, 10.
- Chick, H., Macrae, T. F., Martin, A. J. P. & Martin, C. J. (1938b). *Biochem. J.* **32**, 844.
- Chick, H., Macrae, T. F., Martin, A. J. P. & Martin, C. J. (1938c). *Biochem. J.* **32**, 2207.
- Chick, H., Macrae, T. F. & Worden, A. N. (1940). *Biochem. J.* **34**, 580.
- Daft, F. S. & Sebrell, W. H. (1943). *Publ. Hlth Rep., Wash.*, **58**, 1542.
- Daft, F. S., Sebrell, W. H., Babcock, S. H. & Jukes, T. H. (1940). *Publ. Hlth Rep., Wash.*, **55**, 1333.

- Figge, F. H. J. & Salomon, K. (1941-2). *J. Lab. clin. Med.* **27**, 1495.
- Fouts, P. J., Helmer, O. M. & Lepkovsky, S. (1939). *Proc. Soc. exp. Biol., N.Y.*, **40**, 4.
- Fouts, P. J., Helmer, O. M. & Lepkovsky, S. (1940). *Amer. J. med. Sci.* **199**, 163.
- Fouts, P. J., Helmer, O. M., Lepkovsky, S. & Jukes, T. H. (1938). *J. Nutrit.* **16**, 197.
- Fouts, P. J. & Lepkovsky, S. (1942). *Proc. Soc. exp. Biol., N.Y.*, **50**, 221.
- György, P. (1938). *Proc. Soc. exp. Biol., N.Y.*, **37**, 732.
- György, P. & Eckhardt, R. E. (1940). *Biochem. J.* **34**, 1143.
- György, P., Goldblatt, H., Miller, F. R. & Fulton, R. P. (1937). *J. exp. Med.* **66**, 579.
- Handler, P. & Featherstone, W. P. (1943). *J. biol. Chem.* **151**, 395.
- Hogan, A. G. & Parrott, E. M. (1940). *J. biol. Chem.* **132**, 507.
- Langston, W. C., Darby, W. C., Shukers, C. F. & Day, P. L. (1940). *J. exp. Med.* **72**, 463.
- McKibbin, J. M., Madden, R. J., Black, S. & Elvehjem, C. A. (1939-40). *Amer. J. Physiol.* **128**, 102.
- McKibbin, J. M., Scharfer, A. E., Frost, D. V. & Elvehjem, C. A. (1942). *J. biol. Chem.* **142**, 77.
- Nielsen, E. & Elvehjem, C. A. (1942). *J. biol. Chem.* **145**, 713.
- O'Dell, B. L. & Hogan, A. G. (1943). *J. biol. Chem.* **149**, 323.
- Rhoads, C. P. & Miller, D. K. (1933). *J. exp. Med.* **58**, 585.
- Simmons, R. W. & Norris, E. R. (1941). *J. biol. Chem.* **140**, 679.
- Totter, J. R., Shukers, C. F., Kolson, J., Mims, V. & Day, P. L. (1944). *J. biol. Chem.* **152**, 147.
- Unna, K. & Sampson, W. L. (1940). *Proc. Soc. exp. Biol., N.Y.*, **45**, 309.
- Wills, L. & Stewart, A. (1935). *Brit. J. exp. Path.* **16**, 444.
- Wilson, H. E., Doan, C. A., Saslaw, S. & Schwab, J. L. (1942). *Proc. Soc. exp. Biol., N.Y.*, **50**, 341.
- Wintrobe, M. M., Follis, R. H., Miller, M. H., Stein, H. J., Alcayaga, R., Humphreys, S., Suksta, A. & Cartwright, G. E. (1943). *Johns Hopk. Hosp. Bull.* **72**, 1.
- Wintrobe, M. M., Miller, M. H., Follis, R. H., Stein, H. J., Mushatt, C. & Humphreys, S. (1942). *J. Nutrit.* **24**, 345.
- Wintrobe, M. M., Samter, M. & Lisco, H. (1939). *Johns Hopk. Hosp. Bull.* **64**, 399.
- Woolley, D. W. (1941). *J. biol. Chem.* **139**, 29.

The Inhibition of Amine Oxidase by Amidines

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The enzymic oxidation of diamines such as putrescine, cadaverine or histamine by extracts from pig's kidney was found to be somewhat inhibited by guanidine (Blaschko, 1938, 1939), and Zeller (1938) added the observation that some guanidine derivatives, including synthalin (decamethylene diguanidine), acted as more powerful inhibitors. The trypanocidal action of synthalin was the starting-point of Yorke's (1940) work on diamidines. The action of amidine derivatives on the amine oxidases was therefore more fully studied.

The present paper gives our results with amine oxidase only; the observations on diamine oxidase will be reported separately.

METHODS

Rabbit liver was used throughout. The tissue was ground with sand and its own weight of distilled water then added. After centrifuging, the supernatant fluid was dialyzed against distilled water overnight and one-tenth volume of 0.2M-sodium phosphate buffer pH 7.4 was added. The substrate used was tyramine. The rate of oxidation was followed manometrically. Open manometers and conical flasks fitted with a side-bulb and a centre tube containing 0.3 ml.

N-KOH were used: the gas phase was oxygen and the temperature 38°.

For each experiment four vessels (I-IV) were used, each containing 0.5 ml. extract + 1.1 ml. M/15 sodium phosphate pH 7.5, made up to a total of 2 ml. with water or addition. The additions were: nos. II and IV, 0.2 ml. 0.1M-tyramine hydrochloride; nos. III and IV, 0.2 ml. inhibitor. The inhibitor concentration varied according to the activity of the compound. Final concentrations above 10^{-3} M were not used. The experiments were carried on for 30 or 45 min. and readings were taken every 15 min. For the calculation of the percentage inhibition the O_2 uptake during either the first 15 or 30 min. was taken, whichever was nearer to 100 μ l. in vessel II. In the great majority of the experiments there was no significant difference in the percentage inhibition found at the end of the different periods.

The substances used in these experiments were kindly given to us by Dr A. J. Ewins, F.R.S., and Dr H. King, F.R.S., Boots Pure Drug Co. Ltd., provided those substances previously studied by Dr W. A. Broom (1936). The substances labelled V147, V187 and V335 and 'Marfanil' (p. 349) we obtained through the kindness of Dr J. Walker. Unless stated otherwise the substances were used as hydrochlorides (or dihydrochlorides). Tetramethylene diguanidine (arcsaine) sulphate was a commercial sample from Roche Products Ltd.; for the synthalin B (dodecamethylene diguanidine dihydrochloride) we are indebted to Dr E. M. Lourie.