REFERENCES

- Beadle, G. W. (1945). *Chem. Rev.* **37**, 15.
 Chauvin, R. (1939). *Bull. Histol. Tech. Micr.* **16**, 137.
 Chauvin, R. (1941). *Ann. Soc. ent. Fr.* **110**, 133.
 Daniel, R. J. (1949). (Personal communication.)
 Faure, J. C. (1932). *Bull. ent. Res.* **23**, 293.
 Goodwin, T. W. (1949). *Biochem. J.* **45**, 472.
 Goodwin, T. W. (1950). (Unpublished observations.)
 Goodwin, T. W. & Morton, R. A. (1946). *Biochem. J.* **40**, 628.
 Goodwin, T. W. & Srisukh, S. (1948). *Nature, Lond.*, **161**, 525.
 Goodwin, T. W. & Srisukh, S. (1949). *Biochem. J.* **45**, 263.
 Goodwin, T. W. & Srisukh, S. (1950*a*). *Biochem. J.* **46**, xvii.
 Goodwin, T. W. & Srisukh, S. (1950*b*). *Biochem. J.* **47**, 549.
 Goodwin, T. W. & Srisukh, S. (1950*c*). (Unpublished observations.)
 Hackman, R. H., Pryor, M. G. M. & Todd, A. R. (1948). *Biochem. J.* **43**, 474.
 Husain, M. A. & Mathur, C. B. (1936). *Ind. J. agric. Sci.* **6**, 591.
 Jones, P. H. (1950). (Unpublished observations.)
 Junge, H. (1941). *Hoppe-Seyl. Z.* **268**, 179.
 Pryor, M. G. M. (1940). *Proc. roy. Soc. B*, **128**, 378.
 Strel'nikov, I. D. (1936). *Trav. Inst. zool. Acad. Sci. U.R.S.S.* **2**, 673.
 Uvarov, B. P. (1947). *New Biology*, **3**, 9.
 Uvarov, B. P. (1948). *Trans. R. ent. Soc. Lond.* **99**, 1.
 Volkonsky, M. (1939). *Arch. Inst. Pasteur Algér.* **17**, 194.

Vitamin B₁-like Activity of 2-Amino-5-methylthiazole-4-carboxylic Acid

By G. DI MAGGIO

Pharmacological Institute, University of Catania

(Received 16 January 1950)

During the last few years investigations on the biological activity of vitamin B₁ have taken a new course, especially as a result of information obtained from studies on growth factors. Knight (1937*a, b*) demonstrated that it is not necessary to supply staphylococci with the entire molecule of aneurin, as they are able to satisfy their requirements when supplied with the pyrimidine and thiazole components. Many authors (see Lwoff, 1943) have established the same capability for other micro-organisms and also for higher organisms (Robbins, Bartley, Hogan & Richardson 1937). The last-mentioned workers demonstrated that a mixture of the pyrimidine and thiazole components could replace vitamin B₁ in the diet of polyneuritic pigeons. This has also been demonstrated by Abderhalden & Abderhalden (1938), who have found further (1939) that such synthesizing ability is also possessed, to a limited extent, by extracts from different organs of various animals.

It is further known that the thiazoles stimulate the growth of some organisms (flagellates, Phycomyces) in the same general way as the whole vitamin functions in vertebrates and metazoa in general. Thiazole derivatives have, however, been studied only slightly from the pharmacological standpoint (see Di Maggio, 1946). It seemed of interest therefore to inquire into the action on experimental beriberi of substances containing only the thiazole nucleus.

The present investigation concerns 2-amino-5-methylthiazole-4-carboxylic acid (I).

EXPERIMENTAL

This compound, as the Na salt, was dissolved so that 1 ml. of water contained 0.0166 mg. It was administered orally at a level of 0.0166 mg./100 g. body weight in single daily doses to adult pigeons fed only with rice (12.5 g. in the morning and 12.5 g. in the evening). The doses were given before the morning feed. The pigeons (twelve in number), weighing 450–500 g. and all of the same breed, were obtained from a private farm. Twelve other pigeons from the same farm received no thiazole treatment, but only the rice diet (12.5 g. in the morning and 12.5 g. in the evening) and were used as controls. Water was provided *ad lib.* for all birds.

The gastro-intestinal symptoms (vomiting, diarrhoea) and the reactions to exercise were followed daily. The rectal temperature was measured each day and the body weight every 3 days. Blood samples were taken every 8 days from a wing vein and sugar determined by Hagedorn and Jensen's method. In the terminal phase of the symptoms, daily blood-sugar estimations were carried out.

RESULTS

It was observed (Table 1) that the survival time of the thiazole-treated animals (42–44 days) was almost double that of the untreated controls (22–24 days).

Table 1. *Effect of 2-amino-5-methylthiazole-4-carboxylic acid on course of experimental beri-beri*

Duration of experiment (days)	Body weight (g.)		Body temperature (°)	
	Initial	At death	Initial	At death
Rice diet				
24	460	423	41.7	38.8
24	410	385	42.0	39.0
24	475	435	42.0	39.8
22	480	460	42.0	40.0
24	455	427	41.8	39.9
23	430	405	41.6	38.8
22	500	480	41.7	37.9
24	470	445	41.5	39.6
23	425	400	41.9	38.7
22	425	405	41.8	39.7
22	495	465	42.0	38.8
22	440	420	41.9	38.8
Thiazole treatment (0.0166 g./100 g.)				
42	475	300	41.6	39.5
42	500	330	41.9	38.0
43	495	305	41.7	39.9
42	430	250	42.0	40.0
42	500	325	42.0	38.8
42	480	300	41.6	39.7
41	490	325	41.9	38.8
42	445	285	41.5	38.7
44	470	305	41.7	39.9
42	490	320	42.0	38.8
42	425	260	41.7	38.0
42	485	315	42.0	39.8

Fig. 1. Effect of 2-amino-5-methylthiazole-4-carboxylic acid on beri-beri hyperglycaemia. Untreated pigeons (mean), —; thiazole-treated pigeons (mean), — — — o o o; death of pigeons, •. Ranges are shown by vertical lines, I.

The administration of the thiazole derivative did not noticeably influence the appearance or course of the gastro-intestinal symptoms or the body temperature. In fact vomiting occurred in both the treated and untreated animals after 7–8 days, and diarrhoea also appeared at the same time in both groups of birds. The body temperature at the end of experiment had fallen equally in all animals. The loss of body weight in the thiazole-treated animals at death was greater (about 36 %) than in the control group (about 6 %). However, the beri-beri hyperglycaemia was inhibited until the terminal phase of the avitaminosis (Fig. 1).

DISCUSSION

It is evident, according to these experiments, that 2-amino-5-methylthiazole-4-carboxylic acid exerts a vitamin B₁-like action on the course of experimental beri-beri, although the effect is only temporary.

The mode of action is not yet clear. The partially protective, vitamin-like action of this compound might be due either (1) to the utilization of the substance itself, or (2) to a delaying effect on vitamin B₁ elimination or (3) to a more general protection against draining of the reserves of this vitamin. Another possibility is that the thiazole may contribute towards aneurin synthesis by condensation with pyrimidine bases derived from protein metabolism to an extent limited both by the concentration of the reactants and the synthetic capacities of the organism. This synthetic ability, however, is not usually possessed by vertebrates, and is considered even more unlikely to take place in a deficient animal. Experiments are being continued in an attempt to explain the mechanism of action of these thiazole compounds.

SUMMARY

1. Pigeons fed only with a rice diet and treated daily with 2-amino-5-methylthiazole-4-carboxylic acid live almost double the time of untreated controls.
2. Beri-beri hyperglycaemia in the thiazole-treated animals was inhibited until the terminal phase of the avitaminosis.
3. The administration of the thiazole derivative did not noticeably influence the appearance or course of the gastro-intestinal symptoms or the body temperature.
4. 2-Amino-5-methylthiazole-4-carboxylic acid exerts, therefore, a protective, although temporary, action on the course of experimental beri-beri.

REFERENCES

- Abderhalden, E. & Abderhalden, R. (1938). *Pflüg. Arch. ges. Physiol.* **240**, 746.
 Abderhalden, E. & Abderhalden, R. (1939). *Pflüg. Arch. ges. Physiol.* **242**, 508.
 Di Maggio, G. (1946). *Boll. Soc. ital. Biol. sper.* **22**, 1081.
 Knight, B. C. J. G. (1937*a*). *Biochem. J.* **31**, 731.
 Knight, B. C. J. G. (1937*b*). *Biochem. J.* **31**, 966.
 Lwoff, A. (1943). *L'évolution physiologique*, p. 32. Paris: Hermann.
 Robbins, W. J., Bartley, M. A., Hogan, A. G. & Richardson, L. R. (1937). *Proc. nat. Acad. Sci., Wash.*, **23**, 388.

The Heterogeneity of the Glycerophosphate Fraction of Rat Liver

BY JUNE OLLEY AND M. BLEWETT

Medical Research Council, Human Nutrition Research Unit, M.R.C. Laboratories, Holly Hill, Hampstead, London, N.W. 3

(Received 28 December 1949)

Zilversmit, Entenman & Chaikoff (1948) found that in the liver of the dog, after injection of ^{32}P , a fraction rich in glycerophosphate had specific activity-time curves (S.A./ t) which indicated that glycerophosphate is a precursor of lecithin. Popják & Muir (1949) obtained similar evidence in support of this view from comparison of the S.A./ t curves of a 'glycerophosphate' fraction and those of total phospholipin in rat liver. These conclusions were based on the criteria proposed by Zilversmit, Entenman & Fishler (1943) for the relationships of the S.A./ t curves of a precursor of the ultimate compound.

The validity of these conclusions is dependent on the composition of the 'glycerophosphate' fraction. Several methods have been described for the extraction of 'glycerophosphate' from trichloroacetic acid (TCA) extracts of liver, some of which are outlined in Fig. 1.

Lepage (1944) fractionated TCA extracts of rat liver by the methods of Leva & Rapoport (1943) and Lepage & Umbreit (1943). He found that 58 % of the phosphoric esters present in the 'glycerophosphate' fraction and reacting with periodate could be accounted for as esters of known properties other than glycerophosphate, i.e. pentose-5-phosphate,

Fig. 1. Separation of 'glycerophosphate' by the methods of Zilversmit *et al.* (1948), Lepage (1944) and Kaplan & Greenberg (1944).

* S.A. = specific activity (% injected ^{32}P /mg. P).