diverse procedures as heat-treatment, treatment with inhibitors or activators and addition of exogenous catalase.

4. The enzyme activity of the leaves showed a significant diurnal variation, being minimum at 4 p.m. and maximum at 12 p.m. The total oxalate content also showed a distinct diurnal fluctuation, but in a direction opposite to that of the enzyme.

S.K.S. is thankful to the Council of Scientific and Industrial Research, New Delhi, for the award of a fellowship.

REFERENCES

Arnon, D. I. (1949). *Plant Physiol.* **24**, 1.
Arnon, D. I. & Whatley, F. R. (1954). *Physiol. Plant.* **7**, 602.
Baker, C. J. L. (1952). *Analyst*, **77**, 340.
Bunning, E. (1956). *Annu. Rev. Plant Physiol.* **7**, 71.
Datta, P. K. & Meeuse, B. J. D. (1955). *Biochim. biophys. Acta*, **17**, 602.
Ehrenberg, M. (1950). *Planta*, **38**, 244.
Ehrenberg, M. (1954). *Planta*, **43**, 528.
Feinstein, R. N. (1949). *J. biol. Chem.* **180**, 1197.
Finkle, J. B. & Arnon, D. I. (1954). *Physiol. Plant.* **7**, 614.
Franke, W. & Hasse, K. (1937). *Hoppe-Seyl. Z.* **249**, 231.
Franke, W., Schumann, F. & Bannerjee, B. (1943). *Hoppe-Seyl. Z.* **278**, 24.
Galston, A. W. & Dalberg, L. Y. (1954). *Amer. J. Bot.* **41**, 373.
Hastings, J. W. (1959). *Annu. Rev. Microbiol.* **13**, 297.
Hastings, J. W. & Sweeney, B. M. (1957). *J. cell. comp. Physiol.* **49**, 2009.
Houget, J., Mayer, A. & Plantefol, L. (1927). *C.R. Acad. Sci., Paris*, **185**, 304.
Houget, J., Mayer, A. & Plantefol, L. (1928). *Ann. Physiol. Physicochim. biol.* **4**, 123.
Jakoby, W. B., Ohmura, E. & Hayaishi, O. (1956). *J. biol. Chem.* **222**, 435.
Kimura, T., Katayama, H. & Sasakawa, T. (1956). *Symp. Enzyme Chem., Japan*, **11**, 119.
Laties, G. G. (1950). *Arch. Biochem. Biophys.* **27**, 404.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
Meeuse, B. J. D. & Campbell, J. M. (1959). *Plant Physiol.* **34**, 583.
Mukerji, S. K., Sanwal, G. G. & Krishnan, P. S. (1961). *Nature, Lond.*, **191**, 516.
Niekerk-Blom, C. J. (1946). *Proc. kon. ned. Akad. Wet.* **49**, 1096.
Quayle, J. R., Keech, D. B. & Taylor, G. A. (1961). *Biochem. J.* **78**, 225.
Robbie, W. A. (1948). *Meth. med. Res.* **1**, 307.
Sanwal, G. G. & Krishnan, P. S. (1960). *Nature, Lond.*, **188**, 664.
Shimazono, H. (1955). *J. Biochem., Tokyo*, **42**, 321.
Shimazono, H. & Hayaishi, O. (1957). *J. biol. Chem.* **227**, 151.
Srivastava, S. K. & Krishnan, P. S. (1959). *J. sci. industr. Res.* C, **18**, 146.
Tolbert, N. E. & Cohan, M. S. (1953). *J. biol. Chem.* **204**, 639.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, p. 28. Minneapolis: Burgess Publishing Co.
Venter, J. (1956). *Z. Bot.* **44**, 59.
Viswanathan, P. N. & Krishnan, P. S. (1962). *Nature, Lond.*, **193**, 166.
Viswanathan, P. N., Srivastava, L. M. & Krishnan, P. S. (1962). *Plant. Physiol.* **37**, 283.

Biochem. J. (1962) **85**, 38

Studies on Experimental Thiamine Deficiency

3. GLYOXYLIC ACID, CITRIC ACID AND TISSUE METABOLISM*

By CHI-CHIN LIANG
Department of Physiology, Hong Kong University

(*Received* 26 *January* 1962)

Glyoxylate is toxic to animals (Adler, 1893; Barnes & Lerner, 1943). Kleinzeller (1943) showed that inhibition of oxygen uptake of tissues by glyoxylate was related to a specific inhibition of decarboxylation of pyruvate. The degree of inhibition depended on the concentration of glyoxylate, as found also by Weinhouse & Friedmann (1951), Nakada & Weinhouse (1953), Weinhouse (1955) and D'Abramo, Romano & Ruffo (1957). D'Abramo, Romano & Ruffo (1958) studied the effect of glyoxylate on the oxidation of components of the tricarboxylic acid cycle, and Ruffo, Romano & Adinolfi (1959) suggested that glyoxylate condenses with a C_4 tricarboxylic acid-cycle intermediate to compete for aconitase. Their results showed both inhibition of oxidation and accumulation of citrate, which reached very high values only when oxaloacetate was the substrate. They suggested that both the depression of oxygen uptake and the formation of citrate are due to the two different ways in which oxaloacetate may react in the liver cells with acetyl-coenzyme A to form citrate

* Part 2: Liang (1962*b*).

and a small amount of glyoxylate to give rise to an inhibitor of citrate oxidation. As glyoxylate has been found in the blood of rats after about 3 weeks on a thiamine-deficient diet, and to be sustained throughout their later life on that deficient diet (Liang, 1960, 1962*a*), glyoxylate might well be one of the substances responsible for the biological lesions of thiamine deficiency. To test this possibility, the oxygen uptake and citrate content of tissues from normal and thiamine-deficient rats have been examined.

METHODS

Experimental animals. Albino rats were fed with thiamine-deficient diet (Liang, 1962*a*), and the daily food intake was recorded.

Estimation of citrate in the blood and urine. Blood and urine samples from normal rats and rats subjected to different periods of thiamine depletion were collected and measured according to Liang (1962*a*), and the concentration of citrate was estimated by the method of Stern (1957). Blood citrate was also determined 1–1·5 hr. after intraperitoneal injection of 10 mg. of glyoxylate (neutralized with NaOH)/100 g. body wt.

Estimation of the rate of oxygen uptake. Normal rats were starved for 48 hr. and thiamine-deficient rats for 24 hr. to deplete the liver glycogen, during which period they were fed with cellulose food to satisfy their hunger. The brain, kidney, liver, heart and diaphragm were removed immediately (within 30–90 sec.) after each animal had been killed, and placed in a beaker of ice-cooled Krebs–Ringer solution (Umbreit, Burris & Stauffer, 1957) previously saturated with oxygen. Brain brei and tissue slices were prepared, weighed and placed into the main chamber of the Warburg flask which contained 2 ml. of Krebs–Ringer phosphate solution, pH 7·6, containing 0·1% of glucose. For each tissue, 10 μmoles of cocarboxylase were added to half the flasks used, and graded concentrations of sodium glyoxylate, dissolved in 0·5 ml. of Krebs–Ringer phosphate solution containing 0·1% of glucose, were placed in the side arm. The incubation temperature was 37°, and the gas phase was oxygen. After temperature equilibration, readings were taken at 10 min. intervals for 1 hr. when the side arm contents were tipped and readings continued a further 2 hr. All estimations were in duplicate. The pH value was checked by indicator after the experiment. Other samples of the same tissue were weighed and dried at 100° to constant weight. From these dry weights, the Q_{O_2} values were obtained.

RESULTS

Daily food intake, urine volume and urinary excretion of citrate, based on 100 g. body wt., are plotted in Fig. 1. Rats reared on a thiamine-deficient diet can live for 47–54 days (Liang, 1962*a*). The volume of urine excreted remained constant at 7·5 ml./100 g. body wt./day during the first 3 weeks and began to drop thereafter to a low level of about 1·5 ml./100 g. body wt./day, i.e. one-fifth of the normal value. The urinary excretion of citrate increased slightly during the first 12 days and then decreased for the rest of the period. This change in the urinary excretion of citrate seemed in some way related to the change in the food intake.

The concentration of blood citrate remained constant at 1·6 mg./100 ml. during the first 12 days and increased during the rest of the period of thiamine depletion to a high level of 8·5 mg./100 ml. (Fig. 2). Injection of glyoxylate caused a

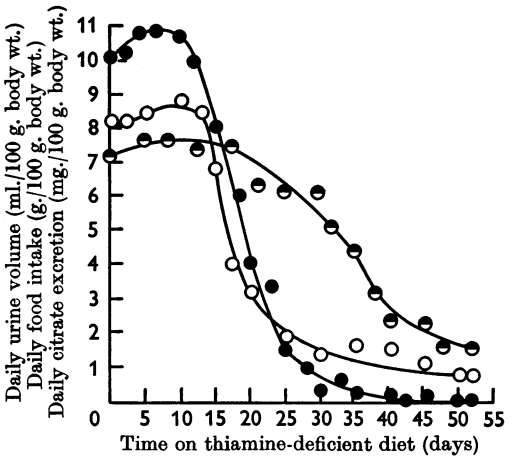

Fig. 1. Daily urinary volume (◓), urinary excretion of citrate (○), and food intake (●) of rats fed on a thiamine-deficient diet. Each reading is the mean value from 6 rats. The zero-time values show no difference from the control animals.

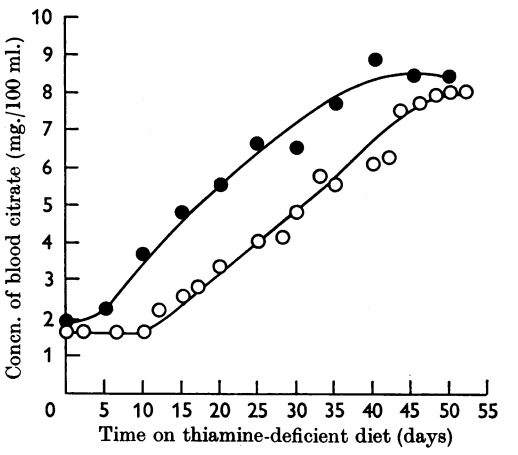

Fig. 2. Concentrations of blood citrate of rats fed on a thiamine-deficient diet (○) and 1–1·5 hr. after the injection of a single dose of 10 mg. of glyoxylate/100 g. body wt. (●). Each reading is the mean value from 6 rats taken at random from a large number of rats. (Some of the rats receiving glyoxylate died after injection of glyoxylate; therefore the last three readings were obtained from 4, 2 and 2 rats respectively.)

Table 1. *Effect of glyoxylate and cocarboxylase on Q_{O_2} of tissues from normal rats and thiamine-deficient rats*

The experimental procedure is described in the Methods section. Readings are expressed as means ±S.E. from 3 normal rats and 6 rats fed on a thiamine-deficient diet for 40 days. The percentages are expressed by reference to the mean value of the first hour obtained from the same sample.

	Final glyoxylate concn. (mM)	Brain					Kidney					Liver				
		1 hr.	2 hr.	%	3 hr.	%	1 hr.	2 hr.	%	3 hr.	%	1 hr.	2 hr.	%	3 hr.	%
Normal rats																
No addition of cocarboxylase	0	11·73 ±0·57	11·37 ±0·51	97·0	10·80 ±0·45	92·2	20·97 ±0·68	20·80 ±0·65	99·2	20·73 ±0·55	98·8	10·82 ±0·49	10·61 ±0·53	98·2	10·55 ±0·57	97·6
	0·5	11·24 ±0·54	10·70 ±0·51	95·2	9·97 ±0·41	88·6	21·07 ±0·70	20·70 ±0·67	98·6	20·31 ±0·58	96·3	9·98 ±0·47	9·53 ±0·47	95·5	9·67 ±0·49	96·8
	1·0	10·82 ±0·61	9·52 ±0·48	88·0	8·44 ±0·49	78·4	20·55 ±0·68	19·41 ±0·65	94·5	19·27 ±0·62	93·8	10·02 ±0·50	9·38 ±0·43	93·8	9·27 ±0·47	92·7
	2·0	11·52 ±0·51	8·99 ±0·45	78·0	7·87 ±0·41	68·3	21·75 ±0·75	19·50 ±0·69	89·6	18·62 ±0·55	85·5	10·17 ±0·53	9·33 ±0·41	91·8	9·20 ±0·43	90·5
Addition of cocarboxylase	0	12·02 ±0·67	11·72 ±0·71	97·5	11·31 ±0·52	94·2	21·22 ±0·78	21·43 ±0·71	101·2	22·20 ±0·76	104·6	10·92 ±0·51	10·94 ±0·56	101·2	11·01 ±0·61	100·8
	0·5	11·84 ±0·62	11·35 ±0·68	95·8	10·87 ±0·56	91·8	22·03 ±0·80	22·01 ±0·76	99·8	22·01 ±0·73	99·8	11·12 ±0·57	10·92 ±0·56	98·3	11·10 ±0·59	99·9
	1·0	11·96 ±0·59	10·65 ±0·73	89·2	10·54 ±0·48	88·2	23·20 ±0·82	22·76 ±0·75	98·2	22·61 ±0·70	97·5	11·08 ±0·54	10·79 ±0·51	97·4	10·73 ±0·57	96·8
	2·0	11·04 ±0·54	9·34 ±0·55	84·6	8·70 ±0·55	78·8	21·84 ±0·79	20·72 ±0·74	94·8	20·24 ±0·78	92·7	10·88 ±0·57	10·32 ±0·48	94·8	10·28 ±0·53	94·5
Thiamine-deficient rats																
No addition of cocarboxylase	0	8·47 ±0·14	8·00 ±0·10	94·5	7·69 ±0·11	90·8	17·22 ±0·31	16·91 ±0·38	98·2	16·77 ±0·30	97·4	8·66 ±0·13	8·45 ±0·16	97·6	8·45 ±0·11	95·4
	0·5	8·23 ±0·13	7·63 ±0·12	92·7	6·27 ±0·16	76·2	16·86 ±0·29	16·10 ±0·32	95·5	15·57 ±0·31	92·3	8·54 ±0·16	7·79 ±0·15	91·2	7·41 ±0·10	86·8
	1·0	8·70 ±0·17	6·97 ±0·10	80·1	5·61 ±0·12	64·5	17·08 ±0·34	14·74 ±0·30	86·3	14·10 ±0·29	82·6	8·30 ±0·18	7·00 ±0·12	84·3	6·83 ±0·11	82·3
	2·0	8·28 ±0·18	5·93 ±0·12	71·6	4·34 ±0·10	52·4	16·96 ±0·34	13·35 ±0·29	78·6	12·59 ±0·25	72·4	8·32 ±0·20	6·55 ±0·12	78·8	6·53 ±0·13	78·5
Addition of cocarboxylase	0	10·08 ±0·22	9·73 ±0·23	96·6	9·55 ±0·26	94·3	19·43 ±0·38	19·84 ±0·39	102·2	20·37 ±0·41	104·8	9·12 ±0·21	9·14 ±0·28	100·3	9·18 ±0·28	100·8
	0·5	9·82 ±0·20	9·07 ±0·20	92·4	8·57 ±0·21	87·3	18·66 ±0·36	18·42 ±0·37	98·8	19·05 ±0·39	102·2	9·34 ±0·17	9·30 ±0·27	100·5	9·35 ±0·23	100·1
	1·0	10·16 ±0·23	8·80 ±0·23	86·6	8·35 ±0·20	82·2	19·96 ±0·40	18·96 ±0·35	95·0	18·43 ±0·35	92·3	8·89 ±0·21	8·48 ±0·27	95·4	8·38 ±0·20	94·3
	2·0	10·34 ±0·25	8·20 ±0·21	79·4	7·47 ±0·23	72·3	19·12 ±0·41	16·06 ±0·38	84·0	15·08 ±0·36	78·0	9·08 ±0·23	7·63 ±0·22	84·0	7·63 ±0·21	84·0

Table 1 (*cont.*)

	Final glyoxylate concn. (mM)	Heart					Diaphragm				
		1 hr.	2 hr.	%	3 hr.	%	1 hr.	2 hr.	%	3 hr.	%
Normal rats											
No addition of cocarboxylase	0	7·68 ±0·37	7·59 ±0·36	98·8	7·42 ±0·30	96·7	5·69 ±0·28	5·44 ±0·20	95·6	5·30 ±0·22	93·2
	0·5	6·84 ±0·32	6·53 ±0·30	95·4	6·42 ±0·32	93·8	5·85 ±0·29	5·52 ±0·22	94·6	5·30 ±0·20	90·8
	1·0	6·96 ±0·38	6·40 ±0·32	91·7	6·10 ±0·30	88·6	6·02 ±0·27	5·53 ±0·26	92·0	5·36 ±0·27	89·2
	2·0	7·22 ±0·41	6·25 ±0·39	86·6	5·84 ±0·39	80·9	5·95 ±0·26	5·01 ±0·21	84·2	4·96 ±0·20	83·4
Addition of cocarboxylase	0	7·88 ±0·42	7·94 ±0·37	100·8	7·97 ±0·37	101·2	5·70 ±0·32	5·67 ±0·28	99·5	5·72 ±0·27	100·3
	0·5	7·68 ±0·45	7·53 ±0·30	98·0	7·50 ±0·40	97·6	5·96 ±0·32	5·86 ±0·26	98·4	5·83 ±0·26	97·8
	1·0	7·79 ±0·49	7·27 ±0·34	93·3	7·32 ±0·41	94·0	5·86 ±0·37	5·48 ±0·30	93·6	5·35 ±0·30	91·4
	2·0	8·01 ±0·49	7·09 ±0·39	88·6	7·00 ±0·37	87·4	6·12 ±0·29	5·41 ±0·32	88·5	5·40 ±0·29	88·3
Thiamine-deficient rats											
No addition of cocarboxylase	0	5·43 ±0·12	5·29 ±0·18	97·4	5·03 ±0·20	92·5	4·82 ±0·12	4·50 ±0·18	93·5	4·43 ±0·14	91·8
	0·5	5·32 ±0·12	5·44 ±0·13	93·5	5·29 ±0·17	90·8	4·66 ±0·12	4·13 ±0·14	88·6	4·01 ±0·10	86·0
	1·0	5·33 ±0·16	4·41 ±0·14	82·7	4·28 ±0·14	80·3	4·39 ±0·13	3·61 ±0·19	82·6	3·16 ±0·10	72·3
	2·0	5·96 ±0·15	4·49 ±0·18	75·4	4·10 ±0·10	68·8	5·02 ±0·18	3·95 ±0·20	78·8	2·74 ±0·09	62·4
Addition of cocarboxylase	0	5·88 ±0·16	5·91 ±0·19	100·5	5·96 ±0·18	101·5	5·07 ±0·14	5·06 ±0·24	99·8	5·10 ±0·12	100·5
	0·5	6·47 ±0·22	6·46 ±0·23	98·5	6·35 ±0·23	98·3	5·13 ±0·13	5·07 ±0·25	98·9	5·01 ±0·12	97·6
	1·0	6·33 ±0·24	5·77 ±0·22	93·4	5·90 ±0·26	91·2	4·99 ±0·12	4·67 ±0·28	93·7	4·55 ±0·10	91·2
	2·0	6·19 ±0·19	5·50 ±0·19	88·8	5·46 ±0·20	88·2	5·26 ±0·26	4·73 ±0·18	90·1	4·59 ±0·16	87·4

slight increase in the concentration of blood citrate of rats in the first few days of depletion and also in rats near the terminal stage of the thiamine depletion. Some rats receiving glyoxylate died within 0·5–1 hr. of injection, so that the records of the terminal stage are incomplete. However, the increase in the concentration of blood citrate was greatest after the initial few days of thiamine depletion.

Table 1 shows the effect of glyoxylate on the Q_{O_2} of different tissues obtained from normal and thiamine-deficient rats in the presence and absence of cocarboxylase. The results are expressed as a percentage of the control, to permit comparison with each other. The inhibitory effect of glyoxylate on tissue metabolism may be expressed in ways depending on (*a*) the extent of inhibition, (*b*) the period the inhibition was maintained, and (*c*) the comparative effect on various organs or tissues.

The extent of the inhibition depended on the concentration of the glyoxylate added to the medium. As a rule, the higher the concentration the greater the degree of inhibition, but the relationship was not a linear one. In low and high concentration, the brain tissue suffered more than other tissues; in the presence of 2·0 mM-glyoxylate, the Q_{O_2} in thiamine-deficient rats was reduced to almost half of that of the control after 2 hr. The effect of glyoxylate on oxygen metabolism of other tissues was less and decreased in the following order: diaphragm, kidney, heart and liver. In all cases the addition of cocarboxylase augmented the oxygen uptake in the tissues of normal and thiamine-deficient rats. The effect measured with the tissues of thiamine-deficient rats does not reach the level characteristic of normal rats.

DISCUSSION

There have been conflicting reports suggesting a connexion between thiamine and the rate of oxidation of pyruvate. Many of them tried to connect B_1-avitaminosis with excretion of citrate. Thus Krebs (1938), Krusius & Simola (1938) and Boothby & Adam (1934) all found increased excretion of citrate in thiamine-deficient rats, but other workers found a decreased urinary excretion of citrate (Lipton & Elvehjem, 1939; Barron & Lyman, 1940; Butler, 1948). Lwoff & Dusi (1937) observed that rats on a thiamine-deficient diet excreted less citrate as the deficiency became acute, but that, on administration of thiamine, the excretion of citrate increased to a maximum after 4–6 days. It was therefore suggested that cocarboxylase was an essential factor in the synthesis of endogenous citrate from the precursors (Sober, Lipton & Elvehjem, 1940). The observation has been in dispute for some time. However, Smith & Meyer (1941) claimed that the decreased excretion of citrate in thiamine deficiency was merely the result of low intake of food and not a direct result of the absence of thiamine. This claim has been partly substantiated by the experiments reported in this paper, as the initial slight increase and subsequent continual decrease in the excretion of citrate ran parallel with the quantity of food intake.

Although the concentration of blood citrate has been studied under many normal and abnormal conditions (Östberg, 1931; Thunberg, 1953), it has been little mentioned in connexion with the blood of thiamine-deficient animals. In the present study the concentration of blood citrate increased after 12 days on a thiamine-deficient diet, and also showed a further increase after administration of glyoxylate. This increased concentration of blood citrate showed that glyoxylate has some influence on the accumulation of citrate as studied by D'Abramo *et al.* (1958) with tissue homogenates. It might be inferred from the study by Liang (1962*b*) that glyoxylate, the concentration of which began to increase in the body of rats after some time on a thiamine-deficient diet, might cause an increase in the concentration of blood citrate on injection of exogenous glyoxylate. In the first few days of thiamine depletion, there might be some residual thiamine in the body so that the injected glyoxylate would be quickly converted into other substances and thus have little influence on the accumulation of citrate; in the terminal stage, the concentration of endogenous glyoxylate is already high, and the metabolic rate is low, so that the injected glyoxylate cannot greatly influence the synthesis of citrate.

Since Ruffo *et al.* (1959) have shown that from glyoxylate and a C_4 tricarboxylic acid-cycle intermediate a complex is formed which competes for aconitase, reversal of isocitratase (isocitrate-lyase) action (Olson, 1954; Smith & Gunsalus, 1955; Saz & Hillary, 1956; Campbell, Smith, & Eagles, 1953), yielding isocitrate and citrate from glyoxylate and succinate is unlikely. However, the aconitase-catalysed reaction is a well-established stage of the tricarboxylic acid cycle leading to citrate (Ochoa, Stern & Schneider, 1951). Whether or not the glyoxylate combines with acetyl-coenzyme A to form malate (Wong & Ajl, 1956; Kornberg & Krebs, 1957) in animal tissues remains to be seen.

It has been suggested that the increase of total non-protein nitrogen and urea in the blood in thiamine deficiency may be due to the anhydraemia produced in this avitaminosis (Sure & Ford, 1942) and in oliguria of infantile beri-beri (Hoobler, 1928). The decrease in the urinary excretion and the accumulation in the blood of glyoxylate and citrate may suggest some inhibiting effects directly on the kidney or indirectly through other organs in ways not yet known.

Injected citrate, if in excess, forms non-ionized complexes with calcium, and therefore produces the toxic effects of acute calcium deficiency (Gruber & Halbeisen, 1948), the symptoms of which comprise increased general activity of muscle and nerve and which may lead to convulsion and tetany. When citrate is given intravenously an overdosage will seriously depress the myocardium, especially in patients with liver disease (Bunker, Stetson, Coe, Grillo & Murphy, 1955; Yendt, 1957). Induced hypothermia probably increases the danger through its depression of all metabolic processes. In this study, the depressive effect of glyoxylate on the metabolism of tissues and the accumulation of citrate in the bodies of thiamine-deficient animals might suggest that citrate plays some part in the symptomatology of the heart and nervous systems in this condition. The possibility remains to be investigated.

Liang (1962*a*) found that the highest concentration of glyoxylate in blood is about 1·2 mg./100 ml. (0·16 mM) in thiamine-deficient rats, and reaches 2·5 mg./100 ml. (0·33 mM) in rats dying from the administration of large doses of glyoxylate (Liang, 1960). However, in the present experiments, the concentration of glyoxylate needed to affect the isolated tissue was rather high, i.e. above 1·0 mM. This discrepancy may be related either to the permeability of the tissue to glyoxylate, as in the intact animals it is well distributed by circulation of the blood, or to the differences in the sustained effect of glyoxylate within the body after its production as compared with its effect in this short-term experiment. Whatever the explanation, further study is still needed.

The recognition of the existence of an inhibitory effect of glyoxylate on the tricarboxylic acid cycle may draw greater attention, not only to the metabolism of carbohydrates, but also to the metabolism of protein, as the excessive breakdown of tissue protein may contribute to the formation of glyoxylate via glycine (Liang, 1960, 1962*a*, *b*). It is also recognized that small amounts of glyoxylate in the presence of oxaloacetate inhibit the oxidation of citrate (Ruffo *et al.* 1959), indicating that in the cell, under physiological conditions, glyoxylate, however formed, must be promptly removed to avoid inhibitory effects.

Thiamine in the form of cocarboxylase participates in several important reactions of carbohydrate metabolism, e.g. in glycolysis, the dismutation, decarboxylation and oxidative decarboxylation of pyruvate, the oxidative decarboxylation of α-oxoglutarate, and, in the pentose phosphate cycle, the transaldolation and transketolation between ketoses and aldoses (Horecker & Smyrniotis, 1953; Racker, Haba & Leder, 1953). Nakada & Sund (1958) found that the oxidative decarboxylation of glyoxylate requires the participation of cocarboxylase, which may explain the accumulation of this acid in the body of thiamine-deficient animals. The increase in oxygen uptake of isolated tissues effected by the addition of cocarboxylase may be due in part to this enzymic effect.

SUMMARY

1. During thiamine depletion of rats the daily urine excretion remained constant in the first 3 weeks and continued to drop to a low level near the terminal stage.

2. The urinary excretion of citrate showed a slight increase during the first 12 days and then decreased for the rest of the period. This is related to the food intake and the anhydraemia produced by thiamine deficiency.

3. The concentration of blood citrate remained at the normal level during the first 12 days and increased during the rest of the period of thiamine depletion. The increase in the concentration of blood citrate may be due to the accumulation of glyoxylate and the decrease in urinary excretion.

4. Oxygen uptake on glucose was reduced in the tissues from the thiamine-deficient rats as compared with that from the normal rats.

5. Glyoxylate showed a greater inhibition of aerobic metabolism of brain brei than on the aerobic metabolism of diaphragm, kidney, and liver slices.

6. Cocarboxylase augmented the oxygen uptake to a greater degree in tissues from thiamine-deficient rats and to a less degree in normal rats.

Part of the work was aided by a grant from the Research Grants Committee of the University of Hong Kong. The author also thanks the University of London for permission to publish the results from a Thesis presented for the degree of Ph.D.

REFERENCES

Adler, O. (1893). *Arch. exp. Path. Pharmak.* **37**, 413.
Barnes, R. H. & Lerner, A. (1943). *Proc. Soc. exp. Biol., N.Y.*, **52**, 216.
Barron, E. S. G. & Lyman, C. M. (1940). *Science*, **92**, 337.
Boothby, W. M. & Adam, M. (1934). *Amer. J. Physiol.* **107**, 47.
Bunker, J. P., Stetson, J. B., Coe, R. C., Grillo, H. C. & Murphy, A. J. (1955). *J. Amer. med. Ass.* **157**, 1361.
Butler, E. C. (1948). *J. Nutr.* **35**, 539.
Campbell, J. J. R., Smith, R. A. & Eagles, B. A. (1953). *Biochim. biophys. Acta*, **11**, 594.
D'Abramo, F., Romano, M. & Ruffo, A. (1957). *Biochim. biophys. Acta*, **24**, 437.
D'Abramo, F., Romano, M. & Ruffo, A. (1958). *Biochem. J.* **68**, 270.
Gruber, C. M., jun. & Halbeisen, W. A. (1948). *J. Pharmacol.* **94**, 65.
Hoobler, B. R. (1928). *J. Amer. med. Ass.* **91**, 307.

Horecker, B. L. & Smyrniotis, P. Z. (1953). *J. Amer. chem. Soc.* **75**, 1009.
Kleinzeller, A. (1943). *Biochem. J.* **37**, 674.
Kornberg, L. H. & Krebs, H. A. (1957). *Nature, Lond.*, **179**, 988.
Krebs, H. A. (1938). *Chem. & Ind.* **57**, 213.
Krusius, F. E. & Simola, P. E. (1938). *Acta chem. fenn.* **11**, 24. Cited in *Chem. Abstr.* **32**, 9195.
Liang, C.-C. (1960). *Nature, Lond.*, **188**, 660.
Liang, C.-C. (1962*a*). *Biochem. J.* **82**, 429.
Liang, C.-C. (1962*b*). *Biochem. J.* **83**, 101.
Lipton, M. A. & Elvehjem, C. A. (1939). *Cold Spr. Harb. Symp. quant. Biol.* **7**, 184.
Lwoff, A. & Dusi, H. (1937). *C.R. Soc. Biol., Paris*, **126**, 771.
Nakada, H. I. & Sund, L. F. (1958). *J. biol. Chem.* **233**, 8.
Nakada, H. I. & Weinhouse, S. (1953). *Arch. Biochem. Biophys.* **42**, 257.
Ochoa, S., Stern, J. R. & Schneider, M. C. (1951). *J. biol. Chem.* **193**, 691.
Olson, J. A. (1954). *Nature, Lond.*, **174**, 695.
Östberg, O. (1931). *Skand. Arch. Physiol.* **62**, 81.
Racker, E., Haba, D. L. & Leder, I. G. (1953). *J. Amer. chem. Soc.* **75**, 1010.
Ruffo, A., Romano, M. & Adinolfi, A. (1959). *Biochem. J.* **72**, 613.
Saz, H. J. & Hillary, E. P. (1956). *Biochem. J.* **62**, 563.
Smith, A. H. & Meyer, C. E. (1941). *J. biol. Chem.* **139**, 227.
Smith, R. A. & Gunsalus, I. C. (1955). *Nature, Lond.*, **175**, 774.
Sober, H. A., Lipton, M. A. & Elvehjem, C. A. (1940). *J. biol. Chem.* **134**, 605.
Stern, J. R. (1957). In *Methods in Enzymology*, vol. 3, p. 426. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Sure, B. & Ford, Z. W., jun. (1942). *J. Nutr.* **24**, 405.
Thunberg, T. (1953). *Physiol. Rev.* **31**, 1.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, p. 149. Minneapolis: Burgess Publishing Co.
Weinhouse, S. (1955). In *A Symposium on Amino Acid Metabolism*, p. 637. Ed. by McElroy, W. D. & Glass, B. Baltimore: The Johns Hopkins Press.
Weinhouse, S. & Friedmann, B. (1951). *J. biol. Chem.* **191**, 707.
Wong, D. T. O. & Ajl, S. J. (1956). *J. Amer. chem. Soc.* **78**, 3230.
Yendt, E. R. (1957). *Canad. med. Ass. J.* **76**, 141.

Biochem. J. (1962) **85**, 44

Sulphate Activation and its Control in *Escherichia coli* and *Bacillus subtilis*

By C. A. PASTERNAK
Department of Biochemistry, University of Oxford

(*Received* 15 *March* 1962)

The first stage in the assimilation of sulphate by micro-organisms is its activation by ATP to form adenosine 5′-sulphatophosphate (APS) (Gregory & Robbins, 1960):

$$\text{ATP} + \text{SO}_4^{2-} \rightleftharpoons \text{APS} + \text{pyrophosphate} \quad (1)$$

In micro-organisms utilizing sulphate as sulphur source only, and not dependent on it as a terminal electron acceptor (Ishimoto & Fujimoto, 1959; Peck, 1959), this is followed by further reaction with ATP to yield adenosine 3′-phosphate 5′-sulphatophosphate (PAPS) (Gregory & Robbins, 1960):

$$\text{APS} + \text{ATP} \rightarrow \text{PAPS} + \text{ADP} \quad (2)$$

These enzymes (adenosine triphosphate–sulphate adenylyltransferase, EC 2.7.7.4, and adenosine triphosphate–adenylylsulphate 3′-phosphotransferase, EC 2.7.1.25, respectively), which have previously been demonstrated in extracts of *Neurospora* (Hilz & Lipmann, 1955; Ragland, 1959), yeast (Bandurski, Wilson & Squires, 1956; Robbins & Lipmann, 1956) and other fungi (Kaji & McElroy, 1958; Spencer & Harada, 1960), have now been shown to be present in two bacterial species, *Escherichia coli* and *Bacillus subtilis*. The control of sulphate activation by cyst(e)ine, the end product of sulphate reduction, has been investigated. When *E. coli* or *B. subtilis* is grown on cyst(e)ine instead of sulphate, the ability of extracts to synthesize PAPS is repressed (Pasternak, 1961). This effect explains the observations of Roberts, Abelson, Cowie, Bolton & Britten (1955) that incorporation of [^{35}S]sulphate into the proteins of *E. coli* is abolished by the presence of cystine in the growth medium.

MATERIALS AND METHODS

Growth of organisms. Stock cultures of *Escherichia coli* A.T.C.C. 9723 and *Bacillus subtilis* N.C.T.C. 1379 were maintained on slopes of Oxoid nutrient agar CM4 at 4°. The medium for growth of *E. coli* was that described by Davis & Mingioli (1950) except that $MgSO_4,7H_2O$ was replaced by $MgCl_2,6H_2O$ (0·12 g./l.) and that $(NH_4)_2SO_4$ was replaced by NH_4Cl (2·5 g./l.). K_2SO_4 (British Drug Houses Ltd.) or