CLXXXVII. STUDIES ON THE METABOLISM OF PYRUVIC ACID IN NORMAL AND VITAMIN B_1-DEFICIENT STATES

IV. THE ACCUMULATION OF PYRUVIC ACID AND OTHER CARBONYL COMPOUNDS IN BERI-BERI AND THE EFFECT OF VITAMIN B_1

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RESULTS are reported in this paper on the amounts of bisulphite-binding substances (B.B.S.), particularly pyruvic acid, in blood, cerebro-spinal fluid, urine and milk obtained from adult subjects. Most of these subjects when investigated were in various stages of vitamin B_1 deficiency; for purposes of comparison, however, some observations have been made on apparently healthy persons. Comparative data have also been obtained by determining the pyruvate and B.B.S. levels after administration of vitamin B_1. Particularly striking results have followed the treatment of cases of fulminating beri-beri with pure vitamin B_1 (thiamin).

Until recently, little attention has been paid to the occurrence in body fluids of carbonyl compounds except the "ketone bodies"—acetone, acetoacetic acid and β-hydroxybutyric acid, which, when they accumulate in the blood, are considered to be responsible for the syndrome called "ketosis" or "ketonaemia". There is, however, a growing interest in such substances in relation to intermediate metabolism in tissues, especially evident in work on muscle, liver and brain, and attention is likely to turn more and more to the origin of metabolites found in unusual amounts in body fluids. Alterations in body fluids such as have been found in the body fluids in beri-beri ought always to be related to the metabolism of the cells of the body which are primarily affected by the vitamin deficiency. An important advance in our understanding of disease processes may be looked for when changes in body fluids are interpreted in terms of what may be called "the chemical pathology of the cell", in place of the conception of a "toxaemia" which is so readily and commonly invoked in medicine.

Observations on the occurrence of B.B.S. substances in body fluids include investigations on (*a*) pyruvic acid in normal and diabetic urine [Fricke, 1922; Pi-Suñer & Farran, 1936], in blood [Westerkamp, 1933] and mammalian sera [Mendel *et al.* 1931], in blood and urine after exercise [Johnson & Edwards, 1936]; (*b*) aldehydes in blood in disease [Stepp, 1920; Stepp & Lange, 1920; Stepp & Feulgen, 1921], including formaldehyde in blood [Stepp & Zumbusch, 1920], in urine [Stepp, 1922; Reisser, 1916], acetaldehyde in blood [Fabre, 1925; Gee & Chaikoff, 1926], in relation to avitaminoses [Kauffman-Cosla & Roche, 1927; Palladin & Utewski, 1928; Kauffman-Cosla *et al.* 1931; Handovsky, 1935], (no acetaldehyde could be detected in the urine of vitamin B_1-deficient rats [Simola, 1936]); (*c*) methylglyoxal in urine [Pi-Suñer & Farran, 1936] and

in relation to the toxic symptoms in vitamin B_1 deficiency [Vogt-Möller, 1931; Geiger & Rosenberg, 1933; Popoviciu & Munteanu, 1934] particularly in breast milk [Asakura, 1932; Takamatsu, 1934]; and (*d*) of α-ketoglutaric acid in blood and urine [Krebs, 1938] and in relation to vitamin B_1 deficiency [Simola, 1936; Krebs, 1937].

The preliminary observations of the occurrence in human subjects in acute beri-beri of an increase of 3–4 times the normal amount of B.B.S. in the body fluids and the isolation of the 2:4-dinitrophenylhydrazone of pyruvic acid from blood, urine and cerebro-spinal fluids have already been reported [Platt & Lu, 1935; 1936]. In a note [Johnson *et al.* 1935] on the level of carbonyl compounds in human blood, values were given showing that in a number of diseases (mainly mental and metabolic) there was no significant increase above the normal level (also determined) and it was considered that carbonyl compounds other than pyruvic acid, acetone and acetoacetic acid were likely to occur in normal blood. The isolation of pyruvic acid from the blood of B_1-deficient animals was reported later by Johnson [1936].

Platt & Lu [1935; 1936] found that in contrast with results from the blood of rats and pigeons, in man only a fraction of the increase of B.B.S. could be accounted for by the increase of pyruvic acid as measured by the hydrazone method. It was also appreciated that B.B.S. might accumulate in conditions other than beri-beri and, whilst normal values for these substances were found in a small group of patients with clinical conditions similar in some respects to beri-beri, it was stated, in view of the evidence of the peculiar relationship of pyruvic acid to metabolism in B_1-avitaminosis, that it might be necessary to search specially for pyruvic acid. Wilkins *et al.* [1937] and Taylor *et al.* [1937] have now found an elevation of B.B.S. in conditions associated with acidosis, ketosis, anoxaemia, "toxaemia" and uraemia as well as in vitamin B_1 deficiency. Wilson & Ghosh [1937] have published evidence of increased values in epidemic dropsy, but the contribution of pyruvic acid to these increases and the effect on them of vitamin B_1 has not yet been determined. Recently Wilkins *et al.* [1938] found values for pyruvic acid by the hydrazone method of only 0·46, 0·72, 0·73 mg./100 ml. in three cases of nutritional deficiency with B.B.S. values 9·6, 13·8 and 6·6 mg./100 ml. calculated as pyruvic acid. The results of Shindo [1937, 1] show increased B.B.S. in beri-beri and a fall in the amounts of these substances after giving vitamin B_1. He believed from the analysis of the hydrazone he isolated from blood that the substance accumulating was acetaldehyde; but later [Shindo, 1937, 2] he reported the isolation of pyruvic acid hydrazone.

Platt & Lu [1936] were able to relate the increase of B.B.S., including pyruvate, to vitamin B_1 by demonstrating that the levels of these substances dropped after the administration of concentrates of vitamin B_1. In July 1935, a "usually fatal" case (no. 2196) recovered after treatment with 750 I.U. of vitamin B_1 in the form of marmite and a fall was recorded of B.B.S., pyruvic acid and "methylglyoxal" in the blood and cerebro-spinal fluid as well as remission of some clinical signs of beri-beri. During the summer of 1936 and 1937, however, pure vitamin B_1 was obtained and its effects on some of the carbonyl compounds in body fluids are recorded in this paper. A number of clinical features of beri-beri have been linked up with biochemical disturbances. In a contribution to a recent discussion [Platt, 1938], it was briefly shown how variations in B.B.S. and pyruvic acid in the blood had led to the elucidation of some factors which appear to contribute to the development of beri-beri.

Experimental

All pyruvate estimations were carried out by the specific hydrazone method as previously described [Platt & Lu, 1936; Lu, 1939, 1]. The vitamin B_1 used for the injection was prepared as described in the following paper of this series.

The patients investigated all showed some evidences of deficiency of vitamin B_1 on clinical examination. For present purposes they have been divided into two groups: (1) a subacute form, and (2) an acute, fulminating form. The latter group comprises patients represented previously [Platt & Lu, 1936] as + + + + deficiency of vitamin B_1; no attempt is made to subdivide the remainder. The patients in the second group are best described as being the "usually fatal cases" before the introduction of potent preparations of vitamin B_1.

Results

Pyruvic acid and B.B.S. in blood and cerebro-spinal fluid

Measurements have been made of the amount of pyruvic acid and B.B.S. in the blood and cerebro-spinal fluid of "normal" subjects (Table I).

Table I. *Summary of "normal" values for B.B.S. and pyruvic acid in blood and cerebro-spinal fluid*

	No. and nature of subjects	Values
mg. pyruvic acid in 100 g. blood	60 resting and "cured" (2–3 tests on each)	All between 0·4 and 0·75
mg. B.B.S. (calc. as pyruvic acid) in 100 g. blood	23 "apparently healthy" Chinese males [Platt & Lu, 1936]	(2·22–4·8) Mean 3·27; standard deviation 0·70
mg. pyruvic acid in 100 g. C.S.F.	12 after adequate dose of vitamin B_1	3 <0·4 6 were 0·4–0·55 3 were 0·70–0·75
mg. B.B.S. (calc. as pyruvic acid) in 100 g. C.S.F.	12 after vitamin B_1	—

Table II. *Pyruvic acid and B.B.S. in blood and cerebro-spinal fluid in subacute and acute beri-beri*

	Subacute beri-beri					Acute beri-beri				
	No. of cases	Mean	Standard error of mean	Standard deviation	Range	No. of cases	Mean	Standard error of mean	Standard deviation	Range
mg. pyruvic acid per 100 g. blood	84	0·93	±0·043	0·40	0·17–1·93	38	2·72	±0·212	1·31	1·0 – 5·77
mg. B.B.S. (calc. as pyruvic acid per 100 g. blood)	65	4·5	±0·14	1·16	2·7 –7·3	36	10·1	±0·49	2·93	5·8 –16·3
mg. pyruvic acid acid per 100 g. C.S.F.	30	0·72	±0·038	0·21	0·41–1·32	29	1·69	±0·18	0·69	0·74– 3·28
mg. B.B.S. (calc. as pyruvic acid) per 100 g. C.S.F.	25	2·81	±0·15	0·73	1·8 –4·2	25	3·7	±0·38	1·92	2·6 – 9·2

A summary of the values for levels of pyruvate and B.B.S. is given in Table II.

It is evident from the results in Tables I and II that (1) the values for pyruvate and B.B.S. in blood and cerebro-spinal fluid in subacute cases are within normal limits or only slightly raised, (2) there are striking increases found in acute beri-

beri and (3) the accumulation is more marked in the blood than in the cerebro-spinal fluid.

The association of the various raised levels is clearer from grouped results; for the subacute cases, these are given in Table III. In 85 subacute cases B.B.S. in blood has also been determined in 68, and the cerebro-spinal fluid examined for pyruvic acid in 30, for B.B.S. in 25 cases.

Table III. *Grouped results for subacute cases*

Blood pyruvic acid level mg./100 g.	No. of cases	B.B.S. in blood, mg./100 g. (calc. as pyruvic acid) 2·5–	5–	7·5–	10–20	Pyruvic acid C.S.F. mg./100 g. 0·4–	0·6–	1·2	B.B.S. in C.S.F. (calc. as pyruvic acid) mg./100 g. 1·5–4·5
0·2–0·6	19	15	4	—	—	2	—	—	4
0·6–1·0	31	19	5	—	—	6	9	1	12
1·0–2·0	35	13	7	4	1	—	10	2	9
		47	16	4	1	8	19	3	25
Totals	85	68				30			25

On account of the need for beginning treatment it was not always possible to secure specimens from these patients under basal metabolic conditions. Values for blood pyruvic acid between 0·6 and 1·0 mg. per 100 g. cannot therefore strictly be regarded as being associated with vitamin B_1 deficiency. In most cases, however, at least half an hour's rest in bed was allowed and it has been found (as will be reported in the following paper in this series) that this period is sufficient for the restoration to normal of increased levels of blood pyruvic acid in non-deficient subjects. Furthermore, when treatment had been withheld, patients with blood pyruvic acid levels of about 1 mg. per 100 g. maintained this increase for many hours under resting conditions until vitamin B_1 was administered. There is little doubt that in the third group, having 1–2 mg. per 100 g. pyruvic acid in the blood, there is an increase which is related to vitamin B_1 deficiency. It is probable that some of the cases included in the 0·6–1·0 mg. per 100 g. group have also increased values resulting from their deficiency in vitamin B_1. In the group with the higher levels it is clear that there is an increase of B.B.S.; this increase is more than can be accounted for by the accumulation of pyruvic acid. In cases with raised levels for pyruvate in the blood there is, in the cerebro-spinal fluid, an increase of pyruvate which, however, is not increased to the same level as that in the blood. All the values for B.B.S. in the cerebro-spinal fluid are below 4·5 mg./100 g. and there are a number between 5·0 and 10 mg./100 g.

In 38 cases of acute beri-beri, values for blood B.B.S. and for B.B.S. and pyruvate in cerebro-spinal fluid have been determined in a number of instances in the same sample of fluid. These are grouped for different values of blood pyruvate in Table IVA and B.

40 % of these cases have values between 1 and 2 mg. per 100 g. pyruvic acid in the blood, the remainder are in groups ranging between 2 and 6 mg./100 g.

The numbers of cases in each group diminish as the value for the group increases. With increase in the level of the blood pyruvic acid, the B.B.S. in the blood and cerebro-spinal fluid and the pyruvic acid in the cerebro-spinal fluid tend to rise; the numbers of cases with these raised values also increase for the higher blood pyruvate groups.

There is a group with blood pyruvic acid values between 1 and 2 mg. per 100 g. in the series of both subacute and acute cases. It is clear from a comparison of the

Table IV A. *Acute beri-beri. Chemical and clinical data*

Record no.	B.B.S. mg./100 g.			Pyruvic acid, mg./100 g.			Methylglyoxal (qual. or mg./100 g.)			C.S.F. pressure mm. water	Blood pressure systolic/ diastolic mm. Hg.	Comments
	Blood	C.S.F.	Urine	Blood	C.S.F.	Urine	Blood	C.S.F.	Urine			
1574	15·3	7·7	—	—	—	—	—	—	—	300	80/50	3 days acute phase; died 15 hr. after admission
1904	16·1	6·71	—	—	—	—	—	—	—	290	58/30	2 days acute phase; died 1¾ hr. after admission
2081	10·9	3·86	—	—	—	—	—	—	—	—	70/40	—
2196	10·16	7·86	—	2·38	1·48	—	+ +	+ +	+ +	260	105/730	4 days acute phase; partly cured. See Fig. 1
2241	9·70	7·06	—	—	—	—	—	—	—	240	—	12 hr. acute phase; died
2472	12·3	10·1	—	6·15	4·2	—	—	—	—	Raised	—	Infantile beri-beri; died 2 hr. after admission
N.T.H. 1293	14·0	5·47	—	7·47	4·6	—	0·27	—	—	300	85/40	1 day acute phase; died 2 hr. after examination
2984	10·8	3·31	—	3·54	2·4		—	—	—	300	90/30	6 hr. acute phase; died 2 hr. after admission
3007	10·7	5·14	—	5·04	3·31	37·0	—	—	—	270	100/50	History not known; unusually marked improvement after venesection. Died 1½ hr.
3089	7·7	—	81·4	—	—	—	—	—	+	—	80/?	2 days acute phase; died 3 hr. after admission
3175	9·9	5·1	—	4·56	3·33	55·3	0	—	0·085	—	90/30	2–3 days acute phase; died 8½ hr. after admission. See Fig. 2
3485	11·18	8·2	21·6	1·29	1·92	—	+ +	—	0·74	—	—	A boy of 6 years (42 lb.) a complicated case; had intermittent pyrexia; evening rise to 103° F. See Fig. 3

Table IV B. *Grouped results for acute cases*

Blood pyruvic acid level mg./100 g.	No. of cases	B.B.S. in blood (calc. as pyruvic acid) mg./100 g. 5–	7–	10	15–20	Pyruvic acid in C.S.F. mg./100 g. 0·6	1	2–3	4	B.B.S. in C.S.F. (calc. as pyruvic acid) mg./100 g. 2·5	5	7·5	10
1–	15	5	5	4	—	3	8	1	—	8	3	—	—
2–	9	2	3	2	—	—	5	—	—	1	3	—	1
3–	7	—	1	4	—	—	3	3	—	2	2	—	2
4–	4	—	1	2	1	—	1	1	1	—	2	—	1
5–	3	—	3	—	—	—	—	2	—	—	1	—	—
	38	7	13	12	1	3	17	7	1	11	11	—	4
Total	38		33				28				26		

values recorded in Tables III and IV that there are increases of pyruvic acid and other carbonyl compounds in blood and cerebro-spinal fluid, more marked in the acute than in the subacute cases. Thus for example more than half of the values for B.B.S. in blood in the subacute series fall below 5·0 mg. per 100 g. while in the acute cases all values are above this level.

Pyruvic acid in urine in vitamin B_1 deficiency

The results of previous work on the occurrence of pyruvic acid in urine have yielded no conclusive results. Kendal & Friedmann [1930] found an "apparent" excess of pyruvic acid (4·5 and 3·9 mg./100 ml.) in morning urine and an apparent deficit in urines obtained after drinking large quantities of water. They were, however, unable to prove that these variations were really due to pyruvic acid. They state further that several workers, notably Fricke [1922], have stated that pyruvic acid may be found in urine. Reference to Fricke's original paper shows, however, that he failed to obtain any evidence of the presence of pyruvic acid in an attempt to isolate it as the phenylhydrazone. His method, moreover, was sensitive only to a concentration of 1 : 1000 and he pointed out that his inability to isolate the compound did not exclude its presence in urine.

Pyruvic acid was isolated as the 2:4-dinitrophenylhydrazone from the urine of patients with acute beri-beri [Platt & Lu, 1935; 1936]; values were obtained of 37·0 and 53·3 mg./l. For 25 apparently healthy Chinese males (college students) values for two samples of freshly passed early morning urines between 0·25 and 0·65 mg./100 ml. were obtained. Occasionally values of 0·7–1·25 mg./100 ml. were found; these on further investigation were attributable to athletic activities some hours before the test.

In a series of 7 healthy subjects on adequate intakes of vitamin B_1 observed over a period of 15–20 days, amounts of "pyruvic acid" by the hydrazone method ranging between 1·25 and 7·5 mg. per day were estimated. Amounts of 10–20 mg. "pyruvic acid" were found to be excreted by subjects in a state of frank vitamin B_1 deficiency.

An attempt was made to confirm the presence of pyruvic acid in urine from "normal" subjects by isolation of the hydrazone from a mixed batch of several litres of urine. A reddish brown preparation was obtained from which no single compound could be isolated. It is likely that there are substances in urine other than pyruvic acid which yield alkali-soluble 2:4-dinitrophenylhydrazones giving a colour like that of the pyruvic compound with alcoholic KOH, e.g. glucuronic acid [Case, 1932], acetoacetic acid [Clift & Cook, 1932], and α-ketoglutaric acid [Krebs, 1938]. Since the hydrazone method does not appear to be satisfactory

for estimating pyruvic acid in normal urine, detailed results obtained and further comment are omitted. The new micro-method recently described [Lu, 1939, 1] would appear to be better suited for further studies of this question.

Pyruvic acid in milk and the peroxidase reaction

It has been possible to obtain a few specimens of human milk for estimation of the pyruvic acid content. The results are given in Table V.

Table V. *Pyruvate in apparently healthy and vitamin B_1-deficient mothers' milk*

State of deficiency	No. of cases studied	mg./100 g. of milk	
		Range	Mean
Normal (Chinese)	8 (11 tests)	0·1 –0·24	0·16
Normal (British)	3	0·12–0·26	0·18
Subacute (Chinese)	5	0·34–0·53	0·41

Tested for peroxidase with the modified guaiac reagent of Arakawa [1930] evidence of peroxidase activity was found in three or four of the first group and in none of four of the second group. The addition of pyruvic acid in amounts required to raise the value to 5 mg./ml. failed to inhibit the reaction in normal milk. In view of the results reported in a series of papers by Japanese workers (some references are given above) an attempt was made to detect the presence of methylglyoxal in milk in which no peroxidase reaction was obtained. A blue colour was obtained with alcoholic KOH with the supposed bis-hydrazone, but it could not accurately be matched against a standard prepared from pure methylglyoxal.

The Arakawa reaction carried out with cow's milk (diluted 1 : 10) to give an intensity similar to that with human milk was inhibited by the addition of milk from a wet nurse with vitamin B_1 deficiency.

In a further series of tests on a deficient subject seven values for pyruvic acid gave a mean of 0·49 mg./100 ml. (0·38–0·66); following the administration of 2 mg. of synthetic vitamin B_1 the value fell to 0·27 mg./100 ml. (see Table VI).

Table VI

Day of observation	Blood	Milk
1	0·96	0·51
2	1·01	0·38
3	0·84	0·46
4	—	0·47
5	—	—
6	0·73	0·66
7	—	0·57
8	0·61	0·39
9	—	0·27

Note. 2 mg. vitamin B_1 given on the 7th day.

The effect of vitamin B_1 on pyruvate and B.B.S. *in the blood, urine and cerebro-spinal fluid in fulminating beri-beri*

In Table VII records of blood and cerebro-spinal fluid changes in four cases after treatment with vitamin B_1 are presented. These are chosen from a number of "usually fatal" cases which have been cured. It has been possible to make several estimations at intervals during recovery.

Table VII. *Pyruvic acid and* B.B.S. *in blood and cerebro-spinal fluid in fulminating beri-beri cases after treatment with vitamin* B_1

		Blood, mg./100 g.		C.S.F., mg./100 g.	
Case no.	Time	B.B.S.	Pyruvic acid	B.B.S.	Pyruvic acid
1398	2.20 a.m.	9·2	4·36	—	1·77
	10.00 a.m.	15·8	2·05	—	—
	4.30 p.m.	8·5	0·74	—	1·13
	5.30 a.m.	9·5	0·42	—	—
2353	9.00 p.m.	14·2	3·97	—	—
	11.40 p.m.	12·4	4·00	—	—
	6.25 a.m.	10·7	1·26	—	—
	3.00 p.m.	13·2	0·95	—	—
2707	12.00	12·2	3·05	8·1	2·34
	2.00 p.m.	11·0	1·84	—	—
	4.00 p.m.	9·8	1·47	—	—
	6.00 p.m.	9·6	1·02	—	—
	9.30 a.m.	8·31	0·84	—	—
2490	2.25 p.m.	11·2	4·76	6·5	2·52
	5.25 p.m.	13·0	5·04	—	—
	8.25 p.m.	7·7	1·94	—	—
	11.30 p.m.	6·3	1·10	—	—
	6.00 a.m.	5·3	0·89	—	—
	10.25 a.m.	5·0	0·47	—	—

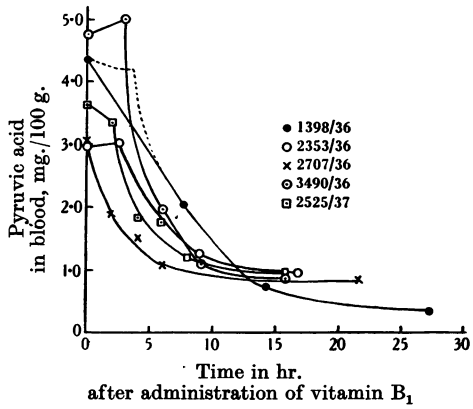

Fig. 1. Blood pyruvate at intervals after treatment with vitamin B_1 in five cases of acute beri-beri.

The results of the blood pyruvate in these cases and that of case no. 2525/37 are plotted in Fig. 1 to show the "lag period".

The initial values for pyruvic acid lie roughly between 3 and 5 mg./100 g. of blood and in all cases they are substantially reduced to 1–2 mg./100 g. 5 hr. after treatment with vitamin B_1. Amounts of vitamin B_1, ranging from 40 to 5 mg. have been given to the different cases. The subject receiving 40 mg. dose showed a slightly more rapid fall of blood pyruvate than in the other cases. In three of the five cases given 5–10 mg. of vitamin B_1 (see Fig. 1) there appears to be a lag period in the restoration of normal values; the data are insufficient in case 1398/36 to determine the presence of a lag but there is a comparatively slow recovery. It is worth mentioning, in regard to the relationship of basic disturbances in cells as against alteration of metabolite levels in body fluids,

that in both cases (1398/36 and 3490/36) in which the urine content of "pyruvic acid" was determined, the levels are less directly related to the improvement in conditions as seen clinically than are the changes in the blood.

From purely clinical considerations it would appear that, in the absence of factors which maintain an increased requirement for vitamin B_1 in the recovery period, 5 mg. are an adequate curative dose for these severe cases. This fact is of much economic importance in countries where beri-beri is endemic and supplies of the vitamin may be low; especially as it has been stated in recent publications (e.g. Sinclair [1939]) that from 20 to 50 mg. are requisite. In instances in which administration of the vitamin failed to bring about a cure there were complications due to concurrent zymotic disease or else death ensued a short time after the treatment had been begun. From the data given several hours would be expected to elapse before conditions approaching normal are established. There appears to be no important difference in the efficiency of the synthetic as compared with the natural crystalline product; a similar result has been obtained for animals by Eckler & Chen [1937]. No untoward effects are observed as a result of giving the vitamin by the intravenous rather than other recommended routes.

Discussion

Raised levels of pyruvate in body fluids

From the data presented above it is clear that increased amounts of pyruvate are found in all cases of fulminating and in a number of cases of subacute beri-beri. Alterations of pyruvate levels in body fluids are no doubt mainly, if not entirely, secondary to an altered chemical state of such tissues (for review of work on tissue changes see Peters & Sinclair [1933]) which require vitamin B_1 for the complete and normal metabolism of pyruvic acid. It will be necessary to investigate the changes in the affected tissues by a direct method before a satisfactory correlation is possible. De Jong [1936] was unable to establish a direct relationship between the clinical changes in vitamin B_1 deficiency conditions and B.B.S. values in the blood for the reason, no doubt, that the blood changes are only a reflexion of pathological disturbances in the cells primarily affected.

Pyruvate in the body does not appear to be toxic in the amounts in which it accumulates in beri-beri [see Lu, 1939, 2]. Thus there is no record of ill-effects in man when levels were raised by intravenous injection or oral administration of Na pyruvate [Wilkins *et al.* 1938; 1939]. Flock *et al.* [1938] report recovery of dogs when their blood levels increased to as much as 70 mg./100 ml. (except when a deleterious alkalosis supervened). Recovery takes place after exercise (see the following paper of this series) even when blood levels similar to those which have been found in acute beri-beri are reached. On the other hand Kermack *et al.* [1927] found a toxic effect of Na pyruvate on injection in rabbits; this, however, may be related to the presence of some toxic substance which is known [Lipschitz *et al.* 1938] to develop in concentrated pyruvic acid solutions after standing.

The possibility must be recognized that pyruvate accumulation is specific for vitamin B_1 deficiency, only in so far as, in vitamin B_1 deficiency, intermediate carbohydrate metabolism is deranged. Also, the interpretation of blood levels may be complicated because of an increase of the metabolites accumulated arising as a result of impaired kidney function, which is known to manifest itself in acute beri-beri as an increase of non-protein nitrogen levels [Platt & Lu, 1936] and possibly as a phosphate retention. Further complications

arise in consequence of the disturbance of metabolism associated with heart failure which contributes to the beri-beri syndrome. The occurrence of creatinuria [Kindler, 1936] in heart failure is of interest in this connexion for it was on account of an observation that increased amounts of creatine were to be found in the urine from patients with beri-beri that attention was first directed to this disease and to an investigation of changes then supposed to be disturbances of metabolism in muscle due to vitamin B_1 deficiency.

Increases of other B.B.S.

Increases of B.B.S. have been observed to be greater than can be accounted for by the increase of pyruvic acid. Furthermore, it is often found that pyruvic acid may be restored to normal levels in the blood while at the same time there is only a slight reduction in the amount of B.B.S. The first question that arises is as to the nature of these substances. From the available evidence acetaldehyde, methylglyoxal and α-ketoglutaric acid have to be included in this group; two other substances which may be mentioned as being of interest in future investigation are glyceraldehyde and dihydroxyacetone. There are at least three possibilities as to their origin. They may be products of deranged metabolism due solely to insufficient amounts of vitamin B_1; they may be derived from pyruvic acid or, thirdly, they may be the products of some associated deficiency or other metabolic error.

In pure vitamin B_1 deficiency produced experimentally in animals it is significant that almost all of the increase of B.B.S. is accounted for by accumulation of pyruvic acid [Thompson & Johnson, 1935]. It appears then, that unless intermediate metabolism in rats and pigeons has important differences from that in man in this respect, the accumulation of B.B.S. other than pyruvic acid is not a direct result of vitamin B_1 deficiency.

The second possibility, that B.B.S. may be formed secondarily from pyruvic acid, has been investigated from time to time [Embden & Oppenheimer, 1912; Annau, 1934; Krebs & Johnson, 1937] and it is known that pyruvic acid can be converted into ketones. The results of Lu (unpublished) on rat and rabbit blood and the results of Wilkins *et al.* [1938] using human blood showed that pyruvate added to blood before or after it was withdrawn from the body was converted into other ketones which in turn are more slowly metabolized. Some years ago Stepp & Behrens [1923] suggested that pyruvic acid was converted by a carboxylase in the blood into acetaldehyde. Later, Simola [1932] claimed to have shown that there was a considerable reduction in the amount of cocarboxylase in the liver and brain of rats after being 30 days on the vitamin B_1-deficient diet. While these results have not been fully confirmed [Lipschitz *et al.* 1938], other measurements of the amounts of cocarboxylase in the tissues of normal and B_1-avitaminous animals have been reported [Ochoa & Peters, 1938] in which diminished amounts of cocarboxylase were found in vitamin B_1 deficiency. Considerable interest in cocarboxylase has developed since Lohmann & Schuster [1937] showed that vitamin B_1 pyrophosphoric ester is the cocarboxylase of alcoholic fermentation by yeast, pyruvic acid being converted into acetaldehyde. In view of these observations and of the occasional reports that acetaldehyde occurs in body fluids it appears that the problem is worth reinvestigation. Lipschitz *et al.* [1938] state that it is not impossible that acetaldehyde is still an intermediate in the dismutation of pyruvic acid in which acetic acid is formed. It should be noted that Briggs [1926] was unable to obtain evidence of conversion into acetaldehyde of pyruvate injected into normal animals; this, however, cannot

be accepted as evidence against the possibility of such a change in vitamin B_1 deficiency.

The third group of possible explanations involves a consideration of the association of other metabolic and nutritional defects. The fasting state is one which needs to be considered. Lipschitz *et al.* have discussed this in relation to delayed removal of pyruvate and have drawn attention to the work of Weil-Malherbe [1937] in support of their contention that other intermediates in carbohydrate metabolism appear to be required to assist in the removal of pyruvate. Considerable increases in B.B.S. in the blood are reported by Wilkins *et al.* [1938] in subjects with a "nutritional deficiency" but who show no increase of blood pyruvate. From reports of the type of subject with which they are working it is likely that in addition to being alcoholics their patients had diets with a higher fat/carbohydrate ratio than ours. It is conceivable that in these circumstances there might be a relative increase of B.B.S. to pyruvic acid in the blood.

An associated insufficiency of glutathione may, according to the work of Gaddie & Stewart [1935], account for the appearance of methylglyoxal which may be one of the substances contributing to the total B.B.S.

Simola [1936] suggested "that at least two components of the B group play an important part in the normal keto-acid metabolism". He found that the excretion of α-ketoglutaric acid in the urine occurred when experimental animals were kept on a diet deficient in all but vitamin B_1 of the B complex. This observation is of particular interest in relation to the observation that vitamin deficiency states as they are seen in China are almost always multiple, especially when their manifestations are of a minor type.

Methylglyoxal needs further consideration. Meyerhof [1934] considers the occurrence of this substance in muscle metabolism to be an artefact, the substance being produced by decomposition (not involving an enzymic mechanism) from triosephosphate. If this interpretation is applicable to our findings then interest is shifted to a study of the accumulation of the precursor of methylglyoxal for, as has been shown, the reaction for this substance is obtained in our beri-beri cases and is no longer detected after treatment with adequate amounts of vitamin B_1. It is unlikely in this instance that the explanation put forward by Müller [1933] that the "methylglyoxal" reaction is due to a trace of glucosone is adequate. An attempt to determine the occurrence of a phosphorylated precursor of methylglyoxal in the blood was complicated by the fact that the involvement of the kidney in beri-beri appeared to have led to a retention of phosphates. There is evidence that methylglyoxal, if present in increased amounts, may have toxic effects [Sjollema & Seakle, 1926; Fischler, 1927; Kermack *et al.* 1927; Takamatsu & Sato, 1934].

The behaviour of "blood sugar", lactic acid and other related substances is of importance but is not discussed further here as the scope of this study has been limited to a consideration of bisulphite-binding carbonyl compounds (glucose has some capacity for binding bisulphite [Clift & Cook, 1932] but only under conditions different from those in the method of estimation employed).

The effects of administration of vitamin B_1

The above results show a marked effect of vitamin B_1 on pyruvate levels in body fluids; there is a reduction of the levels in blood and cerebro-spinal fluids and increased urinary output of pyruvate some time after injection. There is a lag in the fall of the pyruvate after amounts of 5–10 mg. of vitamin B_1. This lag may not accurately represent the changes actually occurring in the affected

tissues, which must be examined directly before the nature of the lag can be determined. The B.B.S. are not so readily restored to normal levels after vitamin B_1 as pyruvate; further investigation is required before this can be explained. As regards the lag just referred to, it is probable that vitamin B_1 has to undergo certain chemical changes (e.g. phosphorylation or combination with a protein) before it can exert its activity. Banga *et al.* [1939] have indeed shown, contrary to what was formerly thought, that the "catatorulin effect" of cocarboxylase is the same as that of the free vitamin.

Summary

1. The results of a comparative statistical study of the B.B.S. and pyruvate in the blood, milk and urine, of normal and vitamin B_1-deficient human beings, are given. The pyruvate level in normal human blood is of the order of 0·5 mg./100 g.

2. Maintained raised levels of blood pyruvate seem to be quite specifically associated with vitamin B_1 deficiency.

3. In the rapidly developing fulminatory types of beri-beri the initial level of blood pyruvate is a definite indication of the degree of the deficiency.

4. Several hours are required for the removal of accumulated pyruvate after administration of vitamin B_1.

5. About 5 mg. of the pure crystalline vitamin are needed in the acute cases to restore normal conditions with respect to pyruvic acid.

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