XCVIII. STUDIES ON THE METABOLISM OF PYRUVIC ACID IN NORMAL AND VITAMIN B₁- DEFICIENT STATES

II. BLOOD PYRUVATE LEVELS IN THE RAT, PIGEON, RABBIT AND MAN

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II. BLOOD PYRUVATE LEVELS IN THE RAT, PIGEON, RABBIT AND MAN

THE physiology and biochemistry of vitamin B₁ have been recently reviewed [Peters, 1938; Cowgill, 1938]. While the role of vitamin B₁ in the mammalian organism remains undefined there is now much evidence in support of the view that the primary biochemical lesion in B₁-deficiency is the inability of the cell to metabolize pyruvic acid [Peters, 1936].

The accumulation of pyruvic acid in the blood in vitamin B₁-deficiency was first shown by Thompson & Johnson [1935] in England, using rats and pigeons, and by Platt & Lu [1935] in China on human beri-beri cases. The above-mentioned authors were able to relate the increase of bisulphite-binding substances (B.B.S.), expressed as pyruvic acid, to B₁-deficiency, by demonstrating that the raised level returns to normal after administration of vitamin B₁. Pyruvic acid was isolated from the blood, cerebrospinal fluid and urine of fulminating beri-beri cases [Platt & Lu, 1935] and from the blood of avitaminous pigeons [Johnson, 1936]. Later, in a more detailed paper, Platt & Lu [1936, 1] showed that contrary to the results obtained in avitaminous animals where the large increase of B.B.S. was probably due entirely to pyruvic acid, only a fraction could be accounted for as pyruvic acid in human cases when the latter is estimated by the hydrazone method [Peters & Thompson, 1934].

In view of the evidence of the peculiar relationship of pyruvic acid to vitamin B₁, Platt & Lu [1936, 1] suggested that further investigations, especially of pyruvic acid itself, were needed before raised B.B.S. values could be reasonably attributed to lack of vitamin B₁. The later investigations of Platt & Lu [1936, 2] on the accumulation of pyruvic acid and the effect of vitamin B₁ on the pyruvic acid and carbonyl compounds in beri-beri blood clearly showed that the initial resting level of blood pyruvic acid gave an indication of the degree of deficiency. Furthermore the B.B.S. value did not so readily return to its normal level after intravenous B₁-injection as compared with pyruvate. In this connexion, it may be mentioned that de Jong [1936] found that the disappearance of symptoms

after the administration of vitamin B₁ to his pigeons sometimes preceded the lowering of bisulphite-binding capacity.

Since then Wilson & Ghosh [1937] have published evidences of increased blood B.B.S. in cases of epidemic dropsy, but the contribution of pyruvic acid to this increase is as yet unknown. Wilkins *et al.* [1937], who found an elevation of B.B.S. in conditions associated with ketosis, anoxaemia and uraemia, as well as B₁-deficiency, also concluded that the estimation of blood B.B.S. is not specific for B₁-deficiency.

Owing to the complicated procedure involved in the quantitative estimation of pyruvic acid by the Neuberg-Case hydrazone method, and in spite of the accumulating evidences of the possible role of vitamin B₁ as its pyrophosphate in the decarboxylation and dehydrogenation of pyruvic acid in the mammalian organism, there exists in the literature very little information with regard to the actual change of pyruvate in the blood or tissues of the B₁-deficient animals. This quantitative conception is of importance for a proper appreciation of the role of pyruvate in the metabolism of the normal and B₁-deficient individuals.

Experimental details

Albino rats, weighing about 70–90 g., were devitaminized with a diet of the following composition: casein 18 %, salt 4 %, agar 2 %, cod liver oil 2 %, arachis oil 3 %, sucrose 71 %, and addition of 60 ml. of autoclaved marmite 100 g. (Marmite solution: mix 5 g. marmite with 10 ml. 43.5 % NaOH and 90 ml. water, autoclave for 1 hr.) Electrocardiograms were taken according to the method of Birch & Harris [1934]. Blood samples were obtained by decapitating the rats and collecting the blood in ice-cold trichloroacetic acid before the estimation. Pigeons were fed on thrice washed polished rice and marmite (3 g. per day) plus 1 drop of radiostoleum. Blood samples were obtained by decapitation after marked head retraction had developed. Rabbit blood was obtained from the ear vein. In view of the effect of muscular activity on blood pyruvate (see Tables III, IV) special care was taken to keep the animals under resting conditions in a dark room for at least 1 hr. before the collection of the blood. The rabbit was therefore kept during this time in a box which was just the size of its body so as to eliminate the possibility of its walking about in the cage. With the exception of a few acute beri-beri cases all specimens of blood from the human subjects were obtained after the patient had more than $\frac{1}{2}$ hr. of rest in bed.

All pyruvate estimations were made by the specific hydrazone method previously described [Platt & Lu, 1936, 1; Lu, 1939].

Pyruvate determinations in the blood of various species of normal and avitaminous states are summarized in Tables I and II.

Table I. *Pyruvate in blood of normal and avitaminous animals*

	mg. pyruvic acid per 100 g. blood	
	Normal	Avitaminous
Pigeons	0.87 (5)	5.39 (5)
Rats	1.09 (5)	3.21 (4)
Rabbit	0.98 (11)	—

The numbers in brackets denote the number of estimations. Subcutaneous injection of 100 I.U. of vitamin B₁ to rats restores the blood pyruvate level to normal in 4–6 hr.; 250 I.U. to pigeons only after 24 hr.

Table II.* *Pyruvate in blood of healthy and vitamin B₁-deficient human subjects*

State of deficiency	No. of cases studied	mg. pyruvic acid per 100 g. blood		
		Range	Mean	Standard deviation
Cured (normal)	60	0.4-0.75	0.55	±0.12
Subacute (chronic)	84	0.77-1.93	0.93	±0.40
Fulminating (acute)	38	1.00-5.77	2.72	±1.13

* The author is much indebted to Dr B. S. Platt in collaboration with whom the data in Table II were obtained.

All the cases studied had at one time shown some degree of deficiency. For the assessment of symptoms in terms of vitamin B₁-deficiency and the relation of the value of this test to clinical studies see Platt & Lu [1936, 1]. Several hours (6-8) are required to initiate the removal of pyruvic acid after intravenous injection of 1000 or 2000 I.U. of vitamin B₁.

Effect of exercise on blood pyruvate

The effect of exercise on blood pyruvate is interesting and important. Food intake and muscular activity are known to be associated with the increase in carbohydrate metabolism which leads to increased requirement for vitamin B₁. In experiments with beri-beri cases, muscular activity appears to be one of the many factors which contribute to the development of acute symptoms. Strenuous exercise in man also leads to increase of pyruvic acid in the blood and an increased heart rate.

Table III. *Blood pyruvate of rabbits under basal and normal conditions*

Rabbit no.	Body weight in kg.	Blood pyruvic acid, mg. per 100 g.		
		Basal	Normal	Difference
1	1.257	1.03	1.43	0.40
2	1.437	1.36	1.89	0.53
3	1.321	0.90	1.48	0.58
Average	1.434	1.09	1.60	0.51

From Table III it can be seen that the basal level (fasting, immobile) rises by 50 % when the animal is allowed to feed and move about. Table IV shows that the average increase of pyruvate in the blood after 3 min. exercise is 4.68 mg. %. It required the injection of 180 mg. of pyruvic acid to produce the same rise. We must conclude, therefore, that at least 180 mg. are liberated during the 3 min. exercise. A normal rabbit can remove this in 30 min. This is in agreement

Table IV. *Blood pyruvate of rabbits under normal conditions and after 3 min. exercise*

Rabbit no.	Blood pyruvic acid, mg. per 100 g.		
	Normal	After 3 min. exercise	Increase
1	1.46	4.98	3.52
2	1.33	2.74	3.41
3	1.23	4.32	3.09
Average	1.34	4.68	3.34

N.B. The exercise consisted of holding the rabbit by the ear and letting it kick: when it refuses to do so it is brought on to a round glass jar where it struggles to find steady footing.

with the results of Johnson & Edwards [1937] on the exercise effect in healthy men, and of Wilkins *et al.* [1937] on pyruvate injected into normal men. Excess pyruvate in the blood up to 100 mg. per kg. body weight can be removed from the blood stream in 30 min. This must mean that 6 g. can be formed in a normal man in 30 min.

DISCUSSION

Pyruvic acid appears in very small quantities under resting normal conditions. Maintained raised levels are found in various states of hypovitaminosis and a temporary raised level after muscular activity. Administration of vitamin B₁ restores the raised blood pyruvate levels to normal as the apparent acute symptoms disappear. Wilkins *et al.* [1938] studied three cases with nutritional deficiency which gave pyruvic acid values by the hydrazone method of 0.73, 0.46 and 0.72 mg. per 100 ml. It will be seen from Table II that these values are in the order of the non B₁-deficiency range in man. So far as is known at the present time pyruvic acid increase in the blood under *resting* conditions has not yet been detected in any pathological state other than B₁-deficiency. We therefore appear to be justified in assuming that pyruvic acid accumulation in the blood is specific for B₁-deficiency. The test has been used in clinical studies of vitamin B₁-deficiency in man [Platt, 1938].

It will be seen from Table II that a maintained raised level of blood pyruvate is associated with vitamin B₁-deficiency in man. The fact that a very small amount of difference is obtained between the normal and the subacute cases and the complicated procedure for estimation of pyruvic acid by the hydrazone method have so far limited the applicability of this finding for assessing the state of B₁-deficiency in clinical studies. However, the latter objection has now been met by the introduction of the new micro-estimation method for pyruvic acid in blood [Lu, 1939] and the former can be overcome by an exercise tolerance test. Platt & Lu [1936, 2] observed an increase of blood pyruvic acid following exercise in both normal and deficient human subjects; the level in the normal rapidly returns to its usual value, while in the deficient subject increased values are maintained or even further raised for some time after. The details of an exercise tolerance test based on this will be reported in the next paper of this series.

However, it must be remembered that there is evidence of increase of pyruvic acid in the blood and urine of healthy individuals after severe exercise [Johnson & Edwards, 1937; Lu & Platt, 1936], and in the urine of uncompensated diabetic patients [Pi Suñer & Farran, 1936]. Furthermore, no significant change of blood pyruvate could be detected in the "atrophic type" of beri-beri where the nerves are primarily involved [Platt & Lu, 1936, 1] and which is supposed to be also due to vitamin B₁-deficiency, although the natural history of this type is not yet clear.

This requires some comment. Careful consideration of the data given in Table II shows that blood pyruvate values of some of the subacute cases come well within the range of values assigned for the fulminating cases. A detailed clinical discussion of some of these has been published [Platt & Lu, 1936, 1], others will be discussed in a separate report. For the present purpose it will suffice to mention that careful analyses of the biochemical and symptomatic syndromes of a large number of beri-beri cases indicate that this lack of identical behaviour in various types of deficiency may well be due to such factors as fever, toxæmia, endocrine disturbances and inanition, which are known to entail abnormal carbohydrate metabolism. Other vitamin deficiencies, e.g. A and C, were in some instances identified as existing in the same patient, especially

among the chronic, subacute type. How far other factors affect the whole syndrome is not known. Thus before it can be concluded whether increased pyruvate in the blood is specific for B₁-deficiency it is necessary to single out characteristic biochemical and symptomatic syndromes and to correlate them as specific conditions for the identification of the deficiency in an organism where other pathological conditions are likely to play an important part. For example, it might be said that the presence of pyruvic acid in diabetic urine gives ground for doubting the validity of using pyruvic acid estimations for research into vitamin B₁-deficiency states. But on the other hand the increase in urinary pyruvate may well be due to secondary causes; thus the altered water metabolism may affect the individual in such a way that his daily requirement of vitamin B₁ is increased: hence his ordinary intake which may be sufficient for a normal individual becomes insufficient, and a state of unrecognized induced or secondary avitaminosis is set up. Of course there is also the possibility of a primary disturbance of the intermediate carbohydrate breakdown which would also involve the accumulation of pyruvic acid.

Studies of the permutations and combinations of the numerous factors which may accompany vitamin B₁-deficiency or be involved in it are urgently needed. In the meantime, inferences drawn from the study of certain biochemical and clinical syndromes are useful as an aid to further advance, though they can only be regarded as preliminary and must be interpreted with caution. Nevertheless estimation of pyruvic acid in the blood forms a practical method for assessing the state of B₁-deficiency in man.

In the following section an examination is made of the blood pyruvate in correlation with the functional manifestation of cardiac disturbance under various conditions of B₁-deficiency with and without inanition or toxæmia and also under normal conditions. In the latter, the increase of blood pyruvic acid was produced from without by injections of Na pyruvate. The results account to some extent for some of the obscure combinations of conditions mentioned above.

III. THE RELATION OF BLOOD PYRUVATE TO CARDIAC CHANGES

In the case of vitamin B₁-deficiency, one of the most important and prominent symptoms is the disturbed function of the heart. The disturbances are not the same in all species; thus in pigeons (fed on polished rice) a vagal heart block seems to occur [Carter & Drury, 1929]; or bradycardia [Sankaran & Krishnan, 1936]; and in rats bradycardia of sinus origin [Drury *et al.* 1930]. While the cardiac changes in pigeons have been attributed to a special pigeon factor the bradycardia in rats is generally held to be specific for B₁-deficiency. Cardiac change in human beri-beri had long been known and was described in detail [Asslmeier & Wenckebach, 1928; Wenckebach, 1934; and Hashimoto, 1937]. Enlargement of the right side of the heart regularly occurs in human cases but has been observed only once in pigeons [McCarrison *et al.* 1928]. Studies on the nature of the electrocardiographic changes in rats [Zoll & Weiss, 1937] and in man [Raman, 1936; Weiss *et al.* 1936; Weiss & Wilkins, 1937] have shown that the cardiac changes in rats are similar to those observed in man. The essential difference is that in man a tachycardia exists. Platt & Lu [1936, 1, 2] reported

that this tachycardia of severe beri-beri cases gave place to a transient bradycardia during recovery after treatment with vitamin B₁. Weiss & Wilkins [1937] have made a systematic study of the nature of the various cardiovascular disturbances and the effect of vitamin B₁ on them. Cardiac changes existing in cases with no B₁-deficiency symptoms did not respond to B₁-administration. They state that, as far as is known, B₁-hypovitaminosis is the only vitamin deficiency which is followed by cardiac disturbances. They think it probable that the myocardial disturbances reported in rickets and scurvy are caused by simultaneous vitamin B₁-deficiency. However, there is also evidence of a tachycardia attributable to vitamin C-deficiency in guinea-pigs [Sankaran & Krishnan, 1936] and a bradycardia in rats attributable to inanition and not curable by vitamin B₁ [Parade, 1937].

Cowgill, on the other hand, has evidence that the heart is not involved in B₁-deficiency in dogs. While it is important to recognize that because tachycardia is attributable to vitamin C-deficiency in guinea-pigs it does not necessarily follow that vitamin C-deficiency would give rise to tachycardia in man or other mammals; yet it is clear that unless coexisting pathological conditions such as other deficiencies are known, the specific biochemical lesion due to the deficiency of one entity cannot be solved. In animal experiments this difficulty is not so serious, although it arises on account of our still relatively imperfect knowledge of synthetic diets. When dealing with human cases, the probability of mixed deficiency is much greater. Knowledge of factors modifying typical biochemical or functional changes is of great importance in recognizing the conditions.

The restoration of cardiac function in human B₁-deficiency can vary a great deal; while the apparent acute symptoms disappear as the pyruvate level in the blood returns to normal, yet cardiac function may or may not be normal even after several days. It was observed in a few of the chronic beri-beri cases where the heart did not immediately resume its normal function after vitamin B₁ treatment, it slowly did so after a period of large vitamin C intake (given for other experiments). Some patients, however, also recovered after various times without this addition of vitamin C. Whether this delayed cure is due to the accompanied dietary change after B₁ or organic changes in the heart tissue itself resulting from chronic deficiency is not yet known.

In an attempt to correlate the biochemical lesion and the clinical manifestation of B₁-deficiency Lu & Dju [1937] studied the relation of blood pyruvate level and the heart rate in rats, and reported that the lowered pulse rate is in most cases related to the accumulation of pyruvate in the blood of the B₁-deficient rats.

In the present study, blood pyruvic acid values of rats kept under different conditions were determined, and are given in the following tables.

Table V. *Pyruvate in the blood of acute deficiency rats*

No. of rats examined	Pulse rate per min.	mg. pyruvic acid per 100 g.	
		Range	Average
5	550-500	0.98-1.19	1.09 (normal)
1	500-450	1.11	1.11
3	450-400	0.99-1.65	1.25
3	400-350	1.58-1.61	1.60
3	350-300	1.82-2.69	2.20
4	300-350	2.59-4.05	3.21

On the diet given above, rats developed the deficiency in 3 weeks. In order to ascertain that the cardiac changes were due to the deficiency, vitamin B₁

was subsequently given in every case to ensure a restoration to normal. All the rats were killed within 10 days from showing the first signs of cardiac change, so that no appreciable loss of body weight occurred during the experiment. This class of animals was regarded as comparable with the fulminating type of beri-beri in man.

The results given in Table V show a clear relation between the content of pyruvate in the blood and the pulse rate; the blood pyruvate increases as the bradycardia of avitaminosis B₁ sets in. From the normal level of about 1 mg./100 g. it rises to 4 mg./100 g. in the most extreme cases.

Table VI. *Pyruvate in blood of chronic deficiency rats*

No. of rats examined	Pulse rate per min.	mg. pyruvic acid per 100 g.	
		Range	Average
1	450-400	2.4	2.4
4	400-350	1.25-4.06	2.75
3	350-300	1.98-3.31	2.65
0	300-250	—	—
1	250-200	5.00	5.00

Table VII. *Pyruvate in blood of infected and hypovitaminous rats*

No. of rats examined	Pulse rate per min.	mg. pyruvic acid per 100 g.	
		Range	Average
1	450-400	1.82	1.82
2	400-350	0.64-3.54	1.91
2	350-300	2.00	2.00
1	250-200	2.00	2.00
3	200-150	1.7-2.38	1.79
1*	380-175†	0.82	0.82

* Moribund rat.

† Taken 40 min. after 0.2 ml. Betaxin.

The effect of long-continued deficiency was next studied in a group of rats maintained for 2 months on the deficient diet, but allowed from time to time a supply of vitamin B₁ to keep them at a pulse-rate level of approximately 400 beats per min. Such rats developed marked polyneuritis, lost about a third of their body weight and were regarded as more comparable with subacute cases of human beri-beri. As Table VI shows, the blood pyruvate, in spite of a rather wide individual variation, varies with the pulse rate though the ratio is not quite the same as in the previous experiment; for a given pulse rate the pyruvate now tends to be higher. This demonstrates that a secondary condition, such as inanition, can have an effect on both the heart and the pyruvate.

Since concurrent fever and infection is not uncommon in human beri-beri, a third group of rats was studied. After a month on the deficient diet, rats become particularly susceptible to cold, and are liable to develop infections. Post mortems on such rats showed infective lesions of liver and lung. Thus the third group had avitaminosis, inanition and toxæmia.

The figures given in Table VII show that now there is little correlation to be seen between blood pyruvate and heart rate. The normal value for pyruvate may even be obtained in moribund rats which have received a dose of vitamin B₁ but which are continuing to show a fall of pulse rate. These observations help to account for some obscure beri-beri cases where, after prolonged illness and infection, even excessive doses of B₁ will not bring about effective cure.

The averages of the foregoing tables are plotted in Fig. 1, which illustrates strikingly what has been said above.

By the use of the newly developed micro-method [Lu, 1939] for the determination of pyruvic acid in small quantities of blood it was possible to follow the blood pyruvate changes in individual rats at various stages of deficiency as indicated

Fig. 1.

by the bradycardia, and at the different stages of recovery after the subcutaneous injection of vitamin B₁. To obtain successive blood samples the following technique was found to be very serviceable: xylol was applied to the rat's tail, causing the blood vessels to dilate; after a minute or two the tail was cleaned and dried under sterile conditions. If 2 mm. of the tail tip from which 1 in. had previously been severed, are suddenly cut off, two or three drops of blood are obtained. The wound is then closed with collodion solution, and the process is repeated when more blood is required. The results from two rats rendered deficient without much loss of weight or any other complication are given in Table VIII. Similar analyses made on chronic and infected rats gave results of the same order as those described above.

Experiments of the type reported in Table VIII clearly confirm the previous experiments and show that the bradycardia of vitamin B₁-deficiency in rats runs parallel with the biochemical changes observed in the blood, and that vitamin B₁ removes the bradycardia and restores the blood pyruvate level to normal only in pure B₁-deficiency, and not in the chronic or infected rats. Birch & Harris [1934] observed the same correlation of bradycardia with lactic acid in the blood.

Table VIII

Pulse rate per min. approx.	Blood pyruvic acid, mg. per 100 g.	
	Rat I	Rat II
500-550	0.98	1.03
450-500	1.17	1.21
400-450	1.54	1.63
350-400	2.07	2.14
300-350	3.24	3.28
*350-400	2.14	2.20
*400-450	1.50	1.59
*450-500	1.21	1.30
*500-550	1.02	0.99

* After administration of vitamin B (100 I.U. injected subcutaneously).

It is worthy of note that the return of the heart rate to normal after injection of B₁ takes place within 4 hr., which seems to indicate that this cardiac disturbance is the result of a "biochemical lesion". Whether the lack of similar response of cardiac disfunction to B₁-therapy in the chronic and infected cases is attributable to organic change of the heart tissue itself is not known. In this connexion, mention may be made of the delayed return to normal function of the heart in chronic beri-beri cases.

Effect of injected pyruvate on heart rate

Next it was thought necessary to know the effect of injected pyruvate on the heart-rate. It appears from injections of pyruvate in man [Wilkins *et al.* 1937] and dogs [Flock *et al.* 1938] that no toxic effects follow, but such effects have been said to occur in the rabbit [Kermack *et al.* 1927]; this was not confirmed in the present work. Toxic effects might perhaps be expected since we know that pyruvic acid polymerides are toxic [Peters, 1936; Lipschitz *et al.* 1938], especially as the heart is a sensitive organ and readily affected by changes in the internal medium. The effect on the heart would however be likely to be transient since the metabolic disappearance of pyruvic acid from the blood stream is so rapid. The pyruvate solution used was prepared in the same manner as described previously [Lu, 1939] and injected as 40 mg./ml. in 0.9% NaCl.

Rabbits were suitable for these experiments because of the possibility of injection into the vein of one ear and the taking of the blood from the other after the shortest interval of time. Electrocardiograph tracings were taken before, during and after injection of pyruvate in amounts of 80 mg. per kg. body-weight. Of the six rabbits studied, with the exception of one which had a skin lesion at the corner of its mouth, and was later proved to be diseased, all showed no change in heart rate, even after the blood pyruvate was raised to 4 or 8 times its normal level. (See Fig. 2.) Thus it seems to follow that pyruvic acid up to 10 mg./100 g. in the blood is not toxic, and is not the cause of the cardiac disturbances in B-deficiency unless the rabbit heart is entirely different from that of the rat and man.

It will be seen from Fig. 2 that the rabbit metabolizes 95 % of the injected pyruvate during the time of injection (2-3 min.). There is a steady fall of blood pyruvate during the first 5 min. until the level reaches that which is found in the deficiency states (1-2 mg./100 g.), then the fall is very slow. These experiments show that a normal rabbit can metabolize 100-150 mg. or more pyruvate in 20 min.

From this it seems to follow that increased blood pyruvate cannot be the direct cause of bradycardia, and hence possibly not of any cardiac disfunction. It is more likely that the two phenomena have some common causative factor.

Since the preliminary communication of the results in this paper was made [Lu, 1938] a paper appeared by Kalaja & Närvänen [1938] in which the conclusion is reached from the effects of subcutaneous injections of various metabolites

Fig. 2. Pyruvate of normal rabbits after injection. ●—● Curve I. 250 mg. pyruvate injected ($2\frac{1}{2}$ min.). ○—○ Curve II. 160 mg. pyruvate injected (2 min.).

which are known to accumulate in B-deficiency on the heart rates of rats, pigeons and rabbits, that the rat heart is singularly susceptible to various compounds, and that pyruvic acid has a powerful effect. 100–150 mg. were the smallest amounts to cause a definite and regular change of heart rate. An example was given of one rat which had 300 mg. of pyruvate and showed a gradual slowing of the heart rate lasting from 30 min. to 3 hr. after injection. These authors also state that in spite of the heavy subcutaneous dose the ketonic acid content of the blood increased relatively little during the experiment. As this finding has a special bearing on the specific relation of the biochemical lesion produced by B_1 -deficiency to the functional change of the heart, the following groups of rats were given subcutaneous injections of different doses and samples of Na pyruvate. Typical data for the cardiac and blood changes are illustrated in Table IX.

The examples cited in Table IX illustrate several important points, the first of which is concerned with pyruvic acid itself. Lipschitz *et al.* [1938] and Peters [1936] observed the toxic effects of pyruvate polymerides on tissues in their *in vitro* experiments. Since the same concentration neutralized under proper conditions when injected gives rise to no toxic symptoms, this fact may in part explain Kamiya's [1937] observations of the action of pyruvic acid on blood vessels.

Table IX

Groups	Amount of pyruvic acid injected as Na salt mg.	Time after the injection when sample obtained min.	Change of heart rate per min.	Blood pyruvate mg./100 g.	B.B.S.* expressed as pyruvic acid mg./100 g.	Remarks
I ₁	180	10	- 45	4.6	17.08	All rats of this group had immediate reactions of shivering which later developed into convulsions. 30 % of the injected rats died within 20 min.
I ₂	180	30	- 105	7.2†	34.16	
I ₃	180	60	- 200	10.0†	59.47	
II ₁	170	5	+ 20 nil	12.6	—	Convulsions at the end of the experiment when the tail is touched
II ₂	68	60	+ 25 nil	9.1	—	
II ₃	34	60	+ 10 nil	5.2	—	
II ₄	102	120	- 175	11.4	—	
II ₅	68	120	- 15 nil	6.4† trace	—	Convulsion on touching the tail after 1½ hr.
III ₁	75	5	+ 20 nil	11.3	18.9	
III ₂	30	10	+ 40	24.0	36.2	
III ₃	30	120	± 0 nil	9.8	14.7	
III ₄	75	120	- 130	14.8†	62.0	

* B.B.S. was not estimated in samples from group II.

† These samples showed intense purple colour of methylglyoxal bishydrazone with alcoholic KOH when tested by the method of Barrenscheen & Dreguss [1931]. Group I. The pyruvic acid solution used was prepared by neutralizing a 1 : 3 dilution of twice freshly redistilled pyruvate to pH 7.4 with 20 % NaOH; this contains 180 mg. per ml. Group II. B.D.H. Na pyruvate, which has 85 % of its B.B.S. as pyruvate. Group III. Pyruvic acid used was prepared by diluting the twice redistilled pyruvic acid and neutralizing to pH 7.4. Just before use it was so diluted that 1 ml. contained 100 mg.

When a pyruvic acid solution neutralized in the concentrated form was diluted and injected into rabbits it produced tremor and vasoconstriction. Examples given in Table IX demonstrate the toxic effect of such preparations in living rats. Since the whole musculature of the rats was involved, the change of heart rate cannot be regarded as due to the specific effect of pyruvate. Furthermore there exists also a much greater proportional change of B.B.S. which also may exert a profound effect. The second interesting point is that the blood pyruvate level can be raised to 2 or 4 times that which was found in B₁-deficiency lasting for 1 or 2 hr. (II₂, III₂ and III₃) without affecting the heart rate. Yet larger doses, lasting for longer periods (compare II₁ and II₄; III₁ and III₄) with practically no difference in blood pyruvate value, had a marked effect on the heart. As I have mentioned above, if the heart change is a chemical effect it should be transient and should respond rapidly to definite levels of pyruvate concentration in the fluid in which the heart is bathed.

DISCUSSION

The *in vitro* experiments of Weil-Malherbe [1937] using brain tissue and Lipschitz *et al.* [1938] using liver and kidneys clearly show that removal of added pyruvate involves other substrates. Wilkins *et al.* [1938] have evidence that pyruvic acid is converted into other ketonic substances by blood both *in vitro* and *in vivo*. My own results, obtained from blood of rabbits and rats, also show that pyruvate is quickly converted into other bisulphite-binding compounds. In some

instances where the B.B.S. value occupied a dominating place in the blood changes, a methyglyoxal-like substance was obtained. In view of the above-mentioned facts it is obvious that pyruvate itself, in the amounts in which it occurs in vitamin B₁-deficiency, has no effect on the rat heart. The slowing of heart rate produced by excessive doses (above 100 mg. for a rat weighing 50 g.) should be interpreted as due to secondary changes caused by the efforts of the organism to remove such an unexpected load of pyruvate.

The experiments reported in these two papers with rats suffering from B₁-hypovitaminosis uncomplicated by any other condition, in which a close correlation was shown to exist between blood pyruvate level and heart rate, demonstrate that these changes are causally associated with B₁-deficiency.

At the same time it is clear that the pyruvate and cardiac changes may be complicated and even obscured by other concurrent conditions. In any given case in man, only the combination of blood pyruvate measurements, exercise tolerance tests and clinical symptoms can reliably differentiate between true vitamin B₁-deficiency and other conditions which in some respects may simulate it. The very rapid return to normality, both of the pulse rate and the blood pyruvate, when the vitamin is given to acute cases, strongly supports the view that the lesion there is biochemical or "functional". If the deficiency is allowed to continue until "organic" or histological changes have set in, then administration of the vitamin may remove the acute symptoms by restoring the blood pyruvate to the normal level, but will not reverse the cardiac changes. If, however, blood pyruvate levels raised many times above the concentration found in deficiency states in rabbits and rats give rise to no change of heart rate. The raised pyruvate levels can hardly, therefore, be the direct cause of the heart rate changes. On the other hand, the results reported in these papers support the view that the pyruvate test is specific for B₁-deficiency, presumably because the deranged metabolism of this deficiency involves the accumulation of pyruvic acid in the blood, and the test can be applied for assessing B₁-deficiency in man.

SUMMARY

1. Blood pyruvate values for normal and avitaminous animals are presented.
2. Blood pyruvate and bradycardia run parallel in rats suffering from vitamin B₁-deficiency.
3. Injection of pyruvate into the circulation of normal rats and rabbits, however, even above the highest levels occurring in vitamin B₁-deficiency, has no effect on the heart rate.
4. It is unlikely therefore that the raised blood pyruvate in B₁-deficiency is the direct cause of the cardiac symptoms; it is itself rather the result of the metabolic derangements characterizing this condition.
5. This is supported by the fact that blood pyruvate in man may reach the normal level long before the pulse rate does so.
6. Even moderate muscular exercise considerably increases the blood pyruvate.

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