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

VI. THE FATE OF INJECTED PYRUVATE IN THE NORMAL RABBIT

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THE results of *in vitro* work have led to the postulation of several possible paths of pyruvate removal in animal tissues; namely (1) the scheme of Toenniesse & Brinkmann [1930] whereby 2 mol. of pyruvic acid are reduced to form 1 mol. of succinic acid, and the latter is then converted successively into fumaric, malic, oxaloacetic and 1 mol. of pyruvic acid, (2) the scheme of Krebs & Johnson [1937] whereby the oxidation of pyruvate is preceded by an anaerobic change; 2 mol. of pyruvate interact to form 1 mol. each of lactic acid, acetic acid and CO₂, (3) the oxido-reduction between pyruvate and an aldehyde, such as triosephosphate, giving lactic acid and an acid such as phosphoglyceric acid, (4) a direct oxidation like that which exists in the brain [Peters, 1936; 1938; Ochoa & Peters, 1938]. It is also relevant that according to Weil-Malherbe [1937] pyruvic acid is removed more effectively from brain slices in the presence of glucose than in its absence. However, little is known about pyruvic acid metabolism *in vivo*. The object of the following experiments is to throw some light on these questions.

Rabbits were used in most of the experiments. Instead of estimating the changes following administration of pyruvate in the blood, urine or excreta after some time, it was decided to load the rabbit suddenly with a definite amount of pyruvate by intravenous injection lasting for 1-2 min. and study the chemical changes resulting from this immediately after the injection, a few minutes later and in some instances 35 min. later, when the blood pyruvate level had returned to the pre-injection level.

Experimental details

All the rabbits were kept under resting conditions for at least 1 hr. before use, as previously described. Anaesthesia was begun by first giving a sedative dose of "Numal Roche" (1 ml. of 10% solution per kg. body weight) and completed by using small amounts of ether. By the combined use of these two substances there was usually no struggle throughout the experimental period; and such methods of anaesthesia have been shown to cause no carbohydrate breakdown [see e.g. Sacks & Sacks, 1933].

Both gastrocnemius muscles of the rabbit were carefully dissected free from connective tissue, leaving the blood and nerve supply intact. One muscle was quickly excised in the resting state, and immediately dipped in liquid air to freeze. Pyruvate was then injected intravenously through the ear vein. Blood samples were taken from the opposite ear. After allowing the time necessary for the metabolism to take place the second gastrocnemius muscle (in the meantime

covered up with its own skin) was quickly excised and immediately dropped into liquid air. About 2-3 sec. elapsed between the excision and the time when the muscle was fully frozen. It never took more than 15 sec. to get the whole gastrocnemius frozen.¹ After the muscle had become very brittle, it was removed to a chilled mortar containing powdered CO₂ snow and ground to a fine powder. The mixture was then transferred to a 100 ml. weighing bottle containing 25 ml. of 5% CCl₃COOH (which was carefully weighed and cooled beforehand) to remove proteins. It was shaken frequently to ensure complete precipitation and extraction. The exact amount of muscle in each specimen was found by weighing the bottle again after room temperature had been reached. The precipitate was filtered and washed twice with 5 ml. 5% CCl₃COOH and the total volume diluted to 40 ml. Aliquot parts were taken for the various estimations. For the estimation of pyruvic acid the micro-method [Lu, 1939, 1] was used and for lactic acid the method of Friedemann & Graesser [1935]; the error caused by using trichloroacetic acid extract in the lactic acid estimations was checked with Zn lactate controls, and found to be negligible under the conditions described. The total reducing sugar in blood was estimated by the method of Folin & Wu [1920] and fermentable sugar, using the same technique except that the proteins were precipitated by 7% CuSO₄ and 10% Na tungstate. For the inorganic phosphate the method of Fiske & Subbarow [1925] was used. The triosephosphate and phosphopyruvate were estimated according to the method of Meyerhof & Lohmann [1934, 1, 2]. With the aid of the photoelectric colorimeter for estimation of small amounts of phosphate and pyruvate it was possible to make all the determinations on the extract from a single gastrocnemius muscle.

The pyruvate used for injection was prepared in the same manner as described previously [Lu, 1939, 1].

RESULTS

Some of the results are given in the following tables. It will be seen from the examples given in Table I that intravenous injection of small doses of pyruvate into rabbits gives rise first to a temporary increase of non-glucose substance (bisulphite-binding substance or B.B.S.) with a simultaneous increase of lactic acid; then a more marked increase of "fermentable sugar" occurs as the non-glucose reducing substances, lactic acid and pyruvic acid levels return to the resting values.

Table I. *Blood changes resulting from injection of small doses of pyruvate*

		All results are expressed as mg. per 100 g.				
Rabbit no.		Total	Ferment-	Non-	Pyruvic	Lactic
		reducing	able	glucose		
		sugar	sugar	sub-	acid	acid
				stances		
1	Resting sample	129	104.2	24.8	0.84	28.8
	150 mg. pyruvic acid	151.8	112.7	39.2	2.16	34.6
	injected intrav.	160.8	128.0	32.8	1.51	38.6
	8 min. later	164.0	143.7	20.3	0.91	31.0
	20 min. later	170.0	148.6	21.4	0.90	28.4
2	Resting sample	103.0	86.2	17.1	1.09	19.0
	200 mg. pyruvic acid	113.0	88.4	29.6	3.08	20.4
	injected intrav.	122.7	105.4	17.4	1.63	24.8
	8 min. later	137.9	118.3	19.6	1.10	21.6
	20 min. later	129.0	111.1	17.9	1.12	21.8
	30 min. later					

¹ This method instead of the technique of freezing *in situ* was adopted to eliminate interference with the circulation, since pyruvate was injected after excision of the control muscle.

Table II. *Pyruvate and lactate changes in blood*

Body wt. kg.	Pyruvic acid injected mg.	Total* blood g.	Pyruvic acid in blood, mg. per 100 g.		Lactic acid in blood, mg. per 100 g.		Increase % of pyruvic acid injected
			Resting	After injection	Resting	After injection	
2.020	450	172	0.925	9.22 (9)	8.29	3.33	23
1.620	450	148	1.792	27.25 (4)	25.46	8.38	26
1.950	450	166	0.828	9.687 (8)	8.85	1.29	—
1.750	340	149	1.082	6.27 (8)	5.19	2.28	—
2.650	510	216	1.025	11.68 (8)	10.55	4.46	13.0
2.200	455	187	—	—	—	—	16.2
2.770	540	236	—	—	—	—	9.8
2.010	420	170	—	—	—	—	34.2
							10.9

* Total blood was calculated by using the value 85 g./kg. body weight.

The numbers inside the brackets denote the time in minutes after the injection when the sample was obtained.

Table III. *Pyruvate and lactic acid changes in muscles*

Body wt. kg.	Pyruvic acid injected mg.	Total* muscle g.	Pyruvic acid in muscle, mg. per 100 g.		Lactic acid in muscle, mg. per 100 g.		Increase % of pyruvic acid injected
			Resting	After injection	Resting	After injection	
2.020	450†	470	0.996	3.06 (8)	2.07	2.17	28.3
1.620	450	377	2.142	8.778 (4)	6.636	5.58	—
1.950	450	452	1.017	3.525 (8)	2.508	2.51	—
1.750	340	407	1.100	3.046 (4)	1.946	2.34	—
2.650	510	616	1.050	3.935 (8)	1.885	2.56	34.8
2.200	455	510	1.177	2.305 (14)	1.125	1.27	—
2.770	540	467	—	—	—	—	—

* Total muscle was calculated by dividing the body weight by 4.3 as found by actual dissecting and weighing of the muscle of two rabbits.

† Injection of 16 ml. of 0.9% NaCl alone did not alter the pyruvate and lactate levels in the blood or the muscles.

It has been shown [Lu, 1939, 2] that over 95 % of the injected pyruvate disappears from the blood stream within the first 5 min. Even after taking into consideration the amount which appeared as additional lactate and glucose, the sum of these does not account for more than 10 % of the total quantity injected. The question then arising was whether the remaining 90 % was actually metabolized or had been retained in the muscles? As this question has considerable bearing on the mode of pyruvate disappearance *in vivo* the lactate and pyruvate changes in the muscle and blood after intravenous injection of small doses of pyruvate were studied. The results of these experiments are summarized in Tables II and III.

The blood pyruvate changes confirm the previous observations. Only 2-3 % of the total injected pyruvate is retained in the muscles. Though a definite increase of the lactate content in the blood occurs, the lactate in the muscles may or may not be raised. If ± 6 mg. per 100 g. are allowed as experimental error then 30 % of the cases studied show a lactate increase. No alteration of sugar content was detected in the muscles.

In view of the role of phosphate in the glycolysis in mammalian tissues the possible change of the various phosphate fractions (e.g. inorganic phosphate, 7 min. acid-hydrolysable, triosephosphate and phosphopyruvate) in the blood or muscle were investigated in samples from two rabbits. Under the conditions of these experiments there was no appreciable alteration in any of the phosphate fractions.

Wilkins *et al.* [1938] and Flock *et al.* [1938] have failed to obtain evidence of lactic acid formation when pyruvate is incubated with blood *in vitro*. Our own preliminary experiments of the same type confirm the above observations. However, in two experiments the defibrinated and fluorized blood from normal rats was able to form lactic acid *in vitro* when hexosediphosphate and adenylic acid as well as the pyruvate were provided for the reaction. 0.33 and 0.47 mg. lactic acid can be produced from 1 ml. blood after 2 hr. at 37°. The reaction takes place with coupled esterification of inorganic phosphate in the same manner as found in muscle extract [Needham & Pillai, 1937] and has already been described for blood by Dische [1934]. This observation suggests that at least one of the paths of pyruvate removal involves the oxidation of triosephosphate by pyruvate. It is intended to make later a comparative study of this system in normal and deficiency states.

The observation of an increased lactate and glucose content in the blood of animals after intravenous injection of large doses of pyruvic acid has been reported before [Meyer, 1912; Dakin & Janney, 1913]. These authors concluded that if conditions do not favour the initial reduction to lactic acid no sugar is formed. Recently Margaria & Ponzio [1937], Simola [1937] and Flock *et al.* [1938] also observed that pyruvate injection was followed by an increase of lactate content of the blood. However, the last-mentioned authors did not find an increase of blood glucose after giving continuous injection of pyruvate to dogs. They claim to have obtained a decrease of blood inorganic phosphate in their dogs. On the other hand Kamiya [1936] concluded that intravenous injection of small doses of pyruvate (0.1-2.5 g.) into normal rabbits gives rise to hypoglycaemia. Nevertheless it may be mentioned that Kamiya's conclusion was based on subsequent estimations of blood sugar on samples obtained not a few minutes but several hours after the injection of pyruvate. Since the rate of pyruvate removal in the rabbit is extremely rapid it appears difficult to ascribe the hypoglycaemia produced 2-4 hr. later to the direct effect of pyruvate.

The finding that the injection of pyruvate is followed by little detectable change in the muscle together with the definite increase of lactate in the blood (involving an intermediate stage which can be estimated as B.B.S. or non-glucose reducing substances followed by an increase of glucose) appears to suggest that some other organ, presumably the liver, is concerned with its removal. It has been shown by Meyer [1912] that the fasting rabbit can utilize injected pyruvate to build up glycogen in the liver. The phenomenon of a slight increase of pyruvate accompanied by a much larger increase of lactate in the blood after injection of pyruvate makes it reasonable to suppose that the ability to rebuild carbohydrate from lactate and pyruvate is one of the factors in the organism limiting the efficiency of pyruvate removal. The well-known decrease of glycogen content in the tissues, the similar small increase of pyruvate accompanied by a much larger increase of lactate in the blood and the delayed removal of these after exercise in avitaminous animals appear to indicate that vitamin B₁ may play an important role in the anabolism as well as the catabolism of glycogen.

SUMMARY

1. Estimations of carbohydrate changes in the blood of normal rabbits immediately after intravenous injection of pyruvate suggest that pyruvate injection gives rise to a sudden increase of non-glucose reducing substance and also lactate which afterwards give rise to glucose.

2. Only 2–3% of the total injected pyruvate can be found in the muscles 8 min. after the injection. There is little or no change in muscle lactate or glucose.

3. When small amounts of pyruvate were injected no appreciable alterations in the phosphate partition were detected in the blood or the muscles.

4. Lactic acid can be formed *in vitro* by incubating blood with pyruvic acid if hexosediphosphate and adenylic acid are also provided. This suggests that pyruvate removal *in vivo* may be closely bound with this important energy-yielding oxido-reduction which is undoubtedly concerned with the regeneration of carbohydrate.

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