CLI. THE METABOLISM OF NORMAL AND TUMOUR TISSUE.

X. THE EFFECTS OF LACTATE, PYRUVATE AND DEPRIVATION OF SUBSTRATE.

By FRANK DICKENS and GUY DRUMMOND GREVILLE.

From the Courtauld Institute of Biochemistry, Middlesex Hospital, London, W. 1.

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THE anaerobic glycolysis of surviving tissue is not only decreased by general enzymic poisons (*e.g.* heavy metals, fluoride) and by more specific inhibitors (*e.g.* halogen-acetic acids, nitric oxide), but it is also affected by certain substances which are possible intermediates in carbohydrate metabolism. Thus glyceraldehyde inhibits the glucolysis of tumours [Mendel, 1929], pyruvic acid accelerates the glucolysis[1] of certain normal tissues [Mendel, Bauch and Strelitz, 1931], and lactate itself has been shown to reduce the anaerobic glycolysis of brain [Meyerhof and Lohmann, 1926, 1, 2]. The alteration of the rate of metabolism without the introduction of substances foreign to the animal body is an experimental method of considerable interest, and in the present paper we describe our experiences with its use.

Effect of lactate on the anaerobic glucolysis.

Meyerhof and Lohmann found that the anaerobic glucolysis of brain was reduced to about one-half by the addition of both *dl*- and *l*-lactates. As *l*-lactate had as great an effect as the natural *d*-lactate, these authors concluded that "bei dieser Beeinflussung der Glykolyse handelt es sich offenbar nicht um einen Prozess, der mit der physiologischen Rolle der Milchsäure zusammenhängt." It was felt that the matter merited further investigation, both because of the remarkable effect of the unnatural optical enantiomorph, and also because the suppression of the glycolytic process by lactate is of interest in the study of tissue respiration in lactate-containing media.

Experimental. The effect of added lactate on the anaerobic glucolysis of testis and of thin slices of rat brain and Jensen sarcoma has been measured (Table I). Zinc *d*- and *l*-lactates[2] were prepared by resolution through the zinc ammonium salts [Purdie and Walker, 1895], seeding with crystals of *d*- and *l*-salts prepared from meat extract and by resolution with morphine [Irvine, 1906] respectively. The purity of the preparations was checked by determinations of rotation and of water of crystallisation. The sodium salts were prepared by treatment of the zinc salts with sodium carbonate [see Meyerhof and Lohmann, 1926, 2]. The glucolysis was measured for periods up to 3 hours; in the first experiment the lactates were present from the start, but in the other experiments the

[1] For definition of terms glycolysis, glucolysis *etc.* see Dickens and Greville [1932, 1].

[2] In this paper the naturally occurring (sarcolactic) acid is referred to as *d*-lactic acid, and its salts as *d*-lactates.

Table I. *Effect of lactate on anaerobic glycolysis of rat tissues.*

1. *Jensen sarcoma.*

Added lactate		0	0·019 *M d*	0·02 *M dl*
$Q_M^{N_2}$	1st hour	27·4	26·6	27·0
	2nd hour	21·6	22·1	22·0
	3rd hour	22·0	18·3	21·5

2. *Jensen sarcoma* (Fig. 1). Lactate added 20 mins. after first reading.

Added lactate		0	0·02 *M d*	0·02 *M l*
$Q_M^{N_2}$	1st period (20 mins.)	46·5	49·5	44·7
	2nd period (40 mins.)	43·5	26·3	34·5
	3rd period (40 mins.)	36·8	23·3	31·5
% decrease	2nd period	6·5	47	23
	3rd period	26	53	29·5

3. *Testis.* Lactate added 20 mins. after first reading.

Added lactate		0	0	0·02 *M d*	0·02 *M l*	0·02 *M dl*
$Q_M^{N_2}$	1st period (20 mins.)	8·4	10·5	9·5	10·8	8·7
	2nd period (40 mins.)	8·1	10·5	8·3	10·3	7·7
	3rd period (60 mins.)	7·8	9·7	6·4	9·1	6·2
% decrease	2nd period	3·5	0	12·5	4·5	11·5
	3rd period	7	7·5	33	16	29

4. *Brain cortex.* Lactate added 15 mins. after first reading.

Added lactate		0	0	0·02 *M d*	0·02 *M l*	0·02 *M dl*
$Q_M^{N_2}$	1st period (15 mins.)	21·2	24·4	20·0	25·2	15·6
	2nd period (60 mins.)	14·9	19·2	13·3	19·3	11·3
	3rd period (60 mins.)	13·1	17·2	10·7	17·4	9·2
% decrease	2nd period	30	21	33	23	28
	3rd period	38	30	47	31	41

5. *Brain cortex.* *dl*-Lactate added 20 mins. after first reading.

Added lactate		0	0	0·02 *M*	0·02 *M*	0·05 *M*	0·10 *M*
$Q_M^{N_2}$	1st period (20 mins.)	12·2	12·9	10·8	14·4	12·3	9·0
	2nd period (60 mins.)	6·2	7·3	5·2	6·4	4·3	2·8
	3rd period (60 mins.)	4·7	5·2	3·2	4·4	2·0	0·8
% decrease	2nd period	49	43	52	56	65	69
	3rd period	61	60	70	69	84	91

lactates were added at the stated time from a side-bulb. In order to eliminate the effect of glycolytic inequalities between different slices of the same tissue, we give for each vessel for various periods after the lactate addition the percentage decrease in glucolysis from the value in the period before the lactate addition. By comparison with the corresponding falling off in the control vessel to which no lactate was added the effect of the lactate may be assessed.

From the results in Table I we conclude that 0·02 *M* *d*-lactate may sometimes cause a decrease of about one-half in the glucolysis (Exp. 2) as in the experiments of Meyerhof and Lohmann; on the other hand, the effect of the added *d*-lactate may be small, as in Exps. 1 and 4. Inhibition occurs with all three tissues. Our experiments, in contrast to those of Meyerhof and Lohmann, do not show that *l*-lactate causes as great an inhibition as does *d*-lactate: in Exps. 2 and 3 the natural salt has about twice the effect of its optical enantiomorph. Exp. 5 shows how the inhibition of brain glucolysis increases with increasing concentration of added lactate. This experiment is

represented in Fig. 2, where corrected values of $Q_M^{N_2}$ are shown, equal for each vessel to the observed $Q_M^{N_2}$ divided by the ratio of the glucolysis in that vessel before lactate addition to the average glucolysis before lactate addition.

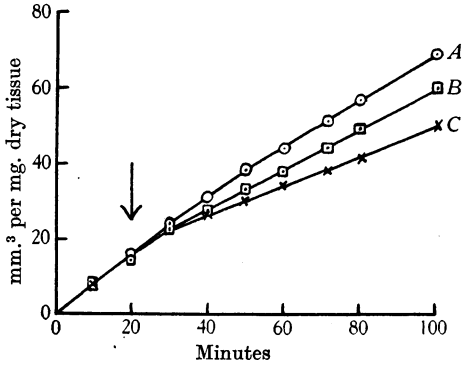

Fig. 1.

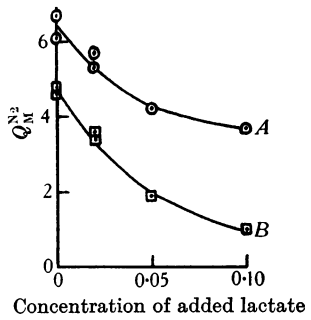

Fig. 2.

Fig. 1. Anaerobic glucolysis of Jensen rat sarcoma. *A*, control; *B*, 0·02 *M* sodium *l*-lactate added after 20 mins.; *C*, 0·02 *M* sodium *d*-lactate added after 20 mins.

Fig. 2. Rat brain cortex in glucose. *A*, second period; *B*, third period.

The inhibition of an enzyme reaction produced by addition of an end-product may be due to acceleration of the reverse reaction or to combination of the added substance with the enzyme system. The former may be excluded with glycolysis, for Lipmann [1927] found no anaerobic disappearance of lactic acid from minced muscle, and Burk [1932] has shown that when the free energy of neutralisation of lactic acid is negligible, the concentration of lactic acid would have to be 10^{20} *M* if an equilibrium with glycogen is to be attained. It thus seems probable that the lactate combines with the enzyme system.

Effect of substrate-deprivation.

Rosenthal [1930] showed that a spontaneous increase in the anaerobic fructolysis of Jensen rat sarcoma occurs about 40 minutes after the manometric vessels are put into the thermostat at 38° (Fig. 9). When glucose is substrate a spontaneous increase in $Q_M^{N_2}$ is not ordinarily observed, but we were able [Dickens and Greville, 1932, 2] to demonstrate its occurrence by introducing a preliminary peiod of anaerobiosis in absence of added glucose. Spontaneous anaerobic activation of glycolysis has so far been reported only in the Jensen sarcoma. In the experiments described in the present paper we applied to other tissues the technique of preliminary anaerobic substrate-deprivation which brought to light activation of glucolysis in the tumour tissue. Adding glucose to give a concentration of 0·2 %, we have tested normal tissues (rat brain, spleen, testis), another tumour tissue (Mill Hill fowl fibro-sarcoma), and an embryonic tissue (rat yolk-sac). With testis a slight spontaneous activation was detected. In the experiment represented in Fig. 6, $Q_M^{N_2}$ before glucose addition was 4·8: for 15 minutes after glucose addition it was 7·6, and then it rose abruptly to 9·8, which was maintained until the end of the experiment, 55 minutes later. No evidence of the occurrence of spontaneous activation in the other tissues was obtained (Figs. 4, 5, 7, 8). The spontaneous increase in fructolysis shown by the Jensen rat sarcoma occurs only within a certain range of fructose concentration

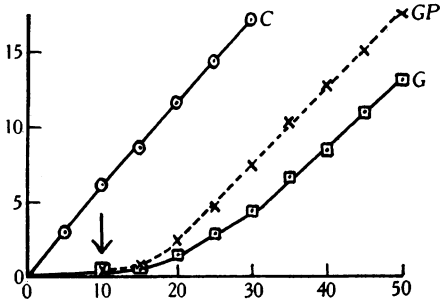

Fig. 3. Jensen rat sarcoma.

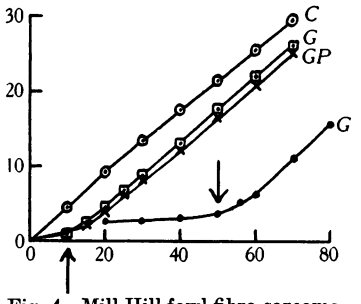

Fig. 4. Mill Hill fowl fibro-sarcoma.

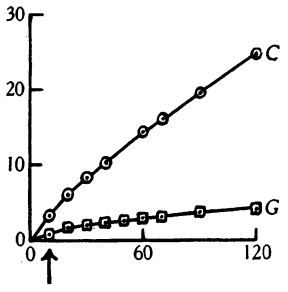

Fig. 5. Rat brain cortex.

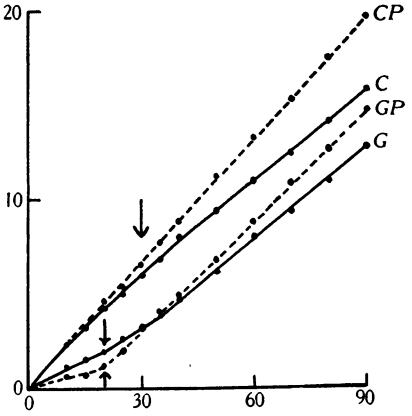

Fig. 6. Rat testis.

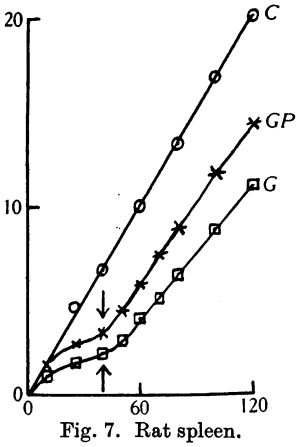

Fig. 7. Rat spleen.

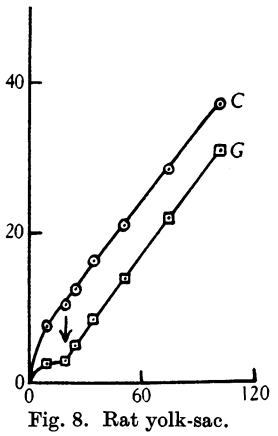

Fig. 8. Rat yolk-sac.

Figs. 3–8. Anaerobic glycolysis. Ordinates, mm.^{3} CO_2 per mg. dry tissue; Abscissae, time in minutes.

C = control (glucose present from beginning of experiment).
G = glucose added at time indicated by arrow.
GP = glucose + pyruvate added at time indicated by arrow.
CP = pyruvate added at time indicated by arrow to control (glucose present from beginning of experiment).

[Dickens and Greville, 1932, 3], and it was not observed at 28°, so that it is possible that the necessary conditions for the other tissues were not realised in these experiments. We therefore do not consider that spontaneous activation is a unique property of the Jensen sarcoma. The two processes which allow the observation of spontaneous activation, initial depletion of "activating" substance in the tissue and slow re-establishment of its optimum concentration, occur under the usual experimental conditions most readily with the Jensen sarcoma in fructose and to a lesser extent with the same tumour and with rat testis in glucose. That they occur in other tissues, that the glycolysis of these tissues also is regulated by an "activating" substance, is a possibility that still remains.

If for each tissue the glucolysis attained after a preliminary period of anaerobiosis in absence of added substrate is expressed as a percentage of the glucolysis of that tissue when glucose is present from the beginning of the experiment, the following values are obtained:

Tissue	Preliminary period in absence of substrate (mins.)	% recovery after glucose addition
Jensen rat sarcoma	20	92
Mill Hill fibro-sarcoma	20	106
Mill Hill fibro-sarcoma	60	112
Rat yolk-sac	30	110
Rat spleen	50	70
Rat testis	20	101
Rat brain cortex	20	12, 12, 11*

* Three different brains.

Very remarkable is the feeble recovery shown by brain cortex. We have indeed found that a very short anaerobic period in the absence of glucose will reduce the subsequent glucolysis of this tissue to one-third of the control value, as shown by the following experiment: brain slices were shaken in the bath for 25 minutes in glucose-free Ringer solution with oxygen and CO_2 in the gas space. Then nitrogen-CO_2 mixture was passed through the vessel for 3 minutes, after which glucose was immediately added from a side-bulb. $Q_M^{N_2}$ in the subsequent 30 minutes was 6·2. In a control experiment with glucose under anaerobic conditions from the start, $Q_M^{N_2}$ was 16·6. The 3-minute anaerobic substrate-deprivation thus caused a reduction of the subsequent glucolysis to 37 %. With the same brain a 25-minute preliminary period caused a reduction to 13 %. The preliminary aerobiosis involved in this experiment caused no reduction of the glucolysis; for when glucose was added immediately before nitrogen was passed, $Q_M^{N_2}$ was subsequently actually higher than in the control.

With brain, a preliminary period in absence of glucose has less effect on subsequent respiration in glucose than on subsequent anaerobic glucolysis. Thus a 40-minute preliminary aerobiosis followed by addition of glucose reduced the respiration in phosphate-Ringer solution to 50 % of the value in a control experiment in which glucose was present throughout. A 25-minute preliminary anaerobiosis in absence of sugar also reduced the respiration after glucose addition to about 50 % of the control value. It is remarkable that a preliminary anaerobic substrate-deprivation should affect subsequent respiration in glucose far less than subsequent anaerobic glucolysis.

The presence of 10^{-3} M sodium pyruvate (see below) does not cause brain glucolysis to recover any better from preliminary anaerobic substrate-deprivation.

Effect of pyruvate.

That sodium pyruvate in small concentrations causes an acceleration of the anaerobic glycolysis of brain, intestinal mucous membrane and liver was shown by Mendel, Bauch and Strelitz [1931]. Rosenthal [1932, 1] compared the activation of the anaerobic glycolysis of liver by pyruvate with the activation produced by preliminary aerobiosis, concluding that the two activation processes depend on the presence of the same factor in the liver, but that the "activator" produced by aerobiosis is not identical with pyruvic acid. Pyruvate also produces an activation with Jensen sarcoma. It was found [Dickens and Greville, 1932, 2] that its addition in 10^{-3} *M* concentration to this tissue before the spontaneous increase of anaerobic fructolysis had occurred caused an immediate increase in fructolysis, $Q_M^{N_2}$ thereafter remaining constant at about the value it would have reached by spontaneous activation, whilst addition of pyruvate after the spontaneous increase had no effect at all (Fig. 9). Also, after preliminary substrate-deprivation, an immediate rise of $Q_M^{N_2}$ to the final value occurs on adding glucose in the presence of pyruvate, the intermediate period being abolished (Fig. 3). It is seen from Fig. 6 that the same occurs with the spontaneous activation of testis glucolysis, the intermediate period being abolished in the presence of pyruvate. Thus under those conditions which allow the spontaneous activation to be observed, an identical activation can be caused prematurely on addition of pyruvate.

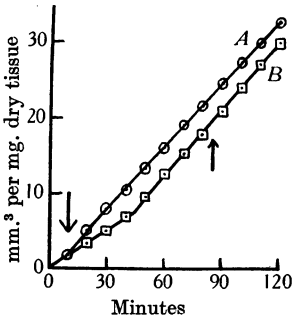

Fig. 9. Anaerobic fructolysis of Jensen rat sarcoma. *A*, pyruvate added at 10 mins. *B*, pyruvate added at 85 mins.

Rosenthal [1932, 2] also found an activation of tumour fructolysis by pyruvate and by certain substances which are reduced by tissues (methylene blue, Lauth's violet, Capri blue and potassium ferricyanide). Rosenthal's results differ from ours in that a greater percentage activation was caused by pyruvate than occurred spontaneously. We, on the other hand, invariably find that pyruvate raises $Q_M^{N_2}$ to practically the same value as is reached by the spontaneous increase, and that pyruvate addition after the occurrence of that increase has no effect. Further, pyruvate addition has no effect on the fructolysis or glucolysis of the Mill Hill tumour, which fails to show spontaneous activation. Nevertheless pyruvate definitely has an effect on other tissues in which spontaneous activation has not been observed. Thus with pyruvate present from the start Mendel, Bauch and Strelitz [1931] observed with brain increases of $Q_M^{N_2}$ up to three times the control values. We have, however, found that addition of 10^{-3} *M* pyruvate to brain after it has been producing lactic acid for some time in the thermostat may merely prevent the glucolysis from falling. Again, with testis (Fig. 6) pyruvate merely prevented $Q_M^{N_2}$ from falling from its initial value, and the addition of pyruvate simultaneously with the glucose after preliminary substrate-deprivation brought $Q_M^{N_2}$ to the same value. With spleen, addition of 10^{-3} *M* pyruvate did not increase the glucolysis (see also Fig. 7). Thus in our experience the effect of pyruvate addition is most often to keep the tissue at its maximum glycolysis. Liver must be regarded as an exception to this: in this tissue pyruvate, aldehyde or a suitable oxidising agent must be present in order that a glycolysis of any considerable magnitude may be attained [Rosenthal, 1932, 1]. One of the factors necessary for the attainment of the maximum glycolysis

seems to be the establishment of a suitable reducible system and possibly of a redox potential in the right range. Most tissues seem to be capable of creating these optimum conditions themselves, but with surviving liver some external agency is necessary.

SUMMARY.

1. The alteration of the glycolysis of tissues by methods not involving the addition of substances foreign to the animal body has been studied.

2. The inhibitory effect of lactates on the anaerobic glucolysis of brain, testis and tumour is very variable. *l*-Lactate has about half the effect of the natural *d*-lactate.

3. After 20 minutes' preliminary anaerobic substrate-deprivation the glucolysis of brain recovers only to about 12 % of the normal value, whilst with the other tissues tested (testis, spleen, rat yolk-sac, tumours) the recovery is almost always complete. With brain, preliminary anaerobic substrate-deprivation affects the glucolysis far more than the respiration.

4. The method of preliminary anaerobiosis in absence of added substrate has been applied to the detection of spontaneous activation of anaerobic glycolysis in various tissues. This has been observed only in the glucolysis and fructolysis of the Jensen rat sarcoma and in the glucolysis of rat testis. It is not supposed however that the potentiality for spontaneous activation exists only in these tissues.

5. The effect of small quantities of pyruvate on the glycolysis of tissues is discussed. In tissues and under conditions in which spontaneous activation is observed, an equal activation is achieved on addition of pyruvate. The view is expressed that the presence of pyruvate tends to keep the glycolysis of tissues at its maximum value.

It is a pleasure to acknowledge the interest taken by Prof. E. C. Dodds in these experiments.

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