CXXXVIII. STUDIES IN TISSUE METABOLISM.

VI. LACTIC DEHYDROGENASE, XANTHINE OXIDASE AND NUCLEOSIDASE IN TUMOUR AND MUSCLE EXTRACTS.

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IN a previous communication [Boyland and Boyland, 1934] two distinct lactic dehydrogenases were described, one present in yeast and one in mammalian muscle. Tumour tissue (Jensen rat sarcoma) extracts made by the method previously used with muscle reduced methylene blue rapidly in the presence of lactic dehydrogenase coenzyme (prepared after Banga *et al.* [1932]) in the absence of any other substrate. Extracts of J.R.S. were also made by the method of freezing and thawing described by Boyland and Mawson [1934] with similar results. No amount of washing of the tissue or dialysis of the extract appeared to reduce the activity. In some cases the time of reduction of methylene blue was slightly decreased by the addition of lactate but the differences appeared to bear no relation to the time of dialysis, which precluded the possibility that the extract was acting on some dialysable substrate which it contained. Similarly extracts of rat and mouse muscle contained some lactic dehydrogenase but also reduced methylene blue without the addition of lactate. Some typical results for muscle extracts are given in Table I. All the results described in this paper were obtained by observation of the time necessary to decolorise methylene blue solution in evacuated Thunberg tubes at 38°.

Table I. *Reduction of methylene blue by frozen muscle extracts.*

1 ml. extract, 0.5 ml. $M/15 \text{ Na}_2\text{HPO}_4$, 0.5 ml. substrate + water, 0.5 ml. $1/5000$ methylene blue p_{H} 8.0–8.2.

Muscle	Rat skeletal		Rat heart		Mouse skeletal		Rabbit heart		Rabbit skeletal	
	T	Activity	T	Activity	T	Activity	T	Activity	T	Activity
Additions	mins.	(100/T)	mins.	(100/T)	mins.	(100/T)	mins.	(100/T)	mins.	(100/T)
None	> 180	0.0	> 800	0.0	> 600	0.0	> 180	0.0	> 180	0.0
Lactate ($M/50$)	> 180	0.0	—	—	> 600	0.0	> 180	0.0	> 180	0.0
Coenzyme (0.4 mg.)	29	3.4	24	4.2	70	1.7	> 180	0.0	> 180	0.0
Coenzyme + lactate	18	5.5	1½	66.0	36	2.8	17	5.9	16	6.2
Adenyl pyrophosphate ($M/1000$)	22	4.3	16	6.2	90	1.1	> 180	0.0	> 180	0.0
Hypoxanthine ($M/6000$)	19	5.2	16	6.2	35	2.9	> 180	0.0	> 180	0.0

It can be seen that extracts of rabbit heart or skeletal muscle will not decolor methylene blue in the presence of coenzyme without added lactate.

Extracts of some tumours (carcinoma 63, sarcoma 37, Crocker 180 and Mal sarcoma of mice and rat sarcoma R. 10) gave results comparable with those obtained with muscle, *i.e.* a lactic dehydrogenase was present but its effect was partly masked by the presence of another enzyme system. It will be shown that

this could be adenine nucleosidase and xanthine oxidase. The optimum reaction for the lactic dehydrogenase of heart muscle is at about p_H 9, while at p_H 7 the enzyme has about half the optimum activity [Boyland and Boyland, 1934]. Experiments showing the effect of p_H on a tumour extract are given in Table II and Fig. 1.

Table II. *Reduction of methylene blue by extracts of frozen J.R.S.*

	p_H	Coenzyme mg.	Lactate ($M/50$)	T mins.	Activity (100/T)
Extract 1	6.0	0.4	—	5	20.0
	6.0	0.4	+	$8\frac{1}{2}$	12.0
	8.5	0.4	—	7	14.0
	8.5	0.4	+	$5\frac{1}{2}$	18.0
Extract 2	8.0	0.4	—	9	11.0
	8.0	0.4	+	8	12.0
	8.0	0.1	—	90	1.1
	8.0	0.1	+	34	2.9

Fig. 1. Reduction of methylene blue by extract of frozen J.R.S. In each tube 1 ml. extract, 1 ml. $M/15$ phosphate, adenylypyrophosphate ($M/1000$), and 1 ml. $M/5000$ methylene blue.

Table II also shows the effect of adding small quantities of coenzyme, sufficient to activate the lactic dehydrogenase but barely enough to act as substrate for xanthine dehydrogenase, thus revealing the effect of lactic dehydrogenase. With sarcomata 37, Crocker 180 and Mal¹ the proportion of the lactic dehydrogenase to the nucleosidase-xanthine oxidase systems is much higher as shown in Table III.

The rate of oxidation of hexosediphosphate in the presence of coenzyme was determined in some extracts and was found to be similar to that of lactic acid in all experiments. It is therefore not tabulated in Table III.

Xanthine oxidase.

The addition of hypoxanthine solution (final concentration 1 in 2000) to the tissue extracts produced decoloration of methylene blue in all cases except those of rabbit skeletal and heart muscle. Figures for xanthine oxidase activity are given in Tables I and III under hypoxanthine.

A measure of the relative amounts of the various enzyme systems is given by the figures in Tables I and III for the "activity" (100/T) with the corresponding substances. The figure for lactic dehydrogenase (column headed lactate

¹ A tumour produced in mice by Mr H. Burrows by subcutaneous injection of sodium 1:2:5:6-dibenzanthracene-9:10-endo- α -succinate [Cook, 1931].

Table III. *Activity of frozen tumour extracts.*

1 ml. extract, 0.5 ml. $M/15$ Na_2HPO_4 , substrate and water 0.5 ml.,
0.5 ml. 1/5000 methylene blue.

In all cases the reduction times with extract alone, and with lactate+extract were long compared with the other times.

(a) and (b) refer to extracts of different batches of tumours.

Substrate Tumour		Activity 100/T		
		Lactate $M/50$	Adenylpyro- phosphate $M/1000$	Hypoxanthine $M/6000$
J.R.S.	(a)	0.0	12.5	33.0
	(b 1)	4.4	20.0	20.0
	(b 2)*	2.2	17.0	18.0
R. 10	(a)	0.0†	17.0	18.2
	(b)	0.0	9.5	15.4
Crocker 180	(a)	3.5	3.2	4.5
	(b)	12.0	5.0	8.0
C 63	(a)	0.9	2.7	10.0
	(b)	0.0	1.4	6.0
S 37	(a)	19.0	12.0	17.0
	(b)	16.0	12.0	12.0
Mal. sarcoma	(a)	3.9	1.0	3.2
	(b)	12.2	6.0	6.0

* Second extraction of same tumour. † With half quantity of coenzyme 100/T was 0.4.

$M/50$) in Table III was obtained by subtracting the "activity" with coenzyme alone from that with coenzyme+lactate. Zero activity therefore implies that the reduction times with coenzyme, with and without lactate, were the same. In some cases the times were actually longer with lactate than without. This may be due to a protection of the coenzyme by lactate. The figures for lactic dehydrogenase are therefore minimum values. The same effect is shown with adenylpyrophosphate and lactate or hexosediphosphate in Table IV.

Table IV. *Reduction of methylene blue by extracts of Mal. sarcoma.*

Additions	Extract 1		Extract 2	
	T mins.	Activity (100/T)	T mins.	Activity (100/T)
Adenylpyrophosphate ($M/1000$)	14	7.2	16	6.2
Adenylpyrophosphate+lactate ($M/50$)	19	5.3	39	2.5
Adenylpyrophosphate+hexosediphosphate ($M/50$)	27	3.7	38	2.6
Coenzyme (0.4 mg.)	27	3.7	37	2.7
Coenzyme+lactate	7	14.0	16	6.2
Coenzyme+hexosediphosphate	6½	15.0	16	6.2

Table IV also shows that adenylpyrophosphate has no activity as lactic dehydrogenase coenzyme. This has been confirmed by testing with the lactic dehydrogenase preparation of rabbit muscle, which contains no xanthine oxidase (see Table I) and does not decolorise methylene blue in the presence of adenylpyrophosphate alone.

Nucleosidase.

Szent-Györgyi's coenzyme, adenylpyrophosphate (prepared according to Lohmann [1932]) and adenylic acid reduced methylene blue in the presence of tumour extracts, but not with rabbit muscle extracts. The effect is probably

due to the combined actions of a nucleosidase (adenine nucleosidase) and xanthine oxidase. It cannot be due to the latter enzyme acting with an impurity in the preparations, for there is a definite optimum p_H at about 7.4 (see Fig. 1), whereas xanthine oxidase has an almost constant activity between p_H 6.5 and 8.5. Levene *et al.* [1924] have shown that nucleosidase has an optimum at p_H 7.5.

A minimum figure for the nucleosidase activity of the extracts is given by the activity in the presence of adenylypyrophosphate, for the enzyme must act on this before the dehydrogenation can occur. That the limiting factor is often the amount of xanthine oxidase present can be seen from the fact that the reduction times are in many cases nearly the same for hypoxanthine and adenylypyrophosphate. On the other hand milk xanthine oxidase reduces methylene blue 100 times more rapidly in the presence of hypoxanthine than in the presence of adenylic acid [Dixon and Lemberg, 1934]. Rabbit muscle extracts do not reduce methylene blue with adenylypyrophosphate or hypoxanthine. The lack of activity with adenylypyrophosphate may be due to the absence of xanthine oxidase only and not of nucleosidase.

Incubation of rabbit muscle extract with coenzyme for 50 minutes did not however destroy the latter, for its power of acting as lactic dehydrogenase coenzyme was unimpaired. If adenine nucleosidase is present it must therefore be only in relatively small amounts.

DISCUSSION.

The foregoing experiments show that the tissue of the tumours examined contains lactic dehydrogenase, which requires a coenzyme for its action and shows optimum activity in alkaline solutions. It therefore appears to be identical with the enzyme present in mammalian muscle. Many tumour extracts however oxidise the coenzyme so rapidly that the lactic acid oxidation is not readily observed. Rat and mouse muscle extracts resemble the tumour extracts to some extent in this respect as they reduce methylene blue in the presence of coenzyme or adenylypyrophosphate. In this they differ markedly from rabbit muscle extract which does not oxidise the coenzyme, nor does it contain xanthine oxidase. It is possibly for this reason that Meyerhof found that rat muscle did not give glycolytically active extracts, as his first experiments were made without addition of coenzyme or adenylypyrophosphate. Boyland and Mawson [1934] were able to prepare glycolytically active rat muscle extracts by adding sufficient adenylypyrophosphate to compensate for its destruction.

Tumour extracts generally attack adenylypyrophosphate and lactic dehydrogenase coenzyme even more rapidly than does rat muscle extract. There is a large variation in the rate of attack with different tumour extracts. Extracts of J.R.S. oxidise the coenzyme so rapidly that it is difficult to determine the lactic dehydrogenase activity with any degree of accuracy. On the other hand some extracts of rapidly growing mouse tumours do not react so rapidly with the coenzyme and therefore show lactic dehydrogenase activity.

SUMMARY.

1. Rat, mouse and rabbit muscle and mouse and rat tumours (C 63, S 37, Crocker 180, Mal. sarcoma, R. 10 and J.R.S.) contain lactic dehydrogenase similar to that found in heart muscle.

2. Rabbit muscle extract does not contain xanthine oxidase or adenine nucleosidase but rat and mouse muscle extracts contain both these enzymes.

3. All the rat and mouse tumour extracts examined contain xanthine oxidase and attack adenylypyrophosphate or lactic dehydrogenase coenzyme rapidly. The rate at which the adenine derivatives are attacked varies with different tumour strains.

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