XXXV. STUDIES IN TISSUE METABOLISM.

VIII. THE EFFECT OF FUMARATE AND SUCCINATE ON TUMOUR RESPIRATION.

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SZENT-GYÖRGYI [1935] has recently emphasised the importance of dibasic acids containing four carbon atoms in the respiration of muscle. He suggests that the reaction $\text{fumarate} \rightleftharpoons \text{oxaloacetate}$ is an essential part of a chain of reactions which brings about the oxidation of foodstuffs in tissues. Oxaloacetate is in part reduced to succinate, which is rapidly converted into fumarate by the powerful succinic dehydrogenase. In the presence of malonate the reaction $\text{succinate} \rightarrow \text{fumarate}$ is inhibited so that the fumarate disappears; the rate of respiration is correspondingly reduced. In most respects Szent-Györgyi's observations on washed pigeon muscle have been repeated upon the mammalian tumours which we have examined. Malignant tissue therefore seems to resemble muscle, kidney and liver in using C_4 dibasic acids as oxygen carriers.

EXPERIMENTAL.

The tumours used were the Jensen rat sarcoma (JRS) and Crocker mouse sarcoma 180. The tissue was sliced by Warburg's method and, in the case of "minced tissue", the slices were minced with scissors to an average size $< 0.5 \times 1 \times 1$ mm. 0.2–0.3 g. moist tissue was placed in the Warburg vessels with 2 ml. $M/15$ phosphate (p_H 7.3) containing, on occasion, glucose or lactate. After 30 min. measured respiration substrates were tipped in (as solutions of Na salts) from the side arms and measurement continued for 1 hour. Q_{O_2} during this hour was expressed as a percentage of Q_{O_2} during the first 30 min. In calculating Q_{O_2} values the dry weight was taken as 1/10 wet weight.

Respiration was increased by addition of succinate or fumarate and decreased by addition of malonate (Table I), but these effects were greater with minced

Table I. *The effects of succinate, fumarate, malate and malonate on oxygen uptake in the absence of other substrates.*

Tissue	Substrates	Initial Q_{O_2}		% of initial Q_{O_2}	
		Slices	Minced tissue	Slices	Minced tissue
Crocker 180 (Exp. I)	—	6.6	6.2	38	53
"	Succinate 4 mg.	6.2	6.7	40	77
Crocker 180 (Exp. II)	—	—	5.7	—	72
"	Succinate 4 mg.	—	5.7	—	95
"	Fumarate 4 mg.	—	6.2	—	82
"	Malonate 2 mg.	—	5.8	—	48
"	Malonate 2 mg. + fumarate 4 mg.	—	5.4	—	71
JRS	—	10.4	10.3	77	70
"	Succinate 4 mg.	9.4	10.1	91	109
"	Fumarate 4 mg.	10.6	9.8	86	89
"	Malate 3.5 mg.	10.2	10.5	80	82
"	Malonate 2 mg.	10.2	10.2	53	40
"	Malonate 2 mg. + fumarate 4 mg.	10.4	9.6	74	69

than with whole slices. The effects were observed irrespective of the presence of glucose or lactate (Table II). This agrees with the suggestion that the C_4 dibasic acids function as oxygen carriers.

Table II. *The effects of succinate, fumarate and malonate on oxygen uptake in presence of glucose or lactate.*

(a) Minced JRS, (b) minced JRS with 4 mg. glucose,
(c) minced Crocker 180 with 4 mg. lactate.

Substrates	Initial Q_{O_2}			% of initial Q_{O_2}		
	a	b	c	a	b	c
—	7.8	8.3	5.8	77	90	62
Succinate 4 mg.	7.6	8.1	6.0	102	112	105
Fumarate 4 mg.	8.2	7.9	6.2	84	98	78
Malonate 2 mg.	7.9	8.0	—	61	67	—
Malonate 2 mg. + fumarate 4 mg.	7.8	8.1	—	74	85	—

In experiments using fumarate and malonate, it was found that though fumarate might counteract the inhibitory effect of malonate by stimulating respiration up to the control value, this value was not exceeded, whereas alone it usually increased the oxygen uptake above that of the control. Since this might be due to the use of insufficient fumarate, experiments were repeated at higher concentrations of fumarate. Table III shows that relatively high fumarate con-

Table III. *The effects of varying fumarate concentrations on oxygen uptake of minced Crocker 180 in the presence of malonate.*

Substrates	Initial Q_{O_2}	% of initial Q_{O_2}
—	7.0	74
4.5 mg. fumarate	6.8	86
2 mg. malonate	7.3	51
2 mg. malonate + 1.5 mg. fumarate	6.3	66
2 mg. malonate + 4.5 mg. fumarate	6.4	78
2 mg. malonate + 15 mg. fumarate	6.7	86

centrations were necessary to restore the respiration in the presence of 2 mg. malonate. The effect of adding C_3 monobasic acids corresponding to succinic, fumaric and malic, *i.e.* propionic, acrylic and lactic acids, was also investigated. Table IV shows that the monobasic acids produced no effects comparable with those of succinic and fumaric acids.

Table IV. *Comparison of the effects of propionate, acrylate and lactate with those of succinate, fumarate and malate on the oxygen uptake of minced JRS.*

Substrates	Initial Q_{O_2}	% of initial Q_{O_2}	Substrates	Initial Q_{O_2}	% of initial Q_{O_2}
—	8.5	69	—	8.5	69
Propionate 1.8 mg.	8.8	66	Succinate 4 mg.	8.2	95
Acrylate 1.8 mg.	8.5	67	Fumarate 4 mg.	8.3	84
Lactate 2.4 mg.	7.9	66	Malate 4.4 mg.	8.5	61

Annau [1935] found that minced liver or kidney tissue produced acetone bodies in the presence of malonate. Similar accumulation of acetone bodies in the presence of malonate has now been shown (Table V) with the minced tumour slices (JRS or Crocker) in phosphate containing glucose. The nitroprusside

Table V. *Nitroprusside reactions.*

Substrates	JRS		Crocker 180
	Acetone bodies	Pyruvate	Acetone bodies
—	—	+	—
Malonate 2 mg.	+	—	+
Fumarate 4 mg.	—	+	—
Fumarate 4 mg. + malonate 2 mg.	—	+	—
Succinate + malonate	+	—	+

tests were performed after 2 hours' incubation with 0.25 g. tumour. Where acetone bodies were not formed by JRS tissue, pyruvate (as indicated by a blue coloration) was detected.

DISCUSSION.

This investigation shows that respiration of isolated tumour slices may be increased by addition of succinate or fumarate. The usual method of preparing tissue slices [Warburg, 1930] not only produces mechanical injury but also facilitates diffusion from the cells of essential substances such as oxygen carriers, which may thus become diluted beyond their effective or optimum concentration. The smaller the pieces of tissue, the more likely this is. Yet if the tissue slices are not sufficiently thin it is impossible for them to have enough oxygen for their needs when suspended *in vitro*.

Druckrey [1935] has suggested that injury is responsible for aerobic glycolysis of tumour slices. It seems important to point out that loss of oxygen carriers by diffusion would, by reducing the effective respiration, tend to increase aerobic glycolysis.

SUMMARY.

Respiration of tumour tissue (JRS and Crocker 180) is increased by addition of succinate or fumarate and decreased by addition of malonate. In this respect tumour resembles muscle, kidney and liver. The possibility that loss of readily diffusible oxygen carriers might affect the respiration of tumour slices is discussed.

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