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STUDIES ON THE METABOLISM OF ORAL TISSUES. THE PRESENCE OF A CITRIC ACID CYCLE IN PALATAL MUCOSA OF NORMAL AND VITAMIN D DEFICIENT RATS

by

L. MENDELSOHN

Joint Dental Research Unit of the C.S.I.R. and the University of the Witwatersrand, Johannesburg

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Until recently the tissues of the oral cavity in mammals were studied mainly by means of histochemical techniques. Biochemical methods have only been applied during the last decade in an attempt to elucidate the metabolic behaviour of these tissues. Glickman *et al.* [1949, 1950] reported that the Qo_2 of normal human and dog gingiva was $1 \cdot 6 \pm 0 \cdot 37$ and $1 \cdot 3 \pm 0 \cdot 07$ respectively. Schrader and coworkers [1957] observed that the Qo_2 of human interdental papilla was $1 \cdot 77 \pm 0 \cdot 16$. Oral tissues have also been shown to contain the following oxidative enzymes:— pyruvic, citric, succinic and malic dehydrogenases [Pincus, 1951]; DPNH-cytochrome c reductase, cytochrome c oxidase, catalase, lactic dehydrogenase and glucose-6-phosphate dehydrogenase [Eichel, 1960].

Recently, Zar and Irving [1958] noted that the Qo_2 of sheep gingiva was $1 \cdot 46 \pm 0 \cdot 33$. In addition, the presence of a complete citric acid cycle was demonstrated in this tissue. This work has now been extended to include the oral tissues of the rat in a similar manner. As part of a general study dealing with the effects of vitamin deficiencies on oral tissues, vitamin D was the first vitamin chosen for investigation. In the present communication it will be shown that a citric acid cycle exists in rat palatal mucosa of both normal and vitamin D deficient animals.

MATERIALS AND METHODS

Dietary regimen

Throughout these studies rats of the Wistar Institute strain were used. Control animals were fed the stock diet of the colony ad libitum and were housed in sunlit quarters.

Rachitogenic diet

Experimental animals were placed on the rachitogenic diet when they weighed 60-80 g. Rickets was induced by feeding these rats a modified Sherman-Pappenheimer diet [Jephcott and Bacharach, 1926]. This diet consisted of:— white flour 85%, egg albumen 10%, calcium lactate 2·8%, iron citrate 0·2% and sodium chloride 2·0%. The animals were given food and water ad libitum. In addition each rat received 1 g. of carrot per day. The animals were reared for 21 days on this diet while housed in separate cages in a room impervious to sunlight. This diet proved most satisfactory, since the rats gained weight rapidly and rickets never failed to develop.

Method of obtaining experimental tissue

Rats were killed by an intraperitoneal injection of 5% Thiopentone Sodium. The palatal mucosa was removed and placed in chilled Krebs-Ringer solution. Since the amount of oral palatal mucosa in each rat was very small, tissues taken from 4-10 rats were pooled depending upon the experimental requirements. A portion of the pooled tissues was used immediately and the remainder stored in Krebs-Ringer solution at 4°C until needed.

Manometric measurements

The direct method of Warburg was used to determine the oxygen consumption of the tissue slices, the procedure and solutions were the same as described by Umbreit *et al.* [1957].

RESULTS

Table I summarizes the basal Qo_2 values (μl. oxygen taken up/mg. dry weight/hour) of normal and rachitic rat palatal mucosa respectively. No significant difference was observed in the endogenous oxygen consumption of both normal and rachitic oral tissues. Upon storage of the palatal mucosa in cold Krebs-Ringer solution a diminution in Qo_2 values was noted in both types of tissue. The reason for including figures for the oxygen consumption of tissues which had been stored for up to three days is mentioned below.

TABLE I
Basal Qo_2 values of normal and rachitic rat palatal mucosa.

Days stored	Qo_2	
	Normal	Rachitic
0	1·96 ± 0·29 (50)	1·92 ± 0·20 (15)
1	1·39 ± 0·30 (20)	1·23 ± 0·17 (12)
2	0·68 ± 0·23 (12)	0·65 ± 0·23 (10)
3	0·59 ± 0·12 (10)	0·60 ± 0·15 (10)

To the main compartment of each Warburg flask was added 2 ml. Krebs-Ringer phosphate solution, pH 7·4; 20-30 mg. wet weight tissues. The actual Qo_2 given represents the mean value of the observations. This is followed by the standard deviation. Figures in brackets indicate number of determinations.

The action of citric acid cycle intermediates on the oxygen uptake of rat palatal mucosa from normal and vitamin D deprived animals is indicated in Table II. It is evident that not all the components of the citric acid cycle significantly enhanced the oxygen consumption of fresh palatal mucosa of both normal and rachitic animals. However, after storage at 4°C a noteworthy increase in the Qo_2 compared with control values was observed in both types of tissue on the addition of each member of the citric acid cycle as substrate. This effect persisted for up to three days which was the longest time the tissues were stored. Therefore, to determine whether this tissue was in fact able to metabolize citric acid cycle components it became necessary to use stored material.

TABLE II

Effect of intermediates of the citric acid cycle on the Qo_2 of normal and rachitic rat palatal mucosa.

Metabolite added	Days stored (Qo_2)			
	0	1	2	3
	Normal	Normal	Normal	Normal
Control	1·96 ± 0·29 (50	1·39 ± 0·3 (20)	0·68 ± 0·23 (12)	0·59 ± 0·12 (10)
0·02M Pyruvate	2·45 (4)	—	1·95 (3)	1·87 (4)
0·01M Oxalacetate	2·51 (4)	—	—	1·18 (4)
0·02M Citrate	2·01 (8)	2·64 (3)	1·30 (6)	1·69 (5)
0·02M Aconitate	2·34 (5)	—	1·46 (4)	1·08 (3)
0·04M Isocitrate	2·84 (4)	—	1·27 (4)	0·96 (5)
0·04M Oxalsuccinate	1·73 (5)	—	1·01 (3)	1·70 (3)
0·02M α-ketoglutarate	1·69 (5)	—	1·24 (4)	1·47 (4)
0·02M Succinate	3·34 (7)	2·58 (3)	1·98 (5)	1·59 (5)
0·02M Fumarate	1·88 (5)	—	1·32 (3)	1·10 (3)
0·02M Malate	1·94 (5)	1·77 (3)	1·53 (4)	1·44 (3)

Metabolite added	Days stored (Qo_2)			
	0	1	2	3
	Rachitic	Rachitic	Rachitic	Rachitic
Control	1·92 ± 0·2 (15)	1·23 ± 0·17 (12)	0·65 ± 0·23 (10)	0·60 ± 0·15 (10)
0·02M Pyruvate	2·47 (5)	1·85 (4)	1·96 (3)	1·22 (3)
0·01M Oxalacetate	1·73 (3)	1·61 (3)	—	—
0·02M Citrate	2·02 (9)	2·94 (6)	2·46 (4)	2·39 (3)
0·02M Aconitate	2·50 (4)	—	—	—
0·04M Isocitrate	1·91 (4)	1·90 (3)	—	—
0·04M Oxalsuccinate	1·76 (5)	1·70 (3)	—	—
0·02M α-ketoglutarate	1·84 (4)	1·43 (3)	—	—
0·02M Succinate	2·43 (4)	3·80 (3)	2·16 (3)	2·81 (3)
0·02M Fumarate	1·72 (3)	1·73 (3)	—	—
0·02M Malate	2·54 (4)	2·16 (4)	2·50 (3)	1·32 (3)

The main compartment of each flask contained 1 ml. Krebs-Ringer phosphate solution, pH 7·4; 1 ml. of the sodium salt of substrate, the final concentration of which is indicated in the Table; 20-30 mg. wet weight tissue. The control flask contained 20-30 mg. wet weight tissue in 2 ml. Krebs-Ringer phosphate solution. Figures in brackets indicate number of observations.

The action of inhibitors of the citric acid cycle on oxygen consumption of normal and rachitic oral tissue is shown in Table III. Both malonate and fluoracetate were found to depress the respiration of fresh and stored normal and rachitic rat palatal mucosa.

TABLE III

The effect of inhibitors of the citric acid cycle on the Qo_2 of normal and rachitic rat palatal mucosa.

Type of tissue	Inhibitor added	Qo_2	
		Days stored	
		0	2
Normal rat Mucosa	Control	1·96 ± 0·29 (50)	0·68 ± 0·23 (12)
	0·02M Malonate	0·82 (4)	0·39 (3)
	0·001M Fluoracetate	0·599 (3)	—
Rachitic rat Mucosa	Control	1·92 ± 0·2 (15)	0·65 ± 0·25 (10)
	0·001M Fluoracetate	0·743 (4)	0·25 (3)

The contents of each flask were the same as described in Table II.

DISCUSSION

Contradictory reports have appeared in the literature concerning the comparison between basal Qo_2 values of normal and rachitic rat tissues. Presnell [1937] reported that the Qo_2 of skin in rachitic rats was approximately 30% lower than that of control rats. These findings were not confirmed by Klungsyör and Pihl [1953] who found no difference in the oxygen consumption of rat skin obtained from animals with well developed rickets and those from a vitamin D supplemented control group. The latter authors suggested that the dissimilarity between the two sets of results might be due to the fact that a different diet was used to induce rickets in each case. In the present investigation no significant difference was detected in the basal Qo_2 values of normal and rachitic rat palatal mucosa. It is interesting to note in this regard that the rachitogenic diet employed in this study was similar to the one used by Klungsyör and Pihl [1953]. The Qo_2 of fresh rat oral tissue was found to be of the same order as that given in the literature for man (1·6 ± 0·37) and sheep (1·46 ± 0·33). Glickman [1950] reported a value of 1·3 ± 0·07 for dog gingiva, a result significantly lower than that demonstrated for rat gingiva in the present study. On recalculating the figures presented in Glickman's paper, however, it would seem that the basal Qo_2 of human, rat, sheep and dog oral tissue is not in fact different.

From Table II it is apparent that not all the intermediates of the citric acid cycle significantly enhanced the oxygen consumption of fresh palatal mucosa from both normal and rachitic animals. However, after storage at 4°C all members of the citric acid cycle were metabolized by both types of tissue, the effect persisting for up to three days. Although as noted in Table I, the basal Qo_2 of both normal and rachitic oral tissues dropped steadily on storage, the addition of each metabolite of the citric acid cycle caused approximately a 50% increase in the respiratory activity of the palatal mucosa. In a number of instances a much larger stimulation of oxygen consumption was observed in both tissues. Similar observations have been made in connection with the action of thyroxine and related compounds on the oxygen consumption of kidney slices from thyroidectomized rats. Thibault [1955] noted that neither thyroxine nor triiodothyronine exerted an immediate effect on the respiration of rat kidney slices suspended in Tyrode buffer. After three days storage at 4°C an appreciable rise in oxygen consumption was observed. Barker [1956] also reported that after storing kidney slices from thyroidectomized rats for one to three days

thyroxine increased the oxygen uptake when compared with pre-incubation values. The reason for this phenomenon is at present obscure.

From the elevation and depression of the oxygen consumption noted on the addition of intermediates and inhibitors of the citric acid cycle respectively, it is suggested that a tricarboxylic acid cycle is present and functioning in rat palatal mucosa of both normal and vitamin D deficient animals.

SUMMARY

Basal Qo_2 values for normal and rachitic rat palatal mucosa are reported. They were found not to differ significantly.

Evidence is presented for the occurrence of a citric acid cycle in the palatal mucosa of both normal and vitamin D deficient rats.

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