Studies on Hypoxia
IV. Differential Response of Respiratory Enzymes in Various Organs of Adult Rats (33715)

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In the course of studying the acute effect of anoxia in fetal and neonatal subjects we found that the incorporation of labeled amino acids into proteins was suppressed in a variety of cells (1, 2). With respect to the acute effect on organs from adult animals several studies have indicated that the protein synthesis was reduced in the liver, pancreas, etc. (3, 4). However, these studies were performed in an atmosphere which contained varying concentrations of oxygen and few employed a totally anoxic environment.

As preliminary effort for studying the effect of total anoxia or protein synthesis in adult organs we decided to probe the possible modifications of respiratory enzyme activities, inasmuch as the results from previous studies indicated that the change in respiratory enzyme activities is a sensitive indicator of metabolic alterations induced by hypoxic treatment (5–9). The objective of this study, therefore, was to study the effect of acute anoxia on succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH) activities in digestive glands and certain other organs that are sensitive to changes in oxygen tension.

Materials and Methods. Animals. Male adult Sprague-Dawley rats, weighing 200–300 g were used. Three days prior to the exposure to anoxia, adrenalectomy was performed in each animal so that the interference of altered corticosteroid output might be minimized. Subsequent to adrenalectomy daily injection of hydrocortisone *tert*-butylacetate (Hydrocortone-TBA, Merck, Sharp and Dohme) in the dose of 5 μg/g of body weight was given. Sodium chloride was added to the drinking water to make a 1% solution.

Anoxic conditions. The adrenalectomized rats were subjected to total anoxia for a period of 6 min by placing them individually into a bell jar flushed with a continuous flow of purified nitrogen. Two min each of anoxic and rest periods were alternated to prevent an early death of animals. The oxygen content in the jar during the anoxia, monitored by using a Westinghouse oxygen analyzer, was in the order of 30–40 ppm. The control animals were treated identically, except that a continuous flow of fresh air was made available. Animals were sacrificed in pairs under light ether anesthesia while tissues were excised. Pieces of the heart, kidney, liver, pancreas, parotid, and submandibular gland were rapidly dissected, sliced to a thickness of approximately 3 mm, and frozen immediately on a slab of dry ice. These were kept in a dry ice chest until analysis of enzymes which was carried out the same day.

Enzyme analyses. The tissues were homogenized manually in a Dounce homogenizer after addition of 0.1 ml of distilled water to 2 mg of tissue for LDH and 5 mg of tissue for SDH, respectively. The homogenate was centrifuged and supernatant was collected. The supernatant solutions used for LDH determination were diluted 10 times with distilled water.

The reagents employed in these analyses were essentially the same as those described elsewhere (10–12). In LDH analyses each cuvette contained the following: 0.5 ml of 0.36 *M* lactate, 0.2 ml of 0.05 *M* NAD, 1.8 ml of 0.05 *N* Tris–HCl buffer at pH 8.9, and 0.5 ml of the tissue homogenate. For SDH analyses each cuvette contained: 0.3 ml of 0.1 *M* potassium cyanide at pH 7.0, 0.3 ml of 0.01 *M* potassium ferricyanide, 0.2 ml of 0.2 *M* sodium succinate, 2.0 ml of 0.3 *M* Tris buffer with pH 7.6, and 0.2 ml of the tissue homogenate. In both cases the homogenate was added at zero time. The reduction of

TABLE I. Immediate Effects of Anoxia in Rats.[a]

Organ	Treatment	Succinate dehydrogenase: No. of animals	Av[b] (±SD)	Significance	Lactate dehydrogenase: No. of animals	Av[b] (±SD)	Significance
Heart	Control	10	3.44 (0.51)	$p < 0.001$	11	39.06 (6.51)	$p < 0.154$
	Anoxic	10	2.36 (0.58)		11	34.97 (6.39)	
Kidney	Control	11	2.26 (0.55)	$p < 0.099$	11	17.02 (3.75)	$p < 0.479$
	Anoxic	11	1.87 (0.50)		11	15.84 (3.90)	
Liver	Control	12	1.40 (0.22)	$p < 0.270$	9	59.14 (16.44)	$p < 0.151$
	Anoxic	12	1.28 (0.29)		9	49.09 (11.23)	
Pancreas	Control	10	0.28 (0.21)	$p < 0.014$[c]	9	12.21 (2.21)	$p < 0.509$
	Anoxic	9	0.07 (0.07)		9	13.28 (4.16)	
Parotid gland	Control	11	0.25 (0.06)	$p < 0.587$	9	11.24 (5.21)	$p < 0.533$
	Anoxic	11	0.26 (0.09)		9	9.95 (3.05)	
Submandibular gland	Control	12	0.81 (0.15)	$p < 0.253$	10	15.05 (2.74)	$p < 0.096$
	Anoxic	12	0.89 (0.18)		10	17.08 (2.40)	

[a] Male adult rats, adrenalectomized 3 days prior to anoxia and given daily injection of Hydrocortone T.B.A. in the dosage of 0.5 mg/100 g of body weight.

[b] Expressed in terms of mμmoles of succinate or lactate converted/min/mg of wet tissue.

[c] Equality of variance was rejected by Bartlett's test; a two-sample t test with unequal variances was employed.

ferricyanide was recorded at 400 mμ for SDH, and the production of NADH was measured at 340 mμ for LDH. The kinetics of enzyme activities were recorded with a Perkin-Elmer UV-visible spectrophotometer (model 202). This was equipped with a multiple cuvette carrier which was maintained at 25°. The linear change for the first 8 min was used for determination of the SDH activity. After conversion of the changes in optical density into the amount of substrate catabolized, the data were subjected to the Student's t test in a program provided by the University of Michigan Terminal System, IBM 7090.

Results. Recorded in Table I are the mean changes in enzyme activity for both SDH and LDH. These values represent the amount of substrate catabolized in millimicromoles per minute per milligram of wet weight of the tissue. In the control animals SDH activity was highest in the heart, followed by kidney, liver, and submandibular gland with the pancreas and parotid gland showing less than 10% of the activity found in the cardiac tissue. On the other hand, LDH activity in the control tissues showed the highest value in the liver, followed by the heart, kidney, and submandibular gland. The pancreas and parotid gland had the least activity, showing only about 20% of that exhibited by the liver.

Immediately following the exposure to anoxia, the enzyme activities for both SDH and LDH were suppressed in the heart, kidney, and liver tissues. However, this characteristic pattern did not prevail in the pancreas, parotid, and submandibular gland. The following summarizes these results.

Heart. Of all the tissues studied, the SDH activity of the heart muscle manifested the greatest change. Compared to the control group the average activity of SDH in the experimental animals amounted to a 32% decrease at the level of $p < 0.001$. Although LDH resulted also in a reduction of 4.09 mμmoles or 15%, the level of significance was rather low ($p < 0.154$).

Kidney. As in the cardiac muscle, both LDH and SDH activities were suppressed in the anoxic animals. The reduction was 0.39 mμ/min per mg or 17% for SDH, and 2.18 mμmoles or 13% for LDH. However, the

level of significance for both SDH and LDH was much lower when compared to that of heart muscles.

Liver. Only a difference of 0.12 mμmoles, or an 8% reduction, was observed in SDH activity of the hepatic tissue, as opposed to a far greater suppression observed in LDH activity. The difference in LDH activity between the experimental and control animals showed a 17% reduction. However, variations among individual animals were high and the level of significance was only $p > 0.151$.

Pancreas. As indicated previously, SDH activity, as related to the wet weight of the organ, was low, possibly due to the large amount of zymogen granules making up a greater portion of the pancreatic acinar cells. Irrespective of this, the pancreas showed the greatest percentage reduction, which amounted to 25% of the control. On the other hand, LDH activity was increased by 9%.

Parotid and submandibular glands. As shown in Table I the level of significance between the experimental and control groups in these two organs was highly variable for both enzymes. The only exception was that in the submandibular gland an increase of 13% in LDH activity was observed, and was significant at the level of $p < 0.096$.

Discussion. The results from this study indicate that a brief exposure to total anoxia brings forth a suppression of SDH activities and that the extent of such suppression is variable, depending on the type of tissue. The suppression of this key enzyme of the tricarboxylic acid cycle, may be expected to produce subsequent disturbances in metabolic steps requiring ATP. On the other hand, the effect was much more variable in the case of LDH, with the exception of an increase of enzyme activity in the submandibular gland.

Turner and Turner (3) reported that radioactive glycine-^{14}C incorporation into the pancreatic cell proteins was depressed with an increasing hypoxia. They attributed this result to the impairment of oxidative phosphorylation due to oxygen deficiency. Similar results were obtained by Sanders *et al.* (4), who reported the reduction of ATP level in the brain and liver under hypoxic condition, with an accompanying decrease of leucine-^{14}C incorporation into the proteins of these organs. These results are consistent with our previous observations, which demonstrated a reduced incorporation of proline-^{3}H and leucine-^{3}H into developing connective tissues (1) and salivary gland cells (2).

Based on the radioautographic quantitation, we demonstrated that these connective tissue cells were differentially affected and that such effects were statistically significant. In this connection it is of interest that SDH activity was most significantly suppressed in such vital organs as the heart and kidney, whereas in glandular tissues the suppression was more moderate and variable. These findings are of value in two different respects. First, we might postulate that, following a brief anoxia, the organs that are vital for the survival of the animals are affected first. Secondly, they provide us with a basis for selecting the tissues that may be used for further experimental studies.

The inconsistent difference observed in the LDH activity, of which the level of significance was rather low, suggests that most of the tissues under study might not be capable of rapidly switching to anaerobic glycolysis, with the exception of the submandibular gland. Indeed, the heart, kidney, and liver, which had the greatest LDH activity in the control groups showed a suppression of 13–17%. The increase of 13%, in LDH activity in the submandibular gland was significant at the level of $p < 0.096$, and therefore deserves further evaluation, in view of the previous reports that suggested an increased anaerobiosis and hence an accumulation of lactic acid under hypoxic conditions (5–7).

Summary. Adult male Sprague-Dawley rats were subjected to acute anoxia of 6-min duration in an environment of purified nitrogen containing 30–40 ppm of oxygen. Six tissues, namely, the heart, kidney, liver, pancreas, parotid, and submandibular gland, were analyzed with respect to the changes in succinate and lactate dehydrogenase activities caused by anoxia. The results show that, while both enzyme activities are suppressed in the organs studied, SDH activity was most

suppressed in the heart, kidney, and pancreas. On the other hand, the submandibular gland showed a significant increase in LDH activity. These results support the view that the reduction of protein synthesis in different organs following exposure to anoxia is variable.

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