cases taking a relatively longer time to establish vascular connections began to degenerate, and that the variation in the number of sense organs in these grafts is a reflection of that circumstance. The fact that all "grafts" which remained free in the body cavity were covered only by membranous epithelium containing no sense organs lends support to this interpretation.

At any rate, the experiment clearly indicates that taste organs can be maintained in the newt for at least 4 months without any trophic influence from nerve fibers of central origin in their immediate vicinity.

Summary. Tongue-tips were transplanted autoplastically to the liver in *Triturus v. viridescens* to effect denervation of the taste organs. All well-vascularized grafts that retained a normally thick epithelium contained histologically normal taste organs when examined 4 months after transplantation. This fact indicates that these taste organs were independent of any trophic action from nerve fibers of central origin. The number of persistent taste organs is correlated with the amount of normal epithelium. It is suggested that differences in the time of reëstablishment of circulation in the grafts may account for the variation found in the type of epithelium (thin, membranous or normal) and, therefore, may also account for the variation in number of taste organs persisting.

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Respiration of Rat Liver Homogenates Following Prolonged Cold Exposure. (23746)

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Although it has been fairly well established (1) that cold exposure leads to an increased tissue oxygen consumption, relatively little is known about the factors responsible for this increase. Work with tissue slices, as shown previously(2), is uninformative in this regard, since the rates observed are for the most part indicative only of endogenous respiration. The work that follows was undertaken in order to elucidate some of the factors responsible for the changes observed with slices. To this end, a variety of substrate oxidations were tested in control and in cold-exposed animals with a period of exposure which has been shown previously(2) to yield a maximal respiratory response in slices.

Methods. Male rats of the Sprague-Dawley strain were used in all experiments. They weighed between 225 g and 275 g at the time of selection. By the time of sacrifice for tissue studies, they were in the range of 275 to 350 g. The selection was made to obtain groups of rats of similar initial weight, being subjected to cold exposure and acting as controls. The exposed animals were placed in individual cages in a cold room maintained at 5 ± 1°C. for intervals of 29 to 33 days, whereas the controls were maintained in the animal colony at about 26°C. Both the control and experimental groups received a diet of "Friskies" dog food and water, given *ad libitum,* up to the time of sacrifice.

Tissue preparation consisted of a liver homogenate made up as follows: The animal was first killed by blow on head and the liver quickly excised and chilled in ice-cold 0.25 M sucrose. Utilizing a Potter-Elvehjem type homogenizer(3), a 10% homogenate was prepared from a portion of the large median lobe. This was carried out in ice-cold 0.25M sucrose containing 0.01M ethylenediamine-tetraacetic acid (pH 7.4). After filtering through 4 layers of cheesecloth to remove any large-size tissue fragments that may have been present, the homogenate was diluted to 3⅓% with

TABLE I. Liver Homogenate Oxidations following Prolonged Cold Exposure.

Substrate	Control		Cold exposed		% increase in cold	P
	No. of animals	qO_2*	No. of animals	qO_2*		
None	15	53.6 ± 1.40‡	12	58.1 ± 1.53‡	8.4	.05
Lactate	8	20.0 ± .70	7	20.9 ± 1.68	4.5	.5
Citrate	15	142.1 ± 3.73	12	169.7 ± 3.69	19.4	.01
Isocitrate	7	100.9 ± 6.90	6	135.1 ± 8.09	33.9	.02
α-Ketoglutarate	15	145.3 ± 4.06	9	187.0 ± 5.09	28.7	.01
Succinate†	8	208.2 ± 9.33	6	285.1 ± 8.70	37.2	"
Fumarate	12	94.6 ± 3.85	13	111.8 ± 2.42	18.2	"
Malate	4	96.6 ± 1.14	6	115.8 ± 1.53	19.9	"
Glutamate	15	153.1 ± 3.52	11	184.9 ± 5.90	20.8	"
β-Hydroxybutyrate	15	130.0 ± 3.80	9	173.3 ± 4.31	33.0	"

* The qO_2 values listed for each of the substrates have been corrected for endogenous respiration; magnitude of the latter appears under substrate heading of "None."
† qO_2 based on the first 10 min. of incubation.
‡ Mean ± S.E.

cold 0.25M sucrose containing 0.01M Tris (hydroxymethyl) amino methane buffer adjusted to pH 7.4 with KOH. From this final dilution, one milliliter aliquots were then placed in each reaction vessel. The tissue preparations were incubated at 38°C utilizing standard Warburg technique(3), allowing 10 minutes for thermoequilibration. The incubation medium had the following concentrations: 0.002M K-adenosinetriphosphate, (pH 7.4), 0.0066M $MgCl_2$, 1.5 x 10^{-5}M cytochrome c, 0.0001M diphosphopyridine nucleotide, 0.01M substrate and 0.01M K-phosphate buffer (pH 7.4). The volume of the reaction mixture including the homogenate was adjusted to 3.0 ml in each incubation flask with 0.25M sucrose buffered at pH 7.4 with 0.01M Tris. All substrates were used as the potassium salts. In addition to the reaction medium, each vessel had 0.2 ml of 5N NaOH in the center well for CO_2 absorption and a gas phase of air. Rate of *tissue oxygen* consumption in the presence of each of the substrates was calculated on a per milligram nitrogen basis and reported as either qO_2 (μl O_2 consumed per mg N per hour) or as μl O_2 consumed per mg N for each 10-minute interval during the course of incubation. In the case of qO_2 measurements the values were corrected for endogenous respiration. Nitrogen was determined by nesslerization. Statistical comparison of the various groups was conducted by the group comparison method as outlined by Snedecor(4). In addition, standard error values were calculated for the mean qO_2 of each group.

Results. A summary of the data in the form of qO_2 values is given in Table I. Here it can be seen that in the absence of added substrate, the respiratory rate is significantly higher ($p < 0.05$) in cold-exposed animals than in controls. This result is in accord with those found previously(2) where the endogenous respiration of liver slices was measured. Table I also shows that all substrate oxidations, with the exception of lactate, proceeded at significantly higher rates in the cold-exposed group than in the control. In the presence of lactate, no difference was detected between the experimental and control groups. In the data on substrate oxidation, it can also be seen that 2 levels of increase are brought about by cold acclimatization. The first includes the oxidation of citrate, fumarate, malate and glutamate where the range of increase is from 18 to 21%; whereas the second includes isocitrate, α-ketoglutarate, β-hydroxybutyrate and possibly succinate where the range of increase is from 28 to 37%.

In addition to the increase in the qO_2 values exhibited by the cold-exposed groups, differences were also found in the pattern of oxygen uptake during the course of the incubation procedure. Examples of this are exhibited in Fig. 1 where the rate of oxygen uptake for successive 10-minute intervals of incubation is plotted against the elapsed time of incubation. The individual values in these curves represent the same number of animals

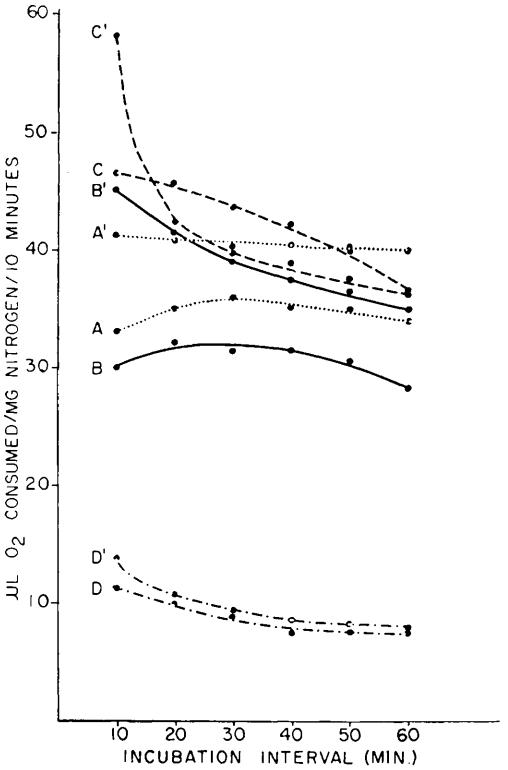

FIG. 1. Effect of duration of *in vitro* incubation on oxidation rates of liver homogenates from control and cold-exposed rats. Curves A, B, C and D represent the rates obtained in the presence of glutamate, β-hydroxybutyrate, succinate and no substrate, respectively, for control animals; A', B', C' and D' are the corresponding curves for cold-exposed animals.

as shown in Table I. The values have not been corrected for endogenous respiration, however.

It can be seen in Fig. 1 that when glutamate is being oxidized, the rate of oxygen consumption is relatively constant from the beginning until the end of incubation in both the control (curve A) and cold-exposed (curve A') groups. In contrast to this, the rate with succinate as substrate decreases very sharply during the first 10 minutes of incubation in the cold-exposed group (curve C') and then more slowly as the incubation is extended to one hour. Succinate controls (curve C) on the other hand, exhibit a more or less linear fall in rate over the entire period of incubation. It should be noted that the sharp initial drop in rate in the cold-exposed group carries the oxygen consumption below the corresponding control values. Consequently, if a comparison of the two groups were made beyond the first 10 minutes of incubation, cold exposure could be interpreted as leading to a lowered rate of succinate oxidation. Measurements of rate change during the course of incubation when β-hydroxybutyrate was present as substrate (curves B and B') gave results that were more or less intermediate to those found with glutamate and succinate. The other substrates listed in Table I also exhibited rate changes during incubation that were quite similar to that shown for β-hydroxybutyrate. In no instance did the rate in the experimental group fall below the control level as was the case in curve C'. In fact, if all the qO_2 values had been calculated on the basis of the first 10 minutes of incubation as was done with succinate, the increases following cold acclimatization would have been much greater than those indicated in Table I.

Discussion. The foregoing data bring out 3 effects of cold acclimatization on *in vitro* liver metabolism: (a) an increase in the rate of endogenous metabolism, (b) an increase in the oxidation rate of a variety of metabolic intermediates, and (c) differences in the pattern of oxygen consumption during *in vitro* incubation.

Insofar as endogenous respiration is concerned, the cause of the increase in the cold-exposed group is unknown. In both the experimental and the control tissue the rates were low with respect to those found in the presence of added substrate. The increased rate could be due to a higher concentration of endogenous substrate in the cold-exposed group than in the control or to an increased enzyme activity or possibly to a combination of an increased enzyme activity and increased level of endogenous substrate. At least part of this question is resolved in the experiments where substrates were used.

As stated earlier, in all cases where substrate was added to the reaction medium, with the exception of lactate, there was a significantly higher qO_2 in the cold-exposed group than in the control. This indicates that cold exposure results in a more or less general increase in activity of the enzymes responsible

for aerobic oxidations. The data also indicate that the magnitude of the increase following acclimatization was greater with some substrates than in others. Since the substrates in the higher category are associated with dehydrogenase enzymes as the initial step in their breakdown, dehydrogenases of the tricarboxylic acid cycle may be more affected by cold exposure than other enzymes of this cycle. It should be noted that one of these in particular, succinic dehydrogenase, has been shown by others(5,6) to have an increased activity following cold acclimatization. However, it should also be noted in the present data that malate oxidation, although associated with a dehydrogenase, is not affected to the same extent by cold exposure as the other substrates associated with dehydrogenases.

Why lactate oxidation alone among all the substrates tested should show no difference between the control and the experimental values is not known. It may be related to the fact that lactate is associated with the soluble glycolytic system of enzymes whereas the other substrates are associated with the particulate enzymes of the tricarboxylic cycle. Both groups were found to have rates that were low in comparison to the other substrates. Therefore, it seems probable that homogenization and subsequent dilution of the tissues in the reaction vessels result in a loss of activity by one or more of those enzymes or cofactors responsible for the oxidation of lactate to the tricarboxylic acid cycle.

In the measurements of rate changes during the course of *in vitro* incubation, it was observed that the experimental tissue, in general, had a more pronounced fall than the control tissue. This was especially true during the early stages of incubation. Two possible explanations might be offered for this phenomenon. The first is that the enzyme systems responsible for the oxidations are more labile following cold acclimatization. The second is that the products of the oxidations in the cold-exposed groups are accumulating at greater rates than in the controls and this in turn is leading to an inhibition of the oxidations themselves.

Summary. Respiration of liver homogenates derived from adult male rats exposed to cold for about one month was studied. Cold exposure led to 1) an increased endogenous metabolism and 2) increased rates when citrate, isocitrate, α-ketoglutarate, succinate, fumarate, malate, glutamate and β-hydroxybutyrate were present as substrates. No change in rate was observed when lactate was present as substrate. Differences were also found in both the magnitude and pattern of *in vitro* oxidations following cold exposure.

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