EFFECT OF HELIUM ON THE RESPIRATION AND GLYCOLYSIS OF MOUSE LIVER SLICES*

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In view of the very definite alteration of the metabolism of various organisms and tissues upon exposure to helium, the desirability of investigating some of the energy-yielding reactions of the metabolic pathways became apparent. The results of such an investigation are given in the present paper.

End (1), Behnke et al. (2, 3), and Behnke (4) showed that helium may exert an effect on living organisms as compared to nitrogen in that the narcosis observed at high atmospheric pressure, when air was breathed, was alleviated upon breathing helium-oxygen mixtures. Lawrence et al. (5) succeeded in establishing a condition of narcosis in mice breathing mixtures of xenon and oxygen ranging from 50 per cent to 78 per cent xenon. Lazarev et al. (6) reported observations on cockroaches and mice which indicated that while xenon was definitely narcotic in nature, krypton was essentially ineffective. In a rather thorough investigation of the effects of xenon and krypton in the case of human beings, Cullen and Gross (7) reached the same conclusion as the former authors. They were successful in inducing surgical anesthesia in two patients. It was demonstrated by one of us (8) that helium might have a metabolic effect in quite another direction. It was found that this element altered the rate of respiratory gas exchange in certain insects and poikilothermic vertebrates as well as accelerated the growth and metamorphosis of *Drosophila melanogaster* and *Tenebrio molitor*. It was also found that helium is capable of increasing both the oxygen consumption and carbon dioxide production of intact mice and the oxygen uptake of diaphragm, liver, ventricle, and probably sarcoma slices (9).

**General Methods**

The experiments herein described are based upon standard methodology utilizing the Warburg apparatus. All reactions were carried on at a temperature of 37.5°C. When used, tissues were manually sliced, washed in isotonic saline solution at 0°C., and blotted on filter paper before placing them in the reaction vessels. Four to six vessels were used in the experiments; one-half of them were filled with a gas mixture containing nitrogen and one-half with a gas mixture containing helium. The results of these experiments are expressed in relative terms, the results obtained from the

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nitrogen mixture being taken as 100 per cent and those from the helium mixture expressed as the corresponding per cent of the former value (e.g., per cent $Q_{O_2} = \frac{Q_{O_2}^{He} - Q_{O_2}^{N_2}}{Q_{O_2}^{N_2}} \times 100$).

The use of relative terms somewhat simplifies the calculation of the critical ratio ($t$ value) in that the results of all control experiments (i.e., those performed in air or $N_2$) are arbitrarily taken as 100 per cent; hence, the standard deviations and the standard errors for the controls equal zero. Therefore it is only necessary to determine these once for any given series of experiments. The equation for the calculation of the $t$ value is simplified by this procedure from:

$$t = \frac{m_2 - m_1}{\sqrt{s.e.}_1^2 + s.e.}_2^2}$$

This method of expression is the same as that used previously by Cook (8) and Cook et al. (9).

While the vessels were immersed in the water bath the gassing of the manometers and vessels was carried out by a considerably modified version of a method described by Burris (10). The gassing was accomplished by alternately evacuating (to 75 mm. of mercury) and filling three times. The shaking rate was 110 4-cm. deflections per minute. An equilibration period of 10 minutes was allowed after gassing. Liver tissue of adult male Swiss mice was used in all experiments except one in which diaphragm was used. The mice were killed by cervical fracture with a hemostat.

The symbols used are as follows:

$Q_{O_2} = \mu l$. $O_2$ consumed/mg. dry weight/hour.

$Q_{CO_2} = \mu l$. CO$_2$ metabolically produced/mg. dry weight/hour.

$Q_{CO_2}$ = $\mu l$. CO$_2$ produced/mg. dry weight/hour, due to the release of CO$_2$ from HCO$_3^-$ by acid formed by the tissue.

A superscript (e.g., $Q_{O_2}^{H_2}$) indicated the type of gas in which the tissue was incubated.

Several pilot experiments established the fact that the effect of helium on the gas exchange of liver slices remained unaltered for at least 1 hour, and probably for a longer period. Consequently as a routine procedure the tissues were incubated for a full 60 minutes. The single exception was that of the experiments in which the respiratory quotient was studied. Here the incubation period was reduced to 30 minutes according to the finding of Laser (12) that the r.q. for tissue slices is valid only during the first half hour.

Details as to suspending media and special methods will be outlined under the appropriate sections dealing with the experimental results. The results are presented in two sections, the first of which has to do with aerobic oxidation and the second with anaerobiosis.
DESCRIPTION OF SPECIFIC PROCEDURES AND RESULTS

1. Aerobic Oxidation

The various reactions studied were (a) oxygen consumption, carbon dioxide production, and respiratory quotient, (b) cyanide inhibition, (c) fluoride inhibition, (d) fluoride inhibition with lactate as a substrate, (e) fluoride inhibition with pyruvate as a substrate, and (f) oxidation of pyruvate by homogenates.

The liver slices were suspended in 2 ml. of Krebs-Ringer phosphate solution with 2.73 parts of 1.3 per cent NaHCO₃ added per 100 parts of solution. 0.2 per cent glucose was used as a substrate unless otherwise indicated. Except when carbon dioxide production or cyanide inhibition was being studied, 0.2 ml. of 10 per cent KOH was placed in the center well together with a folded piece of filter paper (1 × 1 cm.) to increase the surface area. Gas mixtures containing 80 per cent He and 20 per cent O₂ were compared with air.

Carbon Dioxide Production, Oxygen Consumption, and Respiratory Quotient.—The CO₂ production of liver slices was determined by the "direct method" of Warburg (11). Since this method requires that a determination of QO₂ be made as a necessary adjunct to the estimation of QCO₂, the r.q. could be readily calculated. This experiment was done also to confirm previously published results (9). Two sets of vessels consisting of two vessels each were used. One vessel in each set was used for the measurement of QO₂, and contained 0.2 ml. of 10 per cent KOH in the center. In order to measure the CO₂ production the other vessel contained no KOH. One vessel in each set was gassed with the He-0₂ mixture and the other with air in the usual manner. As Laser (12) has recommended, the experiments were terminated after an incubation period of 30 minutes.

Eleven experiments were conducted. The results are summarized in Table I, columns A, B, and C. It is evident that both the QCO₂ and the QO₂ are significantly accelerated to the same magnitude by helium, the respective values in helium being 112.2% and 111.2% of the values in air with a probability of no significance less than 0.01. The mean per cent r.q. is 101.2 per cent, the probability that this value is not different from 100 per cent being greater than 0.4. The r.q. then remains essentially unchanged in an atmosphere in which helium is substituted for nitrogen.

Cyanide Inhibition.—Since Cook (13) found that low barometric pressure had a profound effect on the degree of carbon monoxide inhibition in yeast suspensions, it was deemed desirable to investigate the possibility that helium might exert some influence under similar conditions. Cyanide rather than carbon monoxide was chosen as the inhibitor of the cytochrome oxidase system because of ease of control. Robbie (14, 15) has worked out several convenient methods of cyanide control which yield a constant level of the inhibitor in the...
reaction vessel, one of which was used here. 0.4 ml. of a mixture of 0.5 M KOH and 0.83 M KCN was placed in the center well.

In order to get a variation of cyanide inhibition in different experiments, the length of time the vessels remained evacuated during the usual gassing procedure in any given experiment was varied slightly. This would tend to take some of the hydrocyanic acid out of solution and allow it to escape, thus enabling one to find whether there was a change in the effectiveness of helium with a shift in the degree of cyanide inhibition.

Twenty determinations were made. Both the relative respiratory rates in cyanide and the degree of inhibition were determined for the gases. With an equal concentration of cyanide the $Q_{O_2}$ was significantly increased (122 per cent) by incubation in the He-O$_2$ mixture as compared to air (column A). The mean per cent inhibition by cyanide is 51.2 per cent in the case of air, while for He-O$_2$ it is 43.3 per cent. It was found that the greater the depression due to cyanide, the greater was the effectiveness of helium in alleviating it. This observation may be demonstrated by correlating the difference between the

### TABLE I

*The Effect of Helium on Mouse Liver Tissue*

<table>
<thead>
<tr>
<th></th>
<th>&quot;Normal&quot; slices</th>
<th>Cyanide inhibition</th>
<th>Fluoride inhibition (0.04 M NaF)</th>
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<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
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<tr>
<td></td>
<td>R.Q.</td>
<td>$Q_{O_2}$</td>
<td>$Q_{CO_2}$</td>
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<tr>
<td>No. of experiments</td>
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<td>11</td>
<td>11</td>
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<td>111.2</td>
<td>112.2</td>
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<tr>
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<td>&gt;0.4</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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Abolition of effect with lactate and pyruvate

<table>
<thead>
<tr>
<th></th>
<th>Oxidation of pyruvate by liver homogenates</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>O (0.02 M NaF) (0.022 M Na lactate)</td>
</tr>
<tr>
<td>No. of experiments</td>
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<tr>
<td>Mean</td>
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<td>C.R. (t)</td>
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<tr>
<td>p</td>
<td>&gt;0.7</td>
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per cent inhibition in helium-oxygen and in air with the per cent inhibition in air (the point of comparison). Both the correlation coefficient and the chi-square statistical methods were used in evaluating this point. The correlation coefficient is not very impressive in this respect in that it gives a value of +0.488 which is on the 3 per cent level of significance. Although the chi-square method is not ordinarily used in the analysis of these types of data it was adapted for use by plotting the per cent inhibition in air minus the per cent inhibition in He-O₂ against the per cent inhibition in air, and then examining the scatter of points for randomness, by dividing the plot into quadrants. This was done by placing a horizontal division in such a way that one-half of the total number of points would fall on each side of it. A vertical division was arranged in a like manner. If the scatter was random five points would be expected in each quarter since there were twenty points in all. The observed scatter differed from the expected significantly since the value for chi-square was 7.2 with a $P$ value less than 0.01.

**Fluoride Inhibition.**—In view of the profound effects of helium on cyanide inhibition the desirability became apparent of determining whether there was a comparable effect on the inhibition brought about by another agent at another point. The agent used was NaF which inhibits the enolase reaction of the glycolytic system. The medium, methods of gassing, and liver tissues were the same as those used in the previous experiments.

Each set of vessels (He-O₂ and air) consisted of three flasks. One vessel contained 0.2 ml. of 0.44 $M$ NaCl in the side arm and 0.2 per cent glucose in the medium. The other two had 0.2 ml. of 0.44 $M$ NaF in the side arm, one with glucose (0.2 per cent) in the medium and one with no substrate. 5 minutes before the first reading, the salt solutions were dumped into the reaction space, giving a final molarity of 0.04 $M$. The vessel containing glucose and NaCl acted as a control. The one containing NaF and no glucose was used to determine whether there was any apparent change associated with the presence or absence of an external substrate. The results of the experiments are presented in Table I, columns E and F.

The inhibition, this time due to fluoride, is again alleviated by helium with or without a substrate. When there is no glucose provided the effectiveness of helium seems to be of lesser magnitude and more unstable than is the case if glucose is provided (columns E and F). The former case is represented by a value of 132 per cent on the level of 6 per cent probability, the latter by 140 per cent and lying below the 1 per cent level of probability.

The relevant data were tested by the same statistical methods as in the case of cyanide inhibition for a correlation between the extent of fluoride inhibition and the effectiveness of helium in alleviating it. No significance was apparent in this respect.

**Fluoride Inhibition with Lactate as a Substrate.**—Since fluoride inhibition is
partially released in the presence of helium it would seem probable that the site of action of helium lies above the enolase reaction. To confirm this and to get an indication as to whether helium was also effective in the cycle of Krebs, fluoride was used to inhibit the enolase reaction and sodium lactate was utilized as the substrate. This was done on the theory that if the precursors to the TCA cycle were formed faster in He-O$_2$ than in air the addition of fluoride would keep their formation at low level in an absolute sense; therefore upon the addition of lactate the substrate level just prior to the aerobic phase of tissue metabolism would be essentially the same in both gases and the effectiveness of helium in altering the rate of reactions or series of reactions in the cycle could be readily determined.

In all the vessels 0.2 ml. of a mixture of 0.22 M NaF and 0.242 M Na lactate was placed in the side arm. Upon addition to the reaction the concentrations became 0.02 M and 0.022 M respectively. The arm was emptied into the reaction space 5 minutes prior to the first reading. The manipulations were those common to the previous experiments.

The results are tabulated in Table I, column G. There is no significant change in the oxygen uptake in He-O$_2$ under these conditions; that is, the combined use of fluoride and lactate apparently abolishes the helium effect in aerobiosis.

**Fluoride Inhibition with Pyruvate Only as a Substrate.**—These experiments were performed to verify the results of those in which lactic acid was the substrate and to determine whether the lactic acid dehydrogenase reaction might have masked the effect of helium by a secondary action of the gas at that site. Also, it was thought desirable to confirm the previous results on fluoride inhibition per se.

Three vessels were used in both of the sets. In addition to the usual KOH and suspending medium, one vessel contained 0.2 ml. of 0.22 M NaF in the side arm. The other two vessels had 0.2 ml. of a mixture of 0.22 M NaF and 0.242 M Na pyruvate in the side arm. Upon addition to the reaction the final concentrations were 0.02 M NaF and 0.022 M Na pyruvate. The side arms were emptied into the reaction space 5 minutes prior to the first reading.

The results are summarized in Table I, columns H and I. The oxygen uptake in the flasks containing fluoride and no pyruvate is significantly accelerated by helium while there is no essential difference apparent in those containing both fluoride and pyruvate.

It is apparent upon considering this experiment and the two preceding it that although helium relieves fluoride inhibition to a moderate extent, either lactate or pyruvate can nullify this effect. Presumably lactate is effective through forming pyruvate and the lactic dehydrogenase is not secondarily affected by helium.
Pyruvate Oxidation by Homogenates.—Although the experiments with fluoride would seem to indicate that helium is without effect in the tricarboxylic acid portion of the oxidative pathway it was decided to test this hypothesis in a more direct fashion. Since pyruvate was chosen as the point of departure in the preceding experiments it was felt expedient to use it here.

The following reaction medium was used (cf. 16-18):

- 0.092 M KCl (including AIK)
- 0.0033 M K phosphate buffer (pH 7.4)
- 0.019 M KHCO₃
- 0.0033 M MgCl₂
- 0.133 × 10⁻⁴ M cytochrome C
- 0.001 M Na-ATP
- 0.019 M Na pyruvate

Two flasks were used in each experiment, one of which was gassed with He-O₂ and one with air. The vessels contained 1.8 ml. of the above media, to which was added 0.2 ml. of cold 10 per cent alkaline isotonic KCl (AIK) liver homogenate. 0.2 ml. of 10 per cent KOH and a folded piece of filter paper (1 cm.²) were placed in the alkali cup as usual. The vessels and contents were kept at 0°C. until they were placed in the water bath.

The results are set forth in Table I, columns J, K, and L, and Fig. 1. The per cent Q₀₂ was calculated separately for both one-half hour intervals and for the entire 1 hour incubation time. By this procedure it becomes apparent that the rate of oxidation during the first one-half hour is significantly lower in He-O₂ than in air while the reverse situation holds for the second one-half hour interval. The Q₀₂ for the hour taken as a whole indicates a reduction of slight significance in He-O₂. Considering these data it is apparent that an acceleration is taking place in He-O₂ relative to air. This is made clear by reference to Fig. 1 in which the slope of the oxygen uptake in He-O₂ (0.858) is 42 per cent greater than in air (0.595). By extrapolation these slopes would cross at about 1.5 hours. It is clear, then, that under these conditions the oxidation of pyruvate takes place at an increasing rate in He-O₂ as compared to air, in spite of the fact that the absolute oxygen uptake is less in the former than in the latter during the initial periods.

2. Anaerobic Oxidation

The experiments comprising this section represent an attempt to learn something more of the effect of helium in the glycolytic portion of the oxidative pathway. The reactions investigated were (a) glycolysis in liver slices, (b) glycolysis in sections of diaphragm, and (c) glycolysis in liver homogenates.

Two types of media have been used in the case of liver slices. The first was Krebs-Ringer phosphate solution with 2.73 parts of 1.30 per cent NaHCO₃ added per 100
parts of solution. The second was Krebs-Ringer bicarbonate solution which has a bicarbonate concentration six times that of the Krebs-Ringer phosphate. The bicarbonate solution was also used as a suspending medium for the diaphragm sections. Both types of solutions contained 0.2 per cent glucose as a substrate.

![Graph](https://example.com/graph.png)

**Fig. 1.** Oxygen uptake of mouse liver homogenates incubated in He-O₂ and air, and utilizing pyruvate as a substrate. + = He-O₂ (slope = 0.858). O = air (slope = 0.595). Abscissa, log minutes of incubation. Ordinate, log microliters O₂ consumed/milligram of wet weight.

When homogenized liver tissue was required a Potter-Elvehjem homogenizing apparatus was used (Potter (18, 19)). The tissue was utilized as a cold 10 per cent alkaline isotonic KCl (AIK) homogenate as generally recommended by Potter. 0.2 ml. of the homogenate was used to 1.8 ml. of the medium in the flasks. When lactic acid was to be determined 0.2 ml. of 65 per cent trichloroacetic acid was placed in the side arm and emptied into the reaction space after the appropriate interval in order to
kill the reaction and precipitate the proteins. Lactic acid was determined by the method of Barker and Summerson (20).

The basic glycolytic medium, as adapted from those used by Potter (18, 19) and LePage (21), is as follows:

- 0.05 M KCl (includes A.T.P.
- 0.01 M K phosphate buffer (pH 7.6)
- 0.05 M KHCO₃
- 0.00067 M Na-adenosinetriphosphate (ATP)
- 0.04 M nicotinamide
- 0.00033 M diphosphopyridine nucleotide (DPN)
- 0.0033 M MgCl₂
- 0.004 M K-hexose diphosphate (HDP)

The ATP was obtained from the Sigma Chemical Company, St. Louis, Missouri, as the chromatographically prepared sodium salt. The DPN was obtained from the same source. The hexose diphosphate was purchased from the Nutritional Biochemicals Company, Cleveland, Ohio, as the barium salt. It was prepared as the potassium salt by dissolving it in a minimal amount of N/10 HCl and then passing the solution through a column of potassium-charged ion-X ion exchange resin (dowex 50) supplied by Microchemical Specialties Company, Berkeley, California. This method was found to be very satisfactory.

Anaerobic Glycolysis in Liver Slices.—The rate of glycolysis in liver slices was determined by two methods. The first entailed the use of Krebs-Ringer phosphate solution with added bicarbonate and 0.2 per cent glucose. Two vessels were used in each set, each vessel containing 2 ml. of medium in the reaction space and 0.2 ml. of 6 N H₂SO₄ in the side arm. One vessel of each set acted as a blank to correct for the amount of HCO₃⁻ remaining in the medium between the time the slices were added and the first reading taken. The acid was dumped into the medium of the blank at the time of the first reading and the gas evolution noted. After a 1 hour period the acid was emptied into the medium of the remaining flask of each set. The difference in gas evolved upon addition of the acid was taken as indicative of the extent of glycolysis (i.e. lactic acid formation) carried on by the slices.

The second method utilized Krebs-Ringer bicarbonate with 0.2 per cent glucose added. The rate of glycolysis was determined by direct reading of the manometers and did not entail the use of acid. This second method was used to confirm the results of the first.

The results are contained in Table II, columns A and B. The first method indicates that the lactic acid production is significantly reduced by 41.2 per cent in those liver slices incubated anaerobically in helium (column A). The other method gives a similar reduction of 30.4 per cent and is also highly significant (column B).

Glycolysis in Liver Slices with Added Fluoride and Pyruvate.—In order to determine whether the depression of glycolysis due to helium took place between the enolase reaction and the formation of pyruvic acid, fluoride inhibition was utilized with glucose acting as a substrate and pyruvate as the hydrogen acceptor.

The liver slices were manipulated in the same manner as before. Krebs-Ringer bicarbonate solution with 0.2 per cent glucose was used as the suspending medium. 0.2 ml. of a mixture of 0.44 M NaF and 0.242 M Na pyruvate dissolved in the me-
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dium was placed in the side arm. Upon addition to the reaction (5 minutes before the first reading) the concentration of these salts becomes 0.05 M and 0.022 M respectively.

The rate of glycolysis is again significantly reduced in helium, by 21.8 per cent, in this case (column C of Table II).

Glycolysis in Diaphragm.—The glycolytic rate of mouse diaphragm was tested with respect to the depressing effect of helium in order to determine whether the effect previously noted in liver slices also held for a muscle tissue.

Krebs-Ringer bicarbonate solution with 0.2 per cent glucose was used as the suspending medium. With the exception of tissue manipulation, as noted below, all procedures are the same as in that portion of the previous section in which the same medium was used.

The mouse diaphragms were obtained by transecting the mice at the level of the liver, rapidly dissecting this out, and then carefully cutting around the periphery of the diaphragm with a pair of iridectomy scissors and placing it in cold isotonic NaCl. The central tendon was dissected away from the muscular portion which was then cut into 8 to 12 pieces. The pieces were then divided equally between the flasks. The CO₂ evolution was followed for 1 hour.

Table II (column D) contains the summary of the results. The glycolysis of diaphragm pieces in He-CO₂ is 72 per cent of that in N₂-CO₂. The probability of no significance is less than 0.01.

Liver Homogenates with Glucose and Hexose Diphosphate as Substrates.—

### TABLE II

<table>
<thead>
<tr>
<th></th>
<th>Liver slices</th>
<th>Diaphragm</th>
<th>Liver homogenates</th>
<th>Glucose, HDP, KF, Na pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>No. of experiments</td>
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<tr>
<td>S.E</td>
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<td>C.R. (t)</td>
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<td>p</td>
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</table>
In order to determine whether the depressing effect of helium upon anaerobic glycolysis was due to a surface effect at the cell wall, or to an effect on chemical reactions \textit{per se} it was necessary to utilize the homogenate technique. The first logical step in this course would be to utilize the same substrate that was used in the case of slices, which was glucose. However, glucose cannot be utilized as the only substrate since it cannot be phosphorylated by liver homogenates as was reported by LePage (21) and confirmed in this laboratory. To obtain an observable glycolytic rate hexose diphosphate (HDP) was used as a substrate in conjunction with glucose.

The basic glycolytic medium with concentrations of 0.004 M HDP and 0.01 M glucose was used. Both \( \text{CO}_2 \) evolution and lactic acid product were observed.

The experiments are summarized in Table II (columns E and F). The \( \text{CO}_2 \) evolution in helium is 7 per cent higher than that in nitrogen. The lactic acid production is 5 per cent higher. Both values are statistically valid. It should be emphasized that while anaerobic glycolysis in liver slices shows an inhibition with helium, the opposite occurs with liver homogenates.

\textbf{Liver Homogenates with Hexose Diphosphate Only as a Substrate.---}In the previous experiment both glucose and HDP were used as substrates. It was presumed possible that the small amount of glucose which would be phosphorylated could have been responsible for the difference; that is, the helium might have been affecting the phosphorylation reactions of glucose. Therefore in order to check this possibility, 0.004 M HDP was used as the only substrate in the basic medium.

The results are contained in Table II (columns G and H). The \( \text{CO}_2 \) evolved shows an increase of 4 per cent in helium, the probability of no significance lying between the 1 per cent and 2 per cent level. The lactic acid production was significantly increased in helium by 7 per cent.

\textbf{Liver Homogenates with Glucose and HDP as Substrates, and Added Fluoride and Pyruvate.---}As in the case of slices it again was necessary to rule out any possible influence of helium on the reaction lying between the enolase reaction and the formation of pyruvate in homogenates. The basic glycolytic medium with the addition of 0.01 M glucose, 0.004 HDP, 0.01 M KF, and 0.01 M Na pyruvate was used. The glucose and HDP served as the substrates, the Na pyruvate as the hydrogen acceptor, and the KF as the inhibitor of the enolase reaction. The results of these experiments are indicated in column I of Table II.

Under these conditions the rate of glycolysis is significantly increased by incubation in helium to 128.2 per cent. The extent of the increase is considerably greater than has been met heretofore under similar conditions.

\textbf{DISCUSSION AND CONCLUSIONS}

The experiments here described strongly confirm our previous finding that helium alters the over-all respiratory gas exchange of liver slices \textit{in vitro} (9).
It remains therefore to explore the underlying question concerning where in the oxidation system such an influence is brought to bear.

In order for any agent to alter the final outcome of any chain of reactions it must first affect one or more of the individual rate-limiting reactions of the chain. The experiments reported here were based upon this concept and an attempt has been made to localize as far as possible the specific reactions which might be affected by helium. We may begin with consideration of the terminal oxidative phase and then discuss the preceding, anaerobic glycolytic reactions.

In helium both the oxygen consumption and the carbon dioxide production are increased beyond the corresponding values in air. At the same time the ratio, or the R.Q., remains constant. This fact may be taken as evidence that the same intracellular substrates are metabolized in helium as in air. It is, of course, possible that if there exist alternative pathways for the oxidation of glucose, these pathways could be differentially affected by the two gas mixtures. However, in view of the evidence that the direct oxidative pathways are attacked by helium, it is unnecessary to invoke alternate pathways.

The results of the individual studies concerned with oxygen consumption by liver cells present certain anomalies which have not yet been completely resolved. Thus in those experiments wherein the enolase reaction was inhibited with fluoride, with the subsequent oxidation of either lactate or pyruvate, the results indicate that helium is ineffectual in that portion of the metabolic process which includes the citric acid cycle. On the other hand the fact that cyanide inhibition is affected by helium leads to the conclusion that the tricarboxylic acid cycle is at least indirectly involved. The latter view is supported by the undoubted modification of the oxidation of pyruvate by homogenates in the presence of helium.

It has been noted previously that with an increase in the inhibition of oxygen uptake due to cyanide there is an increase in the effectiveness of helium in alleviating the inhibition. Since cyanide is considered a non-competitive inhibitor of cytochrome oxidase, the extent of inhibition is dependent only upon the concentration of cyanide and independent of the substrate concentration. In order for helium to decrease the extent of the inhibitory action of cyanide it must obviously affect the union between cyanide and cytochrome oxidase. This might occur by an alteration of the cyanide ion in such a way as to decrease its ability to combine with cytochrome oxidase. In addition helium might directly weaken or alter the oxidase-cyanide bond. Another possibility is that the rate at which the electron transfers could be accomplished, i.e. the reaction rates of the cytochrome system, might be changed in such a way that a smaller amount of non-inhibited cytochrome oxidase could function at a higher efficiency. However, the fact that the degree of alleviation offered by helium is inversely altered with respect to the extent of the inhibitory action of cyanide tends to indicate that the latter possibility does not hold. It is impossible to say which of the other hypotheses is closest to reality.
The use of pyruvate in combination with fluoride during the incubation of liver slices, as has been explained previously, is based on the assumption that even though the rate of metabolic formation of pyruvate may be different in He-O₂ as compared to air the inhibition of the enolase reaction will keep the amount of pyruvate formed at a low absolute level. Upon the addition of a relatively high concentration of pyruvate from an external source the pyruvate levels in the two cases will become equal for all practical purposes; hence any difference in the rate of oxygen uptake will be dependent upon whether helium affects the subsequent oxidation of the material. The results of this experimental procedure definitely indicate that the reactions involved in the further oxidation of pyruvate by the liver slices are not subject to any change which might be brought about by helium.

It will be recalled that a similar experiment was performed utilizing lactate as the sole substrate instead of pyruvate. The results of this experiment would depend upon the rate at which lactate was dehydrogenated to form pyruvate as well as the rate at which the pyruvate was metabolized by the citric acid cycle. This procedure revealed nothing which did not confirm the preceding one. The results were similar in all respects in that they confirmed that the rate of oxidation of pyruvate was not altered by helium and further indicated that the lactic acid dehydrogenase reaction was not subject to the influence of helium.

The aerobic oxidation of pyruvate by liver homogenates yielded results which were quite different from those found when slices were used under comparable conditions. The fact that the rate constants, as indicated by the slopes of the oxidation of pyruvate in air and in He-O₂, are so radically different suggests that some basic rearrangement has taken place as a result of homogenization which allows helium to alter modes of reaction if not entire reactions (see Fig. 1). In any case the strong difference in slopes, and the reason why the initial points should be so widely separated, remain obscure since no satisfactory hypothesis can be advanced at present.

In general it can be said that while the oxidation of pyruvate by the citric acid cycle is unaffected by helium in slices, it is very much affected in the case of homogenates. Helium also indirectly affects the Krebs cycle through decreasing the inhibition of cytochrome oxidase due to cyanide. As to whether it has any influence on cytochrome oxidase when there is no cyanide present is not certain.

The fact that the Q₁₀ of slices incubated in a medium containing fluoride both with and without glucose are so greatly increased in an atmosphere of He-O₂ as compared with air (see Table I) tends to indicate that helium is influential above the enolase reaction. Probably the reason for a greater uptake of oxygen under these conditions is due to a greater "pressure" of intermediates prior to the enolase reaction in He-O₂ (i.e., a mass law effect).

Turning therefore to glycolysis we immediately encounter the unexpected fact that the anaerobic glycolytic rates of diaphragm and liver slices showed a
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marked depression in the presence of an atmosphere composed mainly of helium. That this probably was not due to any direct action of helium on the lactic acid dehydrogenase reaction is indicated by the experiment previously discussed in which lactate was used as an aerobic substrate. Hence it appears that the inhibition of glycolysis due to helium is a condition peculiar to anaerobiosis.

Although the depression is peculiar to anaerobiosis it does not mean that all the glycolytic reactions are individually depressed. Indeed, if glucose and HDP, or HDP alone, are used as substrates in homogenates under anaerobic conditions, there is an increase in the glycolytic rate when helium is used instead of nitrogen. The depression, then, is due to one, or a combination of two, possibilities. The first of these is that anaerobic conditions in some way change the properties of the cell wall or plasma membrane in such a way that the presence of helium either interferes with the permeability to glucose or alters the metabolic reactions tied to the cell wall so as to depress them; this would explain in one fashion why there is no depression in the case of homogenates. The second possibility is that anaerobic conditions alter one or more of those reactions preceding the aldolase reactions so that the formation of HDP is depressed. This second possibility would explain the apparently anomalous increase in metabolism under helium when HDP is used as the main substrate. Although essentially the same results are apparent when glucose as well as HDP is used as a substrate, it is not necessarily in conflict with the preceding statements, in that any depression of glucose phosphorylation would not alter the results due to the rate of utilization of HDP unless the ability of homogenates to phosphorylate glucose were much greater than is apparent.

The experiments in which the enolase reaction was inhibited with fluoride, and pyruvate acted as the hydrogen acceptor, very definitely indicate that both the site of the inhibitory action (in slices metabolizing glucose) and of the acceleratory action (in homogenate utilizing glucose and HDP) lie above the enolase reaction.

On the basis of these considerations it is reasonably clear that the inhibition of glycolysis in the presence of helium is due to some specific effect on that portion of the metabolic pathway prior to the formation of HDP. The site or sites of action of helium which result in an increase in glycolytic rate under anaerobic conditions (and probably aerobic as well) lie between the aldolase and the enolase reactions.

SUMMARY

It has been shown that helium has the ability to affect variously the rates of certain metabolic reactions in vitro as compared to nitrogen. An attempt has been made to approximate the sites of action in mouse liver preparations.

The following results have been obtained by the substitution of a mixture of
80 per cent helium and 20 per cent oxygen for air: (a) An increase in the rate of oxygen consumption and carbon dioxide production to the same degree, the respiratory quotient remaining unchanged. (b) A decrease in the magnitude of cyanide inhibition. The effectiveness of helium increases with the degree of the cyanide inhibition. (c) No effect on the activity of slices which have been poisoned with fluoride when either lactate or pyruvate has been added as a substrate. (d) A change in the rate, and the slope of the curve of oxygen consumption in liver homogenates which are utilizing pyruvate as a substrate.

The use of helium relative to nitrogen under anaerobic conditions causes: (a) A depression of the glycolytic rates in both mouse liver slices and diaphragm. (b) An increase in the carbon dioxide evolution and lactic acid production of mouse liver homogenates oxidizing either glucose and hexose diphosphate, or hexose diphosphate alone.

In neither slices nor homogenates does the addition of fluoride and the use of pyruvate as the hydrogen acceptor alter the fundamental response of the preparations.

The following hypotheses have been advanced and discussed in order to explain the observed phenomena:

1. Helium does not alter the substrate utilized by the tissue.
2. The gas interferes in some way with the cyanide-cytochrome oxidase bond, but may not affect cytochrome oxidase in the absence of cyanide.
3. The citric acid cycle is not subject to the influence of helium in tissue slices, but is altered in an unexplained fashion in homogenates. It is postulated that a rearrangement of particulate surfaces may be the significant factor here.
4. The glycolytic cycle is the site of both an inhibitory and an acceleratory effect of helium. The locus of the inhibition lies above the aldolase reaction and that of the acceleration between the aldolase and enolase reactions.

BIBLIOGRAPHY