ARGON, XENON, HYDROGEN, AND THE OXYGEN CONSUMPTION AND GLYCOLYSIS OF MOUSE TISSUE SLICES*

By FRANK E. SOUTH, JR.,‡ AND SHERBURNE F. COOK

(From the Department of Physiology, School of Medicine, University of California, Berkeley)

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Although the influence of helium on the metabolism of animals both *in vivo* and *in vitro* has been extensively explored, and although the ability of the heavier inert gases to exert a narcotic effect upon whole animals has been demonstrated, no investigation has been made of tissue metabolism during exposure to gases other than nitrogen and helium. The experiments reported here attempt to close this gap in our knowledge. We have chosen to inquire into the effects of argon and xenon because of their differences in physical characteristics relative to each other and to helium, and into the effects of hydrogen because of both its physical characteristics and the fact that, like nitrogen, it has a diatomic molecule.

That narcotic effects might be produced by the inhalation of xenon and krypton has been observed by Lawrence *et al.* (1) in mice and human beings, Lazarev *et al.* (2) in cockroaches and mice, and Cullen and Gross (3) in human beings. It was agreed that xenon definitely possessed greater narcotic properties than krypton. The comparative narcotic effects of helium, argon, and nitrogen at elevated pressures were reported by Behnke and Yarbrough (4), and those of nitrogen and hydrogen by Case and Haldane (5). In general, it was found that at these high pressures argon was somewhat more depressant than nitrogen (4), while neither helium (4, 5) nor hydrogen (5) caused an observable intoxication at ambient pressures as high as ten atmospheres. Cook (6) demonstrated that helium, and to a lesser extent argon, were able to accelerate the oxygen consumption and decrease the development time of *Drosophila* and *Tenebrio*. Helium also increased the rate of aerobic gaseous metabolism of various reptiles (6) and whole mice (6, 7).

With respect to activity at the cellular level we have found in a series of experiments that the rate of anaerobic glycolysis of diaphragm and liver slices

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‡ Present address: Department of Physiology and Pharmacology, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico.

is significantly depressed by incubation in helium rather than nitrogen. However, in liver homogenates which are oxidizing either glucose and hexosediphosphate or hexosediphosphate alone, helium increases the glycolytic rate slightly, but significantly (8). A study in which the aerobic and anaerobic effect of helium was compared among slices of mouse liver, brain, sarcoma, and diaphragm revealed that while the rate of oxygen consumption of all tissues studied was increased by the gas, the glycolytic rate was depressed in brain, liver, and diaphragm whereas that of the sarcoma tissue was unaffected (9).

Methods

The methods employed in these experiments were the same as those reported in previous publications (7–9). Standard Warburg techniques have been used with an incubation temperature of 37.5°C. and incubation time of 1 hour. The tissue slices (of liver, brain, and sarcoma) were manually prepared and washed in ice cold isotonic saline before blotting and placing in the reaction vessels. Each vessel contained 2 ml. of Krebs-Ringer phosphate medium with 0.2 per cent glucose in the reaction space, and 0.2 ml. of 10 per cent KOH in the center well along with a piece of folded filter paper measuring 1 cm. on a side. Four vessels were used in each experiment, two of which were gassed with a mixture containing nitrogen and two with a mixture containing either argon, xenon, or hydrogen. For aerobic experiments mixtures containing 20 per cent O_2 and 80 per cent of the inert element were used. When anaerobic glycolysis was to be studied mixtures containing 5 per cent CO_2 and 95 per cent inert gas served. High purity commercial grade nitrogen and argon, and reagent grade xenon were purchased from the Linde Air Products Company. The argon was analyzed by the vapor pressure method by Dr. Carl Garland and Dr. George Jura of the Department of Chemistry, University of California, Berkeley. It was found that the tank of argon contained approximately 2 per cent nitrogen as an impurity. Oxygen and electrolytically prepared hydrogen were obtained from the Stuart Oxygen Company.

Swiss male mice were used as the source of brain and liver tissue, and female *A* strain mice were the source of the sarcoma tissue. Sarcoma A-274 was transplanted to the experimental animals and allowed to maturate for 7 to 13 days before the animals were sacrificed and the tumor removed for slicing. The animals were killed by cervical fracture.

The results have been expressed in relative terms in that those which were obtained by incubating in the nitrogen mixture were taken as an arbitrary 100 per cent and those from the comparison gas mixture were expressed as the corresponding per cent of the former value (*e.g.*, percentage $Q_{O_2} = \frac{Q_{O_2}^{Xe-O_2} \times 100)}{Q_{O_2}^{air}}$. This method of expression and that for calculating the critical ratios (t value) are the same as those previously employed (6–9).

The symbols used are:

Q_{O_2} = μl. O_2 consumed/mg. of tissue dry weight/hour.

Q_A = μl. CO_2 produced/mg. of tissue dry weight/hour, due to the release of CO_2 from HCO_3^- by acid formed by the tissues.

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

RESULTS

The results of these experiments are contained in Table I (oxygen consumption) and Table II (anaerobic glycolysis). To each table, for comparative purposes, have been added the corresponding data for helium, although the latter have been previously published. A general survey of the tables as a whole

TABLE I

Effect of Various Gases on Oxygen Consumption of Mouse Tissues

Tissue	Comparison gas	No. of experiments	Mean $Q_{O_2}^{air}$	Mean Q_{O_2} in comparison gas	Mean percentage Q_{O_2}	S. E.	t(C. R.)	P
Liver	He-O_2*	16	8.10	9.05	111.7	±3.87	3.02	<0.01
	A-O_2	20	9.51	9.79	102.9	±1.01	2.87	<0.01
	Xe-O_2	9	9.82	10.86	110.6	±1.78	5.96	<0.01
	H_2-O_2	10	10.28	9.81	95.4	±1.64	2.80	<0.03
Brain	He-O_2*	9	9.95	10.80	108.5	±1.74	4.92	<0.01
	A-O_2	18	13.33	13.60	102.0	±1.34	1.50	<0.15
	Xe-O_2	9	11.92	13.05	109.2	±2.53	3.64	<0.01
	H_2-O_2	10	12.62	12.25	97.9	±2.07	1.01	<0.30
Sarcoma A-274	He-O_2*	24	4.34	5.56	128.1	±5.29	5.31	<0.01
	A-O_2	17	4.83	5.13	106.2	±3.03	2.05	<0.06
	Xe-O_2	8	4.58	5.04	110.0	±2.47	4.05	<0.01
	H_2-O_2	13	5.06	5.03	99.6	±1.57	0.319	>0.70

C. R. = critical ratio.

The oxygen consumption in air is taken as unity and that in the gas mixture compared to it expressed relative to it on a percentage basis (percentage (Q_{O_2})).

* The results for He-O_2 which have been published previously (9) are included for purposes of comparison.

indicates that the inert gases tend to increase the rate of oxygen consumption of all tissues studied, and depress the glycolysis. However, there are certain deviations in detail which deserve specific mention.

Xenon.—Whatever may be the influence of xenon upon the whole mammal, there is no doubt that its effect upon the individual tissues investigated very closely approximates that of helium. Oxygen consumption by liver, brain, and sarcoma was accelerated significantly and to about the same degree in all three tissues, the percentage increases being 10.6, 9.2, and 10.0 respectively. Glycolytic rates of the tissues are more variously affected by the gas (Table II) in that the activity of liver was depressed by 21.3 per cent and of brain by 13.2 per cent. Both of these values lie below the 1 per cent level of confidence.

Xenon, furthermore, is apparently the only gas which so far has been observed to inhibit the anaerobic metabolism of sarcoma. The depression of 7.7 per

TABLE II

Effect of Various Gases on Anaerobic Glycolysis of Mouse Tissues

Tissue	Comparison gas	No. of experiments	Mean $Q_A^{N_2}$	Mean Q_A in comparison gas	Mean percentage Q_A	S. E.	t(C. R.)	P
Liver	He*	15	3.56	2.48	69.7	±3.72	8.17	<0.01
	A	25	2.37	2.23	94.1	±3.19	1.84	<0.10
	Xe	10	1.69	1.33	78.7	±4.71	4.59	<0.01
	H_2	19	2.35	2.10	89.3	±3.60	2.97	<0.01
Brain	He*	15	11.66	10.48	89.9	±4.29	2.59	<0.03
	A	10	12.78	12.56	98.4	±3.75	0.427	>0.60
	Xe	8	12.37	10.69	86.8	±2.73	4.84	<0.01
	H_2	11	14.07	12.72	90.5	±3.28	2.90	<0.02
Sarcoma A-274	He*	57	25.57	25.88	101.2	±1.72	0.698	>0.45
	A	10	27.48	27.64	100.6	±3.22	0.186	>0.80
	Xe	10	27.27	25.22	92.3	±2.75	2.80	<0.03
	H_2	10	27.68	28.01	101.2	±2.23	0.538	>0.50

The rate of glycolysis in nitrogen is taken as unity and that in the gas mixture compared to it expressed relative to it on a percentage basis (percentage Q_A).

* The results for He which have been published previously (9) are included for comparison purposes.

TABLE III

*Some Physical Constants of Various Gases**

	Helium	Argon	Xenon	Hydrogen	Nitrogen
Molecular weight	4.003	39.944	131.3	2.016	28.016
Density	0.1785	1.7839	5.851	0.0899	1.2506
Atomic No.	2	18	54	1	7
Bunsen coefficient (olive oil, 37°C.)	0.015	0.14	1.7	(Cottonseed oil, 40°C.) 0.05	0.067
Bunsen coefficient (water, 37°C.)	0.0085	0.026	0.085	0.016	0.013
Oil water solubility ratio	1.8	5.4	20.0	3.1	5.2

* The solubility data are taken from Lawrence *et al.* (1).

cent is, however, of only moderate statistical significance since the value for P is slightly below 0.03.

Argon.—Although in many of its physical properties argon lies between helium and xenon (Table III) its influence upon tissue metabolism is less

powerful than that of either of the other two elements. At the same time the results obtained with it fit into the general pattern. Thus the oxygen consumption of liver was accelerated by 2.9 per cent ($P < 0.01$), brain by 2.0 per cent ($P < 0.15$), and sarcoma by 6.2 per cent ($P < 0.06$). The trend appears distinct although the significance of the differences is not as great as would be desired. The effect on glycolysis was even less. The rate of liver was depressed by 5.9, and that of brain by 1.6 per cent, the corresponding values for P being <0.10 and >0.60. With sarcoma there was no effect (increase by 0.6 per cent with a value for P of over 0.80).

Hydrogen.—Since the inert gases employed were monatomic it appeared desirable to compare with nitrogen the effect of another diatomic element. For this purpose hydrogen is most suitable. In general the results indicate that although hydrogen exerts a slight depressant effect upon the rates of oxygen uptake which is of little or no significance statistically, nevertheless it appears to adhere to the usual pattern under anaerobic conditions.

In summarizing the data, it may be noted that under aerobic conditions (Table I) the monatomic, radially symmetrical inert gases (He, A, Xe) behave as a single species insofar as their effects on the rate of oxygen consumption by isolated tissues are concerned, while the two gases which have diatomic, dumb-bell-shaped molecules (N_2 and H_2) behave as another. The former group increases the rates of oxygen uptake relative to the latter.

Under anaerobic conditions (Table II) the situation has been observed to be somewhat different in that both the inert gases *and* hydrogen depress glycolysis of the tissues within a pattern (*i.e.*, liver > brain > sarcoma) relative to nitrogen.

DISCUSSION AND CONCLUSIONS

It is generally held that insofar as the whole animal is concerned the ability of an inert gas to induce a state of narcosis is a more or less direct function of either its molecular weight, its absolute solubility in oil or water, or its oil-water solubility ratio (*cf.* 4, 5, 10). Hence one would expect that the relative degrees of narcosis induced by the various gases would be in the descending order Xe, Kr, A, N_2, H_2, He (see Table III). Regardless of the applicability of these parameters to intact animals it is evident from the tables that none of them may be held responsible for the observations which we have made on isolated tissues. This point is made amply clear if one compares the physical characteristics of helium and xenon noted in Table III with their metabolic behavior as set forth in Tables I and II. Indeed, these considerations as well as what other data are available have led us to believe that there is no *a priori* reason to assume that because, for example, helium and xenon differ widely in their ability to induce a condition of narcosis in the organism in the gross, the two gases must show the same relative deviation in their influence on

metabolism at the cellular level. It is not at all difficult to visualize a situation wherein an inert gas might have a specific action on the central nervous system such as to cause narcosis and at the same time not alter the rate of intrinsic metabolism of the tissues in the same direction. In other words, whereas the total metabolism of the animal might be lowered through a reduction of general muscle tone, mediated by the central nervous system, the endogenous metabolic rates of the constituent tissues might be either increased or decreased, depending upon whether the tissues in question were deriving their energy predominantly from aerobic or anaerobic oxidations. These considerations would apply to brain tissue as well as to any other.

In turning to a discussion of the specific effects of the various gases which have been reported in this paper, it will be remembered that argon failed to exert more than a feeble influence. This may be referable to one of two possible causes: (1) the inherent properties of the element itself, and (2) the fact that our supply of argon was contaminated with 2 per cent nitrogen. The latter alternative would appear the more acceptable. However, this point certainly requires further investigation. Although the individual results with this gas lie at relatively low levels of statistical significance it should be emphasized that in the aggregate they at least appear to adhere to the general pattern established by helium and xenon.

The patterns which were noted above further confirm what has been said in regard to the lack of correlation between the usually cited physical characteristics of the gases and their effects *in vitro*. They also emphasize what is probably a very fundamental difference in their mechanisms of action aerobically and anaerobically—especially is this so when one considers that hydrogen behaves similarly to nitrogen in one case and to the inert gases in another. It might, therefore, be postulated that the diatomic elements may align themselves parallel with active cell surfaces or phase boundaries in such a manner as to occlude them partially and hence alter their ability to carry on their functions with maximum effectiveness. On the other hand the monatomic elements would not have a tendency to so align themselves but would simply pass through the surface interstices as dictated by random movement.

However, if such a situation is important during aerobiosis it would appear that there is some other factor operative during anaerobiosis when the influence of hydrogen is considered in context. Some other, and completely undisclosed, factor must hold sway under such conditions.

SUMMARY

The effects of xenon, argon, and hydrogen on the aerobic and anaerobic metabolism of mouse liver, brain, and sarcoma slices have been investigated. Xenon was found to alter the rates of metabolism of these tissues in a manner almost identical with helium. The gas increased the rate of oxygen consump-

tion in all three tissues and significantly depressed that of anaerobic glycolysis in brain and liver. The depression of glycolysis in sarcoma was less pronounced and not highly significant.

Although both the magnitude and statistical significance of the effects observed with argon were much smaller, there was a seeming adherence to the general pattern established by xenon and helium. Hydrogen while remaining essentially ineffective insofar as oxygen uptake was concerned, depressed glycolysis in both liver and brain slices but did not significantly affect sarcoma slices.

The following points are stressed in the Discussion: (1) the magnitude and direction of effects exerted by helium, argon, xenon, hydrogen, and nitrogen do not conform with the relative values of molecular weight, density, and solubility of these gases; (2) the effect of these gases on tissue metabolism does not necessarily parallel that exerted upon the whole organism.

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