OXYGEN POISONING

IV. THE EFFECT OF HIGH OXYGEN PRESSURES UPON THE METABOLISM OF LIVER, KIDNEY, LUNG, AND MUSCLE TISSUE*

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Since the time of Bert the possibility that the poisonous action of high pressures of oxygen is due to the inhibitory action of oxygen upon the oxidative enzyme systems of tissues has been extensively discussed (1, 2). The number of direct observations in the literature of the metabolism of tissue under high oxygen pressure is, however, quite limited. We have studied the subject by determining the oxygen uptake of tissue preparations *in vitro* by the Warburg technique. By use of an apparatus previously described (3) it is possible to measure the oxygen uptake and CO_2 output of tissues when they are equilibrated with 8 atmospheres of oxygen. In Paper III (4) data from experiments with rat brain have been presented. In this paper we present further data of similar character on isolated surviving liver, kidney, lung, and muscle tissue of the rat.

The technique of the preparation of tissues, equilibration, etc., are fully discussed elsewhere (4).

Liver—In a series of eighteen control determinations of the oxygen uptake of liver slices from normal, fed white rats a mean value of 70 ± 4 micromoles per gm. per hour over a period of time from 0.5 to 5.5 hours was found. The mean R.Q. (thirteen determinations) was 0.72 ± 0.024. An important fact to be noted is that the oxygen uptake did not depart significantly from rectilinearity for 5.5 hours. When the oxygen uptake of liver slices was measured during exposure to oxygen pressures from 5.0 to 8.4 atmospheres, the results recorded in Table I were found.

For better intercomparison the results are reported as percentages of the rate of oxygen uptake of slices from the same liver at 1 atmosphere of oxygen. Two points are established by inspection of the mean of the series: (1) The rate during the 1st hour did not differ significantly from that at 1 atmosphere. (2) As in the case of the brain, the rate of oxygen uptake fell slowly and steadily during exposure. However, the rate has reached 50 per cent of the control rate only after 3.5 hours, as opposed to 1 hour in

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the case of the brain. After 3.5 hours the fall is slower. The respiratory quotient is not significantly different from that of the controls.

TABLE I

Oxygen Uptake and Respiratory Quotients of Liver Slices from Normal Fed Wistar Rats during Exposure of Slices to High Oxygen Pressure

The oxygen uptakes are expressed as percentages of the uptake of parallel slices from the same livers observed simultaneously at 1.0 atmosphere of pO_2, except as indicated. Zero time is taken as the moment O_2 pressure reached a maximum.

Experiment No.	pO_2	Exposure time									R.Q.
		1.0 hr.	1.5 hrs.	2.0 hrs.	2.5 hrs.	3.0 hrs.	3.5 hrs.	4.0 hrs.	5.0 hrs.	6.0 hrs.	
		Oxygen uptake, per cent of controls									
	atmospheres										
94A-1	5.0	133	119	106	100	98	83	72	52	31	
94A-3	6.2	85	68	84	78						
94A-2	6.4	149	149	149	149						
94A-4	6.6	94	64	41	30	23					
		129	114	85	68	53					
94A-5	6.8	127	108	83							
		100	67	58	45	33	25				
94A-7	7.2	129									0.85
		129	100	70							0.80
		108	108	87	69	45					0.92
94A-6	7.3	103	103	103	71						0.48
		103	103	103	64	57	50	43			
94A-9	8.0	103									
		114									
		106									
		123									
96-3	8.3	136									0.50
94A-8	8.4	160									
		118	118								0.96
		120	100	75							0.81
		143	143	125	98						0.84
		116	116	91	80	66	57	48			0.70
Mean.....		119	105	90	77	54	54	54	52	31	0.76
± standard error of mean.....		4	6	7	8	9	12	10			0.06

Some of the rates of oxygen uptake during the 1st hour are higher than those of the control series. However, we do not believe that this signifies a real increase in metabolic oxygen, but was due to a technical error which was eliminated in later experiments. It was found (3) that excessive

amounts of rubber connections in the apparatus took up in physical solution appreciable amounts of oxygen at high pressure. When this source of error was eliminated by glass to glass connections, the oxygen was the same as that under 1 atmosphere (Experiment 94A-9).

This experience was universal with all tissues studied; we have never been able, when the above source of error was minimized or eliminated, to demonstrate conclusively any significant increase of oxygen uptake during the initial period of observation under high oxygen pressures. If such occurs, it must be less than 10 to 15 per cent of the total oxygen uptake; otherwise it would have been detected. However, it requires 2 minutes to raise the pressure in the apparatus and readings cannot be made until physical solution of oxygen in the medium is complete (approximately 8 minutes more); hence the possibility that there is a significant increase of oxygen uptake during this time cannot be rigorously excluded. If such an increase occurs, it must be of short duration so that it does not affect the rate 10 to 15 minutes from the time the maximum pressure is reached. The significance of the point lies in the possibility that reactions absent or minimum at 1 atmosphere of oxygen might be greatly accelerated under high oxygen pressures and be concerned with oxygen poisoning.

In general, the same comment made for the brain slices can be made here: though there is a definite effect by high oxygen pressures on the oxygen uptake of liver slices, its extent is negligible until long after the time required to extinguish the last signs of life in the intact animal under similar conditions.

Kidney—In preliminary experiments kidney slices from rats were exposed to high oxygen pressure, and then the oxygen uptake measured with oxygen at 1 atmosphere. In general relatively long preliminary exposures were required to reduce the oxygen uptake significantly. With oxygen at 7 atmospheres, exposure for 1, 2, and 3 hours resulted in a subsequent oxygen uptake, compared to controls maintained throughout at 1 atmosphere, of 70, 50, and 35 per cent respectively.

When the oxygen uptake of kidney slices was measured during exposure to high oxygen pressures, the data shown in Table II were obtained. With the exception of one experiment (No. 94C-11) the results are reasonably consistent. In general, the sensitivity of the kidney slices to high oxygen lies midway between that of brain and liver, and again it is seen that the falling off in respiration takes place chiefly long after the intact animal would have succumbed. The mean R.Q. value is not significantly different from that observed at 1 atmosphere.

Lungs—Mongrel dogs were used after prolonged exposure to 1 atmosphere of oxygen.

It is well known that animals exposed for long periods to 1 atmosphere

of oxygen develop marked pulmonary symptoms and die as a result. The marked edema and patchy hemorrhage found in such animals have been described by many observers since the first reports of Bert. On account of their obvious interest we studied the metabolism of slices from the lungs of five dogs which had been kept in a chamber containing 0.8 to 1.0 atmos-

TABLE II

Oxygen Uptake and Respiratory Quotients of Kidney Slices from Normal Fed Wistar Rats during Exposure of Slices to High Oxygen Pressure

The oxygen uptakes are expressed as percentages of the uptake of parallel slices from the same kidneys observed simultaneously at 1.0 atmosphere of pO_2 except as indicated. Zero time is taken as the moment O_2 pressure reached a maximum.

Experiment No.	pO_2	Exposure time							R.Q.
		0.5 hr.	1.0 hr.	1.5 hrs.	2.0 hrs.	2.5 hrs.	3.0 hrs.	4.0 hrs.	
	atmospheres								
94C-1	6.4	151	151	151					
94C-2	5.2	100	88	72	50	32	20		
94C-3	5.6	100	87	65	56	48	33	15	
94C-4	5.8	115	69	50					
		143	84	63	50	38	29		
94C-5	5.3		103	94	80				
94C-6	6.0	103	103	59	44	32	24		0.63
94C-7	7.2	84	84	42	32	23	16		0.61
		84	84	42					0.54
94C-8	8.0	139	117	69	57	42			
		116	104	69	60				
94C-9	7.7	127	127						0.53
		131	131	102	82				0.68
		131	131	102	82	61	51		0.65
94C-10	8.6	121	74	64					0.79
94C-11	8.0	41	31						
		40	29						
		65	48						
		61	45						
Total mean ± standard error of mean.....		103 ± 8	90 ± 8	75 ± 8	59 ± 6	39 ± 4	29 ± 3	15	

phere of oxygen from 48 to 116 hours, and which were sacrificed before death.[1] In all cases the oxygen uptake of the slices was determined at 1 atmosphere of oxygen.

All results are expressed on a dry weight basis to avoid error in com-

[1] We are indebted to Dr. John Lockwood of the Harrison Department of Research Surgery, University of Pennsylvania, who supplied and treated these animals.

parison with normal animals, since the variable amount of lung edema in the experimental animals precluded comparison on a wet weight basis. Nineteen determinations on lung slices from normal mongrel dogs gave a mean oxygen uptake of 131 ± 3 micromoles per gm. of dry weight per hour. The mean R.Q. was 0.83 ± 0.02. The data on dogs exposed to oxygen are

TABLE III

Oxygen Uptake and Respiratory Quotients of Slices of Lung from Mongrel Dogs Exposed to Moderate Pressure of Oxygen

Dog No.	Oxygen	Exposure	Dry weight to wet weight ratio	O_2 uptake	R.Q.
	atmosphere	*hrs.*	*per cent of control*	*micromoles per dry gm. per hr.*	
917	0.8	48	82	159	0.86
	0.8	48	66	125	0.63
921	0.8	48	76	108	0.73
926	0.8	48	83	128	0.72
619	1.0	116	78	181	0.82
Mean ± standard error of mean.....				140 ± 15	0.75 ± 0.04

TABLE IV

Oxygen Uptake and Respiratory Quotients at 1 Atmosphere of Oxygen of Slices of Lung from Normal Fed White Rats following Exposure of Slices to High Oxygen Pressures

Oxygen pressure, preliminary period	Exposure time	Subsequent oxygen uptake	Per cent of control rate	R.Q.
atmospheres	*hrs.*	*micromoles per dry gm. per hr.*		
2.2	2	271	114	
		269	109	
4.2	1	292	99	
		212	73	
4.2	2	207	82	
		231	93	
6.9	1	217	93	
		233	81	
6.9	2	170	61	0.82
		167	55	0.87

given in Table III. In all cases, the lungs showed the typical patchy hemorrhage and edema, the latter being reflected in a decreased dry weight to wet weight ratio. However, on the average, there was found no significant change in the oxygen uptake or the R.Q. On the basis of these experiments it is possible to exclude any specific effect of prolonged exposures

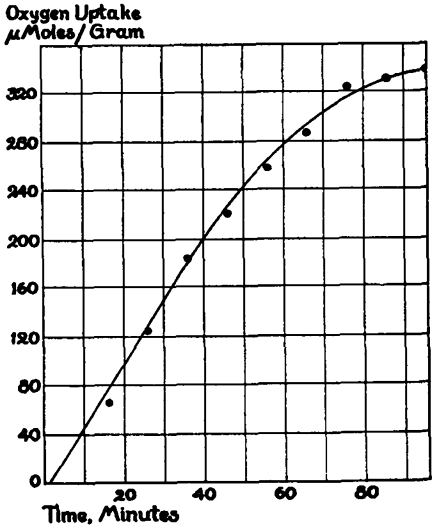

FIG. 1. Oxygen uptake (micromoles per dry gm.) of slices of lung from normal white rat during exposure to 8 atmospheres of oxygen.

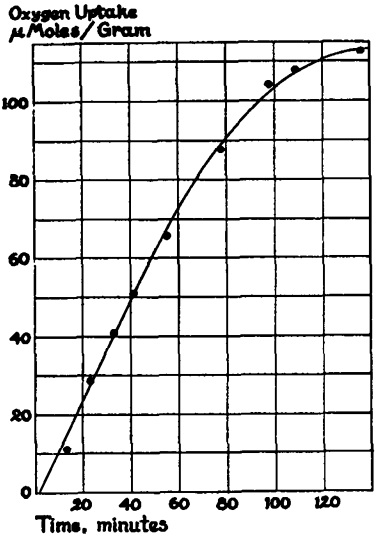

FIG. 2. Oxygen uptake (micromoles per wet gm.) of diaphragm from white rat during exposure to 8 atmospheres of oxygen.

to oxygen at pressures approximating 1 atmosphere causing significant decreases of the metabolic activity of pulmonary tissue.

White rats were used in twenty-two control determinations on the oxygen uptake of slices of lungs; the mean value was 289 ± 3 micromoles per dry gm. per hour and the R.Q. was 0.94 ± 0.01. The oxygen uptake (corresponding to a Q_{O_2} of 6.5) is surprisingly high, considering the histological character of the lung, which would lead one to suppose that the organs contain little metabolically active tissue. The high R.Q. also indicates a predominantly carbohydrate type of metabolism. In a preliminary series lung slices were exposed to high oxygen pressures for 1 or 2 hours, the oxygen uptake being subsequently measured at 1 atmosphere. The data (Table IV) show, as with other tissues, that exposure up to 1 hour at 6.9 atmospheres has relatively little effect upon the subsequent metabolism. Nor is the R.Q. significantly affected. In experiments in which the oxygen uptake was measured during exposure to high oxygen pressure essentially the same results were obtained. Fig. 1 shows the course of oxygen uptake of rat lung slices at 8 atmospheres of oxygen. As repeatedly observed with other organs, the rate of oxygen uptake remains unchanged for a period of time approximating 1 hour, after which it falls off slowly, reaching half the initial rate in about 2 hours.

Muscle—The oxygen uptake of striated muscle (rat diaphragm) was measured during exposure to 8 atmospheres of oxygen (Fig. 2). Muscle is more resistant to oxygen poisoning than all other tissues examined. The initial rate under high oxygen pressure is not significantly different from that under 1 atmosphere. It falls off slowly, reaching half the initial rate in about 3 hours.

DISCUSSION

The tissues of the rat which have been examined are slowly poisoned by high pressures of oxygen. In the course of a relatively long time, considering the high pressures used, the oxygen uptake begins to fall off and eventually ceases. The organs may be arranged roughly in a descending order of susceptibility to high oxygen pressure; *viz.*, brain, kidney, liver, lung, muscle. We have repeatedly made the point, however, that the loss of metabolic activity develops slowly and for that reason we have concluded that it plays little or no rôle in the acute phase of oxygen poisoning or the early death of intact experimental animals. Supportive evidence for this conclusion is found in our experiments on dogs exposed for long periods of time to 1 atmosphere of oxygen. Despite the marked pulmonary changes which in comparable animals leads eventually to death, we observed no significant change of the metabolic activity of lung slices. The significance of these findings to the problem of oxygen poisoning has been fully discussed in Paper III (4).

SUMMARY

1. The effect of high oxygen pressures on the metabolism of slices of liver, kidney, lung, and muscle was determined.

2. The relation of these findings to the problem of oxygen poisoning is discussed.

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