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The Metabolism of the Lungs from Normal Mice and from Mice Exposed to Phosgene

By E. BOYLAND AND F. F. McDONALD, *Experimental Station, Porton, Wilts.*

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The respiration and glycolysis of the lungs of normal mice, and of mice previously exposed to phosgene, have been measured in order to determine if the oedema following exposure is preceded by inhibition of the carbohydrate metabolism. This was done in the hope that knowledge of the nature of the changes might lead to a rational therapy of phosgene poisoning.

EXPERIMENTAL

The measurements were made with the Warburg apparatus at 38°. The lungs were suspended in Ringer-phosphate in the presence of O₂ for respiration measurements and in Ringer-bicarbonate in 95% N₂/5% CO₂ for measurement of the glycolysis. To minimize injury to the lung, the whole lobes from mice of about 20 g. wt. were used for the measurements. Lungs of larger animals would have necessitated the use of minced or sliced tissue. Manometric readings were taken at intervals over a 2 hr. period. The tissue in each experiment was weighed (*a*) before the manometric measurements were made, (*b*) in the wet state at the end of the manometric measurements and (*c*) after drying to constant wt. at 100°. The metabolic quotients (*q*) are expressed in $\mu\text{l./mg. dry wt./hr.}$

RESULTS

Comparison of metabolism of whole lobes and cut lung tissue from normal mice

The lungs of freshly killed normal mice were removed and washed with Ringer solution. The lobes of one lung were weighed, and placed whole in a Warburg vessel, while the lobes of the other lung were minced into fine pieces with scissors before being placed in a second vessel. Some values for metabolism of whole and minced tissue are given in Table 1. The mean values for Q_{O_2} with glucose are 7.3 for whole tissue, 7.4 for minced tissue (eight determinations of each type). Similar experiments have shown that the respiration in the absence of glucose, and the glycolysis in the presence of glucose, are not seriously changed by mincing of the tissue. These results indicate that measurements made on whole lobes of small mice are not affected by the rates of diffusion of O₂ or CO₂ in the tissue and give a true picture of the activity of the enzymes within the tissue. The

slight increase, which is found in the minced tissue, is probably due to the loss of material in the Ringer solution so that the dry weights of tissue are really somewhat low. The values for respiration are similar to those obtained for rat lung by Laser (1932), but the values for glycolysis are higher than those found with rat lung.

Effect of exposure to phosgene

Mice were exposed to air containing phosgene in a chamber as described by Boyland, McDonald & Rumens (1946), the concentrations being checked analytically. The results obtained on the lungs of such exposed mice are set out in Table 2. It is clear

Table 1. *The effect of mincing on the metabolism of normal mouse lung*

Nature of tissue	Wt. of tissue (mg.)		Increase in wet wt. during exp. (%)	Metabolism		
	Before exp. (wet)	Dry		Respiration with glucose Q_{O_2}	Respiration without glucose Q_{O_2}	Glycolysis of glucose $Q_g^{N_2}$
Whole lobes	70	14	52	6.4	—	—
Minced tissue	79	14	133	7.8	—	—
Whole lobes	95	16.2	10	6.9	—	—
Minced tissue	119	18.6	2	6.5	—	—
Whole lobes	65	13.2	48	7.5	—	—
Minced tissue	71	13	12	7.7	—	—
Whole lobes	68	10.6	43	—	7.5	—
Minced tissue	100	17	21	—	8.3	—
Whole lobes	84	17	50	—	6.1	—
Minced tissue	88	15	Nil	—	7.1	—
Whole lobes	74	15	19	—	—	3.0
Minced tissue	69	13	17	—	—	3.8
Whole lobes	85	16	47	—	—	4.0
Minced tissue	120	20	15	—	—	5.4
Whole lobes	82	14	26	—	—	3.5
Minced tissue	89	17	26	—	—	3.6

Measurements of normal lung tissue showed that the respiration is not increased by addition of glucose to the medium. Mean values obtained for Q_{O_2} with glucose were 7.3 (individual values 6.3, 6.3, 7.8, 7.9 and 8.6), and Q_{O_2} without glucose, 7.6 (individual values 6.3, 6.4, 6.5, 6.7, 7.0, 7.2, 7.3, 7.5, 8.0 and 9.1). Similar figures were obtained for the respiration in the presence of DL-alanine, viz. Q_{O_2} without substrate 5.8–7.0, and Q_{O_2} with 0.025M-alanine 6.4–6.8. These results indicate that, if the utilization of glucose or alanine were specifically inhibited by phosgene, such inhibition might not reduce the respiration. The glycolysis values ($Q_g^{N_2}$) found were 2.7, 2.8, 2.9, 2.9, 3.1, 3.1, 3.4 and 3.4, having a mean of 3.0.

In all cases in which measurements were made with lungs from normal mice, the tissue increased in weight over the experimental period. The increase in weight varied from 0 to 133% of the original weight; it is due in part to replacement of air in the lung by Ringer solution, and also to swelling of the tissue.

that there is no significant change in the respiration or glycolysis of the lung tissue following exposure to lethal doses of phosgene.

Most of the lungs of mice exposed to phosgene increased in weight during the measurements, often to the same extent as did normal lungs. In one group of mice which were exposed to a high dosage ($Ct = 4080$ mg. min./m.³, where C = concentration of phosgene in mg./m.³ and t = time in min.), 19 hr. before examination, the wet weights of the lungs decreased during the experiment. This was presumably due to oedema fluid, and possibly other material being washed away with Ringer solution while the manometric measurements were being made. It is possible that a reversal of tissue swelling occurred in the Ringer solution.

The change in weight of the lung following exposure could be seen by comparing the weight of the wet lung with the total body weight of the mice. Some estimations of nucleic acid phosphorus were carried out by the method of Berenblum, Chain & Heatley (1939). The data (Table 3) show how the

Table 2. *Metabolism of lungs from mice exposed to phosgene*

Details of exposure (dosages (<i>Ct</i>) in mg. min./m. ³)	Wt. of tissue (mg.)		Change in wet wt. during exp. (%)	Q_{O_2} with glucose	Q_{O_2} without glucose	Glycolysis of glucose $Q_{O_2}^{N_2}$
	Before exp. (wet)	Dry				
<i>Ct</i> =2100; <i>t</i> =8 min. (less than 1 hr. before measurement)	70	12.8	—	7.1	—	—
	103	20.2	—	7.7	—	—
	61	8.8	—	9.2	—	—
	124	23.0	+24	6.0	—	—
	92	17.0	—	—	6.4	—
	44	8.2	—	—	6.4	—
	122	23.6	+32	—	6.2	—
	122	22.0	+47	—	6.2	—
	150	29.6	—	—	—	2.5
	195	39.6	—	—	—	2.5
	161	32.0	+49	—	—	3.0
	165	31.4	+36	—	—	2.6
	108	16.6	+15	7.1	—	—
	116	17.4	+10	7.3	—	—
	66	12.4	+38	7.6	—	—
<i>Ct</i> =5300; <i>t</i> =8 min. (less than 1 hr. before measurement)	71	11.8	+36	7.7	—	—
	73	10.6	Nil	—	5.9	—
	135	24.9	+42	—	5.9	—
	98	16.0	+19	—	6.6	—
	101	19.0	+18	—	6.6	—
	133	33.4	+56	—	—	3.2
	176	30.8	+ 6	—	—	4.1
	200	35.0	+26	—	—	3.3
	158	29.0	+12	—	—	2.8
	61	10.0	+41	6.3	—	—
	69	10.0	+20	5.1	—	—
	126	22.4	+29	—	7.0	—
<i>Ct</i> =4200; <i>t</i> =2 min. (3 hr. before measurement)	136	20.4	+ 6	—	6.9	—
	177	32.4	+18	—	—	2.9
	207	31.8	Nil	—	—	2.6
	71.3	9.0	-11	5.7	—	—
	155	16.0	-32	5.2	—	—
<i>Ct</i> =4080; <i>t</i> =8 min. (19 hr. before measurement)	84	10.4	- 6	—	6.1	—
	161	16.6	-32	—	4.3	—
	146	18.6	-19	—	—	2.95
	116	12.9	-32	—	—	3.5
	34	5.4	+24	7.6	—	—
<i>Ct</i> =4200; <i>t</i> =2 min. (27 hr. before measurement)	30	4.4	+17	7.7	—	—
	78	13.4	+26	—	7.7	—
	54	8.8	+13	—	7.0	—
	104	19.4	+31	—	—	3.0
	86	25.2	+17	—	—	2.1

weight of lung tissue increases following exposure to phosgene causing a relative decrease in the nucleic acid phosphorus of the lungs.

DISCUSSION

The experiments reveal no great change in the metabolism of the lung following exposure to phosgene. If the oedema of the lung were due to the poisoning of enzymes concerned in carbohydrate metabolism some change should have been observed. Thus, although it may be possible that vesication of the skin by mustard gas is due to poisoning of hexokinase, it seems very improbable that poisoning of this enzyme or of any of the other enzymes con-

cerned with glycolysis in the lung, occurs on exposure to phosgene.

SUMMARY

1. The lungs of young adult mice are sufficiently small to allow of measurement of metabolism without mincing. Mean values of 7.3 for Q_{O_2} and 3.0 for $Q_{O_2}^{N_2}$ were found.

2. No great changes in the metabolism of the lung were observed on exposure of mice to phosgene, and it therefore appears unlikely that the oedema of the lung is due to poisoning of enzymes concerned with glycolysis or respiration in the lung.

We are indebted to the Chief Scientist of the Ministry of Supply for permission to publish these results.

Table 3. *Weight of lung and nucleic acid phosphorus in the lungs of normal and phosgene-poisoned mice*

Details of mouse		Wet wt. of lung (mg.)	Wt. of lung as % of body wt.	Nucleic acid P of lung	
Exposure	Wt. (g.)			(As % of lung wt.)	(As % of body wt.)
None	19	133	0.705	0.115	0.00081
	23	156	0.678	0.109	0.00073
	21	152	0.722	0.114	0.00082
	19.5	148	0.759	0.108	0.00076
	22	141	0.640	0.107	0.00068
	20	161	0.806	0.108	0.00087
	21.8	146	0.650	0.112	0.00075
	22.5	196	0.871	0.110	0.00096
	18	100	0.556	0.098	0.00055
	19.5	135	0.693	0.108	0.00073
	20.5	143	0.698	0.111	0.00077
	19.5	122	0.626	0.115	0.00072
	20.5	312	1.52	0.062	0.00094
	22	301	1.37	0.069	0.00094
Phosgene <i>Ct</i> =3480 (4½ hr. before estimation)	23	310	1.35	0.064	0.00086
	19.5	314	1.61	0.058	0.00094
	19	296	1.56	0.059	0.00092
	21	320	1.52	0.064	0.00098
	20.5	185	0.903	0.087	0.00078
Phosgene <i>Ct</i> =2240 (22 hr. before)	19	277	1.46	0.051	0.00075
	20	213	1.06	0.081	0.00086
	21	269	1.28	0.069	0.00088
	20	305	1.52	0.051	0.00078
	19	164	0.864	0.087	0.00075
	20.5	185	0.903	0.087	0.00078
	20.5	185	0.903	0.087	0.00078

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The Electrophoretic Analysis of Protein Extracts from Striated Rabbit Muscle

2. DENATURATION IN ACETATE BUFFERS

By J. J. C. JACOB, *Laboratoire de Biologie générale, Université de Liège*

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In a previous paper (Jacob, 1947) some interrelations between the classical muscle proteins (myogen, myoalbumin, myosin, globulin X) and the electrophoretic components of various extracts of striated rabbit muscle were established. In order to complete the characterization of components, the analysis of one of these muscle extracts in acetate buffers of decreasing pH is described here. This is necessary since the isoelectric points of several components lie in the pH range covered by acetate buffers, and in an acid medium, most of the muscle proteins are denatured without flocculating

(Finn, 1932; Weber, 1934; Bate Smith, 1937). These new observations are of interest in the study of denaturation processes.

EXPERIMENTAL

The electrophoretic technique and the procedures for the extraction and preparation of muscle extracts have been described in a previous paper (Jacob, 1947). The extraction solution used here contained 0.048 M- Na_2HPO_4 and 0.006 M- NaH_2PO_4 (pH=7.7, $\mu=0.15$); the dialysis solutions, which also served as supernatant in the electrophoretic experiments, contained 0.1 M-sodium acetate, 0.05 M-NaCl