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Oxygen Consumption during Histamine Release by Antigen and Compound 48/80

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Summary. The oxygen consumption of isolated rat mast cells has been determined using a micro capillary respirometer designed by Cunningham and Kirk and modified to permit stirring and addition of reactants. Uptake ranged from 0·5 pl. per cell per minute in 100 per cent O_2 to 0·1 pl. per cell per minute in 1 per cent O_2. It remained steady for at least 1 hour and was not affected by the presence of glucose.

Under a variety of conditions neither histamine release by antigen added to sensitized cells nor by compound 48/80 added to normal cells resulted in any increase in the rate of oxygen uptake. Oxygen does not appear to be a rate limiting factor.

INTRODUCTION

Histamine release in anaphylaxis is inhibited by lack of oxygen. This was first observed in guinea-pig lung by Parrot (1942) who suggested that the anaphylactic reaction was an aerobic reaction. The experimental finding of inhibition of histamine release from sensitized guinea-pig lung by anoxia has been confirmed by several groups of workers (Mongar and Schild, 1957; Moussatché and Danon, 1960; Chakravarty, 1960; Edman, Mongar and Schild, 1964). In rat lung tissue, inhibition of anaphylactic histamine release by lack of oxygen was obtained with difficulty (Diamant, 1962).

Moussatché and Danon (1956) measured the oxygen uptake of guinea-pig lung slices during the anaphylactic reaction using Warburg manometers. They observed a small but significant increase in oxygen consumption after the addition of antigen—horse serum—under normal oxygen tension. Mongar (unpublished) also observed a small but significant increase in oxygen uptake in sensitized chopped guinea-pig lung on addition of antigen. This increase was not due to the contraction of bronchial smooth muscle since it could not be reproduced by applying histamine to the lung. In contrast to these findings Chakravarty (1962) observed no change in the rate of oxygen uptake of sensitized guinea-pig lung after the addition of antigen.

The histamine released in these experiments is probably derived from mast cells which form only a small fraction of the tissue so that the small increase of oxygen uptake on addition of antigen might have been due to the many non-reacting cells present. It seemed that a clearer picture of the role of oxygen in the anaphylactic reaction could be obtained from studies on isolated mast cells. These cannot be obtained readily from the guinea-pig but the rat provides a good source since about a half million cells can be isolated from the peritoneal cavity of each animal.

The oxygen requirements of the anaphylactic reaction of rat mast cells have been studied in this and the subsequent paper. The first paper deals with oxygen uptake before and during the release of histamine by antigen and compound 48/80 under various oxygen tensions. The second paper deals with the effects of anoxia, glucose and thioglycollate. A preliminary account of this work has been published (Mongar and Perera, 1964).

METHODS

The oxygen consumption of isolated rat mast cells was measured by a modified form of the differential capillary respirometer described by Cunningham and Kirk (1940). This consists essentially of a block of brass containing two equal sized cavities. Volume changes of the gas in one cavity in relation to the other are indicated by the movement of a drop of liquid in the capillary connecting tube (Fig. 1). Each cavity had an opening to the

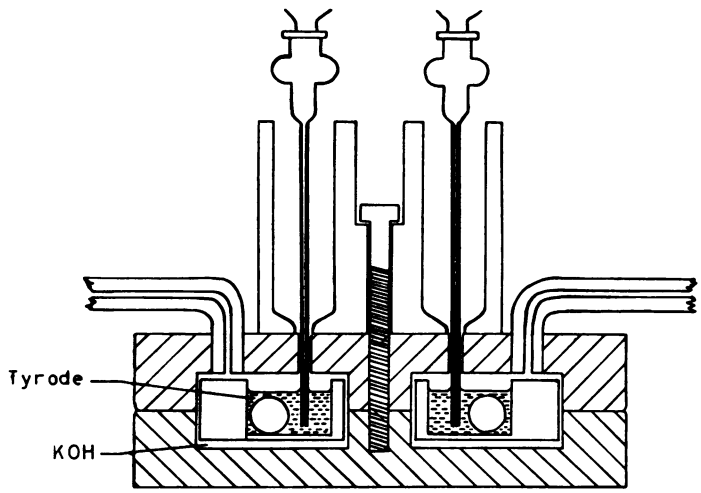

FIG. 1. Modified Cunningham–Kirk micro respirometer.

atmosphere to allow for gassing and introducing reactants. The openings were closed with a rotating Perspex valve. The mast cells suspended in phosphate–Tyrode were placed in one chamber and the suspending medium without cells in the other. The movement of the index is directly proportional to changes in the gas volume. For the capillary used in most experiments a displacement of 1 mm. was equivalent to a volume change of 1·7 µl. Each cavity was fitted with a Perspex disc containing a well for the reactants 1·5 cm. in diameter and 1 cm. deep. The edges of the discs were grooved to allow CO_2 to be absorbed by the filter paper soaked in 10 per cent KOH at the bottom of the cavities.

In order to keep the cells in suspension a glass ball was added to the well of each disc, and the whole apparatus was arranged so that it could be rocked through an angle of 20° about the axis of the capillary sixty times per minute.

Reactants were introduced into the two cavities under identical conditions by a double syringe device consisting of two 2 ml. nylon syringes fitted to a Perspex block with their

pistons driven by a common threaded screw (Fig. 2). The syringes were fitted with long No. 2 hypodermic needles which reached to the bottom of the cavities.

Peritoneal mast cells from normal and sensitized rats were isolated over an albumin gradient (Perera and Mongar, 1963a). Rats were sensitized to horse serum mixed with *pertussis* vaccine as described previously (Perera and Mongar, 1963b). Mast cells isolated from several rats were used in each experiment. The cells were pooled, suspended in ½–1 ml. of phosphate–Tyrode solution (NaCl 8·0, KCl 0·2, $MgCl_2$ 0·1, $CaCl_2$ 0·1, NaH_2PO_4 0·05, glucose 1·0 g. in 1 litre +50 ml. of isotonic phosphate buffer, pH 7·4) and placed in the well in one of the Perspex discs. The apparatus was sealed with stop-cock grease and immersed in a thermostatic bath at 37°. The required gas, oxygen alone or mixed with different concentrations of nitrogen was slowly bubbled through the solutions

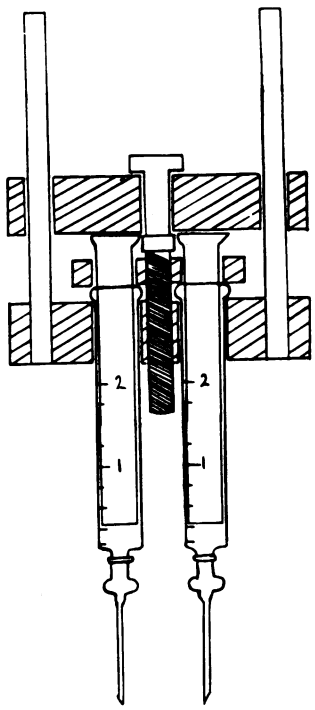

FIG. 2. Twin-syringe device for adding a reactant simultaneously to both vessels of the respirometer.

in both cavities via loosely fitting hypodermic needles. The openings were then closed by rotating the Perspex valve and the basal rate of respiration of the cells established by taking readings of the position of the capillary index every 5 minutes. When a steady baseline was obtained equal volumes of 0·01–0·1 ml. of reactant (antigen or compound 48/80), previously equilibrated to the bath temperature were added to the two cavities using the twin syringe device. The rate of respiration was again determined as before. In some experiments glucose was omitted from the medium.

RESULTS

Control experiments were conducted to determine the characteristics of the apparatus. The opening of the Perspex valve caused a small displacement of the index but 1 minute

after closing the valve the respiration was, on average, unaltered from the initial reading. The addition of saline from the twin-syringe device did not produce any artifact. The introduction of a rocking technique was shown to be necessary; when the apparatus was kept stationary the cells sank to the bottom of the well and the diffusion thus introduced reduced the rate of respiration to about one quarter of the normal rate.

The respiration of mast cells was measured in air in glucose-containing medium. The basal rate of respiration of the cells remained steady for at least an hour at about 1·0 µl. per 5 minutes per million cells (Table 1). The variation in the rate was no greater than to be expected from the sensitivity of the instrument (0·1 µl.).

TABLE 1

OXYGEN UPTAKE OF RAT MAST CELLS: GLUCOSE-CONTAINING TYRODE IN AIR

Time (minutes)	*Respirometer reading*	*Total uptake (µl.)*	*Rate of uptake (µl./10^6 cells/5 min.)*
0	0·95	0	
5	1·50	1·0	1·0
10	2·15	2·1	1·1
15	2·80	3·1	1·0
20	3·30	4·0	0·9
25	3·95	5·1	1·1
30	4·60	6·2	1·1
35	5·20	7·2	1·0
40	5·75	8·2	1·0
45	6·30	9·1	0·9
50	6·80	10·0	0·9
55	7·45	11·1	1·1
60	8·10	12·2	1·1

Effect of Histamine Releasers

When compound 48/80 (10^{-5} and 10^{-6}) or horse serum (2 per cent) was added to normal mast cells there was no increase in the rate of respiration. On the contrary it tended to decrease (Table 2).

Effect of Glucose-Free Medium

In the preceding experiments the respiration was measured in glucose-containing phosphate–Tyrode. The failure under these conditions to obtain an increase in oxygen consumption during histamine release could be due to the presence of glucose since it is known that, in rat tissue at least, glucose can supply the energy required for these reactions. Hence the experiments were repeated in glucose-free phosphate–Tyrode. Glucose was also excluded from the media used for isolating the cells.

The basal rate of respiration showed no signs of falling off for at least an hour nor, in three experiments, was there any increase when glucose was added after the cells had been in glucose-free medium warmed to 37° for at least half an hour.

Effect of Histamine Releasers in Glucose-Free Medium

The oxygen consumption of mast cells in glucose-free phosphate–Tyrode during histamine release by 10^{-6} compound 48/80 or 2 per cent horse serum was measured. There was no increase in oxygen uptake (Table 2), though control experiments showed substantial histamine releases from these cells under these conditions.

TABLE 2

EFFECT OF ADDITION OF HISTAMINE RELEASERS ON OXYGEN UPTAKE IN AIR OF RAT MAST CELLS
(Each row represents a separate experiment)

Releaser	*Concentration*	*Oxygen uptake* ($\mu l./10^6$ *cells/5 min.*)			*Histamine release* (% *of cell contents*)
		Before	*After*	*Mean difference*	
GLUCOSE PRESENT					
48/80	10^{-6}	1·06	0·99		
		1·14	0·80	−0·15	—
		0·90	0·86		
48/80	10^{-5}	1·28	1·14		
		0·97	1·04	−0·02	—
		1·07	1·09		
Antigen: horse serum	2%	1·25	0·91		
		1·05	1·05	−0·18	—
		1·08	0·86		
GLUCOSE ABSENT					
48/80	10^{-6}	0·79	0·80	−0·09	60
		0·71	0·53		55
Antigen: horse serum	2%	0·91	0·80		34
		0·93	0·90	−0·01	49
		0·70	0·81		36

Effect of Varying the Oxygen Concentration

In 100 per cent oxygen the uptake of rat mast cells was steady for at least 1 hour at about 0·5 pl. per minute per cell (Table 3). In 20 per cent oxygen (air) the oxygen consumption of the mast cells fell to about one half and in 5 per cent oxygen to one quarter of that in 100 per cent oxygen.

TABLE 3

OXYGEN UPTAKE OF RAT MAST CELLS IN VARIOUS OXYGEN CONCENTRATIONS: GLUCOSE-FREE TYRODE
(Each value represents a separate experiment)

Oxygen concentration (%)	*Oxygen uptake* ($\mu l./10^6$ *cells/5 min.*)	*Mean* ($\mu l./10^6$ *cells/min.*)
100	3·11, 2·43, 2·20	0·52
20 (air)	1·23, 1·22, 1·13	0·24
5	0·67, 0·50, 0·53	0·11
1	0·72, 0·44, 0·38	0·10

For experiments with 1 per cent oxygen the volume of the mast cell suspension was reduced from 1·0 to 0·5 ml. in order to avoid an appreciable fall of the oxygen concentration during the experiment. The rate of oxygen consumption was rather variable from run to run and tended to decline during an experiment. It averaged 0·1 pl. per minute per cell.

Effect of Antigen at Low Oxygen Concentration

The rate of respiration of sensitized mast cells was determined in 1 per cent oxygen. Antigen—horse serum in a final concentration of 2 per cent—was then added and the rate

of respiration determined again. Even under these conditions of reduced oxygen uptake and in glucose-free medium, the addition of antigen did not cause an increase in the oxygen uptake of the mast cells. Histamine releases under these conditions were not reduced, 55–80 per cent of the total histamine was released by 20 per cent horse serum (Table 4).

TABLE 4

EFFECT OF ADDITION OF ANTIGEN—2 PER CENT HORSE SERUM—ON OXYGEN UPTAKE OF SENSITIZED RAT MAST CELLS IN 1 PER CENT OXYGEN: GLUCOSE-FREE MEDIUM

Oxygen uptake (*μl./10⁶ cells/5 min.*)			*Histamine release* (*% of cell contents*)
Before	*After*	*Mean difference*	
0·34	0·39		80
0·70	0·52	−0·07	71
0·32	0·25		55

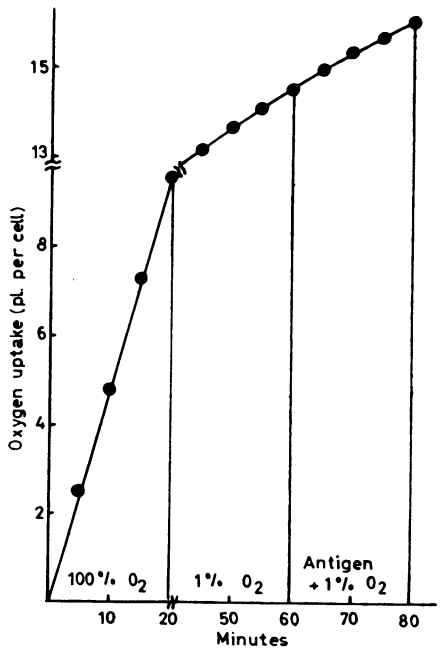

FIG. 3. Effect of reduced oxygen tension and antigen on oxygen uptake of sensitized rat mast cells (mean of three experiments).

The combined effect on oxygen uptake of reducing the oxygen tension from 100 per cent to 1 per cent followed by the addition of antigen is shown in Fig. 3. After a 20 minute period of measurements in 100 per cent oxygen the apparatus was gassed with 1 per cent oxygen and re-equilibrated. The rate was again measured for a 20 minute period. There was a marked slowing of uptake. Addition of antigen without any interruption of readings showed no significant effect on the rate. There was a slight falling off but it was no more than would be expected from a spontaneous decrease of respiration.

DISCUSSION

The differential capillary respirometer of Cunningham and Kirk (1940) has been modified to study respiration of isolated mast cells. The basal rate of respiration of the mast cells in air and in glucose-containing phosphate–Tyrode remained steady for at least an hour. The cells consumed about 0·2 pl. per minute per cell under these conditions. The addition of compound 48/80 in concentrations of 10^{-5} and 10^{-6}, to unsensitized cells did not cause an increase in the oxygen uptake. Nor was any increase observed when antigen —2 per cent horse serum—was added to sensitized cells under the same conditions. The small increase in oxygen uptake previously observed when antigen was added to sensitized guinea-pig lung cannot therefore be explained in terms of increased oxygen requirements of mast cells during the anaphylactic reaction.

The failure to obtain an increase in oxygen uptake of the cells during histamine release in the present experiments was not due to the presence of glucose in the medium which could serve as an alternative source of energy. Experiments conducted in glucose-free media showed that neither compound 48/80 nor antigen caused an increase in oxygen uptake, although parallel experiments showed a substantial release of histamine from these cells of 58 per cent and 40 per cent of their histamine content.

At a concentration of 100 per cent oxygen in glucose-free Tyrode, the oxygen consumption of the mast cells was about 0·5 pl. per minute per cell. When the oxygen concentration in the apparatus was reduced to 1 per cent the oxygen consumption decreased to about 0·1 pl. per minute per cell. Even under these conditions of reduced oxygen uptake, addition of antigen to sensitized cells did not cause an increase in oxygen consumption. Control samples tested for histamine release in 1 per cent oxygen showed normal releases.

The failure to obtain an increase in the oxygen uptake of sensitized rat mast cells during the anaphylactic reaction even when conditions for histamine release were optimal does not support the concept of a direct oxidative mechanism for the anaphylactic reaction. However, there is no doubt that oxygen is required at some stage, for oxygen-lack inhibits anaphylaxis in this preparation (Perera and Mongar, 1965), in other rat tissues (Diamant, 1962) and in other species (Mongar and Schild, 1957).

Even under conditions of reduced oxygen tension and in the absence of glucose as an alternative supply of energy via the anaerobic pathway no stimulation of oxygen uptake was observed. Recently an actual reduction of oxygen consumption during histamine release by a concentration of compound 48/80 sufficient to cause an immediate degranulation in neoplastic mast cells of the mouse has been reported (Fredhohm, 1963) though the onset of action was slow. It seems clear that in the rat, and probably also the mouse, oxygen is not a rate determining factor in the reactions initiated in mast cells by antigen or compound 48/80.

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