RESPIRATION OF RAT PERITONEAL MAST CELLS

NIRMAL CHAKRAVARTY, M.D., and ERIK ZEUTHEN, Ph.D.

From the Biological Institute of the Carlsberg Foundation, Copenhagen, Denmark.
Dr. Chakravarty's present address is Department of Pharmacology, University of Umeå,
Umeå, Sweden

ABSTRACT

Methods for microgasometry of a few hundred mast cells are described. The Cartesian ampulla diver technique is used. The sample size is determined by counting the cells within the diver. The respiration rates at 37°C, expressed in microliters per cell per hour, are 0.29×10^{-6} without substrate and 0.47×10^{-6} with glucose.

INTRODUCTION

The mast cell is generally accepted as carrying the store of histamine which is released in the anaphylactic reaction (29, 3, 20). The release is inhibited by lack of oxygen (27) and when there is interference with the enzyme systems involved in respiration (21, 34, 6) and in glycolysis (8, 28, 7). A relation between energy metabolism and histamine release is thus indicated. The release reaction has been studied both with tissue preparations, usually guinea pig lung (18, 19), and with isolated mast cells from the peritoneal cavity (1, 31, 14) of the rat, but correlated studies involving energy metabolism have been limited to sliced or minced lung tissue (21, 34, 6).

It is desirable that histamine release reactions and energy metabolism be studied on the same sample of isolated mast cells. The histamine release can be studied quantitatively on a fraction of the population of mast cells obtained from the peritoneal cavity of a single rat (32, 33). However, a single Warburg respirometric experiment would require the pooling of cells from about 10 to 20 rats. The thousand times higher sensitivity of the standard Cartesian diver technique (13) focuses attention on this method for respiration measurements of samples of about 10^4 mast cells. However,

in this technique samples are transferred by pipetting. We have encountered the now classical difficulty that standard volumes pipetted from a cell suspension do not deliver standard numbers of cells, because of clumping of the cells and partly because of adherence of the cells to glass surfaces. With the ampulla diver technique (cf. Zeuthen (36)) used in this study the sampling difficulties are circumvented because the diver itself is the pipette, and the cells which enter the diver are later counted directly.

As the first step in the study of the metabolism of mast cells and its relation to histamine release, this paper reports absolute respiration rates of isolated rat peritoneal mast cells. The present experiments are run on an ultramicro scale because this permits accurate counting of all cells in the respirometer.

MATERIAL AND METHODS

The Mast Cells

Sprague-Dawley white rats (males, ⌐300 gm) were obtained from Anticimex, Stockholm, Sweden. In the laboratory (20°C) they were fed rat chow generously, and used later when weighing 400 to

560 gm. The method of isolation of mast cells employed here is a modification of procedures previously described (25, 2, 31). The rat was lightly anesthetized with ether and bled freely from the carotid arteries. Using thermocautery, under aseptic conditions a narrow opening was made into the abdominal cavity. Six milliliters of solution I (see Table I), in which 300 μg heparin was dissolved, was introduced into the abdominal cavity. With the opening closed, the abdomen was gently massaged for $1\frac{1}{2}$ minutes,

TABLE I

Components of the Suspension Media (I, II) for Mast Cells

Component	Solution	
	I	II
NaCl, 0.154 M	116 ml	116 ml
KCl, 0.154 M	4 "	4 "
CaCl$_2$, 0.11 M	3 "	3 "
MgSO$_4$, 0.154 M	1 "	1 "
Na$_2$HPO$_4$ + KH$_2$PO$_4$, pH 7.4, 0.067 M	6 "	6 "
Human albumin		130 mg
pH	7.25	7.4*

* Final pH adjusted with NaOH.

after which the injected fluid was removed with a pipette. Usually 4 ml could be recovered. The suspension was chilled in ice water and further manipulations were done at 0–4°C. The cell suspension thus obtained represents the upper layer in the 10-ml lusteroid centrifuge tube shown in Fig. 1. The lower two layers are 30 per cent and 40 per cent bovine albumin (w/v), 1 ml each. Armour bovine albumin powder (no preservatives) was dissolved in 0.9 per cent NaCl and brought to pH 6.4–6.7 with about 1/16 volume of 1 N NaOH. The tubes were centrifuged for 5 minutes at 1000 RPM (220 g) using the International Refrigerated Centrifuge. The layering shown in Fig. 1 resulted. The layer containing the mast cells was drained off through a hypodermic needle (no. 12) as illustrated in Fig. 1. Leakage around the needle was prevented by gluing a square piece of rubber sheet (not shown in Fig. 1) to the area of the tube to be pierced by the needle. The drops containing the mast cells were collected in a larger centrifuge tube containing 10 to 15 ml of solution I. After mixing followed by centrifugation for 5 minutes at 700 RPM (110 g) the supernatant was decanted off and the cells were washed for a second time with solution II (Table I) with or without sub-
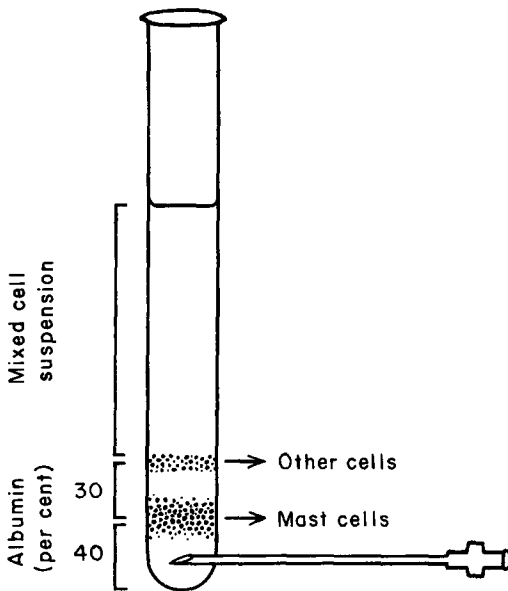

FIGURE 1 Isolation of rat peritoneal mast cells. Layering of the cells in 30 per cent and 40 per cent albumin solution after centrifugation. The cells are drained slowly through the needle.
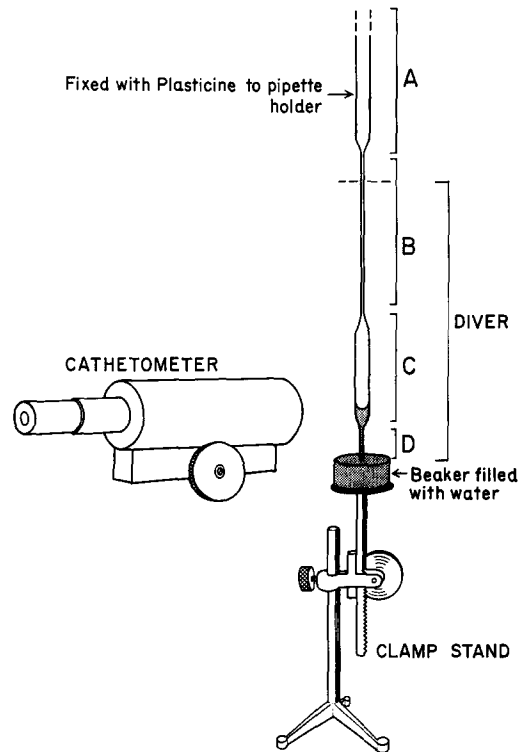

FIGURE 2 Method of measuring the "brake value" of the tail end of the diver "unit" (see text).

strates (see results shown in Table II). Cells to the number of 300,000 to 600,000 were finally collected in 0.1 to 0.2 ml, and pipetted into a siliconed tube. The time from killing of the animal to filling of the diver varied from 1 to 8 hours. Two minutes after killing, the cells were chilled, and were then kept cold until they were taken into the diver. Routinely, a small sample of cells was fixed and stained for microscopy using a mixture containing formalin 10 ml, acetic acid 2 ml, toluidine blue 10 mg, 0.9 per cent NaCl up to 100 ml (15). This caused no measurable change of cell diameter. Counting of the cells in a hemocytometer showed that 0.4 to 1.8 × 10^6 cells could be collected from one rat. The population consisted of 94 to 100 (mean, 98.7) per cent mast cells. The contaminant was mainly small mononuclear cells, probably lymphocytes.

Microgasometry

The ampulla diver technique (36, reviews in 35, 10) was used. Divers were made from Pyrex glass, using as a starting material test tubes with inside/outside diameter ratios of 0.85 to 0.87. From this starting material capillaries (diameter 0.3 to 0.4 mm) were pulled. Using a microflame, "units" (A to D) as shown in Fig. 2 were prepared. These were selected for suitable "brake values" (see below) using the method illustrated in Fig. 2. At this stage the diver (B to D) is still connected through a very narrow channel (B) to the original tube A. The "unit" is held vertically. A horizontal cathetometer is focused on the "ampulla" (C) as shown. A small beaker filled to the brim with water is placed on a clamp stand (see catalogue from the firm Ole Dich, Hvidovre, Denmark) so that the water level is just short of touching the tip (D) of the diver. When the clamp stand is lifted so that the diver makes contact with the water, the ampulla begins to fill by capillarity, and at a rate which is determined by the rate at which air can be expelled through B (the "brake"). This is essentially the principle used for making braking pipettes (see Holter (12)). "Units" for a future supply of divers were selected for lowness of rates (3 to 15 mm/min.) at which water rose by capillarity in the ampulla. Low rates indicate high "brake values." Control divers (0.2 to 0.8 µl gas volume) were stable
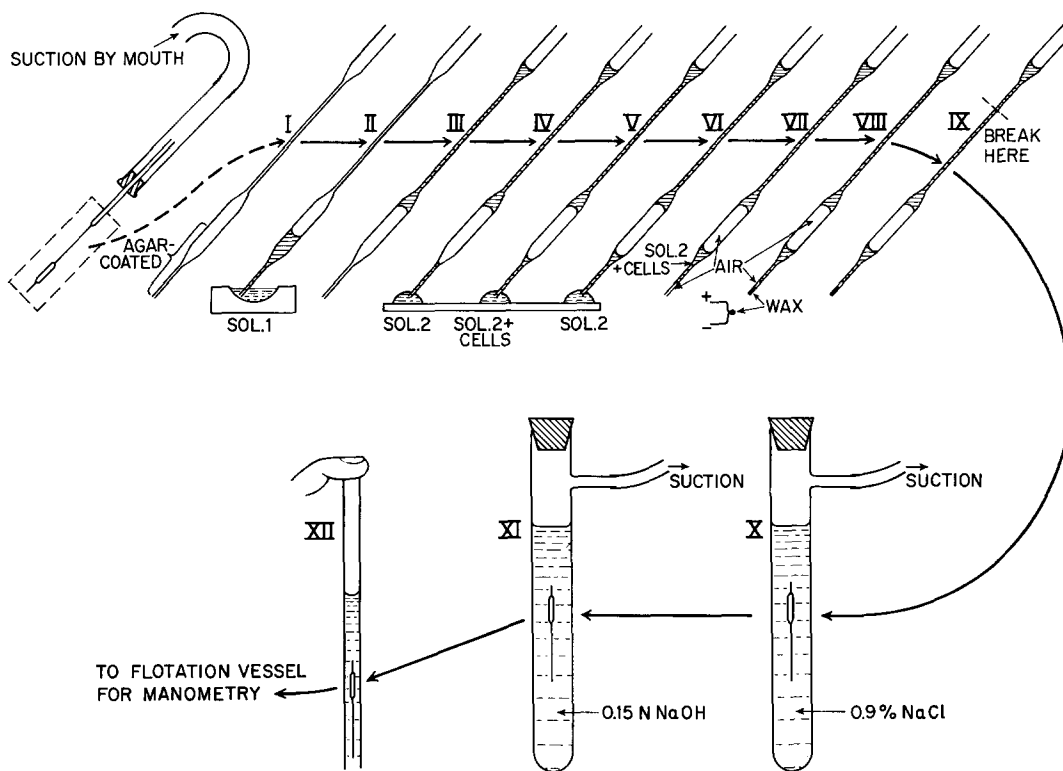

FIGURE 3 Method of filling and preparing the diver for manometry. I shows how the diver is used initially as a pipette. An enlarged view of the part within the dotted lines on no. I has been used to show the subsequent stages from II to IX. In practice the divers were held at a lower angle (nearer to the horizontal plane) than is shown in this schematic drawing. Further details are given in the text.
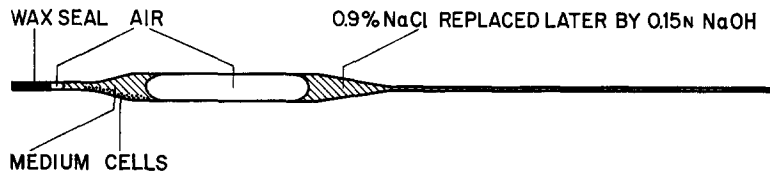
WAX SEAL   AIR   0.9% NaCl REPLACED LATER BY 0.15 N NaOH

MEDIUM CELLS

FIGURE 4   A diver filled for respiration measurement of mast cells
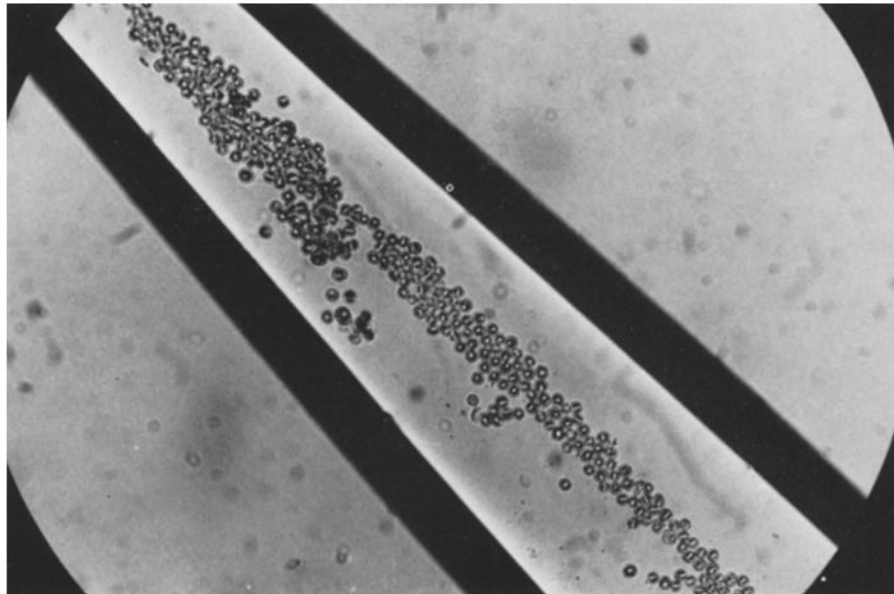

FIGURE 5   Cells in diver, ready for counting. × 1000 (microscopic enlargement × 96)

within $+1 \times 10^{-5}$ to -2.5×10^{-5} μl per hour. "Plus" and "minus" refer to instabilities calculated as gain and loss of gas respectively. In fact, our divers were tight enough to react sluggishly to pressure, and we consider that the recorded instabilities may reflect errors other than gas diffusion through the tail B. The aim is to select divers which are tight to diffusion but not inconveniently sluggish.

Filling the Diver

The diver is filled as illustrated in Fig. 3. The "unit" from Fig. 2 is operated as a pipette (*cf.* Fig. 3, I) after it has been cemented into a wider glass tube connected to the mouth by rubber tubing. It should be noticed that the inside of the tip and the adjacent half of the ampulla is previously agar coated. One per cent agar is sucked halfway into the ampulla; it is blown out before hardening, and the diver is dried by holding it briefly over a 75-watt lamp. The process is repeated, and then the "unit" is heat sterilized for 2 hours or more at 120 to 130°C.

In filling the diver we aim at establishing the situation demonstrated in Fig. 4. At first the brake is

filled with saline as shown in Fig. 3, steps II and III. A tiny amount of suspension medium without cells is then taken into the tip of the diver (Fig. 3, IV). This is immediately followed by a sample ($\sim$0.1 μl) of cell suspension (Fig. 3, V) and this again by a tiny volume of cell-free medium (Fig. 3, VI), no air bubbles separating the three volumes. The purpose is to prevent the cells from coming into contact with the two menisci moving in the diver. The diver is quickly sealed (Fig. 3, VIII) with melted wax, touched the moment gentle suction has cleared (Fig. 3, VII) the outer 0.1 to 0.3 mm of the tip. The wax seal is usually about 1 mm long. The distance from the main gas space of the diver to the farthest cell is maximally 2 mm (quasi steady state established after much less than 100 minutes; see reference 35 and Fig. 6 A). To obtain the situation shown in Fig. 4, essential for the later cell counting, the diver must be held more or less horizontal during the above operations. After the filling, some later displacement of the cells by gravitation can be accomplished by bringing the diver to a vertical position, with repeated observations under the microscope. At this stage the
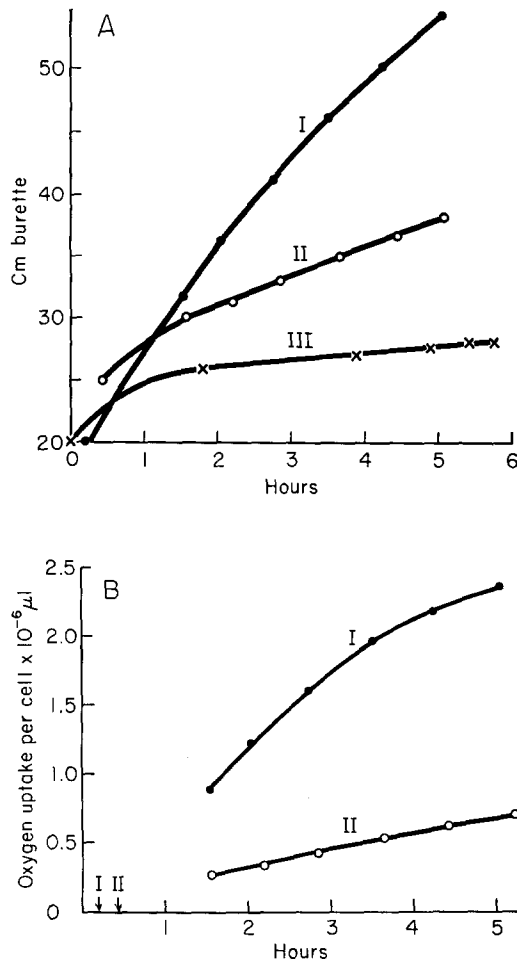
FIGURE 7   414 mast cells; $V_D = 0.41$ μl; substrate, solution II (Table I), pH 7.4; 1 cm burette ~1.54 × 10^{-4} atm. Curve I shows the cumulative gas uptake with live cells. Curve II, diver used for curve I is removed from flotation vessel, frozen ($-20°C$) and thawed three times, and reintroduced into the same flotation vessel and burette-manometer. The interval from end of I to beginning of II is 30 minutes. For both curves zero time is when the burette-manometer is closed to the atmosphere.

FIGURE 6 A AND B   A typical experiment.

I. 750 mast cells; $V_D = 0.44$ μl; medium, solution II (Table I) + glucose 5 mM, pH 7.1; 1 cm burette ~1.65 × 10^{-4} atm.

II. 435 mast cells; $V_D = 0.28$ μl; medium as in I, + sodium azide 5 mM, pH 7.1; 1 cm burette ~1.13 × 10^{-4} atm.

III. No cells; $V_D = 0.33$ μl; medium and pH as in I; 1 cm burette ~1.54 × 10^{-4} atm.

cells are counted, using a Zeiss or Wild dissecting microscope at 100× magnification. For the purpose two salt cellars (or square glass blocks) are placed on the microscope stage, the polished surface upward. One of them has some Plasticine glued to it, and it serves as a movable attachment for the glass tubing to which the diver "unit" is connected. The other is a support over which the diver proper can be gently moved across the microscopic field. Optical conditions are greatly improved if a fairly large droplet of water is placed on the latter salt cellar so that the
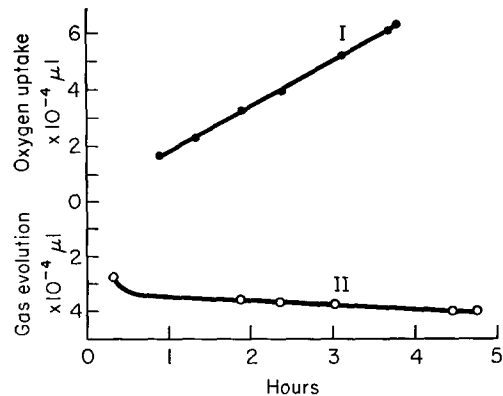
diver can be viewed through water. A suitable grid must be inserted into the ocular of the microscope. If necessary, counting can be done on enlarged photographs like the one shown in Fig. 5. After the diver has been separated by breaking (Fig. 3, IX) the capillary at the level dotted in Figs. 2 and 3, it is balanced (Fig. 3, X) in 0.9 per cent NaCl at 37°C— thus with a tail 10 to 25 mm long. Then the fluid at the tail end is replaced (Fig. 3, XI) by 0.15 N NaOH at 37°C. Finally, the diver is transferred (Fig. 3, XII) to the flotation vessel containing 0.15 N NaOH. To prevent air bubbles from forming on the diver during the actual experiment, CO_2-free air is bubbled through the NaCl and NaOH media kept at 37°C. After the flotation vessel and manometer have been assembled and placed in the bath, CO_2-free air is again bubbled through the flotation medium using a polyethylene tube inserted through the stopcocks. Significant readings can only be made about $\frac{1}{2}$ to 1 hour (the initial period, cf. Fig. 6 A) after the equilibrium pressure is first established. Readings are now taken for several (3 to 6) hours at about half-hour intervals. The time from filling the diver to the first reading is 1 to 2 hours.

Pressure adjustments and readings are done by means of a "burette-manometer" (36) which connects to a closed air space above the floating diver. One millimeter burette reading measures the injection of water into, or withdrawal of water from, the closed air space above the diver, and in different instruments creates pressure changes (Δ p) in this

TABLE II

Respiration of Mast Cells

Group	Wt. of rat (a)	Av. diam. of cells (b)	No. cells in diver (c)	Substrate (d)	Inhibitor (e)	Total O₂ uptake per hr. × 10⁻⁵ μl (f)	O₂ uptake per cell per hr. × 10⁻⁶ μl (g)	Mean value ± SE (h)
	gm	*μ*						
A	446	11.7	242	0	0	6.4	0.22	
	432	13.5	293	0	0	7.8	0.23	0.29 ± 0.04
	432	13.5	583	0	0	21.6	0.35	
	518	12.6	414	0	0	16.2	0.36	
B	481	13.2	611	Glucose, 5 mM	0	29.2	0.46	
	395	12.4	295	"	0	15.0	0.47	
	491	12.3	174	"	0	8.5	0.43	0.47 ± 0.04
	491	12.3	411	"	0	21.8	0.50	
	491	12.3	206	"	0	10.0	0.43	
	560	—	750	"	0	41.0	0.53	
	560	—	435	"	Azide, 5 mM	5.2	0.09	
C	437	12.5	269	Succinate,* 5 mM	0	14.3	0.49	
	437	12.5	298	"	0	9.9	0.30	0.43
	437	12.5	504	"	0	25.6	0.49	
D	506	12.6	323	Pyruvate,* 5 mM	0	13.0	0.37	0.39
	506	12.6	200	"	0	9.4	0.41	
E	518	12.6	584	Isocitrate,* 5 mM	0	16.2	0.26	0.27
	518	12.6	210	"	0	7.0	0.28	

* Sodium salt.

Twenty-four control experiments without cells were done. Controls of A: 6 experiments with 0.9 per cent NaCl or distilled water instead of the biological suspension medium. Controls of B (11 experiments), C (5), D (1), and E (1) were with the respective suspension media shown in the table. The apparent gas exchange in the controls ranged from 2.8×10^{-5} μl/hour (uptake) to 1.0×10^{-5} μl/hour (evolution). There was no indication of differences between control groups A to E. The average, 1.1×10^{-5} μl/hour (uptake), has been subtracted from each of the readings shown in column *f*. The values thus obtained divided by the number of cells in the diver give the values of column *g*.

space equal to 1.15 to 1.65 × 10⁻⁵ atmosphere. If
Δp is the change in equilibrium pressure (atm.) of
the Cartesian diver (gas volume V_D), the oxygen
consumption $\Delta O_2 = \Delta p \times V_D$. The gas volume
(V_D) of divers in the present experiments ranged from
0.2 to 0.8 μl.

Calibration of the manometer and of the diver,
and correction of ΔO_2 to N.T.P., are described in
references 10, 35, and 36.

RESULTS

Fig. 6 A and B shows a typical experiment. In A
the actual burette-manometer readings are shown
for three parallel experiments: with 750 cells
respiring with glucose as a substrate (I); with 435
cells respiring in the same substrate but with
sodium azide (II); and control (III) with the
same medium as I but without cells. In B, curves
I and II are recalculated as cumulative oxygen
uptakes per cell. The arrows indicate the time of
closing the burettes. The control values have not
been deducted. Respiration with glucose is strongly
inhibited by 5 mM azide.

When cells are broken, usually only a few per
cent of the total respiration remains. This finding
has been made use of in an attempt to demonstrate
that the normally recorded slopes do indeed
reflect cellular respiration. Fig. 7 shows the result
of breaking cells by freezing and thawing. Curve
I is the respiration of intact cells, and curve II is
the respiration of cells broken in the diver. The
latter curve is within the range of control values
for divers without cells. The initial gas evolution
observed in experiment II probably exaggerates
the initial period of equilibration which we have
in all our experiments. It probably reflects the fact
that gas previously dissolved in the diver's chilled
media is partly expelled to the gas phase of the
diver when transferred to 37°C. Separate control
experiments gave no indication that the rather
rough treatments resulted in mixing of the two
aqueous compartments in the diver.

Table II gives the respiration values measured
without substrate (A), with glucose (B), with
succinate (C), with pyruvate (D), and with iso-
citrate (E), all substrates as sodium salts in 5 mM
concentration. The necessary controls are de-
scribed in the note to the table. Glucose stimulates
respiration significantly (about 60 per cent). Iso-
citrate seems not to stimulate; succinate and
pyruvate may do so. The endogenous respiration
is 0.29 × 10⁻⁶ μl per hour per cell; the glucose-

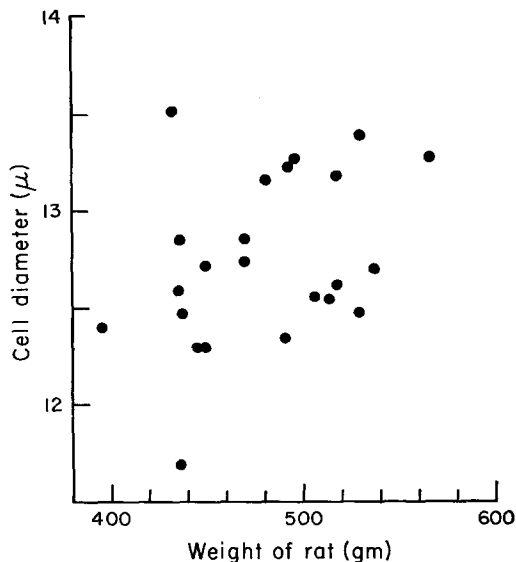FIGURE 8   Relation of mast cell diameter to the weight
of the rat.

stimulated respiration is 0.47 × 10⁻⁶ μl per hour
per cell.

Fig. 8 shows the mean cell diameter for mast
cells from 23 male rats ranging in weight from
400 to 560 gm. The mean value (± SE) for the 23
animals was 12.75 μ ± 0.09. In spite of the small
variation in the individual rats, a relation between
body weight and cell diameter seems to emerge
from the figure—slightly larger cells being ob-
tained from larger rats—as observed earlier by
Padawer and Gordon (26). Considering the errors
involved in the measurement of cell volume from
the diameter (24) and in the gasometric technique,
we have made no attempt to correct the respiration
values for the small variation in the mean diameter
of cells from individual rats.

DISCUSSION

No quantitative data for the respiration of normal
mast cells appear to have been published. Dr.
Jørgen Kieler (personal communication, un-
published data), using standard divers (see Holter,
reference 13) and about 10,000 mast cells, which
were transferred to the diver by a braking pipette,
obtained a respiration rate (with glucose) of 0.4 to
0.8 × 10⁻⁶ μl per hour per cell. This is in fairly
good agreement with our results (0.43 to 0.53 ×
10⁻⁶ μl per hour per cell, Table II). The values re-
ported by us are low (Table III) as compared

TABLE III

Comparison of the Respiration Rate of the Rat Peritoneal Mast Cell with That of Other Related Cells

Type of cell	Species	O_2 uptake* per cell per hr. $\times 10^{-6} \mu l$ (or $\times 10^{-8} \mu mole$)
Mast cells‡	Rat	0.47§ (2.10)
Neoplastic mast cells (calculated from the data of Fredholm (9))	Mouse	1.93 (8.62)
Polymorphonuclear leukocytes (calculated from the data of Oren et al. (22))	Guinea pig	0.34 (1.52)
Polymorphonuclear leukocytes (90–95%) (Martin et al. (16))	Human	0.21|| (0.94)
Myeloid leukemia cells (Bicz (4))	Human	0.22¶ (0.98)

* With 5 to 10 mM glucose in the medium.

‡ After our manuscript was ready for publication a communication by Mongar and Perera (17) appeared. These authors used 1.5×10^6 peritoneal cells in a capillary respirometer, and observed oxygen uptakes of 0.3 to 0.5 picoliter (pl) per cell per minute. For 1 pl ($= 10^{-12}$ liter) this would correspond to 1.8 to $3.0 \times 10^{-5} \mu l$ per cell per hour, which is 40 to 60 times the value found by us.

§ Mast cell volume, calculated (as sphere) from average diameter (12.75 μ), $1.1 \times 10^{-6} \mu l$. Oxygen uptake per volume cell ($\mu l/\mu l$ at 37°C), 0.43.

|| Oxygen uptake per cell per hour in the absence of substrate, $0.34 \times 10^{-6} \mu l$ ($= 1.52 \times 10^{-8} \mu mole$).

¶ Respiration in presence of 0.03 per cent CO_2 (others CO_2-free atmosphere).

with those for mastocytoma cells (Fredholm, reference 9, also personal communication), but are of the same order as those for leukocytes of the granulocytic series, normal (22, 16) or leukemic (4). The lower respiration intensity of normal as compared with neoplastic mast cells seems to conform with the observation of Padawer (23) that Smith's (30) electron micrographs of normal mast cells from hamster and rat show very much fewer mitochondria than do Bloom's (5) sections of mouse mastocytoma cells. Bloom agrees that there is a striking difference in the concentration of mitochondria per unit area section, but suggests that this reflects the overcrowding of granules in the normal mast cell. The respiration data now available invite the suggestion that the number of mitochondria in a mast cell may be much lower than that in a mastocytoma cell. Padawer (24),

using tetrazolium dyes, observed very few sites of succinic dehydrogenase activity in normal mast cells as compared with mastocytoma cells.

Our results (Table II) indicate stimulation of mast cell respiration by glucose, showing absence of the Crabtree effect found by Oren et al. (22) and by Martin et al. (16) for leukocytes. On the other hand, the mast cells resemble the leukocytes (16) in the lack of important stimulation of respiration by pyruvate and Krebs cycle intermediates (see Table II). The operation of the tricarboxylic acid cycle in rat peritoneal mast cells is, however, suggested by the demonstration of succinic dehydrogenase by tetrazolium staining (11, 24).

REFERENCES

1. ARCHER, G. T., Release of histamine from mast cells by tissue extracts, *Nature*, 1958, **182**, 726.
2. ARCHER, G. T., The release of histamine from the mast cells of the rat, *Australian J. Exp. Biol. and Med. Sc.*, 1959, **37**, 383.
3. BENDITT, E. P., ARASE, M., and ROEPER, M. R., Histamine and heparin in isolated rat mast cells, *J. Histochem. and Cytochem.*, 1956, **4**, 419.
4. BICZ, W., The influence of carbon dioxide tension on the respiration of normal and leukemic human leukocytes, *Cancer Research*, 1960, **20**, 184.
5. BLOOM, G. D., Electron microscopy of neoplastic mast cells: A study of the mouse mastocytoma mast cells, *Ann. N. Y. Acad. Sc.*, 1963, **103**, 53.
6. CHAKRAVARTY, N., Aerobic metabolism in anaphylactic reaction *in vitro*, *Am. J. Physiol.*, 1962, **203**, 1193.

7. Chakravarty, N., Inhibition of anaphylactic histamine release by 2-deoxyglucose, *Nature*, 1962, **194**, 1182.

8. Diamant, B., Betydelsen av glykos vid histamin frisättning och mast cell degranulering, thesis, Karolinska Institutet, Stockholm, Sweden, 1962.

9. Fredholm, B., Effects of histamine-liberators on neoplastic mast cells, *in* Proc. 2nd Internat. Pharmacol. Meeting (Prague), *Biochem. Pharmacol.*, 1963, **12**, suppl., 212.

10. Glick, D., Quantitative chemical techniques of histochemistry and cytochemistry, vol. 1. New York, Interscience Publishers, 1961.

11. Glick, D., and Pothapragada, S., Succinic dehydrogenase system in isolated mast cells from the rat, *Proc. Soc. Exp. Biol. and Med.*, 1961, **106**, 359.

12. Holter, H., Technique of the Cartesian diver, *Compt. rend. trav. Lab. Carlsberg*, Ser. chim., 1943, **24**, 399.

13. Holter, H., The Cartesian diver, *in* General Cytochemical Methods, (J. F. Danielli, editor), New York, Academic Press, 1961, **2**, 93.

14. Keller, R., Effect of antibodies against rat γ-globulin or rat albumin on isolated peritoneal rat mast cells, *Nature*, 1962, **193**, 282.

15. Lagunoff, D., and Benditt, E. P., 5-Hydroxytryptophan decarboxylase activity in rat mast cells, *Am. J. Physiol.*, 1959, **196**, 993.

16. Martin, S. P., McKinny, G. R., and Green, R., The metabolism of human polymorphonuclear leucocytes, *Ann. N. Y. Acad. Sc.*, 1955, **59**, 996.

17. Mongar, J. L., and Perera, B. A. V., Oxygen consumption of isolated mast cells in the anaphylactic reaction, *Nature*, 1964, **202**, 93.

18. Mongar, J. L., and Schild, H. O., Inhibition of anaphylactic reaction, *J. Physiol.*, 1957, **135**, 301.

19. Mongar, J. L., and Schild, H. O., Effect of temperature on the anaphylactic reaction, *J. Physiol.*, 1957, **135**, 320.

20. Mota, I., and Vugman, I., Effects of anaphylactic shock and compound 48/80 on the mast cells of the guinea pig lung, *Nature*, 1956, **177**, 427.

21. Moussatche, H., and Prouvost-Danon, A., Influence of oxidative phosphorylation inhibitors on the histamine release in the anaphylactic reaction *in vitro*, *Experientia*, 1958, **14**, 414.

22. Oren, R., Farnham, A. E., Saito, K.,

Milofsky, E., and Karnovsky, M. L., Metabolic patterns in three types of phagocytizing cells, *J. Cell Biol.*, 1963, **17**, 487.

23. Padawer, J., Mast cells and basophils, *Ann. N. Y. Acad. Sc.*, 1963, **103**, 85.

24. Padawer, J., Quantitative studies with mast cells, *Ann. N. Y. Acad. Sc.*, 1963, **103**, 87.

25. Padawer, J., and Gordon, A. S., Isolation of mast cells from other cellular elements of rat peritoneal fluid, *Proc. Soc. Exp. Biol. and Med.*, 1955, **88**, 29.

26. Padawer, J., and Gordon, A. S., Peritoneal fluid mast cells: Their number and morphology in rats of various body weights (ages), *J. Gerontol.*, 1956, **11**, 268.

27. Parrot, J. L., Sur la réaction cellulaire de l'anaphylaxie. Son caractère aerobic, *Compt. rend. Soc. biol.*, 1942, **136**, 361.

28. Prouvost-Danon, A., and Moussatche, H., Influence of anaerobic glycolysis on release of histamine in the guinea pig and rat anaphylactic reaction *in vitro*, *Nature*, 1961, **192**, 361.

29. Riley, J. F., and West, G. B., The presence of histamine in tissue mast cells, *J. Physiol. (London)*, 1953, **120**, 528.

30. Smith, D. E., Electron microscopy of normal mast cells under various experimental conditions, *Ann. N. Y. Acad. Sc.*, 1963, **103**, 40.

31. Uvnäs, B., and Thon, I., Isolation of "biologically intact" mast cells, *Exp. Cell Research*, 1959, **18**, 512.

32. Uvnäs, B., and Thon, I., Evidence for enzymatic histamine release from isolated mast cells, *Exp. Cell Research*, 1961, **23**, 45.

33. Van Arsdel, P. P., and Bray, R. E., On the release of histamine from rat peritoneal mast cells by compound 48/80: Effect of metabolic inhibitors, *J. Pharmacol. and Exp. Therap.*, 1961, **133**, 319.

34. Yamasaki, H., Muraoka, S., and Endo, K., Energy metabolic aspect of histamine release in anaphylaxis, *Japan. J. Pharmacol.*, 1960, **10**, 21.

35. Zajicek, J., and Zeuthen, E., Quantitative determination by a special "ampulla-diver" of cholinesterase activity in individual cells, with notes on other uses of the method, *in* General Cytochemical Methods, (J. F. Danielli, editor), New York, Academic Press, 1961, **2**, 131.

36. Zeuthen, E., Growth as related to the cell cycle in single cultures of *Tetrahymena pyriformis*, *J. Embryol. and Exp. Morphol.*, 1953, **1**, 239.