Polycythemic Mice Produced by Hypoxia in Silicone Rubber Membrane Enclosures: A New Technique.* (31246)

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(Introduced by A. I. Chernoff)
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The polycythemic mouse has been found to be the most sensitive laboratory animal to use for erythropoietin assays(1-3). Polycythemia is ordinarily produced by transfusion, hypoxia, or a combination of the two(4-6). However, transfusions are both expensive and time-consuming; and although keeping animals at reduced atmospheric pressure works well, chambers must be carefully controlled or laboratory accidents can result in the loss of several weeks' supply of animals. A simple, safe method for producing polycythemia which obviates these difficulties became possible with the invention of dimethyl silicone rubber membranes(7). Because of the permeability properties of the membranes, the oxygen concentration within a membrane enclosure is dependent on the oxygen consumption of animals contained. This communication presents results which indicate that specially built cages, covered with dimethyl silicone rubber, offer an improved method of producing polycythemia in laboratory mice.

Materials and methods. The dimethyl silicone rubber was hand-fabricated in the laboratories of General Electric Co. Silicone rubber film 1 mil (0.001-inch) in thickness was bonded on both sides of the membrane with 0.005-inch-thick "Dacron" mat in order to improve the membrane's durability, protect it from abrasion, and to support a pressure differential.

The enclosures were fabricated by modifying 2 standard "Lexan" animal cage bottoms.† One cage bottom, which was subsequently inverted to become the top of the enclosure, was modified by cutting openings (amounting to 143 square inches) in the sides and top. The openings were covered with the "Dacron" backed silicone rubber membrane. Two tube fittings were installed, one in each end of the top of the enclosure, for the purpose of flushing the cage with gas mixtures. An additional hole was cut to allow the positioning of a sensor to monitor the oxygen concentration. A standard plastic animal cage which was easily removable for cleaning was placed in the lower enclosure. Fig. 1 shows the enclosure with the top and bottom sealed together with plastic tape.

The cages were opened 3 times a week for cleaning. Routinely after resealing, they were flushed for approximately 2 minutes with a mixture of 10% oxygen and 90% nitrogen. In one series of experiments the cages were allowed to come to equilibrium without flushing with the gas mixture, and the time to reach equilibrium was noted.

Female A/Jax mice 6-8 weeks old were used in all experiments. For most experiments 11 mice weighing approximately 16 g each were placed in the enclosures for periods up to 3 weeks. In other experiments different numbers of mice were placed in the enclosure in order to measure the effect on the oxygen concentration of the air in the enclosure.

Oxygen content was measured by a Beckman Polarographic Oxygen Analyzer. The CO_2 partial pressure was measured in an Instrumentation Laboratory Inc. pH/gas analyzer. Hematocrits, hemoglobin determinations, and reticulocyte counts were performed by standard methods(8).

The Fe^{59} red blood cell incorporation was

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† Obtained from Maryland Plastics Co.

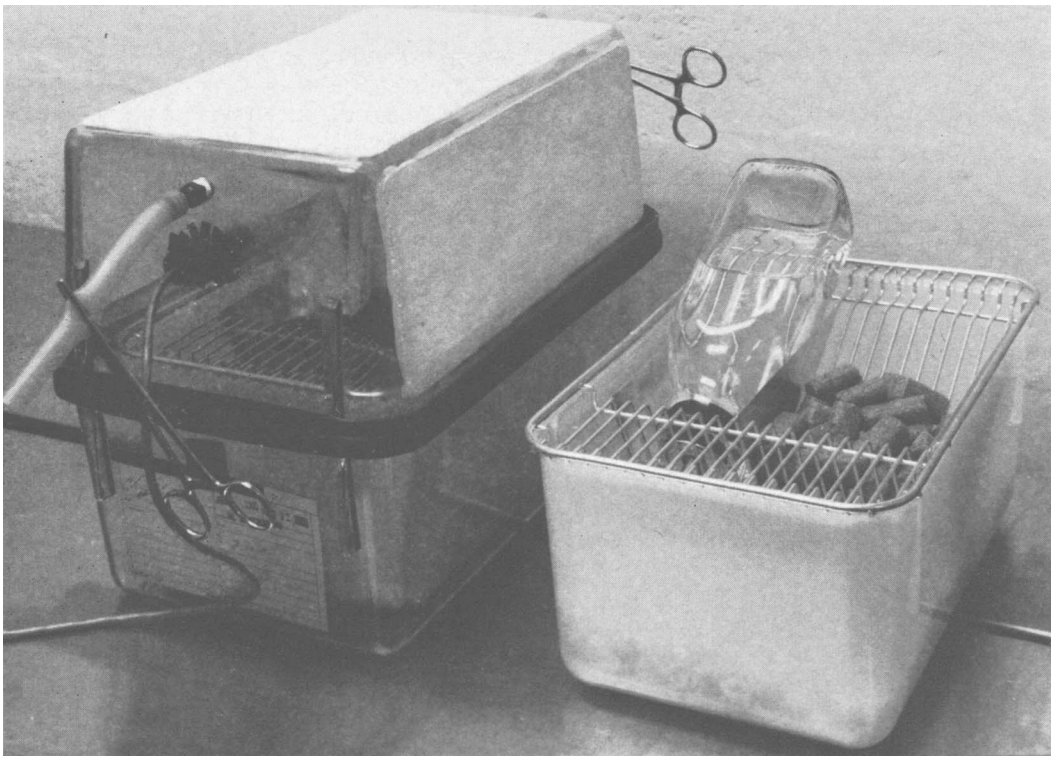

FIG. 1. Two mouse cages; on the left cage is a removable silicone rubber membrane enclosure.

measured by a previously described method (9). On days 4 and 5 after removal of the mice from the enclosure, either 0.5 ml of saline or 0.25 unit of erythropoietin‡ in saline was injected i.p. On day 6, 0.5 μc of Fe^{59}§ in 0.5 ml of saline was given i.p.; the radioiron incorporation in the red blood cells was measured 48 hours later using a Nuclear-Chicago well-type scintillation counter. Blood was obtained by retro-orbital puncture.

The data were analyzed by the one-way classification analysis of variance method and Duncan's Multiple Range test(10).

Results. In a series of 6 experiments, different numbers of animals were allowed to reach oxygen equilibrium without flushing the cages with 10% oxygen. An average of 470.5 minutes (range 442 to 520 minutes) was required for equilibrium to be reached. The subsequent oxygen concentration was found to be dependent on the number of animals in the enclosure (Table I). A diurnal variation occurred; the lowest readings were recorded during nocturnal hours, when the animals showed increased activity. With 11 animals in the enclosure, the range of CO_2 determinations made on air inside the enclosure was 1.5% to 2.1%.

When 11 animals were placed in an enclosure for a period of 3 weeks, there was a progressive rise in hemoglobin and hemato-

TABLE I. Effect of Number of Mice at Different Times of Day on Oxygen Content in Cages Covered with Permselective Membrane.

No. mice in enclosure	O_2 content (%)				Approximate equivalent altitude (ft)
	9 PM	12 M	6 AM	12 N	
1	18.50	18.00	17.87	18.50	3,900
3	15.00	14.00	14.67	16.67	8,800
5	10.50	11.00	11.75	14.00	15,000
7	8.50	8.75	9.25	10.75	21,000
9	8.00	7.50	8.00	9.00	24,000
11	6.75	6.50	6.67	7.50	28,000

‡ Erythropoietin Standard B obtained from Division of Biological Standards, Nat. Inst. for Medical Research, London.

§ Fe^{59} as ferrous citrate specific activity 16.9 to 17.8 mc/mg Fe.

TABLE II. Effect on Hemoglobin, Hematocrit, and Reticulocyte Count After Enclosure of Mice in Cages Covered with Permselective Membrane. Results are recorded ± standard error of mean. Treatment means having different superscript letters are statistically different at 5% level using Duncan's Multiple Range test. Numbers in parentheses refer to number of observations.

	Hematocrit (%)	Hemoglobin (g %)	Reticulocytes (%)
Normal (room air)	$47.65^{e} \pm .64$ (18)	$18.05^{c} \pm .22$ (18)	$3.29^{b} \pm .28$ (18)
In enclosure: 1 wk	$63.90^{c} \pm 1.22$ (5)	$15.76^{e} \pm .42$ (5)	$5.20^{a} \pm .54$ (5)
2 "	$68.60^{b} \pm 1.22$ (5)	$24.35^{a} \pm .42$ (5)	$5.30^{a} \pm .54$ (5)
3 "	$73.50^{a} \pm .96$ (8)	$25.28^{a} \pm .33$ (8)	$4.75^{a} \pm .42$ (8)
8 days post enclosure:			
Saline	$55.89^{d} \pm .91$ (9)	$19.03^{c} \pm .54$ (3)	$.10^{e} \pm .40$ (9)
.5 unit erythropoietin	$57.89^{d} \pm .91$ (9)	$20.65^{b} \pm .47$ (4)	$.94^{c} \pm .40$ (9)

crit values (Table II). The hematocrits rose from 47.65% to 73.5%. Eight days after the animals were removed from the enclosure the mean hematocrit was 56.89%. The average reticulocyte count of normal animals before being placed in the chamber was 3.29%, rose to approximately 5.0% during the period in the enclosure, and was 0.10% eight days following removal from the enclosure.

Fig. 2 shows the iron incorporation of polycythemic animals injected with saline or erythropoietin, as well as the iron incorporation of normal animals. The treatment means are different at the 1% level as shown by Duncan's Multiple Range test.

Discussion. Based on the selective permeability of certain organic materials, the concept of using semipermeable membranes for separation of gases and vapors dates back to the nineteenth century. The term "selective permeability" means that gas A will permeate the membrane faster than gas B and that gas B will permeate the membrane faster than gas C, and so on. These unique properties led to the choice of the term "permselective membranes," which separates these materials from those which are merely porous and pass gases in a nonselective manner. Some of the properties of the dimethyl silicone rubber membranes and their use in a blood oxygenator have been described by Esmond and Dibelius(11).

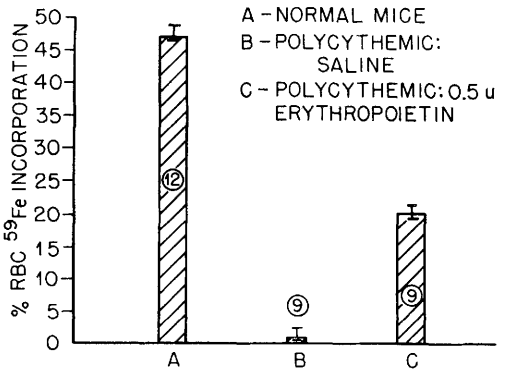

FIG. 2. Radioactive iron incorporation by normal A/Jax mice and mice made polycythemic by enclosure for 3 weeks in membrane-covered cages. Group C received erythropoietin injections. Numbers in circles refer to numbers of mice, and bars represent standard error of mean. Results are combined data of 2 experiments.

In the enclosures described here, as the animals consumed oxygen the partial pressure of oxygen inside the enclosure was lowered below that of ambient air. This produced an oxygen partial pressure difference across the membrane into the enclosure according to the following equation:

$$N_{O_2} = \frac{PrO_2}{t} A(PX_{O_2} - pX_{O_2})$$

where

N = cc per second (NTP)

t = film thickness in cm

PrO_2 = permeability of oxygen

P = pressure of feed gas in cm of mercury

X_{O_2} = mol fraction of O_2 in feed gas

p = pressure of product gas in cm of mercury

x_{O_2} = mol fraction of oxygen in product gas

A = area in cm^2

As the animals produced carbon dioxide, the partial pressure of carbon dioxide built up inside the enclosure, thus producing a partial pressure difference of carbon dioxide across the membrane. Carbon dioxide permeated

the membrane out from the enclosure according to the equation:

$$N_{CO_2} = \frac{PrCO_2}{t} A (PX_{CO_2} - px_{CO_2})$$

Because of the difference of permeability between carbon dioxide and oxygen, the partial pressure difference to drive the carbon dioxide out of the cage is less than 1/5 that necessary to supply the oxygen to the animals. For example, if the partial pressure of oxygen is reduced from 21% outside the cage to 11% inside the cage, the carbon dioxide concentration inside the cage will rise to about 2%.

The enclosures represent a new method for producing polycythemia in experimental animals. The animals appeared healthy and ate well while in the enclosures, but did lose weight and consumed less water than animals kept in room air. The mice regained the weight lost in 4 days after removal; therefore, it was assumed that the weight loss represents primarily fluid loss. The reasons for this weight loss are under continued study. Animals held in a hypobaric chamber also lose weight and after removal regain it in a similar manner (unpublished observations).

The reason for the apparent drop in hemoglobin after 1 week in the enclosure (Table II) is unknown. This drop was inconsistent with hematocrits measured on the same samples which demonstrated the expected increase.

The A/Jax mouse used in these experiments and kept in the membrane enclosures represents a very sensitive assay animal for erythropoietin assays. These polycythemic assay animals injected with 0.5 unit of erythropoietin had a radio-iron red blood cell incorporation of 19.96% as compared to an incorporation of 0.67% in saline-injected animals. The iron incorporations are higher than reported in two recent investigations using different strains of mice(12,13). Complete dose-response curves of animals made polycythemic in the membrane cage enclosures will be reported subsequently.

While the results reported here are concerned with enclosing animals in membrane cages and measuring the effect on red cell parameters, it is obvious that the enclosures would be easily adaptable to other physiological experiments in which a mild to moderate hypoxic stimulus is needed.

Summary. Utilizing enclosures covered with silicone rubber membrane, a new, safe, and simple method for producing polycythemia by hypoxia is presented. The polycythemic animals are excellent for use in erythropoietin assays.

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