MULTICHAMBER APPARATUS FOR MEASURING GAS EXCHANGE OF SMALL LABORATORY ANIMALS

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A modification of the Szent-Györgyi method for simultaneous determination of gas exchange in several small laboratory animals is described.

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A 6-chamber apparatus for studying gas exchange in mice was first used by Szent-Györgyi [1]. The method which he used not only has the advantage that the gas exchange can be determined simultaneously, i.e., in identical conditions, in two series of animals or more, but the necessary calculations are simple.

The essence of the method is that the expired air is passed through a known quantity of alkali and the time required for its neutralization by the carbon dioxide exhaled by the animal is measured. To secure closer contact between the expired carbon dioxide and alkali, the expired air is passed through a fine steel filter, and a 5% aqueous solution of n-butyl alcohol is added to the cylinder containing alkali.

The apparatus proposed by the present author is constructed in accordance with the principle of the apparatus used by Szent-Györgyi, but a number of modifications and additions have been introduced. The fine disc filter of stainless steel is replaced by an ordinary Schott filter. It has been shown experimentally that filter No. 1 is most suitable. To keep the temperature constant throughout the experiment all six chambers are placed in an incubator. The water temperature in the incubator jacket is kept at 20°; a temperature of 22° is thereby maintained in the incubator (because of the heat given off by the mice and by the daylight lamp). The chambers consisted of 3-liter wide-necked flasks with an additional neck (Bunsen flasks). Ten mice were placed into each such chamber. If five mice are placed in a chamber, the variations in total gas exchange calculated from the results of investigations in six chambers as a rule do not exceed 5%, and from data obtained using three chambers 10%. Indices showing variations of not more than 10% of the initial value can be obtained by investigating mice in two chambers, each containing ten animals.

The experiment is performed as follows. The mice are weighed 3 h before the beginning of the experiment and placed in the chambers where they receive no food and become accustomed to the new environment. The chambers are placed in the incubator and connected to a fore-vacuum pump (see figure), which draws air from the chamber through the cylinder A with a 5% aqueous solution of n-butyl alcohol (250 ml). As it passes through the filter C and the aqueous solution of butyl alcohol, the air forms a fine foam, filling from 2/3 to 3/4 of the cylinder. The velocity of air movement and, consequently, the foam level in the cylinder are regulated by the tap D. Ordinary glass taps were found to be unstable in operation and required constant regulation. Taps from gas burners, to which a copper tube is soldered (see figure), are sufficiently stable in operation. To prevent moisture from entering the fore-vacuum pump, a cylinder containing calcium chloride is introduced between the cylinder A and the fore-vacuum pump. A few minutes before the beginning of the experiment, 50 ml of 0.5 N caustic soda with a few drops of indicator (5% alcoholic solution of thymophthalein) is poured into the container B. To remove CO_2 from the air entering the chamber, 15 min before the beginning of the experiment all the chambers are connected to a "trap" containing soda-lime; 3 h after the mice have been placed in the chambers the contents of the containers B (as far as possible at the same time) are poured into the cylinders. Counting the time now begins, and ends when the solution is completely decolorized. The amount of carbon dioxide exhaled by the mice is calculated as follows. It follows from the formula $2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$, that 50 ml of 0.5N NaOH will be neutralized by 550 mg CO_2. Knowing the time taken for neutralizing the alkali, the amount of CO_2 excreted by the mice per minute can be determined. The figure obtained is multiplied by 1000 and divided

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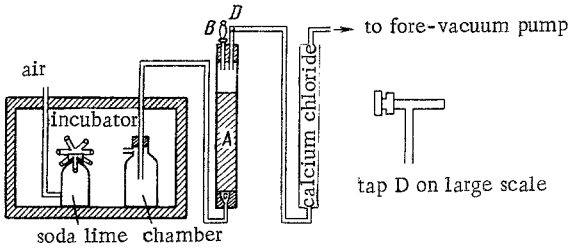

Scheme showing one of the six units of the apparatus for determining gas exchange in small laboratory animals. Height of cylinder 75 cm, diameter 5 cm. Explanation in text.

by the body weight of the mice in the chamber, thereby giving the required CO_2 excretion per kilogram per minute.

If five mice are placed in the chamber the gas exchange of the animals is reduced by approximately 40% compared with the results obtained for one mouse; in our experiment on sexually mature C_3H and C_{57} females it was 61 mg/kg/min. if ten mice are placed in the chamber the CO_2 excretion is reduced to 44 mg/kg/min. The decrease in CO_2 excretion obtained when several mice are placed together in the chamber is in agreement with data in the literature [2] and is due to a decrease in the heat emission surface and also to absence of an orienting reaction in the mice.

LITERATURE CITED

1. A. D. Slonim, Animal Heat and its Regulation in Mammals [in Russian], Moscow-Leningrad (1952).
2. A. Szent-Györgyi, Bioenergetics [Russian translation], Moscow (1960).