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AN APPARATUS FOR THE STUDY OF RESPIRATORY GAS
METABOLISM IN SMALL ANIMALS

by

M.D. Haçaniuk

Translated from

Fiziologichnyi Zhurnal (Ukraine), 1 (1955), 3, 123-129

by

E.R. Hope

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Directorate of Scientific Information Services

DRB Canada

January 1965

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AN APPARATUS FOR THE STUDY OF RESPIRATORY GAS
METABOLISM IN SMALL ANIMALS

M. D. Haçaniuk

1. Introduction

The gaseous exchange apparatus of our design is one of those used for studying respiratory gas metabolism in animals, that is to say, an apparatus whereby the quantity of oxygen consumed by the animals and the quantity of carbon dioxide breathed out by them may be determined.

This apparatus is distinguished from already known apparatus of such type by the fact that its construction is based on a combination of two principles:

- 1) Volumetric determination of the amount of absorbed oxygen, and
- 2) Titration method of determining the amount of released carbon dioxide.

My purpose was to create an apparatus for research on respiratory gaseous metabolism in small animals, since, so far as I am informed, no such equipment is produced in the Soviet Union. In designing the apparatus, the three tasks which I undertook were to simplify procedure in the study of gaseous metabolism, to make possible the dynamic study of gaseous metabolism, and to ensure greater precision.

The apparatus of our design has several economic and technical advantages. In the first place it is to be noted that this apparatus frees the researcher from the necessity of using analytic scales of large capacity (up to 1 kg) and high sensitivity, such as are required for carbon dioxide determination by the weighing method (in this method, one weighs two vessels containing soda lime, which absorbs carbon dioxide). The apparatus proposed by us saves much time as compared with the weighing method.

Since oxygen is determined by a volumetric method and since the volume of a gas depends on the temperature, the whole set-up for the study of respiratory gaseous metabolism is installed in a constant-temperature aquarium.

In the weighing method, two jars with soda lime are placed in the aquarium, after being weighed on an analytic balance. The soda lime absorbs carbon dioxide. When the experimental animal has been kept in the apparatus for a sufficient time to make the determination, the apparatus is shut down; then the soda-lime jars have to be isolated from the apparatus as a whole and the tubes closed off so that no water gets through them, after which the jars are removed from the aquarium and carefully dried until they are in the same state as they were before immersion in the aquarium. They are then allowed to cool to the temperature of the room in which the analytical balance is located, and

are weighed on this balance. This takes so much time that the experimental animal cannot meanwhile be left without any air circulation, since the reserve of oxygen that is in the jar is insufficient; moreover in this time too much carbon dioxide would accumulate, indeed rising to physiologically intolerable concentrations. In other words, if we want to determine the carbon dioxide then we have to take the animal out of the apparatus. Then to continue our carbon dioxide observations after such an interruption, we have to immerse the weighed jars in the aquarium, connect them to the appropriate parts of the apparatus, and wait for some time until the temperature of the cooled jars becomes equal to the temperature of the water in the aquarium. Thus with the weighing method a carbon dioxide determination takes much time.

For determining carbon dioxide by our titration method little time is required, for the jars (Wulf jars) with the barium hydroxide solutions that absorb the carbon dioxide do not have to be taken out of the aquarium. They are merely lifted sufficiently to raise out of the water the [ends of the] closed glass tubes which pass through the central orifice of the jar. These tubes have their lower ends below the surface of the barium hydroxide solution. Through them we take samples of the barium hydroxide for titration. The lifting of the jars, which are mounted on a support, takes 1-2 seconds (automatic lifter). Sampling the solutions in the jars (we take from each jar two 1-2 ml samples) likewise requires very little time (half a minute).

After taking the samples the glass tubes are stoppered with glass rods, the jars are lowered into the aquarium, the blower is turned on, and the experiment is continued. Titration takes less time than weighing. Thus the volumetric method saves much time.

An important advantage of our design is the possibility of making frequent carbon dioxide determinations at short intervals of time, without taking the animals out of the apparatus and without removing the jars containing the barium hydroxide solution. Thus the said design makes it possible to observe continuously the dynamics of the entire course of the gaseous exchange. When working with rats, two jars containing 800 ml each of barium hydroxide solution are sufficient to absorb the carbon dioxide for a period of 3-4 hours, depending on the weight of the rat.

The carbon dioxide determinations may be made with greater precision than in the weighing method, since the weighing method involves several factors that impair its value. The jars removed from the water are difficult to dry, for weighing, to precisely the same state of dryness in which they were before their immersion in the aquarium. Furthermore the jars, after their removal from the aquarium, have to cool to room temperature. If the jars are not fully cooled, another error is introduced in weighing. But if one waits until the temperature of the two jars becomes equal to the room temperature, much time is expended.

II. Description of the Apparatus

The apparatus consists of four main parts:

a) Aquarium tank; in it and on its sides the main gas exchange apparatus is installed;

- b) Heater under the aquarium;
- c) Temperature regulator in the aquarium;
- d) Mixer, for stirring the water in the aquarium.

The drawing shows parts numbered as follows:

1. Manometer filled with some colored liquid, for instance, water colored with methylene blue.
2. Pressure-suction blower (of Leitz pump type).
3. Jar into which a small animal may be placed, for instance a rat.
4. Motor and mixer.
5. Two Wulf jars, each of one liter capacity.

Into each jar, 800 ml of barium hydroxide solution are poured. The central orifice is closed with a rubber stopper through which there passes a glass tube ending 2 cm below the surface of the barium solution. Over the end of the glass tube a rubber tube is fitted, stoppered with a glass rod.

6. Mixer drive-pulley.
7. Trap to prevent sulfuric acid getting into jar 5 from jar 8.
8. Jar (800 ml approximately) containing concentrated sulfuric acid (500 ml) for absorbing water vapor.
9. Jar of capacity approximately 3 l, filled with oxygen.
10. 500 ml burette.
11. Coil for warming the water passing into jar 9 from burette 10.
12. Jar containing water, approximately 2-3 l.
13. Rubber bulb for pumping water from jar 12 to burette 10.
14. Connection to local electric power supply.
15. Relay winding.
16. 4-volt accumulator, or equivalent rectifier of local power supply.
17. Platinum contact of temperature regulator.
18. Toluol.
19. Heater coil under the aquarium.
20. Mixer blades.

Beckman thermometer, not shown in the drawing.

Items 1, 2, 3, 5, 7, 8, 9, 10, 12 and 13 make up the gaseous exchange apparatus proper (Fig. 3).

Items 4, 6 and 20 constitute the mixer.

Items 14 and 19 supply heat.

Items 15, 16, 17 and 18 constitute the temperature regulator (Fig. 4).

III. Arrangement and Operation of the Apparatus

The gaseous exchange apparatus constitutes a closed system in which there is a blower-driven circulation of air. From the pressure-suction blower 2 air passes into jar 3 in which the small experimental animal is placed. The jar has a wide neck, closed with a rubber stopper. Through the stopper there pass two glass tubes. The air passes through the inlet tube, which ends just below the stopper. The outlet tube is bent over a little, so as not to impede the movements of the experimental animal. This tube extends nearly to the bottom of the jar, for carbon dioxide is heavier than air.

The apparatus operates as follows. First the mixer is set going. The motor (4), mounted behind the aquarium, is switched on. It turns the drive-pulley (6) of the mixer. The blades of the mixer (20) stir the water, which is heated from below, and maintain an identical water temperature throughout the aquarium tank.

At the same time the heater is turned on. Current from the local power supply passes from 14 through the heating coil (19) located beneath the aquarium, and the water is rapidly heated. Further heating is handled circumspcctly, by shutting off the heater and using the subsidiary heater and regulator. On the right-hand wall of the aquarium is mounted the temperature regulating part of the apparatus, in the form of a cupboard that may be closed. It is shown in Figure 2.

The temperature regulator consists of a coiled metal tube filled with toluol and located on the bottom of the aquarium. As the water heats up, the toluol expands and raises the level of the mercury in the temperature regulator. Contact is made between the mercury and the platinum contact 17, which switches on the current from the accumulator (16). This current goes to the relay winding and turns off the power switch (14), thus stopping the heating.

The temperature in the aquarium is regulated and maintained with a precision of as much as 0.01°C . To keep watch on the variations of temperature there is a Beckman thermometer (not shown in the drawing, but visible in photos 1 and 2).

When the temperature in the aquarium rises to the required level (for rats, 29°C), the gaseous exchange apparatus is put into action, first without any experimental animal, so that the whole apparatus may as soon as possible come to the same temperature. Meanwhile the barium hydroxide absorbs the carbon dioxide from the air in all parts of the gaseous exchange apparatus.

When the temperature of all the jars of the gaseous exchange apparatus has become equal to the temperature of the aquarium (which may be seen from the absence of further change in the water level in the manometer connected to the gaseous exchange apparatus), then the air circulation in the apparatus is stopped, and the two barium hydroxide solutions are titrated. After this, the experimental animal is inserted and observations commenced.

IV. Determination of the Amount of Absorbed Oxygen

Determination of the amount of absorbed oxygen is carried out as follows. By the means of the bulb 13, air is pumped into jar 12 and water from this jar fills burette 10. By opening cock B, we also fill coil 11 with water. Then we open cock A leading to the manometer. Water from the burette and coil passes into jar 9 and forces oxygen out of it, which passes into the jar which is to contain the experimental animal, and the excess of oxygen above barometric pressure, if stop-cock E is now opened, passes through it and thus brings the jar containing the experimental animal to barometric pressure, while the liquid in the two legs of the manometer is brought to the same level. Now the experimental animal is placed in jar 3, stop-cock F is closed, and the blower is turned on. Air is circulated continuously through the system. The amount of oxygen in the system gradually decreases, being consumed by the experimental animal, and the pressure falls.

The carbon dioxide eliminated by the experimental animal is carried by the air stream into the two jars 5, where it is absorbed by the barium hydroxide. If now, after a certain time, we stop the blower and open cock A leading to the manometer and cock D connecting the suction side of the blower with the pressure side, then the manometer shows a rise of the liquid level (reduced pressure) in the nearer leg, on account of the absorption of oxygen by the experimental animal. Then, by opening cocks B and C, we let enough water into the jar to bring the two legs of the manometer to the same level. In this way we admit into the system as many cubic centimeters of oxygen as were consumed by the experimental animal.

That is, we read the number of cubic centimeters of oxygen absorbed during a given interval of time by determining with the burette the quantity of water that is let in, displacing a corresponding number of cubic centimeters of oxygen. Ordinarily the reading may be taken whenever desired, at any intervals of time.

With small rats a reading may be taken every five minutes; with medium-sized and large rats it is better to make a determination twice during the five minute interval. Then we calculate the amount of oxygen consumed by the experimental animal per hour and per kg of body weight.

V. Determining the Amount of Carbon Dioxide Eliminated

When the experimental animal (a rat) has remained in the apparatus for the necessary period of time, for instance an hour, the motor is stopped. The carbon dioxide eliminated by the animal during this time is absorbed by the barium hydroxide solution contained in the two jars 5. These two solutions are titrated, and from the difference between this titer and the titer before insertion of the experimental animal we calculate the amount of carbon dioxide absorbed. The titration of the potassium hydroxide solutions is

carried out with a N/100 solution of oxalic acid. Samples of the barium hydroxide solutions are taken with a 2 ml cylindrical micropipette. From each jar a sample is taken at least twice.

We give the data from one experiment.

TABLE.

June 11. Rat No.4, weight 204 g.

	<u>Time-interval</u>	<u>Oxygen taken up, cm³</u>
1 hr.	13.0 min (rat inserted)	
1 "	15.0 "	8.7
1 "	17.5 "	17.6
1 "	20.0 "	18.7
1 "	22.5 "	22.5
1 "	25.0 "	22.7
1 "	27.5 "	18.7
1 "	30.0 "	19.7
1 "	32.5 "	18.0
1 "	35.0 "	16.0
1 "	37.5 "	16.2
1 "	40.0 "	17.0
1 "	42.5 "	20.0
1 "	45.0 "	17.5
1 "	47.5 "	15.0
1 "	50.0 "	16.7
1 "	52.5 "	18.4
1 "	55.0 "	19.0
1 "	57.5 "	18.1
2 "	00.0 "	20.0
2 "	03.0 "	23.0
	In 50 minutes	364.0
	in 60 minutes	437.0

CARBON DIOXIDE DETERMINATION

	<u>Jar I</u>	<u>Jar II</u>
Volume of barium hydroxide after initial titration:	794 ml	794 ml

Amount of N/100 oxalic acid expended per 2 ml of barium hydroxide before insertion of the experimental animal:

31.97	31.67
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Amount of N/100 oxalic acid expended per 2 ml of barium hydroxide after insertion of the experimental animal:

27.4	30.05
27.3	29.92
Mean <u>27.35</u>	Mean <u>29.98</u>

The barium hydroxide jars have lost:

$$\frac{794 \times 4.62}{2} = 1834.14 \text{ ml N/100 (COOH)}_2$$

$$\frac{794 \times 1.69}{2} = 670.93 \text{ ml N/100 (COOH)}_2$$

The two jars have lost:

$$\begin{array}{r} 1834.14 \text{ ml} \quad \text{N/100 (COOH)}_2 \\ + 670.93 \text{ ml} \quad \text{N/100 (COOH)}_2 \\ \hline 2505.07 \text{ ml} \quad \text{N/100 (COOH)}_2 \end{array}$$

The loss of strength in the barium hydroxide solutions is explained by absorption of carbon dioxide by the barium hydroxide; 1 ml N/100 (COOH)₂ is equivalent to 1 ml N/100 CO₂.

1 ml of N/100 CO₂ contains 0.22 mg of CO₂, and since 1 cm³ of CO₂ at normal barometric pressure and at normal temperature weighs 0.508 mg, then 1 ml of N/100 (COOH)₂ corresponds to 0.11176 cm³ of CO₂ (0.22 × 0.508).

It is evident that the two barium hydroxide jars have absorbed, in 50 minutes, 2505 × 0.11176 = 280 cm³ of CO₂; thus for 60 minutes 336 cm³ of CO₂.

The respiratory coefficient, $\frac{\text{CO}_2}{\text{O}_2}$, is thus $\frac{336}{437} = 0.768$.

The consumption of oxygen per kg of body weight per hour becomes $\frac{437 \times 1000}{204} = 2142 \text{ cm}^3$, and the carbon dioxide eliminated = 1647 cm³.

The apparatus of our design may be set up in any laboratory. All that is needed is an aquarium tank or a large open glass vessel. One may dispense with the mechanical mixer and with electrical heating, for the mixing may be done manually, with a wooden paddle, and the temperature may be maintained by pouring in cold or hot water.

The apparatus shown in the photographs was assembled and the electrical devices made up at the workshops of the Bohomoleç Institute of Experimental Biology and Pathology in Kiev.

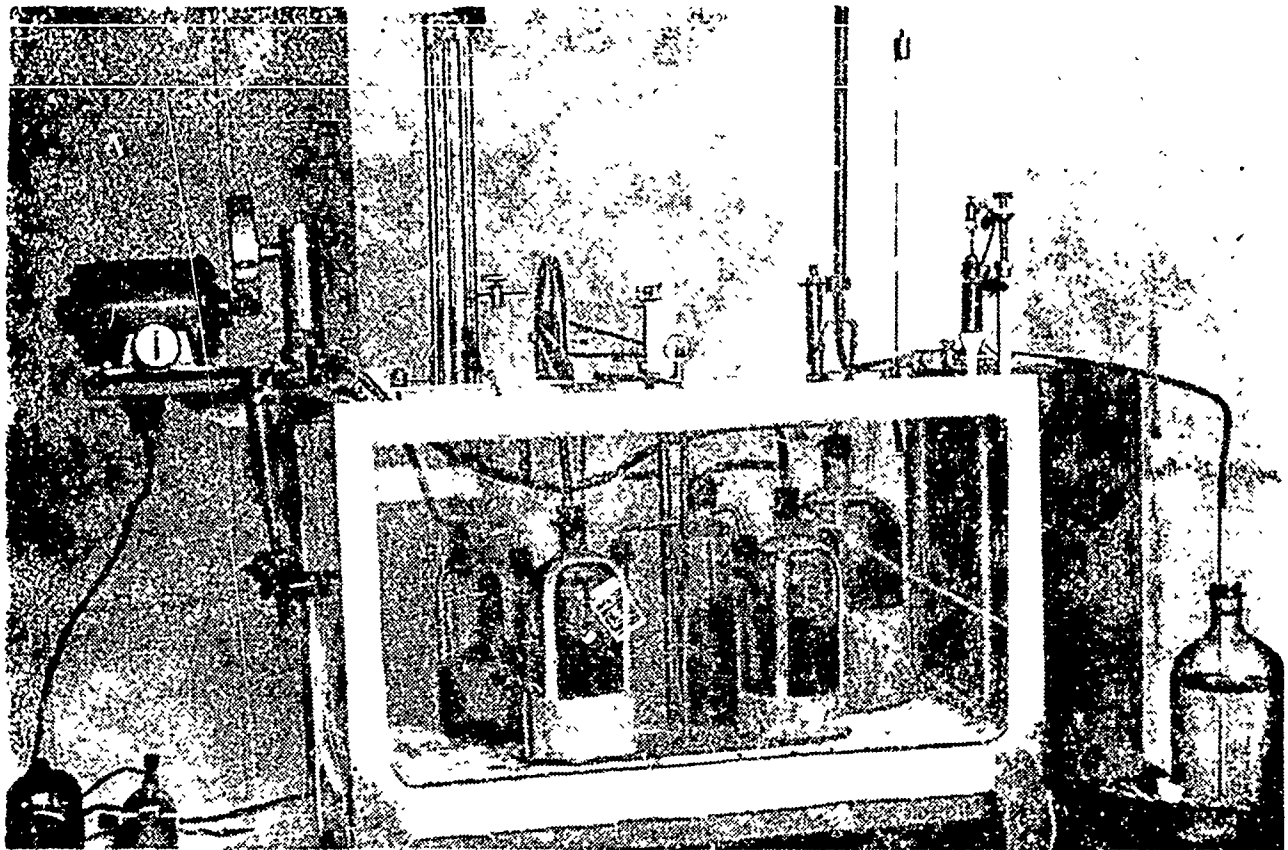


Fig. 1. General view of respiratory gaseous metabolism apparatus for small animals.

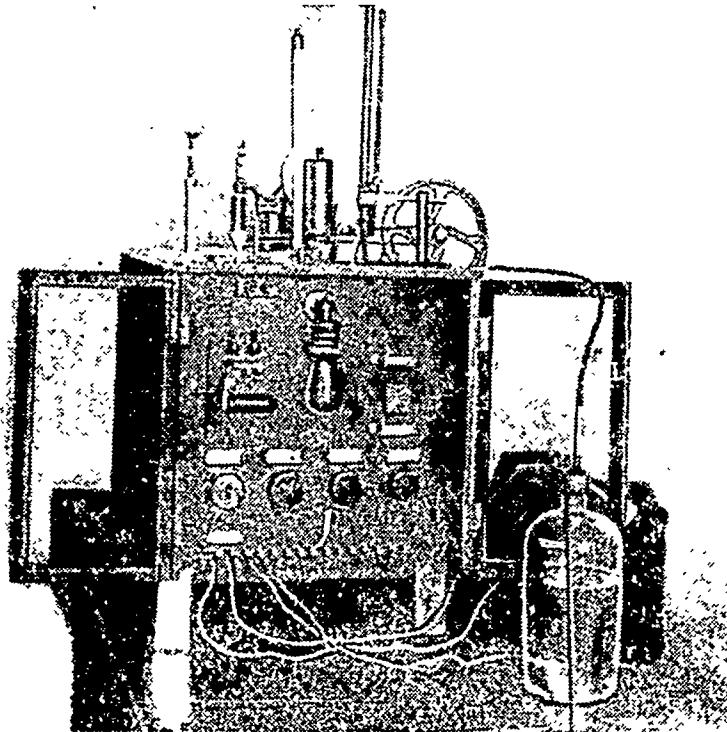


Fig. 2. Control panel of temperature regulating unit.

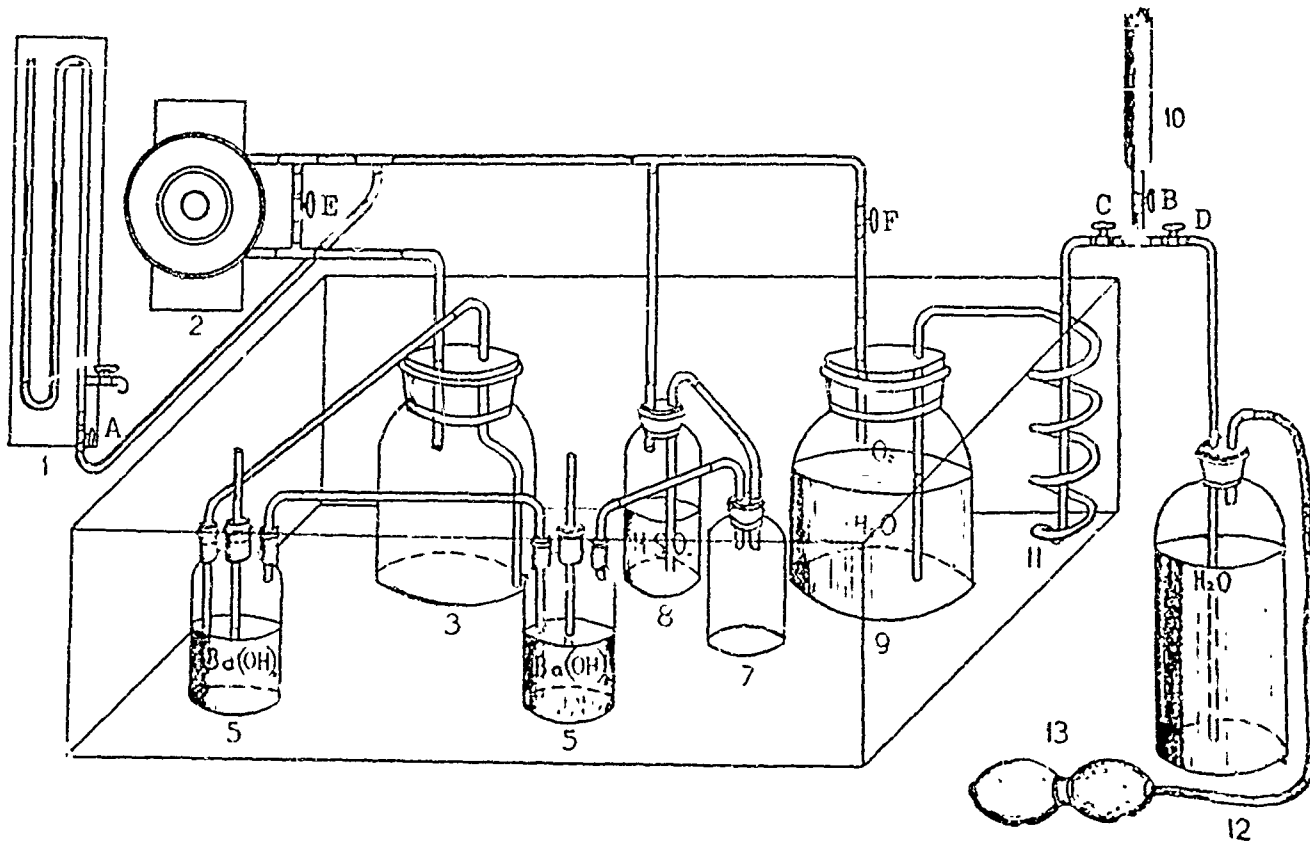


Fig. 3. Diagram of gaseous exchange set-up.

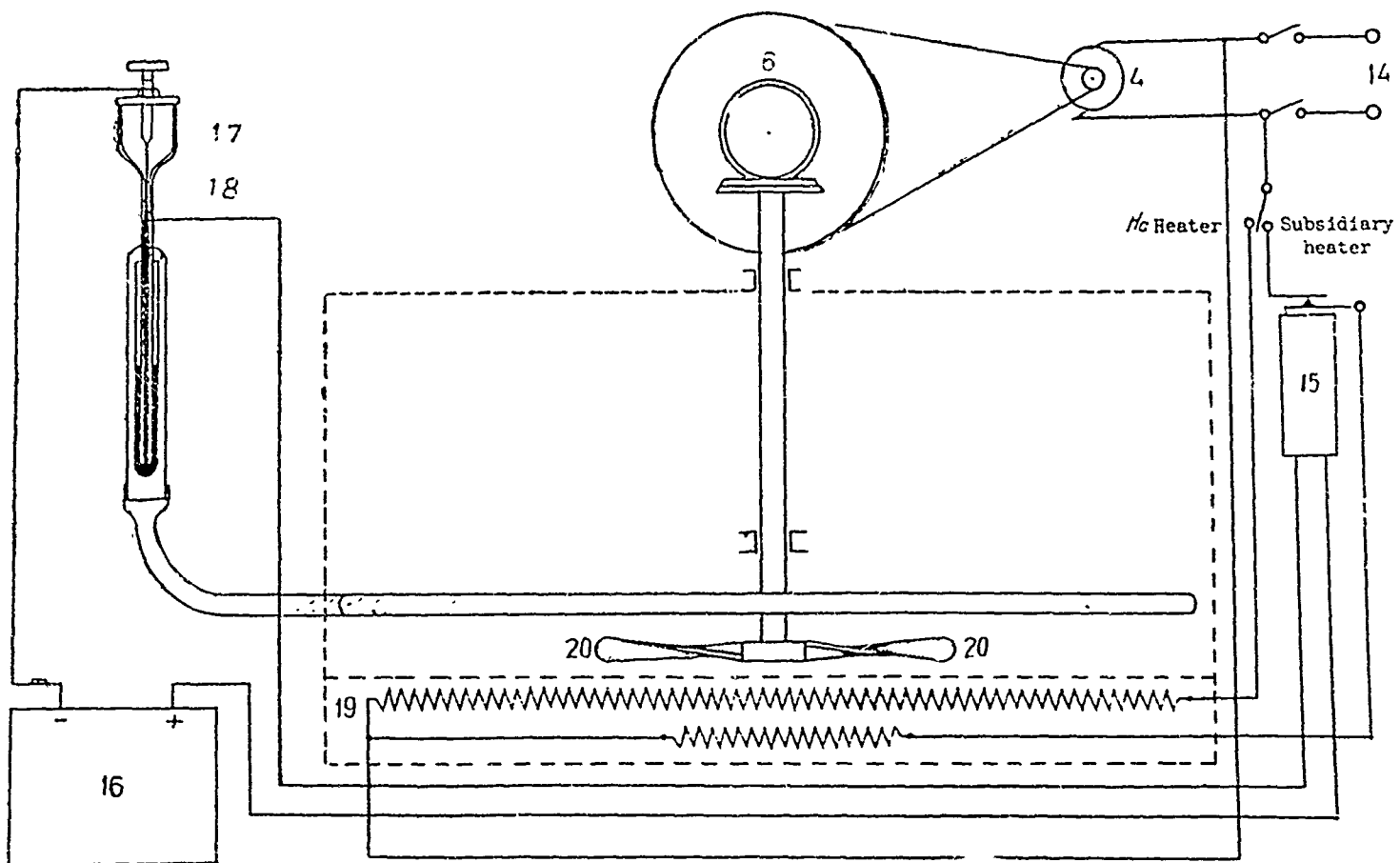


Fig. 4. Diagram of heating and temperature regulation.