XVIII. A TECHNIQUE FOR STUDYING THE METABOLISM OF RATS.

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It is very important that the following conditions should be observed if accurate results are to be obtained in metabolism experiments. First, during the whole experimental period the daily intake of food must be the same, secondly, the food given must be of constant chemical composition, and, thirdly, all the excretions must be accurately collected.

The technique used for studying the metabolism of rabbits and dogs has been described elsewhere [Korenchevsky, 1925, 1, 2]. In this paper are summarised the technical requirements necessary for obtaining accurate results with the rat.

It was necessary to overcome the following difficulties of technique.

1. Rats are small animals, therefore their metabolism is readily influenced by changes in the temperature of their environment. Our experience has shown that a difference of 2° in room temperature affects the metabolism of the rat considerably.

2. Rats are easily excited by sounds and movements in their environment. Differing degrees of physical activity during different periods of the experiment influence particularly the gaseous metabolism.

3. Rats usually scatter their food over the floor of the cage. Therefore in the metabolism cage food is dropped into the urine and faeces containers, making accurate investigation of the metabolism impossible because (a) the ration will not have been entirely eaten, (b) the food mixed with urine or faeces will render the analysis of excreta inexact.

4. Rats do not usually consume their food in a limited time, but when feeding naturally eat at intervals during the whole 24 hours. The time of feeding, however, influences the metabolic rate.

5. The exact separation of the urine and faeces of the different periods of the experiment is difficult since it is not possible to use the catheter or to stain the food with colouring matter and through the food the faeces of alternate experimental periods.

To overcome the first two difficulties mentioned a special room is set aside for metabolism experiments only. A room in the middle of the building and having no windows is used. This room is kept at a constant temperature of

25° by means of a thermoregulator connected with an electric radiator. The ventilation is reduced to such an extent that, whilst the minimal requirements of ventilation are met, the temperature is kept constant even when the outside temperature changes very considerably. The room is entered only at special times each day and only for the purposes of experiment, so that the rat is disturbed as little as possible. The lack of daylight is repaired by exposing the rats twice a week to the ultra-violet rays of a mercury vapour lamp.

To overcome the difficulties mentioned in §§ 3 and 4, only those rats which can be trained to consume their food in about 5 hours from the time the food is given are chosen for metabolism experiments. During the whole period of the experiment the food is given daily at the same time, in the same amount and of the same composition. In order to prevent the rats from dropping their food in the cage they are given a semi-liquid paste. By this simple device the rats are able to lick the paste from the dish but are not able to take it in their paws and scatter it in the cage. The paste used lately is of the following composition: caseinogen 18, starch 49, butter 13, salt mixture 5, dried yeast 10, sugar 3, cod-liver oil 2, lemon juice 5, water 130.

We do not consider the composition of this paste ideal for feeding rats for an indefinite period. However, we have kept rats very easily on this paste for several months, sometimes nearly a year. We are at the present moment trying to improve the composition of this paste and to overcome the monotony of the diet.

Rats which are being used for nitrogen metabolism experiments only, so that only urine and faeces are collected, are kept in cages as described by Ackroyd and Hopkins [1916]. The food containers are placed in these cages in such a way that the rats are unable to remove them and overturn them or excrete urine or faeces into them. The glass separator of Gross and Connell [1923] and the washing of the cages were described in the paper of Chick and Roscoe [1930]. We, however, do not use dilute acetic acid for washing, distilled water giving the same results.

In order to overcome the difficulty of separating the urine and faeces of each period of the experiment accurately, sufficiently long periods of experiment are chosen. Our experience has shown that 3-day, or if possible 4-day periods are long enough since the rat excretes urine and faeces frequently. As can be seen from a sample experiment (see Table I) the data obtained are sufficiently accurate even if the periods are of two days' duration only.

The method described by Pashoutin [1886] and in this country by Haldane [1892] was used for studying the gaseous metabolism. The method is based on Pettenkofer's principle of the direct determination of carbon dioxide and moisture and indirect estimation of oxygen calculated from the changes obtained in weight of the animal and the excretion of CO_2 and H_2O . The metabolism chamber was constructed according to our design. This consists of a strong cylindrical glass jar having a flanged top edge ground on the upper surface. The lid is a thick brass plate with a rubber washer on the under side making

an air-tight joint with the edge of the cylinder. Immediately below the flange is fitted a brass ring having four swinging bolts and wing nuts which slip into slots in the lid, thus enabling it to be closed firmly. Through the lid are soldered several brass tubes one of which passes to the bottom of the jar. Through this tube the air stream passes to the bottom of the chamber thus providing perfect ventilation without allowing the rat to be in a direct draught. A zinc funnel to which a cylindrical wall is soldered, making a small cage for the rat, is placed in the lower three-quarters of the chamber. Below the tube of the funnel a small glass vessel is placed for the collection of the urine. The bottom of the zinc cage consists of two nettings resting in the funnel, an upper one of large mesh on which the rat rests and through which the faeces pass on to the under one of fine mesh. The urine passes through both nettings into the container below the funnel.

Woulff's bottles with absorbents for water (H_2SO_4) and carbon dioxide (soda-lime or KOH in sticks, moistened with water) are connected as follows, the numbers indicating the order of the bottles in the chain: No. 1 soda-lime, No. 2 KOH, No. 3 H_2SO_4 , No. 4 H_2SO_4 , No. 5 chamber, No. 6 H_2SO_4 , No. 7 H_2SO_4 , No. 8 soda-lime, No. 9 H_2SO_4 , No. 10 H_2SO_4 , No. 11 KOH, No. 12 H_2SO_4 . The bottles Nos. 4, 7, 10, 11 and 12 are extra check bottles to indicate the completeness of the absorption in the first bottles. Bottles Nos. 1 to 4 are to remove CO_2 and H_2O from the air entering the metabolism chamber; Nos. 6 to 12 to catch H_2O and CO_2 excreted by the rat.

In the metabolism room are kept large balances, having a precision of 0.02 g., on which all the bottles and the metabolism chamber are weighed. In order to obtain accurate results the metabolism is taken for 17 hours, such a long period giving very constant figures (see Table I) covering all the fluctuations of such a small and excitable animal as the rat.

Table I. *Metabolism of a normal growing rat (per 200 g. rat per week).*

Date	Wt. of rat g.	Nitrogen g.			H_2O g.	CO_2 g.	O_2 g.
		In urine	In faeces	Balance			
5 May	366	—	—	—	119.1	61.3	50.6
11 May	371	1.37	0.43	+0.24	106.0	62.5	51.2
12 May	371				117.9	62.4	52.0
18 May	375	1.33	0.37	+0.30	105.9	61.5	51.4
19 May	375				108.1	61.3	51.0
Average		1.35	0.40	+0.27	111.4	61.8	51.2

Note. In this Table nitrogen metabolism was estimated for periods of 2 days only.

The usual procedure of an experiment is as follows. The rat (weighed) is placed in the nitrogen metabolism cage in the morning and food is given. A little dry boric acid is placed in the urine container. The chain is connected as given above (without the rat in the chamber) and air is drawn through for one hour before the gaseous metabolism experiment is started. The aim of the preliminary ventilation is to obtain a standardised condition of bottles and

chamber and also to remove the traces of sulphurous acid produced by fresh impregnation of pumice stone with sulphuric acid. The bottles are weighed and re-connected, the rat is placed in the chamber, which is closed during the weighing. The chamber containing the rat is weighed, after which it is connected in the chain and air passed for 17 hours. At the end of this time the chamber with the rat in it and then the bottles are re-weighed. The urine and faeces excreted by the rat while in the metabolism chamber are added to those which were excreted while the rat was at rest and feeding in the nitrogen metabolism cage so that the whole 24 hours' excreta are collected. Table I gives typical results of metabolism investigations carried out with this technique.

Before starting the experiment the amount of paste given to the rat is so adjusted that a constant weight in adult rats and only a very slow increase in growing rats is obtained ; this adjustment takes about 3 weeks.

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REFERENCES.

- Ackroyd and Hopkins (1916). *Biochem. J.* **10**, 551.
Chick and Roscoe (1930). *Biochem. J.* **24**, 1780.
Gross and Connell (1923). *J. Physiol.* **57**, lx.
Haldane (1892). *J. Physiol.* **13**, 419.
Korenchevsky (1925, 1). *Quart. J. Exp. Phys.* **15**, 259
— (1925, 2). *Brit. J. Exp. Path.* **6**, 21.
Pashoutin (1886). *Vrach* (Russian), No. 18.