Calculations of percentage stimulation (Table I) were based on the assumption that had no DNP been added the rate of decrease of oxygen consumption after the initial control period would have been the same as that observed in the longer control experiments. Controls showed this assumption valid within ± 5%. Accordingly, no change in oxygen consumption was considered significant unless it exceeded 10%.

If undissociated DNP is the active agent in the stimulation or inhibition of tissue respiration by this substance, one would expect to find the optimum total concentration to be a function of pH, while the optimum concentration in terms of the undissociated form should be quite constant. Furthermore, it should be possible to find a total DNP concentration which would stimulate respiration at one pH level and inhibit at another. It is shown in Table I that these expectations were realized. Such results are most directly explicable on the assumption that, over the pH range investigated, undissociated DNP is the active agent in the stimulation or inhibition of oxygen consumption of rabbit striated muscle (diaphragm).

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Metabolism of Anesthetized Rats.

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The experiments reported in this paper were undertaken chiefly in order to find out whether or not anesthesia could be used with advantage in metabolism studies as a means of reducing the variability of metabolic rates between different rats.

Female rats weighing from 65 to 163 gm. were anesthetized with a suspension of one per cent sodium amytal in 0.9% NaCl solution. The effect of intraperitoneal injection of the drug was tested in 15 rats. When 2 out of 3 rats injected intraperitoneally with 1.5 to 2 cc. of the suspension per 100 gm. of body weight died, the dosage was reduced, yet the mortality among the intraperitoneally injected rats remained high, so that only 6 results of this group could be used for comparison. The dosage of amytal for these intraperitoneally injected rats varied from 7.6 to 10.4 with an average of 9.2

* The authors are indebted to C. F. Winchester for the measurement of the respiratory exchange of the rats.

mg. per 100 gm. of body weight. The drug was injected subcutaneously into 11 female rats in doses of 5.8 to 9.8 with an average of 7.4 mg. per 100 gm. of body weight. Five litter mates served as controls for the intraperitoneally injected rats and 7 for the subcutaneously injected rats. The controls were so selected, that their body weights matched those of the experimental rats.

Prior to the injection of the anesthetic the experimental rats as well as the controls were fasted for 24 hours. The respiratory exchange of each rat was determined individually in a closed respiration apparatus of the Regnault-Reiset type. Seven such apparatuses are combined in a thermostatic cabinet and are operated simultaneously. The respiration trials were started approximately one-half hour after injection of the experimental rats and lasted over a period of 6 hours during which time the oxygen consumption was recorded every half hour. The CO_2 production was determined at the end of the trial for the entire period.

In order to eliminate the influence of body size on the results, the amount of oxygen consumed per half hour was divided by the 3/4 power of body weight. The results thus obtained with the individual rats of each of the 4 groups (2 groups of injected rats and 2 corresponding groups of controls) were averaged for each of the half-hour periods of the respiration experiment. The standard deviation for one result expressed in per cent of the respective mean is the coefficient of variability for the metabolism of different rats of one group within one period. The average of these coefficients of variability for all half-hour periods was $\pm 12.4 \pm 1.2\%$ for the intraperitoneally injected and $\pm 12.5 \pm 1.0\%$ for the corresponding control rats. The average coefficient of variability for the subcutaneously injected rats was $9.0 \pm 0.5\%$, that for the corresponding controls amounted to $13.3 \pm 1.1\%$.

Anesthesia decreased the metabolism of the rats considerably. During the first half-hour period of the respiration experiment, the rate of O_2 consumption of the intraperitoneally injected rats was only 68%, that of the subcutaneously injected rats only 80% of the rate of oxygen consumption of the respective controls. This depressing influence of the drug gradually disappeared in time and during the last 3 periods of the respiration experiment, *i. e.*, later than 5 hours after the injection the metabolic rates of the injected rats were practically equal to those of the controls. In order to eliminate individual differences in the metabolic level the results for each rat in the various periods of the experiment were expressed in per cent of the average results of the last 3 periods for the same rat. The relative figures thus obtained for the injected rats in each

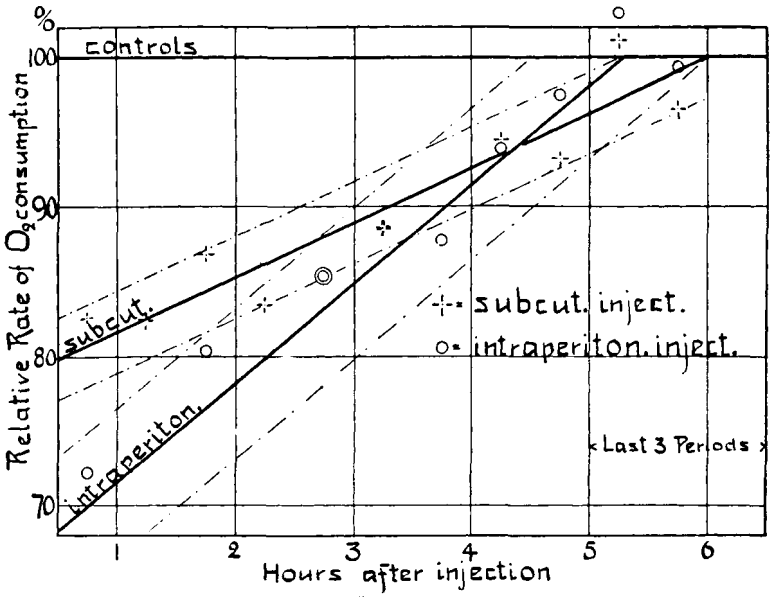

FIG. 1.

Relative rate of oxygen consumption of anesthetized rats in per cent of the corresponding rate of control rats.

period were in turn expressed in per cent of the average for the control rats in the same period. This calculation was carried out in order to eliminate possible systematic influences of time as such on the metabolic rates of the rats. The results obtained are plotted in Fig. 1. The crosses indicate the relative oxygen consumption of the subcutaneously injected rats; the circles represent the corresponding data for the intraperitoneally injected rats. The double cross and the double circle indicate data obtained during two periods together. The heavy straight lines in the figure illustrate the result of linear interpolation of the data by the method of least squares which led to the following equations:

$$M_i = 68.2 + 6.66\ (t - 0.5)$$
$$M_s = 79.9 + 3.64\ (t - 0.5)$$

where M_i = oxygen consumption of the intraperitoneally injected rats in per cent of the corresponding values for the controls

M_s = corresponding results for the subcutaneously injected rats

t = time in hours after the injection

The difference in the regression coefficients ($6.66 \pm 0.72 - 3.64 \pm 0.40$) which determine the slope of the two lines in the figure is statistically highly significant. Its random probability is below one per cent. The dash point lines on the figure indicate the boundaries of the standard deviation of the lines.

The results indicate that amytal injected intraperitoneally had a more pronounced effect on metabolism but lost this effect faster than did the drug injected under the skin.

The total effect of the drug on metabolism may be measured as the product of the decrease in metabolic rate and the time. This total effect is indicated on the figure as the area of the triangle formed by the ordinate at the start of the experiment, the inclined straight line and the horizontal line which represents the metabolic rate of the controls. This total effect contains an element of uncertainty because the respiration trials started approximately half an hour after the injection of the drug. The total effect on the subcutaneously injected rats amounted to 73% of the effect on the intraperitoneally injected rats. The dosage of amytal per unit of body weight in the subcutaneously injected rats amounted to 80% of the dosage applied intraperitoneally. This difference may partly explain the difference in the total effect. The differences in dosage within each group, however, did not systematically affect the rate of metabolism per unit of the 3/4 power of body weight, measured during the entire period of 6 hours, which averaged to 93.8 ± 4.3 Cals. daily for the intraperitoneally injected, and to 91.8 ± 2.0 Cals. for the subcutaneously injected rats.

Conclusions. Intraperitoneal injection of amytal did not decrease the variability in the rate of oxygen consumption between different rats. Subcutaneous injection of the drug decreased this variability so that average results of a given standard deviation of the mean could be obtained with approximately half as many rats under anesthesia as would be required with non-treated rats. It is questionable whether or not this decrease in variability justifies the application of anesthesia in respiration trials since this application may introduce influences on metabolism tending to make the results less conclusive in many cases even though the variability may be decreased. The application of the anesthetic also makes the metabolic rate dependent on the time after injection so that a result on an anesthetized rat is not conclusive unless the time after the injection and its influence on the rate of metabolism are defined.