17228. Relationship Between Spontaneous Activity and Metabolic Rate as Influenced by Certain Sympathomimetic Compounds.

F. A. WATERMAN. (Introduced by A. C. Kuyper.)
From the Department of Biology, Wayne University, Detroit, Mich.

The measurement of spontaneous activity in small animals appears to be dependent upon the method used, many of which have been described in the literature. Results reported by the author,[1] based upon change of resistance in an electrical circuit when an electrode is moved in and out of a salt solution, differ from those obtained by others. Schulte *et al.*,[2] using a work adder type of kinisimeter, reported that d-N-methyl-α-methyl-β-phenylethylamine HCl (desoxyephedrine) was a more powerful stimulant than dl-benzedrine HCl. They also found that the l-isomer of N-methyl-β-cyclohexyl-isopropylamine HCl had a greater stimulating effect than the d-isomer. The writer was unable to confirm either of these results. The results obtained with all kinisimeters may be criticized on the basis that they do not record the very small movements produced by compounds, such as desoxyephedrine which causes the animal to swing his head continuously in a rotary manner. Since oxygen is consumed in making these movements, it was considered that a measurement of oxygen consumption and determination of the metabolic rate would be of use in determining spontaneous activity effects.

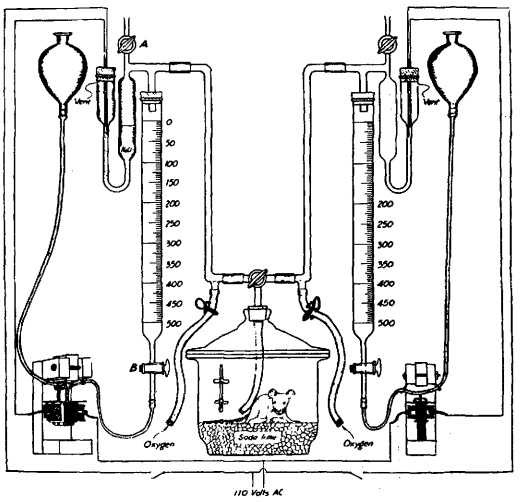

FIG. 1.
Apparatus for the continuous estimation of the metabolic rate of small animals.

Apparatus. The types of apparatus used for oxygen consumption determination have been reviewed by Tabor and Rosenthal.[3] The apparatus used in this study is shown in Fig. 1. The tops of two 500 ml dispensing burettes were connected to a large pyrex desiccator. A thermometer was fastened to the inside wall of the desiccator in order that the vapor tension could be corrected. The upper ends of the burettes were connected to specially constructed manometers filled with a saturated solution of sodium chloride containing a small amount of aerosol to decrease surface tension. Two expansion chambers are included in each of the manometers to prevent the electrolyte from being accidentally drawn into the burettes or spilled to the outside when the apparatus is being filled with oxygen. It will be noted that a small vent is blown in the side wall of the expansion chamber containing the electrodes. These electrodes control the current passing through Allen and Bradley solenoids, which, in turn, control the flow of water from the 500 ml leveling bulbs into the burettes. A simple switch is included in each solenoid circuit in order that the current may be broken at will, to permit filling the burettes with oxygen. The base of the desiccator is filled with soda lime and a disc of heavy screen wire (hardware cloth) replaces the desiccator plate.

All measurements are made in a constant temperature room. The animal is placed in the chamber and the apparatus washed out

1 Waterman, F. A., *Science*, 1947, **106**, 499.

2 Schulte, J. W., Reig, E. C., Bacher, J. A., Jr., Lawrence, W. C., and Tainter, M. L., *J. Pharm. Exp. Therap.*, 1941, **71**, 62.

3 Tabor and Rosenthal, *Am. J. Physiol.*, 1947, **149**, 449.

several times with oxygen. As the animal consumes oxygen the side arm of the manometer falls and breaks the electrical circuit, causing the solenoid to drop and allowing water to enter the burette. When the water has replaced the consumed oxygen, the original level of the electrolyte in the manometer is restored, automatically closing the circuit and the solenoid again pinches the rubber tube which stops the flow of water. When one burette is depleted of oxygen, the 3-way stopcock is turned and the second burette is thrown into service while the first one is being refilled. Thus the oxygen consumed can be determined continuously.

In the calculations an R.Q. of 0.725 was assumed.[4] The metabolic rate in this investigation is defined as calories of heat per hour per gram of body weight. All measurements were made with animal in the post absorptive state. This method is particularly useful for the rapid screening of drugs. The accuracy of such methods as that of Haldane[6] and accurate surface area determinations are unnecessary.

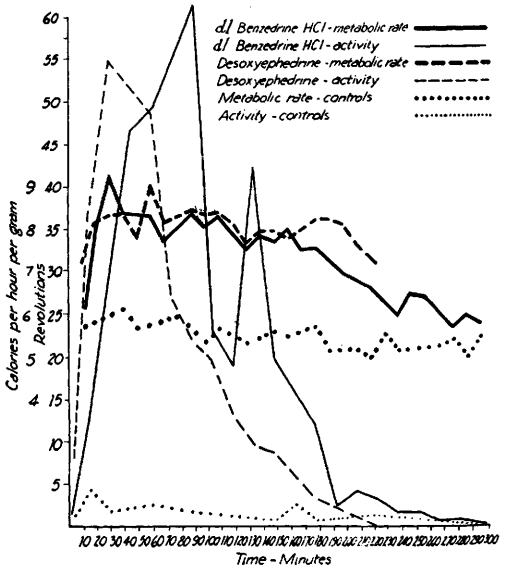

FIG. 2.
Graph showing the effect of dl-benzedrine and desoxyephedrine on the metabolic rate and central nervous excitation.

Experimental. Control animals were injected with normal saline solution and their metabolic rate determined at 10-minute intervals over a period of 6 hours. The experimental animals were injected with the compound being studied and determinations of oxygen consumption in 10-minute intervals were begun at once. The control and experimental data obtained were plotted along with the data previously obtained from activity studies[1] in order that the relationship between metabolic rate and activity could be readily seen. The following compounds were studied:

	No. of rats	Dosage (mg/kg)
d-N-methyl-α-methyl-β-phenylethylamine HCl (desoxyephedrine)	6	5
dl-benzedrine HCl	6	5
1(3',4'-dihydroxyphenyl)-2-isopropylaminoethanol HCl (Isuprel)	6	1
N-methyl-β-cyclohexylisopropylamine HCl		
d-isomer	6	5
l-isomer	6	5
Neosynephrine HCl	6	0.07

Fig. 2 shows the effects of both benzedrine and desoxyephedrine (d-N-methyl-α-methyl-β-phenylethylamine HCl) on the metabolic rate and activity of the animals. The maximum activity as well as the maximum metabolic rate are higher in the case of benzedrine

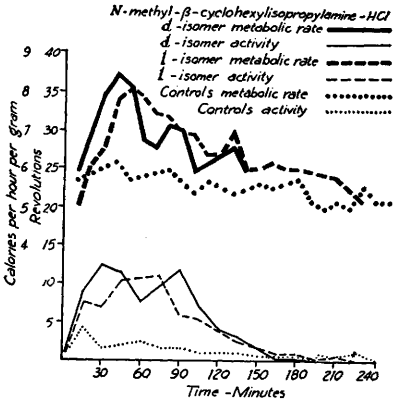

FIG. 3.
The relationship between the d- and l-isomers of N-methyl-cyclohexylisopropylamine HCl as regards their effect on metabolic rate and central nervous excitation.

[4] Griffith and Farris, 1942, The Rat in Laboratory Investigation, p. 186.

[5] Diack, Samuel L., *J. Nutrition*, 1930, **3**, 289.

[6] Haldane, J. B. S., *J. Physiol.*, 1892, **13**, 419.

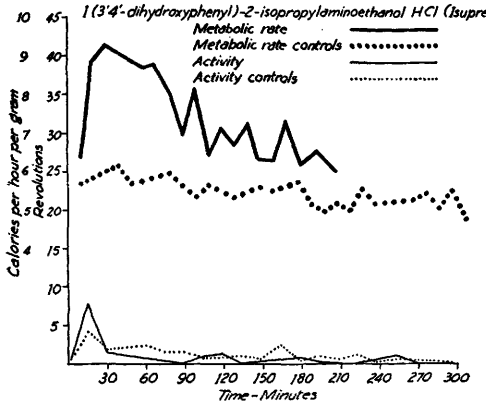

FIG. 4.

The effect of Isuprel on the metabolic rate and central nervous excitation.

than in the case of desoxyephedrine.

The effects produced by the d- and l-isomers of N-methyl-β-cyclohexylisopropylamine HCl are shown in Fig. 3. The d-isomer is more active than the l-isomer as shown by effects on both activity and the metabolic rate. All of the above-mentioned compounds show parallel increases in both activity and metabolic rate.

The injection of 1(3′,4′-dihydroxyphenyl)-2-isopropylaminoethanol HCl (Isuprel), Fig. 4, produces, if anything, a slight decrease in the activity of the normal animal. However, the metabolic rate is markedly increased, being even higher than that produced by dl-benzedrine. The animal is in a state of complete collapse and breathing rapidly. His heart rate is greatly increased, the peripheral vessels are greatly dilated and the temperature is elevated. The effect on the metabolic rate is clearly not due to movements produced by central nervous stimulation. This compound has been shown to have a relaxing effect on bronchiole spasm induced by pilocarpine and to markedly decrease the blood pressure.[7] The increased oxygen consumption is attributable in part to increased heart rate, respiratory movements, and possibly to increased tissue oxidation.

Summary. Comparisons were made between the oxygen consumption and increased movements induced by the central nervous excitatory effects of a number of physiologically active organic compounds. After injection of several of these compounds, benzedrine, desoxyephedrine and N-methyl-β-cyclohexylisopropylamine HCl, oxygen consumption and voluntary movements varied in direct relationship to each other. After the injection of another compound (Isuprel), oxygen consumption increased greatly, but there was a decrease in activity. Oxygen consumption can be used as a measure of voluntary muscular activity only under limited conditions.

[7] Lands, A. M., personal communication.

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17229. A Simplified Tissue Culture Technic.

H. GROSSFELD.* (Introduced by J. B. Murphy.)

In a single chicken embryo, we find all that is required for preparing tissue cultures: the ingredients of the culture medium as well as the explant. Salt-glucose mixtures are replaced by amniotic fluid from the same embryo from which the tissues are obtained. An egg shell sector about 1¼ inches in diameter is cut out of the large egg pole on the 8-9th day of incubation, and the contents are poured cautiously into a Petri dish so as to avoid bursting the very delicate amniotic membrane. The membrane is then punched with a thin, sharp cannula and 2-3 cc of amniotic fluid are aspirated. The shell sector should not be larger than indicated, otherwise the amniotic membrane may be easily damaged. Only completely transparent fluid should be used; turbid fluids are mixtures of yolk and amniotic fluid and will inhibit growth. Tissue for explants is then cut out

* Fellow of the World's Health Organization.