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THE ACTION OF NITROPHENOLS ON THE METABOLIC RATE OF RATS

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The effect of the mono- and di-nitrophenols and certain related compounds has been determined on the rate of oxygen consumption, the rate of carbon dioxide output and the rectal temperature of the Wistar albino rat.

Of the compounds examined, only 2: 4-dinitrophenol and its derivative, 3: 5-dinitro-*o*-cresol, stimulated metabolic rate. 2-Nitrophenol and 2: 3-, 2: 6-, and 3: 5-dinitrophenol produced no change in metabolic rate; 3-nitrophenol and 2: 5-dinitrophenol had no action on carbon dioxide production although they caused a decrease in oxygen consumption. 4-Nitrophenol and 3: 4-dinitrophenol increased only the rate of carbon dioxide output; 2-amino-4-nitrophenol increased the rate of carbon dioxide output and decreased the rate of oxygen consumption ; 4-amino-2-nitrophenol caused depression of metabolic rate.

It was confirmed that neither rectal temperature nor carbon dioxide output could replace rate of oxygen consumption as a reliable index of metabolic stimulant action. An apparatus is described which facilitates measurement of the oxygen consumption of small mammals.

The actions of the mono- and di-nitrophenols on metabolic rate are obscure; previous investigators reached inconsistent conclusions. 3-Nitrophenol, for example, is described in turn as a rectal temperature depressant (Gibbs and Reichart, 1894), without action on rectal temperature (Magne, Mayer and Plantefol, 1932; Tainter, Bergstrom and Cutting, 1935), a stimulant of yeast respiration (Field, Martin and Field, 1935) and a stimulant of Arbacia egg respiration (Clowes and Krahl, 1937; Tyler and Horowitz, 1937). Similarly, 2: 5-dinitrophenol has been reported as a rectal temperature depressant (Magne *et al.*, 1932), without action on rectal temperature (Tainter *et al.*, 1935), a depressant of yeast respiration (Genevois and Saric, 1932) and a depressant of oxygen consumption of dogs (Magne *et al.*, 1932).

The present investigation was undertaken to re-examine systematically the effect of the mono- and di-nitrophenols and three related compounds on the metabolic rate of the intact mammal. In the course of the work an attempt was made to establish a practical method for determining the metabolic rate of small mammals.

The investigation was carried out in two stages. Each nitrophenol was initially examined for action on carbon dioxide output, an index recommended by Benedict and Homans (1911) for preliminary metabolic studies, and for effect on rectal temperature. The action of each compound on rate of oxygen consumption was then determined.

METHODS

Carbon Dioxide Output.—The apparatus was based on the open-chain circuit of Haldane (1892). Ventilation was by a centrifugal blower (Thermotank Ltd.) with a maximum output of 22,000 cu. ft./hr., driven by a $\frac{1}{4}$ h.p., 1,425 rev./min., continuously-rated electric motor. The air was led from a 3.5 l. plenum box, through soda-lime towers, to a copper manifold with outlet pipes of $\frac{1}{2}$ in. diameter. The animal chambers were Kilner jars, 800 ml. capacity; metal lids fitted with rubber gaskets and two copper pipes of $\frac{1}{8}$ in. diameter replaced their usual glass tops. The absorption trains were three glass tubes (12 in. by 1 in.) connected in series; the first and last tubes were filled two thirds with 8 to 14 mesh calcium chloride granules and one third with magnesium perchlorate; the middle tube contained small grain "Indicarb" soda-lime.

In each run, 6 male and 6 female rats of 160 to 200 g. body weight were used; 2 were controls and 10 were treated. Each rat was weighed, treated as required and placed in the animal chamber, which was then connected to a manifold outlet. The chambers were ventilated for 10 min., the weighed absorption trains connected and the effluent passed through

the trains for 1 hr. The absorption tubes were then removed, the rectal temperature of the rats taken and the tubes re-weighed. The weight increase in the last two tubes was the weight of CO_2 absorbed.

Oxygen Consumption.—A manometric air-filled apparatus was constructed. The circuit (Fig. 1 (*b*)) consisted of a gas-tight reservoir (R), animal chamber (A) and soda-lime container (S) ventilated by a pump (P) with an open manometer (M) attached between pump and reservoir. The reservoirs were 45 l. steel drums, to which connecting pipes of $\frac{3}{8}$ in. copper tubing were fixed by brazing to a $\frac{1}{4}$ in. steel plate, which was in turn soldered to the end of the drum with a wiped joint. Kilner jars with modified lids were used for the animal chamber and soda-lime container. The pump (C. F. Palmer Ltd.) was an electrically-driven reciprocating rubber bellows respiration pump with an output of $3\frac{1}{2}$ l./min. at 40 strokes/min., rendered gas-tight by careful reassembly, replacing the original rubber gaskets with neoprene gaskets. Connexions were made with glass Y-tubes, heavy rubber tubing and $\frac{3}{8}$ in. copper tubing. A

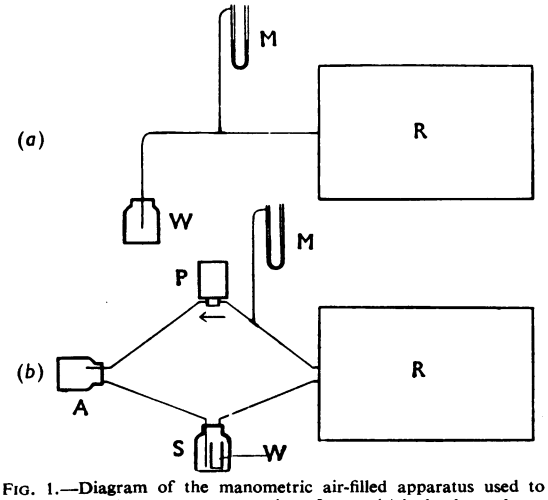

FIG. 1.—Diagram of the manometric air-filled apparatus used to measure the oxygen consumption of rats. (*a*) is the thermobarometer, (*b*) the experimental system. R, 45 litre reservoirs; M, manometers; P, pump; A, animal chamber; W, water containers; S, soda-lime jar.

second reservoir with manometer was set up as a thermobarometer (Fig. 1 (*a*)) to correct for variations in ambient conditions. The manometers, graduated in mm., were filled with Brodie-Krebs fluid (Krebs, 1951). Containers with 20 ml. water (W) were introduced into both systems to stabilize the contribution of water vapour pressure to the internal pressure.

The weighed rat was introduced into the animal chamber, both manometer taps closed, and the pump operated for 5 min. with the circuit open between pump and reservoir, to ventilate the entire system. A pressure of about 120 mm. fluid was built up in the reservoir by blocking the air outlet, the circuit reconnected and the airtightness of the apparatus checked. The pump was then operated for 10 min. to establish equilibrium, before taking the first reading of the room temperature thermometer and manometers with the pump in the closed position. Subsequent readings of thermometer and manometers were made at 15 min. intervals for 1 hr. Multiplication of the corrected pressure decrement by the calibration constant, k, gave the oxygen consumed (ml./hr.) at N.T.P. k was evaluated by calibration of the apparatus from $k=273V/TP_o$, where V=gas volume (ml.), T=room temperature (°A.), P_o=10,025 mm.; V was determined by the method of Gerhartz (1926).

Room temperature was maintained at 18 to 20° in all experiments.

The nitrophenols were dissolved in 0.1N-NaOH and administered intraperitoneally to the rats as solutions of *p*H 7 to 9.

RESULTS

Carbon Dioxide Output.—Six estimates of carbon dioxide output and rectal temperature were obtained with each of 4 doses of the drug, from an arbitrarily selected low dose to the maximum dose tolerated by most rats under the experimental conditions. Individual estimates were rejected if the rat convulsed or died during the experiment, if less than 0.30 g. carbon dioxide was absorbed in 60 min. except when respiratory movement was barely perceptible, or if the rat was unduly restless.

The action of each nitrophenol on carbon dioxide output was established by testing the hypothesis that there is a between-dose component of variance in carbon dioxide output; if the null hypothesis was rejected, the regression of carbon dioxide output on dose was calculated, and its significance tested. In each instance it was found that, where there was significant regression, it was linear. Table I summarizes the results of experiments in which the null hypothesis was accepted, Table II where it was rejected. Fig. 2 shows the

TABLE I

ANALYSES OF VARIANCE OF RATE OF CO_2 OUTPUT OF RATS RECEIVING NITROPHENOLS, WHERE THERE WAS NO SIGNIFICANT BETWEEN-DOSE VARIANCE

Within-dose variance was accepted as homogeneous when Bartlett's test gave $P>0.05$. Measurements were made at 4 doses within the given range, and 6 estimates of CO_2 obtained for each dose.

Compound	Dose Range (mg.)	*P* Bartlett's Test	Mean Squares	
			Between Doses d.f.=3	Within Doses d.f.=20
2-Nitrophenol ..	80–120	>0·50	0·0096	0·0065
3- ,, ..	30–45	>0·05	0·0063	0·0047
2 : 3-Dinitrophenol ..	15–24	>0·50	0·0138	0·0054
2 : 5- ,, ..	10–25	>0·50	0·0030	0·0054
2 : 6- ,, ..	1–6·5	>0·10	0·0037	0·0054
3 : 5- ,, ..	2–7	>0·50	0·0033	0·0057
4-Amino-2-nitrophenol	10–50	>0·10	0·0037	0·0017

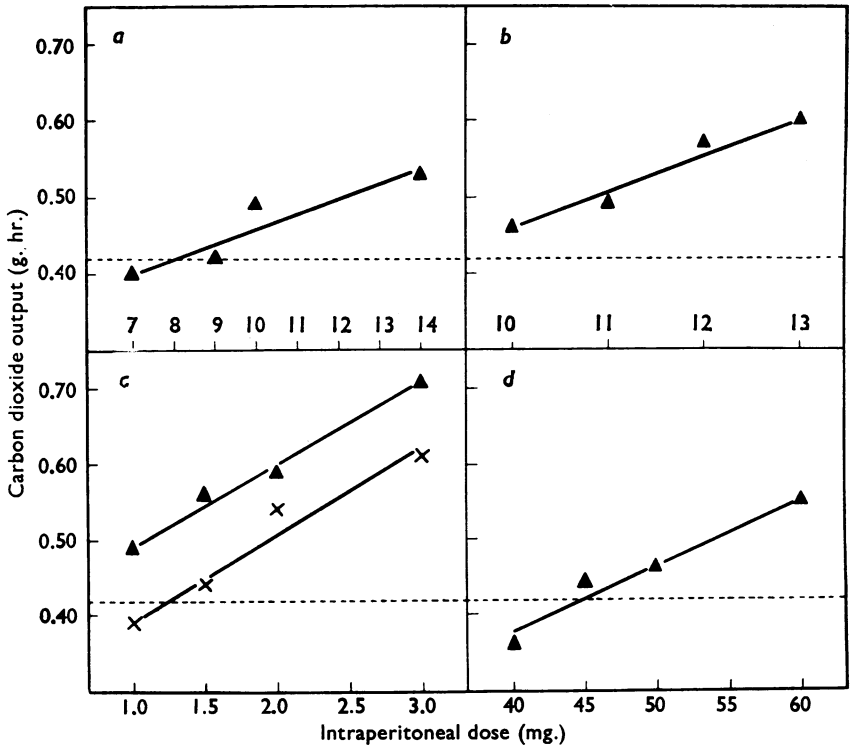

FIG. 2.—The relation between the CO_2 output of Wistar albino rats and intraperitoneal dose of (*a*) 4-nitrophenol, (*b*) 3 : 4-dinitrophenol, (*c*) 2 : 4-dinitrophenol (▲) and 3 : 5-dinitro-*o*-cresol (X), and (*d*) 2-amino-4-nitrophenol. The dotted lines represent the mean carbon dioxide output of 96 control rats.

regression lines of dose on carbon dioxide output for 4-nitrophenol, 3:4-dinitrophenol, 2:4-dinitrophenol, 3:5-dinitro-*o*-cresol, and 2-amino-4-nitrophenol.

4-Amino-2-nitrophenol had a peculiar effect. An irregular decrease in carbon dioxide output occurred, with a mean of 0.33 g./hr. for the 24 observations, but, although the difference between this mean and that of control rats was highly significant according to the *t* test ($P<0.001$), no significant between-dose component of variance was found.

The carbon dioxide outputs of 96 control rats had an apparently normal distribution, with a mean of 0.42 g./hr. and standard deviation 0.05. In the course of these experiments a quality control chart was used, and confirmed that the variables were in statistical control.

Rectal Temperature.—The effect of each nitrophenol on rectal temperature was determined by testing the significance of the difference between the mean temperature of the 6 rats which received the maximum non-fatal dose and the grand mean temperature of control rats. The rectal tempera-

TABLE II

ANALYSES OF VARIANCE OF RATE OF CO_2 OUTPUT OF RATS RECEIVING NITROPHENOLS, WHERE THERE WAS A SIGNIFICANT BETWEEN-DOSE VARIANCE

Linear regression of CO_2 output upon dose was established in each instance; the regression equations are shown. Within-dose variance was accepted as homogeneous when Bartlett's test gave $P>0.05$. Measurements were made at 4 doses within the given range, and 6 estimates of CO_2 output obtained for each dose.

Compound	Dose Range (mg.)	*P* Bartlett's Test	Mean Squares: Due to Regression d.f. = 1	Mean Squares: Deviation from Regression d.f. = 2	Mean Squares: Within Doses d.f. = 20	[Regression Equation x = Dose (mg.) y = CO_2 Output (g./hr.)
4-Nitrophenol	7–14	>0·05	0·0585	0·0032	0·0035	y=0·0194x + 0·27
2 : 4-Dinitrophenol	1– 3	>0·10	0·1585	0·0007	0·0033	y=0·1100x + 0·38
3 : 4- ,,	10–13	>0·10	0·0686	0·0016	0·0053	y=0·0478x − 0·02
3 : 5-Dinitro-*o*-cresol	1– 3	>0·10	0·1801	0·0040	0·0051	y=0·1171x + 0·27
2-Amino-4-nitrophenol	40–60	>0·50	0·0986	0·0024	0·0021	y=0·0087x + 0·03

tures of 90 control rats were apparently normally distributed, with a mean temperature of 100.3° F. and standard deviation 0.8. Table III shows that the mean temperatures of rats receiving all but 3:4- and 3:5-dinitrophenol are unlikely to be drawn from a control population. It was concluded that only 2:4-dinitrophenol and 3:5-dinitro-*o*-cresol increased rectal temperature; the

TABLE III

MEAN RECTAL TEMPERATURES OF GROUPS OF SIX RATS RECEIVING THE MAXIMUM NON-FATAL DOSE OF NITROPHENOLS

P is the probability, by the *t* test, that there is no significant difference between the mean temperature of each group of treated rats and the grand mean control temperature (100·3° F.).

Compound	Dose (mg.)	Mean Temperature (° F.)	*P*
2-Nitrophenol	120	96·6	<0·001
3- ,, ...	45	95·7	<0·001
4- ,, ...	14	98·5	<0·001
2 : 3-Dinitrophenol ..	24	98·0	<0·01
2 : 4- ,, ..	3	103·0	<0·01
3 : 5-Dinitro-*o*-cresol ..	3	104·2	<0·02
2 : 5-Dinitrophenol ..	25	95·6	<0·001
2 : 6- ,, ..	6·5	96·8	<0·001
3 : 4- ,, ..	13	99·6	>0·05
3 : 5- ,, ..	7	100·1	>0·10
2-Amino-4-nitrophenol ..	60	96·7	<0·01
4-Amino-2-nitrophenol ..	50	97·1	<0·01

mononitrophenols, 2:3-, 2:5- and 2:6-dinitrophenol, 2-amino-4-nitrophenol and 4-amino-2-nitrophenol all depressed rectal temperature.

Oxygen Consumption.—The effect of the nitrophenols on rate of oxygen consumption was determined with paired rats, one receiving saline and the other the maximum tolerated dose of the nitrophenol. The rats, which weighed 150 to 210 g., were paired for weight and sex; pairs of males and females were used alternately. Decisions were reached by a non-linear Probability Ratio Sequential test (Wald, 1947) of the hypothesis $H_0: |\mu| : < \delta\sigma$ against $H_1: |\mu| : \geqslant \delta\sigma$ where μ is the mean and σ^2 the variance of $\triangle O_2$, the difference in oxygen consumption of the paired rats. δ was chosen as 1, and 0.05 assigned to α and β, the maximum probabilities of errors of the first and second kind. Table IV summarizes the results. The fiducial limits of the mean $\triangle O_2$ establish that 2:4-dinitrophenol and 3:5-dinitro-*o*-cresol stimulated, while 3-nitrophenol, 2:5-dinitrophenol, and both aminonitrophenols depressed, oxygen consumption.

The oxygen consumptions of 120 control rats had an apparently normal distribution, with a mean of 345 ml./hr. and standard deviation 56. A quality control chart confirmed that the variables were in statistical control.

TABLE IV

EFFECT OF NITROPHENOLS ON RATE OF OXYGEN CONSUMPTION OF RATS, DETERMINED BY A NON-LINEAR PROBABILITY RATIO SEQUENTIAL TEST

$\triangle O_2$=Difference in oxygen consumption of paired treated and control rats. The acceptance of H_0 decides that there is no effect on oxygen consumption, H_1 that there is action on oxygen consumption. The probability of accepting the wrong hypothesis is 0·05.

Compound	Dose (mg.)	No. of Trials for Termination of Test	Hypothesis Accepted	Mean $\triangle O_2$ and 95% Fiducial Limits (ml./hr.)
2-Nitrophenol	105	7	H_0	− 2·9 ± 49·3
4- ,, ...	12	6	H_0	+ 2·0 ± 43·1
2 : 3-Dinitrophenol ..	24	7	H_0	+ 4·0 ± 52·1
2 : 6- ,, ..	6	12	H_0	− 17·0 ± 56·7
3 : 4- ,, ..	13	9	H_0	− 6·0 ± 36·6
3 : 5- ,, ..	6	10	H_0	− 4·8 ± 16·7
3-Nitrophenol	40	10	H_1	− 70·7 ± 49·3
2 : 4-Dinitrophenol ..	3	7	H_1	+ 92·3 ± 51·6
2 : 5- ,, ..	20	21	H_1	− 57·5 ± 36·1
3 : 5-Dinitro-*o*-cresol ..	3	6	H_1	+252·5 ± 39·2
4-Amino-2-nitrophenol	50	6	H_1	− 79·2 ± 40·6
2-Amino-4-nitrophenol	50	11	H_1	− 52·5 ± 35·0

DISCUSSION

In this investigation the inherent variation in biological material was recognized and accepted. No attempt was made to impose "basal" conditions or to enforce total inactivity; variations in feeding, activity, etc., were assumed to be random in accordance with a definite but hypothetical distribution. That the variables were in statistical control was confirmed by satisfactory quality control charts.

Magne *et al.* (1932) classified nitrophenols as metabolic stimulants, metabolic depressants or compounds without action on metabolic rate. This classification may now be regarded as inadequate, since the nitrophenols in this series can be assigned to no less than 6 groups in respect of action on the respiratory exchange of rats (Table V). Nitrophenols with different actions on carbon dioxide output and oxygen uptake were previously unsuspected.

TABLE V

NITROPHENOLS ASSIGNED TO GROUPS FOR ACTION ON RATES OF CO_2 OUTPUT AND OXYGEN CONSUMPTION

Group	Compound	Action on: CO_2 Output	Action on: O_2 Uptake
1	2 : 4-Dinitrophenol 3 : 5-Dinitro-*o*-cresol	Stimulant	Stimulant
2	2-Nitrophenol 2 : 3-Dinitrophenol 2 : 6- ,, 3 : 5- ,,	No action	No action
3	3-Nitrophenol 2 : 5-Dinitrophenol	,,	Depressant
4	4-Nitrophenol 3 : 4-Dinitrophenol	Stimulant	No action
5	2-Amino-4-nitrophenol	,,	Depressant
6	4-Amino-2-nitrophenol	Depressant	,,

Of the nitrophenols tested, only those with nitro-groups in both the 2- and 4- positions produce metabolic stimulation in the intact mammal, in spite of former claims that stimulant activity was possessed by 4-nitrophenol, 2:6-dinitrophenol and 3:4-dinitrophenol. The claims for 4-nitrophenol (Gibbs and Reichert, 1894; Magne *et al.*, 1932) and 2:6-dinitrophenol (Tainter and Cutting, 1933), based solely on rectal temperature observations, are weakened by the varying response of this index between species. For example, 2:6-dinitrophenol increases the rectal temperature of pigeons but not of rats (Tainter *et al.*, 1935). Similarly, little significance can be attached to the claim of Magne *et al.* (1932) that 3:4-dinitrophenol increases the rectal temperature and oxygen consumption of dogs, because they admit that the temperature-changes were inconsistent; they measured the oxygen consumption of only one dog treated with 3:4-dinitrophenol.

It appears premature to attempt to correlate the physico-chemical properties and metabolic stimulant activity of the nitrophenols, in spite of previous generalizations by Magne *et al.* (1932) and Clowes and Krahl (1937), since in this series activity is specific for 2:4-dinitrophenol and its methyl derivative, 3:5-dinitro-*o*-cresol.

The stimulation of carbon dioxide output by nitrophenols which are not metabolic stimulants (4-nitrophenol, 3:4-dinitrophenol and 2-amino-4-nitrophenol), although of lesser importance, cannot be ignored. With these nitrophenols, a linear relationship was established between dose and carbon dioxide output, within the dose range examined. Moreover, within the present series, the presence of a nitro-group in the *para*-position to the hydroxyl-group was a necessary and sufficient condition for increasing the carbon dioxide output of rats.

4-Amino-2-nitrophenol and 2-amino-4-nitrophenol were examined as known metabolites of 2:4-dinitrophenol (Guerbet and Mayer, 1932), but their metabolic effects bore no resemblance to those of 2:4-dinitrophenol. Reduction of the nitro-group in the 2-position of 2:4-dinitrophenol has converted a powerful stimulant to a compound which stimulates carbon dioxide output and depresses oxygen consumption; reduction of the nitro-group in the 4-position of 2:4-dinitrophenol has produced a depressant of metabolic rate. The reduction of 2:4-dinitrophenol in the body is therefore a true detoxication.

This investigation has shown that neither rectal temperature nor carbon dioxide output can replace rate of oxygen consumption as a reliable index of metabolic stimulant action. Considerable depression of rectal temperature occurred with compounds having no action on metabolic rate, and it is difficult to avoid concluding that only a temperature increase may have significance. Similarly, the carbon dioxide output of rats can be increased over short periods irrespective of action on metabolic rate, since increased carbon dioxide output was observed with unchanged or depressed oxygen consumption. The use of carbon dioxide output as a simple index of metabolic action, suggested by Benedict and Homans (1911), is therefore not reliable. The apparatus developed in this work measures the oxygen consumption of small mammals simply and accurately, reducing further the importance of carbon dioxide output and rectal temperature as indices of metabolic action.

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