"Studies of Nitrophenols"

by

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Contents.

Preface.

The recognition of the metabolic stimulant action of certain nitrophenols by Gibbs & Reichert (1) in 1894 aroused little interest at the time, but within the last twenty five years many investigations have been made, in vitro and in vivo, chiefly with the aim of correlating their chemical and biological properties.

Interest in the nitrophenols was aroused during the 1914-18 war when frequent poisoning and several deaths occurred amongst workers in munition factories. The accidents were initially attributed to small amounts of impurities produced during the manufacture of explosives, but the investigations of Magne, Mayer and Plantefol (2) proved the dinitrophenols responsible. Intensive pharmacological studies of the nitrophenols followed, leading to the recognition of the high toxicity and metabolic stimulant action of 2,4-dinitrophenol (DNP) and some related compounds.

Two dinitrophenols, DNP and 6-methyl-2,4-dinitrophenol (DNOC) found immediate application in medicine as metabolic stimulants (3,4). By the mid 1930's they had gained popularity in the treatment of obesity

(5), but the indiscriminate use of these drugs caused many cases of poisoning with several fatalities (6), and their clinical use was abandoned.

Dinitrophenols were also employed in agriculture and are now extensively used as insecticides (7) and selective herbicides (8,9). In addition, dinitrophenol solutions are applied to thin the blossom of fruit trees and to ensure the regular opening of dormant buds in hot climates (10).

The mode of action of the nitrophenols has been the subject of many investigations. Studies on sea urchin eggs and on yeasts showed that concentrations of DNP stimulating respiration also inhibited cell division and growth; this was not understood until Loomis and Lipmann (11) found that DNP in low concentrations reversibly inhibits oxidative phosphorylation in certain biochemical preparations. Such an effect accounts for the inhibition of energy-requiring processes (e.g. growth and cell division), for the increased oxygen consumption and for the dissipation of more energy as heat, by DNP.

Interest in potential metabolic stimulants has been revived by the recent recognition of the metabolic stimulant action of the salicylates, perhaps the most widely used of household drugs.

Introduction.

The effects of nitrophenols on metabolic rate have been studied over a wide biological field. Among the systems which have been investigated are whole animals (1, 2, 12-21), yeasts (22-33), eggs of marine animals (34-42), bacteria (43), tissue slices (44-49), homogenates (50,51) and grasshopper embryos (52). Comparison of the relative metabolic actions of the nitrophenols on these materials is not possible, however, since the nitrophenols can produce diverse effects on different systems; for example, the same concentration of DNP stimulates the respiratory exchange of tissue slices, and inhibits the respiratory exchange of tissue homogenates (46). In spite of numerous investigations in search of metabolic stimulants which are less toxic than DNP and DNOC, no clear picture emerges of the relative metabolic actions of the various mono and dinitrophenols.

The literature on the metabolic activity of the mono and dinitrophenols is summarised in Table I, which demonstrates the divergence of previous results and emphasises the absence of thorough investigations of 2,3-, 2,5-, 2,6-, 3,4- and 3,5-dinitrophenol.

Table I.

Summary of nitrophenol literature.

Material	Intact vertebrates							Vertebrate Tissues	
	Dog, Rabbit	Dog		Rat	Pigeon		Guinea pig	Kidney slices	Brain slices
Reference	(1)	(18)		(21)	(20)	(15)	(12)	(45)	(47)
Index of action	Temp.	Temp.	O_2 cons.	Temp.	Temp.		O_2 cons.	O_2 cons.	O_2 cons.
Compound									
2-NP	-	-		0				0	
3-NP	-	0		0	0				
4-NP	+	+		0	+			+	
DNOC				+		+	+		
2,4-DNP	+	+		+		+	+	+	+
2,3-DNP		-							
2,5-DNP		-	-	0	+				
2,6-DNP		0		0	+				
3,4-DNP		+	+						
3,5-DNP		0	0						

Table continued overleaf.

Table I (continued).

Material	Invertebrates		Yeasts		Homogenates
	Arbacia eggs				Particulate system
Reference	(35)	(41)	(22, 23, 27)	(30)	(50)
Index of action	O_2 cons.	Respirn.	Respirn.	Respirn.	O_2 cons.
Compound					
2-NP	0	0	0		0
3-NP	+	+	+		
4-NP	+	+	+		+
DNOC	+		+		+
2,4-DNP	+	+	+	-	+
2,3-DNP					
2,5-DNP			+	-	
2,6-DNP	+	+	+	-	
3,4-DNP					
3,5-DNP					

Key:- NP = nitrophenol; DNP = dinitrophenol; respirn. = respiration; O_2 cons. = oxygen consumption; temp. = temperature.

Only a selection of the many investigations on 2,4-dinitrophenol and DNOC is represented in the table, since these compounds have been subjected to numerous studies. Consideration of the results in this table may be effected under the following headings:-

(1) Intact vertebrates.

The metabolic effects of nitrophenols have been studied on rabbits (1), guinea pigs (12), pigeons (13-15, 20, 21), rats (19-21), and dogs(1, 2, 17, 18) by determining changes produced in respiratory exchange or rectal temperature. The use of temperature as the sole index of metabolic stimulation has had great popularity, despite the variations reported between species (1, 18, 20, 21).

(2) Tissues of vertebrates.

2,4-dinitrophenol increases the oxygen consumption of tissue slices (44-47, 49) but the actions of the other nitrophenols on these materials have been less adequately examined. Mudge (45) did measure the oxygen consumption of rabbit kidney slices treated with 2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol, but his method must be questioned owing to the exceptionally low values of his controls.

(3) Invertebrates.

A complete parallel exists between the results of Tyler et al. (41) and Clowes & Krahl (35) for the stimulation by nitrophenols of the respiration of eggs of marine animals.

(4) Yeasts.

The physiological activities of some nitrophenols have been compared by their effect on yeast respiration, but the table shows that the results are contradictory. Field et al. (24) explain this contradiction by demonstrating the capacity of DNP to inhibit or stimulate yeast respiration depending on the pH of the medium.

(5) Tissue homogenates.

Clowes et al. (50) demonstrated that the metabolic action of some nitrophenols on a cell-free particulate system from Arbacia eggs paralleled their action on intact sea urchin eggs (35). The metabolic actions of the nitrophenols on homogenates must, however, be interpreted with caution since the results are dependent on experimental conditions, and do not always parallel results in vivo; for example, Peiss & Field (46) have shown that the respiration

of certain homogenates is subject only to inhibition, and not to stimulation, by DNP.

The present investigation was therefore undertaken to elucidate the actions of all the mono and dinitrophenols, and of certain related compounds, on metabolic rate. The investigation was to be thoroughly and systematically performed on the intact mammal, by reliable and accurate techniques.

It was hoped that the results of this study would suggest a relation between the metabolic effects and physico-chemical properties of these compounds, and would thus be a guide to a new series of biologically active compounds.

Methods.

Metabolic stimulants increase the heat production and respiratory exchange of animals; metabolic depressants have the opposite effects. The action of drugs on the metabolic rate of intact animals can therefore be determined by measurement of either heat production or respiratory gaseous exchange, i.e., by direct or indirect calorimetric methods.

In direct calorimetry the total heat liberated by the animal is measured directly with a calorimeter. Hill & Hill (53) adopted this technique for general metabolic studies, but its accuracy for rapid metabolic changes is questionable, since errors arise through changes in animal body temperature during the experimental period (Krogh, 54). Indirect methods are therefore preferable for studying the metabolic effects of drugs.

Indirect calorimetry is by measurement of the rate of respiratory exchange. The best indirect estimate of the heat production of animals is obtained

from the rate of oxygen consumption; the rate of carbon dioxide output is dependent to an appreciable extent on the proportions of fat, carbohydrate and protein metabolised at the particular time (54,55). Determinations of carbon dioxide output were nevertheless recommended by Benedict & Homans (56) for preliminary surveys in metabolic problems, as a simple method of recognising changes in heat production.

Before examining methods of measuring respiratory gaseous exchange, an experiment was conducted to determine if the closed jar method of Smith, Emmens & Parkes (57), devised for thyroid assays, was suitable for studying the action of other compounds on metabolic rate. Four groups of twelve mice, six males and six females, were treated with varying doses of salicylic acid and their survival time in closed Kilner jars measured. No relationship was found between dose and survival time (Table II) due, no doubt, to the restlessness of mice receiving high doses; this technique was therefore abandoned and attention directed towards more conventional methods.

Table II.

The Survival time of Salicylate-treated mice in sealed Kilner jars.

Salicylic acid Dose (mg.)	6.31	7.94	10.00	12.60
Survival time (min.)	85 70 70 72 74 63 115 98 79 181 89 -*	118 63 60 107 92 59 78 85 126 160 166 101	89 117 71 89 91 84 81 84 90 112 90 118	109 81 74 94 100 93 77 117 103 107 89 127
Mean	91	101	93	98

* Jar improperly sealed.

In the present investigation it was proposed to examine each nitrophenol initially for effect on rectal temperature and rate of carbon dioxide production, since these indices are very convenient for dealing with large numbers of animals. The action of each compound on rate of oxygen consumption would then be determined. The literature was reviewed for suitable apparatuses to measure the carbon dioxide output and oxygen uptake of small mammals over one hour periods.

Countless techniques have been developed for measuring the respiratory exchange of animals (see reviews by Zuntz (58), Krogh (54), Abderhalden (59), Atwater & Benedict (60) and Benedict & Macleod (61)) based on the open circuit, closed chamber or closed circuit types of apparatus described below.

In the open circuit apparatus, the animal is contained in a ventilated gas-tight vessel with inlet and outlet tubes. Pettenkofer's method (54, 62, 63) for large animals involves the analysis of representative samples of air entering and leaving the animal chamber, coupled with the accurate measurement of the

ventilation current. In Haldane's method (64) for small animals, where a sampling procedure is unnecessary, the animal chamber is ventilated with carbon dioxide-free dry air or oxygen and the resultant air stream treated as a whole with absorbants.

In the closed chamber type of apparatus, used for example by Mattill (65) and Wesson (66), the animal is contained in an airtight vessel and its respiratory exchange followed by periodically analysing the carbon dioxide and oxygen contents of the confined air. This method is clearly limited by animal size, suitable sampling techniques, and the accuracy of the analysis of small gas samples. Alternatively, as in Krogh's microrespiration apparatus (67) for very small animals (< 2 g. weight), the experimental vessel is connected to a compensated manometer by which the oxygen consumed and carbon dioxide produced can be measured.

In the closed circuit system of Regnault & Reiset (54, 59) or Dewar & Newton (68), the airtight circuit consists of a pump or blower for ventilation, an animal chamber, an absorption system, and a device for introducing oxygen to maintain the system at constant volume or pressure. The oxygen consumption

of the animal is found by measurement of the oxygen introduced, by volumetric or gravimetric methods.

None of the existing apparatuses were completely acceptable for this investigation so suitable systems for measuring the carbon dioxide output and oxygen uptake of small mammals were devised, in the light of the available resources, as described below. Salicylic acid was selected as a known metabolic stimulant for use in preliminary experiments to develop the apparatuses and experimental methods.

For multiple estimations of carbon dioxide production a circuit based on Haldane's open-chain system (64) was considered most satisfactory and the apparatus was therefore designed on this principle. The circuit was initially ventilated by oxygen from a cylinder but it was considered more economical to use a silent, durable, easily maintained pump or blower with a suitable output. The water pump of Haldane (64) and Goto (69), is noisy and may result in irregular ventilation from water pressure fluctuations; Atwater & Benedict's rotary blower (60) causes noise, oil contamination and heating of the air stream; vapour from Wesson's mercury pump (66) is detrimental to surrounding equipment and

may be toxic to the animal; noise restricts the use of compressed air in situations where the compressor is nearby. A centrifugal blower was therefore indicated for ventilating the system since it is relatively silent in operation, needs no maintenance and provides good ventilation. The output of such a blower may be reduced by resistance to the air flow, but this need present no difficulties if the circuit is designed with this in mind.

A centrifugal blower (manufactured by Thermotank Ltd., Glasgow) of maximum output 22,000 cu.ft./h. was therefore procured to ventilate the system, and was driven by a 1/8 h.p. continuously rated electric motor at 1425 rev./min. The air was conducted from the blower as follows:- a plenum box ($3\frac{1}{2}$ l. capacity) with two copper outlet pipes of 1 in. diameter, was attached to the blower outlet. The two resulting air streams were passed through 15 in. x $2\frac{1}{2}$ in. gas towers containing soda lime, to a copper T-tube of 1 in. diameter. In this way a constant supply of carbon dioxide-free air was obtained.

To enable the carbon dioxide production of several rats to be determined simultaneously a manifold

was used. This consisted of a copper pipe, 40 in. x 3 in., with twenty five regularly-spaced outlet pipes of $\frac{1}{2}$ in. diameter, which split the ventilating air stream into subsidiary streams for ventilating a maximum of twenty five animal chambers.

The animal containers were Kilner jars, 800 ml. capacity, with the glass tops replaced by metal lids, fitted with rubber gaskets and two copper pipes of 3/8 in. diameter for ventilation.

In preliminary experiments with the mouse as experimental animal, aqueous potassium hydroxide was used to absorb the carbon dioxide produced, and the carbon dioxide content of the resultant solution determined volumetrically by titration with hydrochloric acid in an atmosphere of oxygen, with phenolphthalein and bromophenol blue as indicators. Absorption of the carbon dioxide produced was incomplete even with a fine bubbler and a fifty-fold excess of alkali, since carbon dioxide was absorbed in a second potash solution in series with the first (Table III).

Table III.

The partial absorption, by 5N KOH, of the carbon dioxide produced in one hour by individual mice.

Expt. No.	1st Absorption		2nd Absorption in series.	
	Vol. (ml.) 5N KOH	CO_2 absorbed (ml./h.)	Vol. (ml.) 5N KOH	CO_2 absorbed ml./h.)
1	40	106	40	16
2	50	98	40	13
3	50	105	16	10

The difficulties of handling and titrating a more alkaline solution were considered too great to warrant further investigation of liquid absorbants; gravimetric methods of estimating carbon dioxide output were next considered. The Wistar albino rat was used in all subsequent experiments.

The absorption train used consisted of three pyrex glass tubes, 12 in. x 1 in., joined in series. Tubes 1 and 3 contained desiccants and were two thirds filled with 8-14 mesh calcium chloride, and one third with magnesium perchlorate; tube 2 contained "indicarb" soda lime as the carbon dioxide absorbant. The absorption tubes were plugged at each end by a small pad of glass wool. The weight of carbon dioxide produced by the rat during the experimental period was the weight increase in the second and third absorption tubes. The efficiency of this absorption train was demonstrated by the absence of an increase in weight in a second train in series with the first.

The whole assembly was kept compact by placing the animal jars on top of the absorption tubes lying side by side on a table. The room temperature was maintained at 18-20°C.

In each run it was found convenient to use twelve rats, six males and six females, of 160-200 g. weight; two were controls and ten treated. Each rat was weighed, injected intraperitoneally as required, and placed in the animal chamber connected to a manifold outlet. The chamber was ventilated for ten minutes before the weighed absorption train was connected to the animal chamber. The effluent was passed through the train for sixty minutes. The absorption tubes were then removed, the rectal temperatures of the rats taken and the absorption train reweighed to find the weight increase in the second and third absorption tubes. In initial experiments the rat was sacrificed at this stage to estimate the serum drug concentration.

Measurements of carbon dioxide output were made with the treated rats receiving four doses of the drug, ranging from a low dose to the maximum dose tolerated by most rats under experimental conditions. The determinations on each compound terminated when six estimates of carbon dioxide output were obtained for each dose. Individual estimates of carbon dioxide production were rejected if the rat convulsed or died during the experimental period; if the value

for the carbon dioxide produced in sixty minutes was less than 0.30 g., unless the respiratory movements of the rat were barely perceptible; or if the rat was excessively restless. The results rejected are included in Tables X-XXIV.

An experiment carried out with salicylate demonstrated that there was no advantage in using the drug concentration in serum, instead of dose administered, as the independent variable. In this experiment, the drug concentration was estimated colorimetrically by Trinder's method (70).

For determining the oxygen consumption of rats, consideration was given to the use of the closed circuit apparatuses described in the above-mentioned reviews (54, 58-61). Volumetric methods, used for example by Benedict (71), Joel (59), and Bargeton & Krumm-Heller (72), were considered unsuitable because of the difficulty of attaining precision in measuring small volumes of gases. The gravimetric methods of Atwater & Benedict (60) and Cohnheim (59) were rejected because of the difficulty of weighing the oxygen cylinder quickly and sufficiently accurately.

It was therefore decided to construct an

air-filled apparatus in which the oxygen consumed could be simply and accurately measured manometrically. The circuit used was as follows. Air was drawn from a gas-tight reservoir by a pump, passed through the animal chamber and soda lime container, and returned to the reservoir. A manometer was attached to the circuit between the pump and reservoir, and a second reservoir with manometer set up as a thermobarometer to correct internal pressure changes for variations in room temperature and barometric pressure.

An electrically driven reciprocating rubber bellows respiration pump (manufactured by C.F.Palmer Ltd., London), with an output of $3\frac{1}{2}$ l./min. at 40 strokes/min., ventilated the system. This pump is preferable to the rotary blower (60), centrifugal (73), mercury (66) or membrane pumps (72) used previously, since it operates silently, is easily maintained, has a large capacity and produces no oil contamination of the enclosed air. The pump was easily rendered gas-tight by careful reassembly and by replacing the original rubber gaskets with neoprene gaskets; its high efficiency was maintained by periodic cleaning of the metal mushroom valves. The possibility of an appreciable error arising from the elasticity of the pump bellows was

eliminated by comparing the pressure changes produced in the system on introducing 300 ml. volumes of water with the experimental system at high and low pressures alternately. The results of this experiment (Table IV) show that no perceptible error arises from this source.

The reservoirs were steel drums, capacity 45l., to which connecting pipes of 3/8 in. copper tubing were fixed by brazing to a 1/4 in. thick steel plate soldered to the drum end with a wiped joint.

The animal chamber and soda lime container were Kilner jars with the lids modified as previously described; the latter was three quarters filled with medium grain "indicarb".

The manometers, graduated in mm., were filled with Brodie-Krebs fluid, density 1.03 (85).

Connections were made with glass Y-tubes, heavy rubber tubing and as much 3/8 in. copper tubing as possible to prevent heating of the internal air by the rat. A 2 ft. length of copper pipe was included between the two Kilner jars. The copper tubing and metal reservoir contribute to the maintenance of temperature equilibrium of the system. Although a heat increment occurred over one hour periods in the air directly after the animal, i.e. before the reservoir, the air emerging from the reservoir had a temperature almost identical

Table IV.

Pressure changes produced on introducing 300 ml. water, with the experimental system at high and low pressures, to determine the effect of pump elasticity.

Th.B. = Thermobarometer.

Expt. S. = Experimental System.

ΔP = difference in pressure change of the paired observations.

Expt. No.	Initial Reading (mm. fluid)		Final Reading (mm. fluid)		Pressure change (mm.fluid)	ΔP mm. (fluid)
	Th.B.	Expt. S.	Th.B.	Expt.S.		
1	+41.0 +48.5	-131.0 +64.5	+41.5 +49.5	-77.0 +119.5	53.5 54.0	+0.5
2	+1.5 +9.5	-79.5 +29.5	+7.0 +12.0	-20.0 +86.0	54.0 54.0	0
3	+14.5 +18.0	-75.5 +20.0	+17.0 +19.5	-18.5 +75.5	54.5 54.0	-0.5
4	+20.5 +22.5	-78.5 +30.0	+23.0 +22.5	-20.5 +85.0	55.5 55.0	-0.5
Mean						-0.1

to that of the air in the thermobarometer (Table V).

A container with 20 ml. water was introduced into both systems to stabilise the contribution of water vapour pressure to the internal pressure. In the experimental system the water was contained in a boiling tube inside the soda lime vessel; in the control system, in a Kilner jar between the reservoir and manometer.

The volume of oxygen consumed was found by calibrating the apparatus to obtain a value for k, the constant relating pressure and volume changes in the equation $x = kh$, at constant temperature, where x = volume change (ml.) at NTP and h = resultant manometer change (mm. fluid). Since the volume of liquid present was negligible compared with the gas volume, k was evaluated as $k = 273V/TPo$, where V = gas volume, ml.; T = room temperature, ^{o}A; Po = 10,025 mm. The apparatus volume was calculated by the method of Gerhartz (59), by observing the pressure change produced in the system on introducing a known volume of water. The results are given in Table VI. The

Table V.

Temperature changes produced in one hour in the experimental system, during determinations of oxygen consumption of rats.

T_c = temperature change in thermobarometer, °C.

T_1 = temperature change before reservoir, °C.

T_2 = temperature change after reservoir, °C.

Expt. No.	T_c	Experimental System		T_2-T_c, °C.
		T_1	T_2	
1	+0.3	+0.9	0	-0.3
2	+0.3	+2.5	+0.3	0
3	+0.3	+0.6	+0.5	+0.2
4	+0.3	+1.7	+0.6	+0.3
5	0	+0.5	0	0
6	+0.2	+2.2	+0.5	+0.3
Mean				+0.1

Table VI.

Calibration of oxygen consumption apparatus with 500 ml. volumes of water.

p_1 = initial internal pressure, mm. fluid.

p_2 = final internal pressure, mm. fluid.

V = calculated apparatus volume, ml.

k = calibration constant.

Expt. No.	Ambient Press. (mm.Hg.)	Room Temp. (^{o}A)	p_1	p_2	V	k
1	764.20	292.7	-66.0	+26.0	54,848	5.10
2	764.20	292.9	-59.5	+32.0	54,848	5.10
3	764.30	292.9	-81.5	+11.0	54,263	5.05
4	764.40	292.8	-68.5	+23.0	54,848	5.10
5	764.50	293.0	-85.5	+6.0	54,848	5.10
6	764.50	292.9	-90.5	+1.5	54,848	5.10
Mean						5.09

circuit was modified for this by connecting the animal jar to a 1 l. glass separating funnel. Measurements were made of the pressure change produced on introducing 500 ml. volumes of water from the funnel, with the system at sub-atmospheric pressure; room temperature and barometric pressure were recorded at each manometer reading. The sensitivity of the apparatus was confirmed and the value of k verified, by detecting an internal pressure change of 1 mm. on the introduction of 5 ml. volumes of water (Table VII).

The airtightness of this system can be accepted with confidence, since even small leaks were immediately detected by the rapid fall of manometer fluid. This surely is preferable to Krogh's (54) idea of maintaining the internal pressure at atmospheric, in the hope of preventing leakage!

The apparatus, consisting of the experimental circuit and the thermobarometer, was maintained in a room of temperature 18-20°C.

Table VII.

Pressure change produced on introduction of 5 ml. water to oxygen consumption apparatus.

Th.B. = thermobarometer.

Expt. S. = experimental system.

Expt. No.	Initial reading (mm. fluid)		Final reading (mm. fluid)		Pressure change (mm.fluid)
	Th.B.	Expt.S.	Th.B.	Expt.S.	
1	+3.5	-46	+5.5	-43	+1
2	+5.5	-42.5	+6.5	-40.5	+1
Mean					+1

To measure oxygen consumption, the weighed rat was introduced into the jar, both manometer taps closed and the pump operated for five minutes with the circuit open between pump and reservoir to ventilate the system to the air. A pressure of about 120 mm. fluid was then 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 ten minutes to establish equilibrium before taking the first reading. Subsequent readings of manometers and the room temperature thermometer were made at fifteen minute intervals for one hour. The manometers were read as soon as the fluid reached a steady state, with the pump stopped in the closed position. The decrease in pressure was found by subtracting the final from the initial corrected manometer readings; multiplication of this pressure decrement by k gave the volume of oxygen consumed (ml./h.) at standard temperature and pressure.

The effect of the nitrophenols on oxygen consumption was determined with paired rats, one of which

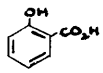
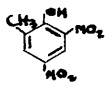
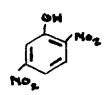
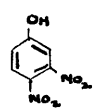
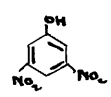
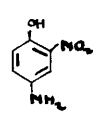
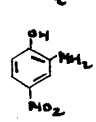
was given saline and the other the maximum non-fatal dose of the nitrophenol, both by intraperitoneal injection. The rats, weight 150-210 g., were paired for body weight and sex, and pairs of male and female rats used alternately. Determinations were continued with each compound until the termination of the sequential test of the hypothesis that the mean difference between the oxygen consumption of the paired rats was zero.

The compounds included in this investigation were salicylic acid, the three mononitrophenols, the six dinitrophenols, DNOC, 2-nitro-4-aminophenol and 2-amino-4-nitrophenol. All were dissolved in N/10 NaOH and administered intraperitoneally as solutions with pH 7-9.

Table VIII contains the reference for source, the solvent for recrystallisation, m.p. of the purified compound and the concentration of the solution administered in each instance. The solutions of 2,3-, 3,4-, and 3,5-dinitrophenols administered in the first experiment required dilution as in the table.

Table VIII.

Reference for source, solvent for recrystallisation, m.p. of purified compound and concentration of solution administered.

Compound	Ref., for source	Solvent	m.p.	m.p. literature	Conc. of soln. administered (g./100 ml.)
	commercial	water	158°C.	158°C.(80)	20
	commercial	aq. ethanol	45°C.	45°C.(80)	3
	commercial	ether	96°C.	96°C.(80)	1
	commercial	water	114°C.	114°C.(80)	1
	commercial	water	86°C.	86°C.(80)	1
	commercial	water	114°C.	114°C.(80)	1
	(74) (79)	water	144°C.	145°C.(80)	6;3
	(74)	methanol	105°C.	96°C. (80) 104°C.(81)	1
	commercial	water	63°C.	64°C.(80)	1
	(74) (79)	benzene	134-5°C.	129-132°C. (74) 134°C.(80)	4;2
	(75) (76)	dil.HCl	124-5°C.	126°C.(76) 122°C.(80)	2;0.2
	(78)	ethanol	127°C.	128°C.(78) 128°C.(80)	1
	(77)	benzene-toluene mixt.	143.5°C.	142-3°C. (77) 146°C.(80)	2

Results.

The results are presented in three sections: (1) carbon dioxide production, (2) rectal temperature, and (3) oxygen consumption.

(1) Carbon dioxide production.

Control rats: The histogram in Fig. 1 shows a near normal distribution of the values of carbon dioxide produced per hour by ninety six control Wistar albino rats, weight 160-200 g.; the mean carbon dioxide output = 0.42 g./h., standard deviation = 0.05, and coefficient of variation = 12%.

In the quality control chart in Fig. 2 each batch consists of three consecutive determinations of control carbon dioxide outputs. It is seen that no significant difference occurs in batch means since only one mean lies outside the inner control lines at 0.37 and 0.47 g. This is evidence, that, although feeding and movement were not controlled, the measurements of carbon dioxide output were experimentally valid, with the variables in a state of statistical control.

Fig. 1.

Histogram: distribution of carbon dioxide output of ninety six control Wistar albino rats.

Abscissa: CO_2 output (g./h.). Ordinate : frequency.

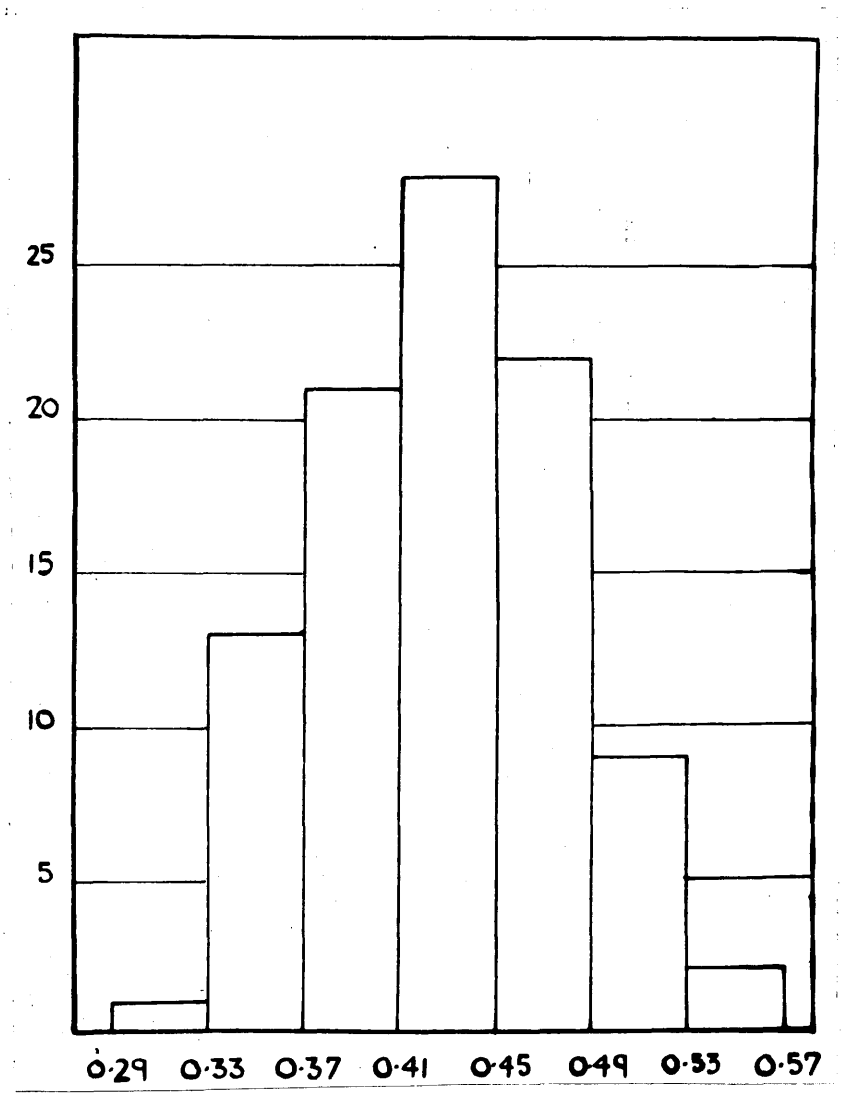
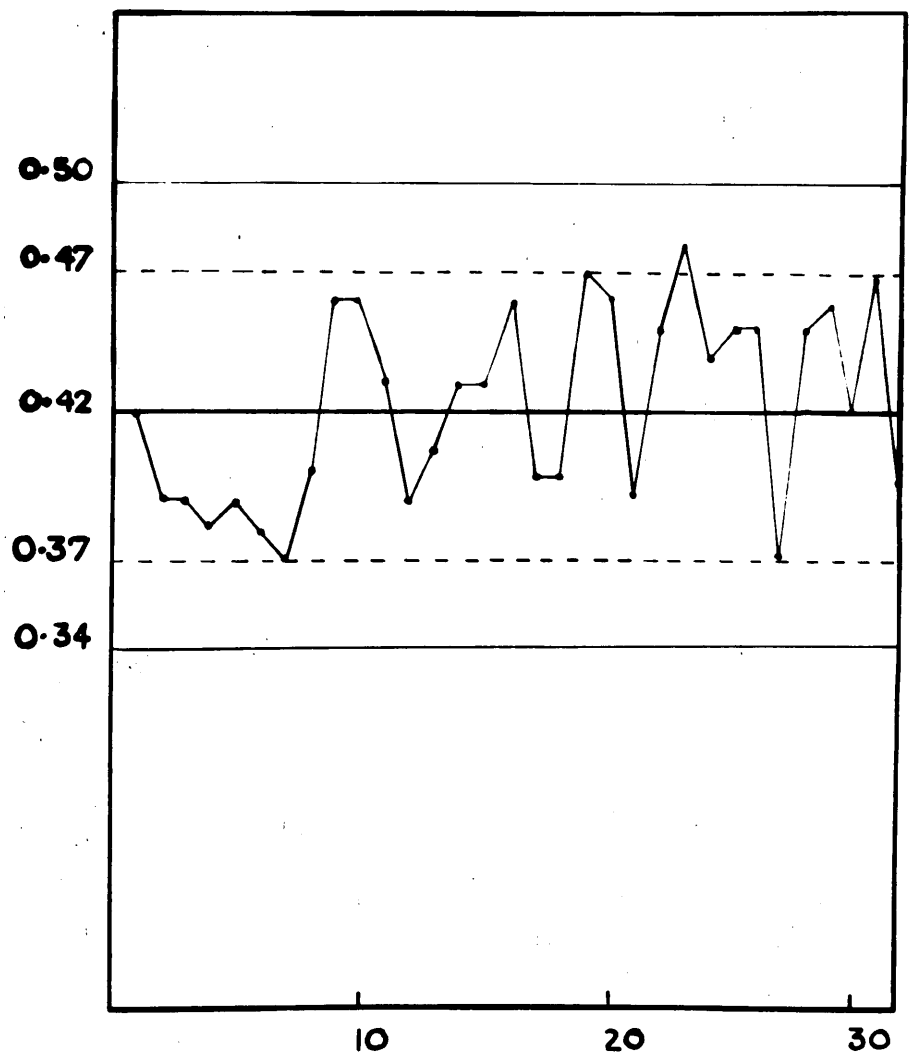


Fig. 2.

Control chart for carbon dioxide outputs of ninety six control Wistar albino rats. Each batch represents three consecutive determinations. Abscissa: batch number. Ordinate: CO_2 output (g./h.).



Treated rats: The effect of each compound on carbon dioxide output was determined by testing the hypothesis that a component of variance exists between the four doses; if the null hypothesis was rejected the linear regression of carbon dioxide output on dose was calculated, and its significance tested by an analysis of variance (82).

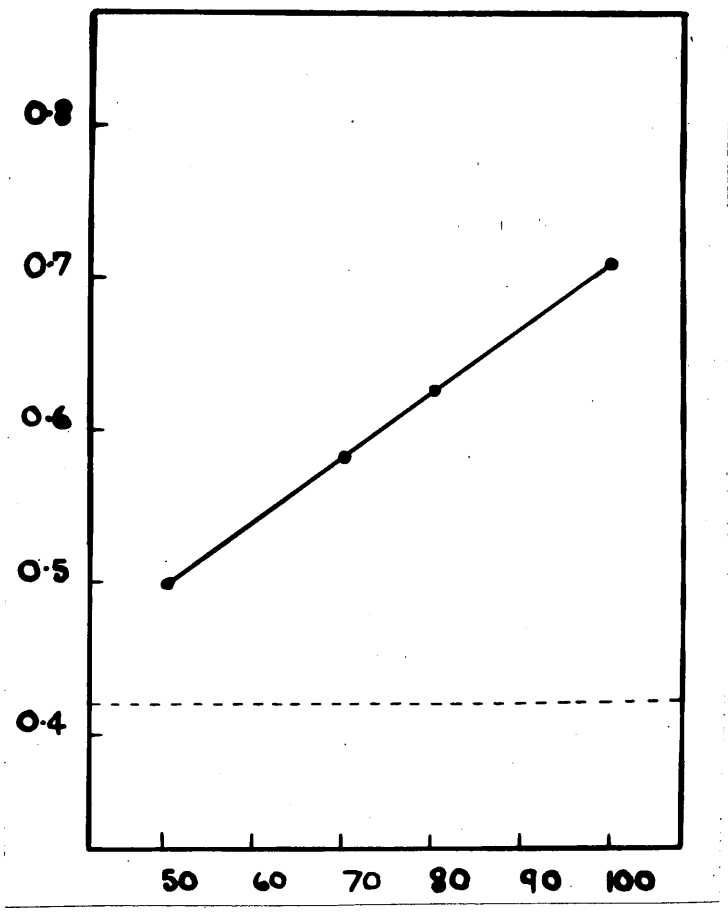
The results for salicylic acid and the various nitrophenols (Table X-XXIV) are summarised below. Interesting signs observed with treatment are recorded.

Salicylic Acid. Table X.; Fig. 3.

The carbon dioxide production of rats receiving doses of 50, 70, 80 and 100 mg. salicylic acid was linearly dependent on dose; the regression equation was $y = 0.0043x + 0.28$, where x = dose (mg.) and y = carbon dioxide output (g./h.). Bartlett's test gave $P > 0.50$. The analysis of variance in the following table shows a highly significant

Fig. 3.

Dose response curve of salicylic acid on the carbon dioxide output of Wistar albino rats. Abscissa: dose (mg.). Ordinate : CO_2 output (g./h.).



linear regression of dose on carbon dioxide output.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses:			
Regression	0.1469	1	0.1469
Deviation from regression	0.0029	2	0.0015
Within Doses	0.1312	20	0.0066
Total	0.2810	23	

The results of the experiment with blood concentration of the drug as the independent variable (Table XI), gave the regression equation $y = 0.0034x + 0.36$, where x = concentration in serum (mg./100 ml.) and y = carbon dioxide output (g./h.). In this instance the residual variance about the regression line $\sigma_{\bar{x}}$, is 0.077. An analysis of variance of these data, with dose as the independent variable gave $\sigma_{\bar{x}} = 0.068$, so there is obviously no advantage to be gained from using the drug concentration in the serum.

2-nitrophenol: Table XII.

Deaths resulting from high doses of 2-nitrophenol were preceded by a great decrease in respiratory rate.

The carbon dioxide output of rats was unaffected with 80, 90, 105 and 120 mg. doses. Bartlett's test gave $P > 0.50$. The following table shows that no significant effect was found.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses	0.0289	3	0.0096
Within Doses	0.1301	20	0.0065
Total	0.1590	23	

3-nitrophenol : Table XIII.

All rats treated with 3-nitrophenol jerked occasionally; moribund animals showed a marked decrease in respiratory rate; the bodies of rats which died from high doses remained limp.

The carbon dioxide output of rats was unaffected with doses of 30, 35, 40 and 45 mg. 3-nitrophenol. Bartlett's test gave $P > 0.05$. The analysis of variance, given in the following table, confirms the failure to demonstrate a significant effect.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses	0.0188	3	0.0063
Within Doses	0.0948	20	0.0047
Total	0.1136	23	

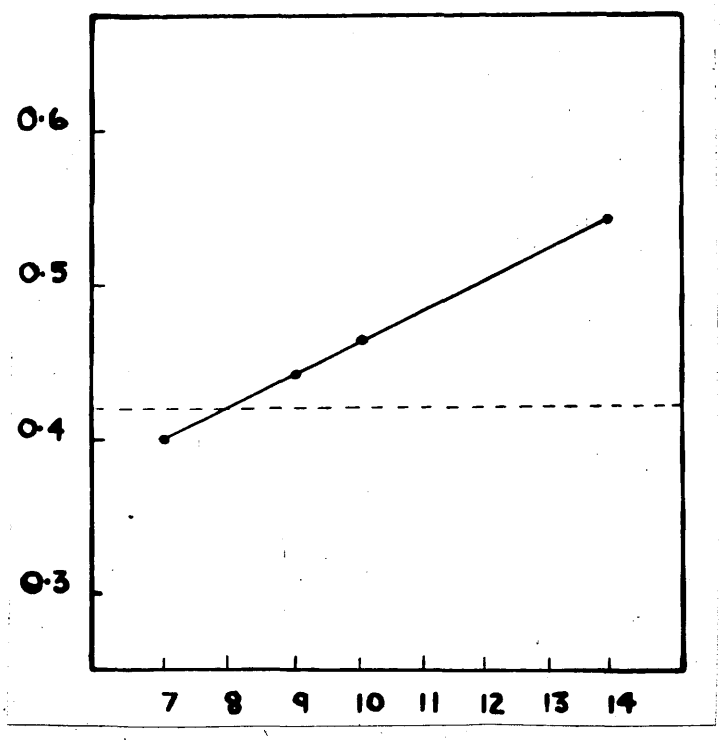
4-nitrophenol: Table XIV, Fig. 4.

An apparent increase occurred in the rate of respiratory movement of rats receiving $>$ 10 mg. The carbon dioxide output of rats receiving 7, 9, 10 and 14 mg. 4-nitrophenol was dependent on dose; the regression equation was $y = 0.0194x + 0.27$, where x = dose (mg.) and y = carbon dioxide output (g./h.). Bartlett's test gave $P > 0.05$. The analysis of variance, given in the following table establishes a highly significant linear regression of dose on carbon dioxide output.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses:			
Regression	0.0585	1	0.0585
Deviation from regression	0.0063	2	0.0032
Within Doses	0.0707	20	0.0035
Total	0.1355	23	

Fig. 4.

Dose response curve of 4-nitrophenol on the carbon dioxide output of Wistar albino rats. Abscissa: dose (mg.). Ordinate : CO_2 output (g./h.).



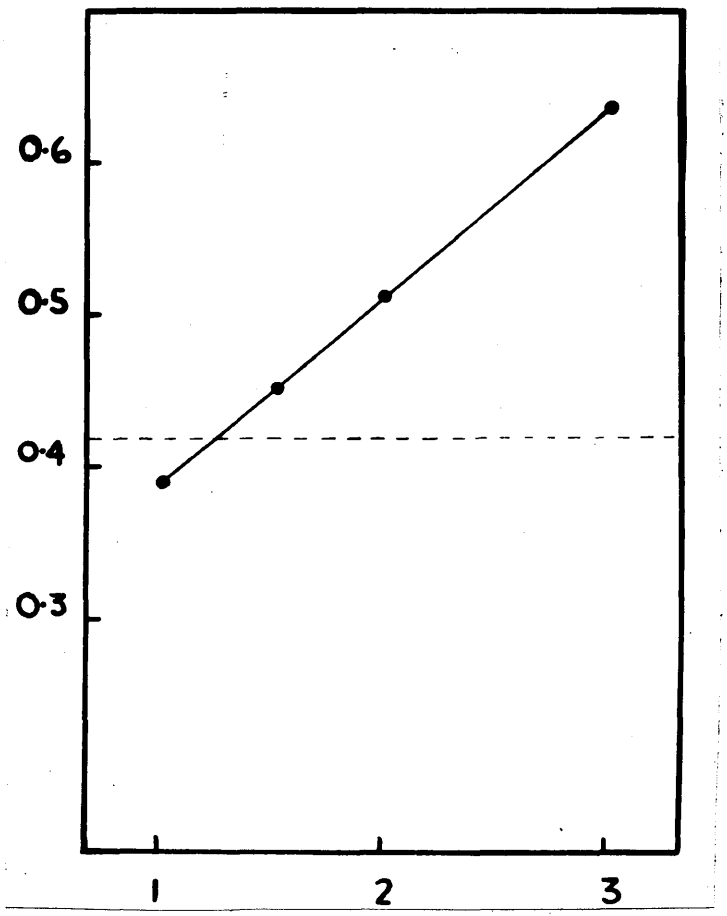
DNOC: Table XV, Fig. 5.

The carbon dioxide output of rats receiving 1.0, 1.5, 2.0 and 3.0 mg. DNOC was dependent on dose; the regression equation was $y = 0.1171x + 0.27$, where x = dose (mg.) and y = carbon dioxide output (g./h.). Bartlett's test gave $P > 0.10$. The analysis of variance shows a highly significant linear regression of dose on carbon dioxide output.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses:			
Regression	0.1801	1	0.1801
Deviation from regression	0.0079	2	0.0040
Within Doses	0.1012	20	0.0051
Total	0.2892	23	

Fig. 5.

Dose response curve of DNOC on the carbon dioxide output of Wistar albino rats. Abscissa : dose (mg.). Ordinate : CO_2 output (g./h.).



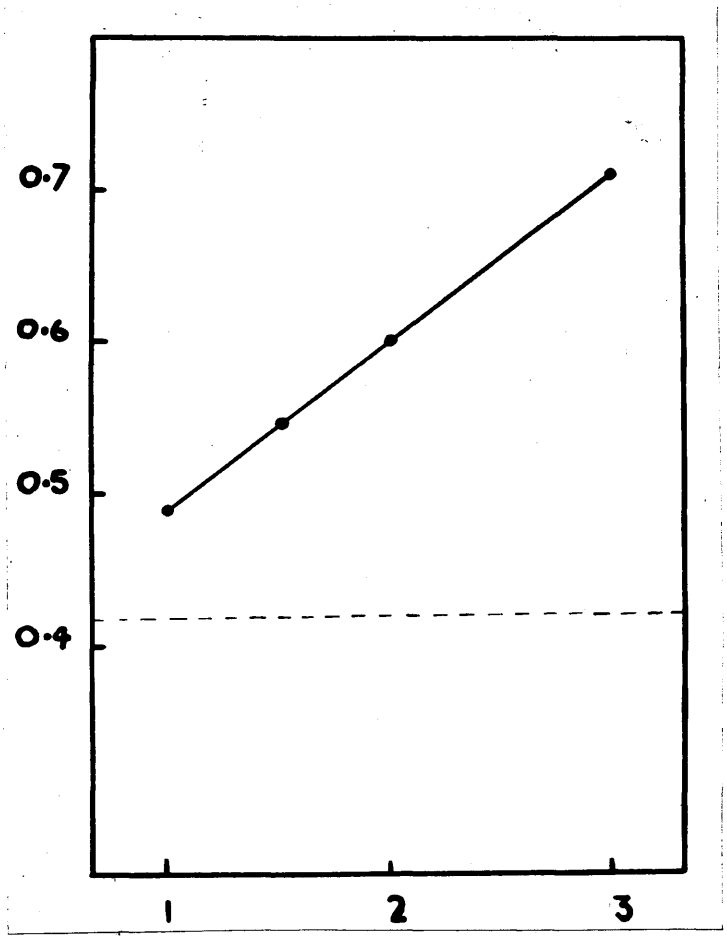
2,4-dinitrophenol: Table XVI, Fig. 6.

The carbon dioxide output of rats receiving 1.0, 1.5, 2.0 and 3.0 mg. 2,4-dinitrophenol was dependent on dose; the regression equation was $y = 0.1100x + 0.38$, where x = dose (mg.) and y = carbon dioxide output (g./h.). Bartlett's test gave $P > 0.10$. The analysis of variance in the following table establishes a highly significant linear regression of dose on carbon dioxide output.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses:			
Regression	0.1585	1	0.1585
Deviation from regression	0.0013	2	0.0007
Within Doses	0.0667	20	0.0033
Total	0.2265	23	

Fig. 6.

Dose response curve of 2,4-dinitrophenol on the carbon dioxide output of Wistar albino rats. Abscissa: dose (mg.). Ordinate : CO_2 output (g./h.).



2,3-dinitrophenol: Tables XVII, XVIII.

Table XVII contains the results of the first experiment to determine the effect of 2,3-dinitrophenol on carbon dioxide output; the carbon dioxide output of rats receiving doses of 15, 18, 21 and 24 mg., gave $P < 0.001$, in Bartlett's test, and the results were discarded.

Table XVIII contains the results of the repeated experiment which showed that the carbon dioxide output of rats was unaffected with doses of 15, 18, 21 and 24 mg. Bartlett's test gave $P > 0.50$. The analysis of variance, given in the following table, shows that no significant effect was established.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses	0.0415	3	0.0138
Within Doses	0.1087	20	0.0054
Total	0.1502	23	

2,5-dinitrophenol: Table IXX.

All treated rats developed very dark-coloured eyes and blood. The carbon dioxide output of rats

was unaffected by doses of 10, 15, 20 and 25 mg. 2,5-dinitrophenol. Bartlett's test gave P $>$ 0.50. No significant effect was found between dose and carbon dioxide output as seen from the analysis of variance:-

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses	0.0089	3	0.0030
Within Doses	0.1088	20	0.0054
Total	0.1177	23	

2,6-dinitrophenol: Table XX.

With fatal doses of 2,6-dinitrophenol the respiratory rate of the rats decreased considerably. Doses of 1.0, 3.0, 5.0 and 6.5 mg. had no effect on the carbon dioxide output of rats. Bartlett's test gave P $>$ 0.10. The analysis of variance in the following table confirms the failure to demonstrate a significant effect.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses	0.0112	3	0.0037
Within Doses	0.1083	20	0.0054
Total	0.1195	23	

3,4-dinitrophenol: Table XXI, Fig. 7.

Convulsions preceded the deaths of rats receiving > 16 mg. 3,4-dinitrophenol; all deaths were followed by the immediate stiffening of the rat body.

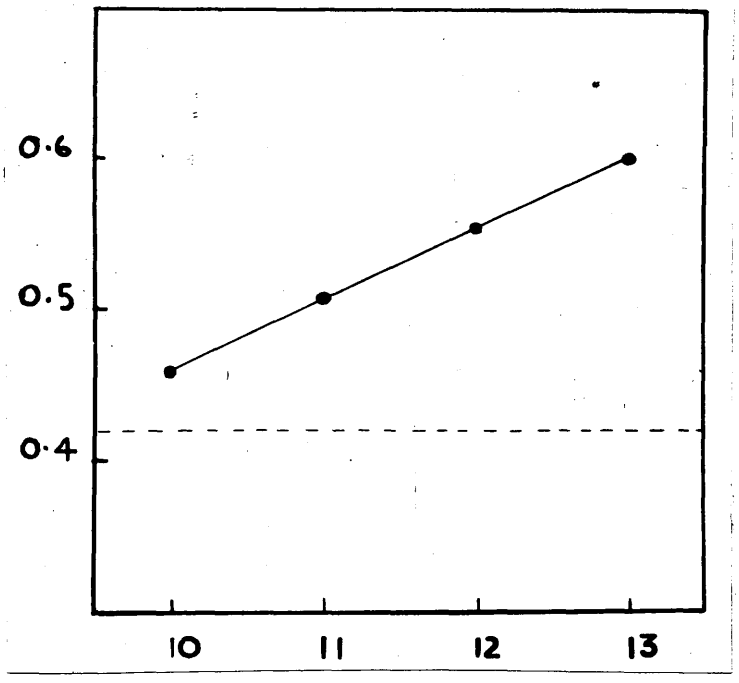
The carbon dioxide output of rats receiving doses of 10, 11, 12 and 13 mg. 3,4-dinitrophenol was dependent on the dose; the regression equation was $y = 0.0478x - 0.02$ where x = dose (mg.) and y = carbon dioxide output (g./h.). Bartlett's test gave $P > 0.10$. The analysis of variance given below shows a highly significant linear regression of dose on carbon dioxide output.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses:			
Regression	0.0686	1	0.0686
Deviation from regression	0.0031	2	0.0016
Within Doses	0.1068	20	0.0053
Total	0.1785	23	

Fig. 7.

Dose response curve of 3,4-dinitrophenol on the carbon dioxide output of Wistar albino rats.

Abscissa : dose (mg.). Ordinate : CO_2 output (g./h.).



3,5-dinitrophenol: Table XXII.

The carbon dioxide output of rats is unaffected by 2, 4, 6 and 7 mg. 3,5-dinitrophenol. Bartlett's test gave $P > 0.50$. The following table shows that no significant effect was found.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses	0.0100	3	0.0033
Within Doses	0.1134	20	0.0057
Total	0.1234	23	

2-nitro-4-aminophenol: Table XXIII.

Because of the insolubility of the compound the maximum dose administered was 50 mg.; at this dose no deaths occurred.

The carbon dioxide output of rats was depressed by doses of 10, 30, 40 and 50 mg. 2-nitro-4-aminophenol. Bartlett's test gave $P > 0.10$. The analysis of variance shows that no significant effect was established between doses.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses	0.0112	3	0.0037
Within Doses	0.0335	20	0.0017
Total	0.0447	23	

2-amino-4-nitrophenol: Table XXIV. Fig. 8.

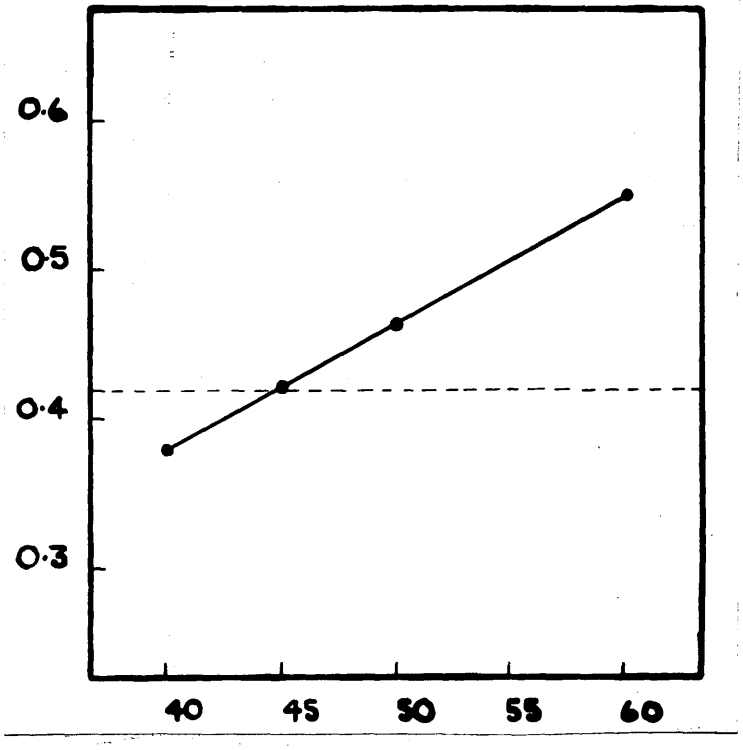
All treated rats developed a peach tint in their eyes, ears, nose, tail and paws; an apparent increase occurred in their depth of breathing.

The carbon dioxide output of rats receiving 40, 45, 50 and 60 mg. 2-amino-4-nitrophenol was dependent on dose; the regression equation was $y = 0.0087x + 0.03$, where x = dose (mg.) and y = carbon dioxide output (g./h.). Bartlett's test gave $P > 0.50$. The analysis of variance in the following table, shows a highly significant linear regression of dose on carbon dioxide output.

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares
Between Doses:			
Regression	0.0986	1	0.0986
Deviation from regression	0.0047	2	0.0024
Within Doses	0.0415	20	0.0021
Total	0.1448	23	

Fig. 8.

Dose response curve of 2-amino-4-nitrophenol on the carbon dioxide output of Wistar albino rats.
Abscissa : dose (mg.). Ordinate : CO_2 output (g./h.).



(2) Rectal Temperature.

Control rats: The mean rectal temperature of ninety Wistar albino control rats was 100.3°F., with a standard deviation of 0.812. 95% of control rats have temperatures between 98.7°F. and 101.9°F. It therefore follows that (i) a group cannot be distinguished from a control population if a proportion of the order of 1 in 20 has a temperature outwith this control range, (ii) if 10 or more rats in 20 have temperatures outside this range, then, a priori, the group is unlikely to be drawn from a population of control rats.

Treated rats: The effect of salicylic acid and the nitrophenols was therefore determined by deciding whether the rectal temperatures of the six rats receiving the maximum non-fatal dose could be, a priori, drawn from a control population. The results (Table X-XXIV) are summarised below.

Salicylic Acid: Table X.

The six rats receiving 100 mg. salicylic acid had temperatures above the control range, with a mean of 103.2°F. 100 mg. salicylic acid therefore increases rectal temperature.

2-nitrophenol: Table XII.

Temperatures of the six rats receiving 120 mg. 2-nitrophenol were below the range for control rats, with a mean of 96.6°F. 120 mg. 2-nitrophenol consequently depresses rectal temperature.

3-nitrophenol: Table XIII.

The six rats receiving 45 mg. 3-nitrophenol had temperatures below the range for control rats, with a mean of 95.7°F. 45 mg. 3-nitrophenol is therefore a rectal temperature depressant.

4-nitrophenol: Table XIV.

Four of the six rats receiving 14 mg. 4-nitrophenol had temperatures below 98.7°F.; the mean temperature was 98.5°F. 14 mg. 4-nitrophenol has thus a depressant action on rectal temperature.

DNOC: Table XV.

Five of the six rats receiving 3 mg. DNOC had temperatures above the control range with a mean

of 104.2°F. 3 mg. DNOC accordingly elevates rectal temperature.

2,4-dinitrophenol: Table XVI.

Five of the six rats receiving 3 mg. 2,4-dinitrophenol had temperatures above 101.9°F.; the mean temperature was 103.0°F. 3 mg. 2,4-dinitrophenol is consequently a rectal temperature stimulant.

2,3-dinitrophenol: Table XVIII.

Five of the six rats receiving 24 mg. 2,3-dinitrophenol had temperatures below the control range, with a mean of 98.0°F. 24 mg. 2,3-dinitrophenol accordingly depresses rectal temperature.

2,5-dinitrophenol: Table IXX.

The temperatures of the six rats receiving 25 mg. 2,5-dinitrophenol were below the range for control rats, with a mean of 95.6°F. 25 mg. 2,5-dinitrophenol has therefore a depressant effect on rectal temperature.

2,6-dinitrophenol: Table XX.

All six rats receiving 6.5 mg. 2,6-dinitrophenol had temperatures below 98.7°F., with a mean of 96.8°F. 6.5 mg. 2,6-dinitrophenol therefore depresses rectal temperature.

3,4-dinitrophenol: Table XXI.

Five of the six rats receiving 13 mg. 3,4-dinitrophenol had temperatures within the control range, with a mean of 99.6°F. 13 mg. 3,4-dinitrophenol has thus no demonstrable effect on rectal temperature.

3,5-dinitrophenol: Table XXII.

The temperatures of five of the six rats receiving 7 mg. 3,5-dinitrophenol were inside the range for control rats; the temperature mean was 100.1°F. 7 mg. 3,5-dinitrophenol accordingly has no apparent effect on rectal temperature.

2-nitro-4-aminophenol: Table XXIII.

Five of the six rats receiving 50 mg. 2-nitro-4-aminophenol had temperatures below 98.7°F.; the temperature mean was 97.1°F. 50 mg. 2-nitro-4-

aminophenol therefore is a rectal temperature depressant.

2-amino-4-nitrophenol: Table XXIV.

Temperatures of five of the six rats receiving 60 mg. 2-amino-4-nitrophenol were below the control range, with a mean of 96.7°F. 60 mg. 2-amino-4-nitrophenol consequently depresses rectal temperature.

(3) Oxygen Consumption.

Control rats: The histogram in Fig. 9 shows the near normal distribution of values of oxygen consumed per hour by one hundred and twenty Wistar albino control rats, weight 150-210 g. The mean oxygen consumption = 345 ml./h., standard deviation = 56, and coefficient of variation = 16%.

The quality control chart in Fig.10, where each batch represents three consecutive determinations of oxygen consumption of control rats, shows that there is no significant difference in batch means. No batch mean lies outside the inner control lines at 281 and 408 ml.; the experiment is thus in a state of statistical control.

Fig. 9.

Histogram : distribution of oxygen consumptions of one hundred and twenty control Wistar albino rats. Abscissa: O_2 consumption (ml./h.). Ordinate : frequency.

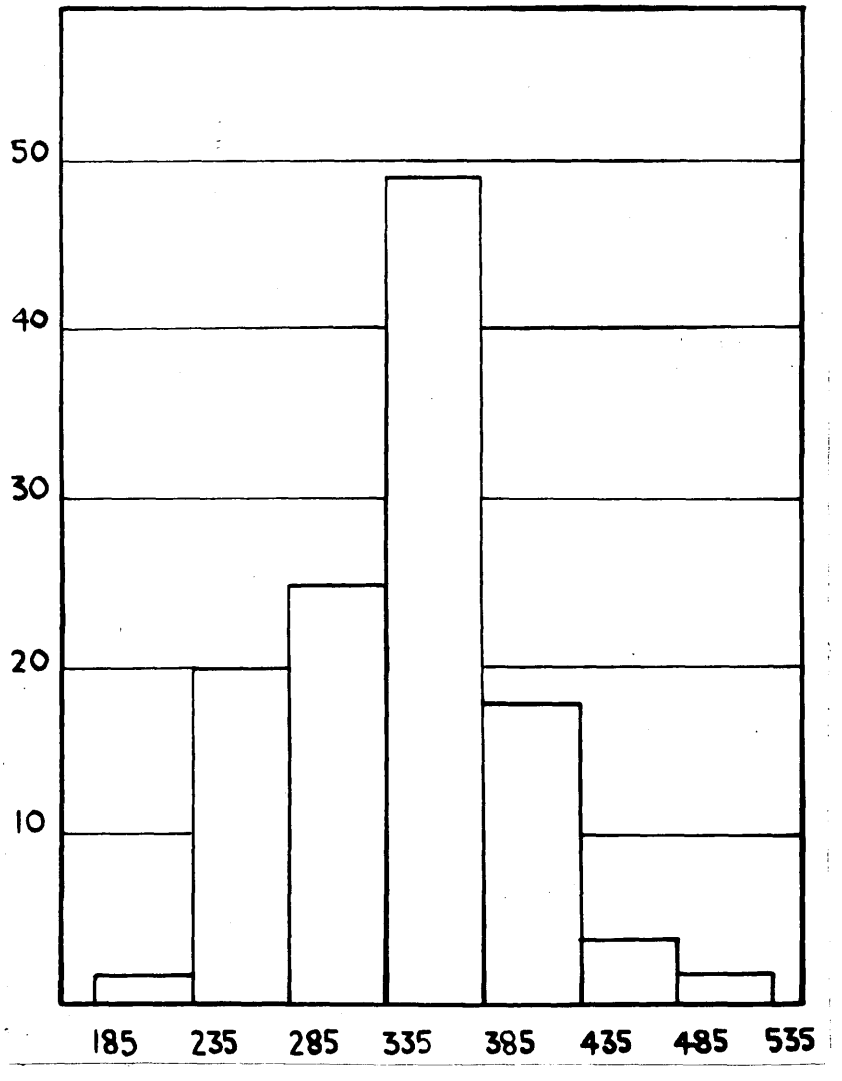
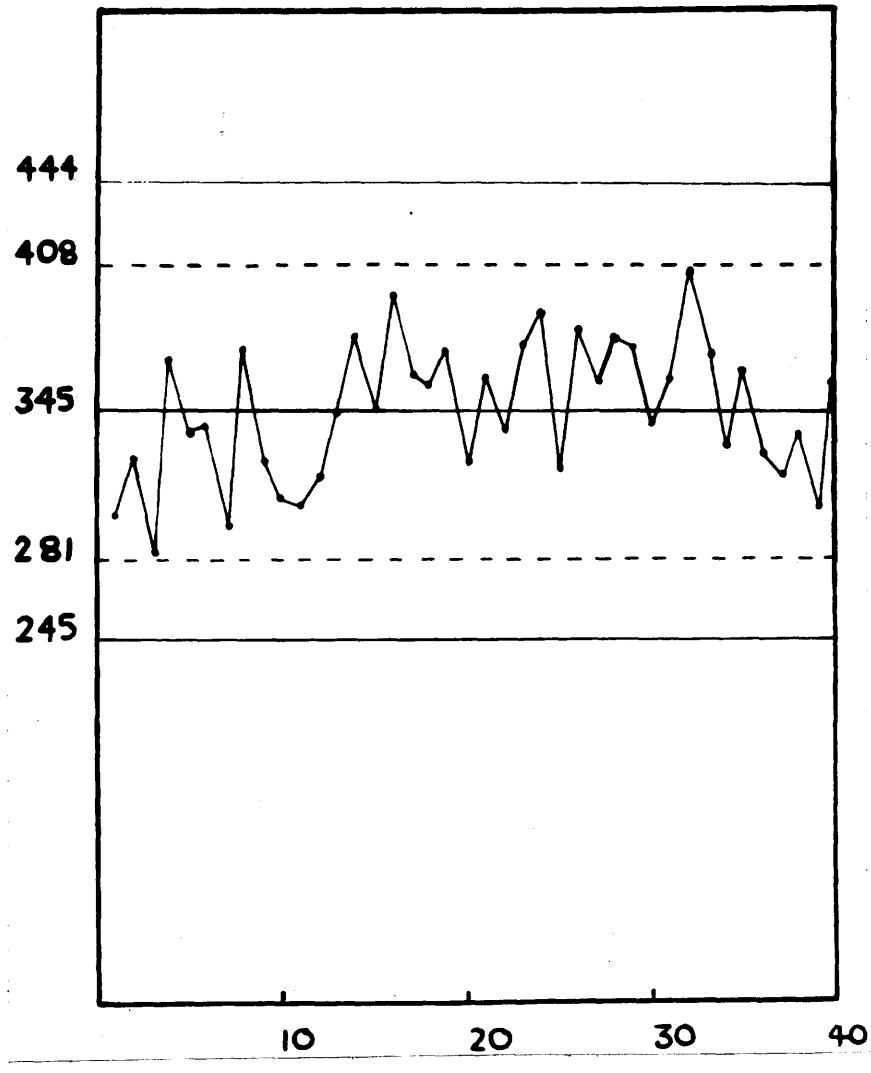


Fig. 10.

Control chart for oxygen consumptions of one hundred and twenty control Wistar albino rats. Each batch represents three consecutive determinations. Abscissa: batch number. Ordinate : O_2 consumption (ml./h.).



Treated rats: The action on oxygen consumption was decided by a sequential test (83) of the hypotheses whether the mean difference between the paired rates of oxygen consumption of treated and control rats was or was not zero. Consecutive determinations of oxygen uptake were made on treated and control rats paired for sex and weight, and ΔO_2, the difference in oxygen uptake of the paired rats, evaluated. $Z = \left(\Sigma \Delta O_2\right)^2 / \Sigma (\Delta O_2)^2$ was calculated sequentially after each trial and the hypothesis $H_1 : |\mu| > \delta\sigma$ tested against $H_0 : |\mu| < \delta\sigma$, where μ is the mean and σ^2 the variance of ΔO_2; δ was selected as 1 and the value of 0.05 assigned to α and β, where α = maximum probability of error of the first kind and β = maximum probability of error of the second kind. The critical limits for z were obtained from the U.S. Bureau of Standards Tables (84).

The results of salicylic acid and the various nitrophenols on oxygen consumption (Tables XXV-XXXVII) are summarised below.

Salicylic Acid: Table XXV.

100 mg. salicylic acid produced a significant effect on the oxygen consumption of rats; the mean ΔO_2 and its 95% fiducial limits were +77.2 ±49.0 ml. 100 mg. salicylic acid therefore stimulates consumption.

2-nitrophenol: Table XXVI.

105 mg. 2-nitrophenol had no effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were -2.9± 49.3 ml.

3-nitrophenol: Table XXVII.

40 mg. 3-nitrophenol produced a significant effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were -70.7 ± 49.3 ml. 40 mg. 3-nitrophenol therefore depresses oxygen consumption.

4-nitrophenol: Table XXVIII.

12 mg. 4-nitrophenol had no effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were +2 ± 43.1 ml.

DNOC: Table IXXX.

3 mg. DNOC had a significant effect on oxygen

consumption; the mean ΔO_2 and its 95% fiducial limits were +252.5 $\pm$ 39.2 ml. 3 mg. DNOC therefore produces marked stimulation.

2,4-dinitrophenol: Table XXX.

3 mg. 2,4-dinitrophenol had a significant effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were +92.3 $\pm$ 51.6 ml. 3 mg. 2,4-dinitrophenol consequently increases oxygen consumption.

2,3-dinitrophenol: Table XXXI.

24 mg. 2,3-dinitrophenol produced no effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were +4 $\pm$ 52.1 ml.

2,5-dinitrophenol: Table XXXII.

20 mg. 2,5-dinitrophenol produced a significant effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were -57.5 $\pm$ 36.1 ml. 20 mg. 2,5-dinitrophenol has thus a depressant action.

2,6-dinitrophenol: Table XXXIII.

6 mg. 2,6-dinitrophenol had no effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were -17 ± 56.7 ml.

3,4-dinitrophenol: Table XXXIV.

13 mg. 3,4-dinitrophenol produced no effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were -6 ± 36.6 ml.

3,5-dinitrophenol: Table XXXV.

6 mg. 3,5-dinitrophenol had no effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were -4.8 ± 16.7 ml.

2-nitro-4-aminophenol: Table XXXVI.

50 mg. 2-nitro-4-aminophenol had a significant effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were -79.2 ± 40.6 ml. 50 mg. 2-nitro-4-aminophenol accordingly depresses oxygen consumption.

2-amino-4-nitrophenol: Table XXXVII.

50 mg. 2-amino-4-nitrophenol produced a significant effect on oxygen consumption; the mean ΔO_2 and its 95% fiducial limits were -52.5 ± 35 ml. 50 mg. 2-amino-4-nitrophenol therefore lowers oxygen consumption.

A summary of the results of this investigation, for the effects of the nitrophenols on rate of carbon dioxide output, rectal temperature and rate of oxygen uptake, is contained in Table IX. In the table a positive sign indicates stimulant action; a negative sign depressant action; a zero no action.

Table IX.

Summary of Results.

Compound	Dose Range (mg.)	Action on		
		CO_2 output	Rectal Temp.	O_2 Uptake
Salicylic Acid	50-100	+	+	+
2-nitrophenol	80-120	0	-	0
3-nitrophenol	30- 45	0	-	-
4-nitrophenol	7- 14	+	-	0
DNOC	1 - 3	+	+	+
2,4-dinitrophenol	1 - 3	+	+	+
2,3-dinitrophenol	15-24	0	-	0
2,5-dinitrophenol	10- 25	0	-	-
2,6-dinitrophenol	1 - 6.5	0	-	0
3,4-dinitrophenol	10- 13	+	0	0
3,5-dinitrophenol	2 - 7	0	0	0
2-amino-4-nitrophenol	40- 60	+	-	-
2-nitro-4-aminophenol	10- 50	-	-	-

Discussion.

The present investigation shows that the nitrophenols tested can be allotted to the following six groups in respect of action on metabolic rate:-

(1) 2,4-dinitrophenol and DNOC, which increase carbon dioxide output, oxygen consumption and rectal temperature.

(2) 2-nitrophenol, 2,3-, 2,6- and 3,5-dinitrophenol, which have no action on either carbon dioxide output or oxygen consumption. Of these compounds, all but 3,5-dinitrophenol depress rectal temperature.

(3) 3-nitrophenol and 2,5-dinitrophenol, which have no effect on carbon dioxide output but a depressant action on oxygen consumption and rectal temperature.

(4) 4-nitrophenol and 3,4-dinitrophenol, which stimulate carbon dioxide output but have no action on oxygen uptake. 4-nitrophenol depresses rectal temperature.

(5) 2-amino-4-nitrophenol, which stimulates carbon dioxide output and depresses oxygen consumption and rectal temperature.

(6) 2-nitro-4-aminophenol, which decreases carbon dioxide output, oxygen consumption and rectal temperature.

Some of these findings are not novel but the salient feature of the literature in this field is the diversity of previous results for certain nitrophenols (see Table I). In the present investigation these nitrophenols have, for the first time, been examined for their actions on metabolic rate, by reliable methods in properly designed experiments.

To appreciate the value of the present results it is essential to consider carefully the factors contributing to the inconsistency of previous conclusions. It is evident that the use of unsuitable materials and techniques, together with the presentation of inadequate and unconvincing data, is the rule rather than the exception.

Previous investigations on the metabolic action of the nitrophenols failed primarily to observe the basic principles of experimentation. Conclusions were formed from observations on individual animals (15, 18); standard conditions were not imposed (18); tests of significance were not applied to the results. With undesigned or badly-designed experiments, false conclusions readily result from systematic and inherent variations in biological

material, and it is considered that most of the previously reported effects of the nitrophenols on metabolic rate could be fallacious on this ground alone.

The diversity of preparations used, e.g., intact mammals, (1, 2, 12-21), mammalian tissues (44-47, 49), sea urchin eggs (34-42), yeasts (22-33) and homogenates (50, 51), presents further problems of interpretation. Yeasts, sea urchin eggs, tissues and homogenates are economically attractive materials, but the effects produced are often peculiar to the material and may even lack consistency for a particular preparation. The confusion resulting from the use of unsuitable materials is now exemplified.

(1) The actions of nitrophenols on Arbacia egg respiration reported independently by Clowes & Krahl (35), and Tyler & Horowitz (41), are in complete agreement, but Krahl & Clowes (86) have shown that the respiratory stimulation of Arbacia eggs is a doubtful index for general metabolic stimulation. They demonstrate respiratory stimulation of Arbacia eggs by halophenols which are incapable of increasing the rectal temperature or respiratory exchange of rats.

(2) On yeasts the reported metabolic actions

of the nitrophenols differ. Field et al. (22, 23, 27) claim that yeast respiration is stimulated by 2,4-dinitrophenol, 2,5-dinitrophenol, and 2,6-dinitrophenol; Genevois & Saric (30), on the other hand, claim that these dinitrophenols inhibit respiration. Yeasts are therefore another unsuitable preparation for metabolic studies since the results markedly depend on the choice of medium, substrate and pH.

(3) Experimental conditions likewise influence respiratory stimulation of isolated animal tissues, and stimulation can be produced by drugs which are inactive on the whole animal.

(4) The use of homogenates is not advisable for studying the metabolic actions of drugs since the action of even 2,4-dinitrophenol on cell-free preparations may differ from its action on intact cells (47).

The intact mammal is therefore of most value for investigating the effect of drugs on metabolic rate. If a drug directly stimulates the metabolic rate of the intact mammal it will generally stimulate the respiration of tissue slices and unicellular preparations, over a wide range of experimental conditions; per contra, stimulation in vitro can occur

with drugs which have no such action on the intact mammal.

Closely linked with this use of unsuitable materials is the use of questionable techniques. The detection of stimulation in intact mammals solely by the index of rectal temperature lacks sensitivity and is acceptable only with powerful stimulants; moreover, there is a diversity of response between species to individual drugs (see Table I).

A final criticism must be made of certain authors who do not present sufficient data to justify their conclusions. Gibbs & Reichert (1) fail to state the temperatures of the experimental animals and even leave doubt as to whether the conclusions are based on observations on dogs or rabbits, or both. Magne et al. (18) claim that 3,5-dinitrophenol has no effect on the oxygen consumption of the dog but give no data; their conclusions on the effect of 3,4-, 3,5-, and 2,5-dinitrophenol on oxygen consumption must also be questioned, since they base their conclusion on observations of single animals; for valid inference it is obvious that determinations must be carried out in replicate.

The current investigation therefore differs

from most of its predecessors in its use of designed experiments, tests of significance, a suitable subject and reliable methods. These features are discussed below.

The inherent variation in biological material was recognised and accepted; no attempt was made to impose stringent "basal" conditions or to enforce total inactivity (cf., the doubtful claims of Mørch (87,), and Forbes, Kriss & Miller (88) for constant results in animal metabolic studies, by alleged rigorous control of activity and feeding). In the present work, variations in feeding, activity, etc. were simply assumed to be random, in accordance with a definite hypothetical distribution, and no claim is made that these inevitable sources of variance were rigidly controlled. The near normal distributions of the carbon dioxide outputs and oxygen consumptions of the control rats (Fig. 1 & 9), and the quality control charts (Fig. 2 & 10) confirm that the variables were in statistical control.

The principal methods of statistical inference adopted were the analysis of variance (82) for effect on rate of carbon dioxide output, and a sequential test for effect on rate of oxygen consumption (see Statistical Methods). Parenteral dose was adopted

as the independent variable in the analysis of variance of carbon dioxide outputs, since it was shown with salicylic acid that no advantage was gained by using the actual concentration of drug in the serum. Moreover, difficulties would arise in the estimation of the serum concentration of certain nitrophenols. The actions of the nitrophenols on oxygen consumption were determined by a non-linear probability ratio sequential test of Student's hypothesis (83). This enabled definite conclusions, including the acceptance of the null hypothesis as a positive inference, to be reached from a minimum number of observations. Little importance was attached to the rectal temperature changes because of the insensitivity of this index; these data were therefore treated less formally.

In choosing a subject for studying overall metabolic effects it was considered that the mammal, although at first sight the least practical, was definitely best. The Wistar albino rat was therefore chosen for this investigation. In this study the indices used to determine the metabolic action of the nitrophenols on the rat were rate of carbon dioxide output, rate of oxygen consumption and, incidentally, rectal temperature. Suitable apparatuses were therefore devised for measuring the carbon dioxide output and

oxygen consumption of the rat, or other small mammals, over one hour periods.

The measurements of carbon dioxide output were made rapidly and accurately with an apparatus based on Haldane's open-circuit system (64), modified for simultaneous individual gravimetric estimations on a maximum of twenty five rats. This is a conventional type of apparatus which requires no discussion.

The closed circuit apparatus for measuring the oxygen consumption of single rats was developed from first principles and met the requirements of economy, simplicity and accuracy. In the development of this apparatus, several theoretical points arose which warrant some discussion. Firstly, there was the possibility of oxygen deficiency. The large volume of the enclosed air (56 litres) eliminated this danger and ensured an adequate supply of oxygen for the rat, since the oxygen content of the system was unlikely to fall below 18.7%, even with 100% respiratory stimulation. Secondly, there arose the problem of controlling water vapour pressure. This was solved by introducing vessels with water into both the experimental and control systems to stabilise the

contribution of water vapour pressure to the internal pressure. A system was thus obtained which was more physiological than one in which all moisture was removed, and the difficulty of drying the air completely was avoided. Brody (62) claims that a saturated atmosphere in closed circuit apparatus upsets the heat regulation of the animal. This seems unlikely since the temperature of the expired air is approximately twice that of the system temperature, and substantial evaporation can still occur (v.p. of water at 20°C. = 17.5 mm. Hg., at 40°C. = 55.3 mm. Hg.). Thirdly, the requirement of temperature equilibrium was met. The thermal conductivity of the metal tubes and reservoir prevented a significant rise in temperature of the enclosed air, and was responsible for the attainment of temperature equilibrium between the system and its surroundings. The efficient circulation of the enclosed air ensured that no marked temperature gradient occurred within the system. Fourthly, the use of the thermobarometer was permitted since there was virtually no difference in the internal temperatures of the control and experimental systems. It must be emphasised that the omission of a thermobarometer in the constant pressure apparatuses of Cohnheim (59), Joel (59), Bargeton & Krumm-Heller

(72) and Groebbels (59) must have resulted in gross errors from failure to correct for slight variations in ambient conditions. Finally, there was the question of temperature control. A temperature variation of 2°C. produces no appreciable change in the value of k, the calibration constant. A state of statistical control was attained in all the present experiments in a room of almost constant temperature, i.e. 18-20°C. It was therefore decided that former investigators have overemphasised the importance of rigid temperature control in metabolic studies.

The technical validity of the present investigation has now been defined and the results, obtained in a systematic manner, are seen to be preferable to those of previous investigations. The importance of the present results can therefore be considered in their own right.

The results show that the nitrophenols tested fall into six groups which were described above. The group of most general interest is that composed of the metabolic stimulants (Group 1) since these compounds have many applications and are comparatively rare, (only DNP and some substituted derivatives, tri-iodo-thyronine and related compounds, and salicylic acid are authentic metabolic stimulants in the intact

mammal); no new stimulant has, however, emerged from this study. Compounds with the actions of Groups 2, 4 and 6 are well-established, but the existence of compounds with the actions of Groups 3 and 5 was previously unsuspected.

An outstanding feature of the results is that changes in rate of carbon dioxide output are not always accompanied by parallel changes in rate of oxygen consumption; the carbon dioxide output of rats is therefore a very unreliable index of metabolic stimulation. This point is best demonstrated with a specific example, the compounds of Group 4. Here, the carbon dioxide output of rats was significantly increased by 4-nitrophenol and 3,4-dinitrophenol, while the oxygen uptake was unaffected. Such increased carbon dioxide output in the absence of increased oxygen uptake points to a change in respiratory quotient (R.Q.). A "false" change in R.Q., resulting from hyperventilation, could account for the results of this group, since a component of carbon dioxide output from hyperventilation would be commensurate with the observed values. Alternatively, 4-nitrophenol and 3,4-dinitrophenol could produce a true change in R.Q.

It is also obvious that changes in rectal temperature bear little relation to changes in metabolic rate, except with powerful metabolic stimulants or depressants, since most of the nitrophenols depress rectal temperature. Several compounds, 2- and 4-nitrophenols, 2,3- and 2,6-dinitrophenol, depressed rat rectal temperatures without affecting the rate of oxygen consumption - an effect probably due to a disturbance of the thermal equilibrium of the rat. Determinations of rectal temperature are consequently only of value when a significant increase occurs, since almost any compound in sufficient dose will be a depressant.

This investigation has therefore confirmed that rate of oxygen uptake is the only valid indirect index for establishing, over one hour periods, metabolic stimulant action of rats. Measurements of carbon dioxide output and rectal temperature, even in preliminary surveys, must be regarded as unprofitable. Furthermore, with the apparatus described here, measurements of the oxygen consumption of small mammals are now a feasible proposition and these preliminary surveys are superfluous.

Although the rate of carbon dioxide output can no longer be regarded as a genuine index of metabolic stimulation, these results still deserve some comment. In this investigation all the nitrophenols which stimulated carbon dioxide output exhibited, within the given range of dose, a linear relationship between dose and carbon dioxide output. This is a common form of dose response curve in the whole animal, and warrants no further comment.

In the determinations of carbon dioxide output an example occurs of heterogeneity of variances for the different doses of 2,3-dinitrophenol (Table XVII). This is regarded as an error of the first kind, and as emphasising the stochastic nature of biological data.

The results for the two aminonitrophenols are of interest since 2-amino-4-nitrophenol and 2-nitro-4-aminophenol are well-known metabolites of 2,4-dinitrophenol(89). Their metabolic effects have no resemblance to the powerful stimulant action of 2,4-dinitrophenol; reduction of the 2-nitro group of DNP to the amino group has converted a metabolic stimulant to a compound capable of stimulating carbon dioxide output, and depressing rectal temperature and

oxygen consumption; reduction of the 4-nitro group of DNP has converted the stimulant to a true metabolic depressant. The reduction of 2,4-dinitrophenol in the body therefore leads to a loss of stimulant action, and is a true detoxication.

Before considering a relation between biological activity of the nitrophenols and molecular structure or physical properties, former theories were examined. Magne, Mayer & Plantefol (18) maintained that all nitrophenols with a nitro group para to the hydroxyl possessed metabolic stimulant action. Clowes & Krahl (35) concluded from studies on Arbacia eggs that a nitro group para to the hydroxyl enhanced the metabolic stimulant activity which they found in their limited series of nitrophenols (Table I).

In the light of the present results, neither of these theories is wholly acceptable. Of the nitrophenols tested, only those with nitro groups in both the 2- and 4- positions produced metabolic stimulation; all those with a nitro group in the 4-position increased carbon dioxide output. It may therefore be concluded that, within the present series of compounds, the presence of a 4-nitro group is a

necessary and sufficient condition for the effect of increasing the carbon dioxide output of rats. No such deduction can be made for metabolic stimulation, and any correlation of physico-chemical properties and metabolic stimulant activity of the nitrophenols would be premature.

The main aim of this study has been fulfilled with the definition, by systematic experiment, of the actions of the mono and dinitrophenols on the metabolic rate of the intact mammal. The present data form an essential foundation for more detailed studies of the mode of action of the nitrophenols.

A development of importance in this investigation has been the construction of the apparatus for measuring the uptake of oxygen by small mammals. This apparatus will be of value in future metabolic studies, since it enables estimations of oxygen consumption to be performed economically, rapidly, and accurately.

Appendix.

Statistical Methods.

Carbon dioxide outputs: Each experiment comprised the carbon dioxide outputs (y_i) of a number of groups of animals receiving different doses (x_j) of the compound. For the analysis (82), each dose was regarded as a different treatment, and the hypothesis was tested that there exists a component of variance between treatments. If the null hypothesis was not rejected, it was concluded that no effect on carbon dioxide output had been demonstrated. If the null hypothesis was rejected, the linear regression of carbon dioxide output on dose was calculated, and its significance tested by analysis of variance. In no case was it necessary to proceed further, and consider curvilinear regression or the transformation of variables.

Oxygen consumptions: The determination of the effect of a given dose of a certain compound on the rate of oxygen uptake was relatively costly in time and space. It was therefore essential to employ a powerful and efficient method of statistical inference. A sequential test of a composite hypothesis was therefore indicated, and the non-linear probability ratio sequential test proposed by

Wald (83) seemed most suitable. This test distinguishes only two hypotheses, but in the present application if ΔO_2 is appreciable its fiducial limits were sufficient to decide whether the resultant effect is positive or negative. The great advantage of this test is the availability of a complete set of tables (84).

In each experiment the observations were paired, one rat receiving the drug, the other acting as control, and the algebraic difference in oxygen consumption (ΔO_2) determined. The hypothesis $H_0: |\mu| < \delta\sigma$ was tested against $H_1: |\mu| > \delta\sigma$ where μ is the mean ΔO_2 and σ^2 its variance; δ, which determines the critical limit, was arbitrarily selected as 1; the value of 0.05 was assigned to α and β, where α is the maximum probability of error of the first kind and β is the maximum probability of error of the second kind.

The virtue of this powerful and efficient test is that values of α, β and δ can be freely selected to suit the particular experimental conditions.

Protocols.

Carbon dioxide output and rectal temperature.

The results of the experiments to determine the effects of salicylic acid and the nitrophenols on the rate of carbon dioxide production and rectal temperature of Wistar albino rats are given in Tables X-XXIV.

Oxygen consumption.

The results of the experiments to determine the actions of salicylic acid and the nitrophenols on the rate of oxygen consumption of Wistar albino rats are given in Tables XXV-XXXVII.

Table Xa.

Carbon dioxide output and rectal temperatures of rats after injection of salicylic acid.

Salicylic acid Dose (mg.)	Date May 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
100	4th	190	0.74	102.6	
	4th	195	0.81	103.0	
	8th	178	0.66	103.4	
	8th	188	0.78	102.2	
	8th	170	0.76	105.0	
	12th	188	0.59	103.2	
	12th	192	0.68	103.0	Result redundant.
	12th	182	-	104.6	Died*.
80	4th	166	0.73	103.0	
	4th	162	0.53	103.0	
	4th	160	0.76	101.4	
	8th	188	0.52	103.0	
	8th	165	0.55	103.0	
	12th	185	0.56	103.8	
	12th	181	0.49	102.6	Result redundant.
70	4th	193	0.53	104.0	
	4th	173	0.69	103.6	
	4th	168	0.54	103.0	
	8th	160	0.63	101.0	
	8th	180	0.61	103.0	
	12th	188	0.53	101.0	
50	4th	192	0.50	100.0	
	4th	174	0.58	100.0	
	8th	188	0.55	100.0	
	8th	178	0.50	101.0	
	8th	175	0.47	102.0	
	12th	181	0.41	100.0	
	12th	195	0.29	98.0	Result discarded.
0	4th	172	0.42	100.4	
	4th	185	0.45	100.2	
	8th	185	0.40	99.6	
	8th	177	0.43	100.4	
	12th	165	0.38	101.6	
	12th	190	0.37	99.8	
	12th	188	0.45	100.0	
	12th	178	0.36	100.8	

* Convulsions preceded death.

Table Xb.

Summary of Table Xa.

Salicylic acid Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
100	0.72	103.2
80	0.61	102.9
70	0.59	102.6
50	0.50	100.5
0	0.41	100.4

Table XIa.

Carbon dioxide output and drug concentration in blood of rats after injection of salicylic acid.

Salicylic acid (mg.)	Date May, 1955.	Rat Weight (g.)	CO_2 output (g./h.)	Serum Concn. (mg./100 ml.)
85	20th	185	0.73	74
	20th	186	0.59	77
	20th	170	0.60	78
	24th	180	0.69	74
	24th	182	0.66	82
	26th	175	0.53	85
65	20th	200	0.62	54
	20th	190	0.51	57
	20th	180	0.53	54
	24th	180	0.67	48
	24th	195	0.59	57
	24th	195	0.52	55
50	20th	184	0.58	49
	20th	174	0.59	52
	24th	185	0.47	52
	24th	186	0.44	59
	26th	178	0.47	51
	26th	168	0.56	48
25	20th	194	0.35	32
	20th	182	0.43	30
	24th	188	0.58	31
	24th	200	0.39	28
	24th	186	0.45	33
	26th	200	0.49	32
0	20th	178	0.37	-
	20th	184	0.39	-
	24th	190	0.41	-
	24th	177	0.35	-
	26th	200	0.41	-
	26th	184	0.39	-

Table XIb.

Summary of Table XIa.

Salicylic acid Dose (mg.)	Mean CO_2 output (g./h.)	Mean Serum Concn. (mg./100 ml.)
85	0.63	78
65	0.57	54
50	0.52	52
25	0.45	31
0	0.39	-

Table XIIa.

Carbon dioxide output and rectal temperature of rats after injection of 2-nitrophenol.

Dose (mg.)	Date Jan. 1956	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
135	18th	190	-	95.2	Died.
	18th	184	-	-	Died.
120	18th	198	0.66	97.8	
	18th	182	0.50	97.0	
	18th	173	-	-	Died.
	24th	166	-	-	Died.
	24th	196	0.43	96.8	
	27th	182	0.48	96.6	
	27th	183	0.37	95.2	
	27th	184	0.60	96.0	
105	18th	166	-	-	Died.
	18th	200	0.51	97.0	
	24th	195	0.38	95.0	
	24th	174	0.37	96.6	
	27th	198	0.55	96.2	
	27th	180	0.44	95.4	
	27th	168	0.44	95.2	
90	18th	181	0.33	95.4	
	18th	193	0.34	95.8	
	24th	178	0.46	96.2	
	24th	168	0.49	95.2	
	24th	174	0.48	97.2	
	27th	166	-	-	Died.
	27th	160	0.44	97.8	
80	18th	193	0.48	95.2	
	24th	189	0.44	95.6	
	24th	176	0.31	96.4	
	24th	187	0.38	96.6	
	27th	185	0.49	96.4	
	27th	191	0.42	96.8	
0	18th	190	0.49	101.2	
	18th	175	0.43	100.4	
	24th	198	0.51	100.8	
	24th	160	0.42	101.4	
	27th	170	0.43	101.4	
	27th	168	0.47	100.8	

Table XIIb.

Summary of Table XIIa.

2-nitrophenol Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
120	0.51	96.6
105	0.45	95.9
90	0.42	96.3
80	0.42	96.2
0	0.46	101.0

Table XIIIa.

Carbon dioxide output and rectal temperature of rats after injection of 3-nitrophenol.

Dose (mg.)	Date 30th Nov.- 15th Dec. 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
50	30th	184	0.60	96.6	
	30th	176	-	96.4	Died.
	1st	160	-	100.4	Died.
	7th	179	-	97.8	Died.
	15th	198	-	97.6	Died.
45	30th	199	-	96.4	Died.
	30th	186	-	97.2	Died.
	1st	174	0.36	95.2	
	1st	181	0.50	95.2	
	7th	191	0.51	96.0	
	7th	184	0.53	96.4	
	15th	196	0.52	95.4	
	15th	172	0.37	95.8	
	15th	186	-	96.4	Died.
40	30th	195	0.46	96.0	
	30th	199	0.53	97.2	
	1st	187	0.31	96.6	
	1st	176	0.36	96.8	
	7th	181	0.46	96.0	
	7th	185	0.39	96.0	
	7th	171	0.51	96.0	Result redundant.
	15th	169	-	96.6	Died.

Table continued overleaf

Table XIIIa. (continued).

Dose (mg.)	Date 30th Nov.- 15th Dec. 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. (°F.)	Notes
35	30th	169	0.40	96.4	
	30th	187	0.37	96.0	
	30th	192	0.41	96.4	
	1st	179	0.41	96.2	
	1st	198	0.37	95.4	
	15th	174	0.36	95.4	
30	30th	179	0.54	97.4	
	1st	186	0.39	97.0	
	1st	193	0.41	96.2	
	7th	170	0.32	96.2	
	15th	183	0.43	96.2	
	15th	190	0.49	99.0	
25	1st	163	0.33	95.6	
	7th	178	0.44	95.8	
	7th	169	0.33	96.0	
	7th	180	0.22	95.8	Result discarded.
	15th	183	0.44	96.0	
	15th	165	0.33	95.8	
0	30th	171	0.37	100.4	
	30th	200	0.53	99.8	
	1st	186	0.40	100.2	
	1st	179	0.42	100.0	
	7th	190	0.51	100.0	
	7th	185	0.42	100.0	
	15th	193	0.50	99.0	
	15th	181	0.41	99.8	

Table XIIIb.

Summary of Table XIIIa.

3-nitrophenol Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
45	0.47	95.7
40	0.43	96.4
35	0.39	96.0
30	0.43	97.0
25	0.37	95.8
0	0.45	99.9

Table XIVa.

Carbon dioxide output and rectal temperatures of rats after injection of 4-nitrophenol.

Dose (mg.)	Date 27th June-18th July 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
50	27th	180	-	-	Died.
50	27th	188	-	-	Died.
40	28th	176	-	-	Died.
30	28th	160	-	-	Died.
20	28th	165	-	-	Died.
16	11th	191	-	--	Died.
15	11th	186	-	-	Died.
15	18th	200	-	-	Died.
14	11th	185	0.46	99.0	
	11th	178	0.51	98.4	
	11th	187	0.57	97.8	
	11th	194	0.51	98.2	
	18th	161	0.62	98.4	
	18th	166	-	98.6	Died.
	18th	165	0.51	99.2	
12	11th	186	0.39	99.8	
	11th	182	0.47	99.6	
	18th	164	0.51	99.8	
10	28th	190	0.39	100.0	
	28th	168	0.40	99.8	
	11th	165	0.47	100.2	
	11th	185	0.62	99.4	
	18th	179	0.55	100.4	
	18th	177	0.48	98.5	

Table continued overleaf

Table XIVa. (continued).

Dose (mg.)	Date 27th June-18th July 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
9	27th	165	0.33	99.8	
	27th	180	0.44	100.0	
	28th	166	0.42	99.4	
	18th	200	0.42	101.0	
	18th	165	0.43	100.2	
	18th	195	0.49	100.2	
7	27th	166	0.40	100.0	
	27th	178	0.40	99.6	
	27th	160	0.36	99.8	
	27th	185	0.42	101.0	
	28th	197	0.42	100.8	
	28th	190	0.39	99.0	
5	27th	200	0.44	100.2	
	27th	190	0.45	99.8	
	28th	164	0.38	100.4	
	28th	170	0.40	100.0	
0	27th	170	0.43	99.8	
	27th	190	0.45	99.0	
	28th	200	0.42	100.0	
	28th	174	0.38	100.4	
	11th	182	0.41	101.6	
	11th	175	0.35	100.0	
	18th	185	0.48	99.6	
	18th	195	0.42	100.2	

Table XIVb.

Summary of Table XIVa.

4-nitrophenol Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
14	0.53	98.5
12	0.46	99.7
10	0.49	99.7
9	0.42	100.1
7	0.40	100.0
5	0.42	100.1
0	0.42	100.1

Table XVa.

Carbon dioxide output and rectal temperature of rats after injection of DNOC.

DNOC (mg.)	Date June 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
3.0	3rd	200	0.67	107.0	
	3rd	175	0.65	100.2	
	3rd	174	0.69	105.0	
	3rd	200	0.57	103.0	
	6th	168	0.49	102.8	
	6th	194	-	103.8	Died.
	9th	166	0.61	107.0	
	9th	175	-	-	Died.
2.0	3rd	180	0.56	101.8	
	3rd	184	0.42	102.0	
	3rd	200	0.76	-	Distressed; result discarded.
	6th	184	0.53	104.0	
	6th	164	0.67	104.0	
	6th	180	0.63	103.4	
	9th	180	0.43	101.6	
1.5	3rd	198	0.50	100.0	
	3rd	200	0.39	100.8	
	6th	185	0.46	99.8	
	6th	167	0.45	101.0	
	9th	171	0.41	100.6	
	9th	180	0.42	101.2	
	9th	163	0.27	99.6	Result discarded.
1.0	3rd	185	0.46	102.0	
	6th	170	0.41	102.0	
	6th	175	0.42	102.2	
	6th	194	0.35	101.0	
	9th	195	0.33	101.0	
	9th	166	0.34	101.4	
0	3rd	187	0.36	100.0	
	3rd	180	0.40	100.2	
	6th	170	0.36	99.6	
	6th	178	0.38	100.0	
	9th	187	0.41	100.8	
	9th	195	0.35	98.4	
	9th	185	0.36	101.8	
	9th	170	0.34	99.6	

Table XVb.

Summary of Table XVa.

DNOC Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
3.0	0.61	104.2
2.0	0.54	102.8
1.5	0.44	100.6
1.0	0.39	101.6
0	0.37	100.1

Table XVIa.

Carbon dioxide output and rectal temperature of rats after injection of 2,4-dinitrophenol.

Dose (mg.)	Date June 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. (°F.)	Notes
3.0	16th	200	0.79	102.0	
	16th	200	-	100.2	Died*.
	21st	200	0.77	105.2	
	21st	198	0.59	103.0	
	21st	195	0.63	103.0	
	23rd	160	0.74	102.8	
	23rd	200	0.77	101.8	
	23rd	163	1.04	102.0	Rat very restless; result discarded.
2.0	16th	200	0.62	101.2	
	16th	200	0.57	102.0	
	21st	170	0.53	102.6	
	21st	176	0.70	101.8	
	21st	178	0.57	103.4	
	23rd	164	0.56	103.4	
	23rd	167	0.80	101.4	Rat very restless; result discarded.
1.5	16th	170	0.56	101.0	
	16th	171	0.53	101.6	
	16th	167	0.53	100.6	
	21st	165	0.56	101.2	
	21st	192	0.61	100.8	
	23rd	200	0.56	99.8	
	23rd	190	0.54	100.0	Result redundant.
1.0	16th	192	0.48	101.6	
	16th	185	0.53	101.0	
	16th	184	0.53	100.0	
	21st	200	0.51	101.6	
	21st	180	0.42	101.4	
	23rd	184	0.47	101.8	
0	16th	170	0.42	101.8	
	16th	172	0.43	98.4	
	21st	164	0.43	100.8	
	21st	160	0.45	100.0	
	23rd	187	0.51	101.0	
	23rd	178	0.44	99.8	
	23rd	199	0.45	101.6	
	23rd	163	0.48	100.6	

* Convulsions preceded death.

Table XVIb.

Summary of Table XVIa.

2,4-DNP Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^oF.$)
3.0	0.72	103.0
2.0	0.59	102.4
1.5	0.56	100.8
1.0	0.49	101.2
0	0.45	100.5

Table XVIIa.

Carbon dioxide output and rectal temperature of rats after injection of 2,3-dinitrophenol.

Dose (mg.)	Date 14th Sept.- 3rd Oct. 1956	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. (°F.)	Notes
300	14th	168	-	-	Died.
60	14th	160	-	100.4	Died.
48	27th	185	-	100.4	Died.
30	14th	175	-	102.8	Died.
30	14th	174	-	102.6	Died.
30	14th	166	-	101.4	Died.
27	14th	182	-	103.2	Died.
27	3rd	180	0.68	97.2	
27	3rd	184	-	103.2	Died.
	14th	170	-	100.6	Died.
	25th	176	0.57	98.4	
	25th	193	0.52	98.6	
	27th	164	0.53	96.2	
24	27th	162	0.66	98.6	
	27th	190	0.36	98.2	
	3rd	183	0.72	98.2	
	3rd	160	-	104.2	Died.
	25th	178	0.49	99.2	
	25th	170	0.46	98.8	
	27th	178	0.52	99.8	
21	27th	173	0.40	98.0	
	27th	178	0.46	97.6	
	3rd	184	0.48	99.2	

Table continued overleaf

Table XVIIa. (continued).

Dose (mg.)	Date 14th Sept.- 3rd Oct. 1956	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
18	14th	182	0.46	97.8	
	25th	182	0.46	98.2	
	25th	169	0.45	98.0	
	25th	165	0.45	100.6	
	27th	173	0.44	98.2	
	3rd	185	0.49	98.4	
15	14th	184	0.40	99.0	
	27th	179	0.40	98.0	
	27th	192	0.50	100.2	
	3rd	176	0.44	98.8	
	3rd	192	0.51	97.6	
	3rd	170	0.43	98.8	
12	14th	160	0.36	98.6	
	25th	164	0.41	100.2	
	25th	178	0.52	99.8	
	25th	174	0.50	100.2	
0	14th	188	0.40	98.6	
	14th	187	0.38	98.6	
	25th	160	0.47	100.0	
	25th	162	0.40	99.6	
	27th	171	0.43	97.2	
	27th	162	0.37	97.8	
	3rd	160	0.46	100.8	
	3rd	185	0.46	101.2	
	3rd	172	0.46	99.8	

Table XVIIb.

Summary of Table XVIIa.

2,3-DNP Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
24	0.56	98.0
21	0.47	98.8
18	0.46	98.5
15	0.45	98.7
12	0.45	99.7
0	0.43	99.3

Table XVIIIa.

Carbon dioxide output and rectal temperature of rats after injection of 2,3-dinitrophenol.

Dose (mg.)	Date Feb. 1957	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. (°F.)	Notes
24	22nd	190	0.52	98.4	
	22nd	174	0.63	97.4	
	25th	175	0.51	96.4	
	25th	173	0.62	98.2	
	25th	171	0.63	98.4	
	26th	171	0.66	99.0	
	26th	175	0.54	97.8	Result redundant.
21	22nd	194	0.55	99.0	
	22nd	180	0.50	98.4	
	22nd	165	0.50	96.0	
	25th	198	0.60	99.2	
	25th	170	0.45	96.0	
	26th	185	0.47	99.8	
	26th	178	0.37	98.2	Result redundant.
18	22nd	175	0.50	98.8	
	22nd	167	0.63	98.0	
	22nd	161	-	99.0	Leak present.
	25th	194	0.61	99.8	
	25th	164	0.45	100.6	
	25th	178	0.58	98.2	
	26th	182	0.54	100.0	
	26th	166	0.49	98.4	Result redundant.
15	22nd	180	0.58	100.2	
	22nd	173	0.41	98.8	
	25th	166	0.32	95.0	
	25th	167	0.52	98.0	
	26th	166	0.52	100.2	
	26th	165	0.56	97.6	
	26th	163	0.38	97.8	Result redundant.
0	22nd	174	0.49	100.0	
	22nd	173	0.40	101.4	
	25th	168	0.26	95.4	Result discarded.
	25th	164	0.32	100.0	
	26th	178	0.40	98.2	
	26th	166	0.40	101.4	
	26th	174	0.47	100.2	

Table XVIIIb.

Summary of Table XVIIIa.

2,3-DNP Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
24	0.59	98.0
21	0.49	98.1
18	0.54	99.2
15	0.47	98.3
0	0.41	100.2

Table IXXa.

Carbon dioxide output and rectal temperature of rats after injection of 2,5-dinitrophenol.

Dose (mg.)	Date Nov. 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. (°F.)	Notes
50	13th	179	-	97.0	Died.
40	13th	188	-	96.0	Died.
30	13th	191	0.32	96.4	Rat ill.
30	14th	187	-	97.0	Died.
25	11th	176	0.32	95.2	
	11th	189	0.48	95.8	
	11th	190	0.44	95.0	
	13th	171	0.24	96.0	Result discarded.
	14th	184	0.36	95.6	
	14th	192	0.43	96.8	
	14th	192	0.46	95.4	
20	11th	174	0.30	98.2	
	13th	184	0.52	96.6	
	13th	181	0.54	97.0	
	14th	176	0.38	98.4	
	14th	169	-	-	Died.
	14th	173	0.33	95.0	
	14th	180	0.44	95.5	
15	11th	187	0.49	95.0	
	11th	169	0.38	101.2	
	13th	198	0.54	98.6	
	13th	179	0.52	98.4	
	14th	181	0.37	99.0	
	14th	190	0.47	98.8	
10	11th	169	0.33	98.0	
	11th	187	0.49	97.4	
	11th	192	0.44	98.4	
	11th	189	0.41	98.2	
	13th	189	0.39	97.4	
	13th	176	0.45	98.2	
0	11th	184	0.43	100.4	
	11th	191	0.42	98.8	
	13th	183	0.40	100.2	
	13th	168	0.38	99.8	
	14th	179	0.45	101.2	
	14th	194	0.47	100.8	

Table IXXb.

Summary of Table IXXa.

2,5-DNP Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
25	0.42	95.6
20	0.42	96.8
15	0.46	98.5
10	0.42	97.9
0	0.43	100.2

Table XXa.

Carbon dioxide output and rectal temperature of rats after injection of 2,6-dinitrophenol.

Dose (mg.)	Date Nov. 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
30	21st	197	-	98.8	Died.
20	21st	189	-	98.0	Died.
15	21st	173	-	98.0	Died.
10	21st	181	-	97.8	Died.
7	21st	193	-	99.0	Died.
7	22nd	189	-	99.2	Died.
	21st	191	0.50	95.8	
	21st	187	0.46	95.8	
6.5	22nd	198	0.57	98.0	
	23rd	199	0.55	97.4	
	23rd	178	0.53	97.2	
	23rd	169	0.59	96.8	
	21st	183	0.46	96.4	
	22nd	187	0.40	97.6	
5	22nd	193	0.64	98.2	
	23rd	199	0.60	98.8	
	23rd	189	0.62	100.2	
	23rd	191	0.48	99.2	
	21st	173	0.44	99.6	
	22nd	199	0.63	98.8	
3	22nd	186	0.43	98.6	
	22nd	176	0.43	99.0	
	23rd	194	0.54	100.8	
	23rd	191	0.42	99.8	
	21st	190	0.47	99.6	
	22nd	193	0.57	100.6	
1	22nd	189	0.54	99.4	
	22nd	197	0.52	101.6	
	23rd	178	0.49	100.0	
	23rd	180	0.44	99.8	
	21st	183	0.49	100.6	
	21st	187	0.49	100.0	
	22nd	192	0.45	102.0	
0	22nd	187	0.43	101.2	
	23rd	186	0.47	99.8	
	23rd	173	0.34	100.2	

Table XXb.

Summary of Table XXa.

2,6-DNP Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
6.5	0.53	96.8
5.0	0.53	98.4
3.0	0.48	99.4
1.0	0.51	100.2
0	0.45	100.6

Table XXIa.

Carbon dioxide output and rectal temperature of rats after injection of 3,4-dinitrophenol.

Dose (mg.)	Date May 1956	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
200	24th	185	-	106.0	Died*.
130	24th	170	-	100.0	Died*.
40	24th	185	-	102.0	Died*.
20	24th	170	-	100.6	Died*.
16	25th	175	-	101.8	Died*.
16	28th	184	-	-	Died.
15	28th	175	-	103.6	Died.
14	25th	160	-	101.2	Died.
14	25th	190	0.48	100.0	Died.
14	28th	163	-	103.6	Died.
14	28th	189	0.64	100.8	
14	29th	180	-	102.2	Died.
14	29th	190	-	108.0	Died.
14	29th	184	-	-	Died.
14	29th	197	-	103.2	Died.
	25th	174	0.60	100.4	
	28th	160	-	102.4	Died.
	29th	191	0.60	99.8	
13	29th	184	0.25	-	Result discarded.
	29th	185	0.72	99.5	
	31st	175	0.46	99.6	
	31st	174	0.50	99.9	
	31st	190	0.70	98.4	
	25th	184	0.62	99.6	
	25th	186	0.59	97.0	
12	25th	164	0.47	99.0	
	28th	197	0.60	100.4	
	31st	178	0.49	99.0	
	31st	183	0.64	99.4	

Table continued overleaf

Table XXIa.(continued).

Dose (mg.)	Date May 1956	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
	25th	180	0.51	100.8	
	28th	174	0.39	100.4	
	28th	173	-	100.4	Died.
11	29th	167	0.49	100.0	
	29th	170	0.50	100.2	
	31st	168	0.52	97.0	
	31st	198	0.53	97.0	
	31st	195	0.51	100.2	Result redundant.
	24th	193	0.47	98.0	
	25th	160	0.43	99.2	
	25th	160	-	101.4	Died.
10	28th	168	0.47	99.6	
	28th	175	0.38	100.0	
	29th	166	0.49	99.2	
	31st	197	0.54	100.4	
	31st	181	0.54	99.6	Result redundant.
9	24th	168	0.47	100.0	
8	24th	165	0.42	99.8	
8	24th	184	0.45	99.4	
6	24th	190	0.51	101.0	
6	24th	192	0.44	100.0	
4	24th	167	0.41	101.0	
	24th	165	0.35	101.0	
	25th	184	0.35	99.8	
	25th	160	0.48	100.8	
	28th	160	0.44	99.6	
0	28th	170	0.42	98.4	
	29th	198	0.53	101.8	
	29th	188	0.45	101.0	
	31st	163	0.38	100.8	
	31st	171	0.48	101.2	

* Convulsions preceded death.

Table XXIb.

Summary of Table XXIa.

3,4-DNP Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
13	0.60	99.6
12	0.57	99.1
11	0.49	99.2
10	0.47	99.4
9	0.47	100.0
8	0.44	99.6
6	0.48	100.5
4	0.41	101.0
0	0.43	100.5

Table XXIIa.

Carbon dioxide output and rectal temperature of rats after injection of 3,5-dinitrophenol.

Dose (mg.)	Date March 1956	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. (°F.)	Notes
100	9th	184	-	100.0	Died*.
80	9th	166	-	100.0	Died*.
20	9th	164	-	100.8	Died*.
8	14th	163	-	100.4	Died.
8	14th	175	-	102.4	Died.
	9th	185	0.45	98.4	
	12th	188	0.54	101.0	
	12th	180	0.58	101.4	
7	12th	170	-	103.0	Died.
	14th	172	0.44	100.2	
	14th	186	0.43	99.2	
	14th	197	0.66	100.4	
	9th	178	0.45	99.4	
	9th	195	0.58	99.4	
6	12th	165	0.42	98.6	
	12th	190	0.53	99.2	
	14th	190	0.54	99.4	
	14th	162	0.42	101.4	
	9th	174	0.39	99.2	
	9th	165	0.40	99.4	
4	12th	164	0.51	100.2	
	12th	180	0.46	98.4	
	14th	176	0.52	101.8	
	14th	189	0.50	100.4	
	9th	168	0.48	99.4	
	9th	160	0.40	99.2	
2	12th	170	0.53	100.0	
	12th	190	0.64	100.0	
	12th	168	0.51	100.0	
	14th	165	0.49	99.8	
	9th	175	0.48	100.4	
	9th	175	0.40	100.0	
0	12th	190	0.40	99.4	
	12th	160	0.50	100.8	
	14th	166	0.47	101.4	
	14th	169	0.53	100.8	

* Convulsions preceded death.

Table XXIIb.

Summary of Table XXIIa.

3,5-DNP Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
7	0.52	100.1
6	0.49	99.6
4	0.46	99.9
2	0.51	99.7
0	0.46	100.5

Table XXIIIa.

Carbon dioxide output and rectal temperatures of rats after injection of 2-nitro-4-aminophenol.

Dose (mg.)	Date 18th Oct.- 5th Nov. 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. ($^{o}F.$)	Notes
50	18th	184	0.38	100.0	
	18th	197	0.37	96.2	
	18th	200	0.35	96.0	
	21st	193	0.32	97.2	
	5th	197	0.33	96.2	
	5th	198	0.38	97.0	
45	18th	189	0.34	95.6	
	21st	160	0.30	96.8	
	5th	200	0.33	96.4	
40	18th	197	0.35	97.2	
	18th	185	0.30	96.8	
	21st	163	0.23	97.4	
	21st	188	0.32	96.0	
	5th	197	0.29	96.8	
	5th	189	0.31	94.8	
30	18th	190	0.29	97.6	
	18th	175	0.27	97.0	
	21st	198	0.32	96.0	
	21st	198	0.36	96.8	
	21st	184	0.28	95.4	
	5th	191	0.31	95.0	
20	21st	160	0.30	98.4	
	5th	200	0.37	97.2	
	5th	190	0.27	96.6	
10	18th	193	0.37	99.1	
	18th	188	0.29	99.2	
	21st	164	0.26	99.2	
	21st	170	0.27	98.4	
	5th	196	0.37	100.2	
	5th	200	0.39	99.0	
0	18th	189	0.44	99.2	
	18th	164	0.45	101.0	
	21st	172	0.48	100.2	
	21st	200	0.44	100.4	
	5th	186	0.44	100.6	
	5th	160	0.33	100.4	

Table XXIIIb.

Summary of Table XXIIIa.

2-nitro-4-aminophenol Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^oF.$)
50	0.36	97.1
45	0.32	96.3
40	0.30	96.5
30	0.31	96.3
20	0.31	97.4
10	0.33	99.2
0	0.43	100.3

Table XXIVa.

Carbon dioxide output and rectal temperature of rats after injection of 2-amino-4-nitrophenol.

Dose (mg.)	Date Oct. 1955	Rat Weight (g.)	CO_2 output (g./h.)	Rectal Temp. (°F.)	Notes
90	11th	200	-	-	Died.
80	11th	179	-	-	Died.
80	11th	192	-	-	Died.
70	11th	173	-	-	Died.
70	11th	185	-	-	Died.
60	7th	186	0.57	96.2	
	7th	179	0.45	95.4	
	11th	182	0.59	95.2	
	11th	163	-	-	Died.
	13th	173	0.60	100.0	
	13th	180	0.53	96.0	
	13th	190	0.55	97.2	
50	7th	191	0.43	95.8	
	7th	171	0.49	96.2	
	11th	187	0.42	96.4	
	11th	190	0.50	98.6	
	13th	190	0.43	95.4	
	13th	188	0.46	95.8	
45	7th	166	0.44	98.0	
	7th	160	0.41	97.4	
	7th	199	0.43	95.8	
	11th	188	0.55	97.0	
	13th	191	0.41	96.6	
	13th	183	0.42	97.2	
40	7th	184	0.38	97.0	
	7th	173	0.42	97.6	
	7th	190	0.38	98.2	
	13th	192	0.33	97.8	
	13th	160	0.34	98.0	
	13th	188	0.33	97.4	
0	7th	164	0.36	100.2	
	7th	178	0.40	99.6	
	11th	192	0.42	100.2	
	11th	162	0.47	99.8	
	13th	173	0.39	100.8	
	13th	183	0.45	99.8	

Table XXIVb.

Summary of Table XXIVa.

2-amino-4-nitrophenol Dose (mg.)	Mean CO_2 output (g./h.)	Mean Rectal Temp. ($^{o}F.$)
60	0.55	96.7
50	0.46	96.4
45	0.44	97.0
40	0.36	97.7
0	0.42	100.1

Table XXV.

The Effect of 100 mg. salicylic acid on rate of oxygen consumption of rats.

Trial No.	Date Nov. 1956.	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2 = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	1st	F	184	372	188	+184
2	1st	M	198	489	349	+140
3	1st	F	191	372	417	-45
4	2nd	M	160	374	361	+13
5	2nd	F	190	415	364	+51
6	3rd	M	160	389	349	+40
7	5th	F	163	318	257	+61
8	5th	M	190	420	351	+69
9	7th	F	161	387	257	+130
10	7th	M	173	445	316	+129
Mean				398	321	+77

Inference : H_1 accepted ($z = 5.850$)

Table XXVI.

The Effect of 105 mg. 2-nitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Dec. 1956	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2 = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	3rd	M	206	361	463	-102
2	3rd	F	166	316	285	+31
3	4th	M	188	361	316	+45
4	5th	F	183	361	344	+17
5	5th	M	193	333	303	+30
6	7th	F	180	285	326	-41
7	7th	M	193	346	346	0
Mean				338	340	-3

Inference : H_o accepted ($z = 0.025$).

Table XXVII.

The Effect of 40 mg. 3-nitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Dec. 1956	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2 = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	12th	M	185	303	415	-112
2	12th	F	157	305	285	+20
3	14th	M	176	247	405	-158
4	14th	F	165	318	308	+10
5	15th	M	193	349	369	-20
6	17th	F	170	280	328	-48
7	17th	M	193	333	361	-28
8	18th	F	172	252	333	-81
9	19th	M	210	331	512	-181
10	19th	F	159	280	389	-109
Mean				300	371	-71

Inference: H_1 accepted ($z = 5.392$).

Table XXVIII.

The Effect of 12 mg. 4-nitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Oct. 1956	Sex	Weight (g.)	ml. O_2 consumed/hour		$\triangle O_2$ = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	17th	F	155	290	260	+30
2	19th	M	157	293	232	+61
3	19th	F	194	366	425	-59
4	20th	M	184	318	326	-8
5	22nd	F	194	344	361	-17
6	23rd	M	160	280	275	+5
Mean				315	313	+2

Inference : H_o accepted ($z = 0.017$).

Table IXXX.

The Effect of 3 mg. DNOC on rate of oxygen consumption of rats.

Trial No.	Date Nov. 1956	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2 = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	8th	F	169	428	280	+148
2	8th	M	165	677	285	+392
3	9th	F	157	687	280	+407
4	9th	M	180	555	351	+204
5	12th	F	164	499	275	+224
6	12th	M	202	522	382	+140
Mean				561	309	+253

Inference : H_1 accepted ($z = 5.071$).

Table XXX.

The Effect of 3 mg. 2,4-dinitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Oct. 1956	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2= (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	24th	F	186	491	389	+102
2	24th	M	182	333	344	-11
3	25th	F	189	481	354	+127
4	25th	M	157	433	280	+153
5	26th	F	181	428	379	+49
6	27th	M	189	445	344	+101
7	27th	F	190	471	346	+125
Mean				440	348	+92

Inference : H_1 accepted ($z = 5.330$).

Table XXXI.

The Effect of 24 mg. 2,3-dinitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Oct. 1956	Sex	Weight (g.)	ml. O_2 consumed/hour		$\triangle O_2$ = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	10th	F	190	379	295	+84
2	11th	M	192	277	249	+28
3	11th	F	200	326	356	-30
4	11th	M	160	331	305	+26
5	12th	F	188	333	397	-64
6	15th	M	167	321	277	+44
7	16th	F	165	293	354	-61
Mean				323	319	+4

Inference : H_o accepted ($z = 0.038$).

Table XXXII.

The Effect of 20 mg. 2,5-dinitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Dec. 1956	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2 = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	21st	F	160	257	237	+20
2	21st	M	209	407	387	+20
3	22nd	F	176	351	333	+18
4	24th	M	205	336	478	-142
5	24th	F	170	280	318	-38
6	26th	M	194	361	349	+12
7	26th	F	167	191	382	-191
8	27th	M	168	305	344	-39
9	27th	F	180	422	338	+84
10	29th	M	192	351	333	+18
11	29th	F	182	282	364	-82
	Jan. 1957					
12	4th	M	175	260	430	-170
13	4th	F	162	267	364	-97
14	5th	M	177	310	356	-46
15	5th	F	172	173	397	-224
16	7th	M	175	277	344	-67
17	7th	F	164	328	336	-8
18	7th	M	162	262	331	-69
19	8th	F	164	338	346	-8
20	8th	M	160	293	382	-89
21	9th	F	176	247	356	-109
Mean				300	357	-58

Inference: H_1 accepted ($z = 7.480$).

Table XXXIII.

The Effect of 6 mg. 2,6-dinitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Jan. 1957	Sex	Weight (g.)	ml. O_2 consumed/hour		$\triangle O_2$ = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	10th	M	191	379	504	-125
2	10th	F	166	377	300	+77
3	14th	M	184	364	417	-53
4	14th	F	161	280	285	-5
5	15th	M	190	310	438	-128
6	15th	F	162	328	387	-59
7	16th	M	180	354	344	+10
8	16th	F	173	372	277	+95
9	18th	M	197	321	359	-38
10	21st	F	187	282	420	-138
11	21st	M	186	394	361	+33
12	22nd	M	179	450	323	+127
Mean				351	368	-17

Inference : H_o accepted ($z = 0.457$).

Table XXXIV.

The Effect of 13 mg. 3,4-dinitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Nov. 1956	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2 = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	13th	F	162	323	346	-23
2	14th	M	204	389	374	+15
3	14th	F	177	323	316	+7
4	14th	M	185	333	417	-84
5	16th	F	166	313	338	-25
6	16th	M	192	326	379	-53
7	17th	F	194	389	392	-3
8	19th	M	182	364	328	+36
9	21st	F	164	394	318	+76
Mean				350	356	-6

Inference: H_o accepted ($z = 0.159$).

Table XXXV.

The Effect of 6 mg. 3,5-dinitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Nov. 1956	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2 = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	23rd	M	195	387	389	-2
2	24th	F	167	333	372	-39
3	24th	M	188	405	440	-35
4	25th	F	174	359	359	0
5	25th	M	208	384	387	-3
6	26th	F	171	366	338	+28
7	26th	M	162	349	338	+11
8	28th	F	183	336	349	-13
9	28th	M	191	351	374	-23
10	29th	F	190	392	364	+28
Mean				366	371	-5

Inference : H_o accepted ($z = 0.448$).

Table XXXVI.

The Effect of 50 mg. 2-nitro-4-aminophenol on rate of oxygen consumption of rats.

Trial No.	Date Feb. 1957	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2 = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	12th	M	174	227	272	-45
2	13th	F	183	234	313	-79
3	13th	M	205	257	298	-41
4	14th	F	179	239	369	-130
5	15th	M	194	199	257	-58
6	15th	F	162	199	321	-122
Mean				226	305	-79

Inference : H_1 accepted ($z = 5.003$).

Table XXXVII.

The Effect of 50 mg. 2-amino-4-nitrophenol on rate of oxygen consumption of rats.

Trial No.	Date Feb. 1957	Sex	Weight (g.)	ml. O_2 consumed/hour		ΔO_2 = (1) - (2)
				(1) Treated Rat	(2) Control Rat	
1	2nd	M	173	298	407	-109
2	2nd	F	177	288	252	+36
3	3rd	M	168	280	318	-38
4	3rd	F	163	234	323	-89
5	5th	M	175	280	282	-2
6	6th	F	173	219	341	-122
7	6th	M	188	272	359	-87
8	7th	F	206	310	379	-69
9	8th	M	176	272	280	-8
10	8th	F	205	298	300	-2
11	9th	M	175	239	326	-87
Mean				272	324	-53

Inference : H_1 accepted ($z = 5.800$).

Bibliography.

1. Gibbs, W. & Reichert, E.T. Amer. Chem. J. 16, 443, 1894.

2. Magne, H. Mayer, A. & Plantefol, L. Ann. physiol. physicochim. biol., 8, 1, 1932.

3. Cutting, W.C., Mehrtens, H.G. & Tainter, M.L. J. Amer. Med. Assoc., 101, 193, 1933.

4. Dodds, E.C. Lancet, (i), 1048, 1934.

5. Tainter, M.L., Stockton, A.B. & Cutting, W.C. J. Amer. Med. Assoc., 101, 1472, 1933.

6. Current Comment. J. Amer. Med. Assoc., 101, 1080, 1933.

7. Tattersfield, T., Gimingham, C.T. & Morris, H.M. Ann. Appl. Biol., 12, 218, 1925.

8. Norman, A.G., Minarik, C.E. & Weintraub, R.L. Ann. Rev. Pl., Physiol., 1, 141, 1950.

9. Blackman, G.E., Templeman, W.G. & Halliday, D.J. Ann. Rev. Pl. Physiol., 2, 199, 1951.

10. Simon, E.W. Biol. Reviews, 28, 453, 1953.

11. Loomis, W.F. & Lipmann, F. J. Biol. Chem. 173, 807, 1948.

12. Dodds, E.C. & Pope, W.J. Lancet, (ii), 352, 1933.

13. Heymans, C. & Casier, H. Physiol. Abstr., 19, 275, 1934.

14. Heymans, C. J. Pharmacol., 51, 144, 1934.

15. Heymans, C. & Casier, H. Arch. internat. pharmacodyn. et. therap., 50, 20, 1935.

16. Magne, H. Mayer, A. & Plantefol, L. Ann. physiol. physicochim. biol., 7, 269, 1931.

17. Magne, H., Mayer, A. & Plantefol, L. Ann. physiol. physicochim. biol., 8, 51, 1932.

18. Magne, H. Mayer, A. & Plantefol, L. Ann. physiol. physicochim. biol., 8, 157, 1932.

19. Hall, V.E., Field, J. (2nd), Sahyun, M., Cutting,W.C. & Tainter, M.L. Amer. J. Physiol., 106, 432, 1933.

20. Tainter, M.L. & Cutting, W.C. J. Pharmacol., 49, 187, 1933.

21. Tainter, M.L., Bergstrom, F.W. & Cutting, W.C. J. Pharmacol., 53, 58, 1935.

22. Field, J. (2nd), Martin, A.W. & Field, S.M. J. Cell. Comp. Physiol., 4, 405, 1934.

23. Field, J. (2nd) Proc. Soc.Exp. Biol., 32, 1342, 1935.

24. Field, J., Martin, A.W. & Field, S.M. Proc. Soc.Exp. Biol., 31, 56, 1933-34.

25. Field, J., Martin, A.W. & Field, S.M. Proc. Soc.Exp. Biol., 31, 996, 1933-34.

26. Field, J. Martin, A.W. & Field, S.M. Proc. Soc.Exp. Biol., 32, 1043, 1935.

27. Field, J. Martin, A.W. & Field, S.M. J.Pharmacol. 53, 314, 1935.

28. Martin, A.W. & Field, J. Proc. Soc. Exp. Biol., 32, 54, 1934.

29. Martin, A.W. & Field, J. Proc. Soc.Exp. Biol., 36, 375, 1937.

30. Genevois, L. & Saric, R. Compt. rend. soc. de biol., 111, 181,1932.

31. Genevois, L. & Creac'h, P. Compt. rend.soc. de biol., 118, 1357,1935.

32. De Meio, R.H. & Barron, E.S.G. Proc.Soc.Exp.Biol., 32, 36, 1935.

33. Simon, E.W. J. Exp. Botany, 4, 377, 1953.

34. Clowes, G.H.A., Krahl, M.E. & Keltch, A.K. Biol. Bull., 69, 341, 1935.

35. Clowes, G.H.A. & Krahl, M.E. J. Gen. Physiol., 20, 145, 1936.

36. Keltch, A.K., Clowes, G.H.A. & Krahl, M.E. Biol. Bull., 71, 399, 1936.

37. Clowes, G.H.A., Krahl, M.E. & Keltch, A.K., Biol. Bull., 73, 376, 1937.

38. Krahl, M.E. & Clowes, G.H.A. J. Cell. Comp. Physiol., 11, 1, 1938.

39. Krahl, M.E. & Clowes, G.H.A. J. Cell. Comp. Physiol., 11, 21, 1938.

40. Krahl, M.E. Biol. Bull., 98, 175, 1950.

41. Tyler, A. & Horowitz, N.H. Biol. Bull., 73, 377, 1937.

42. Tyler, A. & Horowitz, N.H. Proc. Nat. Acad. Sc., 23, 369, 1937.

43. Shoup, C.S. & Kimler, A. J. Cell. Comp. Physiol., 5, 269, 1934-5.

44. Krahl, M.E. & Clowes, G.H.A. J. Biol. Chem., 111, 355, 1935.

45. Mudge, G.H. Amer. J. Physiol., 167, 206, 1951.

46. Peiss, C.N. & Field, J. J. Biol. Chem. 175, 49, 1948.

47. Peiss, C.N. & Field, J. Arch. Biochem., 36, 276, 1952.

48. Plantefol, L. Ann. physiol. physicochim. biol., 8, 127, 1932.

49. Terner, C. Biochem. J., 50, 145, 1951.

50. Clowes, G.H.A., Keltch, A.K., Strittmatter, C.F. & Walters, C.P. J. Gen. Physiol., 33,555, 1949-50.

51. Cross, R.J., Taggart, J.V., Covo, G.A. & Green, D.E. J. Biol. Chem., 177, 655, 1949.

52. Bodine, J.H. & Boell, E.J. J. Cell. Comp. Physiol., 11, 41, 1938.

53. Hill, A.V. & Hill, A.M. J. Physiol., 46, 81, 1913.

54. Krogh, A. Monographs on Biochemistry, 1916.

55. Benedict, F.G. Publ. Carneg. Inst., 503, 67, 1938.

56. Benedict, F.G. & Homans, J. Amer. J. Physiol., 28, 29, 1911.

57. Smith, A.U., Emmens, C.W. & Parkes, A.S. J. Endocrinol., 5, 186, 1947.

58. Zuntz, N., Hermann's Handbuch der physiologie 4, part 2, 86.

59. Abderhalden's Handbuch d. biol. Arbeitsmethoden.

60. Atwater, W.O. & Benedict, F.G. Publ. Carneg. Inst., 42, 1905.

61. Benedict, F.G. & MacLeod, G. J. Nutrit., 1, 343, 1928-29.

62. Brody, S. Bioenergetics & Growth, 1945.

63. Paechtner, J. Handbuch der Ernährung und des Stoffwichsels der Landwirtschaftlichen Nutztiere, 1931.

64. Haldane, J. J. Physiol., 13, 422, 1892.

65. Mattill, H.A. J. Biol. Chem., 55, 717, 1923.

66. Wesson, L.G. J. Nutrit., 3, 503, 1931.

67. Krogh, A. Biochem. Zeitschr., 62, 266, 1914.

68. Dewart, A.D. & Newton, W.H. Brit. J. Nutrit., 2, 123, 1948.

69. Goto, K. Biochem. Zeitschr., 135, 107, 1923.

70. Trinder, P. Biochem. J., 57, 301, 1954.

71. Benedict, F.G. J. Nutrit., 3, 161, 1930-31.

72. Bargeton, D. & Krumm-Heller, C. J. Physiologie, 41, 119, 1949.

73. Arvay, A. P flüg. Arch. 214, 421, 1926.

74. Holleman, A.F. Rec. Trav. chim., 21, 432, 1902.

75. Reverdin, F. Org. Synth., col. vol. I, 219.

76. Sidgwick, N.V. & Taylor, T.W.J. J.C.S., 121. 1853, 1922.

77. Hartman, W.W. & Silloway, H.L. Org. Synth., 25, 5, 1945.

78. Girard, A. Bull. Soc. chim., 35, 772, 1924.

79. Holleman, A.F. Rec. Trav. chim., 21, 434, 1902.

80. Rodd, E.H. Chemistry of Carbon Compounds, 3, 758, 1956.

81. Bantlin, A. Ber., 11, 2103, 1878.

82. Fisher, R.A. Statistical Methods for Research Workers. Oliver & Boyd, 1946.

83. Wald, A. Sequential Analysis. New York, Wiley, 1947.

84. Appl. Math. Ser. U.S. Bur. Stand., No. 7, 1951.

85. Dixon, M. Manometric Methods. Third Edition, Cambridge University Press, 1951.

86. Krahl, M.E. & Clowes, G.H.A. J. Gen. Physiol., 20, 173, 1936-37.

87. Mørch, J.R. J. Physiol., 67, 224, 1929.

88. Forbes, E.B., Kriss, M. & Miller, R.C. J. Nutrit., 8, 535, 1934.

89. Guerbet, M. & Mayer, A. Ann. physiol. physicochim. biol., 8, 136, 1932.