"The Biological Activity of Salicylate and Related Compounds".

by

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Thesis submitted for the degree of Doctor of Philosophy in the Faculty of Science of the University of Glasgow.

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"The Biological Activity of Salicylate and Related Compounds".

Summary. Ph.D. Thesis.

M.M. Andrews. July, 1958.

It is well established that sodium salicylate in moderate dosage, increases the metabolic rate of experimental animals and man. The effect on this property of alterations in the chemical structure of salicylate was the subject of the present investigation.

The first part of the present work was to determine which compounds within a series of eighteen substituted benzoates were active as metabolic stimulants in the intact rat. These eighteen compounds included the complete series of both the mono and dihydroxybenzoates and the cresotinates.

Wistar albino rats were used throughout the investigation and the individual rates of oxygen consumption were measured in a closed circuit manometric apparatus.

In these experiments the results were

expressed in terms of the difference in rates of oxygen consumption between paired, treated and control rats and the mean difference in rate of oxygen consumption was estimated for each compound.

The treated rats were given, by intraperitone injection, the sodium salts of the test compounds in the maximum practical doses tolerated. The control animals were given a corresponding volume of normal saline.

2:3-dihydroxybenzoic acid, phthalic acid and 6-methylsalicylic acid were, at the doses used, inactive. Meta- and parahydroxybenzoic acid, 2:4-, 2:5-, 2:6-, 3:4- and 3:5-dihydroxybenzoic acid, o-aminobenzoic acid, salicyluric acid, salicylami and 5-aminosalicylic acid decreased the metabolic rate. Only the three cresotinic acids, i.e. 3, 4, and 5-methylsalicylic acid possessed the metabolic stimulant property of salicylate.

The relative efficacy of the three cresotina and salicylate as metabolic stimulants was determined by comparison of their respective dose-response curves, and molar potency ratios of the cresotinates relative to salicylate were calculated. Ortho-

cresotinate was the most powerful with a ratio of 2.61, meta- and para-cresotinate were of the same order with values of 1.78 and 1.89 respectively.

Two possible explanations of the higher potencies of the cresotinates were considered. No difference in the primary action of the drugs was established by determining the effect on rate of oxygen consumption of a mixture of ortho-cresotinate and salicylate.

The other possibility considered was that the rates of detoxication and excretion of the cresotinates differed among themselves and from salicylate. No differences in rates of disappearance of the drugs from the blood were found. This finding implies that the relative potencies of the cresotinates and salicylate as metabolic stimulants in the intact rat are a reflection of true potency differences at the tissue level.

The reports of previous workers have been presented and the significance of the present results discussed.

Acknowledgments.

I would like to express my appreciation to Dr. D.H. Sproull of the Clinical Chemotherapeutic Research Unit of the Medical Research Council for constant advice and help throughout, and to Dr. J. Reid and Professor J.M. Robertson for undertaking the supervision of this work.

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The advice on Statistical Methods given by Dr. S.D. Silvey of Glasgow University Mathematics Department is also gratefully acknowledged.

Contents.

Introduction.

The salicylates, which were introduced as plant extracts centuries ago, were among the first drugs to be synthesised for use therapeutically (Kolbe, 1874). In the late 19th century, Buss (1875) and Stricker (1876) reported on their use as anti-pyretic and analgesic agents, and in the same year MacLagan (1876) published his observations on the use of salicin and salicylate in the treatment of rheumatic fever. Salicylic acid in the form of its sodium salt or its acetyl derivative is now recognised as one of the best remedies for rheumatic diseases (M.R.C. Joint Trials, 1955 and 1957).

Among the numerous pharmacological properties of salicylate studied in the last fifty years its action as a metabolic stimulant is outstanding. This effect has been studied on man, dogs, cats, rats, rabbits and mice; in vitro, the following preparations are sensitive to this action

of salicylate:- rat liver, kidney and brain slices, mouse liver slices, rat diaphragm, tubercle bacilli and rat tissue homogenates and mitochondria.

The action on metabolic rate in vivo and in vitro of some compounds chemically related to salicylate has also received some attention.

The first clinical report of salicylate as a metabolic stimulant was that of Denis and Means (1916), who studied the influence of therapeutic doses of sodium salicylate (up to 6.6 grams per day) on the basal metabolic rate of adult men. Using a Benedict's Universal Respiration Apparatus they found an increase of 15% in the basal metabolic rate of only one of the three subjects; they did not comment on this finding.

Barbour (1919) and Barbour and Devenis (1919) reported that 1 gm. of acetylsalicylic acid produced in most normal subjects a definite increase, of 9%, in carbon dioxide excretion, and an overall increase in heat production; in febrile patients, the dissipation of heat increased by about 38%, with a slight decrease (of 3.5%) in the production of heat. This last finding was not confirmed by Dodd, Minot and Arena

(1937), who studied one subject and found that the effect of aspirin was an increase in metabolic rate during fever as well as when the subject was afebrile.

In 1935, Sylla observed that the metabolic rate of a woman suffering from salicylate poisoning was increased, which he considered a result of the muscular exertion of the deep and rapid respiration shown by his patient.

Rossier and Buhlman (1950), studying the acid-base equilibrium of the blood and respiratory function following the administration of sodium salicylate and salicylamide, found that sodium salicylate in the usual doses of 8-10 gms. per day led to "definite hyperactivity of the respiratory function" and a rise in the basal metabolic rate. They presented tables showing an increase of approximately 30% in the basal metabolic rate of normal individuals treated with sodium salicylate, but attached little significance to, and made no comment on these results. Salicylamide in comparable doses did not produce a change in the basal metabolic rate.

The above findings made no impact on

pharmacologists and clinicians in the first half of the present century; the hypothesis of a direct central action of salicylate prevailed but recent years have seen the adoption of broader, less dogmatic and more constructive views on the pharmacology of salicylate.

Cochran (1952) measured the oxygen consumption, carbon dioxide output and depth and rate of respiration of six subjects by a closed circuit Knipping type spirometer. Three sub-acute rheumatic fever patients were treated with oral doses of aspirin and three normal adults were given sodium salicylate intravenously. In all cases, a marked and progressive increase in oxygen consumption was observed. In 1954, Cochran repeated this work on acutely ill febrile patients, and, contrary to the findings of Barbour and Devenis (1919), observed an increase in the rate of oxygen consumption. He also demonstrated that whereas a single 3 gm. dose of sodium salicylate or aspirin was a powerful metabolic stimulant, 5 gm. doses of sodium meta- or para-hydroxybenzoate did not significantly increase the metabolic rate of convalescent rheumatic fever patients.

Alexander and Johnson (1958) measured the oxygen consumption of normal and hypothyroid patients receiving the full, therapeutic doses of aspirin, recommended by Coburn (1943) and practiced by Reid (1948) in this department, and established linear relationships between serum salicylate concentration and rate of oxygen consumption for both classes of patients.

Clinically salicylate is now well established as a metabolic stimulant.

Meanwhile there have been occasional reports on the effect of salicylate on the rate of oxygen consumption of laboratory animals.

As early as 1901, Singer had reported an increase in the oxygen consumption of rabbits to whom toxic doses of acetylsalicylic acid had been given. There were no other reports in this field until 1937 when Dodd, Minot and Arena (1937), gave five normal unanaesthetized dogs large doses (0.2 - 1.5 gms./kilo) of salicylate by mouth, or intravenous or subcutaneous injection, and measured the changes in respiratory rate and gaseous exchange in the Benedict-Roth apparatus. The immediate response was an abrupt increase

in the rate of oxygen consumption, followed by an increase in the depth of respiration and in the basal metabolic rate. These authors felt that "the changes in temperature, the sensation of heat, and the increase in gaseous exchange induced by salicylate therapy, were of much greater importance in producing an increase in the respiratory rate and depth than any direct central action of the drug".

Baloch, Donhoffer, Mestyan, Pap and Toth, (1952), Meade (1954) and Hall, Tomich and Woollett (1954) confirmed these results on rats, and Tenney and Miller (1955) have demonstrated a similar increase in the rate of oxygen consumption of dogs. More recently, Reid (1957) has reported a direct proportionality between the dose of sodium salicylate and the rate of oxygen consumption of rabbits.

Baloch et al (1952) have also studied the effect of salicylamide on the rate of oxygen consumption and rectal temperature of rats. A subcutaneous injection of 100 mgms. salicylamide was followed by a marked lowering of body temperature (0.5 - 3.5^{0}F) and a decrease in oxygen consumption of 0-47%.

Meade (1954) gave single 50 mgm. doses of certain mono- and dihydroxybenzoic acids intraperitoneally to rats and found that salicylic acid was the only compound studied which increased the rate of oxygen consumption significantly, meta-hydroxybenzoic acid was a depressant and the remaining mono- and dihydroxybenzoic acids were, in this dosage, without effect.

Hall, Tomich and Woollett (1954), investigated a number of antirheumatic compounds and others chemically related to salicylic acid. They found that salicylic and acetylsalicylic acid were the only compounds which increased the rate of oxygen consumption of rats or mice; 2:5- and 2:6-dihydroxybenzoic acid, meta- and para-hydroxybenzoic acid and salicylamide were ineffective.

In vitro studies have shown that tissue slices, homogenates and micro-organisms are all sensitive to this stimulating action of salicylate.

Alwall (1939) reported an increase in the oxygen consumption of liver and kidney slices at concentrations of 1-14 millimolar salicylic acid in the presence of citrate buffer but not in the presence

of phosphate buffer.

Fishgold, Field and Hall (1951) could detect no stimulation of respiration of thick liver slices at any concentration of sodium salicylate but observed an increase in rate of oxygen consumption of brain slices at concentrations of 0.06-0.56 mM./L. followed by a progressive fall at higher concentrations.

Using much higher concentrations - M/20 or M/10 sodium salicylate, Lutwak-Mann (1942) found a considerable decrease in the rate of oxygen consumption of liver slices. She could detect no stimulation at any of the concentrations used. M/10 sodium ortho-cresotinate was similar in action to M/10 sodium salicylate but M/10 benzoate or anthranilate had no effect. The respiration of liver slices of rats killed after salicylate treatment sometimes showed a small increase after four hours; inhibition was never observed.

More recently, Sproull (1954) has studied the effect of salicylate on the rate of oxygen consumption of mouse liver slices, measured by the Warburg direct method. He established definite and reproducible dose-response curves in the presence of

graded concentrations of sodium salicylate from 3.5×10^{-4}M to 7.5×10^{-3}M. There was a progressive increase in respiratory rates from 3.5×10^{-4}M to 2×10^{-3}M followed by a fall, the mean oxygen consumption of the treated tissues becoming less than that of the controls by a concentration of 5×10^{-3}M sodium salicylate.

Smith and Jeffrey (1956) produced a marked increase in the rate of oxygen uptake of isolated rat diaphragm at a concentration of 5×10^{-3}M sodium salicylate.

Patel and Heim (1954) studied the effect of the mono-hydroxybenzoic acids on the respiration of rat brain homogenates. Respiration in the presence of glucose, pyruvate or glutamate was markedly increased by 1.5×10^{-2}M sodium salicylate, but when succinate or lactate was used the same concentration inhibited respiration. The meta- and para-hydroxybenzoic acids in similar concentrations only showed stimulation when glucose was used as the substrate.

Kaplan, Kennedy and Davis (1954) found that salicylate, meta- and para-hydroxybenzoate, gentisate

and γ-resorcylate all inhibited the oxygen uptake of rat kidney and liver homogenates in the presence of succinate or α-ketoglutarate. They also found that, contrary to the claims of Alwall (1939) concerning tissue slices, 6.7×10^{-3}M salicylate inhibited the oxygen uptake of rat kidney homogenates in the presence of citrate.

Brody (1956) has shown that sodium salicylate will stimulate the oxygen consumption of rat brain mitochondria; he also showed that salicylate has an action similar to 2:4-dinitrophenol, which stimulates oxygen consumption by inhibition of oxidative phosphorylation (Loomis & Lipmann, 1948, and Simon, 1953). Sodium salicylate, aspirin, methyl salicylate and 2:3-dihydroxybenzoic acid were the only compounds examined by Brody (1956) which were found to depress oxidative phosphorylation, but he did not study the effect of these compounds on respiratory rate.

Penniall, Kalnitsky and Routh (1956) studied the effect of salicylic acid, salicyluric acid, acetylsalicylic acid and gentisic acid on the in vitro respiration of rat brain homogenates and

mitochondria at concentrations of 2×10^{-7}M to 2×10^{-2}M. They found that salicylic acid at concentrations of 2×10^{-4}M to 2×10^{-3}M decreased the uptake of inorganic phosphate of rat brain homogenates while the oxygen uptake proceeded essentially unchanged with the net result that the P:O ratios were thus progressively decreased. Similar results were obtained with rat brain mitochondria. Gentisic and salicyluric acids both showed a slight inhibition of the rate of oxygen uptake of rat brain mitochondria at concentrations less than 2×10^{-3}M.

Experiments with tissue homogenates must always be interpreted with caution, since the results are often determined by experimental conditions. It is noted that Peiss and Field (1948), who are very experienced workers, failed to show that 2:4-dinitrophenol, a powerful metabolic stimulant to tissue slices, could stimulate the respiration of tissue homogenates.

Bernheim (1940) has shown that sodium salicylate increased the rate of oxygen consumption of tubercle bacilli. The addition of 1.0 mgms. sodium salicylate to the bacteria suspended in 2 ccs. M/20

phosphate buffer (pH6-7) more than doubled the oxygen uptake; 1.0 mgm. sodium meta- or para-hydroxybenzoate had no action and sodium anthranilate had only a slight one. He suggested that the salicylate was being oxidised as a substrate, and that salicylate, or compounds of similar configuration, might be important as normal metabolites of tubercle bacilli and that they might play a part in bacterial metabolism.

Although this interpretation of Bernheim's is probably wrong in the light of this review, it did lead to the important discovery of PAS as an effective drug in the treatment of tuberculosis; that however, is another story (Lehmann, 1946).

Hitherto only preliminary studies of compounds chemically related to salicylic acid have been reported. Certain workers have investigated the effect on the rate of oxygen consumption of a few mono- and dihydroxybenzoic acids (Meade, 1954 and Hall, Tomich and Woollett, 1954) on the whole animal but they used small doses of these drugs and did not pursue their studies long enough to give any

decisive evidence. The present investigation was undertaken to obtain comprehensive data on the pharmacological activity of compounds related to salicylic acid.

The measurement of oxygen consumption of whole animals is one of the few pharmacological properties of salicylic acid which can be very easily examined in the laboratory, and was therefore considered appropriate to use in the present investigation. The rat was chosen as the most practical experimental animal, the smallest in which metabolic rate changes can be easily detected over one hour periods.

The compounds studied can be divided into three groups:-

(1) Mono and dihydroxybenzoic acids. These included the three hydroxybenzoic acids:- ortho-, meta-, and para-hydroxybenzoic acid, and the six dihydroxybenzoic acids:- 2:3-, 2:4-, 2:5-, 2:6-, 3:4-, and 3:5-dihydroxybenzoic acids.

(2) Substituted salicylic acids. The compounds included in this group were 5-aminosalicylic acid, salicylamide, salicyluric acid and the four methyl substituted salicylic acids, i.e. 3-methyl, 4-methyl,

5--methyl and 6--methylsalicylic acid.

(3) Ortho--substituted benzoic acids. o--aminobenzoic acid and phthalic acid.

The second part of the present investigation was to determine, and if possible account for, the relative potencies of any compounds found to increase metabolic rate.

Methods.

Oxygen consumption measurements were made using a closed circuit manometric method described by Cameron (1958). Air was pumped from a gas tight reservoir by an electrically driven reciprocating rubber bellows respiration pump, through the animal chamber - a Kilner jar - and returned to the reservoir via a soda lime container. A manometer was attached to the circuit between the reservoir and the pump and a second reservoir and manometer assembly was set up as a thermobarometer. The glass tops of the Kilner jars were replaced by metal lids fitted with rubber gaskets and two copper pipes for ventilation. All connections were made with glass Y-tubes, rubber tubing or 3/8 ins. copper tubing. The machines were kept in a room whose temperature where possible was kept between 18 and 20°C.

The calibration constants, k, of the two machines were found by measuring the pressure changes

produced in the system on introducing a known volume of water. k is the constant relating pressure and volume changes in the equation $x = kh$ where x = volume change (mls.) at N.T.P., and h = resultant manometer change (mms. fluid). k was evaluated from $k = \frac{273V}{TP_o}$ where V = gas volume (mls.) calculated from Boyle's Law, T = room temperature (^oA) and P_o = 10,025.

The manometers, graduated in mms., were filled with Brodie-Krebs fluid, density 1.03.

The weighed rat was placed in the Kilner jar, which was then connected to the apparatus and air pumped through the system for five minutes. A pressure of 100-120 mms. was then built up in the reservoir and the air circulated for ten minutes before taking the first manometer reading. Readings of the two manometers and the room temperature were recorded at fifteen minute intervals over a one hour period. From the final corrected pressure change, the volume of oxygen consumed was calculated. The results of oxygen consumption measurements were expressed in mls./hour at N.T.P.

In the first experiments, Wistar albino rats of weight range 230-290 gms. were used. The animals were paired for sex and weight and, in each run, one animal received the test solution while the other received an identical volume of normal saline. Equal numbers of male and female rats were used.

The drugs were administered by intraperitoneal injection as solutions of the sodium salts, pH7-9; the doses administered were the highest practical doses tolerated by the rats. Table I shows the compounds used, the source from which they were obtained, the concentration of the solution injected and the dose administered.

Dose-response experiments. The compounds used in these experiments were salicylic acid and the three cresotinic acids i.e. 3, 4, and 5 methylsalicylic acid. Wistar albino rats of 170-250 gms. weight were used. The animals were paired for sex and weight; as before one animal was given the drug while the other was given the same volume of normal saline. For each pair the difference in rate of oxygen consumption, (ΔO_2) expressed in mls./hr. at N.T.P. was determined.

The compounds were injected intraperitoneally as solutions of their sodium salts (pH6-9). Fresh solutions were made up every fortnight and were kept at $4^{o}C$ in the dark when not in use. Four doses, ranging from an arbitrary low dose to the maximum dose generally tolerated by the rats, were administered.

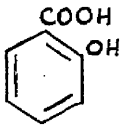
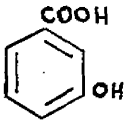
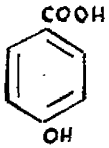
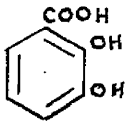
The observations were randomised, in respect of drug and dose, within a 4 x 4 Graeco-Latin square and twelve results of ΔO_2 (6 males, 6 females) were obtained for each drug at each dose.

The combined action of salicylate and ortho-cresotinate was studied on Wistar albino rats of 200-250 gms. weight. The rats were paired for sex and weight and male and female rats were used alternately. Six rats (i.e. three pairs of the same sex) were used for each trial, one from each pair received normal saline while the other received an intraperitoneal injection of salicylate, or the equivalent dose of ortho-cresotinate, or a mixture of half of both. The difference in rates of oxygen consumption between the treated and control rats of each pair was determined.

Time-concentration experiments. Wistar albino rats of weights varying from 150 to 350 gms. were injected with the highest dose of the drug used previously, and killed by decapitation at varying times after the injection over a sixteen hour period. The doses used for injection were 150 mgms. salicylate and 100 mgms. of the three cresotinates. The times of sampling were 15 minutes, 30 minutes, 1, 2, 4, 6, 8 and 16 hours and determinations of the plasma concentration from eight rats (4 males, 4 females) were made for each drug at each time. The blood was collected in heparinized tubes and the plasma separated by centrifuging. Plasma concentrations of salicylate and the three cresotinates were determined by the method of Trinder (1954), which was found to be applicable to the methyl substituted salicylic acids as well as to salicylic acid itself.

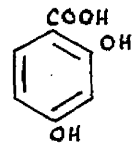
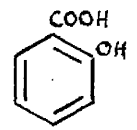
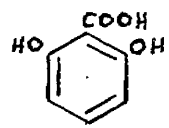
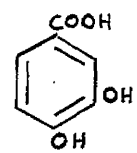
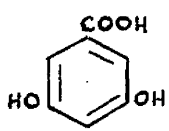
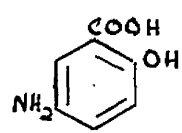
Table I.

Compounds used in the first experiments with their source, melting points, concentration of solution used, and dose.

Compound	Structure	Source	M.Pt. Obs. °C	M.Pt. Lit. °C	Conc. of Solution gms./100 ml.	Dose mgms.
Salicylic Acid		H. & W.	158	159	10	120
m-hydroxybenzoic Acid		Light's	199-200	201	10	500
p-hydroxybenzoic Acid		Light's	212-213	213	10	500
2:3-dihydroxybenzoic Acid		C.T.U.	203-204	204	4	100

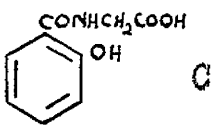
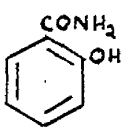
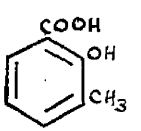
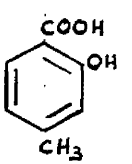
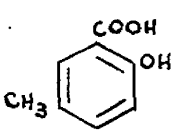
contd.

Table I.(contd.).

Compound	Structure	Source	M.Pt. Obs. °C	M.Pt. Lit. °C	Conc. of Solution gms./100 ml.	Dose mgms.
2:4-dihydroxybenzoic Acid		C.T.U.	213-216	213	6	300
2:5-dihydroxybenzoic Acid		Light's	199	200	10	500
2:6-dihydroxybenzoic Acid		C.T.U.	160-164	167	10	200
3:4-dihydroxybenzoic Acid		C.T.U.	199-201	199	10	500
3:5-dihydroxybenzoic Acid		C.T.U.	223	232	10	500
5-amino-salicylic Acid		H. & W.	280	283	2	100

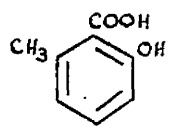
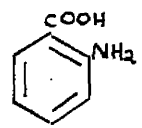
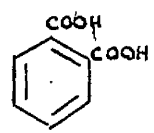
contd.

Table I. (contd.).

Compound	Structure	Source	M.Pt. Obs. °C	M.Pt. Lit. °C	Conc. of Solution gms./100 ml.	Dose mgms.
Salicyluric Acid		C.T.U.	163-165		4	100
Salicylamide		H. & W.	137-138	140	5	50
3-methylsalicylic Acid i.e. ortho-cresotinic Acid		H. & W.	162	163-164	5	100
4-methylsalicylic Acid i.e. meta-cresotinic Acid		C.T.U.	168-170	178	4	100
5-methylsalicylic Acid i.e. para-cresotinic Acid		C.T.U.	147	152	3	105

contd.

Table I. (contd.).

Compound	Structure	Source	M.Pt. Obs. °C	M.Pt. Lit. °C	Conc. of Solution gms./100 ml.	Dose mgms.
6-methylsalicylic Acid		C.T.U.	169-170	168	3	24
o-aminobenzoic Acid		H. & W.	144-145	145	10	100
Phthalic Acid		H. & W.	206-207	206-208	5	100

H. & W. - Hopkin & Williams chemicals. Light's - Light's chemicals.

C.T.U. - Chemotherapy Department Chemistry Laboratory.

The melting points were obtained from the Handbook of Chemistry and Physics, 35th Ed.: Chemical Rubber Publishing Co. 1953-1954.

Results.

The first experiments were to determine which of eighteen compounds, related in their chemical structure to salicylate, could increase the rate of oxygen consumption of rats.

ΔO_2, the difference in rate of oxygen consumption of each pair of treated and control rats, was determined and the composite hypothesis that the mean difference was zero (H_0) was tested against the single alternative that it differed from zero (H_1), using the sequential test proposed by Wald (1947). Formally, in each case, where the mean ΔO_2 was μ and the variance σ^2 it was decided whether $|\mu| < \delta\sigma$ (i.e. H_0) or whether $|\mu| \geq \delta\sigma$ (i.e. H_1). In these experiments δ, which determines the critical ΔO_2, was assigned the value 1. The maximum probability of a decision in favour of either hypothesis when in fact the other was true was chosen as 0.05. $Z = \left(\sum \Delta O_2\right)^2 \Big/ \sum \left(\Delta O_2\right)^2$ was calculated

after each trial; the trials were continued till the value of Z fell outwith the region of indecision defined in the tables of Arnold (1951). The number of trials required naturally varied from one test to another. Where H_1 was accepted the sign of the mean ΔO_2 was formally established from its 95% fiducial limits, since in no instance did these limits include zero.

A histogram of the rates of oxygen consumption of the 164 control rats (weight range 230-290 gms.) shows a near normal distribution (Fig. 1). The mean rate of oxygen consumption was 420.9 mls./hr. and the standard deviation was 60.3. A quality control chart, in which each batch was four consecutive control results, confirmed that the experiments were in statistical control (Fig. 2).

The results of these experiments are tabulated in Table II. In each case the 95% fiducial limits of the mean ΔO_2 are given with the mean.

The main findings (detailed in Tables XVII - XXXIII) were as follows: 100 mgms. 2:3-dihydroxybenzoic acid, 100 mgms. phthalic acid and 24 mgms. 6-methylsalicylic acid were without effect on the rate of oxygen consumption; 500 mgms. meta- and

Fig. 1.

Histogram: distribution of rate of oxygen consumption of one hundred and sixty four control Wistar albino rats.

Abscissa: oxygen consumption (mls./hr.).

Ordinate: frequency.

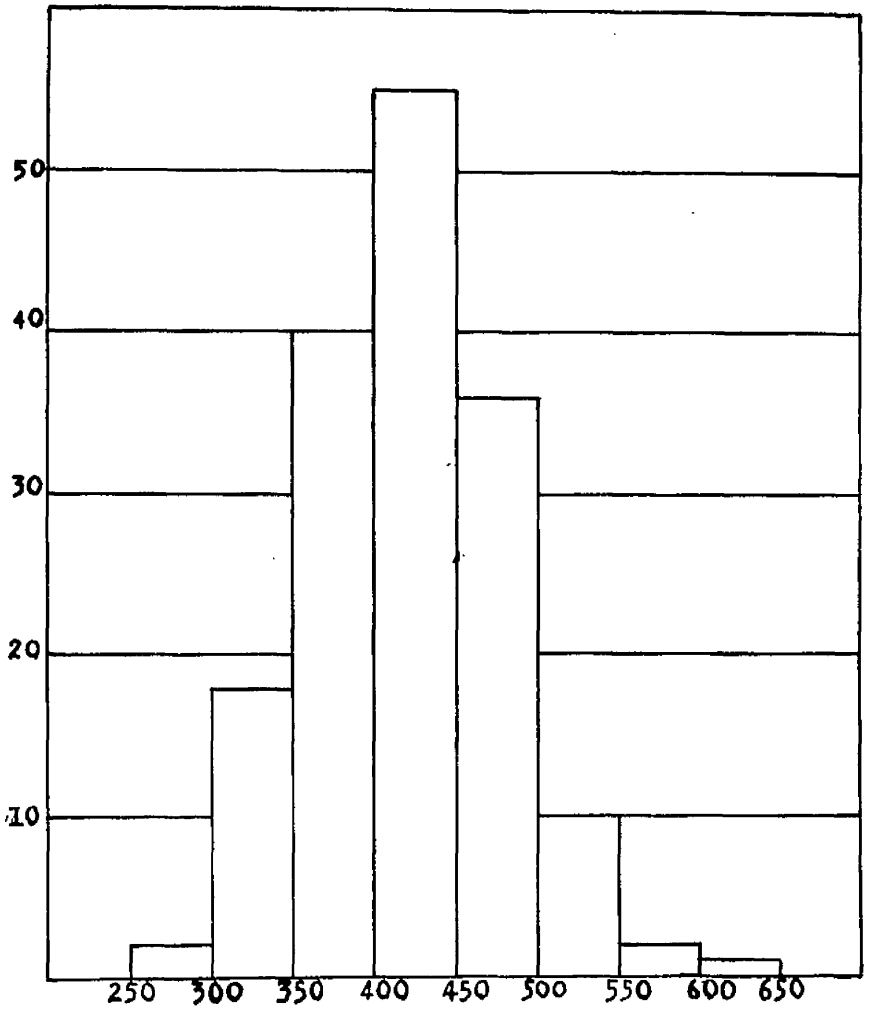
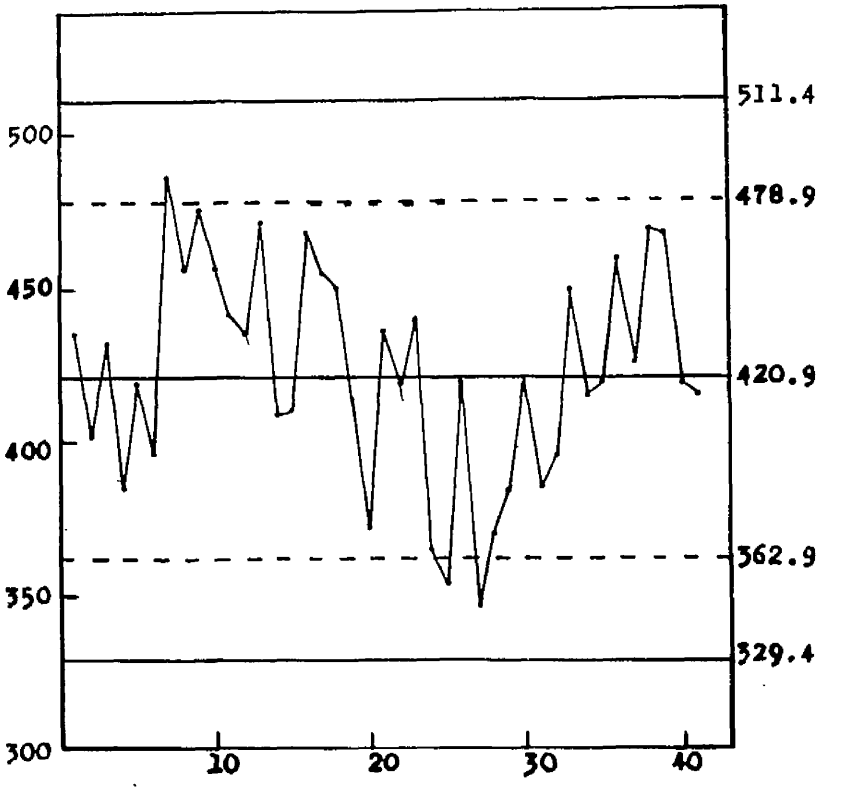


Fig. 2.

Control chart of the rates of oxygen consumption of one hundred and sixty four control Wistar albino rats. Each batch consisted of four consecutive determinations.

Abscissa: batch number.

Ordinate: oxygen consumption (mls./hr.).



para-hydroxybenzoic acid, 2:5-, 3:4- and 3:5-dihydroxybenzoic acid, 300 mgms. 2:4-dihydroxybenzoic acid, 200 mgms. 2:6-dihydroxybenzoic acid, 100 mgms. 5-aminosalicylic acid, salicyluric acid and ortho-aminobenzoic acid and 50 mgms. salicylamide decreased the rate of oxygen consumption; 120 mgms. salicylic acid, 105 mgms. para-cresotinic acid and 100 mgms. ortho- and meta-cresotinic acid markedly increased the rate of oxygen consumption.

Only four compounds were found to increase the metabolic rate of rats. These compounds were salicylic acid and the three cresotinic acids, 3, 4, and 5-methylsalicylic acids. Qualitatively these acids were very similar, the maximum doses tolerated were of the same order and the toxic effects observed with higher doses such as hyperventilation and convulsions, were the same.

The next step was therefore to determine whether there were any differences in the potencies of these substances as metabolic stimulants in the intact rat.

Table II.

Effect of salicylate and related compounds on the rates of oxygen consumption of Wistar albino rats.

ΔO_2 is the difference in rates of oxygen consumption between paired treated and control rats (mls./hr.). H_o is the hypothesis that the mean ΔO_2 is zero, H_1 the alternative. n is the number of trials required for termination of the sequential test of H_o against H_1.

Compound	Dose mgms.	Mean ΔO_2 and its 95% Fiducial Limits	(n)	Hypothesis accepted
Salicylic Acid	120	+53.5 ± 34.6	(10)	H_1
m-hydroxybenzoic Acid	500	-173.0 ± 103.9	(6)	H_1
p-hydroxybenzoic Acid	500	-78.1 ± 42.1	(8)	H_1
2:3-dihydroxy-benzoic Acid	100	-25.5 ± 55.9	(12)	H_o
2:4-dihydroxy-benzoic Acid	300	-84.4 ± 44.5	(7)	H_1
2:5-dihydroxy-benzoic Acid	500	-332.9 ± 71.6	(6)	H_1
2:6-dihydroxy-benzoic Acid	200	-121.5 ± 71.0	(7)	H_1

contd.

Table II. (contd.).

Compound	Dose mgms.	Mean ΔO_2 and its 95% Fiducial Limits	(n)	Hypothesis accepted
3:4-dihydroxy-benzoic Acid	500	-102.9 ± 67.4	(10)	H_1
3:5-dihydroxy-benzoic Acid	500	-104.5 ± 69.9	(7)	H_1
5-aminosalicylic Acid	100	-95.1 ± 55.4	(7)	H_1
Salicyluric Acid	100	-46.9 ± 29.9	(8)	H_1
Salicylamide	50	-128.6 ± 46.8	(6)	H_1
3-methylsalicylic Acid	100	+176.1 ± 76.7	(7)	H_1
4-methylsalicylic Acid	100	+109.9 ± 33.6	(6)	H_1
5-methylsalicylic Acid	105	+186.6 ± 62.9	(8)	H_1
6-methylsalicylic Acid	24	+21.6 ± 61.7	(10)	H_o
o-aminobenzoic Acid	100	-68.7 ± 40.7	(7)	H_1
Phthalic Acid	100	-13.6 ± 48.9	(9)	H_o

The relative potencies of the sodium salts of these cresotinates as metabolic stimulants were compared with sodium salicylate. Rats were injected intraperitoneally with four doses of each drug; these doses ranged from an arbitrary low dose to the highest dose generally tolerated by the rat. The concentrations of the solutions used for injection were 5 gms./100 mls. for the three cresotinates and 6 gms./100 mls. for salicylate. Table III gives the doses of each drug injected.

Table III.

Drug	Dose mgms.
Salicylic Acid	30, 90, 120, 150
o-Cresotinic Acid	25, 50, 75, 100
m-Cresotinic Acid	
p-Cresotinic Acid	

Initially, eight determinations (4 males, 4 females) of ΔO_2 were made for each drug at each dose. Preliminary examination of the results showed

an apparently linear relationship between ΔO_2 and dose within the dose range 50-100 mgms. for the three cresotinates and within the dose range 30-150 mgms. for salicylate (Fig. 3). Formal confirmation of this hypothesis involved further determinations of ΔO_2 at doses of 62.5 and 87.5 mgms. for ortho-, meta- and para-cresotinate, and at 60 and 135 mgms. for salicylate.

Consideration of all the results (Fig. 4) suggested that there was indeed a linear relationship between ΔO_2 and dose between the doses 62.5 and 100 mgms. of the three cresotinates and 90 and 150 mgms. salicylate, therefore further observations were made within these dose ranges.

Twelve determinations of ΔO_2 were finally made for each drug at each of the doses given in Table IV and these results were used for the formal analysis. The mean ΔO_2 for each drug at each dose is given in Table V. Fuller details of the results are presented in Tables XXXIV - XXXVII.

Fig. 3.

Dose-response curves of salicylate (o), ortho-cresotinate (□), meta-cresotinate (Δ·····), and para-cresotinate (+) on the rate of oxygen consumption of Wistar albino rats.

Abscissa: dose (mgms.).

Ordinate: ΔO_2 (mls./hr.).

ΔO_2 = the difference in the rate of oxygen consumption between paired treated and control rats. Each point is the mean of eight determinations of ΔO_2.

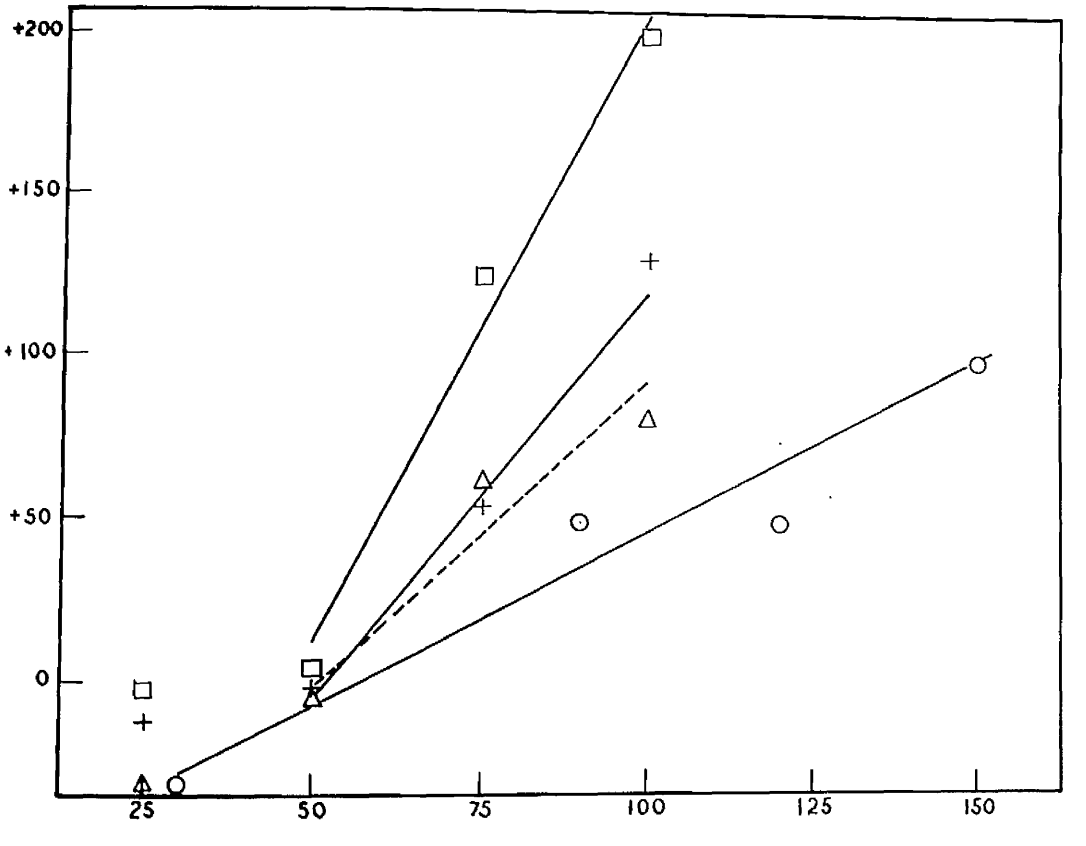


Fig. 4.

Dose-response curves of salicylate (O), ortho-cresotinate (□), meta-cresotinate (Δ----) and para-cresotinate (+) on the oxygen consumption of Wistar albino rats.

Abscissa: dose (mgms.).

Ordinate: ΔO_2 (mls./hr.).

ΔO_2 = the difference in the rate of oxygen consumption between paired treated and control rats.

Each point is the mean of eight determinations of ΔO_2.

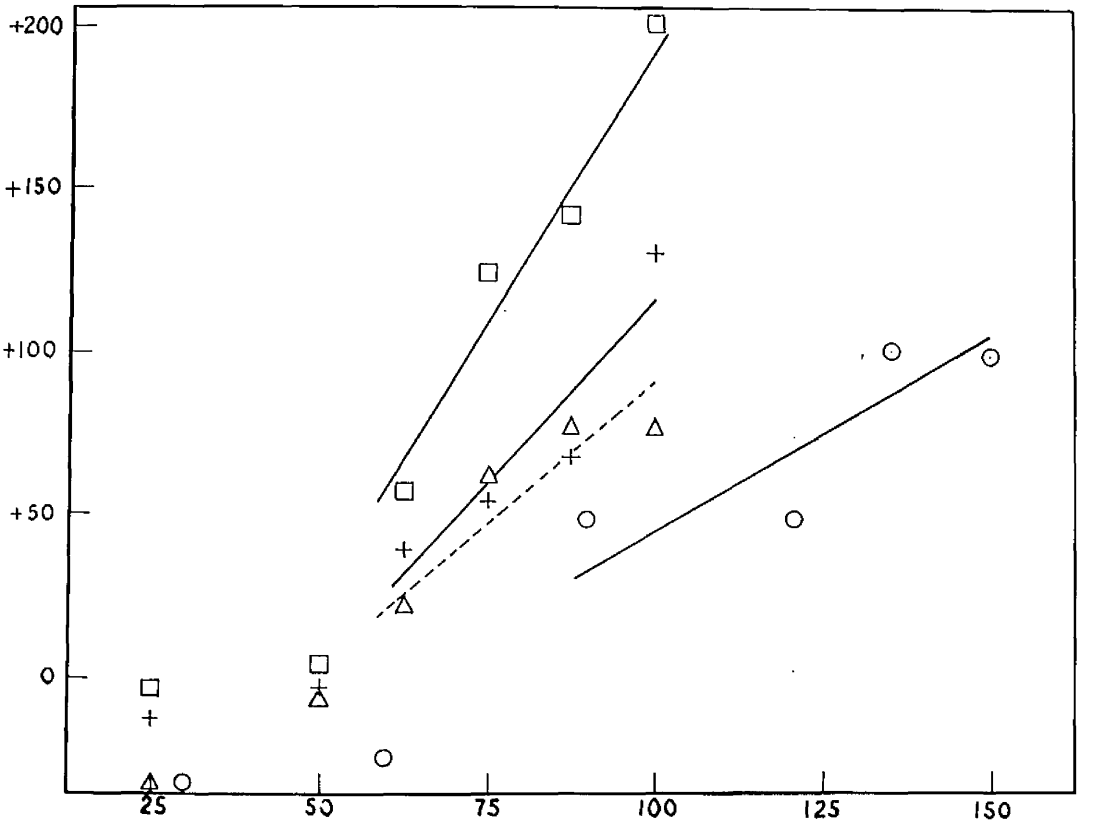


Table IV.

Drug	Dose mgms.
Salicylic Acid	90, 120, 135, 150
o-Cresotinic Acid m-Cresotinic Acid p-Cresotinic Acid	62.5, 75, 87.5, 100

Before proceeding to the analyses of variance of these data, homoscedasticity was established by Bartlett's test; $0.50 > P > 0.10$.

In each case, within the dose range finally selected, the dose-response curves fitted regression equations of the form $y_i = a_i + b_i x_i$ where y_i was ΔO_2 (mls./hr.) and x_i was the dose (mgms.) (Fig. 5). This, therefore, made possible a "comparative slope-ratio assay", in which the potency ratios were obtained from the regression coefficients. Thus, where any two of the regression equations were $y_1 = a_1 + b_1 x_1$ and $y_2 = a_2 + b_2 x_2$, $P_1 = b_1/b_2$ gave P_1, the potency ratio of b_1 with respect to b_2.

Table V.

The mean differences in rates of oxygen consumption between paired treated and control rats given various doses of salicylate and the three cresotinates.

Differences in rates of oxygen consumption (ΔO_2) are expressed in mls./hr.

Dose (mgms.)	Salicylic Acid	Ortho-Cresotinic Acid	Meta-Cresotinic Acid	Para-Cresotinic Acid
62.5		+82.2	+19.8	+51.9
75.0		+127.4	+70.0	+57.9
87.5		+134.7	+91.7	+89.4
90.0	+34.7			
100.0		+177.8	+97.8	+114.4
120.0	+62.7			
135.0	+92.5			
150.0	+105.2			

Fig. 5.

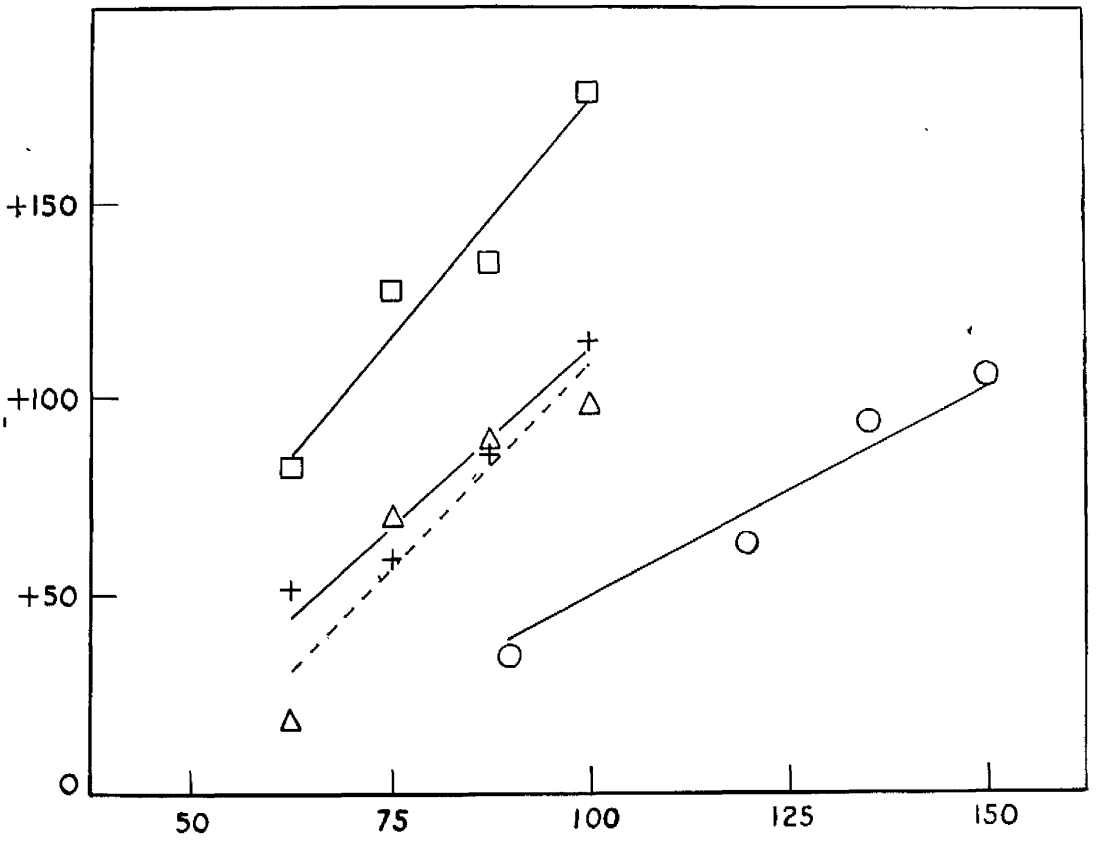
Dose-response curves of salicylate (O), ortho-cresotinate (□), meta-cresotinate (Δ····) and para-cresotinate (+) on the rate of oxygen consumption of Wistar albino rats.

Abscissa: dose (mgms.).

Ordinate: ΔO_2 (mls./hr.).

ΔO_2 = the difference in the rate of oxygen consumption between paired treated and control rats.

Each point is the mean of twelve determinations of ΔO_2.



Variance was divided into that due to (i) regression, (ii) deviation from regression, (iii) between doses and (iv) residual.

The requirements for a valid slope-ratio assay are that there must be linearity i.e. the regression of response on dose must be linear for each drug and that the intercepts made on the Y axis by the four regression lines must be the same, within sampling error (Bliss, 1946). The second point in analogous to parallelism in parallel line assays since it derives from the very existence of a potency ratio. These conditions were met in the analyses of variance summarised in Tables VI and VII. Fuller details of the analyses can be found in the "Statistical Methods" section. Application of the F-test to the between intercepts mean square, the deviation from regression mean square and the residual mean square showed that there was neither significant deviation from linearity nor difference in intercept.

The potency ratios of the cresotinates relative to salicylate, and the 95% confidence limits, presented in Table VIII were then calculated as described in the "Statistical Methods" section. To

Table VI.

Analyses of variance of ΔO_2 and the regression equations of ΔO_2 on dose for salicylate and the three cresotinates.

ΔO_2 is the difference in rate of oxygen consumption of paired treated and control rats.
$y = \Delta O_2$ (mls./hr.), x = dose (mgms.).

Salicylic Acid. Dose range 90-150 mgms.
Regression equation: $y = 51.95x - 32.30$

Source of variance	Sum of squares	d.f.	Mean squares
Between doses.			
Due to regression	28,792.43	1	28,792.43
Deviation from regression	6,914.41	2	3,457.21
Within dose	192,202.16	44	4,368.23
Total	227,909.00	47	

o-Cresotinic Acid. Dose range 62.5 - 100 mgms.
Regression equation: $y = 146.95x - 60.54$

Source of variance	Sum of squares	d.f.	Mean squares
Between doses.			
Due to regression	51,823.39	1	51,823.39
Deviation from regression	3,297.56	2	1,648.78
Within dose	207,559.04	44	4,717.25
Total	262,679.99	47	

contd.

Table VI. (contd.).

m-Cresotinic Acid. Dose range 62.5 - 100 mgms.
Regression equation: $y = 127.74x - 96.24$.

Source of variance	Sum of squares	d.f.	Mean squares
Between doses.			
Due to regression	39,160.49	1	39,160.49
Deviation from regression	5,945.44	2	2,872.72
Within dose	117,908.08	44	2,679.73
Total	163,014.00	47	

p-Cresotinic Acid. Dose range 62.5 - 100 mgms.
Regression equation: $y = 109.39x - 63.80$

Source of variance	Sum of squares	d.f.	Mean squares
Between doses.			
Due to regression	28,717.50	1	28,717.50
Deviation from regression	1,704.44	2	852.22
Within dose	186,888.75	44	4,247.47
Total	217,310.68	47	

Table VII.

Slope ratio assay of salicylate and the cresotinates: general analysis of variance.

Source of variance	Sum of squares	d.f.	Mean squares
Due to linear regressions	148,493.81	4	37,123.45
Deviation from regressions	17,861.85	8	2,232.73
Between intercepts	3,672.35	3	1,224.12
Residual	704,831.22	176	4,004.72

Table VIII.

Molar potency ratios of the three cresotinates relative to salicylate as metabolic stimulants in the intact rat.

Drug	Potency Ratio	95% Confidence Limits
o-Cresotinic Acid	2.61	2.50 - 2.72
m-Cresotinic Acid	1.78	1.69 - 1.87
p-Cresotinic Acid	1.89	1.79 - 1.99

allow for chemical equivalence of the drugs these potency ratios were calculated in molar units.

Significant differences in potency were found, in comparing the three cresotinates with salicylate, as metabolic stimulants in the intact rat.

In these experiments the rats were of a lower weight range than in the previous experiments. The histogram of 252 control results of the rates of oxygen consumption of rats of this weight range (170-250 gms.) showed a near normal distribution (Fig. 6) and the quality control chart in which each batch was four consecutive control results showed that the experiment was in statistical control (Fig. 7). The mean rate of oxygen consumption was 389.2 mls./hr. and the standard deviation was 48.5.

The distributions of the rates of oxygen consumption for the two series of experiments were similar. In the first series the weight range was 230-290 gms. and the mean rate of oxygen consumption was 420.9 mls./hr. (S.D. 60.3), while in the dose-

Fig. 6.

Histogram: distribution of rate of oxygen consumption of two hundred and fifty two control Wistar albino rats.

Abscissa: oxygen consumption (mls./hr.).

Ordinate: frequency.

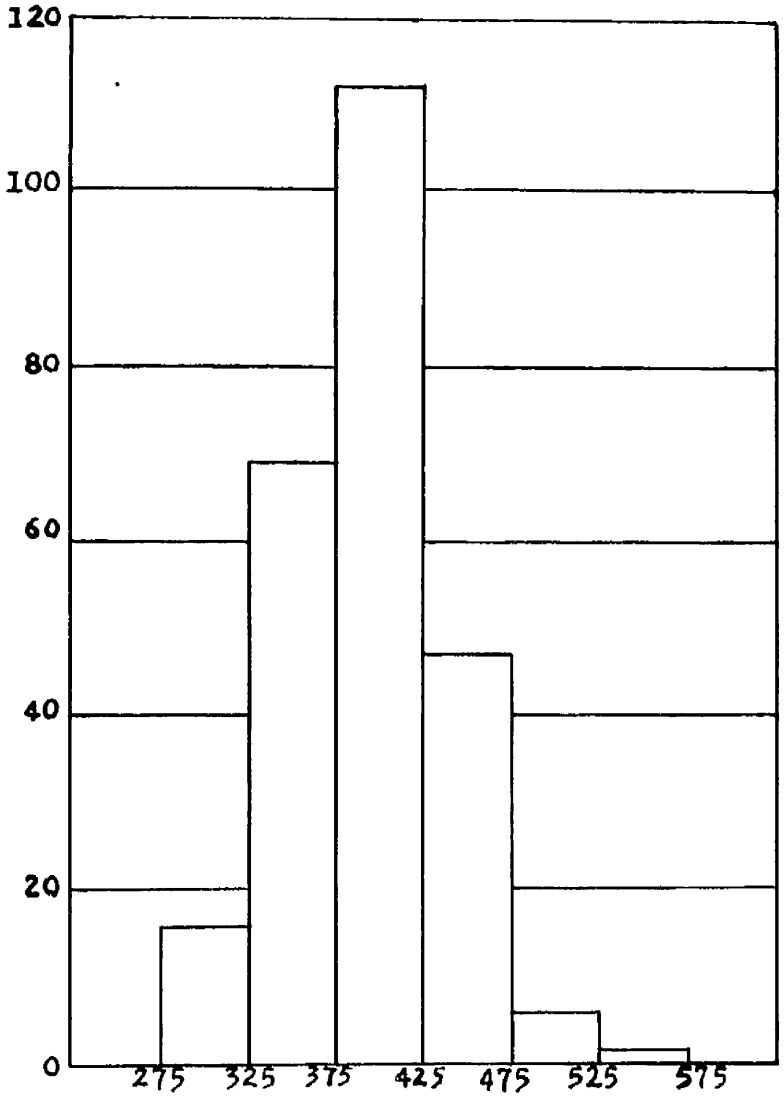
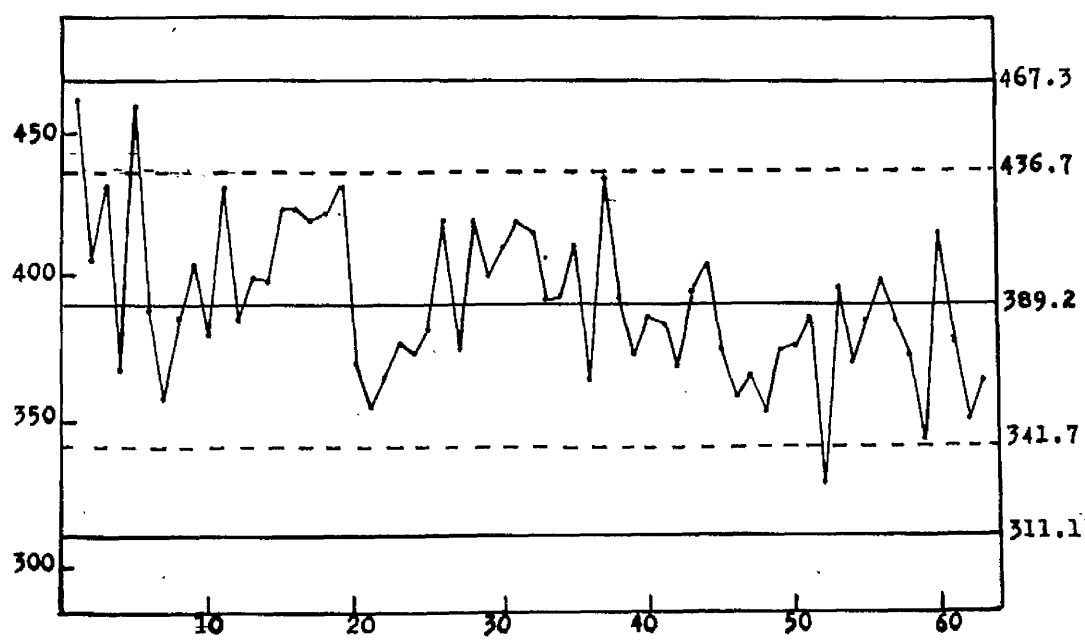


Fig. 7.

Control chart of the rates of oxygen consumption of two hundred and fifty two control Wister albino rats. Each batch consisted of four consecutive determinations.

Abscissa: batch number.

Ordinate: oxygen consumption (mls./hr.).



response experiments the weight range was 170-250 gms. and the mean rate of oxygen consumption was 389.2 mls./hr. (S.D. 48.5). Although these figures imply correlation between body weight and rate of oxygen consumption, body weight did not perceptibly influence the values of ΔO_2. For example, using the twelve results of ΔO_2 obtained after injection of 135 mgms. salicylate no correlation was found between body weight and ΔO_2. ($r = 0.02$, $P > 0.50$). This implies that other factors overshadow and obscure any possible relationship between ΔO_2 and body weight.

One possible explanation of the greater potencies of the cresotinates as metabolic stimulants was that they differed from salicylate in their primary action. This possibility was next investigated.

The nature of the combined action of salicylate and ortho-cresotinate was determined as described by Gaddum (1953). Such experiments might point to a difference in the primary action of the two drugs, which in turn might explain the greater potencies of the cresotinates, particularly the ortho-cresotinate.

The effects on the rate of oxygen consumption of a selected dose of salicylate, an equipotent dose of ortho-cresotinate, and a mixture of half of each dose of the two drugs, were compared. ΔO_2, the difference in rates of oxygen consumption for paired, treated and control rats was determined for 150 mgms. salicylate (S), 70 mgms. ortho-cresotinate (O) and a mixture of 75 mgms. salicylate and 35 mgms. ortho-cresotinate (SO). The results were put simultaneously to three sequential tests; these tests were to determine whether or not the effect on the mean ΔO_2 of the mixture of 75 mgms. salicylate and 35 mgms. ortho-cresotinate was distinguishable from the effect of twice the dose of either of the two drugs administered separately. The differences tested were therefore ΔO_2 (S) -

$\Delta O_2(O)$, $\Delta O_2(S) - \Delta O_2(SO)$, $\Delta O_2(O) - \Delta O_2(SO)$. In each case, the hypothesis (H_o) that the mean difference was zero was tested against the single alternative (H_1) that it differed from zero. Formally, in each case, it was decided whether $H_o : |\mu| < \delta\sigma$ or $H_1 : |\mu| \geq \delta\sigma$ where μ was the mean difference and σ^2 was its variance; the value of δ was taken as 0.7. The maximum probability of accepting a wrong decision was, in each case, 0.05.

In these experiments, controls could have been omitted and the direct differences in rates of oxygen consumption of rats receiving the three treatments considered. For this purpose, the rats would have been used in triplets, which in terms of practical convenience offered no advantage over working with ΔO_2.

The results are summarised in Table IX. Fuller details are presented in Tables XXXVIII - XLIII. In every case the hypothesis H_o was accepted. Thus, administration of a mixture of salicylate and ortho-cresotinate gave a response which was indistinguishable from an additive effect, and therefore no difference in the primary actions of the two drugs was established

by this experiment.

The other possibility considered was that the relative potencies of the cresotinates might be dependent on variation in rates of degradation and excretion; the present investigation was concluded with an examination of this problem.

Table IX.

Combined action of salicylate and ortho-cresotinate.

Results of the sequential tests of the hypothesis that the mean difference in ΔO_2 of rats treated with 150 mgms. salicylate (S), 70 mgms. ortho-cresotinate (O) and a mixture of 75 mgms. salicylate and 35 mgms. ortho-cresotinate (SO) were equal. Formally where each mean difference was μ and its variance σ^2 the hypothesis $H_0: |\mu| < 0.7\sigma$ was tested against $H_1: |\mu| > 0.7\sigma$; the probability of accepting the wrong hypothesis was 0.05.

Difference tested	No. of trials required	Hypothesis accepted	Mean difference ± 95% fiducial limits of the mean
(S) - (O)	14	H_0	+1.6±38.5
(S) - (SO)	14	H_0	+6.0±41.6
(O) - (SO)	14	H_0	+4.4±43.5

The possibility that differences in rates of degradation and excretion between salicylate and the three cresotinates might account for the differences in potencies has been put forward. It was examined by comparing the rates of disappearance of the drugs from the blood after single intraperitoneal injections. Rats were given the previous maximum dose of the drugs, and sacrificed at varying times after the injection. Determinations of the plasma drug concentration were made on eight animals for each drug at each time. The method for estimating salicylate, described by Trinder (1954), was applicable to the three cresotinates. In each case, Beer's Law was obeyed over the range 100-600 μg. per ml. (Fig. 8).

The mean plasma concentrations for each drug at each time are given in Table X. Fuller details are presented in Tables XLIV - XLVII. Blume and Plum (1935) have shown that, in rabbits, salicylate appears in the blood 2½ minutes after an intraperitoneal injection and after reaching a maximum falls gradually for six hours. Similarly, in the present results the time-concentration curves showed

Fig. 8.

Trinder's method for the estimation of salicylate in biological fluids: standard graphs for salicylate (o), ortho-cresotinate (□), meta-cresotinate (Δ) and para-cresotinate (+).
Abscissa: concentration of solution (μgms./cc.).
Ordinate: optical density.

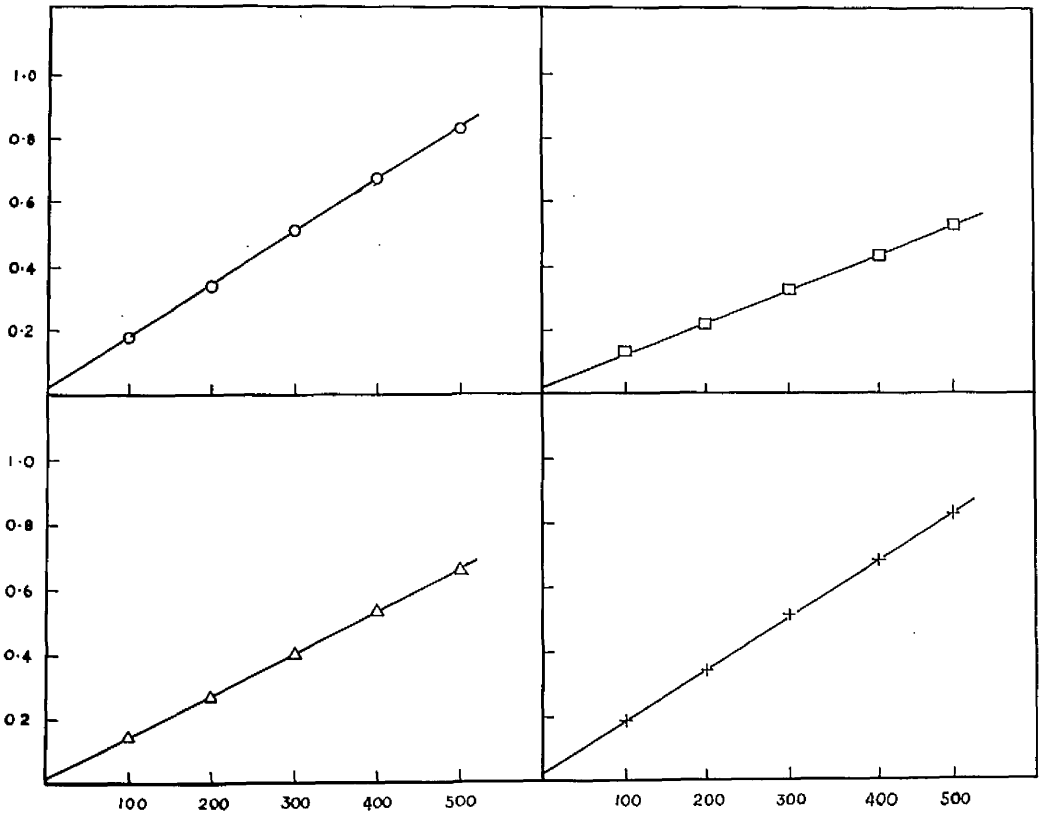


Table X.

The mean drug plasma concentrations in Wistar albino rats at the various times after injection of salicylate and the cresotinates.

Plasma concentrations are expressed in m.Eq./L.

Time (hrs.)	Salicylic Acid	Ortho-cresotinic Acid	Meta-cresotinic Acid	Para-cresotinic Acid
.25	7.95	6.89	6.41	6.09
.50	8.11	6.71	7.05	6.21
1.0	7.37	6.61	5.72	6.15
2.0	6.43	6.60	5.58	5.67
4.0	5.98	5.12	4.75	5.20
6.0	5.19	4.82	4.60	4.37
8.0	4.53	4.16	4.14	4.15
16.0	3.02	2.76	2.20	2.59

in all cases an apparently exponential decline (Fig. 9). Transformation of the concentrations to a logarithmic scale conferred linearity to these data (Fig. 10). After this transformation the curves fitted regression equations of the form $Y_j = a_j + b_j x_j$ where Y_j was the log of the plasma drug concentration expressed in milli-equivalents per litre and x_j was the time after injection in hours.

The homoscedasticity of the transformed data was confirmed by Bartlett's test which gave $0.50 > P > 0.10$; analyses of variance failed to show a significant deviation from linear regression for any of the drugs (Table XI). Formal comparisons of these regression lines were then undertaken.

Any linear regression is fully defined in terms of two parameters, elevation and gradient. In the present experiment the elevations were mainly dependent on the initial dose of the drug injected; the gradients, however, were characteristic of each time-concentration relationship and any differences in the rates of degradation or excretion would appear as differences in gradient.

An analysis of covariance which is

Fig. 9.

Time-concentration curves of salicylate (o), ortho-cresotinate (□), meta-cresotinate (Δ) and para-cresotinate (+).

Abscissa: time after injection (hours).

Ordinate: drug plasma concentration (m.Eq./L.).

Each point is the mean of eight determinations.

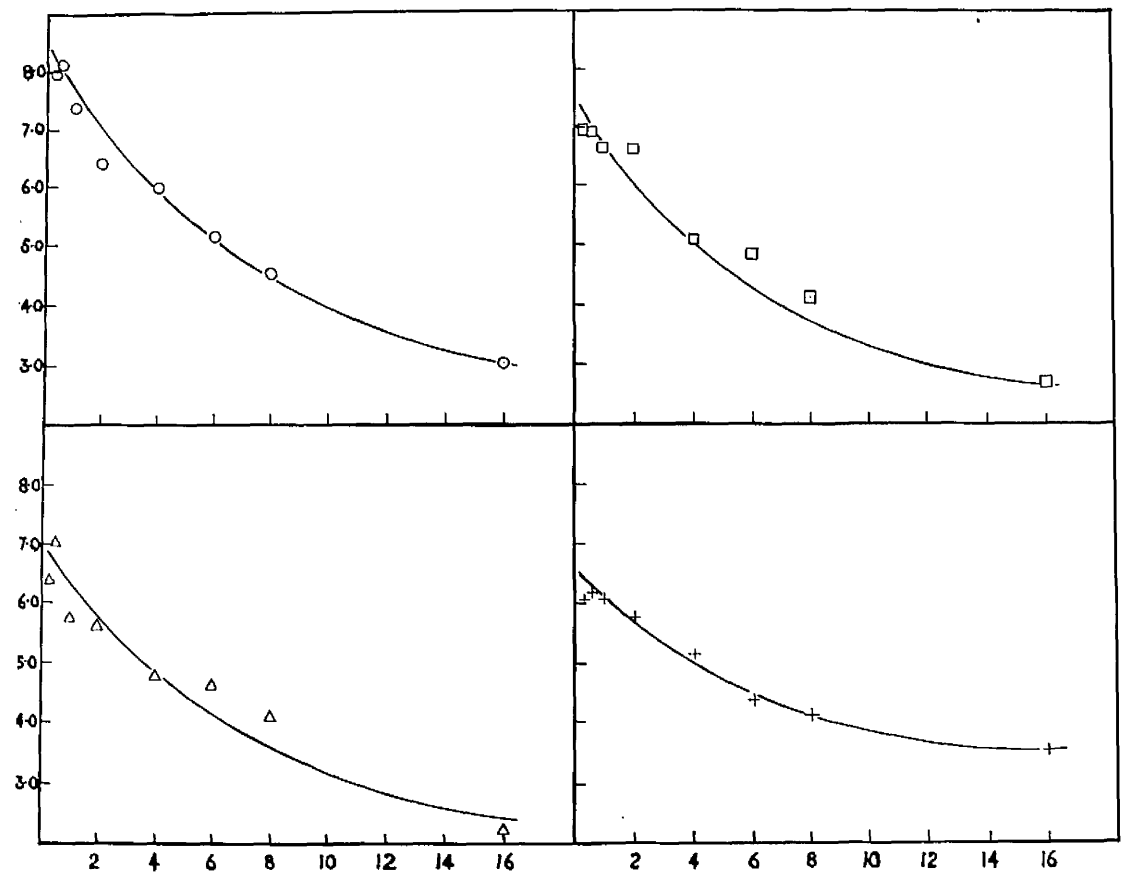


Fig. 10.

The transformed time-concentration curves of salicylate (o), ortho-cresotinate (□), meta-cresotinate (Δ) and para-cresotinate (+).
Abscissa: time after injection (hours).
Ordinate: log drug plasma concentration (m.Eq./L.).
Each point is the mean of eight determinations.

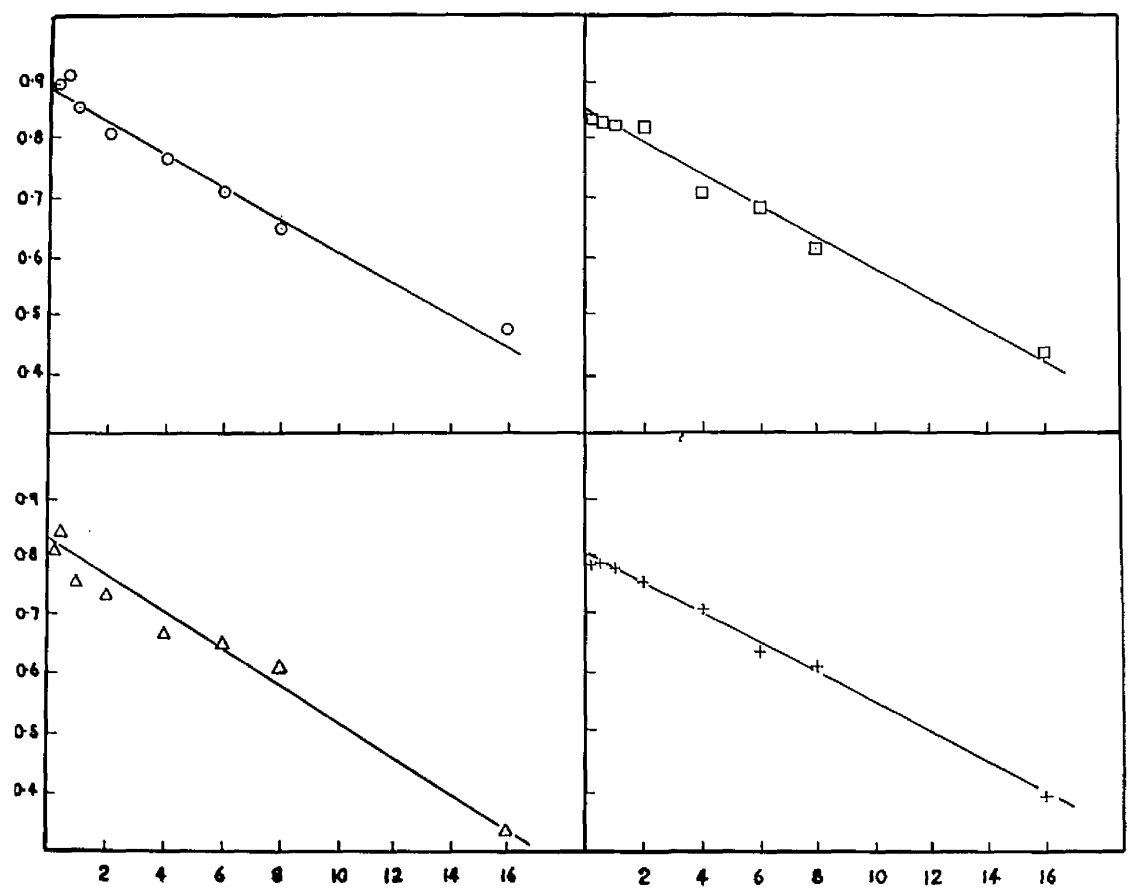


Table XI.

Analysis of variance of the log plasma drug concentrations and the regression equations of log plasma concentration on time after injection for salicylate and the three cresotinates.

x = time (hours), Y = log plasma drug concentration (m.Eq./L.).

Compound	Mean Squares: Due to Regression d.f. = 1	Deviation from Regression d.f. = 6	Residual d.f.=56	Regression equation
Salicylic Acid	1.1433	0.0047	0.0055	Y = 0.89 - 0.027x
o-Cresotinic Acid	1.0823	0.0027	0.0045	Y = 0.84 - 0.026x
m-Cresotinic Acid	1.3777	0.0070	0.0051	Y = 0.82 - 0.029x
p-Cresotinic Acid	1.0210	0.0058	0.0067	Y = 0.80 - 0.025x

summarised in Table XII, confirmed that there was no significant difference between the four regression coefficients. Application of F-tests to the common line mean square, the regression coefficient mean square and the within sample mean square, gave $P>0.50$. This established that the four regression lines did not come from different populations.

There was thus no evidence for any difference in the rates of degradation or excretion.

In the preceding experiment, the dose administered was not based on the animal's body weight; instead, the distribution of body weights was randomised. There was, of course, a correlation between the rat's body weight and the plasma concentration attained at a given time after a single injection. This was shown by an analysis of covariance, in which covariance was divided into "within" and "between" classes. In this analysis the four classes were the four drugs at the first time i.e. 15 minutes; within each class, there was a significant correlation between body weight and plasma drug concentration, $P < 0.001$ (Table XIII).

New regression equations were calculated

Table XII.

The disappearance from the blood of salicylate and the cresotinates. The regressions of log plasma drug concentrations on time after injection: analysis of covariance.

Source of variance	Regression Coefficient	Deviation from Regression Sum of squares	d.f.	Mean squares
Salicylic Acid	-0.0268	0.3342	62	0.00539
o-Cresotinic Acid	-0.0261	0.2674	62	0.00431
m-Cresotinic Acid	-0.0294	0.3299	62	0.00532
p-Cresotinic Acid	-0.0253	0.3787	62	0.00611
Within		1.3102	248	0.00528
Regression Coefficient		0.0151	3	0.00503
Common Line	-0.0269	1.3253	251	0.00528
Elevation		0.2764	3	0.092
Total		1.6017	254	0.00631

Table XIII.

Analysis of Covariance of rat body weight and plasma drug concentration for salicylate and the cresotinates.

The four classes were the four drugs at 15 minutes after injection.

Source of variation	d.f.	Sum of squares	Sum of products	Coefficient of regression	Coefficient of correlation and P.
Between classes	3	3,575	165.7	$b = 0.0463$	$r = 0.724$; P not significant.
		14.67			
Within classes	28	60,675	-1072.45	$b' = -0.1768$	$r' = 0.768$; $P < .001$
		32.12			
Total	31	64,250	-906.75		
		46.79			

in which the log plasma drug concentration was linearly related to both body weight and time i.e. regression equations of the form $Y = a + b_1 x_1 + b_2 x_2$ where Y was the log of the concentration in milli-equivalents per litre, x_1 was the weight in grams, and x_2 was the time in hours. For each drug an analysis of multiple correlation showed that there was a significant component of variance due to weight. The results of the F-tests gave $P < 0.001$ (Table XIV).

Inclusion of body weight in the regression equation therefore altered the regression coefficients of log concentration on time slightly, but not significantly. Table XV shows the values of these regression coefficients; b was the regression coefficient when weight was not included, b' was the value when weight was included. For each compound the value b was tested against an arbitrary value (b') by a t-test described by Fisher (1954). No significant differences were found.

This confirms the successful randomisation of the rat's body weight and justifies the practice of fixed doses not based on body weight.

It is concluded that there is no gross difference in the rates of degradation or excretion of the drugs.

Table XIV.

Multiple Correlation Analysis of log plasma drug concentration on weight of Wistar albino rats and time after injection, of salicylate and the three cresotinates.

Y = log drug plasma concentration (m.Eq./L.).
x_1 = weight (gms.).
x_2 = time (hrs.).

Drug	Mean Squares: Due to time d.f.=1	Due to weight d.f.=1	Residual d.f.=61	Regression equation
Salicylic Acid	1.1433	0.0802	0.00416	$Y = 1.12 - 0.00093x_1 - 0.027x_2$
o-Cresotinic Acid	1.0823	0.0935	0.00285	$Y = 1.08 - 0.00099x_1 - 0.025x_2$
m-Cresotinic Acid	1.3777	0.1062	0.00367	$Y = 1.09 - 0.0011x_1 - 0.031x_2$
p-Cresotinic Acid	1.0210	0.1847	0.00303	$Y = 1.21 - 0.0017x_1 - 0.022x_2$

Table XV.

Regression coefficients of log plasma drug concentration on time after injection for salicylate and the cresotinates: t-tests of the differences between these values.

b is value when rat body weight was not included.
b′ is value when weight was included.

Drug	b	b′	t and P
Salicylic Acid	0.0268	0.0266	0.11, P > 0.50
o-Cresotinic Acid	0.0261	0.0245	0.98, P > 0.10
m-Cresotinic Acid	0.0294	0.0306	0.66, P > 0.50
p-Cresotinic Acid	0.0253	0.0217	1.82, P > 0.05

Discussion.

Before discussing the results of this investigation in detail, the experimental conditions and theoretical background will be briefly considered.

In accordance with the practice in this laboratory (Cameron, 1957), the variability of biological material was accepted; variations in exercise, feeding etc. were assumed to be randomly distributed. The animals were, however, paired for sex and weight, and all the animals were chosen within an arbitrary weight range. The near normal distribution of the rates of oxygen consumption (Figs. 1 and 6) of the control rats and the quality control charts (Figs. 2 and 7) showed that the experiments were in statistical control.

The room temperature was, as far as possible, maintained at 18-20°C, because variations in the ambient temperature could affect the rate of oxygen consumption

of the rats. In the preliminary experiments, for a few days at the height of summer, the room temperature rose to 23-25°C and the batch mean oxygen consumption fell below the inner control line (Fig. 2).

The sequential test devised by Wald (1947) was a most appropriate method of statistical inference for many of the experiments. The theory of this sequential "t-test" has only been developed in respect of two exhaustive and mutually exclusive composite hypotheses; the sample size is, of course, not fixed in advance but is determined by the nature of the data themselves, in relation to the degree of discrimination required, and the maximum acceptable probability of a wrong decision.

There are certain advantages, apart from economy of observations required, in such a test. The parameter δ, which determines the critical limits of the test can be freely chosen to fit the requirements of a particular experiment. Thus, in the first series of the present experiments, δ was taken as 1, so that the test would not distinguish between differences in the mean rates of oxygen consumption less than one standard deviation of the

mean difference. In the combined action experiments, where greater discrimination was desirable, δ was taken as 0.7.

There are two types of statistical error possible in such a test. We may reject H_0 when it is in fact true - Type I error, or we may accept H_0 when it is false - Type II error. The maximum probabilities of these errors have been designated α and β. This test, unlike Student's classical t-test, takes account of both types of statistical error, so that a negative conclusion (H_0) may be positively asserted with as much confidence as the alternative (H_1). A further advantage of Wald's test is the wide range of choice of values for α and β. Throughout this investigation the value of 0.05 was chosen for α and β.

Thus, by choosing suitable values of the parameters of this test, it is possible to set up realistic and appropriate tests for a very wide range of experimental situations.

The dose-response curves of the three cresotinates and salicylate exhibited a linear relationship between dose and difference in rate of

oxygen consumption between paired, treated and control rats (ΔO_2). There was, therefore, no need to transform dose to a logarithmic scale. The present dose-response relationship is consistent with the results of Alexander and Johnson (1956) on human subjects and of Reid (1957) on rabbits. This present assay was therefore a comparative slope-ratio assay and differed from the common parallel line assays in that the potency ratios depended on the gradients of the lines.

The first recorded use of a slope-ratio assay appears to be that of Birch and Harris (1934), although their discussion did not explicitly recognise the nature of the analysis. They found that the duration of cure of bradycardia in vitamin B_1 deficient rats was directly proportional to the dose of vitamin B_1 given, and proposed to estimate the potency of a test preparation by adjusting the dose scale until its response curve corresponded to that of the standard. Since then there have been a few other cases cited in the literature, concerning work on whole animals, in which the effect metameter has shown a linear relationship to dose e.g. Levin and Tyndale

(1937) on the quantitative assay of follicle stimulating substance; Bergmann and Turner (1939) studying the guinea pig and chick thyroid in the assay of thyrotropic hormone; O'Brien and Morgareidge (1939) on the effect of phosphorus on the biological estimation of vitamin D activity in rats with rickets; Emmens (1939) on the effect of androgens on the comb length of capons; and Bates, Riddle and Miller, (1940) on the assay of adrenotropic extracts on two day old chicks.

In recent years, increased interest in microbiological assays, based on linear dose-response relationships, has encouraged the derivation of analyses of a special case of slope-ratio assay, where both test and standard active substance are the same and where linearity extends down to a common positive response at zero dose (Wood, 1946). Various statistical methods of analysing such data have been developed, using the method of maximum likelihood and fitting the data to multiple regression equations (Wood and Finney, 1946).

The assay of the four metabolic stimulants described in the present investigation was novel; because (i) the common intercept on the Y axis was

negative, i.e. the dose-response curves must approach the X axis asymptotically (vide Fig. 4), (ii) it was a comparative assay, and therefore better not treated in terms of multiple regression.

A procedure derived by Silvey (1958), by the method of maximum likelihood, met the present requirements. The main features of this analysis were as follows:- if m drugs were given to n individuals at p doses, drug i being applied at doses x_{i1}, x_{i2}, ... x_{ip} then the response of the n individuals who received dose x_{ij} of drug i were denoted by y_{ij1}, y_{ij2} ..... y_{ijn}. The equation of the line for drug i was $y_i = a_i + b_i x_i$. Before estimating potency, compliance with the two fundamental conditions, linear dose-response relations and common intercepts, was first tested by a general analysis of variance. In testing for a common intercept, since the doses used were not the same for the four drugs, a weighted variance was used. It was shown that, in the present assay, the variability of the a_i's was due simply to the inherent variability of the experimental material and thus the regression equation of drug i was of the form

$y = \alpha + \beta_i x$ where α did not depend on the drug. This fact was used to calculate for each drug an estimate of β_i better than b_i and from these better estimates of b_i the potencies and the 95% confidence limits were calculated. The theory of this step was based on the assumption that the distribution of response to a particular dose of a particular drug was normal, and that the variance of this distribution depended neither on dose nor drug. The estimate of confidence limits was only valid when the standard deviation of b_i was small relative to β_i (Silvey, 1958).

The theory of the combined action experiments is well known and is amply discussed by Gaddum (1953).

The rates of degradation and excretion were compared from the time-concentration experiments. In every case, transformation of the drug plasma concentration to a logarithmic scale gave a linear relation with time, consistent with exponential time-concentration curves. Theoretically, this indicated that the type of curve obtained was similar to that exemplified classically by Newton's Law of Cooling.

Expressing this in the form of an equation $dc/dt = -kc$ i.e. the rate of fall in concentration, c, with time, t, was proportional to the concentration. By integrating, $\log c = -Kt + C$ i.e. the log of the concentration is indirectly proportional to time, where K and C are constants of the linear equation. The time-concentration curves thus fitted regression equation of the form $Y_j = a_j + b_j x_j$ where Y_j was the log of the plasma concentration and x_j was the time after injection. The only parameter necessary to define the rate of disappearance of the drug from the blood was therefore the regression coefficient, b_j; the intercepts on the Y axis were dependent on the initial dose injected.

Brodie, Soberman, Levy, Axelrod, Hollander and Steele (1949) in their studies on the degradation of antipyrine in the measurement of total body water and King and Harvey (1953) studying the absorption and excretion of dinitro-ortho-cresol have used a similar transformation and Gaddum (1944) has stated that, with the exception of alcohol, most drugs show this exponential type of decay.

The effect of body weight in relation to

the time-concentration experiments merits some discussion since it is a common practice to adopt a system of dosage in units of drug per unit body weight; this assumes, between these factors, an explicit relationship, the existence of which is not self evident. An analysis of covariance established that the drug plasma concentration of each drug was dependent on the rat's body weight. Including body weight in the regression analysis, new regression coefficients due to time were calculated; the regression coefficients of log concentration on time were not altered significantly by this inclusion. In the present circumstances it would have been pointless to make any allowance, in the dose injected, for body weight, when randomisation was clearly sufficient to control this factor.

Having considered the experimental conditions and theoretical background, the results of this investigation and their significance can now be discussed in more detail.

The compounds tested in the first experiments

fall into three groups:

(1) Inert. The drugs which at the dose administered did not alter the rate of oxygen consumption of rats were 2:3-dihydroxybenzoic acid, phthalic acid and 6-methylsalicylic acid.

Although Brody (1956) found that 2:3-dihydroxybenzoic acid was an inhibitor of oxidative phosphorylation of rat mitochondria he did not investigate its effect on the rate of oxygen uptake. In keeping with the present results, Meade (1954) found that the small dose of 50 mgms. 2:3-dihydroxybenzoic acid failed to increase the metabolic rate.

6-methylsalicylic acid was much more toxic than any of the other compounds which were tested but, in doses at which it was tolerated, it did not alter the metabolic rate.

(2) Drugs which decreased the rate of oxygen consumption of rats ("metabolic depressants"). Many of these compounds were tolerated at high doses. At such doses meta- and para-hydroxybenzoic acid, 2:4-, 2:5-, 2:6-, 3:4- and 3:5-dihydroxybenzoic acid depressed the rate of oxygen consumption of rats.

5-aminosalicylic acid, salicylamide, salicyluric acid and o-aminobenzoic acid were also metabolic depressants when given in doses similar to salicylic acid.

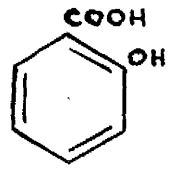
Meade (1954) used very much smaller doses of the hydroxybenzoic acids and found that only the meta substituted derivative was a metabolic depressant. 50 mgms. salicylate was a metabolic stimulant, and the same dose of para-hydroxybenzoate, 2:4-, 2:5-, 2:6-, 3:4- and 3:5-dihydroxybenzoate were inactive. In a preliminary report Hall, Tomich and Woollett (1954) stated that meta- and para-hydroxybenzoic acid, 2:5- and 2:6-dihydroxybenzoic acid and salicylamide had no effect on the rate of oxygen consumption of rats or mice but they did not state the doses used.

Gentisic acid i.e. 2:5-dihydroxybenzoic acid, and salicyluric acid have been isolated in the urine as metabolites of salicylic acid (Kapp and Coburn, 1942). Thus, in the body, some salicylic acid is converted from a metabolic stimulant to a depressant, which suggests that these two acids, salicyluric and gentisic, are true detoxication products in respect of their metabolic stimulant action.

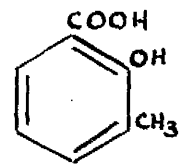
It was already known that the antituberculous drug, para-aminosalicylic acid i.e. 4-aminosalicylic acid, depressed the rate of oxygen consumption in man (MacGregor and Sommer, 1954) and, for this reason, the effect of 5-amino-salicylic acid on the metabolic rate was investigated. The 5-aminosalicylic acid was also a metabolic depressant.

(3) Drugs which increased the rate of oxygen consumption of rats ("metabolic stimulants"). The only compounds which increased the metabolic rate of rats were salicylic acid and the ortho, meta- and para-cresotinic acids.

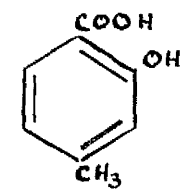
These acids have many chemical, physical and pharmacological properties in common. Chemically, they are all phenolic acids with the hydroxyl group in the ortho position relative to the carboxyl group.



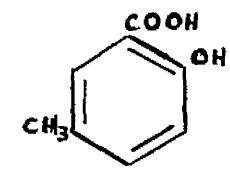
Salicylic Acid



o-Cresotinic Acid



m-Cresotinic Acid



p-Cresotinic Acid

The above acids are all volatile in steam, soluble in hot water, alcohol, ether and chloroform, (Lange, 1946) and all give a purple colour with ferric chloride. May (1909) found that the cresotinic acids resembled salicylic acid in their action as antifermentatives, as bactericides, as antipyretics and as specifics in acute rheumatism.

The qualitative similarity of these active acids both chemically and pharmacologically led naturally to the question of relative potency. Does the introduction of a methyl group into the benzene molecule alter the potency of salicylate as a metabolic stimulant? To answer this question the comparative assay of the three cresotinates against salicylate was undertaken.

The cresotinates were found to be more powerful metabolic stimulants than salicylate when administered in single doses intraperitoneally to rats; ortho-cresotinate was the most powerful with a potency ratio of 2.61, meta-cresotinate had a value of 1.78, and para-cresotinate had a value of 1.89. The fiducial limits of the ratios showed that these differences

in the potencies of the three cresotinates relative to salicylate were significant at the 95% level. The preceding potency ratios were calculated on a molar basis. The values would be numerically lower, but equally significant, computed as weight for weight, but this would take no account of the chemical equivalence of the drugs.

The introduction of the methyl group into the benzene ring therefore increased the potency of salicylate as a metabolic stimulant; the ortho position was the most effective. This raised the question, which will be fully investigated in due course, of the effect of other alkyl or aryl substituents in the ortho position. It is of interest to note, that preliminary observations with 3-phenyl salicylate suggest that it is even more potent than ortho-cresotinate. The mean ΔO_2 of six rats who received single 50 mgm. doses of 3-phenyl salicylate intraperitoneally was +162.1 mls./hr. (Table XLVIII). This is approximately equivalent to the effect produced by 95 mgms. ortho-cresotinate. Higher doses of 3-phenyl salicylate killed the animals in hyperventilation and convulsions - the same toxic

effects as salicylate and the cresotinates.

There were two obvious explanations to account for the higher potency of the cresotinates. The first of these was that the drugs differed in their primary actions.

It could be argued that if the conditions for the comparative assay were met then the drugs probably had a similar action. It was desirable, however, to put this to an experimental test. A study of the separate and combined actions of the drugs failed to establish a difference; administration of a mixture of salicylate and ortho-cresotinate gave a response indistinguishable from an additive effect. Thus, in the intact rat there was, potency excepted, pharmacologically no appreciable or significant distinction between the two acids.

The other main possibility was that the greater potency of the cresotinates was due to higher drug concentrations in the blood, which might easily occur as a result of different rates of degradation and excretion. The analysis of covariance of the regressions failed to show any difference in the rate

of degradation or excretion.

This inference is, in itself of some interest, since it would have been reasonable to suppose that the incorporation of a methyl group into the benzene ring of salicylate would have at least altered the proportions of the various detoxication products, and hence the overall rate of degradation and excretion.

It is concluded that the tissues themselves must, therefore, be more sensitive to the cresotinates, and that there must be true potency differences at the tissue level among the four acids. The explanation of this awaits further investigation.

Minor changes in the benzene molecule obviously alter the pharmacological activity. Points of interest which arose from this investigation were (i) for metabolic stimulant action, the carboxyl group must be free or at least not conjugated with glycine as in salicyluric acid nor with ammonia as in salicylamide, (ii) the ortho-hydroxyl group cannot be replaced by an amino or carboxyl group without destroying the metabolic stimulating property, and

(iii) the presence in the ring of a methyl group only destroyed the activity of salicylic acid when it occupied the 6-position i.e. the position ortho to the carboxyl group. It may be that the presence of the methyl group in the 6-position is introducing some important steric effects. In the present series, a necessary condition for stimulant action was, therefore, the presence of a hydroxyl group in the ortho position only, relative to the carboxyl group; the addition of a second hydroxyl group to any other position in the benzene ring eliminated this action.

It is interesting to note that several of the above compounds have been used or tried therapeutically in the treatment of rheumatic fever. In 1875, Buss announced his discovery of the therapeutic value of salicylic acid in acute rheumatic fever, and in the following year Buss (1876) published a further paper on the action of the closely related cresotinic acids showing that they exercised similar curative effects in this disease. Koranyi (1877) and Gatte (1879) (quoted by Demme, 1890) confirmed the

antipyretic properties of the cresotinic acids and suggested their use in medical practice for feverish conditions. In 1890, Demme prepared pure samples of ortho-, meta and para-cresotinic acids for physiological and therapeutic use and found sodium para-cresotinate to be harmless but therapeutically effective in the treatment of febrile diseases. He stated that meta-cresotinate was less effective, and ortho-cresotinate, although acting quicker in smaller doses, was unsuitable because it caused "paralysis of the heart muscles".

May (1909) has shown that the toxic effects of salicylate and the three cresotinates are similar. Comparable doses of the four drugs produced death due to convulsions and hyperventilation. Stockman (1912) using the sodium salts of the three cresotinic acids in the treatment of rheumatic fever concluded that, for practical purposes, they were inferior to sodium salicylate. He felt that, therapeutically sodium meta-cresotinate had the same action and much the same value as sodium salicylate, the para- compound was distinctly less active and the ortho-compound, although very active, had an undesirable "slowing

and depressing influence" on the heart.

The work of Stockman and others has not been followed up, and apart from the introduction (Dobner, 1930 and Reischel, 1930) of Amatin, i.e. acetyl meta-cresotinic acid, as a new antipyretic and anti-neuralgesic, alleged to cause "no irritation of the stomach and no marked perspiration" the cresotinic acids have remained almost unknown as therapeutic agents.

The isomers of salicylic acid - meta- and para-hydroxybenzoic acid are ineffective in the treatment of rheumatic fever (Stockman 1920), and although salicylamide (Litter, Moreno and Donin, 1951) sodium gentisate, (Meyer and Ragan, 1948), sodium γ-resorcylate (Reid, Watson, Cochran and Sproull, 1951) and 2:3-dihydroxybenzoic acid (Michotte and Danaux, 1952) have all been reported as effective in the treatment of acute rheumatism, none has yet replaced the old established drugs - aspirin and sodium salicylate. The present results show that many of these antirheumatic drugs fail to increase the metabolic rate of rats. Many of them are, in fact, depressants. In the past, several workers (Cochran, 1952, Hall, Tomich and Woollett, 1954, Reid,

1957, Adams and Cobb, 1958) have discussed the possible mode of action of salicylate in rheumatic fever and related it to its metabolic stimulating property without producing any conclusive evidence. It is clear, however, from the present results, that ability to increase metabolic rate is not essential, although, within this series, it may be sufficient for antirheumatic activity.

Salicylic acid and 2:4-dinitrophenol are well known metabolic stimulants; slight changes in the salicylate molecule can eliminate this effect. Similarly, Cameron (1958), in her study of the nitrophenols, found that 2:4-dinitrophenol and its methyl derivative, dinitro-ortho-cresol, were the only compounds which increased the oxygen consumption of rats.

No generalisations have yet been made for the phenolic acids but it appears that they are as specific as the nitrophenols, since only ortho-hydroxybenzoic acid and the 3, 4, and 5 methyl substituted derivatives had metabolic stimulating properties.

The immediate importance of the present investigation is that it forms a foundation for further

studies by physical chemists and pharmacologists. What physical properties of salicylate and the cresotinates account for the present potency ratios of these drugs as metabolic stimulants in the intact rat? This fundamental question is posed rather than answered by the present results.

Appendix.

Statistical Methods.

Comparative dilution assay of four metabolic stimulants.

(Silvey, 1958).

(1). Analysis of variance.

Source of variation	Sum of squares	d.f.
Between intercepts	$np\ S_I^2$	m-1
Deviation of mean response from linear regression	$n\ S_D^2$	m(p-2)
Residual	S^2	mp(n-1)

m = Number of drugs.

p = Number of doses for each drug.

n = Number of individuals receiving dose x_{ij} of drug i.

$$S^2 = \sum_{ijk}(y_{ijk} - y_{ij})^2 = \sum_{ijk} y_{ijk}^2 - n\sum_{ijk} y_{ij.}^2$$

$y_{ij.}$ is the mean response to dose x_{ij} of drug i.

$$S_D^2 = S_{D_1}^2 + S_{D_2}^2 \cdots\cdots S_{Dm}^2$$

$$S_{Di}^2 = \sum_j y_{ij.}^2 - p y_{i..}^2 - b_i^2 \left(\sum_j x_{ij}^2 - p x_{i.}^2 \right)$$

$$b_i = \frac{\sum_j x_{ij} y_{ij.} - p x_{i.} y_{i..}}{\sum_j x_{ij}^2 - p x_{i.}^2}$$

$x_{i.}$ = mean dose of drug i.

$y_{i..}$ = overall mean response to drug i.

A comparison of $\frac{1}{m(p-2)} n S_D^2$ with $\frac{1}{mp(n-1)} S^2$ by means of an F-test decides whether the regressions of response on dose are linear.

$$S_I^2 = w_1 a_1^2 + w_2 a_2^2 \cdots\cdots w_m a_m^2 - w \bar{a}^2$$

S_I^2 is a weighted sum of squares for the a_i's.

$$w_i = 1 - \frac{p x_{i.}^2}{\sum_j x_{ij}^2}$$

$$w = w_1 + w_2 \cdots\cdots w_m$$

$$\bar{a} = \frac{1}{w} \left(w_1 a_1 + w_2 a_2 \cdots w_m a_m \right)$$

A comparison of $\frac{1}{m-1} npS_I^2$ with $\frac{1}{mp(n-1)} S^2$ decides whether the intercepts are all the same.

(2) Estimation of potency ratio.

The regression of drug i is of the form $y_i = \alpha + \beta_i x_i$ where α does not depend on i. Using this fact we obtain an estimate of β_i better than b_i

$$b_i' = \frac{\sum_j x_{ij} y_{ij.} - p\bar{a} x_{i.}}{\sum_j x_{ij}^2}$$

Estimate of the variance of b_i' is

$$v_{ii} = \frac{\hat{\sigma}^2}{n}\left[\frac{1}{\sum_j x_{ij}^2} + \frac{p}{w}\left(\frac{x_{i.}}{\sum_j x_{ij}^2}\right)^2\right]$$

and the covariance of b_i' and b_k'

$$v_{ik} = \frac{\hat{\sigma}^2}{n} \frac{x_{i.} x_{k.}}{\sum_j x_{ij}^2 \sum_j x_{kj}^2} \frac{p}{w}$$

$\hat{\sigma}^2$ is the residual mean square in the analysis of variance above.

Potency ratio $P = \dfrac{b_i'}{b_k'}$

(3) 95% confidence limits (θ_1, θ_2)

$$\theta^2\left(b_k'^2 - v_{kk}t^{*2}\right) - 2\theta\left(b_i'b_k' - v_{ik}t^{*2}\right) + \left(b_i'^2 - v_{ii}t^{*2}\right) = 0$$

θ_1 and θ_2 are the smaller and larger roots of this equation. t^* is the 5% value of a t-distribution with mp(n-1) degrees of freedom.

Index to Tables.

Tables XVI - XXXIII give the results of the experiments to determine the effect of eighteen substituted benzoates on the rate of oxygen consumption of Wistar albino rats.

Tables XXXIV - XXXVII give the results of the dose-response experiments for salicylic acid, ortho-, meta- and para-cresotinic acid.

Tables XXXVIII - XLIII give the results of the combined action experiments of salicylic acid and ortho-cresotinic acid.

Tables XLIV - XLVII give the results of the time concentration experiments for salicylic acid, ortho-, meta- and para-cresotinic acid.

Table XVI.

The effect of 120 mgms. salicylic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	29.11.56.	F	242	537.0	614.8	+77.8
2	30.11.56.	M	290	420.4	454.4	+34.0
3	3.12.56.	F	256	357.2	386.4	+29.2
4	3.12.56.	M	284	332.9	498.2	+165.3
5	4.12.56.	M	269	354.8	420.4	+65.6
6	4.12.56.	F	240	461.7	444.7	-17.0
7	5.12.56.	M	290	393.7	471.4	+77.7
8	6.12.56.	M	268	430.1	456.8	+26.7
9	7.12.56.	F	250	435.0	493.3	+58.3
10	7.12.56.	F	265	466.6	483.6	+17.0
	Average			418.9	472.4	+53.5±34.6

H_1 accepted (Z = 5.61).

Table XVII.

The effect of 500 mgm. m. hydroxybenzoic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	11.12.56.	M	270	418.0	308.6	-109.4
2	12.12.56.	M	256	425.3	252.7	-172.6
3	13.12.56.	M	255	420.4	308.6	-111.8
4	14.12.56.	F	275	418.0	260.0	-158.0
5	14.12.56.	F	256	617.2	284.3	-332.9
6	17.12.56.	F	270	447.1	294.0	-153.1
	Average			457.7	284.7	-173.0±103.9

H_1 accepted ($Z = 5.05$).

Table XVIII.

The Effect of 500 mgms. p. hydroxybenzoic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	2.2.57.	M	238	342.6	323.2	-19.4
2	3.2.57.	M	260	478.7	364.5	-114.2
3	3.2.57.	M	264	410.7	315.9	-94.8
4	6.2.57.	M	258	405.8	330.5	-75.3
5	7.2.57.	F	230	296.5	255.2	-41.3
6	7.2.57.	F	233	442.3	279.5	-162.8
7	8.2.57.	F	262	342.6	325.6	-17.0
8	8.2.57.	F	260	558.9	459.3	-99.6
	Average			409.8	331.7	-78.1±42.1

H_1 accepted (Z = 5.86).

Table XIX.

The effect of 100 mgms. 2:3-dihydroxybenzoic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	10.9.57.	F	253	498.2	495.7	-2.5
2	10.9.57.	F	246	431.0	612.0	+181.0
3	10.9.57.	F	243	330.5	267.3	-63.2
4	10.9.57.	F	236	479.4	288.2	-191.2
5	11.9.57.	M	242	420.4	371.8	-48.6
6	11.9.57.	M	260	448.8	385.1	-63.7
7	11.9.57.	M	240	471.8	397.8	-74.0
8	11.9.57.	M	232	376.7	430.1	+53.4
9	12.9.57.	F	232	392.7	336.6	-56.1
10	12.9.57.	M	230	381.5	376.7	-4.8
11	12.9.57.	M	248	447.1	405.8	-41.3
12	12.9.57.	M	260	482.0	487.1	+5.1
	Average			430.0	404.5	-25.5 ± 55.9

H_o accepted ($Z = 1.01$).

Table XX.

The effect of 300 mgms. 2:4-dihydroxybenzoic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	24.12.56.	F	260	471.4	354.8	-116.6
2	24.12.56.	F	258	413.1	320.8	-92.3
3	27.12.56.	F	267	459.3	437.4	-21.9
4	27.12.56.	M	264	493.3	388.8	-104.5
5	28.12.56.	F	241	464.2	301.3	-162.9
6	28.12.56.	M	252	478.7	432.5	-46.2
7	4.1.57.	M	242	415.5	369.4	-46.1
		Average		456.6	372.2	-84.4 ± 44.5

H_1 accepted ($Z = 5.42$).

Table XXI.

The effect of 500 mgms. 2:5-dihydroxybenzoic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	9.1.57.	M	265	471.4	102.1	-369.3
2	10.1.57.	M	280	539.5	114.2	-425.3
3	14.1.57.	F	263	476.3	150.7	-325.6
4	15.1.57.	M	262	507.9	160.4	-347.5
5	16.1.57.	F	235	398.5	150.7	-247.8
6	16.1.57.	F	260	452.0	170.1	-281.9
	Average			474.3	141.4	-332.9±71.6

H_1 accepted (Z = 5.83).

Table XXII.

The effect of 200 mgms. 2:6-dihydroxybenzoic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	12.3.57.	M	261	495.7	345.1	-150.6
2	13.3.57.	M	243	364.5	340.2	-24.3
3	13.3.57.	M	260	517.6	257.6	-260.0
4	14.3.57.	F	232	425.3	376.7	-48.6
5	14.3.57.	F	234	442.3	332.9	-109.4
6	15.3.57.	F	234	371.8	255.2	-116.6
7	15.3.57.	F	240	452.0	311.0	-141.0
	Average			438.4	316.9	-121.5±71.0

H_1 accepted ($Z = 5.22$).

Table XXIII.

The effect of 500 mgms. 3:4-dihydroxybenzoic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	18.1.57.	F	263	522.5	320.8	-191.7
2	21.1.57.	F	247	398.5	522.5	+124.0
3	22.1.57.	M	253	449.6	308.6	-141.0
4	23.1.57.	F	240	451.4	369.8	-81.6
5	23.1.57.	M	243	430.1	342.6	-87.5
6	24.1.57.	F	238	410.7	291.6	-119.1
7	24.1.57.	M	268	469.2	451.4	-17.8
8	25.1.57.	F	248	545.7	369.8	-175.9
9	25.1.57.	M	236	396.1	238.1	-158.0
10	2.2.57.	M	260	473.9	303.8	-170.1
	Average			454.8	351.9	-102.9±67.4

H_1 accepted ($Z = 5.70$).

Table XXIV.

The effect of 500 mgms. 3:5-dihydroxybenzoic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	18.2.57.	M	244	546.8	340.2	-206.6
2	18.2.57.	M	265	483.6	413.1	-70.5
3	19.2.57.	F	270	447.1	349.9	-97.2
4	21.2.57.	F	248	401.0	342.6	-58.4
5	21.2.57.	M	243	493.3	340.2	-153.1
6	22.2.57.	F	278	418.0	401.0	-17.0
7	22.2.57.	F	255	476.3	347.5	-128.8
	Average			466.6	362.1	-104.5±69.9

H_1 accepted (Z = 5.31).

Table XXV.

The effect of 100 mgms. 5-aminosalicylic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO^2 mls./hr. (1)-(2)
1	3.4.57.	M	254	422.8	401.0	-21.8
2	3.4.57.	F	230	366.9	298.9	-68.0
3	3.4.57.	M	270	428.4	267.7	-160.7
4	3.4.57.	F	235	369.8	280.5	-89.3
5	1.5.57.	M	232	461.7	352.4	-109.3
6	1.5.57.	F	230	321.3	285.6	-35.7
7	2.5.57.	M	255	425.9	244.8	-181.1
	Average			399.5	304.4	-95.1±55.4

H_1 accepted ($Z = 5.22$).

Table XXVI.

The effect of 100 mgms. salicyluric acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	4.4.57.	M	254	427.7	432.5	+4.8
2	4.4.57.	F	272	418.0	352.4	-65.6
3	5.4.57.	M	252	357.0	321.3	-35.7
4	5.4.57.	F	257	344.3	275.4	-68.9
5	30.4.57.	M	240	386.4	357.2	-29.2
6	1.5.57.	F	253	374.2	325.6	-48.6
7	1.5.57.	M	260	479.4	367.2	-112.2
8	1.5.57.	F	240	321.3	300.9	-20.4
	Average			388.6	341.7	-46.9±29.9

H_1 accepted ($Z = 5.32$).

Table XXVII.

The effect of 50 mgms. salicylamide on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	1.6.57.	M	242	335.3	296.5	-38.8
2	1.6.57.	M	260	354.5	196.4	-158.1
3	17.7.57.	F	244	449.6	308.6	-141.0
4	17.7.57.	M	282	494.7	372.4	-122.4
5	18.7.57.	F	245	388.8	240.6	-148.2
6	18.7.57.	M	285	433.5	270.3	-163.2
	Average			409.4	280.8	-128.6±46.8

H_1 accepted (Z = 5.43).

Table XXVIII.

The effect of 100 mgms. 3-methyl salicylic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	21.3.57.	F	246	390.2	487.1	+96.9
2	21.3.57.	F	236	403.4	565.0	+161.6
3	21.3.57.	M	245	257.6	558.5	+300.9
4	22.3.57.	F	236	418.2	520.2	+102.0
5	22.3.57.	M	250	405.8	661.0	+255.2
6	22.3.57.	M	278	497.3	599.3	+102.0
7	22.3.57.	F	238	398.5	612.4	+213.9
	Average			395.9	572.0	+176.1±76.7

H_1 accepted (Z = 5.89).

Table XXIX.

The effect of 100 mgms. 4-methyl salicylic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	18.7.57.	M	268	366.9	488.3	+121.4
2	18.7.57.	M	285	329.0	456.5	+127.5
3	19.7.57.	F	240	413.1	532.2	+119.1
4	19.7.57.	M	232	380.0	515.1	+135.1
5	29.7.57.	F	232	403.4	439.8	+36.4
6	29.7.57.	F	236	418.2	538.1	+119.9
	Average			385.1	495.0	+109.9±33.6

H_1 accepted (Z = 5.49).

Table XXX.

The effect of 105 mgms. 5-methyl salicylic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	6.5.57.	M	250	369.4	583.2	+213.8
2	23.5.57.	F	238	313.5	459.3	+145.8
3	6.5.57.	M	246	318.8	632.4	+313.6
4	23.5.57.	F	230	441.2	589.1	+147.9
5	6.5.57.	M	230	447.1	524.9	+77.8
6	24.5.57.	F	230	318.3	532.2	+213.9
7	24.5.57.	M	285	545.7	678.3	+132.6
8	24.5.57.	F	238	311.1	558.5	+247.4
	Average			383.2	569.8	+186.6±62.9

H_1 accepted (Z = 5.25).

Table XXXI.

The effect of 24 mgms. 6-methyl salicylic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	30.7.57.	F	232	377.4	364.7	-12.7
2	30.7.57.	F	250	444.7	376.7	-68.0
3	9.9.57.	F	275	461.6	405.5	-56.1
4	9.9.57.	F	250	393.7	609.9	+216.2
5	13.9.57.	M	230	359.6	390.2	+30.6
6	13.9.57.	M	255	393.7	481.1	+87.4
7	16.9.57.	M	268	486.0	544.3	+58.3
8	16.9.57.	M	235	494.7	510.0	+15.3
9	18.9.57.	F	246	397.8	410.6	+12.8
10	18.9.57.	F	256	617.2	549.2	-68.0
	Average			442.6	464.2	+21.6±61.7

H_o accepted (Z = 0.65).

Table XXXII.

The effect of 100 mgms. o-aminobenzoic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	28.5.57.	M	265	345.1	252.7	-92.4
2	28.5.57.	F	232	366.9	332.9	-34.0
3	31.5.57.	M	283	359.6	295.8	-63.8
4	31.5.57.	M	286	380.0	334.1	-45.9
5	31.5.57.	F	230	379.1	323.2	-55.9
6	31.5.57.	M	265	437.4	281.9	-155.5
7	1.6.57.	M	247	385.1	351.9	-33.2
	Average			379.0	310.3	-68.7 ± 40.7

H_1 accepted ($Z = 5.12$).

Table XXXIII.

The effect of 100 mgms. Phthalic Acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	13.9.57.	F	255	517.7	408.0	-109.7
2	13.9.57.	F	232	461.7	469.0	+8.3
3	19.9.57.	M	240	476.3	435.0	-41.3
4	19.9.57.	M	232	357.0	374.9	+17.9
5	20.9.57.	F	250	473.9	435.0	-38.9
6	20.9.57.	F	245	428.4	382.5	-45.9
7	25.9.57.	M	240	469.2	436.1	-33.1
8	25.9.57.	M	270	487.0	609.9	+122.9
9	26.9.57.	F	232	405.4	402.9	-2.5
	Average			452.9	439.3	-13.6±48.9

H_o accepted ($Z = 0.44$).

Table XXXIV.

Oxygen consumption of rats after injection of salicylic acid.

No.	Date	Dose mgms.	Wt. gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff ΔO_2 mls./hr. (1)-(2)
1	1.10.57.	30	225	F	428.4	451.4	+23.0
2	15.10.57.	30	210	M	444.7	388.8	-55.9
3	18.10.57.	30	225	F	372.3	395.3	+23.0
4	5.11.57.	30	210	F	284.3	403.4	-119.1
5	7.11.57.	30	210	M	362.1	367.2	+5.1
6	12.12.57.	30	207	F	422.8	398.5	-24.3
7	13.12.57.	30	210	M	402.9	397.8	-5.1
8	18.12.57.	30	203	M	408.2	301.3	-106.9
1	20.12.57.	60	202	F	390.2	321.3	-68.9
2	9.1.58.	60	220	M	418.0	345.1	-72.9
3	13.1.58.	60	212	F	436.1	385.1	-51.0
4	16.1.58.	60	232	M	415.5	369.4	-46.1
5	20.1.58.	60	202	M	364.7	385.1	+20.4
6	21.1.58.	60	225	F	408.2	408.2	0
7	22.1.58.	60	200	M	334.1	321.3	-12.8
8	13.2.58.	60	175	F	359.6	386.4	+26.8
1	2.10.57.	90	238	M	427.9	418.0	-9.9
2	7.10.57.	90	229	M	413.1	433.5	+20.4
3	10.10.57.	90	208	F	340.2	454.4	+114.2
4	14.10.57.	90	205	F	323.9	374.9	+51.0
5	8.11.57.	90	205	F	452.0	454.1	+2.1
6	27.11.57.	90	205	M	352.4	403.4	+51.0
7	28.11.57.	90	244	F	346.8	446.3	+99.5
8	2.12.57.	90	203	M	364.7	433.5	+68.8
9	17.2.58.	90	180	F	374.9	482.0	+107.1
10	28.2.58.	90	230	M	383.9	403.4	+19.5
11	4.3.58.	90	240	M	441.2	431.0	-10.2
12	7.3.58.	90	230	F	447.1	349.9	-97.2

contd.

Table XXXIV. (contd.)

Salicylic Acid.

No.	Date	Dose mgms.	Wt. gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	30.9.57.	120	238	M	454.4	478.7	+24.3
2	8.10.57.	120	235	F	374.2	388.8	+14.6
3	9.10.57.	120	205	F	402.9	428.4	+25.5
4	17.10.57.	120	223	F	382.5	448.8	+66.3
5	6.11.57.	120	210	F	422.8	396.1	-26.7
6	14.11.57.	120	208	M	308.6	500.6	+192.0
7	26.11.57.	120	215	M	329.0	415.7	+86.7
8	11.12.57.	120	210	M	349.4	357.0	+7.6
9	19.2.58.	120	205	F	349.4	385.1	+35.7
10	26.2.58.	120	230	M	437.4	486.0	+48.6
11	5.3.58.	120	190	F	328.1	486.0	+157.9
12	6.3.58.	120	175	M	362.1	482.0	+119.9
1	19.12.57.	135	224	F	342.6	503.0	+160.4
2	10.1.58.	135	215	M	392.7	487.1	+84.4
3	14.1.58.	135	244	M	420.4	476.3	+55.9
4	15.1.58.	135	204	M	357.0	502.4	+145.4
5	17.1.58.	135	203	M	430.1	486.0	+55.9
6	10.2.58.	135	204	F	339.2	499.8	+160.6
7	11.2.58.	135	200	F	357.2	522.5	+165.3
8	12.2.58.	135	195	F	354.5	364.7	+10.2
9	18.2.58.	135	235	M	391.2	413.1	+21.9
10	20.2.58.	135	200	M	336.6	346.8	+10.2
11	25.2.58.	135	230	F	386.4	444.7	+58.3
12	27.2.58.	135	205	F	321.3	502.4	+181.1
1	4.10.57.	150	225	F	369.8	436.1	+66.3
2	11.10.57.	150	220	F	364.5	556.5	+192.0
3	21.10.57.	150	230	F	408.2	432.5	+24.3
4	4.11.57.	150	205	M	323.9	471.8	+147.9
5	11.11.57.	150	205	M	339.2	494.7	+155.5
6	29.11.57.	150	203	M	323.2	473.9	+150.7
7	16.12.57.	150	220	M	401.0	469.0	+68.0
8	17.12.57.	150	240	F	433.5	418.2	-15.3
9	14.2.58.	150	225	M	381.5	583.2	+201.7
10	21.2.58.	150	205	F	349.9	478.7	+128.8
11	24.2.58.	150	215	M	387.6	517.7	+130.1
12	3.3.58.	150	210	F	420.8	431.0	+10.2.

Summary of Table XXXIV.

Salicylic Acid.

Dose mgms.	Mean ΔO_2 mls./hr.
30	-32.5
60	-25.6
90	+34.7
120	+62.7
135	+92.5
150	+105.2

Table XXXV.

Oxygen consumption of rats after injection of ortho-cresotinic acid.

No.	Date	Dose mgms.	Wt. gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	4.10.57.	25	243	F	323.2	320.8	-2.4
2	11.10.57.	25	210	F	405.5	402.9	-2.6
3	21.10.57.	25	215	F	397.8	415.7	+17.9
4	4.11.57.	25	215	M	413.1	349.9	-63.2
5	11.11.57.	25	204	M	323.2	466.6	+143.4
6	29.11.57.	25	215	M	400.4	400.4	0
7	16.12.57.	25	238	M	456.5	397.8	-58.7
8	17.12.57.	25	218	F	452.0	398.5	-53.5
1	1.10.57.	50	240	F	473.9	449.6	-24.3
2	15.10.57.	50	205	M	428.4	448.8	+20.4
3	18.10.57.	50	230	F	418.0	425.3	+7.3
4	5.11.57.	50	208	F	438.6	415.7	-22.9
5	7.11.57.	50	201	M	405.8	422.8	+17.0
6	12.12.57.	50	230	F	405.5	346.8	-58.7
7	13.12.57.	50	205	M	403.4	471.4	+68.0
8	18.12.57.	50	215	M	436.1	459.0	+22.9
1	19.12.57.	62.5	240	F	479.4	484.5	+5.1
2	10.1.58.	62.5	232	M	374.2	403.4	+29.2
3	14.1.58.	62.5	230	M	382.5	441.2	+58.7
4	15.1.58.	62.5	204	M	381.5	486.0	+104.5
5	17.1.58.	62.5	203	M	385.1	408.0	+22.9
6	10.2.58.	62.5	200	F	383.9	435.0	+51.1
7	11.2.58.	62.5	200	F	331.5	469.2	+137.7
8	12.2.58.	62.5	190	F	337.8	388.8	+51.0
9	19.2.58.	62.5	245	F	320.8	473.9	+153.1
10	26.2.58.	62.5	225	M	318.8	474.3	+155.5
11	5.3.58.	62.5	185	F	341.7	420.8	+79.1
12	6.3.58.	62.5	195	M	301.3	439.8	+138.5

contd.

Table XXXV. (contd.)

o-Cresotinic Acid.

No.	Date	Dose mgms.	Wt. gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff.ΔO_2 mls./hr. (1)-(2)
1	30.9.57.	75	220	M	476.9	571.2	+94.3
2	8.10.57.	75	220	F	359.6	558.5	+198.9
3	9.10.57.	75	215	F	318.3	529.7	+211.4
4	17.10.57.	75	230	F	376.7	478.7	+102.0
5	6.11.57.	75	235	F	436.1	479.4	+43.3
6	14.11.57.	75	225	M	316.2	420.8	+104.6
7	26.11.57.	75	201	M	422.8	622.1	+199.3
8	11.12.57.	75	205	M	454.1	490.9	+36.8
9	17.2.58.	75	175	F	359.6	422.8	+63.2
10	28.2.58.	75	220	M	377.4	548.3	+170.9
11	4.3.58.	75	250	M	381.5	520.0	+138.5
12	7.3.58.	75	205	F	392.7	558.5	+165.8
1	20.12.57.	87.5	203	F	352.4	510.3	+157.9
2	9.1.58.	87.5	203	M	346.8	436.1	+89.3
3	13.1.58.	87.5	215	F	376.7	456.8	+80.1
4	16.1.58.	87.5	200	M	400.4	540.6	+140.2
5	20.1.58.	87.5	202	M	315.9	563.8	+247.9
6	21.1.58.	87.5	222	F	374.9	515.1	+140.2
7	22.1.58.	87.5	215	M	413.1	505.4	+92.3
8	13.2.58.	87.5	195	F	367.2	550.8	+183.6
9	14.2.58.	87.5	220	M	372.3	546.3	+174.0
10	21.2.58.	87.5	180	F	397.8	420.8	+23.0
11	24.2.58.	87.5	240	M	376.7	532.2	+155.5
12	3.3.58.	87.5	215	F	439.8	571.1	+131.3
1	2.10.57.	100	210	M	420.8	734.4	+313.6
2	7.10.57.	100	248	M	495.7	731.4	+235.7
3	10.10.57.	100	230	F	385.1	721.7	+336.6
4	14.10.57.	100	220	F	379.1	590.9	+211.8
5	8.11.57.	100	210	F	415.7	507.5	+91.8
6	27.11.57.	100	215	M	392.7	624.8	+232.1
7	28.11.57.	100	244	F	425.3	529.7	+104.4
8	2.12.57.	100	200	M	461.7	546.8	+85.1
9	18.2.58.	100	220	M	410.6	487.1	+76.5
10	20.2.58.	100	235	M	420.4	624.5	+204.1
11	25.2.58.	100	200	F	385.1	561.0	+175.9
12	27.2.58.	100	215	F	422.8	488.4	+65.6

Summary of Table XXXV.

o-Cresotinic Acid.

Dose mgms.	Mean ΔO_2 mls./hr.
25	-2.4
50	+3.7
62.5	+82.2
75	+127.4
87.5	+134.7
100	+177.8

Table XXXVI.

Oxygen consumption of rats after injection of meta-cresotinic acid.

No.	Date	Dose mgms.	Wt. gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	2.10.57.	25	240	M	439.8	403.4	-36.4
2	7.10.57.	25	238	M	464.1	441.2	-22.9
3	10.10.57.	25	246	F	415.5	422.8	+7.3
4	14.10.57.	25	210	F	438.6	367.2	-71.4
5	8.11.57.	25	213	F	425.3	386.4	-38.9
6	27.11.57.	25	203	M	328.1	357.2	+29.1
7	28.11.57.	25	233	F	377.4	339.2	-38.2
8	2.12.57.	25	203	M	459.0	369.8	-89.2
1	4.10.57.	50	215	F	362.1	339.2	-22.9
2	8.10.57.	50	248	F	388.8	374.2	-14.6
3	9.10.57.	50	205	F	349.4	326.4	-23.0
4	17.10.57.	50	220	F	397.8	308.6	-89.2
5	11.11.57.	50	210	M	390.2	385.1	-5.1
6	14.11.57.	50	203	M	388.8	391.2	+2.4
7	26.11.57.	50	216	M	346.8	400.4	+53.6
8	11.12.57.	50	210	M	293.3	349.4	+56.1
1	20.12.57.	62.5	202	F	385.1	433.5	+48.4
2	9.1.58.	62.5	232	M	347.5	418.0	+70.5
3	13.1.58.	62.5	202	F	545.7	482.0	-63.7
4	17.1.58.	62.5	202	M	349.9	357.2	+7.3
5	20.1.58.	62.5	230	M	397.8	433.5	+35.7
6	21.1.58.	62.5	202	F	376.7	430.1	+53.4
7	22.1.58.	62.5	225	M	448.8	451.4	+2.6
8	13.2.58.	62.5	175	F	364.5	391.2	+26.7
9	18.2.58.	62.5	245	M	403.4	454.1	+50.7
10	20.2.58.	62.5	185	M	425.9	420.8	-5.1
11	25.2.58.	62.5	225	F	408.2	437.4	+29.2
12	27.2.58.	62.5	195	F	390.2	372.3	-17.9

contd.

Table XXXVI. (contd.)

m-Cresotinic Acid.

No.	Date	Dose mgms.	Wt. gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	1.10.57.	75	210	F	380.0	402.9	+22.9
2	15.10.57.	75	208	M	452.0	512.7	+60.7
3	18.10.57.	75	208	F	341.7	443.7	+102.0
4	5.11.57.	75	243	F	466.6	558.9	+92.3
5	7.11.57.	75	220	M	433.5	448.8	+15.3
6	12.12.57.	75	212	F	418.0	493.3	+75.3
7	13.12.57.	75	240	M	402.9	484.5	+81.6
8	18.12.57.	75	205	M	388.8	437.4	+48.6
9	14.2.58.	75	190	M	376.7	522.5	+145.8
10	21.2.58.	75	210	F	383.9	452.0	+68.1
11	24.2.58.	75	200	M	372.3	466.7	+94.4
12	3.3.58.	75	185	F	397.8	431.0	+33.2
1	19.12.57.	87.5	204	F	371.8	452.0	+80.2
2	10.1.58.	87.5	202	M	341.7	372.3	+30.6
3	14.1.58.	87.5	238	M	383.9	442.3	+58.4
4	15.1.58.	87.5	204	M	380.0	474.2	+94.2
5	16.1.58.	87.5	202	M	388.8	469.0	+80.2
6	10.2.58.	87.5	215	F	377.4	476.9	+99.5
7	11.2.58.	87.5	200	F	374.2	507.9	+133.7
8	12.2.58.	87.5	185	F	390.2	428.4	+38.2
9	17.2.58.	87.5	185	F	367.2	405.5	+38.3
10	2.3.58.	87.5	225	M	315.9	495.7	+179.8
11	4.3.58.	87.5	225	M	308.6	512.6	+204.0
12	7.3.58.	87.5	185	F	337.8	401.0	+63.2
1	30.9.57.	100	220	M	456.8	471.4	+14.6
2	11.10.57.	100	220	F	418.0	503.0	+85.0
3	21.10.57.	100	232	F	405.8	469.0	+63.2
4	4.11.57.	100	228	M	492.2	553.4	+61.2
5	6.11.57.	100	212	F	437.4	449.6	+12.2
6	29.11.57.	100	202	M	386.4	495.7	+109.3
7	16.12.57.	100	203	M	422.8	561.3	+138.5
8	17.12.57.	100	223	F	367.2	428.4	+61.2
9	19.2.58.	100	180	F	318.8	466.7	+147.9
10	26.2.58.	100	245	M	401.0	670.7	+269.7
11	5.3.58.	100	218	F	393.7	524.9	+131.2
12	6.3.58.	100	230	M	431.0	510.0	+79.0

Summary of Table XXXVI.

m-Cresotinic Acid.

Dose mgms.	Mean ΔO_2 mls./hr.
25	-32.6
50	-5.3
62.5	+19.8
75	+70.0
87.5	+91.7
100	+97.8

Table XXXVII.

Oxygen consumption of rats after injection of para-cresotinic acid.

No.	Date	Dose mgms.	Wt. gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	30.9.57.	25	204	M	456.6	372.3	-84.3
2	8.10.57.	25	237	F	425.9	415.7	-10.2
3	9.10.57.	25	220	F	364.5	364.5	0
4	17.10.57.	25	228	F	379.1	388.8	+9.7
5	6.11.57.	25	220	F	382.5	367.2	-15.3
6	14.11.57.	25	210	M	418.2	410.6	-7.6
7	26.11.57.	25	212	M	364.5	362.1	-2.4
8	11.12.57.	25	208	M	403.4	415.5	+12.1
1	2.10.57.	50	220	M	466.7	461.6	-5.1
2	7.10.57.	50	248	M	464.1	449.6	-14.5
3	10.10.57.	50	220	F	400.4	392.7	-7.7
4	14.10.57.	50	220	F	379.1	405.8	+26.7
5	8.11.57.	50	230	F	428.4	402.9	-25.5
6	27.11.57.	50	218	M	436.1	431.0	-5.1
7	28.11.57.	50	233	F	349.9	374.2	+24.3
8	2.12.57.	50	203	M	393.7	398.5	+4.8
1	19.12.57.	62.5	216	F	377.4	464.1	+86.7
2	10.1.58.	62.5	215	M	347.5	374.2	+26.7
3	14.1.58.	62.5	220	M	385.1	377.4	-7.7
4	15.1.58.	62.5	232	M	374.2	405.8	+31.6
5	16.1.58.	62.5	202	M	339.2	344.3	+5.1
6	10.2.58.	62.5	200	F	396.1	393.7	-2.4
7	11.2.58.	62.5	200	F	369.8	461.6	+91.8
8	12.2.58.	62.5	180	F	379.1	464.1	+85.0
9	14.2.58.	62.5	175	M	367.2	482.0	+114.8
10	21.2.58.	62.5	195	F	349.4	448.8	+99.4
11	24.2.58.	62.5	245	M	398.5	469.0	+70.5
12	3.3.58.	62.5	205	F	401.0	422.8	+21.8

contd.

Table XXXVII. (contd.).

p-Cresotinic Acid.

No.	Date	Dose mgms.	Wt. gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff.ΔO_2 mls./hr. (1)-(2)
1	4.10.57.	75	227	F	415.5	486.0	+70.5
2	11.10.57.	75	204	F	428.4	451.4	+23.0
3	21.10.57.	75	215	F	377.4	443.7	+66.3
4	4.11.57.	75	245	M	461.7	473.9	+12.2
5	11.11.57.	75	203	M	430.1	490.9	+60.8
6	29.11.57.	75	210	M	415.7	591.6	+175.9
7	16.12.57.	75	205	M	359.6	380.0	+20.4
8	17.12.57.	75	240	F	425.3	427.7	+2.4
9	18.2.58.	75	230	M	341.7	479.4	+137.7
10	20.2.58.	75	215	M	401.0	490.9	+89.9
11	25.2.58.	75	220	F	380.0	413.1	+33.1
12	27.2.58.	75	195	F	354.8	357.2	+2.4
1	20.12.57.	87.5	202	F	447.1	490.9	+43.8
2	9.1.58.	87.5	215	M	530.4	550.8	+20.4
3	13.1.58.	87.5	243	F	398.5	456.8	+58.3
4	17.1.58.	87.5	202	M	369.8	425.9	+56.1
5	20.1.58.	87.5	210	M	398.5	498.2	+99.7
6	21.1.58.	87.5	202	F	420.8	494.7	+73.9
7	22.1.58.	87.5	245	M	415.5	410.7	-4.8
8	13.2.58.	87.5	180	F	321.3	510.0	+188.7
9	19.2.58.	87.5	190	F	330.5	442.3	+111.8
10	26.2.58.	87.5	230	M	380.0	459.0	+79.0
11	5.3.58.	87.5	195	F	336.6	408.0	+71.4
12	6.3.58.	87.5	250	M	359.6	634.2	+274.6
1	1.10.57.	100	214	F	342.6	551.6	+209.0
2	15.10.57.	100	204	M	405.5	502.4	+96.9
3	18.10.57.	100	240	F	459.3	590.5	+131.2
4	5.11.57.	100	220	F	385.1	507.5	+122.4
5	7.11.57.	100	212	M	483.6	507.9	+24.3
6	12.12.57.	100	240	F	425.9	624.8	+198.9
7	13.12.57.	100	225	M	391.2	605.1	+213.9
8	18.12.57.	100	205	M	388.9	437.4	+48.5
9	17.2.58.	100	200	F	398.5	410.7	+12.2
10	2.3.58.	100	200	M	295.8	438.6	+142.8
11	4.3.58.	100	235	M	371.8	551.6	+179.8
12	7.3.58.	100	205	F	408.0	400.4	-7.6.

Summary of Table XXXVII.

p-Cresotinic Acid.

Dose mgms.	Mean ΔO_2 mls./hr.
25	-12.2
50	-0.3
62.5	+51.9
75	+57.9
87.5	+89.4
100	+114.4

Table XXXVIII.

The effect of 150 mgms. salicylic acid on the oxygen consumption of rats.

No.	Date	Weight gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	18.3.58.	205	M	284.3	466.6	+182.3
2	18.3.58.	235	F	387.6	545.7	+158.1
3	19.3.58.	228	M	362.1	534.6	+172.5
4	20.3.58.	205	F	390.2	550.8	+160.6
5	21.3.58.	205	M	427.7	449.6	+21.9
6	21.3.58.	245	F	395.3	550.8	+155.5
7	24.3.58.	206	M	347.5	437.4	+89.9
8	25.3.58.	205	F	374.9	423.3	+48.4
9	26.3.58.	229	M	366.9	544.3	+177.4
10	26.3.58.	245	F	288.2	517.7	+229.5
11	27.3.58.	210	M	345.1	437.4	+92.3
12	28.3.58.	218	F	374.9	504.9	+130.0
13	31.3.58.	205	M	364.5	442.3	+77.8
14	1.4.58.	205	F	420.8	400.4	-20.4

Table XXXIX.

The effect of 70 mgms. orthocresotinic acid on the oxygen consumption of rats.

No.	Date	Weight gms.	Sex	O_2 consumption mls./hr. Control (2)	O_2 consumption mls./hr. Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	18.3.58.	200	M	266.2	471.8	+205.6
2	19.3.58.	250	F	519.7	590.5	+70.8
3	19.3.58.	205	M	362.1	499.8	+137.7
4	20.3.58.	245	F	408.2	588.1	+179.9
5	21.3.58.	200	M	323.3	453.9	+130.6
6	24.3.58.	214	F	439.8	454.4	+14.6
7	24.3.58.	202	M	362.1	476.9	+114.8
8	25.3.58.	226	F	413.1	478.7	+65.6
9	26.3.58.	218	M	349.4	540.6	+191.2
10	27.3.58.	235	F	325.6	568.6	+243.0
11	27.3.58.	200	M	357.0	476.9	+119.9
12	31.3.58.	200	F	437.4	466.6	+29.2
13	31.3.58.	200	M	392.7	507.5	+114.8
14	1.4.58.	245	F	461.7	498.2	+36.5

Table XI.

The effect of a mixture of 75 mgms. salicylic acid and 35 mgms. orthocresotinic acid on the oxygen consumption of rats.

No.	Date	Weight gms.	Sex	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	18.3.58.	200	M	286.7	337.8	+51.1
2	19.3.58.	245	F	390.2	545.7	+155.5
3	20.3.58.	210	M	362.1	515.2	+153.1
4	20.3.58.	205	F	298.4	420.8	+122.4
5	21.3.58.	200	M	349.9	386.4	+36.5
6	24.3.58.	200	F	377.4	448.8	+71.4
7	25.3.58.	202	M	359.6	461.7	+102.1
8	25.3.58.	200	F	377.4	497.3	+119.9
9	26.3.58.	245	M	320.8	583.2	+262.4
10	27.3.58.	210	F	413.1	530.4	+117.3
11	28.3.58.	202	M	456.8	590.5	+133.7
12	31.3.58.	200	F	441.2	510.0	+68.8
13	1.4.58.	205	M	386.4	510.3	+123.9
14	1.4.58.	205	F	397.8	471.8	+74.0

Table XLI.

A comparison of the effect of 150 mgms. salicylic acid and of 70 mgms. orthocresotinic acid on the oxygen consumption of rats.

Trial No.	Salicylic Acid ΔO_2 mls./hr.(S)	Orthocresotinic Acid ΔO_2 mls./hr.(O)	Diff. mls./hr. (S)-(O)
1	+182.3	+205.6	-23.3
2	+158.1	+70.8	+87.3
3	+172.5	+137.7	+34.8
4	+160.6	+179.9	-19.3
5	+21.9	+130.6	-108.7
6	+155.5	+14.6	+140.9
7	+89.9	+114.8	-24.9
8	+48.4	+65.6	-17.2
9	+177.4	+191.2	-13.8
10	+229.5	+243.0	-13.5
11	+92.3	+119.9	-27.6
12	+130.0	+29.2	+100.8
13	+77.8	+114.8	-37.0
14	-20.4	+36.5	-56.9
Average	+119.7	+118.1	+1.6 $\pm$38.52.

H_o accepted (Z = 0.008).

Table XLII.

A comparison of the effect of 150 mgms. salicylic acid and of a mixture of 75 mgms. salicylic acid + 35 mgms. o-cresotinic acid on the oxygen consumption of rats.

Trial No.	Salicylic Acid ΔO_2 mls./hr.(S)	Mixture ΔO_2 mls./hr.(SO)	Diff. mls./hr. (S)-(SO)
1	+182.3	+51.1	+131.2
2	+158.1	+155.5	+2.6
3	+172.5	+153.1	+19.4
4	+160.6	+122.4	+38.2
5	+21.9	+36.5	-14.6
6	+155.5	+71.4	+84.1
7	+89.9	+102.1	-12.2
8	+48.4	+119.9	-71.5
9	+177.4	+262.4	-85.0
10	+229.5	+117.3	+112.2
11	+92.3	+133.7	-41.4
12	+130.0	+68.8	+61.2
13	+77.8	+123.9	-46.1
14	-20.4	+74.0	-94.4
Average	+119.7	+113.7	+6.0 $\pm$41.59.

H_o accepted (Z = 0.103).

Table XLIII.

A comparison of the effect of 70 mgms. orthocresotinic acid and of a mixture of 75 mgms. salicylic acid + 35 mgms. o-cresotinic acid on the oxygen consumption of rats.

Trial No.	Orthocresotinic Acid ΔO_2 mls./hr.(O)	Mixture ΔO_2 mls./hr.(SO)	Diff. mls./hr. (O)-(SO)
1	+205.6	+51.1	+154.5
2	+70.8	+155.5	-84.7
3	+137.7	+153.1	-15.4
4	+179.9	+122.4	+57.5
5	+130.6	+36.5	+94.1
6	+14.6	+71.4	-56.8
7	+114.8	+102.1	+12.7
8	+65.6	+119.9	-54.3
9	+191.2	+262.4	-71.2
10	+243.0	+117.3	+125.7
11	+119.9	+133.7	-13.8
12	+29.2	+68.8	-39.6
13	+114.8	+123.9	-9.1
14	+36.5	+74.0	-37.5
Average	+118.1	+113.7	+4.4 $\pm$43.46.

H_0 accepted (Z = 0.052).

Table XLIV.

Plasma concentration after injection of 150 mgms. salicylic acid to rats.

Date	Weight gms.	Sex	Time hrs.	Plasma Conc. mgms.%	Plasma Conc. m.Eq./L.
16.10.57.	258	M	.25	103	7.46
16.10.57.	236	F	.25	114	8.26
23.12.57.	208	M	.25	127	9.20
23.12.57.	265	F	.25	124	8.99
20.2.58.	370	M	.25	83	6.01
20.2.58.	270	F	.25	102	7.39
25.3.58.	247	M	.25	123	8.88
25.3.58.	244	F	.25	102	7.39
28.10.57.	290	M	.50	100	7.25
28.10.57.	216	F	.50	130	9.42
15.11.57.	276	M	.50	101	7.32
15.11.57.	252	F	.50	114	8.26
25.2.58.	280	M	.50	100	7.25
25.2.58.	260	F	.50	105	7.61
26.2.58.	280	M	.50	98	7.10
26.2.58.	185	F	.50	148	10.72
23.12.57.	260	M	1.0	60	4.35
23.12.57.	280	F	1.0	114	8.26
19.2.58.	330	M	1.0	89	6.45
19.2.58.	215	F	1.0	120	8.70
26.3.58.	270	M	1.0	100	7.25
26.3.58.	235	F	1.0	112	8.12
2.4.58.	295	M	1.0	95	6.88
2.4.58.	200	F	1.0	124	8.99
27.12.57.	306	M	2.0	82	5.94
27.12.57.	236	F	2.0	60	4.35
28.12.57.	233	M	2.0	103	7.46
28.12.57.	237	F	2.0	104	7.54
27.3.58.	315	M	2.0	76	5.51
27.3.58.	220	F	2.0	87	6.30
1.4.58.	290	M	2.0	90	6.52
1.4.58.	205	F	2.0	108	7.83

contd.

Table XLIV. (contd.)

Salicylic Acid.

Date	Weight gms.	Sex	Time hrs.	Plasma Conc. mgms.%	Plasma Conc. m.Eq./L.
6.12.57.	205	M	4.0	94	6.81
6.12.57.	238	F	4.0	77	5.58
14.1.58.	380	M	4.0	65	4.71
14.1.58.	205	F	4.0	82	5.94
26.2.58.	290	M	4.0	75	5.43
26.2.58.	210	F	4.0	88	6.38
27.3.58.	260	M	4.0	88	6.38
27.3.58.	230	F	4.0	91	6.59
25.10.57.	245	F	6.0	65	4.71
24.12.57.	230	M	6.0	78	5.65
24.12.57.	203	F	6.0	95	6.88
30.12.57.	243	M	6.0	80	5.80
30.12.57.	274	F	6.0	54	3.91
25.3.58.	257	M	6.0	59	4.27
25.3.58.	234	F	6.0	64	4.64
26.3.58.	208	M	6.0	78	5.65
26.3.58.	216	F	6.0	Dead.	
29.10.57.	312	M	8.0	54	3.91
29.10.57.	256	F	8.0	65	4.71
14.1.58.	301	M	8.0	54	3.91
14.1.58.	226	F	8.0	67	4.85
25.2.58.	280	M	8.0	64	4.64
25.2.58.	255	F	8.0	40	3.33
1.4.58.	290	M	8.0	70	5.07
1.4.58.	220	F	8.0	80	5.80
29.10.57.	300	M	16.0	35	2.54
29.10.57.	172	F	16.0	Dead.	
18.2.58.	200	M	16.0	43	3.12
18.2.58.	205	F	16.0	44	3.19
20.2.58.	350	M	16.0	42	3.04
20.2.58.	255	F	16.0	45	3.26
1.4.58.	200	F	16.0	50	3.62
2.4.58.	290	M	16.0	34	2.46
2.4.58.	230	F	16.0	41	2.97

Summary of Table XLIV.

Salicylic Acid.

Time hrs.	Mean Plasma Conc. mgms.%	Mean Plasma Conc. m.Eq./L.
.25	110	7.95
.50	112	8.11
1.0	102	7.37
2.0	89	6.43
4.0	83	5.98
6.0	72	5.19
8.0	62	4.53
16.0	42	3.02

Table XLV.

Plasma concentration after injection of 100 mgms. ortho-cresotinic acid to rats.

Date	Weight gms.	Sex	Time hrs.	Plasma Conc. mgms.%	Plasma Conc. m.Eq./L.
28.10.57.	326	M	.25	84	5.53
28.10.57.	192	F	.25	108	7.10
15.11.57.	225	M	.25	100	6.58
15.11.57.	240	F	.25	99	6.51
26.2.58.	180	M	.25	119	7.83
26.2.58.	203	F	.25	100	6.58
27.3.58.	325	M	.25	88	5.79
27.3.58.	200	F	.25	140	9.21
16.10.57.	228	M	.50	97	6.38
16.10.57.	253	F	.50	90	5.92
23.12.57.	261	M	.50	96	6.32
23.12.57.	268	F	.50	102	6.71
20.2.58.	265	M	.50	92	6.05
20.2.58.	235	F	.50	102	6.71
25.3.58.	234	M	.50	112	7.37
25.3.58.	216	F	.50	125	8.22
27.12.57.	220	M	1.0	104	6.84
27.12.57.	256	F	1.0	98	6.45
28.12.57.	294	M	1.0	92	6.05
28.12.57.	236	F	1.0	105	6.91
25.2.58.	297	M	1.0	82	5.39
25.2.58.	205	F	1.0	114	7.50
1.4.58.	315	M	1.0	87	5.72
1.4.58.	215	F	1.0	122	8.03
23.12.57.	205	M	2.0	94	6.18
23.12.57.	296	F	2.0	96	6.32
19.2.58.	320	M	2.0	70	4.61
19.2.58.	230	F	2.0	90	5.92
26.3.58.	234	M	2.0	110	7.24
26.3.58.	196	F	2.0	Died.	
26.3.58.	245	F	2.0	105	6.91
2.4.58.	200	M	2.0	116	7.63
2.4.58.	215	F	2.0	121	7.96

contd.

Table XLV. (contd.).

o-Cresotinic Acid.

Date	Weight gms.	Sex	Time hrs.	Plasma Conc. mgms.%	Plasma Conc. m.Eq./L.
25.10.57	247	F	4.0	80	5.26
24.12.57.	205	M	4.0	92	6.05
24.12.57.	240	F	4.0	78	5.13
14.1.58.	310	M	4.0	70	4.60
25.3.58.	258	M	4.0	86	5.66
25.3.58.	268	F	4.0	65	4.22
26.3.58.	320	M	4.0	70	4.61
26.3.58.	215	F	4.0	83	5.46
25.10.57.	233	F	6.0	75	4.93
6.12.57.	212	M	6.0	68	4.47
6.12.57.	245	F	6.0	80	5.26
14.1.58.	290	M	6.0	66	4.34
26.2.58.	295	M	6.0	67	4.41
26.2.58.	205	F	6.0	Died.	
27.3.58.	325	M	6.0	77	5.07
27.3.58.	240	F	6.0	Died.	
3.4.58.	240	F	6.0	70	4.60
3.4.58.	215	F	6.0	83	5.46
29.10.57.	298	M	8.0	47	3.09
29.10.57.	265	F	8.0	64	4.21
18.2.58.	320	M	8.0	52	3.42
18.2.58.	210	F	8.0	66	4.34
20.2.58.	285	M	8.0	58	3.82
20.2.58.	240	F	8.0	57	3.75
2.4.58.	240	M	8.0	90	5.92
2.4.58.	235	F	8.0	72	4.73
29.10.57.	322	M	16.0	33	2.17
29.10.57.	192	F	16.0	Died.	
30.12.57.	232	M	16.0	28	1.84
30.12.57.	268	F	16.0	46	3.03
25.2.58.	310	M	16.0	40	2.63
25.2.58.	245	F	16.0	49	3.22
1.4.58.	280	M	16.0	50	3.29
1.4.58.	230	F	16.0	47	3.09
2.4.58.	245	F	16.0	43	2.83

Summary of Table XLV.

o-Cresotinic Acid.

Time hrs.	Mean Plasma Conc. mgms.%	Mean Plasma Conc. m.Eq./L.
.25	105	6.89
.50	102	6.71
1.0	100.5	6.61
2.0	100	6.60
4.0	78	5.12
6.0	73	4.82
8.0	63	4.16
16.0	41	2.76

Table XLVI.

Plasma concentration after injection of 100 mgms. meta-cresotinic acid to rats.

Date	Weight gms.	Sex	Time hrs.	Plasma Conc. mgms.%	Plasma Conc. m.Eq./L.
23.12.57.	260	M	.25	88	5.79
23.12.57.	254	F	.25	93	6.12
19.2.58.	360	M	.25	72	4.74
19.2.58.	275	F	.25	90	5.92
26.3.58.	218	M	.25	102	6.71
26.3.58.	210	F	.25	117	7.70
2.4.58.	225	M	.25	91	5.99
2.4.58.	200	F	.25	126	8.29
27.12.57.	208	M	.50	113	7.43
27.12.57.	232	F	.50	107	7.04
28.12.57.	252	M	.50	98	6.45
28.12.57.	248	F	.50	106	6.97
27.3.58.	330	M	.50	88	5.79
27.3.58.	200	F	.50	135	8.88
1.4.58.	260	M	.50	93	6.12
1.4.58.	210	F	.50	117	7.70
16.10.57.	288	M	1.0	72	4.74
16.10.57.	230	F	1.0	90	5.92
23.12.57.	230	M	1.0	90	5.92
23.12.57.	246	F	1.0	94	6.18
20.2.58.	320	M	1.0	76	5.00
20.2.58.	230	F	1.0	99	6.51
25.3.58.	258	M	1.0	85	5.59
25.3.58.	266	F	1.0	90	5.92
28.10.57.	292	M	2.0	68	4.47
28.10.57.	204	F	2.0	103	6.78
15.11.57.	272	M	2.0	75	4.93
15.11.57.	264	F	2.0	78	5.13
25.2.58.	310	M	2.0	72	4.74
25.2.58.	215	F	2.0	88	5.79
26.2.58.	285	M	2.0	81	5.33
26.2.58.	177	F	2.0	Died.	
3.4.58.	210	F	2.0	114	7.50

contd.

Table XLVI. (contd.).

m-Cresotinic Acid.

Date	Weight gms.	Sex	Time hrs.	Plasma Conc. mgms.%	Plasma Conc. m.Eq./L.
29.10.57.	306	M	4.0	66	4.34
29.10.57.	198	F	4.0	87	5.72
30.12.57.	244	M	4.0	68	4.47
30.12.57.	243	F	4.0	80	5.26
25.2.58.	248	M	4.0	69	4.54
25.2.58.	245	F	4.0	72	4.74
1.4.58.	345	M	4.0	60	3.95
1.4.58.	205	F	4.0	76	5.00
29.10.57.	282	M	6.0	56	3.68
29.10.57.	206	F	6.0	69	4.54
18.2.58.	285	M	6.0	54	3.55
18.2.58.	205	F	6.0	76	5.00
20.2.58.	225	M	6.0	70	4.60
20.2.58.	225	F	6.0	72	4.74
2.4.58.	240	M	6.0	72	4.74
2.4.58.	210	F	6.0	90	5.92
6.12.57.	203	M	8.0	49	3.22
6.12.57.	270	F	8.0	65	4.28
14.1.58.	232	M	8.0	59	3.88
14.1.58.	206	F	8.0	73	4.80
26.2.58.	220	M	8.0	56	3.68
26.2.58.	225	F	8.0	84	5.53
27.3.58.	285	M	8.0	57	3.75
27.3.58.	245	F	8.0	61	4.01
24.12.57.	238	M	16.0	29	1.91
24.12.57.	242	F	16.0	34	2.24
14.1.58.	220	M	16.0	30	1.97
14.1.58.	218	F	16.0	24	1.58
25.3.58.	222	M	16.0	31	2.04
25.3.58.	220	F	16.0	28	1.84
26.3.58.	314	M	16.0	40	2.63
26.3.58.	234	F	16.0	52	3.42

Summary of Table XLVI.

m-Cresotinic Acid.

Time hrs.	Mean Plasma Conc. mgms.%	Mean Plasma Conc. m.Eq./L.
.25	97	6.41
.50	107	7.05
1.0	87	5.72
2.0	85	5.58
4.0	72	4.75
6.0	69	4.60
8.0	63	4.14
16.0	33	2.20

Table XLVII.

Plasma concentration after injection of 100 mgms. para-cresotinic acid to rats.

Date	Weight gms.	Sex	Time hrs.	Plasma Conc. mgms.%	Plasma Conc. m.Eq./L.
27.12.57.	215	M	.25	102	6.71
27.12.57.	220	F	.25	96	6.32
28.12.57.	252	M	.25	84	5.53
28.12.57.	258	F	.25	80	5.26
25.2.58.	270	M	.25	86	5.66
25.2.58.	235	F	.25	88	5.79
1.4.58.	245	M	.25	90	5.92
1.4.58.	220	F	.25	114	7.50
23.12.57.	274	M	.50	84	5.53
23.12.57.	268	F	.50	82	5.39
19.2.58.	270	M	.50	88	5.79
19.2.58.	260	F	.50	88	5.79
26.3.58.	225	M	.50	96	6.32
26.3.58.	200	F	.50	83	5.46
2.4.58.	220	M	.50	104	6.84
2.4.58.	200	F	.50	130	8.55
28.10.57.	274	M	1.0	78	5.13
28.10.57.	204	F	1.0	102	6.71
15.11.57.	274	M	1.0	78	5.13
15.11.57.	231	F	1.0	97	6.38
26.2.58.	215	M	1.0	96	6.32
26.2.58.	185	F	1.0	111	7.30
27.3.58.	325	M	1.0	76	5.00
27.3.58.	205	F	1.0	110	7.24
16.10.57.	240	M	2.0	81	5.33
16.10.57.	236	F	2.0	93	6.12
23.12.57.	239	M	2.0	84	5.53
23.12.57.	262	F	2.0	95	6.25
20.2.58.	270	M	2.0	75	4.93
20.2.58.	250	F	2.0	90	5.92
25.3.58.	257	M	2.0	78	5.13
25.3.58.	234	F	2.0	94	6.18

contd.

Table XLVII. (contd.).

p-Cresotinic Acid.

Date	Weight gms.	Sex	Time hrs.	Plasma Conc. mgms.%	Plasma Conc. m.Eq./L.
29.10.57.	264	M	4.0	64	4.21
29.10.57.	222	F	4.0	98	6.45
18.2.58.	315	M	4.0	64	4.21
18.2.58.	205	F	4.0	83	5.46
20.2.58.	275	M	4.0	67	4.41
20.2.58.	215	F	4.0	88	5.79
2.4.58.	265	M	4.0	71	4.67
2.4.58.	205	F	4.0	97	6.38
29.10.57.	316	M	6.0	72	4.74
29.10.57.	248	F	6.0	65	4.28
24.12.57.	257	M	6.0	50	3.29
24.12.57.	240	F	6.0	72	4.74
25.2.58.	305	M	6.0	54	3.55
25.2.58.	190	F	6.0	Died.	
1.4.58.	290	M	6.0	64	4.21
1.4.58.	210	F	6.0	70	4.60
3.4.58.	230	F	6.0	84	5.53
30.12.57.	253	M	8.0	54	3.55
30.12.57.	282	F	8.0	46	3.03
14.1.58.	248	M	8.0	58	3.82
14.1.58.	204	F	8.0	Died.	
25.3.58.	230	M	8.0	58	3.82
25.3.58.	182	F	8.0	Died.	
26.3.58.	260	M	8.0	63	4.14
26.3.58.	228	F	8.0	74	4.87
3.4.58.	235	F	8.0	84	5.53
3.4.58.	235	F	8.0	68	4.47
6.12.57.	297	M	16.0	30	1.97
6.12.57.	260	F	16.0	44	2.89
14.1.58.	325	M	16.0	19	1.25
14.1.58.	251	F	16.0	38	2.50
26.2.58.	320	M	16.0	34	2.46
26.2.58.	210	F	16.0	Died.	
27.3.58.	275	M	16.0	38	2.50
27.3.58.	280	F	16.0	50	3.29
27.3.58.	200	F	16.0	58	3.82.

Summary of Table XLVII.

p-Cresotinic Acid.

Time hrs.	Mean Plasma Conc. mgms.%	Mean Plasma Conc. m.Eq./L.
.25	93	6.09
.50	94	6.21
1.0	93	6.15
2.0	86	5.67
4.0	79	5.20
6.0	66	4.37
8.0	63	4.15
16.0	39	2.59

Table XLVIII.

The effect of 50 mgms. 3-phenylsalicylic acid on the oxygen consumption of Wistar albino rats.

No.	Date	Sex	Weight gms.	O_2 consumption mls./hr. Control (2)	Treated (1)	Diff. ΔO_2 mls./hr. (1)-(2)
1	6.2.58.	M	255	415.5	617.2	+201.7
2	6.2.58.	M	245	392.7	441.2	+48.5
3	7.2.58.	M	280	444.7	510.3	+65.6
4	7.2.58.	M	235	339.2	637.5	+298.3
5	7.2.58.	M	260	486.0	656.1	+170.1
6	7.2.58.	M	275	357.0	545.7	+188.7
	Average			405.9	568.0	+162.1

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