RESEARCH PAPERS

THE MEASUREMENT OF OXYGEN CONSUMPTION IN SMALL ANIMALS*

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An apparatus is described which can be employed for the measurement of the oxygen consumption of one to six small animals in the weight range 25–500 g. The normal oxygen consumption of mice, rats and guinea pigs at 25° has been determined. The values obtained agree adequately with those of others. Evidence is given to support a claim that the guinea pig is, in some ways, a more suitable animal for the assay of metabolic stimulants than the rat or the mouse. Preliminary experiments have been made on the stimulant action of dinitrophenol and thyroxine on the oxygen consumption of guinea pigs.

During investigations of the toxicities of the dinitrophenols the need arose to measure their effects on the oxygen consumption of small animals.

A characteristic feature of the stimulant action of 2:4-dinitrophenol on laboratory animals is that it reaches and loses its maximum effect within the short time of 3–4 hours. In this it differs from thyroxine which shows a slower falling off of activity. Thus the apparatus used for assaying any metabolic stimulant must satisfy three basic requirements. First, it must be comfortable enough for an animal to settle down quickly to a resting or "basal" condition. Secondly, it must be sensitive to small changes in the oxygen consumption, and thirdly, its design should permit individual and group measurements to be made on several animals under nearly identical conditions[2,3].

The main objects of this communication are to describe an apparatus that has been designed to meet these requirements, to record its use in the measurement of the normal oxygen consumption of mice, rats and guinea pigs, and to report preliminary investigations on the measurement of the stimulating effects of 2:4-dinitrophenol and L-thyroxine on the oxygen consumption of the guinea pig.

Construction of the Apparatus (Figs. 1 and 2)

A tank, approximately 3 ft. × 1 ft. × 1 ft. is constructed of Perspex sheet $\frac{3}{8}$ in. thick. Six Perspex cylinders are set horizontally between two long sides, with the ends just short of the outer faces. These cylinders form the outer walls of the six metabolism chambers (1–6) and are covered by circular Perspex plates 5 in. in diameter. The plates are held in place by two brass 4 BA bolts embedded in the tank walls and two wing nuts. Gas tightness is ensured by means of Vaseline or preferably a silicone grease smeared on the tank walls and on the plates. Each plate

* A demonstration of the apparatus described in this communication was given at the Physiological Society meeting held December 14–15, 1956[1].

is fitted with a glass tap (A–F, A1–F1) held in place by a rubber sleeve. The taps are connected by pressure tubing to the outlets of the two brass manifold tubes (M, M1). Each bank of taps is firmly fixed to a rigid wood frame (WF). This permits all plates to be removed rapidly in one operation. One of the manifold tubes is fitted with a master tap (G). The other is connected to an 80 ml. floatmeter and recorder by way of a CO_2 trap (PT) and a water trap (RT). Each metabolism chamber contains a smaller Perspex cylinder (6 in. × 3 in.) with a long slit (4 in. × $\frac{3}{8}$ in.)

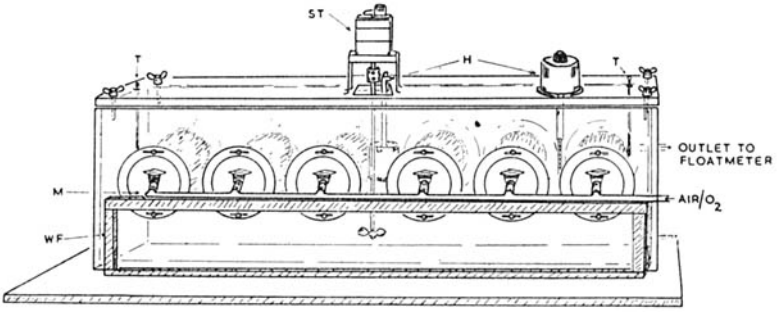

FIG. 1. Apparatus for the measurement of oxygen consumption. Floatmeter not shown. See text for description.

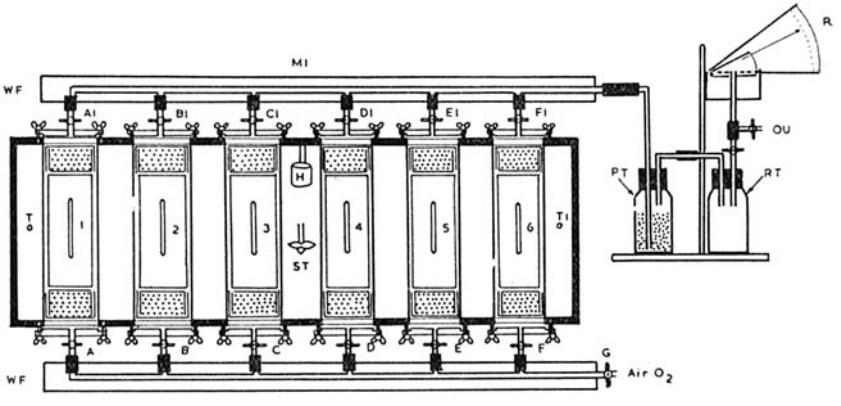

FIG. 2. Diagrammatic representation of the apparatus. Circular sealing plates are drawn just clear of side plates.

and fitted with four feet to keep a space between the two cylinders. A narrower (1 in.) cylinder is necessary for small rats and mice. In the spaces between the ends of the sealing plates and the inner cylinders are two "cartridges" containing Green 6–10 mesh Protosorb for trapping the CO_2. Each cartridge is $3\frac{3}{4}$ in. dia. × $1\frac{1}{2}$ in. deep and is constructed of perforated zinc in two close fitting halves. The cartridges fit snugly but not too tightly. The whole tank is filled with water. It is covered with a Perspex lid in which are fitted two thermometers (T, T), a stirrer (ST), and a thermostatically controlled heating device (H).

Floatmeter Recorder

The manifold Ml is attached by rubber pressure tubing to the CO_2 trap (PT). This is a small glass bottle (150 ml.) fitted with a two holed rubber bung and containing 20–30 g. Protosorb. Through one hole of the bung is a connection to the floatmeter trap (RT). Through the other is a large bore glass trap (OU) that can be opened to permit free current of air or oxygen through the apparatus without filling the floatmeter chamber. The floatmeter is fitted with a Perspex scale and the whole apparatus is calibrated in 10 ml. divisions. This is similar to that described by Tainter[4].

Supply of Air or Oxygen

Air is supplied by double bulb hand bellows, or oxygen through a bubbler from a cylinder.

While many methods require the use of oxygen or air enriched with oxygen[2,5,6] it was considered that air alone might be suitable for short time exposures. Clearly the total volume of air available would have to be liberal and the exposure time short for larger (300–500 g.) animals. In other words, it was essential to reduce the volume of oxygen consumed to a minimum. After some trials 10 ml. was adopted as a convenient volume. Consideration of the capacity of the apparatus indicates that each animal has available about 750 ml. of air. Therefore, if 10 ml. of oxygen is consumed the reduction of the total amount available is about 6 per cent. This can be replaced easily and rapidly by frequent aeration. No evidence has been obtained to suggest that this diminution of the total available oxygen is harmful to the animal.

Method of Operation

The sealing plates on one side of the apparatus (A–F) are removed and one animal put into each chamber. The plates are replaced and the whole apparatus aerated thoroughly. Times are not taken until the animals have settled and the temperatures inside the metabolism chambers have reached 25°. Temperature equilibrium is established within 5 minutes although the settling time varies. As a rule it was found that rats and mice settled in 20–30 minutes[5] and guinea pigs in 5–10 minutes. During the settling period aeration is carried out frequently for the group and for the individuals.

Group Determination on Six Animals

After thorough aeration the apparatus is charged with sufficient air to bring the floatmeter arm to its maximum elevation. The master tap is closed. Timings by stop watch are taken for the consumption of three or four alternate 10 ml. volumes. The procedure is repeated until 12–16 values have been obtained that show less than 5 per cent variation.

Single Animal Determinations

The apparatus is aerated thoroughly and the floatmeter put into circuit as described previously. The master tap is closed. In order to measure the oxygen consumption of an animal in say chamber 1, Tap A is turned

off, taps B–F are turned on, and taps B1–F1 are turned off. The time taken for the consumption of a single 10 ml. volume is measured. Immediately after this the whole apparatus is thoroughly aerated and the procedure repeated for the remaining chambers. While individual measurements are made the remaining chambers are opened to the air.

Temperature

After some trials it was found that the most suitable environmental temperature was 25°. Lower temperatures (18–20°) appeared to cause restlessness in the animals and therefore delayed their settling time.

TABLE I

CORRELATION OF OXYGEN CONSUMPTION AND BODY WEIGHT OF GUINEA PIGS

Sex	Regression coefficient			Equation
	D.F.	Value	S.E.	
Male ..	46	0·625	0·034	$y = 0{\cdot}625x - 0{\cdot}796$
Female ..	46	0·604	0·033	$y = 0{\cdot}604x - 0{\cdot}738$
Combined ..	92	0·614	0·023	$y = 0{\cdot}614x - 0{\cdot}765$

y = log oxygen consumption (ml. O_2/min.) and
x = log weight (g.)

Higher temperatures were also unsuitable because some of the compounds under investigation, for example, 2:4-dinitrophenol, 4:6-dinitro-*o*-cresol are strongly hyperthermic and their effects on metabolism are enhanced markedly by high environmental temperatures[7].

Time Taken to Carry Out a Series of Determinations

12–16 group and six individual determinations on six guinea pigs take about 0·3 hours depending on the weight of the animals. For six mice the same number of determinations takes about 1·5 hours.

Treatment of Animals

Animals are maintained on normal diets or as required. Food (but not water) is removed 1·5–2·0 hours before measurements are made.

DETERMINATION OF OXYGEN CONSUMPTION

All results are calculated on a metabolism chamber temperature of 25°. No corrections have been made for atmospheric pressure.

Normal Values

Preliminary experiments with rats and mice demonstrated that their natural activity was high, and that it was difficult to get good resting values of oxygen consumption. Thus it was found that relatively mild exercise caused as much as a 50 per cent, and walking up to 150 per cent increase in the oxygen consumption of mice. Therefore, more attention was paid to the guinea pig. These are naturally quiet animals and have been employed by other workers for the assay of thyroid compounds[8]. Normal measurements were made on 96 guinea pigs (48 male and 48

female) in 16 groups of 6 animals per group. The weight range was 88–494 g. Twelve rats and twelve mice were also included in the study.

The normal oxygen consumption of guinea pigs measured over a wide weight range revealed a good relation between body weight and oxygen consumption. No significant difference in the response of the sexes was noted. Group and mean values are summarised statistically in Table I.

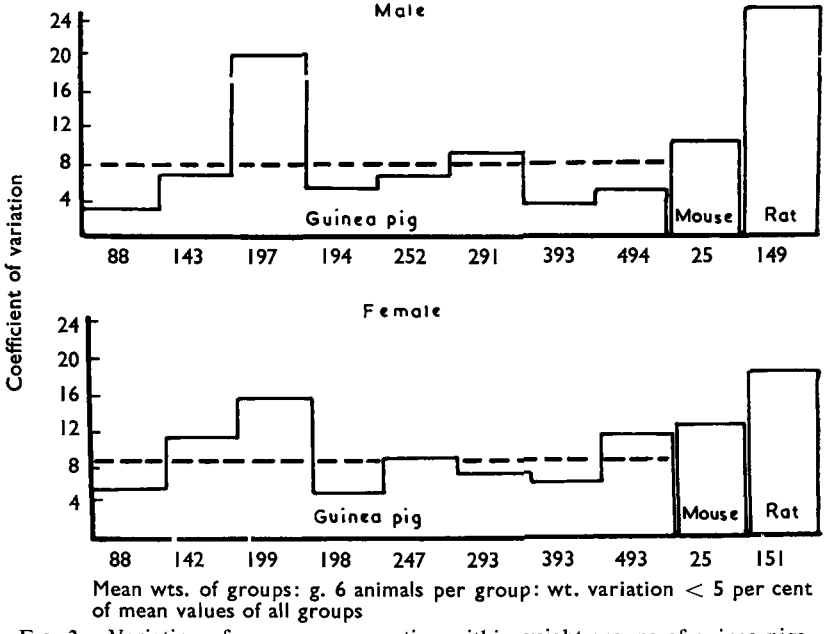

FIG. 3. Variation of oxygen consumption within weight groups of guinea pigs.

These results are in good agreement with those of Gaddum[9] and of Reineke and Turner[8]. In most weight groups there was reasonable uniformity in the oxygen consumption of the individuals. Only those animals in the weight range about 150–250 g. showed marked variation (Fig. 3).

Comparison of the mean of six individual determinations and the value of the group value revealed a fairly constant difference of about 15 per cent. No immediate explanation of this phenomenon can be offered but it may lie in the physical structure and operation of the system. From the point of view of assay methods the variation is not unreasonable provided that it remains constant, and preliminary studies on the assay of dinitrophenol suggest that such group measurements may be preferable.

SERIAL DETERMINATION AND THE EFFECT OF EXCESS OXYGEN

Two experiments were made. In the first, air was supplied initially and the oxygen consumption of each of six guinea pigs was determined serially until six values per animal were obtained. The animals were removed, allowed to rest for two hours, and then replaced in the apparatus when they were supplied with air enriched with oxygen (air 1 pt.: oxygen

8 pts.), and six serial values obtained in a similar manner. In the second experiment twelve serial determinations were made on six guinea pigs before and after 4 daily injections of 120 μg. L-thyroxine sodium. Air only was supplied. The results are summarised in Table II and Figure 4.

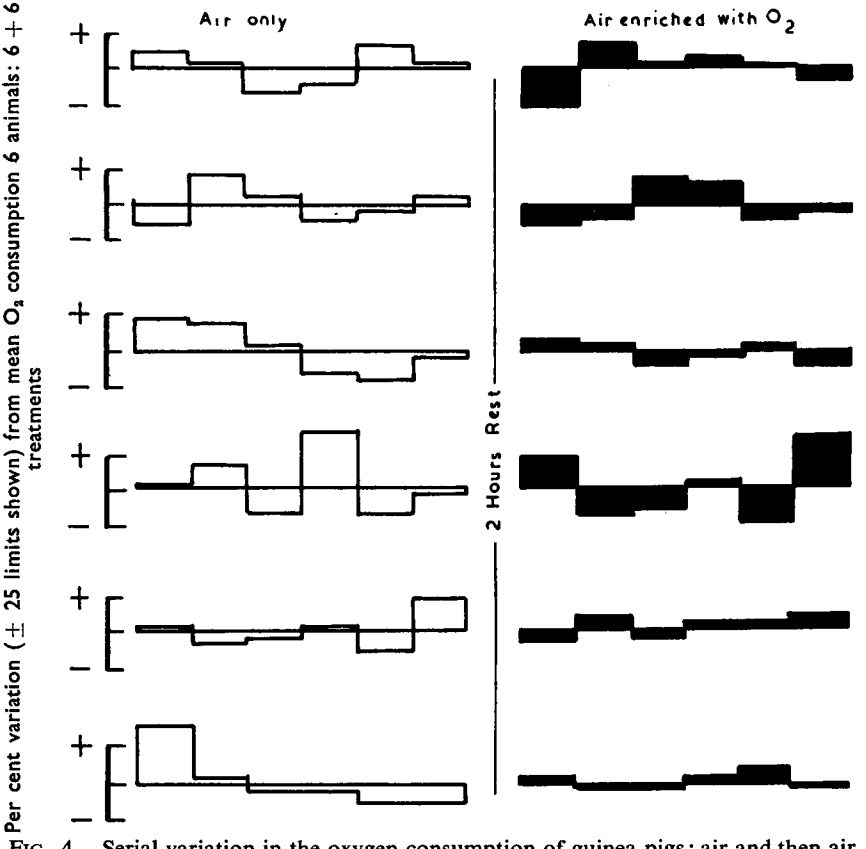

FIG. 4. Serial variation in the oxygen consumption of guinea pigs: air and then air heavily enriched with oxygen supplied.

The main result of this experiment is the demonstration that random fluctuations occur in the oxygen consumption of the guinea pig when serial measurements are made. In all probability this accounts for the fact that a series of group determinations made on six animals never shows much variation. In other words, it is likely that in any chronological series of observations random fluctuations of the individuals will cancel out to give homogeneous group values.

Variations greater than 20 per cent about the mean value of any one animal were rare and the average was 10 per cent. Only slight movements were noted at any time and the animals appeared almost motionless. By increasing the supply of oxygen there was an average increase in the consumption of about 20 per cent. Individual variations were less, but differences between animals were greater than when air alone was supplied.

METABOLIC STIMULANTS

2:4-*Dinitrophenol*

In each of the two simple dose response experiments single doses of dinitrophenol were administered to guinea pigs by intraperitoneal injection of a 1 per cent aqueous solution (w/v) as its Na salt. The doses given were

TABLE II

SERIAL VARIATION IN OXYGEN CONSUMPTION OF SIX GUINEA PIGS (285–380 G.)

Treatment	Oxygen consumption			
	ml. O_2/min.		Coefficients of variation of the six mean values	
	Range of 6 mean serial values	Mean	Range	Mean (*a*)
Air	4·19–5·71	4·72	7·1–19·0	14·7± 4·2
Air–O_2	5·42–6·61	5·97	5·1–23·5	11·4±7·2

Note.—(*a*) If the S.D. of the mean coefficient of variation is accepted as a measure of scatter between the six animals mean serial values, then it is clear that air plus oxygen results in a more variable response than air alone in the ratio $\frac{7{\cdot}2}{11{\cdot}4} : \frac{4{\cdot}2}{14{\cdot}7}$ or 2·1 : 1·0.

5·0, 10·0, 20·0, 25·0, 30·0 and 35·0 mg./kg. Single determinations were made on each animal before and 1·25 hours after administration. Guinea pigs weighing about 300 g. were used. Dosing and determinations were carried out as follows:—

Experiment	Total animals used	Dosing		Determinations	
		No. of levels	Animals/dose	Total	Type
1. (Fig. 5) ..	36	6	6	34	Group and individual
2.*	18	6	3	18	Individual

* The response in the second experiment was similar to that in the first.

Thyroxine

Two experiments were made. The first was a simple dose response, making single determinations. The second was to investigate the results obtained from many determinations.

Thyroxine was administered daily for four days to guinea pigs by the subcutaneous injection of L-thyroxine sodium dissolved in 0·1 per cent aqueous (w/v) sodium carbonate. Determinations and weighings were made on each animal before the first dose and after the last. Dosing and determinations were made as follows:—

Experiment	Total animals used	Dosing		Determinations	
		μg. thyroxine/ animal/day	Animals/dose	Before treatment	After treatment
1. (Fig. 6) ..	15	15, 30, 60, 120, 240	3	1	1
2. (Table III)	6	120	1	12	up to 12

Dinitrophenol administered intraperitoneally to guinea pigs caused an increase in the oxygen consumption which showed a general relation of log dose to log per cent increase in oxygen consumption per 100 g. body weight. Figure 5 illustrates this. On the whole, better results were obtained when 6 animals were used per dose, and close agreement was obtained between the group and mean individual determinations.

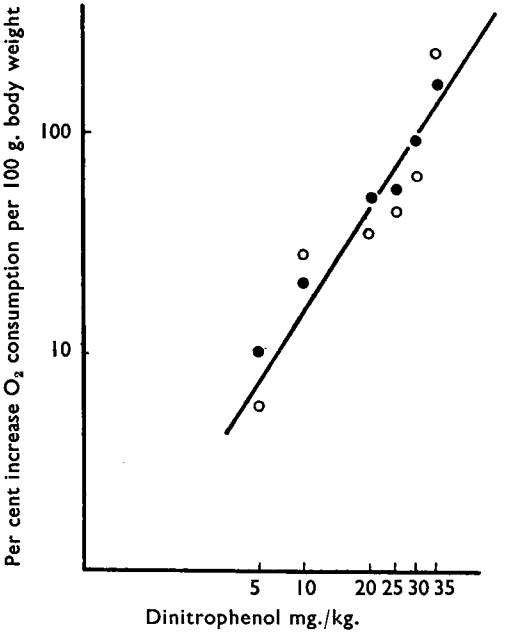

FIG. 5. Effect of dinitrophenol on oxygen consumption of guinea pigs. Single doses given by intraperitoneal injection. –●—●–group determinations on six animals per dose,–O—O–means of six individual determinations per dose. Line by observation.

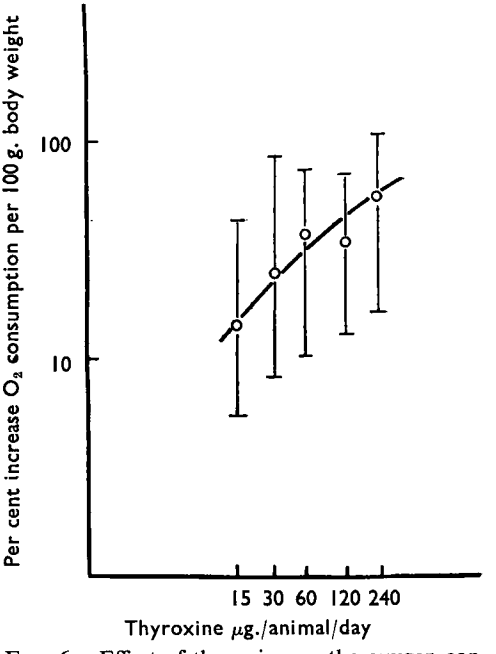

FIG. 6. Effect of thyroxine on the oxygen consumption of guinea pigs. Four animals per dose level. Mean and scatter shown.

Administration of graded doses of thyroxine revealed a similar relation between body weight and dose although there was a fairly wide scatter (Fig. 6).

In the second experiment (Table III) the administration of four daily doses of 120 μg. of thyroxine to each of six guinea pigs gave expected results, although three of the animals became very weak during treatment.

These three died before twelve readings could be taken. Therefore, responses were calculated on the mean values derived from the first three as well as from all available determinations. The former gave more homogeneous results particularly for the animals that died when progressive respiratory failure resulted in a slow respiration rate.

DISCUSSION

"Basal metabolism or the basal metabolic rate is an expression of the body in complete mental and physical repose and in the post absorbtive

state."[10] As this condition is difficult if not impossible to obtain and to assess in small animals it is better to aim at achieving a less absolute value.

If very small numbers of animals are being used in an assay of a metabolic stimulant then more strict adherence to true metabolic conditions will be necessary. However, preliminary investigations on designed assays using larger numbers of animals and a wide range of dose levels suggest that it is more important to ensure, as far as possible, a reasonable degree of physical inactivity in the animals throughout. The design of apparatus described appears to favour this requirement.

TABLE III

THE OXYGEN CONSUMPTION OF GUINEA PIGS BEFORE AND AFTER FOUR DAILY DOSES OF THYROXINE (120 μG./ANIMAL/DAY)

	Before treatment		After treatment		Difference (response) (per cent) (*b*)		
		Mean		Mean		Oxygen consumption	
Animal	wt. (g.)	ml. O_2/min. (*a*)	wt. (g.)	ml. O_2/min. (*a*)	wt.	On all values	On first three values
1	305	4·39 (12)	276	4·85 (10)	− 10·6	+ 25·2	+ 25·5
2	352	4·76 (12)	313	5·97 (12)	− 11·1	+ 27·6	+ 26·4
3	500	5·91 (12)	429	5·95 (5)	− 14·2	+ 16·0	+ 13·7
4	352	5·39 (12)	323	4·27 (12)	− 5·3	− 14·4	+ 22·5
5	410	6·37 (12)	394	5·93 (3)	− 3·9	− 2·0	+ 10·1
6	355	4·79 (12)	317	5·97 (12)	− 10·6	+ 40·5	+ 25·5

Notes:

(*a*) The total number of values obtained are in brackets beside the mean value of the oxygen consumption.
(*b*) In calculating the increase in oxygen consumption allowance is made for the weight loss in each case.

Of the animals studied, guinea pigs have proved to be the most suitable for assays, as even stimulation by dinitrophenol caused little disturbance to their natural quietness.

In selecting the best weight, consideration must be given to the variations in the normal oxygen consumption of those animals within the weight range of 150–250 g. As a general rule, it is suggested that heavier animals (300–400 g.) should be used. It is interesting to note that Reineke and Turner[8] record few values of animals weighing 150–250 g. Therefore, it seems likely that they encountered similar variations and rejected these animals as unsuitable for thyroxine assays.

At this stage it is not proposed to deal with the best methods of assay involving oxygen consumption measurements, but with an apparatus of the type described the procedure for group determination must be made as an initial routine procedure. This allows the animals time to settle and permits the efficiency of the apparatus to be checked. In addition, if all the animals have received the same dose of metabolic stimulant then the procedure will give added information on the response of the group.

Work is now in hand to determine the minimum number of measurements that must be made on individual animals in an assay, and preliminary experiments suggest that three may be sufficient. Clearly one

determination per animal has several disadvantages, not the least being that it necessitates the use of a large number of animals.

The results of the experiments described in this communication lend weight to the arguments that many animals are necessary for the assay of metabolic stimulants[2]. Although much information exists on the assay of thyroxine and its analogues some of the methods are not altogether satisfactory and require improvement. Assay methods for dinitrophenol and related compounds, for example, 4:6-dinitro-*o*-cresol have not been satisfactorily worked out, and as a rule observations have been made on very small numbers of animals. In view of the risks to man and to animals exposed to this type of chemical it is essential that these omissions are rectified and these complementary problems are now being studied in more detail.

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