THE QUANTITATIVE RESPONSE OF THE OXYGEN CONSUMPTION AND WEIGHT OF GUINEA PIGS TO SOME METABOLIC STIMULANTS. WITH A NOTE ON (±)-5-IODOTRYPTOPHAN

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Regression coefficients for dose and oxygen consumption have been derived for 2,4-dinitrophenol (2,4-DNP), 4,6-dinitro-*o*-cresol (DNC), 2,6-dinitrophenol (2,6-DNP), and (−)-thyroxine. (±)-5-Iodotryptophan has no significant effect on the oxygen consumption or weight of guinea pigs.

A modified form of equipment for the measurement of oxygen consumption of small animals has been described in a previous communication[1]. It can be used to demonstrate a significant relation between increase in oxygen consumption and dose of various metabolic stimulants[1,2]. When log per cent increase in oxygen consumption was plotted against log dose for 2,4-dinitrophenol (2,4-DNP), and 4,6-dinitro-*o*-cresol (DNC), the relation appeared to be linear. A similar response was noted with (−)-thyroxine.

Because several investigators[3–5] have measured metabolic activities by plotting increase in oxygen consumption against time it seemed reasonable to investigate the possibility of extracting a linear relationship from the response. Included in this communication, therefore, are regression coefficients calculated on previous data[1,2] and on fresh experiments, including assays on 2,6-DNP and (−)-thyroxine. Special attention has been paid to 2,6-DNP because not only did it cause moderate increases in the oxygen consumption of guinea pigs, but it also caused a hypothermia in rats, particularly at high dose levels[2].

In addition a note has been included on a new synthetic amino acid, (±)-5-iodotryptophan[6], to report experiments that have demonstrated its inability to affect the oxygen consumption or body weight of guinea pigs. Although this amino acid was prepared for another biological investigation, it was considered desirable to test its possible effects on the overall basal metabolism of guinea pigs particularly as it contained an aromatic nucleus containing iodine.

Methods

Animals. When possible equal numbers of male and female guinea pigs weighing 250 to 350 g. were used for all dose levels of all substances.

Number of doses. Since earlier studies[2] confirmed that maximum levels of the dinitrophenols occurred 1 to 2 hours after administration, oxygen consumption values were obtained 1·25 to 1·5 hours after *single* intraperitoneal doses.

A different procedure was adopted for thyroxine, and a preliminary experiment demonstrated that within dose limits the hormone exercised a cumulative action after several daily doses. In consequence of this *four* daily doses of thyroxine were always given before the final oxygen consumption was measured 24 hours after the last dose.

First and last values for oxygen consumption *were calculated on the weight of the animal at the time of the measurement.* This is particularly important when thyroxine is used for several days since the weight loss may be 20 per cent or more. When dinitrophenols are assayed a single weight is sufficient because these substances reach their maximum effects on oxygen consumption very rapidly.

All substances were administered as aqueous solutions of their sodium salts.

Plan of Dosing

Substance	Number of animals	Number of animals per dose	Dose mg./kg.
2,4-*DNP*			
Scheme A (*a*)	18	3	5, 10, 20, 25, 30, 35.
Scheme B (*a*)	36	6	5, 10, 20, 25, 30, 35.
DNC	24	6	5, 10, 15, 20.
2,6-*DNP*	24	6	5, 10, 20, 30.
(−)-*thyroxine*	30	5	0·125, 0·25, 0·50, 1·0, 2·0(*b*).
(±)-5-*iodotryptophan*	18	3	1·4 × 2 mg./kg. thyroxine. 2·4 × 2 mg./kg. thyroxine plus 4 × 2 mg./kg. (±)-5-iodo-tryptophan. 3·4 × 2 mg./kg. (±)-5-iodotryptophan.

Note.—(*a*) See Harvey[1]. (*b*) See note on Table III.

Results

The cumulative effects of four daily doses of 1 mg. of thyroxine per kg. on the oxygen consumption and weights of guinea pigs are shown in Figure 1. Regression coefficients and equations for 2,4-DNP and DNC are shown in Table I, and comparison of their residual variances in Table II. Equations have not been derived for any of the male or female regression

TABLE I

Regression coefficients and equations for 2,4-dnp and dnc

Type of measurement	*n*	*b*±S.D.	P	Equation
2,4-*DNP, Scheme A.*				
All doses, all animals	18	+1·20±0·37	>0·001 <0·01	$y=0.123\pm1.20x$
All doses, males	9	+0·84±0·12	<0·001	(*a*)
All doses female	9	+1·89±0·79	>0·01 <0·05	(*a*)
Group 1	6	+1·65±0·91	>0·01 <0·05	(*a*)
Group 2	6	+0·57±0·74	N.S.	—
Group 3	6	+1·32±0·06	<0·001	(*a*)
2,4-*DNP, Scheme B*				
All doses, all animals	35	(*b*)+1·12±0·17	<0·001	$y=0.253+1.12x$
5, 10, 20 mg./kg. (*c*)	17	(*b*)+0·99±0·23	~0·01	$y=0.371+0.99x$
10, 20, 35 mg./kg. (*c*)	18	+1·65±0·25	<0·001	$y=1.75x-0.514$
25, 30, 35 mg./kg. (*c*)	18	+3·45±0·85	<0·001	$y=3.45x-3.167$
All doses, males	17	(*b*)+1·48±0·24	<0·001	(*a*)
All doses, females	18	+0·93±0·23	<0·001	(*a*)
Group	6	+1·48±0·16	<0·001	$y=1.48x-0.216$
Mean of six values per dose	6	+1·10±0·17	> 0·01 >0·001	$y=0.324+1.10x$
DNC				
Group	4	2·23±0·34	>0·01 <0·05	$y=2.33x-1.282$

Notes—
(*a*) Variation between four sex values or small group values too great for satisfactory equations.
(*b*) One negative value disregarded.
(*c*) Overall regression for these three values 1·42±0·19 (see Table II) giving equation: $y=1.42x-0.119$.

values because of significant differences, or for the three small group values in Scheme A. Some of the significant regressions have been plotted graphically on Figure 2.

Because of the apparently anomalous behaviour of 2,6-DNP in causing limited increases in oxygen consumption and also a significant hypothermia[2] a fuller investigation was made on this substance. The results of this experiment are shown in Figure 3. From this it is seen that the mean per cent increases in oxygen consumption per group were, at 5 mg./kg. + 25·7 per cent, at 10 mg./kg. + 28·3 per cent, at 20 mg./kg. + 14·1 per cent and at 30 mg./kg. + 23·1 per cent.

One large increase (76·7 per cent) made the last average disproportionately great. Omitting this value the mean would have been 10·1 per cent. These results suggested two phases of activity, first, an initial stimulation, significantly positive, but of short duration, and secondly, a negative response reducing the first initial increase to within normal limits. This trend was in accordance with the observations on the effects of this dinitrophenol on body temperature.

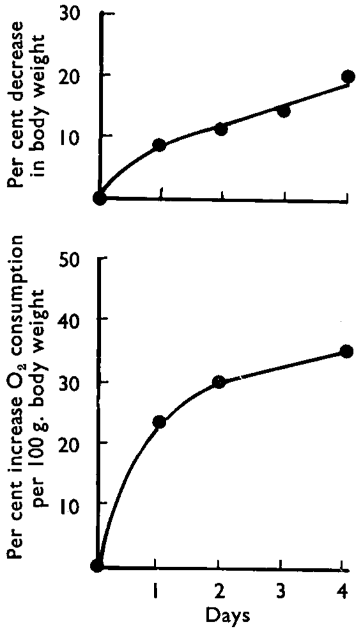

FIG. 1. Increasing oxygen consumption and decreasing weight of guinea pigs. Mean value of 3 male and 3 female animals each given 4 daily doses of 1 mg/kg. Na-(−)-thyroxine.

A regression coefficient was calculated for all doses except the first.

Dose levels	n	b ±S. D.	P
10, 20, 30 mg./kg.	17 (*a*)	−0·87 ± 0·52	N.S. at 0·20 (*b*)

(*a*) One reading lost.
(*b*) For $n_1 = 1$, $n_2 = 17$, F = 1·8 at P = 0·20.

TABLE II
COMPARISON OF REGRESSIONS BY RESIDUAL VARIANCES 2,4-DNP

Type of measurement	D.F.	Variance, ratios and significance
2,4-*DNP*		
All doses, all animals		
Scheme A	16	0·219 } 2·54 Nearly significance at P=0·01
Scheme B	33	0·086 }
5, 10, 20 mg./kg. ..	15	0·1123 } 1·9 N.S. } 2·6, P<0·05
10, 20, 30 mg./kg. ..	16	0·0591 } 1·4 N.S.
25, 30, 35 mg./kg. ..	16	0·0432

The overall regression for the last three regressions is 1·42±0·19 with a residual variance of 0·070. This does not vary significantly from the All doses all animals or Group Regressions variances of Scheme B. (P<0·01).

Regression coefficients and equations for relations between dose of thyroxine, oxygen consumption and weight are given in Table III, and the residual variances in Table IV. The relation between oxygen consumption and dose is also illustrated in Figure 4. It is noted that the

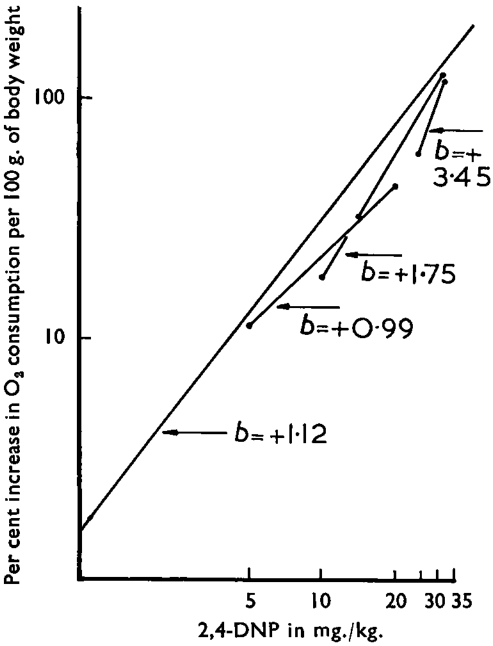

FIG. 2. Various regressions of oxygen consumption and dose of DNP in the guinea pig. (Scheme B).

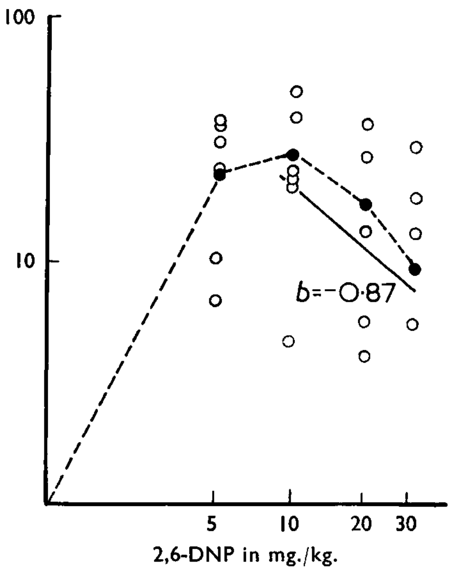

FIG. 3. Partial stimulation of oxygen consumption of guinea pigs by 2:6-DNP. ●---● Mean values.

dose:weight response is more significant than the oxygen consumption dose relation.

TABLE III

REGRESSION COEFFICIENTS AND EQUATIONS FOR THYROXINE

Type of measurement	*n*	*b*±S.D.	P	Equation
Thyroxine—oxygen consumption (*c*)				
All doses, all animals	22	(*a*) +0·59±0·26	>0·01<0·05	$y=0{\cdot}022+0{\cdot}59x$
All doses, males	15	+0·25±0·13	>0·05<0·20	(*b*)
All doses, females	7	(*a*) +1·66±0·61	>0·02<0·05	(*b*)
Group	4	+0·63±0·19	>0·05<0·20	$y=063x-0{\cdot}167$
Mean of six values per dose ..	4	+0·60±0·20	>0·05<0·20	$y=0{\cdot}073+0{\cdot}60x$
Thyroxine—body weight (*c*)				
All doses, all animals	24	+0·34±0·08	<0·001	$y=0{\cdot}240+0{\cdot}34x$
All doses, males	15	+0·29±0·01	>0·01<0·05	$y=0{\cdot}77+0{\cdot}29x$
All doses, females	9	+0·39±0·04	<0·001	$y=0{\cdot}092+0{\cdot}39x$
Group	4	+0·36±0·11	>0·05<0·20	$y=0{\cdot}192+0{\cdot}36x$

Notes.

(*a*) Two negative values disregarded.
(*b*) Regressions not significant enough for good equations.
(*c*) Regressions calculated on 4 dose levels only (0·125, 0·25, 0·5, 1·0 mg./kg.) because 2 mg./kg. did not cause any increases in oxygen consumption greater than those caused by 1 mg./kg.

To investigate further the scatter of individual values within dose groups the coefficients of variation for the mean oxygen consumption for each dose were determined for 2,4-DNP (Scheme B), and for (—)-thyroxine. These are shown in Table V.

The results of the experiments on 5-iodotryptophan are given in Table VI.

Discussion

Three general observations may be made from the results shown in Table V. First, the response is more homogeneous at higher than at lower dose levels of 2,4-DNP, in fact, there is a significant regression between the coefficient of variation and dose (b — 1·05 ± 0·15, $P < 0·01 > 0·001$). Secondly, there is a similar *trend*, but not very significant, in the case of thyroxine ($b = -29·1$, $P > 0·20$). Thirdly, the weight changes do not vary significantly (11·9 ± 3·0).

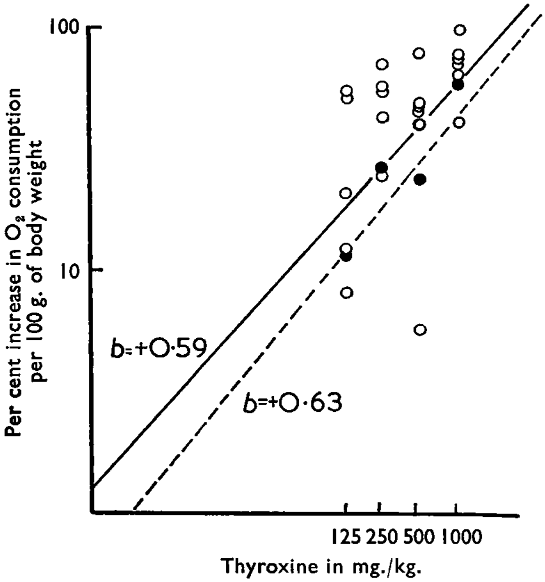

FIG. 4. Regression of oxygen consumption and doses of Na(—)-thyroxine in the guinea pig. All doses all animals, ○——○. Group, ●- - -●.

From the experiments with 5-iodotryptophan (Table VI) it is clear that this amino acid has no significant effect on increasing the oxygen consumption or lowering the body weight of guinea pigs. Neither does it appear to possess anti-thyroxine action, since it does not diminish the response of guinea pigs given simultaneous doses of thyroxine.

A completely satisfactory method for the assay of metabolic stimulants, particularly thyroxine, has not yet been evolved, but these results show sufficient promise to warrant further investigation. The measurement of oxygen consumption in large numbers of small animals presents many technical difficulties, especially that of achieving thoroughly sound "basal" conditions, but it is considered that an approach to the problem along the lines developed in the present studies should eliminate many of these obstacles.

It has proved possible in these experiments to derive a significant relation between dose and oxygen consumption, particularly when synthetic stimulants are employed. Similar but less significant relations

TABLE IV

COMPARISON OF REGRESSIONS BY RESIDUAL VARIANCES—THYROXINE

Type of measurement	D.F.	Variance, ratios and significance
Thyroxine—oxygen consumption		
All doses, all animals	20	0·169 } 9·0 N.S. (with Group)
All doses, male	13	a
All doses, female	5	a
Group	4	0·0352 } 1·0 N.S.
Mean six values per dose ..	4	0·0359
Thyroxine—weight		
All doses, all animals	22	0·0193 } 1·1 N.S. (with male); } 12·0 P 0·01 (with female); } 3·1 N.S. (with Group)
All doses, male	13	0·018 } 11·2 P 0·01 (with female)
Al doses, female	7	0·0016
Group	4	0·0061

a. See data in Table III; as no equation derived, no significance or ratios calculated.

TABLE V

SCATTER OF OXYGEN CONSUMPTION AND BODY WEIGHT VALUES FOR DIFFERENT DOSE LEVELS OF 2,4-DNP AND THYROXINE

Coefficient of variation				
2,4-DNP		(−)-Thyroxine		
Dose mg./kg.	Oxygen consumption	Dose µg./kg.	Oxygen consumption	Body weight
5	34·5	125	42·7	11·9
10	28·8	250	10·5	16·1
20	18·4	500	26·1	9·2
25	17·7	1000	6·6	10·4
30	7·9			
35	6·6			

TABLE VI

(±)-5-IODOTRYPTOPHAN ON THE OXYGEN CONSUMPTION AND BODY WEIGHT OF THREE MALE AND THREE FEMALE GUINEA PIGS

Na-(−)-thyroxine only, 4 daily doses, 2 mg./kg.		Na-(−)-thyroxine, and (±)-5-iodotryptophan 4 daily doses, 2 mg./kg. each substance		(±)-5-iodotryptophan 4 daily doses, 2 mg./kg.			
					Oxygen consumption		
					Actual ml./100 g. min.		
Weight per cent change	Oxygen per cent change	Weight per cent change	Oxygen per cent change	Weight per cent change	Before	After	Per cent change
−14·7	+44·5	−13·1	+4·3	+2·7	2·17	2·04	−5·9
−14·2	+47·5	−24·5	+48·8	−2·1	1·90	2·25	+18·4
−8·3	+27·0	−11·0	+53·5	0·0	1·75	2·05	+17·1
−20·5	+22·5	−14·8	+82·2	−2·6	1·55	1·62	+4·5
−15·2	+30·5	−9·9	+48·7	+1·0	2·19	1·70	−22·3
−8·4	+39·0	11·7	+27·7	+1·5	1·96	1·82	−7·1
Averages							
−13·6	+35·1	−14·2	+44·2	0·1	1·92	1·91	+0·8

Tests on Data in Table VI

Since there are n observations there are n-1 (5) degrees of freedom and for P=0·05 t should be 2·57.

1. *Comparison between thyroxine and thyroxine plus 5-iodotryptophan treatments*
   (*a*) Between weight changes; t = 0·25, N.S.
   (*b*) Between changes in oxygen consumption t = 2·02, N.S.

2. *Comparison between treatments—before and after 5-iodotryptophan only*
   (*a*) Between individual values, oxygen consumption t = 0·03 N.S.
   (*b*) Between weights, differences very small, no t test applied.

can be derived for (—)-thyroxine. Extrapolation of the regressions to the intersection of the x and y axes (Figs. 2 and 4) suggests that over a wide dose range the linear relation holds good. For, although the regressions do not pass exactly through the intersections, the variations are small—usually about 5 per cent.

The minimum number of animals necessary for a satisfactory regression appears to lie between 20 and 30 with at least 4 dose levels. Small numbers (for example, Scheme A, 2,4-DNP) are likely to give variable results. Evidence in support of these suggestions may be obtained by examining the regressions resulting from random selection of 1,2 . . . 5 increased oxygen consumption values for each dose level of 2,4-DNP in Scheme B. Thus:

Number of animals selected at random from each group	Total number of animals	$b \pm$S.D.	Coefficient of variation
1	6	+1·49±0·40	26·9
2	11	+1·14±0·24	21·1
3	18	+1·19±0·25	20·9
4	24	+1·15±0·25	21·6
5	30	+0·96±0·20	20·7
	Average	+1·17±0·27	
6 (*vide supra*)	35	+1·12±0·17	15·1

The average regression from 1 to 5 random selections is seen to be only 5 per cent greater than that obtained from the all doses, all animals regression; also the coefficient of variation is greater with a smaller than with a larger number of animals.

It appears that equal numbers of male and female animals are essential if sufficient numbers of either sex are not available. With 2,4-DNP the variations between the four sex regressions from Schemes A and B are highly significant, and therefore mean values for males and females, namely, 1·41 and 1·16 cannot be used in calculating and overall regression.

The selection of appropriate dose levels for 2,4-DNP type compounds presents fewer problems and the overall regression calculated on the three dose groups in Scheme B does not vary significantly from all the doses, all animals and Group regressions. On the whole, Group responses are reliable (see Maclagan and Sheahan[4]) and they have the advantages of speed, simplicity and probably the elimination of individual variations[1].

The experiments on thyroxine show that the weight decrease: dose relation is better than the oxygen consumption: dose relation. This is in general agreement with the observations of Reinecke and Turner[7], and it is difficult to appreciate why this simple technique has not received further attention (see Burn[8] commenting on Kreitmair[9]).

One other feature that requires further investigation is the observation that increasing doses of thyroxine often cause a reduction in response. Maclagan and Shehan[4] report steady increases with doses of thyroxine above 2 mg./kg. on mice, but in my experiments the maximal dose for guinea pigs is 4 daily doses of 1 mg./kg. A fuller study is desirable to provide more basic information for the design of an adequate assay technique.

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