" I dedicate this thesis to my Parents,
who have unselfishly sacrificed their
energies and interests on my behalf
during these long years of studies. "

ACKNOWLEDGEMENTS

I wish to thank personally Dr. André DesMarais for his guidance, personal understanding and encouragement for making this thesis possible. I shall not only remember Dr. DesMarais as a teacher and director but as an esteemed and dear friend whose keen insight and friendly advice directed this work during my stay at the University of Ottawa.

I thank all the staff members of the department for their encouragement especially Dr. Bernard Belleau of the Department of Chemistry for his help.

My gratitude also goes to my fellow graduate students, and to the members of the technical staff especially Mr. A. Boudria and R. D'Aoust for their various contributions brought to this research, and G. Ben-Tchavtchavadze for the photographs.

I wish also to express not only my gratitude but also my sincere thanks to the Defence Research Board of Canada for its generous financial support through grant Number 9310-66 to Dr. André DesMarais.

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ABSTRACT

Experiments were designed to find whether thyroxine and an anti-thyroxine compound (DDIH) administrations had any effects on the oxygen consumption of both warm- and cold-acclimated animals. Further experimentation was conducted to find if this anti-thyroid compound was competitive in nature with thyroxine in both warm- and cold-acclimated animals.

The results obtained may be summarized as follows:

1. The dose of 2 mgm/kg of L-thyroxine produced a maximal increase in oxygen consumption in warm-acclimated animals.

2. The inhibitory effect of DDIH, as observed by Serif and Seymour (1961) was confirmed.

3. This inhibitory effect being reversed by increasing the level of injected thyroxine would appear to be competitive in nature.

4. Exogenous thyroxine had very little calorigenic effect in cold-acclimated animals.

5. DDIH did not show any inhibitory effect in cold-acclimated animals, neither on the elevated metabolic rate nor on the slight increase produced by thyroxine administration.

These results are discussed along with current literature references and it is concluded that these small findings do emphasize the importance of the thyroid hormones in cold-acclimated animals. Further, these observations suggest reliability of our

experimental model on calorigenic effect of thyroid hormones where whole animals were used instead of cell fractions.

STATEMENT OF THE PROBLEM

In an attempt to study the peripheral activity of thyroid hormones in cold acclimation, it appeared proper to try to obtain an experimental model of the calorigenic effect of the thyroid hormones in which the whole animal would be used instead of cell fractions.

In 1961 a report was published on the effects of a specific anticalorigenic compound better known as the diacetyl derivative of 2,6, diiodohydroquinone (DDIH) (Serif and Seymour 1961). This compound (DDIH) was shown to inhibit the calorigenic action of injected thyroxine in mice. Comparison of the structure of this compound with that of thyroxine, along with evidence obtained by various workers in this field as reviewed by Barker (1962), suggested that it could act as a competitor of thyroxine for acceptor sites of deiodination. This problem of deiodination appears to be at the very heart of the peripheral activity of the thyroid hormones on calorigenesis. However, the anticalorigenic effect of DDIH had not, to our knowledge, yet been shown to be competitive in nature.

It thus appeared necessary to verify the possibility of such competition and to find at the same time whether the inhibitory

effect of this substance would be the same in both warm- and cold-acclimated animals.

REVIEW OF THE LITERATURE

Part One

I. Introduction

All homeotherms must, at great costs to themselves, maintain a constant internal temperature. This requires that their heat production increases in order to compensate for the heat lost to the environment when the external temperature decreases. Since there is no way of avoiding cold, homeotherms have to fight a physiological battle (physical and chemical actions and their interactions) against body cooling in order to ensure their survival. There is ample evidence to the fact that these animals become acclimated to cold in the sense that continuous or repeated exposure to cold is accompanied by specific physiological changes which tend to minimize or lessen the cost of maintenance of their body temperature. It is not our intention to elaborate extensively on the Physiology of Cold Acclimation since this subject has recently been reviewed thoroughly by Smith and Hoijer (1962) as well as by participants to both the XXI International Congress of Physiological Sciences, Buenos Aires, 1959 and the Proceedings of the International Symposium on Temperature Acclimation in Leiden, Netherlands, 1963. Since a review, at this time, of the literature on Cold Physiology is both impractical and not asked

for, be it sufficient to give the readers a general but yet concise outline of what occurs to homeotherms that become acclimated to a cold environment. It is now well established that the development of cold acclimation requires a period of time usually estimated to be from two to six weeks. During this time there is a gradual increase in cold resistance (Blair, Dimitroff, Hingeley 1951; Hart 1953 and 1961), an increase in food consumption (Sealander 1952; Cottle, Carlson 1954), an elevation of the Metabolic Rate (Thibault 1949; Hart 1953 and 1958), an elevation of peripheral (Carlson 1954, Héroux 1959) and sometimes central (Gelineo 1934) temperatures, a decrease in shivering (Hart et al 1956) and an increase in non-shivering heat production (Tanche et al 1962) and other associated physiological and biochemical changes. We shall thus briefly limit ourselves to the role of thyroid hormones in cold acclimation, together with a discussion of the effects of antithyroid substances.

II. The thyroid hormones in cold acclimation.

A considerable amount of attention has been directed towards the hormonal regulation of the metabolic response to cold, particularly by the thyroid, whose role has been more than stressed in the elevated metabolic rate of animals chronically exposed to cold. A convincing body of evidence has been accumulated to show that thyroid activity is increased in many different animals (Thibault 1949; Rand 1952; Hart 1953; Cottle 1956). Total

thyroidectomy has been shown to lower the BMR by 40% and consequently, to induce a decrease in body temperature (Spence 1959). The intravenous injection of single doses of either triiodothyronine (T_3) or tetraiodothyronine (T_4) increases considerably the BMR after a latent period of six to eight hours for T_3, while it is much longer for T_4 (Thibault 1957). Such results substantiate the assumption that the thyroid hormones regulate to a great extent the BMR although they cannot be considered as the only agents of such a regulation, since other substances, like the catecholamines, have been shown to increase the BMR in the presence of a fixed amount of thyroid hormone (Ring 1942; Thibault 1949). The essentiality of the thyroid hormones to survival in cold as well as their calorigenic abilities has since become a well-documented fact which has been recently reviewed (Carlson 1960; Cottle 1960; Smith and Hoijer 1962). One could give a detailed analysis of other factors interrelated with the action of thyroid hormones; be it sufficient to cite one of these factors which has been closely scrutinized in this laboratory: ascorbic acid. Some evidence has been provided that this substance somehow reduces the needs of thyroid hormones in rats exposed to cold (DesMarais 1953; 1956; 1957; 1963). One of the conclusions derived from such studies is that at least a minimum amount of thyroid hormones is essential for the animal to increase its metabolic rate in order to maintain thermal equilibrium.

The mode of action of the thyroid hormones on the release of energy at the cellular level has recently been reviewed (Ingbar and Galton 1962; Barker 1963; Smith and Hoijer 1962) and is believed to be related to its uncoupling effect on oxidative phosphorylation. This cellular activity of thyroid hormones will be dealt with accordingly throughout the length of this thesis. Another interpretation of this role of the thyroid hormones, recently suggested by Potter 1958 and elaborated by Beyer 1962, is related to the synthesis of enzymes activating the TPNH-regulated calorigenic shunt.

III. Inhibitors of the synthesis and metabolism of thyroid hormones.

One may say that there are two central points of attack for the different thyroid inhibitors: 1. The Site of Production of the thyroid hormones (i.e. the gland); 2. The Site of Action of the thyroid hormones (i.e. at the cellular level).

1. Inhibitors of Production.

We shall give only a brief outline of inhibitors acting at the gland level, since it is only indirectly related to this research. One can classify these inhibitors into two main categories:

a) those interfering with the Iodide Trapping Mechanism.

b) those interfering with the Binding of Iodine to Protein* and with hormone synthesis.

* By the interference with the Binding of Iodine to Protein, one infers that they block the formation of iodinated amino acids (MIT and DIT) or thyroid hormones).

1a) In the case of Iodide Trapping Mechanism one is well aware of the fact that the thyroid shares its ability to concentrate iodide with a series of other anions of different chemical behaviour and of no physiological use for the gland. Still these anions such as thiocyanate (Barker 1936; Halmi 1960; Wollman 1956); perchlorate (Ways et al 1952; Standbury 1953; Lewitus 1960; Crooks et al 1960); and nitrate (Ways and Wyngaarden 1952) are selectively accumulated in the thyroid similarly to Iodide, and when present in comparable concentrations, they compete with iodide ions for the trapping sites within the gland. In all cases the T/S ratio of iodide is lowered, thereby showing that the thyroidal trapping of iodide has been reduced.

1b) On looking at the different agents which interfere with the Binding of Iodine to protein and with hormone synthesis, one finds volumes of literature dealing with hundreds of different chemicals which offer minimum to maximum inhibitory properties at this level. We shall limit our description of such inhibitory chemicals to but the best knownones, such as Thioureas, Propylthiouracil, Resorcinol; additional information can easily be obtained in different reviews (Selenkow 1955; Danowski 1962). Thioureas, inhibitors of the organic binding, were abandonned when Thiouracil became available, more specifically Propylthiouracil (PTU). This substance, although a more effective suppressor of thyroid function, has some drawbacks interferring with the results of experimentation: toxicity to animals, damage to kidneys,

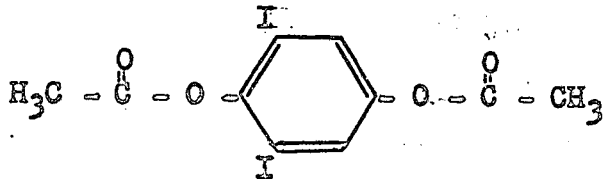
impairment to peripheral metabolism of T_4 (Escobar 1960). Resorcinol showed diminished I^{131} uptake which seemed to result from decreased binding of iodine (Bull and Fraser 1950). Many other agents have been shown to interfere at this level and as one reads this literature one cannot but notice that the mechanisms of action of these many agents have not as yet been fully described, defined or even understood.

2. Inhibitor of the action of thyroid hormones.

The second point of attack for the different thyroid inhibitors is the Site of Action of the thyroid hormones (i.e. the cellular level). Less work has been done at this level because of the complex nature of experimentation at these different sites. It has been hinted before and even well established that P.T.U. and derivatives do have effects on the peripheral metabolism of T_4 (Escobar 1960), thus pointing positively to this second point of attack. It has been theorized that T_4 is converted to T_3 at the cell level of different tissues (Standbury 1960; Werner et al 1961) either at the cell wall or within the cell. A group of agents supposedly interfere with or inhibit such a conversion, thus having antithyroidal properties. One such chemical which has been used is B.H.D.B., n-butyl 3,5, diiodo-4-hydroxy benzoate (Sheahan et al 1951), which has shown definite antithyroid properties. This agent has been shown to prevent the calorigenic action of T_4 in mice (Sheahan et al 1951) and potentiate the calorigenic action of T_3 (McClagan et al 1952); further

it has been shown to uncouple oxidative phosphorylation when added to isolated mitochondria (Mudd, Lippman 1955). Grief (1960) has shown that B.H.D.B. completely prevents the swelling of isolated mitochondria produced by T_4 (Tapley 1956). This phenomenon of mitochondrial swelling is highly complex and far from being satisfactorily explained. It seems likely that B.H.D.B. has multiple effects and is an antithyroid compound acting at least in part, at the site of action of T_4.

Ubiquinones (CoEnzymes Q) have also been considered as a site of action of antithyroid substances, because of the close chemical relationship between these substances and the phenolic portion of the thyroid hormones (Barker 1963). One such agent which has attracted our attention is known as DDIH i.e. the diacetyl derivative of 2,6, diiodohydroquinone (D.I.H.).



The D.I.H. form has been suggested as a potential degradation product of T_4 (Allegreti 1954). Preliminary studies of the effect of D.I.H. on oxygen consumption in mice revealed that its toxicity interfered with an evaluation of its anti-hormonal activity. It was then found that DDIH, the diacetyl derivative of D.I.H., proved to be relatively non-toxic and exhibited antithyroid effects. This substance was shown to inhibit the elevation of oxygen uptake in mice injected with T_4, but not in those injected with T_3 (Serif and Seymour 1961). It acted like B.H.D.B., which had been theorized

to be antithyroid in nature by virtue of its ability to prevent deiodination of T_4 to T_3. It remained to be shown whether this agent (D.D.I.H.) acted as a competitive inhibitor of T_4 and whether its effects were the same in cold-acclimated animals.

EXPERIMENTAL

Part Two

1. Description of Methods:

I. Animals and Diet

Male albino mice of the Wistar strain weighing initially between 20 and 30 grams were placed separately in metal cages with wire screen bottoms (Plate 1, Figure 1) at room temperature and at 30°C (±1°C). In the cold environment, the animals were kept in individual plastic cages containing sawdust. All animals were fed Purina Lab Chow and tap water ad libitum.

2. Temperatures of exposure:

The animals were left at room temperature in the animal quarters for one week to familiarize them with instruments and handling. Groups of animals were then placed in a constant temperature room of 30°C (± 1°C). Experimentation was started on this group after one month of acclimation. Other groups of mice were placed in a cold room at 20°C and were acclimated by gradually lowering the temperature by 2°C every 3-4 days until the required temperature of 10°C was reached. Experimentation was then undertaken after three weeks of acclimation to this temperature (10°C ± 1°C).

3. Treatments:

a) Thyroxine injections: (l-Thyroxine Sodium):

Thyroxine was first administered subcutaneously at a dose level of 2 mg/kg or 4 mg/kg at zero time, followed by three doses of 0.5 or 1 mg/kg every twelve hours. Since it was found that similar results were obtained if a second dose of 1.0 or 2.0 mg/kg was given 24 hours after the first injection, instead of the three doses, this last procedure was followed:

b) DDIH (Diacetyl derivative of 2,6-diiodohydroquinone).

The finely ground DDIH, suspended in a 0.5% saline solution of gelatin and well shaken before administration so as to ensure uniform distribution of the micromolecules, was injected intraperitoneally. (The 2,6-diiodohydroquinone (DIH) was diacetylated at the Chemistry Department of the University).

c) Oxygen consumption measurements:

At both zero time and forty-eight hours after the first injection of either Thyroxine or DDIH or both, the oxygen consumption was measured according to the technique described in section 4.

4) Oxygen Consumption:

The method of measurement involved the use of an improved closed circuit apparatus of the type of Maclagan et al (1950). The changes made were brought about so as to obtain measures of oxygen consumption of resting animals instead of non-resting ones. The eight animals were placed in an ordinary vacuum desiccator (9 litre capacity) (Plate I, Figure 1) but confined within respective areas by wire mesh partitions (Plate I-II, Figure 2-3) which prevented fighting and excessive movements.

Ascarite or Soda Asbestos instead of Soda lime was used because this is recognized to absorb carbon dioxide more efficiently (Plate I, Figure 1). Since it was found that accumulation of water vapor does affect the readings unfavourably, magnesium perchlorate (Plate II, Figure 4) was used to absorb any water vapour present in the system. The experiment was started by weighing each of eight mice to the nearest gramme, placing them in the desiccator (Plate II, Figure 3) and placing the lid over the top without closing it completely. A 50% oxygen-nitrogen mixture was circulated through the desiccator at the rate of 8 l/min. for 3 minutes to ensure a 50% enriched atmosphere. The desiccator was then sealed with special care, the tap opened to connect the desiccator to the manometer, the circuit closed by clamping the T-juncture (Plate II, Figure 5) and the desiccator was then placed in a water bath with stirrer. At either temperature of measurement a 30 minute period was allowed for temperature equilibration within the chamber. At the end of this time the zero reading was recorded and further readings were taken at intervals of 10 minutes each for one hour. It was found that readings recorded for one hour instead of three hours were sufficiently constant for correct measurements of oxygen consumption. It was necessary to tap the manometer before each reading to prevent the mercury from sticking. The calculation of oxygen consumption is very simple, for it depends merely upon the pressure and the volume and temperature of the system. Calculation of oxygen consumption is as follows:

PLATE I

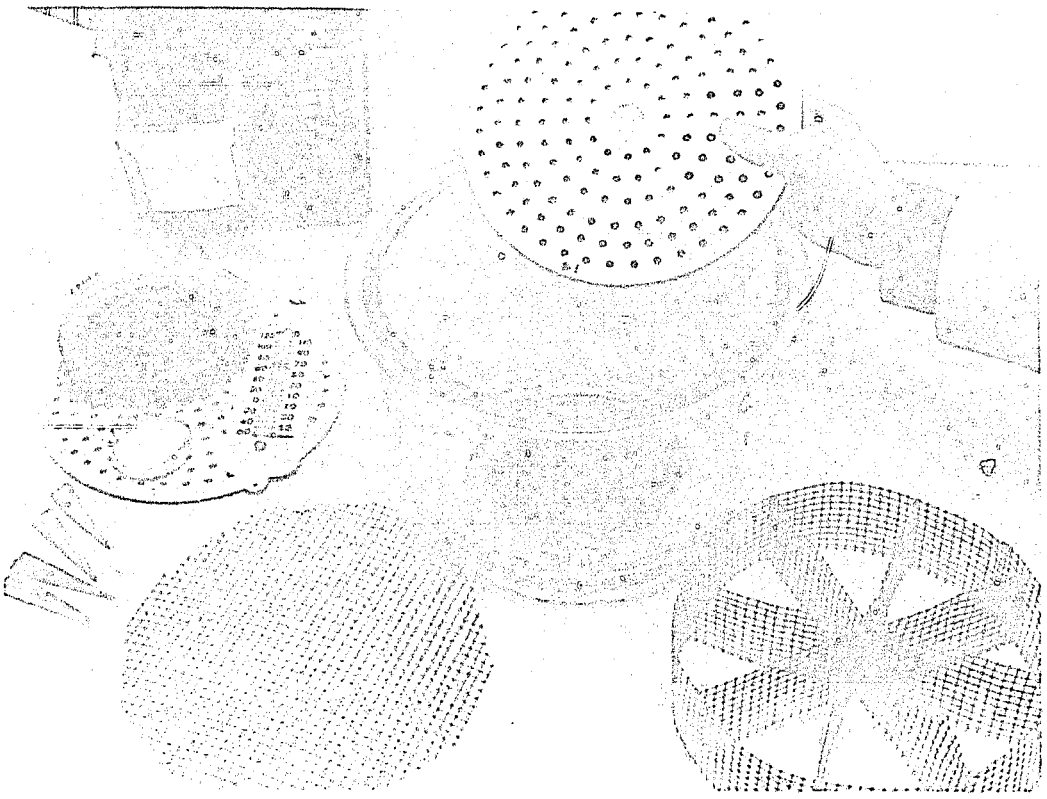


Fig. 1

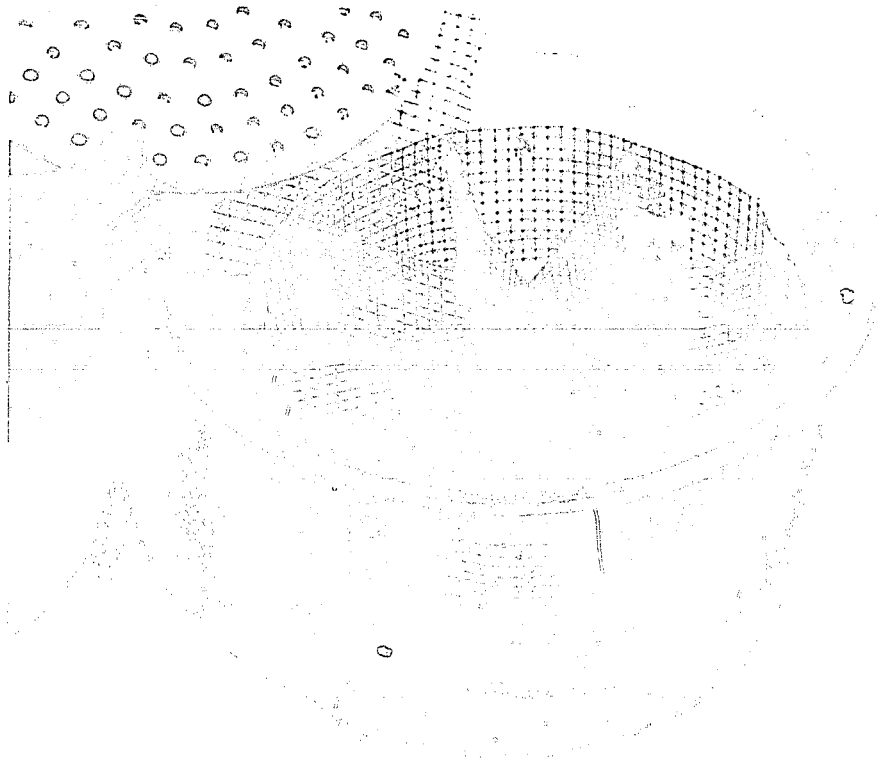


Fig. 2

PLATE I

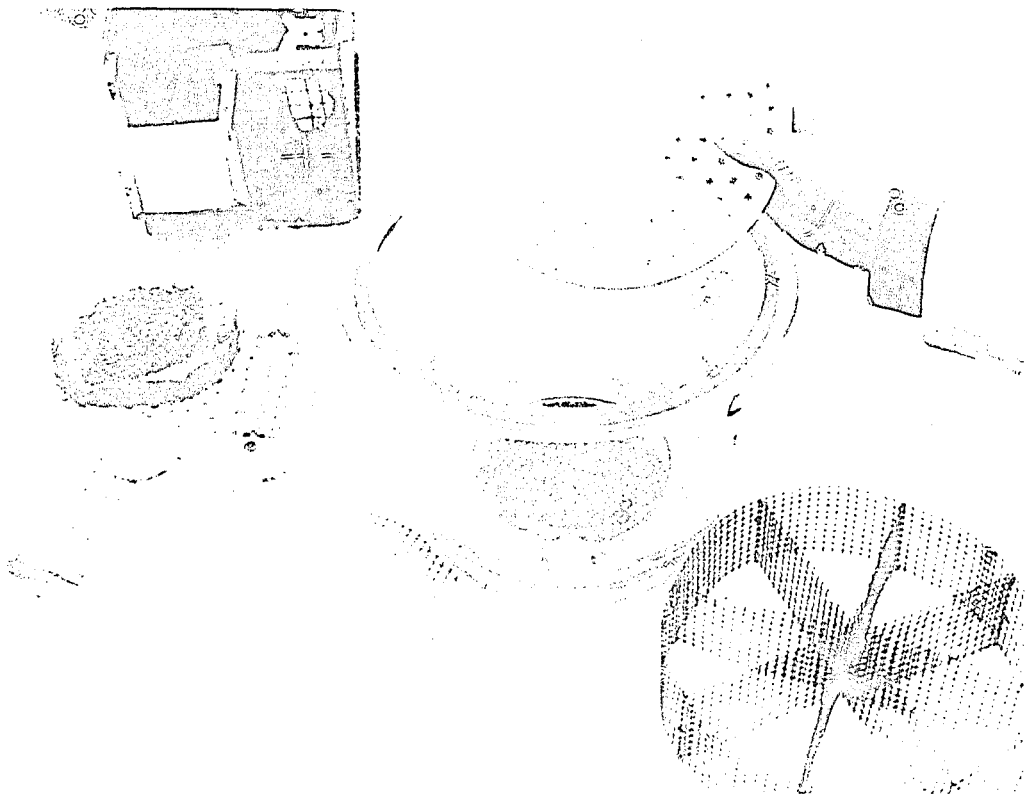


Fig. 1

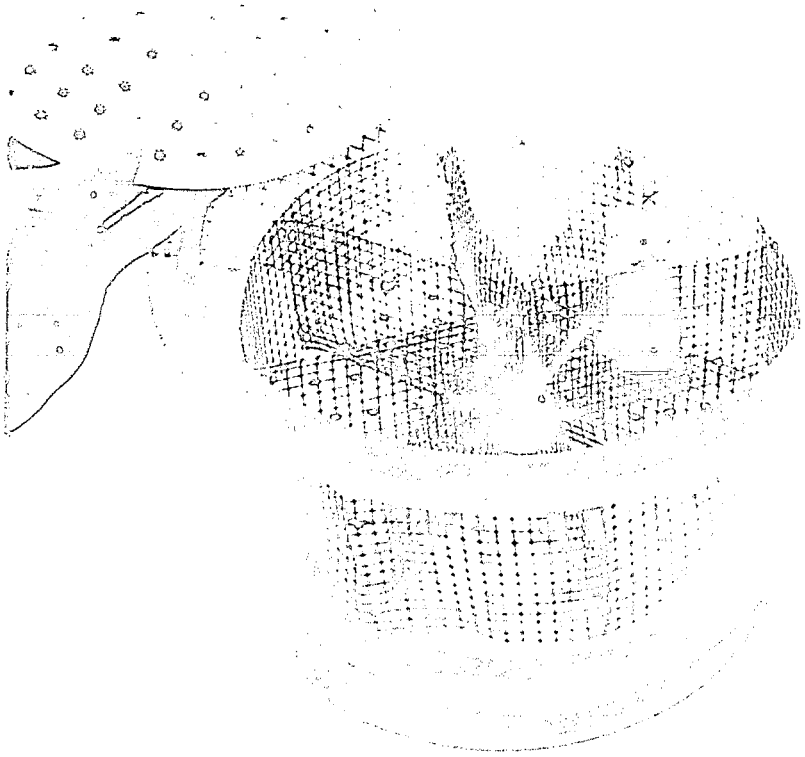


Fig. 2

PLATE II

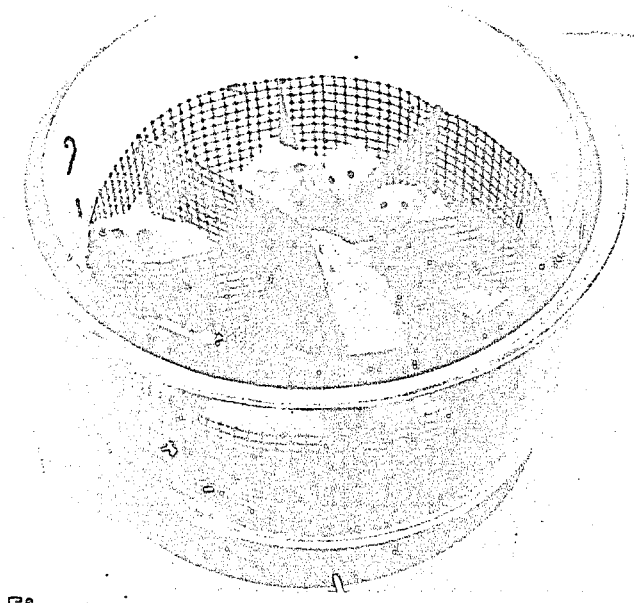


Fig. 3

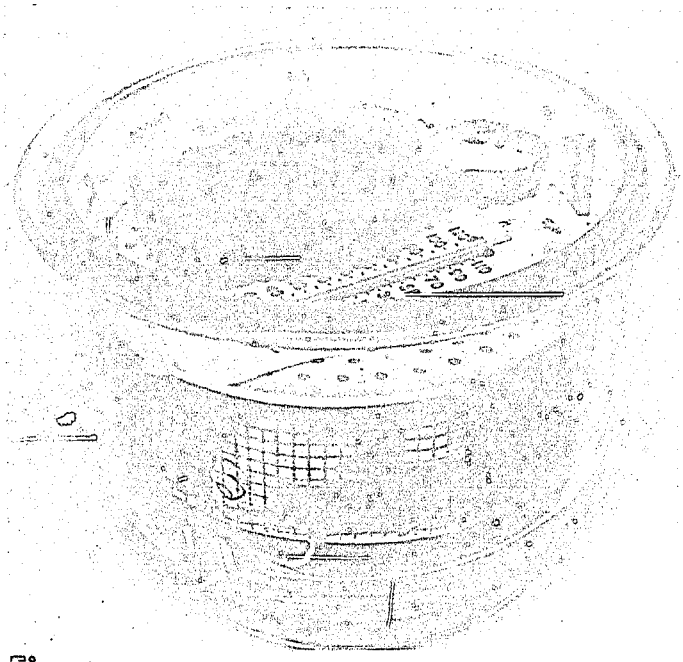


Fig. 4

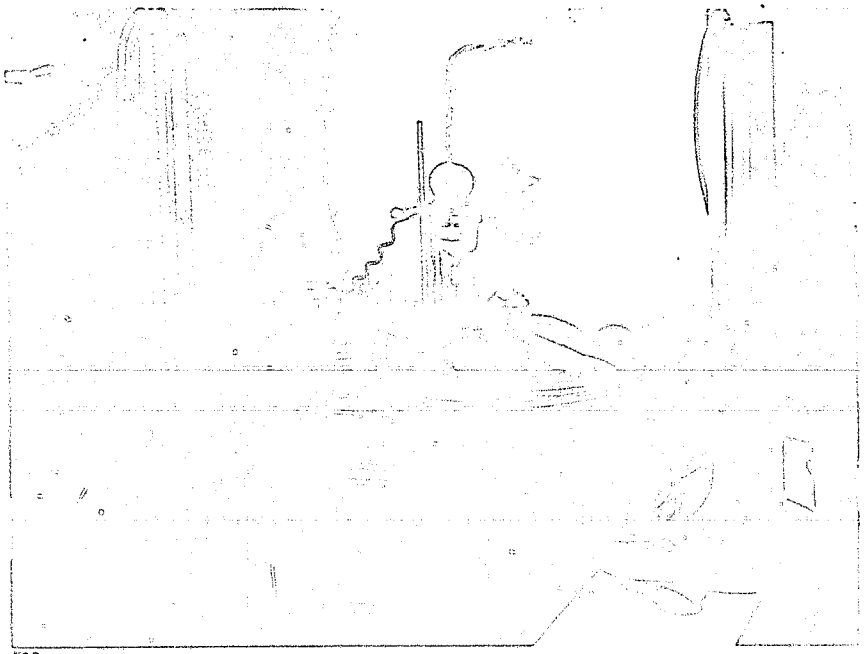


Fig. 5

PLATE II

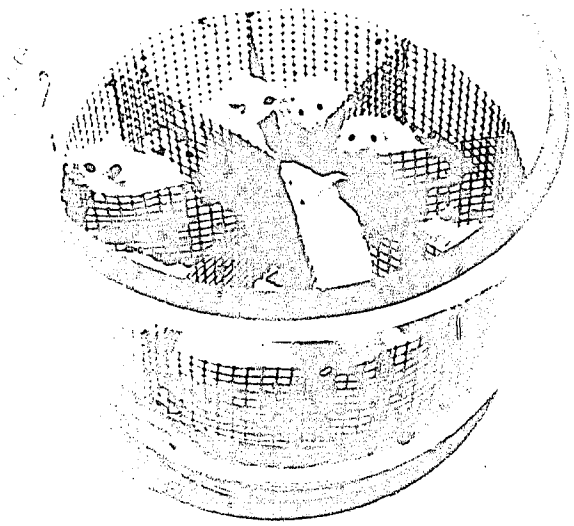


Fig. 3

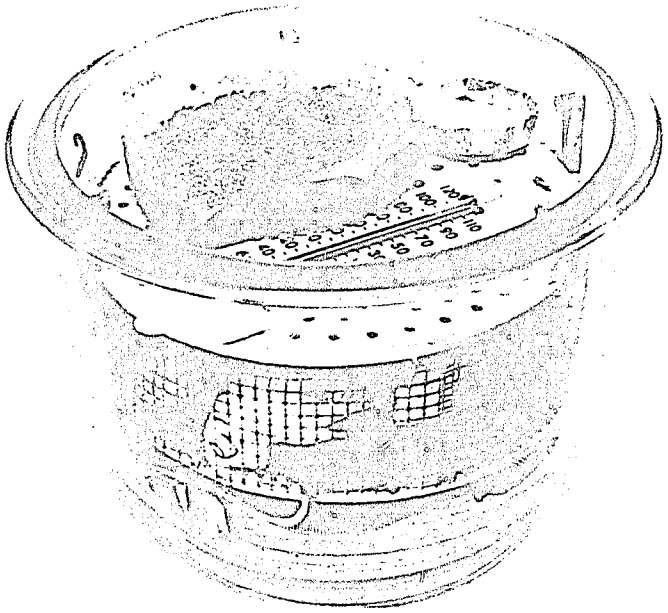


Fig. 4

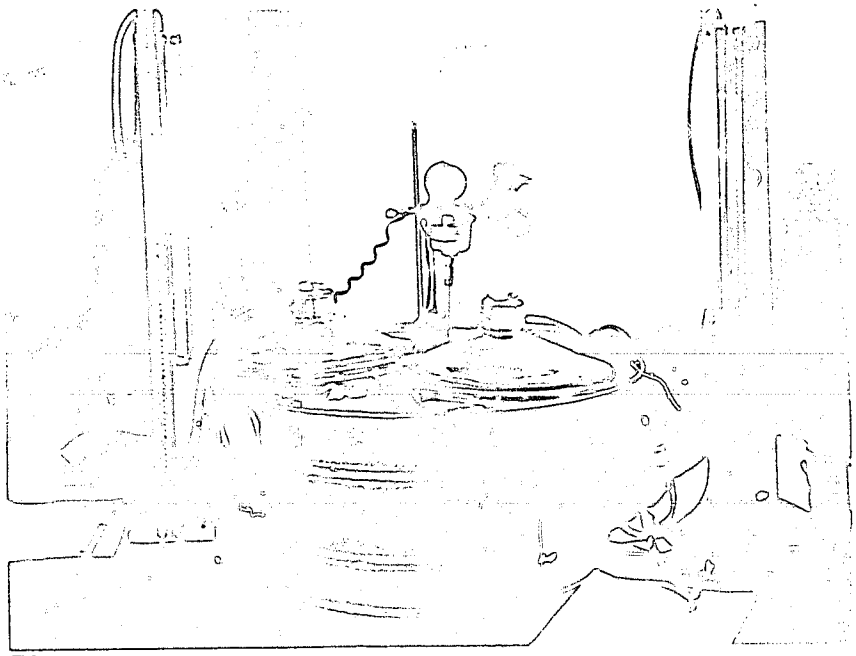


Fig. 5

$$\text{Oxygen Consumption in ml/hr} = \frac{V \times p \times 273 \times 1}{P \times 273 + T \times t}$$

V = Vol. of Desiccator (ml)
t = Time of observation (hr.)
p = Fall in pressure (mm Hg.)
P = Atmospheric Pressure
T = Final temperature (°C)

The results are expressed as ml. oxygen at N.T.P.

Calibration of Desiccators was done similarly to MacClagan's method. The volume of contained objects was subtracted from the volume of the desiccators. The Ascarite or Soda Asbestos (80 cc.) and Magnesium perchlorate (10 cc.) occupy approximately 100 cc. and 1 ml. was also allowed for each gram of mouse. Because of the fact that the temperature rose slightly (0.0°C - 1.6°C) during most of the measurements, we introduced a correction factor which takes into consideration the effect of this increase in temperature-pressure in a closed circuit system. This correction factor is as follows:

$$C = \frac{P \times (T_2 - T_1)}{T_1}$$

P = Atmospheric pressure (mm. Hg.)
T_1 = Initial temperature at zero reading (°A)
T_2 = Final temperature (°A)

Every value of metabolic rate in this paper is for groups of eight animals and expressed as ml. of oxygen consumed/hr./gm. body weight.

II. Results

1. Assessment of Reliability of Method:

a) Constancy of a fall in pressure.

A typical curve of oxygen consumption showing a linear relationship between fall in pressure and time, one hour after 30 minutes of equilibration, is shown in Figure 1. Any leak in the system can easily be noticed by comparing the differences of every 10 minutes interval readings of the fall in pressure.

b) Correlation between chamber temperature and Oxygen consumption.

As stated previously we had noticed an increase in temperature within the desiccators throughout the experiments for both cold and warm acclimated animals. We thus plotted oxygen consumption against the increment in temperature within the desiccators. (Text-Figure 2). It is fairly apparent that the degree of temperature changes occuring during experimentation in the desiccators does not influence to any extent the oxygen consumption measurements.

c) Oxygen consumption and duration of acclimation:

It is well known that oxygen consumption varies with age of any animal. This factor had to be investigated so as to be certain that our results would not be proportionally altered. The oxygen consumption has been plotted against duration of acclimation in months. (Text Figure 3). From these results one can readily conclude that oxygen consumption was not significantly modified by the duration of acclimation of our experimental animals.

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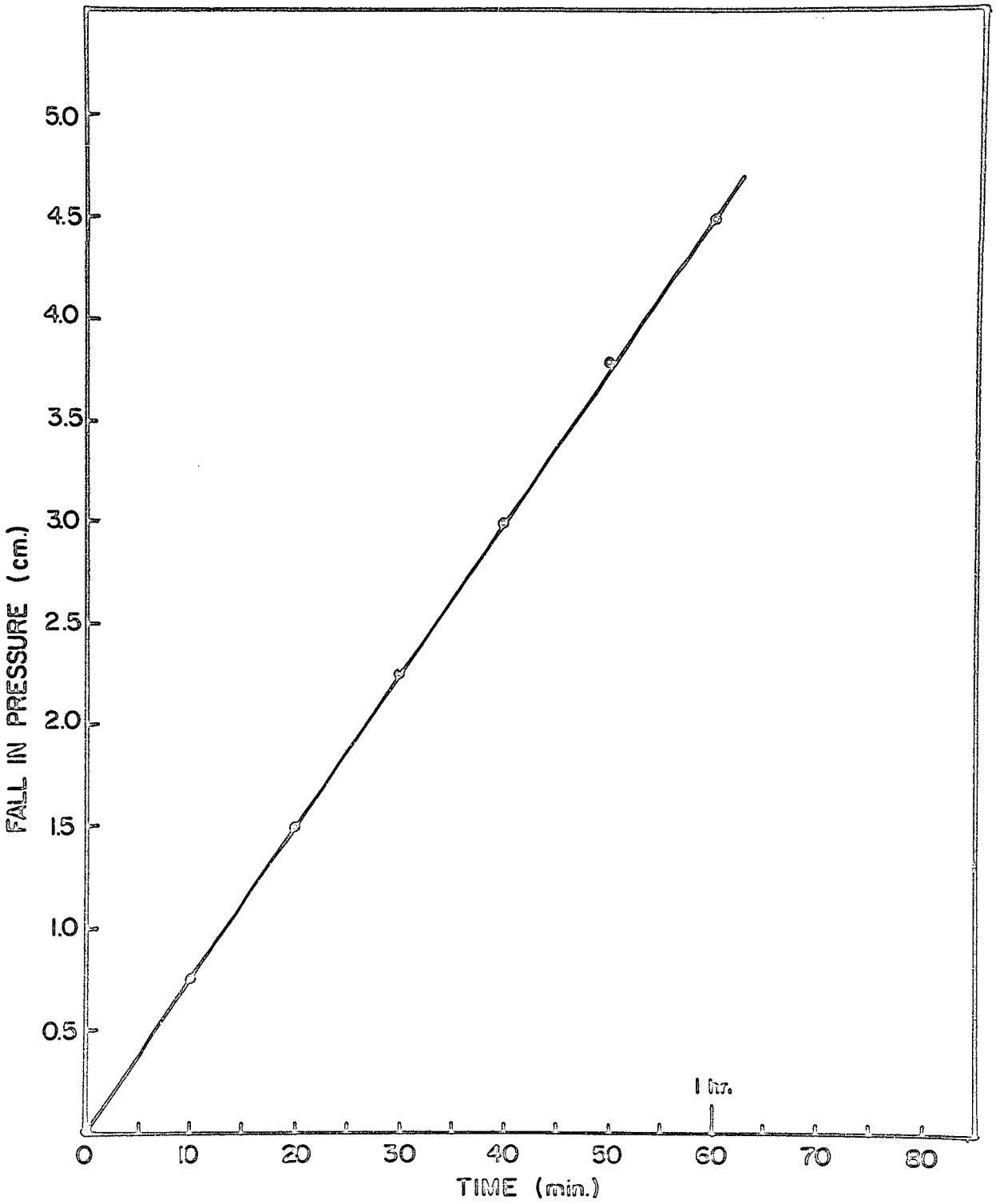
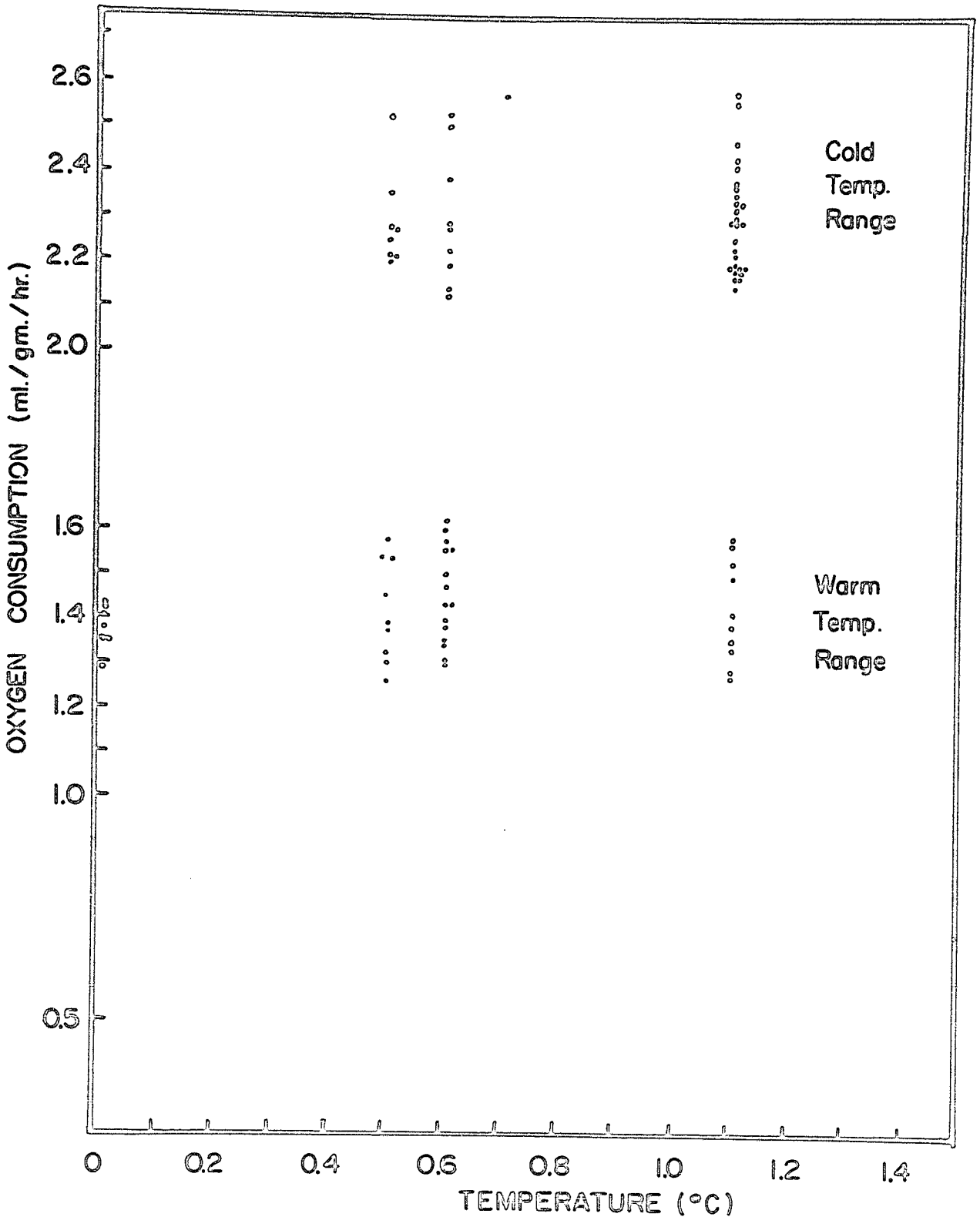
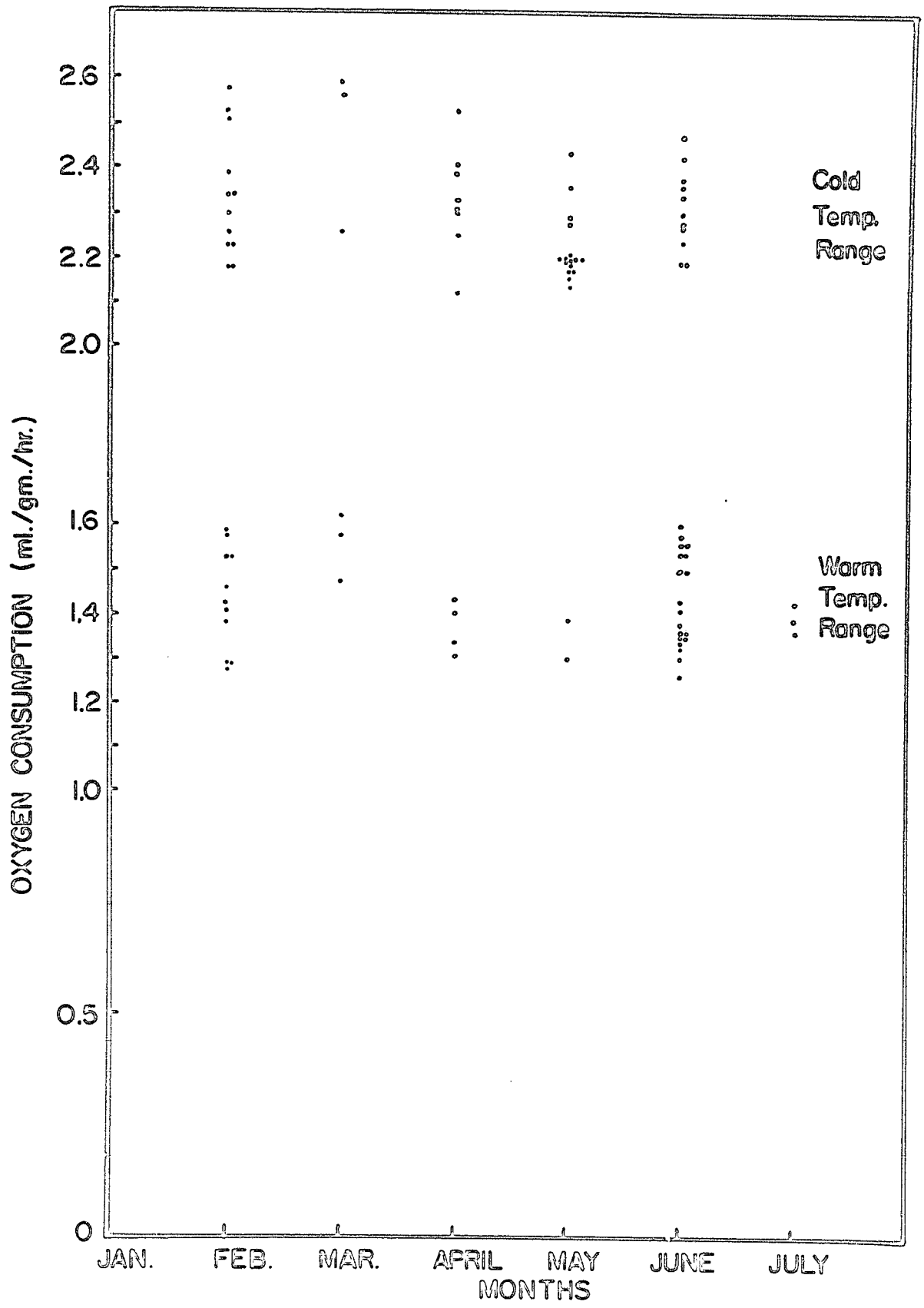


Text-fig. 1. A typical experiment on Eight Control mice in a single dosiccator.



Text-fig. 2. Effect of temperature changes within desiccators on Oxygen Consumption Measurements.



Text-fig. 3. Effect of duration of acclimation on Oxygen Consumption.

d) The effect of different number of animals in desiccators on Oxygen Consumption.

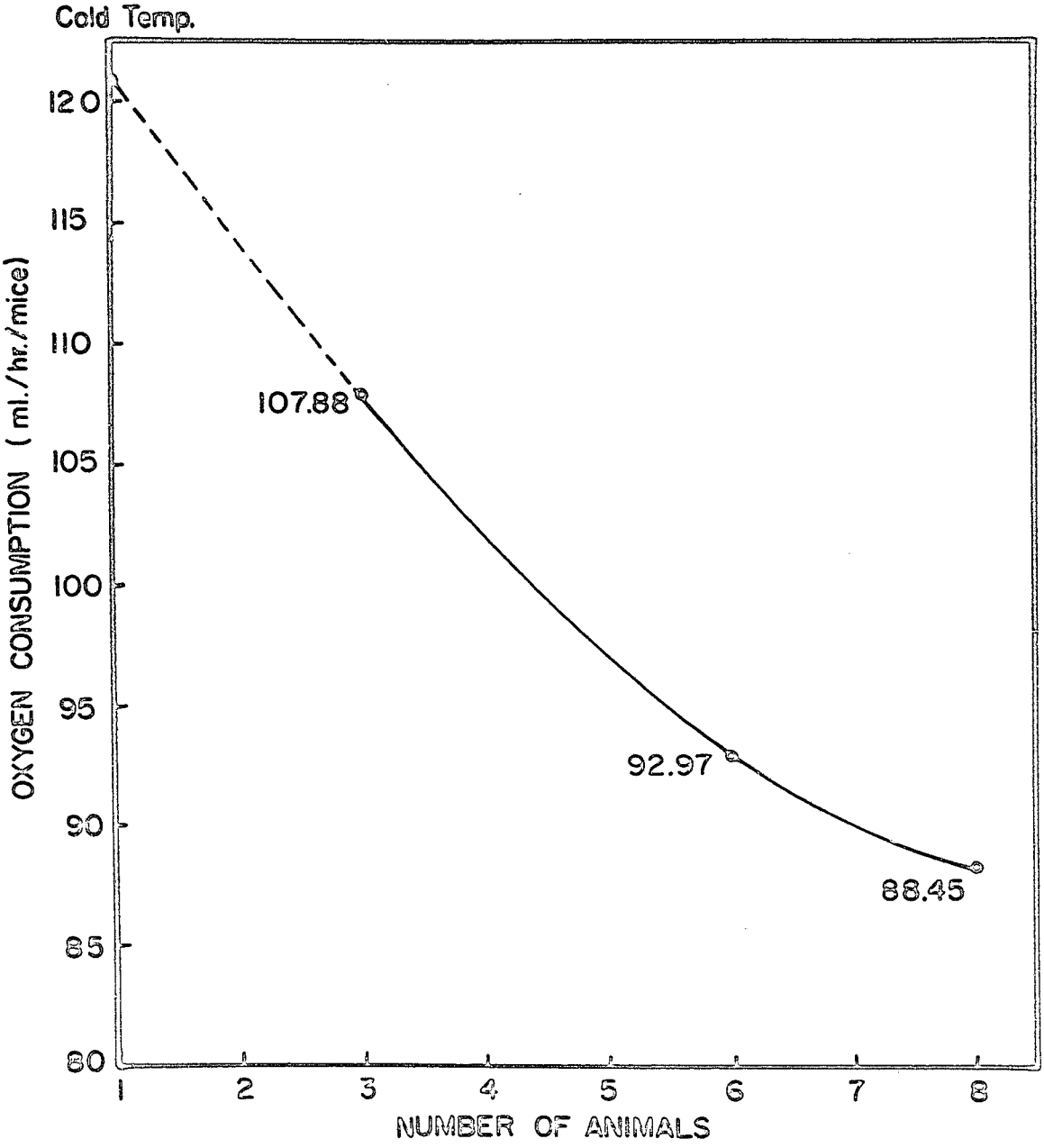
Our results on oxygen consumption in the warm temperature were found to be well within predicted measures for one single mouse (Hart 1953). However, when measurements were done in the cold, the values found were on the average, only 62% of those reported by Hart (1953). We suspected that the grouping of animals for oxygen consumption measurements could have been responsible for this discrepancy. To verify this fact we proceeded to find the oxygen consumption of 3, 6 and 8 animals of the same groups, at the temperature of acclimation. The results obtained for these different numbers of mice were then computed for each subgroup in ml/hr/average weight of mouse of the same sub-group, and are shown in Table 1.

It can be seen that the values obtained for warm-acclimated animals at 30° ± 1°C were nearly the same whatever the number of animals. In the cold-acclimated ones, measured at 10°C, significant differences were obtained. The exponential curve (Text Figure 4) drawn through these values gave us, by extrapolation, the oxygen consumption of one single mouse, which is similar to the value obtained by Hart (1953) from single mice. Our animals thus appear to be acclimated to the same extent as those of Hart (1953), but the same increase in oxygen consumption could not be observed under our conditions of measurement.

Table 1

The effect of different number of animals in desiccators on Oxygen Consumption measured at the temperature of acclimation.

Temp.	Groups	No. of mice	Av. O_2(ml/gm/hr)	Av. Wt(gm)	Av. O_2 consumption per mouse
	A,C,E,F.	3	1.43	37.92	54.35
30°C	A,C,E,F.	6	1.51	37.92	56.97
	A,C,E,F.	8	1.52	37.66	57.36
	I,II,V.	3	2.95	36.57	107.98
10°C	I,II,IV,V.	6	2.55	36.50	92.97
	I,II,IV,V.	8	2.35	37.62	88.45



Text-fig. 4. Average Oxygen Consumption of different number of mice.

2. Oxygen consumption of mice at different temperatures without treatments:

The results of these experiments are shown in Table 2 for animals acclimated at both 30°C and 10°C. Included in the number of experiments are those done at zero hour which in themselves are readings not influenced by the treatments.

Table 2

Oxygen Consumption of untreated mice acclimated to 30° and 10°C.

Temp.	No. of experiments	Mean O_2 consumption	F	P
A (30°C)	43	1.42 ± .02	3.92	< .01
B (10°C)	49	2.30 ± .02		

It was found that the average oxygen consumption for warm-acclimated animals was 1.42 ± .02, which compared favourably with the results obtained by Hart (1953), while in the cold the mean O.C.R. was 2.30 ± .02 ml/gm/hr, a value significantly different from that of warm-acclimated animals, despite the fact that the O.C.R. of grouped cold-acclimated mice was only 62% of that expected for single animals.

Table 3

Effects of treatments on Oxygen Consumption of mice at the two temperatures of acclimation.

Treatments	30° temperature				10° temperature			
	No.	O_2 consumption before treatment	Increase in O_2 consumption after treatment	% increase	No.	O_2 consumption before treatment	Increase in O_2 consumption after treatment	% increase
2 mg/kg L-thyroxine	1	1.44 (9)	0.58 ± 0.034	40.8	2	2.30 (10)	0.27 ± 0.026	12.5
4 mg/kg L-thyroxine	3	1.42 (4)	0.59 ± 0.026	41.5	4	2.25 (4)	0.23 ± 0.013	10.1
400 mg/kg DDIH	5	1.41 (4)	0.06 ± 0.017	4.1	6	2.22 (5)	0.06 ± 0.002	2.8
800 mg/kg DDIH	7	1.47 (2)	0.00 ± 0.000	0	8	2.23 (4)	0.04 ± 0.002	1.6
2 mg/kg L-thyroxine + 400 mg/kg DDIH	9	1.43 (4)	0.33 ± 0.002	23.2	10	2.22 (4)	0.29 ± 0.030	13.1
4 mg/kg L-thyroxine + 400 mg/kg DDIH	11	1.32 (4)	0.58 ± 0.040	44.2	12	2.27 (4)	0.24 ± 0.017	10.4

Note: Oxygen consumption expressed in ml/hr/ per g of mouse; No.: treatment number; numbers in parentheses: number of measurements (each obtained on eight mice); increase in oxygen consumption represents the value obtained in the same groups 48 hours after the onset of treatment.

3. Effects of treatments

The results are shown in Table 3. These data were submitted to the R X 2 analysis of variance (Yates 1934), the result of which appears in Table 4.

Table 4

Analysis of variance (R X 2)

Source of variation	df	SS	MS	F	P
Treatments	5	1.427	0.285	4.99	<0.01
Temperature	1	0.359	0.359	6.29	<0.05
Interaction	5	0.371	0.374	1.30	>0.05
Error	46	2.627	0.057		

Since the interaction was not significant, the temperature effect was further tested by the "t" test, giving us a value of 4.5 which showed us that our groups of animals could be separated into two distinct populations. We were thus justified to apply the One-way analysis of variance to each of the temperature populations. This One-way analysis, when resulting in a significant F, was further completed by a Duncan (1955)-Kramer's (1956) test in order to locate the significant differences between treatments.

(i) Warm-acclimated animals.

The differences in effects of treatments on this population are recorded in Table 5 at 1% level of significance.

Table 5

Effects of treatments on warm-acclimated mice

Treatment No.	7	5	9	11	1	3
	.000	.060	.330	.582	.586	.588

These results show no significant difference between treatment nos. 7, 5, 9 nor between nos. 9, 11, 1, 3, but a significant difference between treatments nos. 7, 5 and 11, 1, 3. Treatment number 9 lies between these above significantly different treatments, thus indicating an inhibitory effect.

(ii) Cold-acclimated animals.

The differences in effects of treatments on this population are recorded in Table 6 at 1% level of significance.

Table 6

Effects of treatments on cold-acclimated mice.

Treatment No.	8	6	4	12	2	10
	.037	.060	.227	.237	.270	.290

From these results we notice two groups of treatments which are distinctly different from each other i.e. treatments nos. 8, 6 are significantly different from treatments nos. 4, 12, 2, 10.

III. Discussion and Conclusions.

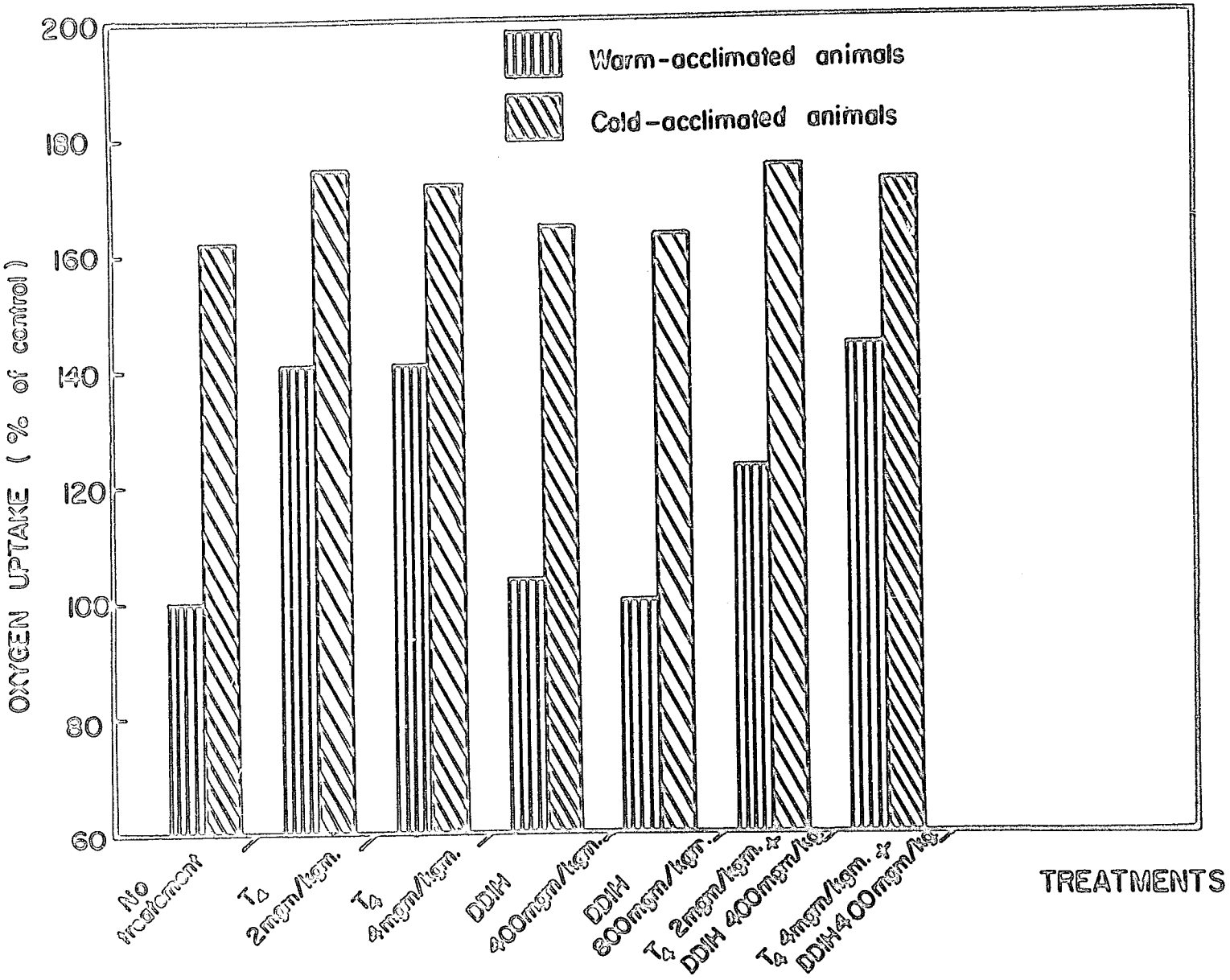
Many types of apparatus for the measurement of oxygen consumption have been devised and used by researchers. Closed circuit types of apparatus have been used by Chapman et al. (1944), Morrison (1947), Farmer (1948), Hart (1950), Sellers and You (1950), MaClagan (1950), and Dawson (1955), to name but a few. Since we wanted to work on groups of animals, we used the closed circuit apparatus described by MaClagan (1950), also utilized by Serif and Seymour (1961). It is felt that the slight modifications introduced in the determination of oxygen consumption is a definite improvement over the method of MaClagan et al (1950), since our data agree with those of Hart (1950 and 1953) obtained on single warm-acclimated mice. Further the mice in this new apparatus were relatively normal in behaviour and no erratic results in oxygen consumption were obtained as seen in Table 1. The discrepancy observed in cold-acclimated mice could be explained in terms of heat transfer between grouped mice, as noted by Pearson (1960), and in a different air-body surface ratio; but since it was shown that the values obtained could be extrapolated back to the rate of oxygen consumption for single mice at the same acclimation temperature, as seen in Fig. 4, and since we used identical experimental procedures throughout, it would seem that the fraction of the expected oxygen consumption measured in these experiments is a reliable index of the actual metabolic rate.

It appears that the dose of thyroxine used in this experiment was sufficient to produce a maximum increase in oxygen consumption at both temperatures, since the administration of twice as much was not followed by any increase as seen in the Histogram and Table 3. The dose of thyroxine administered represented a fifty to one hundred fold increment over the suspected normal secretion rate of thyroxine, although the secretion rates reported in the literature have been obtained by methods the reliability of which is opened to question.

Our results confirmed the observations of Serif and Seymour (1961) on the anti-thyroid activity of DDIH, and also revealed that this substance was a competitive inhibitor of the calorigenic activity of thyroxine; its effect is reversed by an increase in the dosage level of injected thyroxine. (Histogram 1). This observation appears very important, since it gives support to the suggestion of Serif and Seymour (1961) that this substance inhibits some enzymatic reaction prerequisite to the calorigenic effect of thyroxine. Whether this is a problem of cell permeability to thyroxine, associated or not with the proposed deiodination of T_4 to T_3 as an activation mechanism of thyroxine, remains to be determined. But the test as such appears to be a valid experimental model for the study of the effects of other substances on the peripheral activity of the thyroid hormones.

It is a well documented fact that in cold environments, animals increase their food consumption greatly, increase their activity, etc.

resulting in increased fecal (Intoccia et al, 1959) and urine (Kot et al, 1959) excretion with increased depletion of thyroxine. Many observations of this type now point more and more to the fact that the thyroid secretes its active principle more rapidly in cold surroundings (Dempsey 1943; Knigge 1963). We find that in cold-acclimated animals, thyroxine administration had a very limited effect, 12.5% increase as compared to 40.8% at room temperature (Histogram 1). It could therefore be theorized from such results that partial saturation of thyroxine acceptor sites in cold-acclimated animals impairs the efficiency of exogenous hormone, on the grounds that liver mitochondria of refrigerated animals have been shown to bind less thyroxine in vitro (Tonoye 1961). This might also explain the failure of DDIH to show any inhibitory effect as found in warm-acclimated animals. These findings once again emphasize the importance of the thyroid hormones in cold-acclimated animals, and the opportunity of an experimental model, in vivo, for studying the peripheral activity of these hormones.



Histogram showing the percentage oxygen uptake according to treatments.

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