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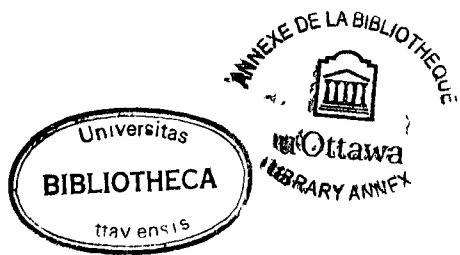
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**STUDIES ON THE REGULATION OF TISSUE RESPIRATION**

**By**

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**A THESIS  
submitted in partial fulfilment of the  
requirement for the degree of  
Master of Science**



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## INTRODUCTION

It is a basic physiological fact that the metabolism of animal organisms, as measured by oxygen consumption or caloric production, is not a constant magnitude but is dependent on, and controlled by, many different factors. Body size, hormonal and environmental influences are recognized as being important factors in this respect.

It is known for more than 100 years that weight-specific basal metabolic rate in mammals decreases with increasing body-size. This is evidenced by the surface rule which states that metabolic rate per unit weight decreases with increasing body size, but is constant per unit surface. The surface rule is found to apply to many invertebrate and vertebrate classes (Bertalanffy, 1951 (a)). As far as mammals are concerned, it has been shown recently (Racine, 1951, unpublished) that the surface rule applies to growing rats, in so far as the general trend of the dependence of basal metabolism on body weight is concerned, although in detail the relation is more complex. In man the well-known DuBois formula, as applied in clinical tests, amounts dimensionally to the surface rule.

Similarly, it is obvious that hormonal factors belong to the most important regulators of metabolism, e.g., the thyroid gland and its hormone, thyroxine. It appears that a combination of many hormones is involved in the regulation of physiological processes, instead of the individual hormones being concerned with the adjustment of one particular process in the animal body. This is especially true in the

case of the pituitary hormones, as they appear to influence the physiological processes of metabolism, growth, reproduction, lactation, ovulation and many other functions. Changes in the activity of the thyroid by way of hyper- and hypothyroidism manifest themselves in corresponding changes in basal metabolism; these changes are important for diagnosis of thyroid disorders.

The metabolism of the organism as a whole is obviously composed of the metabolism of its organs, tissues and ultimately of its cells. Thus, the problem presents itself: What is the relation between the metabolism of the entire animal, as measured by its basal metabolic rate, and the metabolism of the tissues composing it? Further, we must ask whether the factors regulating metabolism in the entire animal are also active in its isolated tissues, and whether the action of these factors *in vivo* is reproducible *in vitro*.

There is, of course, a large amount of experimental material pertinent to these questions. Closer inspection, however, shows that these data are rather scattered, unconnected and often contradictory. The importance of *in vitro* study cannot be minimized, for there are certain advantages in the study of isolated tissues: Constant conditions can be maintained and controlled, and indirect influences which affect *in vivo* investigations can be eliminated. The importance of this problem is well acknowledged. Thus, Kleiber (1947) states: "the analysis of these factors controlling the metabolic level *in vitro* and *in vivo* appears to be a most fruitful field for future research on tissue metabolism." The present

study aims at giving more information on some of the questions connected with the above problems.

CHAPTER ISURVEY OF PREVIOUS INVESTIGATIONSTissue Respiration and Body Size

The question of the dependence of metabolism on body size can be studied in two respects, namely interspecifically, i.e., comparing nature animals of different species of animals, and intraspecifically, i.e., comparing animals of the same species but of different body size. These aspects are applicable to the size-metabolism relation in the metabolism of entire animals as well as to their tissue metabolism.

There are numerous older investigations on the relation of tissue respiration ( $Q_{O_2}$ ) and body size which, however, are inconclusive and often contradictory; this older work is reviewed by Bertalanffy and Pirozynski (1953). Recent investigations of this problem were carried through, in intraspecific comparison by Bertalanffy and Pirozynski (1951 (a)), 1953); Pirozynski and Bertalanffy (1952), and interspecific comparison, by Krebs (1950).

Bertalanffy and Pirozynski have investigated the question whether the systematic decline of weight-specific basal metabolic rate with increasing body size, as expressed in the surface rule and related formulations (Kleiber, 1947), can be explained in terms of a corresponding decline of tissue respiration. This work led to the result that, in intraspecific comparison, among 7 characteristic tissues of rats in a series from new-born to adult (9 gm. to 392 gm. body weight), only the

diaphragm shows a variation in  $Q_{O_2}$  comparable to that of weight-specific metabolic rate. With respect to heart, there is a slight decrease in  $Q_{O_2}$  with increasing size, but the correlation coefficient is low. The  $Q_{O_2}$  correlation of lungs is similar to that of heart. The data obtained from liver seem to show a break in the allometric line of the  $Q_{O_2}$  somewhere in the vicinity of 100 gm. The  $Q_{O_2}$  values of kidney-cortex remain practically constant over the range of body sizes investigated. Brain-cortex seems to show a slight increase in the  $Q_{O_2}$  with increasing body size. As far as liver and kidney are concerned, recent work on tissue respiration of growing chickens (Grandall and Smith, 1952) has essentially confirmed these results.

A thorough study of the interspecific relation of tissue metabolism and body size was made by Krebs (1950). Investigating 5 tissues (lung, liver, kidney-cortex, brain-cortex and spleen) of 9 mammalian species, ranging from the mouse to the horse, he found that in general, the  $Q_{O_2}$  values of the larger species were somewhat lower than the homologous values of small species. However, there is no parallel decrease in the different tissues, nor a consistent relation to the decrease of weight-specific basal metabolic rate with increasing size. Not finding a systematic difference in tissue respiration which would correspond to the differences in total metabolism in the mammalian series, Krebs attributes these differences to variation in the  $Q_{O_2}$  of musculature tissue, which was not studied in his experiments.

To a certain degree, Krebs hypothesis is supported by Bertalanffy

and Pirozynski's findings, since in their experiments, the diaphragm as the only striated muscle investigated, was also the only one to show a decrease in  $Q_{O_2}$  comparable to that of weight-specific metabolic rate. This was emphasized by Schmidt-Nielsen (1951). Bertalanffy and Pirozynski (1951 (b)) replied that, on the basis of a quantitative estimate, a decline in tissue respiration of musculature, even if it would be of the same order as that found in the diaphragm, would not be sufficient to account for the decline of metabolic rate of the entire animal.

Thus, part of the present work is concerned with a further study of the size-dependence of tissue respiration of musculature, posing the following questions:

- 1) What is the relation of the  $Q_{O_2}$  of skeletal muscle to body size?
- 2) Is the relation between  $Q_{O_2}$  of tissue and body size which is established for the diaphragm, as highly significant, dependent on external factors applicable in vitro?

Part of the results obtained in these studies has already been published (Bertalanffy and Estwick, 1953).

## B. METABOLISM AND THE THYROID GLAND

### 1. Influence of Thyroid on Total Metabolism ( $T.M.R.$ )

The influence of the thyroid hormone on total metabolism is well known. There is a tremendous amount of literature on this subject, as

reviewed, e.g., by Selye (1952). In this thesis only a few recent findings shall be mentioned, which have a possible relation to the problem embodying the subject matter of this investigation.

The fact that thyroxine administration or hyperthyroidism leads to an increased basal metabolic rate is familiar, and the latter is used as a clinical criterion in diagnosing thyroid disorders. It must be borne in mind, however, that the influence of thyroxine on basal metabolic rate is not absolutely consistent. Balogh et al. (1951), having given doses of thyroxine, 0.1 to 0.5mgm. subcutaneously to normal, thyroidectomized and hypophysectomized rats, observed that subcutaneous injection is usually followed by a rise in basal metabolic rate within 2 hours. However, normal and hypophysectomized rats respond occasionally with a decline of oxygen consumption. Werner and Hamilton (1951) have indicated a number of cases of hyperthyroidism without apparent hypermetabolism. These observations should be of special interest to clinicians who rely mainly on B.M.R. in diagnosis of thyroid hypo- and hyperfunction.

It is to be noted further, that the effect of thyroxine varies relative to the mode of administration. Grad and Leblond (1950) indicated the effect of thyroxine on oxygen consumption and heart rate, following bile duct ligation and partial hepatectomy. Their results revealed, "that thyroxine was less effective by the intraperitoneal than the subcutaneous route. Presumably, this was due to the fact that the material absorbed from the peritoneal cavity through the portal veins

has a better chance of coming in direct contact with the liver than that absorbed from under the skin through the systemic circulation. Thus, the decreased activity of thyroxine after intraperitoneal injection suggested that the liver reduced the amount available to the tissues. The effectiveness of thyroxine was not reduced by the liver as completely as that of most steroids, for, even after intraperitoneal injection, thyroxine has a definite effect on the heart rate, while steroids were almost completely inactivated when administered by the same route." Their results were taken to indicate that the liver excreted and inactivates excess amounts of thyroid hormone.

Variation in response to thyroxine is not limited to different tissues, but seems to be based also on species differences. Zarrow and co-workers (1951) compared the effects of experimental hyperthyroidism and hypothyroidism on resistance to anoxia in rats and mice. Their results showed that the effect of thyroxine on the degree of anoxia to which the animal could be exposed varied quite markedly in the rat and the mouse. They attribute this difference to the difference in the output of thyroxine by the two species. If one considers the mouse as a species secreting less than optimal amount of thyroxine, it is apparent that the initial effect of thyroxine administration would be to increase resistance; as the dosage increases, the optimum would be passed and a decreased resistance obtained. Conversely, in the rat with an optimal normal output of thyroxine, the initial effect must be to decrease survival time. In similar manner the failure to obtain good protection with thiouracil

in the mouse may be explained by the fact that this species is already in a hypothyroid state.

## 2. Respiration of Isolated Tissues from Thyroxine-Injected Animals.

Administration of thyroxine in vivo seems to increase not only basal metabolism of the entire animal, but also tissue metabolism as observed in vitro by the Warburg technique, although the results do not seem very consistent. Conversely, thyroidectomized animals show not only lower basal metabolism but also show lower tissue metabolism. A survey of the older literature on this subject has been given by Krebs and Johnson (1948), and Rappert, Cansanelli and Guild (1946). It was shown by the latter, that thyroidectomy causes a depression in oxygen consumption of the tissues (liver and kidney) investigated and rendered the liver sensitive to subsequent thyroxine administration. In the case of the kidney, while previous thyroidectomy decreases the oxygen consumption, it does not apparently sensitize the tissue to the effects of thyroxine.

Some recent work, (Balegh et al. (1951), seems to emphasize that the time factor has to be considered as playing an important role with respect to thyroxine action. This was further indicated by Barker and Klitzner (1952) who pointed out that no tissue studied exhibited any metabolic change at 6 hours after injection of thyroxine. However,  $CO_2$  of heart, skeletal muscle, smooth muscle, diaphragm, liver kidney and gastric mucosa was elevated in varying amounts by 12 hours after injection. These values continue to increase for 4 days and started downward after

six days. Change in metabolism of cardiac muscle increased from 100 to about 160% in two days, 220% in 4 days; this was the maximum increase. The maximum increase for skeletal musculature was about 190% in 6 days. Another important observation in their experiments is the fact that spleen, brain and testis showed lack of change in tissue metabolism following a single injection of thyroxine, while other tissues investigated showed varying changes in metabolism. The maximum increase in  $Q_{O_2}$  is observed from 4 to 6 days after administration of thyroxine. Skeletal musculature showed only slight increase as compared with cardiac musculature and other tissues. The result of Anselmine et al. (1929) was somewhat different. They observed no effect or slight increase in oxygen consumption of liver, spleen and kidney 24 hours to 7 days after injection of thyroxine. Paol (1933), also indicated that tissue treated with thyroxine in vitro over a 24 hour period showed significant increase in  $Q_{O_2}$ . More recent work by Ullrich and Whitehorn (1952), who administered desiccated thyroid powder per os, has indicated that certain tissues are more subject to the influence of thyroxine than others, a further indication of variation in thyroxine action.

### 3. Respiration of Isolated Tissue Treated Directly with Thyroxine

The influence of direct application of thyroid hormone on in vitro metabolism (oxygen consumption), has received wide attention. However, some of the results obtained were not in agreement with the general concept of the thyroid as activator of metabolism. On one hand,

there are a number of experiments which indicate a positive influence on in vitro metabolism when thyroxine is added directly to the tissues investigated. On the other hand, there are experiments which indicate no effect of thyroxine. To mention a few workers whose results fall in the latter group, Hicks (1932), has worked with minced skeletal musculature of rabbits under similar experimental conditions as the present work. Hopping (1930), has investigated the direct application of thyroxine (Squibb) to alligator blood, and in another series of experiments injected thyroxine in the animal. After 3 days oxygen consumption of blood from injected animals showed an increase of from 150 to 190 percent above control, while none of the experiments where thyroxine was applied directly showed increase in oxygen consumption. A complete survey of the older literature is given by Rossiter (1940) and need not be repeated here. A few workers, Ebina (1932), Verebely (1932), Davis and Hasting (1936), have indicated some positive effect, but their results have failed to receive more recent support in spite of improved working conditions and techniques.

#### 4. Respiration of Isolated Tissues Treated Directly with Thyroglobulin

Reviewing some of these in vitro experiments, the work of Rossiter (1940), is very significant. He added thyroxine to brei and dispersion preparations in the presence of glucose or sodium pyruvate. Many experiments were done covering a wide range of thyroxine concentrations. The author observed that in vitro addition of thyroxine did not cause an increase in oxygen uptake of brei and dispersion preparations of rat brain in the

presence of glucose or sodium pyruvate. A number of in vitro experiments were conducted with thyroglobulin and here the effect was very consistent being present in all experiments except in experiments where thyroglobulin used had been kept in solution for three weeks at 0°C. There was an increase in oxygen uptake from 1.5 to 34%, an average of 15.2% when glucose (0.01 M) was used as substrate in brief preparations; and from 5.5 to 7.5%, an average of 6% when Na-pyruvate (0.02 M) was used. In dispersion preparation the increase was lower with glucose (0.01 M) as substrate, being from 3 to 16%, an average of 9.56%. The increase in oxygen uptake with thyroglobulin was maximal the first 30 minutes, and usually very small by the end of 60 minutes.

Caszanelli et al. (1937, 1939) have conducted a number of experiments with testis, brain, kidney, heart, and liver of guinea pig, in an attempt to establish the role of thyroglobulin on tissue metabolism. The duration of experiments was one hour, and three different types of medium were used in the respiratory flasks: a) 0.85% NaCl in a Sørensen buffer mixture with  $M_{180}$  glucose, the  $PO_4$  being in either  $M_{150}$  or  $M_{75}$  concentrations; b) Ringer's solution in similar concentrations of  $PO_4$  and glucose; c) horse serum, made almost bicarbonate free by being shaken with HCl at a pH of 6.0 to 6.5, then brought to physiological pH with NaOH. This serum contained no fermentable sugar, and had almost 50 mg. % reduced substances. In some experiments glucose was added to the serum to a concentration of M

180. Thyroglobulin and thyroxine were used in iodine concentrations of a similar order and magnitude. About 200 gamma of

thyroxine was usually added to each flask, the amount actually in solution at physiological pH as shown by  $I_2$  determinations, varying with the medium, being greatest in serum.

The conclusion drawn from this previous work with thyroglobulin, is that under specific conditions thyroglobulin acts as an activator of aerobic oxidations in kidney, testis, heart and liver. The conditions appear to be that the metabolic rate of the tissue before the addition of thyroglobulin shall not be at a very high level. It was suggested that there seems to be a ceiling above which thyroglobulin cannot further elevate metabolism.

Thyroxine, in contrast to thyroglobulin, caused no unequivocal rise in oxygen consumption in any of the tissues in salt buffer mixture; though in one observation carried through in serum, thyroxine was shown to have elevated  $QO_2$  of heart and kidney. The authors believe that there was the possibility that under certain conditions the physiological properties of thyroxine may be altered by contact with serum.

The present study offers some more material on the action of thyroxine and thyroglobulin on tissue respiration after their application in vitro. The present work has been conducted with commercially highly purified thyroglobulin in contrast with the older experiments where the thyroglobulin used was subject to bacterial contamination because of the method of extraction.

### C. METABOLISM AND PITUITARY

#### 1. Pituitary as a Central Regulator of Hormone Balance

A question which often arises when considering the changes in function

produced by administration of pituitary extracts or related procedures, is whether the effects in whole or in part are a result of direct action of some constituents of the extract, or whether they are the consequence of the stimulation of some other endocrine gland for which the extract employed contains a specific agent.

So far as influence on tissue and total metabolism are concerned, pituitary activity may be especially considered in relation to the thyroid gland, by way of the pituitary-thyroid relationship. "The thyrotropic hormone secreted by the basophilic cells of the anterior pituitary stimulates synthesis of the thyroid hormone by the gland, and it is also necessary for the release of this hormone from the thyroid gland. The cycle of reciprocal regulation is completed by the circulating thyroxine acting back on the anterior pituitary, large amounts depressing small amounts stimulating the secretion and release of the thyrotropic hormone. There is a delicate equilibrium between the manufacture, storage and discharge of this secretion of the thyroid gland." (Maqsood, 1952). Although there are many gaps in our knowledge of the pituitary-thyroid interrelationship, this concept seems plausible at the present time. Experimental evidence showing a decrease of basal metabolic rate after thyroidectomy and hypophysectomy may be interpreted in terms of the pituitary-thyroid relationship. Further, hypophysectomized animals also show atrophic thyroids and adrenals (Long, 1943).

## 2. Influence of Growth Hormone on Tissue Respiration

Another pituitary factor possibly connected with metabolism is the growth hormone. The association of the anterior pituitary with growth

promotion was noted in individuals suffering from pituitary tumors. Early experimental demonstration that the growth rate of mammals can be accelerated by injection of anterior pituitary extracts was carried out by Evans and Long (1921), who showed that true gigantism could be produced in rats by this means. Apart from the clinical evidence (gigantism, acromegaly in hyperfunction of the pituitary, etc.), it seems that the effect of growth hormone is connected with protein synthesis in the liver. Lee and Freeman (1940) have found that, although the average liver weight was significantly greater in animals treated with growth hormone, the number of cells per gram of tissue remained unchanged. They concluded that the liver exhibits a true hyperplasia under the influence of the extracts used and that all elements of the organ participate in it.

The effect of growth hormone on total metabolism and tissue metabolism has received less attention. Some experimental work was conducted by Kleiber and Cole, (1939), to investigate body size and energy metabolism in growth hormone rats. The metabolic rate per unit body weight of rats made giant by chronic injections of growth hormone was significantly smaller than the metabolic rate per unit body weight of their litter-mate controls which had normal size. The metabolic rate in vitro per unit dry weight of the diaphragm of the giant rats was also lower than the corresponding rate of the diaphragm of the controls. It seems evident by these experiments that chronic doses of pituitary extract result in a depression of basal metabolic rate and of oxygen consumption in the isolated diaphragm.

The present study offers some data on the relation of the hypophysis

to tissue metabolism, investigation being made of  $Q_{O_2}$  of tissues from hypophysectomized rats and pituitary dwarf mice in comparison with their total metabolism. The complicated interrelationship of the pituitary hormones is illustrated in Fig. 5.

D. OTHER INFLUENCES ON RESPIRATION (OXYGEN CONSUMPTION)

A few other factors possibly influencing tissue respiration were also investigated in the present study. Since these experiments are more in the nature of a first orientation some pertinent literature will be quoted in connection with the results.

## CHAPTER II

### MATERIAL AND METHODS

In the present experiments male and female albino rats (Wistar strain) were used ranging from 10 to 384 gm. body weight. They were maintained on Purina Fox-chow diet and water given ad libitum. The animals were starved for 12 to 18 hours prior to sacrifice. They were killed by a blow at the nape of the neck.

Hypophysectomized animals and normal controls of the same age and weight were purchased from Hormone Assay Laboratory, Chicago. The animals were fed on diet consisting of bread, milk, raw carrots, and meat (canned dog food), and were sacrificed within one week to 18 days after hypophysectomy. The growth rates and other factors of hypophysectomized animals employed in these experiments were similar to those characteristic of hypophysectomized rats as shown by Shaw and Greep (1949).

Other experiments were carried through with mice. Two different strains of mice were used. One was a strain of white mice inbred for generations at this laboratory, and hereafter referred to as "Ottawa strain". The other was a strain of pituitary dwarf mice, carrying the recessive gene "d<sub>w</sub>" which, due to hypofunction of the hypophysis, causes dwarfism. This strain was obtained from Jackson Memorial Laboratory, Bar Harbour, Maine. The gene for dwarfism is a recessive gene (d<sub>w</sub>) carried by phenotypically normal breeders (d<sub>w</sub> d<sub>w</sub><sup>0</sup>) in heterozygous condition. Our strains also contained, beside the dwarf gene, genes for different colours (spotted

black, agouti, albino) and gene for brashy tail. Mating of two breeders gives a Mendelian ratio of 1/4 dwarfs ( $dw\ dw$ ), 2/4 phenotypically normal carriers ( $dw\ dw^*$ ), and 1/4 normal homozygous animals ( $dw^* dw^*$ ). In this way, 3/4 normal litter-mates appear. The homozygous animals stop growth at about 10 gm. body weight. "Dwarfism is caused by a hypofunction of the anterior lobe. Injection of anterior lobe extract stimulates the growth of the dwarfs so that they reach almost normal weight. It was shown that the dwarfs are lacking the growth hormone which is formed by the eosinophilic cells of the anterior lobe." (After Bertalanffy, 1951 (b)).

The required organs were excised immediately after sacrifice and placed in vessels containing ice-cold saline surrounded by ice-cubes. Slicing of the tissues was done by means of Stadie Riggs Microtome (1940). In the case of cardiac musculature and liver, slices were made about 0.4 mm. in thickness. It is of great importance that slices be as much as possible, uniformly thick. Marked variation in the thickness of slices results in variation in  $Q_{O_2}$  values.

Skeletal muscles from the thigh and leg were used at random from animals between 10 and 30 gm. In animals above this weight, fibers were selected from the musculus rectus femoris by way of isolating muscle strips from the connective tissue and carefully teasing the muscle. As control experiments revealed, fibers isolated in this way give consistent  $Q_{O_2}$  values for a longer time than slices.

Diaphragms were cut in small pieces ranging from 3.0 to 10.0 mg. dry weight. Cutting into smaller pieces was favourable in order to obtain maximum

$Q_{O_2}$  (oxygen consumption).

$Q_{O_2}$  (ml  $O_2$ / mg. dry weight/ hour) was determined by Warburg's direct method according to Umbreit et al. (1949), at a temperature of  $37 \pm 0.1^\circ C$ . and in pure oxygen. It was calculated on the basis of the dry weight of tissue slices according to the formula:

$$x = h \left[ \frac{V_g \frac{273}{T} + V_{fa}}{P_0} \right] = h k$$

$x$  = Amount of gas exchanged =  $h$   $k$  flask constant  
alteration  
in reading  
on open arm  
of manometer

$h$  = Observed change in manometer reading in mm.

$V_g$  = Volume of gas phase in flask including connecting tubes.

$V_f$  = Volume of fluid in vessel.

$P_0$  = 760 mm. Hg. (standard pressure) expressed in terms of the manometer fluid.

Thus  $P_0 = 760 \times 13.60$  (where 13.60 is the specific gravity of mercury) / specific gravity of manometer fluid.

$T$  = Temperature of bath in absolute degrees  
 (273 + Temp. in  $^\circ C$ .)

$a$  = Solubility of liquid in vessel of gas involved expressed as ml. gas/ml. liquid when gas is at a pressure of one atmosphere (760 mm. Hg.) at temperature  $T$ .

In the large well of each manometric flask was placed 1 cc. of medium with glucose, 5.4%. The slices were then placed into the large wells. In the small central wells were placed 0.2 ml. of 10% potassium

hydroxide solution, into which small pieces of filter paper were saturated. The flasks were oscillated in the water bath for about 10 minutes with stop-cock open. After this equilibration period, the measurement was started. The manometers were oscillated about 70 times per minute. Readings were taken at interval of 10 minutes during a period of 60 minutes. The pH (7.4) of the medium measured after the completion of experiments showed only slight change. At the end of the 60 minute period the tissue slices were washed in aqua destillata and dried in an oven of 110°C. for about 12 to 16 hours.

The time from sacrifice to the beginning of equilibration was about 15 to 20 minutes. The medium generally applied was Krebs-Ringer-solution, Medium II, type A, according to Krebs (1950), with glucose and with or without metabolites as indicated in the individual experimental series.<sup>x</sup> The medium is composed as to correspond to the ionic composition of the mammalian serum.

In the case of the thyroxine experiments, thyroxine was added directly to the medium after gassing with pure oxygen for one hour. It was found expedient to add from about 0.1 to 0.5 mgm. in 100 cc of medium or more, in order to obtain the desired concentration of hormone per cc of medium. More specific concentrations will be indicated in Chapter III, Results. The pH measured after the addition of thyroxine showed no change.

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x) 83 parts 0.9% NaCl, 4 parts 1.15% KCl, 1 part 2.11%  $\text{KH}_2\text{PO}_4$ , 1 part 3.82%  $\text{MgSO}_4$ , 3 parts 1.3%  $\text{NaHCO}_3$ , 18 parts buffer (100 parts 1.78%  $\text{Na}_2\text{HPO}_4$  + 25 parts 1.38%  $\text{NaH}_2\text{PO}_4$ ), 5 parts 5.4% glucose, 4 parts 0.16M Na-pyruvate, 7 parts 0.1M Na-fumarate, 4 parts 0.16M Na-glutamate.

Generally, thyroglobulin was given in concentration of 0.4 mg/20cc of Medium, from which 1 cc was placed in each manometric flask. However, in one experiment where the effect of thyroxine and thyroglobulin was compared, the concentration of thyroglobulin was made so as to correspond to the I<sub>2</sub> content of thyroxine employed. This was done according to the estimate given by Canzanelli and Rapport (1937). However, lower concentrations of thyroglobulin has also shown similar effect. Thyroglobulin was found to be soluble in 0.9% NaCl solution.

The statistical evaluation of the data was carried through in various ways according to the nature of the experiments. In the case of the experiments on tissue respiration in relation to body size, the data were plotted according to the allometric expression:

$$Y = b x^a \text{ or } \log y = \log b + a \log x, \quad y \text{ being } QO_2,$$

$x$  body weight,  $a$  the slope of the regression line,

$b$  the integration constant for  $x = 1$ . The values of  $a$ ,  $b$ ,

$s$   $\frac{\log y - \log x}{\log x}$  (standard error) and  $r$  (correlation coefficient) were

calculated according to the method indicated by Brody (1945, p. 378 ff.).

When necessary, the standard error was calculated according to the usual formulas:

$$[s_x^2] = \frac{(\sum x)^2}{n} = S_x^2$$

$$v = \frac{\sum x^2}{n-1}$$

$$s = \sqrt{v}$$

$$M = \frac{\sum x}{n}$$

When necessary, the  $t$ -test was applied using the formula:

$$\sigma'_{\bar{x}_1 - \bar{x}_2} = \frac{(N_1 + N_2) (\sigma_1^2 + \sigma_2^2)}{N_1 N_2 [(N_1 - 1) + (N_2 - 1)]}$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sigma'_{\bar{x}_1 - \bar{x}_2}}$$

CHAPTER XIIRESULTS1. Tissue Respiration and Body Sizea) Skeletal musculature

The relation of tissue metabolism of skeletal muscle to body size was studied in the two species, rat and mouse.

In rats, animals of the Wistar strain were used, as mentioned previously. The determinations comprise a complete series of weights from 10 gm. to 384 gm., a total of 36 animals and about 144 individual determinations. The results are indicated in Table 1 which also gives the statistical correlation of the data. The averages of 4 - 6 individual determinations of tissue of the same animal are plotted double-logarithmically in fig. 1, which also shows the logarithmic regression and standard error.

As these experiments show, tissue respiration of skeletal muscle of growing rats shows but slight variation with increasing body size, the slope of the logarithmic regression line being  $\alpha_x = 0.06$ , while according to the surface or  $3/4$  power rule of metabolism, the slope would be  $-0.33$  or  $0.25$ , respectively.

In mice, determinations of  $Q_{O_2}$  of skeletal muscle were made with two groups of animals, small (ca. 10 gm.) and large ones (ca 25 gm.), of two different strains. One strain investigated were white mice, inbred for generations in our laboratory and referred to as Ottawa Strain.

The other series of animals used was phenotypically normal individuals of the strain Pituitary dwarf, which gives rise to normal animals as well as homozygous dwarfs, as indicated in Chapter II under Material and Methods. The metabolic behavior of the dwarfs will be considered later.

The values obtained are plotted in fig. 2, and the statistical evaluation is given in Table II.

From the determinations mentioned, and so far as the relation of  $Q_{O_2}$  and body size can be established from a regression line drawn through the means of two groups investigated, it appears that the relation mentioned is much more pronounced in mice than in rats. The regression lines of both strains are nearly parallel,  $r$  being 0.245 for normal, Ottawa strain, and 0.340 for the pituitary strain. The values of both weight groups lie somewhat higher in the normal individuals of the pituitary strain than in the Ottawa strain, but the parallelism of the  $Q_{O_2}$  - body size relation is obvious.

As far as interspecific comparison of rats and mice is concerned it appears that no definite relation between  $Q_{O_2}$  and body size of the species in question can be established. With respect to mature animals the  $Q_{O_2}$  of adult mice (25-30 gm. body weight) is  $3.49 \pm 0.17$  (28 experiments). This is much smaller than the corresponding value for rats of the same size, and similar to that of adult rats (cf. fig. 1). The  $Q_{O_2}$  of skeletal muscle of young normal dwarf strain mice (6 to 10 gm.), and Ottawa strain, (6 to 8 gm.), is  $5.7 \pm 0.27$  (11 experiments) and  $4.9 \pm 0.23$  (16 experiments) respectively. These values are in the range of values obtained with rats

weighing 10 gm.

b) Diaphragm

As mentioned previously, the diaphragm was the only tissue among those studied in rats by Bertalanffy and Pirozynski (1951 (a), 1953) to show a definite correlation of  $Q_{O_2}$  with body size, the slope of the regression line being  $\alpha = -0.26$  with a high correlation coefficient  $\rho = 0.93$ . This has been further investigated in the present studies.

Bertalanffy and Pirozynski's determinations were carried through in Krebs-Ringer-phosphate solution without glucose. It was of interest to investigate whether a similar  $Q_{O_2}$  - size relation, or a consistent change of it, can be found under different nutritional conditions. For this reason, determinations over the entire range of body weight were carried through in Krebs Medium II, type A, with addition of glucose and further, with addition of the metabolites of the Krebs Cycles (Na-pyruvate, Na-fumarate, Na-glutamate).

A total of about 116 individual determinations were carried through. The results are given in fig. 3, which also shows the regression line of the data of Bertalanffy and Pirozynski. The statistical evaluation is given in Table 3.

As can be seen from these data, the earlier results are confirmed. As is to be expected, the absolute  $Q_{O_2}$  values are higher when glucose and metabolites are added. The slope of the regression line,  $\alpha$ , also the correlation coefficient,  $\rho$ , are, however, similar in all three

series in different media. There are differences in the slope of the regression line, ( $\alpha$ ), but it does not seem that improved nutritional conditions as would correspond to the series (saline alone, addition of glucose, addition of metabolites of the Krebs cycle), would lead to a consistent change of the  $Q_{O_2}$  - body size relation.

In interspecific comparison, the  $Q_{O_2}$  of diaphragm of rats was compared with that of adult mice (weight 25-30 gm.). The  $Q_{O_2}$  of the diaphragm of adult mice in the Medium mentioned is  $9.04 \pm 0.27$  (15 experiments). This corresponds to diaphragm of rats weighing ca. 30 gm., and is about twice the value of adult rats (over 300 gm.), as has previously been stated by Meyerhof and Himwich 1924. Thus, the interspecific relation between  $Q_{O_2}$  and body size is different with respect to skeletal muscle and to diaphragm.

## 2. Influence of Thyroid Hormone on Tissue Respiration in Vitro

In view of the contrasting results mentioned previously in this paper, the influence in vitro of thyroid hormone on tissue respiration was studied in different ways.

Thyroxine (Squibb) was added to medium into which tissue for Warburg determination were placed as described in Chapter II. The range of hormone concentration was from 0.01 to 0.05 mg./ cc. of Medium.

### a) Diaphragm

Diaphragm of rats with body weight from 11 to 290 gm. was placed

into Medium II, type A with glucose, and with addition of thyroxine as described. The results of these experiments are given in Table 5. As can be seen, the  $t$ -test does not reveal a significant change of  $Q_{O_2}$  with or without thyroxine.

b) Musculature from H. adrenalectomized animals

It could be assumed that an influence of thyroxine on tissue respiration in vitro, although not present in normal animals, would be enhanced by a condition of hypothyroidism. As mentioned previously (Zarrow and co-workers, 1951), it seems that rats are normally in a state of saturation, so to speak, with the thyroid factor or factors. The additional administration of thyroxine in vitro, therefore, would show little effect. If, however, the animal is in a state of hypothyroidism it is possible that thyroxine administration would show a greater effect on tissue respiration. This has been demonstrated with the liver of thyroidectomized rats by Rapport, Cansanelli and Guild (1946). The animals were thyroidectomized 4 to 6 weeks prior to experiment and d-l thyroxine, 20 mgm. per kilo., was injected subcutaneously 48 hours before the experiment, in a single dose. There was an increase in  $Q_{O_2}$  of 21% in liver from normal injected animals, and an increase of 48% in liver from thyroidectomized animals.

As will be discussed later in this thesis, hypophysectomy leads to a depression of both total and tissue respiration. This effect could be attributed, at least partly, to an influence of the thyroid via the

thyretropic hormone of the anterior lobe. Atrophy of the thyroid (Long, 1943), and the pituitary-thyroid relationship as described by Maqsood (1953) further emphasize the relationship of these glands. If this reasoning is correct, an influence of thyroxine administration in vitro on tissue respiration would be detectable in hypophysectomized animals which is not observed in normal specimens.

In order to verify this expectation, experiments were carried through with hypophysectomized animals. The experiments were conducted from a week to 18 days after hypophysectomy. Tissue respiration of musculature was determined according to the procedure indicated in Chapter II, and in Medium II, type A. (Krebs, 1950). Thyroxine was applied in concentration of 0.05 mgm./cc. Medium. The results are given in Table 9. The values obtained compare favourably with those obtained in thyroxine-free medium. No significant effect of thyroxine administration in vitro on musculature from hypophysectomized animals was detected, while according to Barker and Klitgaard (1952), skeletal musculature has shown positive response to subcutaneous administration of thyroxine. In one pilot experiment where higher concentration of thyroxine was applied (100 gamma/ cc Medium), the result was also negative, giving a  $Q_{O_2}$  value of 2.45 with thyroxine and 2.65 without thyroxine.

The experiments mentioned show that in different tissues, such as heart, diaphragm, and skeletal musculature, under normal conditions as well as after hypophysectomy, thyroxine administration in vitro is unable to show significant influence on tissue respiration. The tissues investigated

were chosen with a view that such influence should be especially manifest in the tissues and conditions chosen. The diaphragm is a tissue which facilitates a minimum of experimental injury for purpose of tissue metabolism study, since it is only cut into small pieces and no slicing nor piercing is necessary. As the studies on size-dependence of  $Q_{O_2}$  show, this tissue seems to respond to regulating influences more definitely than others. Skeletal musculature was used because the influence of thyroxine on basal metabolism of the entire animal is doubtless due to a large extent, to increased oxidation in the musculature. Diaz et al. (1947) has shown that muscular activity in hyperthyroidism is considerably affected, inferring a strong influence of the thyroid on mammalian skeletal muscle. The authors concluded that hyperthyroid state is associated with general muscular weakness and increased susceptibility to fatigue. The failure to maintain tetanus tension from nerve stimulation together with normal response to direct stimulation suggests that the factor of neuromuscular transmission may be involved. Thyroidectomized animals were used because of the conditions previously mentioned.

It appears, therefore, that the tissues and conditions chosen were such as to provide particularly favourable conditions for a possible action of thyroxine on tissue respiration in vitro, so that other tissues and conditions would even be less favourable to demonstrate such action. No positive results were obtained in any of these series.

This, of course, corresponds to the negative results obtained

by other investigators, as indicated previously in the review of literature.

c) Influence of Thyroglobulin on Tissue Respiration

Different results were obtained with thyroglobulin. Although the percentage of increase varied, the trend was consistent.

Thyroglobulin, pure, obtained from Delta Chemical Works, was applied in concentration of 0.5 mgm/20 cc. of Medium, from which 1 cc. was placed in each manometric flask, and in one series 2.5 mgm. per cc. was applied. Thus, according to Cansanelli and Rappert (1937), 2.5 mgm. of thyroglobulin would contain about 15 gamma of  $I_2$ ; this amount of  $I_2$  would be present in about 22.5 gamma of thyroxine. Thyroglobulin was found soluble in 0.9% NaCl solution as was previously mentioned by Resiter (1940).

Most of the experiments were conducted with cardiac muscle of rats, and slices were obtained by employing the Stadie Riggs microtome according to the procedure indicated under Material and Methods, Chapter II. Heart tissue was chosen because according to experiments by Barker and Klitgaard (1952), Ullrick and Whitehorn (1952), cardiac musculature seems to be quite sensitive to injected thyroxine, and thyroxine administration

END

As seen from Table 7, thyroglobulin applied to tissues in vitro leads to an increase of  $QO_2$  which is statistically significant, the value of  $\bar{x}$  being 3.57 when compared with untreated tissues and 8.92 when compared with thyroxine treated tissues. It seems probable that thyroxine has a

slight depressing effect according to the conditions of this experiment; correspondingly, the values of  $p$  are  $< 0.01$  for both series. As mentioned previously, Cansanelli et al. (1937, 1939) have also found an in vitro action of thyroglobulin on  $Q_{O_2}$  of tissues, as contrasted to the lack of such action of thyroxine. However, relatively few experiments with thyroglobulin were done, doubtless due to the fact that the preparation was not obtainable commercially for a long time. The present experiment seems to confirm that, while thyroxine is ineffective in vitro in influencing tissue respiration of those investigated, thyroglobulin is effective in this respect.

### 3. Hypophysis and Tissue Respiration

#### a) Tissue Respiration in Hypophysectomized Rats

It is a well known fact that hypophysectomy causes a considerable decrease in total (basal) metabolism of the entire animal (Selye, 1947 (a)). This was confirmed by some experiments conducted in this laboratory by I. Bickis. These determinations give basal metabolism values for hypophysectomized rats as being about one half the values of normal animals of the same size.

As already mentioned, hypophysectomized rats were obtained from Hormone Assay laboratory. The animals were in good state of health and fed as indicated under Material and Methods, Chapter II. In another series of experiments where experimental animals were fed fox-chow diet

supplemented by raw carrots once weekly, and also on a special synthetic diet described by Griffin et al. (1948),<sup>x</sup> it has been observed that survival time for hypophysectomized animals fed with Fox-chow diet as indicated here has been well over 120 days in contrast to 112 days reported by Shaw and Greep (1949). We have found that Fox-chow supplemented by carrots once weekly, is not inferior to the synthetic diet proposed by the authors mentioned above. The death of animals over a period of 120 days was 17 percent as against 30 to 40 percent for 112 days as reported by these authors. Feeding with synthetic diet did not seem to improve the general state of health nor alter the weight-loss which is unavoidable with hypophysectomized animals.

The  $Q_{O_2}$  of tissues from hypophysectomized animals was determined in the usual manner and comparison made with normal controls of the same strain. The values with tissues from hypophysectomized animals compared favorably with those indicated in Table 9, which represent an independent series of experiments conducted to determine the influence of thyroxine on tissues from hypophysectomized animals.

x Casein 18% glucose monohydrate 73%, corn oil (Masola) 5%. Salt mixture<sup>1</sup> 4%. To each Kilo of the diet so prepared add thymine hydrochloride 3.0 mg., riboflavin 2.0 mg., calcium pantothenate 7.0 mg., pyridoxine hydrochloride 2.5 mg., and choline 0.5 mg.

1 Salt mixture (parts by weight):  
 NaCl 1470, Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 2086, MgSO<sub>4</sub>·7H<sub>2</sub>O 588, KCl 1680,  
 CaCO<sub>3</sub> 2940, FePO<sub>4</sub>·4H<sub>2</sub>O 206, KH<sub>2</sub>PO<sub>4</sub> 4340, MgCO<sub>3</sub> 672,  
 MnSO<sub>4</sub>·H<sub>2</sub>O 3.2, K<sub>2</sub>AL<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O 1.2, CuSO<sub>4</sub>·5H<sub>2</sub>O 5.4,  
 NaF 7.4, and KI 1.2.

**b) Cardiac and Skeletal Musculature**

The effect of hypophysectomy on oxygen consumption of cardiac and skeletal musculature is quite definite when compared with similar tissues from normal rats. This is indicated in Tables 6 and 10 respectively. Not only was there a sharp decline in oxygen consumption, but this decline became more pronounced when the interval between hypophysectomy and beginning of manometric procedure for determination of  $Q_{O_2}$  values is extended. Thus, there was a decrease of 36.5 percent in  $Q_{O_2}$  of cardiac musculature 11 days after hypophysectomy, and a total decrease of 48.5 percent 18 days after hypophysectomy. The values obtained with skeletal musculature of 11 days post hypophysectomy are compared with musculature of normal animals, and indicated in Table 10. There is a decrease of 43.8% which is 7.3% higher than the figures indicated for cardiac muscle. The difference between hypophysectomized and normal animals is so obvious that the  $t$ -test was unnecessary to indicate significance.

**c) Tissue Respiration in Pituitary Dwarf Mice**

While hypophysectomy is an experimental and phenotypical condition, pituitary dwarf mice present hypofunction of the hypophysis as a genotypical condition. Thus, comparison of hypophysectomized rats and pituitary dwarf mice offers a valuable opportunity to study the influence of the pituitary on metabolism

It appears that there are no data in the literature as to total (basal) and tissue metabolism of pituitary dwarf mice. For this reason basal metabolism determinations were conducted in our laboratory by I. Bickis, the results of which are given in Table 8. It appears from these data that pituitary dwarf mice show only about half the basal metabolism of normal animals of the same strain and same body weight. This indicates that the cessation of growth, as found in the dwarf animals, is correlated with a correspondingly low total metabolism.

As indicated previously, the normal heterozygous individuals of pituitary strain show somewhat higher values of  $Q_{O_2}$  than individuals of corresponding sizes of the Ottawa strain, probably because of genetical differences. Comparison has to be made, therefore, between normal animals and dwarfs (homozygous) of the pituitary strain. As can be seen, the dwarfs have lower  $Q_{O_2}$  values ( $Q_{O_2} = 4.5 \pm 0.17$ ) than the normal animals of the same size ( $Q_{O_2} = 5.7 \pm 0.27$ ). The difference is significant,  $t$  being 5.35,  $p$  being  $< 0.01$ . However, the  $Q_{O_2}$  of musculature of the dwarfs (6 to 9 gm.) corresponds roughly to the  $Q_{O_2}$  of musculature of adult heterozygous animals (16 to 20 gm.), as shown in Table II.

#### 4. Other Influences on Tissue Respiration

Some pilot experiments have been conducted with respect to a few other factors which may have some influence on metabolism. These results are not considered conclusive, but are reported here because they present some material relative to the problem in question.

a) Insulin

A review of older literature given by Krebs and Johnson (1948), indicates that the effect of insulin on oxygen consumption of pigeon breast muscle is small in plain suspension of minced muscle, but is marked if boiled muscle extract or citrate is present, and greatest if both extract and citrate are added. Insulin was, according to Cori et al. the first example of a hormone interfering with a known and simple enzymatic reaction. The phosphorylation of glucose by hexokinase,  $\text{glucose} + \text{A.T.P.} \longrightarrow \text{glucose - 6 - phosphate} + \text{A.D.P.}$  is inhibited by the Housay factor of the anterior pituitary and adrenal cortex hormone; this inhibition is removed in vitro as well as in vivo by insulin. Recent experiments by Eyer and Marlin (1951) have shown that there are some effects of insulin on metabolism in normal rabbits. The authors concluded that insulin,  $\frac{1}{2}$  U/Kg. produces an increased heat production over basal in the first fully effective hour, of 26%, part of which may be attributed to induced epinephrin secretion. Selye (1947 (b)) has indicated that the B.M.R. is usually not significantly altered either by pancreatectomy or by insulin overdose, although pancreatectomy decreases the respiratory quotient (R.Q.) to about 0.7, probably as a result of decreased sugar utilization, increased gluconeogenesis or both.

In the present experiments Protamine zinc insulin, 40 units per cc. was used. 0.05 cc. of insulin was added to each manometric flask containing 1 cc. medium. The experiments were conducted with liver slices and diaphragm as described under Chapter II, Material and Methods, in

order to investigate the action of insulin in vitro.

As shown in Table 12, the results are negative, having shown no influence of insulin on tissue respiration in vitro.

b) Respiration in Regenerating Liver

It is well established that there exist some relationship between mitotic activity and cell respiration. An increase in oxygen consumption has been reported in cleavage by Runnstrom (1933), Brachet (1950). An excellent presentation has been given by the latter in his book; "Chemical Embryology", and needs no further elaboration here.

Since regenerating rat liver is a favorite object in cell-metabolism studies, some determinations were carried through in order to compare the  $Q_{O_2}$  of normal liver (which is practically free of mitosis) and regenerating liver (which shows a relatively high mitotic activity).

The animals were anesthetized with ether and their livers removed by partial hepatectomy; they were sacrificed within three days after operation, for  $Q_{O_2}$  determination.

The term regenerating liver used here, may more appropriately be described as "compensatory hyperplasia", since it is the intact lobes which undergo a process of rapid growth. It was pointed out by Novikoff and Petter (1948) that during the first 24 hours after partial hepatectomy the remaining mass increases markedly by increase in cell size without cell division. The most active phase, however, occurs about the third day after operation when mitotic figures are most numerous.

Table 13 shows no difference between the  $Q_{O_2}$  of normal and regenerating liver. This indicates that, either the energy requirements of mitotic activity are too small to significantly influence the resting metabolism of tissue, or that the energy for mitotic activity is not provided by respiratory processes but rather by anaerobic glycolysis; this was found by O'Connor (1950), on the basis of determinations of the number of dividing cells, rate of respiration and rate of aerobic glycolysis in the mid-brain of chick embryo.

e) Hydergine-Methansulfonate

Favourable clinical and experimental results in various peripheral disorders such as hypertension, Reynaud's and Buerger's disease, post traumatic vascular disturbances, gangrene, frostbite, etc. (J.H. Hafkenschiel et al., (1950), A Kappert and W. Madern (1950), R. J. Popkin (1950) and L.L. A. Hurley et al. (1951), of recently developed ergot derivatives lead particular interest to their mode of action.

According to Rothlin (1946/47) the following actions of ergot alkaloids can be distinguished:

1. Effects with point of attack in the central nervous systems: respiration, circulation, relation between respiration and circulation, thermoregulation, emesis, sedative effects. It is possible that the depressive action on metabolism (Oxygen consumption) J.E. Roberts, L. C. Massepust, Jr., and A.R. Buchanan) may be also added to this group.

2. Effects with peripheral points of attack.

a) Patent effects: direct stimulation of smooth musculature, e.g., the uterus; b) latent effects: sympathicolytic action, i.e., inhibition of functions controlled by the sympathetic system of adrenaline.

Some experimental work was done in this laboratory with an ergot derivative, Hydergine (trade name for a combination in equal amounts of dihydroergocristine, dihydroergokriptine, and dihydroergocornine), which seems to point at a direct depressing, but not necessarily toxic action of this drug on tissue respiration in vitro. This would open the perspective that, besides the action quoted, sympathicolytic substances may have a direct action on cellular metabolism. In view of the importance of this problem, some results are included in the present paper, although the results are not conclusive and would need much further elaboration. As seen from Table 14, 14a hydergine leads to a depression of  $QO_2$ . Results obtained by J. Krywiensyk in this laboratory seem to show further, that the depressive action of hydergine on tissue respiration can be compensated or even over-compensated by addition of metabolites of the Krebs cycle.

This would indicate that, besides the effect of ergot alkaloids on the central and peripheral nervous system, there is a direct action on cellular metabolism which seems to be based on some interference in the Krebs cycle, and can, therefore, be compensated by an abundance of metabolites of the Krebs cycle. This, however, is only in the way of a

tentative hypothesis, and would need more experimental material to be firmly established. The data referred to, are given because they seem to be indicative of an interesting and theoretically important effect, but it is felt that they are not conclusive and that further investigation is needed.

Hydergine was applied in concentration of 4.5 mg. percent by adding 0.1 cc. of solution of 4.5 mg. hydergine in 10 cc. of double distilled water, to 1 cc. medium. The values represent mean  $Q_{O_2}$  values of about 4 individual determinations of animals between 120 and 200 gm.

Table 1.  $Q_{O_2}$  of skeletal musculature from normal rats (10 to 384 gm.) using Medium II type A with glucose. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate). Values represent Mean  $Q_{O_2}$  of 4 to 6 individual determinations.

Body Weight	$Q_{O_2}$	Body Weight	$Q_{O_2}$
10	5.10	120	4.27
11	5.32	146	4.30
13	4.99	158	4.22
15	5.59	168	4.05
16	4.69	168	4.18
18	5.60	170	4.15
18	4.93	170	4.12
18	4.55	170	4.56
20	5.23	172	3.76
22	4.74	174	4.50
24	4.76	282	4.76
26	4.80	300	4.24
28	4.76	300	3.83
30	4.93	300	4.59
36	4.21	306	3.88
40	4.15	362	4.21
68	4.25	364	3.44
84	4.77	384	4.39

Statistical Evaluation

$$\alpha = 0.065$$

$$h = 5.95$$

$$S(\log y, \log x) = 0.039$$

$$r = 0.664$$

Fig. 1.  $Q_{O_2}$  of skeletal muscle of rat in relation to body size. Medium II, type A (Krebs, 1950) with glucose, 0.9% NaCl substituted for Na-pyruvate, Na-fumarate, and Na-glumate. The central line indicates the logarithmic regression, the two parallel lines give the standard error in percent, including 2/3 of the cases. Each point represents the average of 4-6 individual determinations of tissues of the same animal.

42 (a)

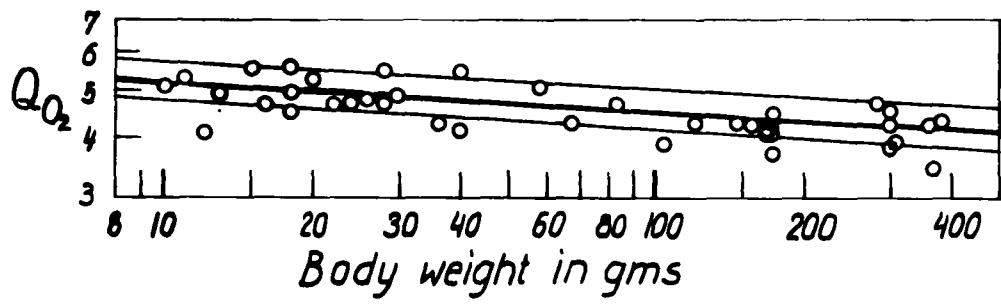


Table 2.  $Q_{O_2}$  of skeletal musculature from normal mice (25 to 30 gm.), using Medium II type A with glucose. .9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate). Values represent Mean  $Q_{O_2}$  of 4 to 6 individual determinations.

Body weight	$Q_{O_2}$
26	3.23
28	3.02
28	3.98
30	3.07
32	3.96
28	3.82

Mean 3.492 0.17

Fig. 2.  $Q_{O_2}$  of skeletal musculature from mice of normal strain, and Pituitary dwarf strain using Medium II type A with glucose. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate). Each point represents an average of 12 individual determinations. - - - - -: Pituitary strain animals 8 and 18 gm. —————: Normal strain animals 7 and 28 gm.

44 (a)

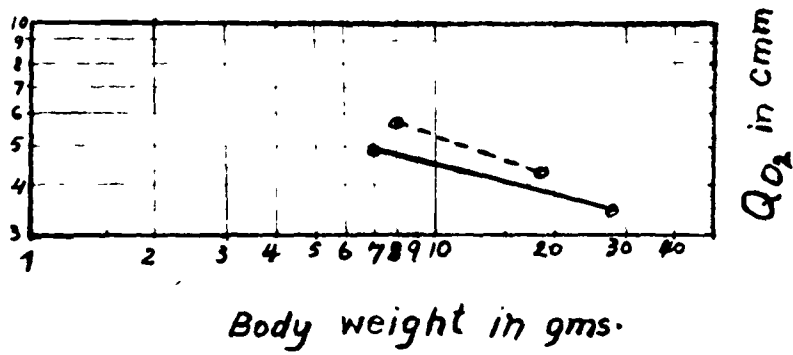


Table 3.  $Q_{O_2}$  of diaphragm from normal rats (11 to 364 gm.), using Medium II type A with glucose. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate); also Medium II type A with above metabolites. Values represent Mean  $Q_{O_2}$  of 4 to 6 individual determinations.

With glucose			With glucose and with metabolites		
Body Weight	$Q_{O_2}$	Body Weight	$Q_{O_2}$	Body Weight	$Q_{O_2}$
11	9.87			11	14.03
13	9.67			20	13.80
16	9.10	110	6.97	26	11.62
18	9.55	150	7.17	31	12.25
26	8.37	208	6.30	41	13.84
30	8.69	220	6.34	72	11.45
40	7.81	220	5.09	90	11.75
48	7.87	290	6.97	110	12.03
78	8.07	317	5.79	180	8.55
82	7.20			220	8.69
96	7.98			364	7.51

Statistical Evaluation

$$\alpha = 0.148$$

$$b = 14.14$$

$$s(\log y \cdot \log x) = 0.034$$

$$r = 0.909$$

$$\alpha = 0.171$$

$$b = 22.84$$

$$s(\log y \cdot \log x) = 0.047$$

$$r = 0.876$$

Fig. 3.  $Q_{O_2}$  of diaphragm of rats in relation to body size and in different media. ---: Krebs-Ringer phosphate solution, data from Bertalanffy and Pirozynski; ⊙ : Medium II, type A, with glucose; ⊗ : Medium II, type A, with Na-pyruvate, Na-fumarate, Na-glutamate and glucose (Krebs, 1950). Points, regression lines and standard error as in Fig. 1.

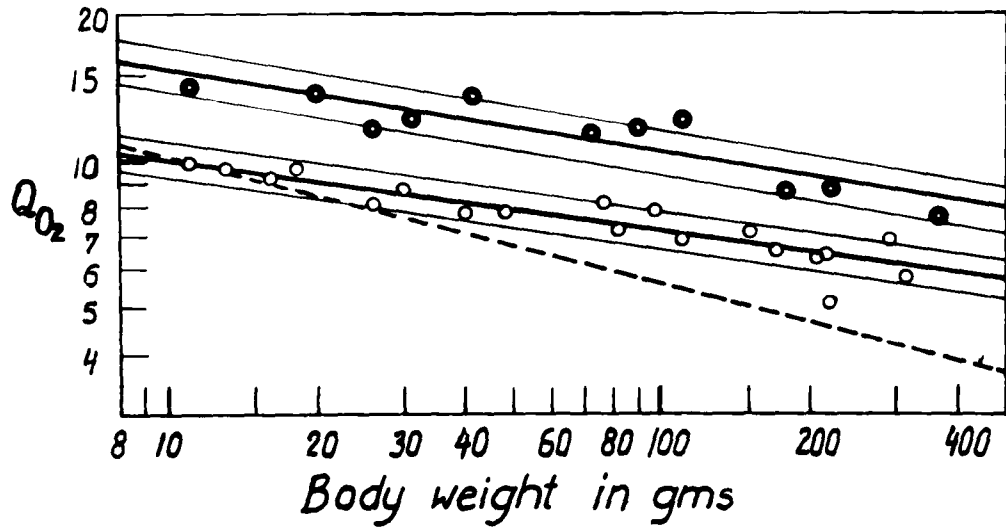


Table 4.  $Q_{O_2}$  of diaphragm from normal mice (25 to 30 gm.) using Medium II type A with glucose. 0.9% NaCl substituted for metabolic (Na-pyruvate, Na-fumarate and Na-glutamate). Values represent individual determinations.

	$Q_{O_2}$	
	9.54	-----
	8.45	8.33
	11.95	8.86
Body Weight: 25 to 30 gm.	8.18	8.58
	8.29	8.01
	9.21	10.54
	9.07	9.84
	8.36	8.40

Mean 9.04 ± 0.27



Fig. 4.  $\dot{V}O_2$  of diaphragm from normal rats (11 to 290 gm.) using Medium II type A with glucose, with and without addition of thyroxine. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate). Each point represents individual  $\dot{V}O_2$  value. ●●●●: Thyroxine treated. ○○○○: Without thyroxine.

49 (a)

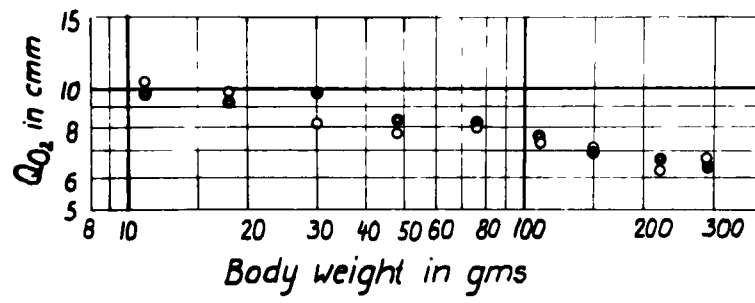


Table 6.  $QO_2$  of cardiac musculature from normal control animals using Medium II type A with glucose, and with glucose and metabolites; also cardiac musculature of hypophysectomized animals of 11 and 18 days post hypophysectomy. Values represent individual determinations. Weight range of animals: 104 to 220 gm.

Control with metabolites	Control with glucose	11 days post hypophysectomy	18 days post hypophysectomy	
12.49	10.14	6.84	5.08	5.08
10.47	10.59	6.22	6.22	3.98
10.45	9.16	6.08	6.56	4.43
12.67	9.91	5.58	6.20	4.72
10.90	7.44	6.81	5.87	5.17
11.53	11.38	6.38	5.38	3.81
12.25	9.90	5.26	4.03	4.35
11.28	9.19	- -	5.68	3.09

Mean  $11.40 \pm 0.35$

$9.71 \pm 0.41$

$6.16 \pm 0.22$

$5.00 \pm 0.24$

Table 7.  $QO_2$  of cardiac musculature using Medium II type A with glucose, with the addition of thyroxine and thyroglobulin respectively. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate). The two parts of table (A & B.) represent different independent series of experiments which, nevertheless, give the same results. Thyroglobulin was applied in concentration of 0.5 mg. in 20 cc. Medium, from which 1 cc. was placed into each manometric flask. Thyroxine was applied in concentration of 0.05 mgm. / 20 cc. The values represent mean values of 3 individual determinations.

	A.		B.	
	(a) Control	(b) With Thyro- globulin	(c) With Thyroxine	(d) With Thyro- globulin
	8.87	10.50	8.55	9.52
	10.56	10.91	9.20	10.90
	8.53	9.65	8.51	9.41
	9.52	11.32	9.83	11.58
	10.98	11.29	8.05	10.67
	9.21	10.08	10.00	11.20
Mean	9.61	10.62	9.02	10.54

$$t \text{ (a and b) } = 3.57$$

$$p < 0.01$$

$$t \text{ (a and c) } = 2.58$$

$$p = 0.39$$

$$t \text{ (c and d) } = 8.92$$

$$p < 0.01$$

**Fig. 5. Diagram after H. B. Van Dyke (1943) indicating the complicated interrelationship of the pituitary hormones.**

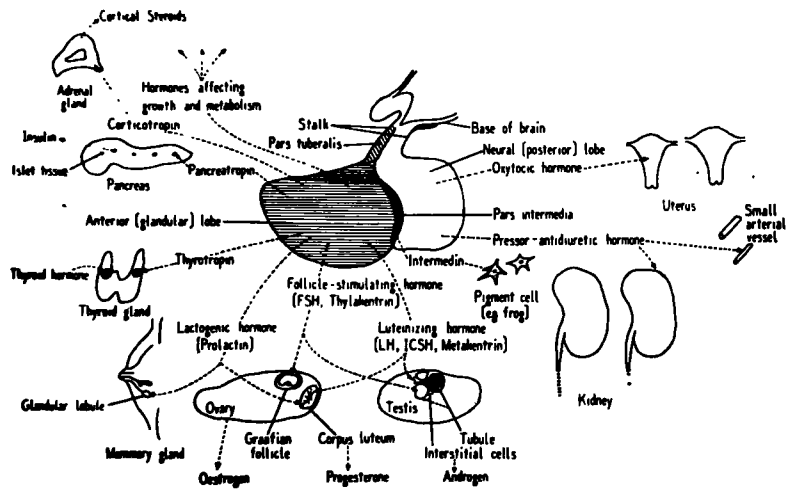


Table 8. Oxygen consumption under basal conditions, of homozygous dwarf (10 gm.), heterozygous dwarf strain (10 and 20 gm. respectively) and normal Ottawa strain (8 gm.) : Experiments by L. Pickie.

Strain	Weight in gm.	R. Q.	CO <sub>2</sub> in cmm.	O <sub>2</sub> in cmm.	Mean $\dot{V}_O_2$
<u>dwarf</u> <u>dwarf</u>	9.4 10.0	0.77 0.78	10.1 10.1	13.1 12.9	13.0
<u>dwarf</u> •	10.0 9.5	0.77 0.74	16.8 15.7	21.8 21.0	21.4
<u>dwarf</u> •	20.0 21.0	0.75 0.76	29.0 31.4	38.6 41.5	40.0
<u>Ottawa</u> <u>strain</u>	8.0 8.0	0.80 0.78	17.9 17.9	21.8 23.8	22.4

**Table 9.  $\dot{V}O_2$  of skeletal musculature from hypophysectomized rats using Medium II type A with glucose, with and without addition of thyroxine. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate.) Values represent individual determinations. Thyroxine was applied in concentration of 0.05  $\mu$ g./cc.**

Thyroxine	Without Thyroxine
2.01	2.42
3.04	3.18
2.81	2.53
3.28	1.81
2.01	3.44
2.44	2.83
3.07	2.46
2.60	2.66
Mean 2.65 $\pm$ 0.16	2.66 $\pm$ 0.17

Table 10.  $Q_{O_2}$  of skeletal musculature from hypophysectomized rats compared with normal rats of same strain, and maintained under identical conditions, using Medium II type A with glucose. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate). Values represent individual determinations.

Hypophysectomized	Normal Control
2.85	3.74
2.83	3.61
1.51	4.84
2.03	5.25
2.25	5.31
3.28	6.49
3.78	3.68
Mean 2.64 ± 0.29	4.70 ± 0.41

Table 11.  $\dot{V}O_2$  of skeletal musculature from a) adult pituitary dwarf mice (6 to 9 gm.), b) normal adult of pituitary strain (16 to 20 gm.), c) young normal Ottawa strain (6 to 8 gm.), d) young normal of pituitary strain (6 to 10 gm.), using Medium II type A with glucose. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and  $N_2$ -glutamate). Values represent individual determinations.

(a) Adult pituitary dwarf		(b) Normal adult pituitary strain		(c) Young normal mice		(d) Young normal pituitary strain	
4.60	4.43	3.59	4.56	6.31	6.00	5.25	6.04
4.39	3.28	5.85	3.58	5.25	6.30	6.99	5.59
4.80	4.82	4.74	5.89	4.61	4.72	5.68	6.34
3.93	5.77	3.03	4.03	5.73	4.78	5.40	4.98
3.91	4.39	3.74	4.58	5.26	5.31	6.68	6.27
4.37	5.03	4.36	4.04	4.72	4.27	3.47	• --
--	--	--	--	5.28	3.09	--	--
--	--	--	--	4.11	3.21	--	--

Mean  $\dot{V}O_2$  4.51 ± 0.17

4.32 ± 0.25

4.93 ± 0.23

5.72 ± 0.27

$\bar{x}$  (a & d) = 5.35

$P < 0.01$

**Fig. 6. Size relationship of Normal and Pituitary strain mice.**  
A): Phenotypically normal pituitary strain mouse (18 gm.) of six weeks. B): Normal strain mouse (30 gm.) of 20 weeks. C): Pituitary dwarf, littermate of A). (9 gm.).

57 (a)



(A)

(B)

(C)

Table 12.  $Q_{O_2}$  of diaphragm and liver slices from normal rats (134 to 330 gm.) using Medium II type A with glucose, with and without addition of Insulin. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate). Values represent individual determinations. Insulin was applied in concentration of 0.05 cc. in 1 cc. Medium.

Diaphragm		Liver	
Control	Insulin	Control	Insulin
- --	- --	8.95	8.16
- --	- --	8.54	7.88
- --	- --	9.15	8.45
6.46	5.11	10.46	6.17
6.34	5.14	6.78	6.14
6.93	6.67	7.75	7.42
7.42	7.89	7.59	6.94
8.40	6.84	7.17	6.91
6.53	4.90	6.13	6.42
7.05	6.64	7.62	7.10
5.75	6.36	6.52	7.86

Mean 6.86<sup>±</sup>0.28 6.19<sup>±</sup>0.36 7.87<sup>±</sup>0.38 7.22<sup>±</sup>0.24

Table 13.  $Q_{O_2}$  of regenerating and normal liver 3 days after partial hepatectomy, using Medium II type A with glucose. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate). Values represent individual determinations.

Normal liver	Regenerating liver
7.87	7.01
8.15	9.03
6.73	7.46
10.26	10.76
9.94	10.40
8.95	9.73
6.73	7.46

Mean  $8.37 \pm 0.53$   $8.83 \pm 0.57$

$\bar{x} = 0.967$

$\bar{y} = 0.46$

**TABLE 14**Restitution of normal respiration of the rat diaphragm.By addition, after one hour, Na-fumarate:Experiments by J. Frydenczyk

$Q_{O_2}$ , 1st hr.		$Q_{O_2}$ , 2nd hr.	
Controls	With Hydergine	Controls	With Hydergine, Na-fumarate added
-8.79	-10.08	-11.89	-12.11 <sub>x</sub>
-10.22	-11.12	-10.62	-12.68 <sub>x</sub>
- 7.88	- 8.64	- 6.23	- 6.52 <sub>x</sub>
- 6.01	- 6.54	- 7.04	- 6.25
- 8.65	- 9.21	- 9.13	- 7.84
- 9.08	- 9.96	- 7.85	- 7.56
- 6.85	- 7.86	- 6.43	- 6.23
- 9.15	-10.00	- 6.79	- 5.36

The  $Q_{O_2}$  values are averages of 4 determinations (x 3 determinations)

$$\bar{x} = 4.47$$

$$s < 0.001$$

$$\bar{x} = 0.276$$

$$s = 0.78$$

Table 14 (a).  $Q_{O_2}$  of diaphragm from rats 120 to 200 gm. with and without addition of Hydergine and using Medium II type A with glucose. 0.9% NaCl substituted for metabolites (Na-pyruvate, Na-fumarate and Na-glutamate). The values represent mean  $Q_{O_2}$  for 4 individual determinations. Hydergine was applied in concentration of 4.5 mg. percent by adding 0.1 cc. of solution of 4.5 mg. hydergine in 10 cc. of double distilled water, to 1 cc. medium.

Hydergine	Without hydergine
5.96	6.90
5.73	5.86
6.11	6.85
4.74	5.21
4.22	5.06
4.89	6.32

Mean 5.27                      6.03

$\bar{x} = 8.798$

$R < 0.01$

CHAPTER IVDISCUSSION1. Tissue Respiration and Body Size

The relation between metabolism and size, as expressed in the decrease of weight-specific basal metabolic rates with increasing body size, and found intra-specifically (comparison of animals of the same species and different size) as well as interspecifically (comparison of adult animals of different species) is known well over hundred years; but none of the explanations given for this phenomenon is satisfactory.

(Kleiber, 1947, Bertalanffy, 1951 (a)). Bertalanffy and Pirozynski (1953) came to the conclusion that none of the conventional explanations of the decrease of weight-specific metabolic rate with increasing body size, such as explanation in terms of thermoregulation, of muscular activity, of the relative decrease in "metabolically active" organs during growth, and of age, is considered to be sufficient. They emphasize that no decline in tissue respiration of the organs investigated can be found which would correspond to the decline in basal metabolic rate of the entire animals. It appears, therefore, that the latter depends upon regulative factors lying in the organism as a whole.

The present experiments were conducted with a special view on a recently propounded theory, viz., that the "characteristic differences in the basal rate of heat production in animals of different size are

to be attributed mainly to variation in  $Q_{O_2}$  of the musculature." (Krebs, 1950).

Krebs did not investigate the  $Q_{O_2}$  of muscles in animals of different size. His conclusion is based upon the fact that the muscles play a leading part in thermoregulation which, according to the classical explanation given by Rubner, is at the base of the surface rule. Krebs offers this explanation for interspecific comparison of metabolic rates in the series of mammals, but naturally it could also be applied to intraspecific comparison.

This hypothesis was rejected by Bertalanffy and Pirozynski mainly for theoretical reasons. They emphasize that the surface or  $3/4$  power rule of metabolism applies not only to mammals, but also cold blooded vertebrates and certain invertebrate classes (Bertalanffy, 1951 (a); Bertalanffy and Krywienczyk, 1953). For this reason, homeothermy and muscular activity in its service cannot be considered the basic principle in the size dependence of metabolism. A simple calculation shows, further, that even if a decline of the  $Q_{O_2}$  of musculature according to the surface or  $3/4$  power rule is assumed, this factor would not be sufficient to account for the decline of basal metabolic rate of the entire animal.

Compare two rats of a) 270 gm., b) 10 gm. body weight. a) According to Donaldson (1924, table 129, p. 184), musculature is about one half (45%) of total body weight in adult animals. According to Field et al., (1937), respiration of musculature accounts for about one half of summed tissue respiration in mature animals. Hence it is fair to assume

that, in the large animal, the average  $Q_{O_2}$  for "rest of body" and for "musculature" are roughly equal. Denote the metabolic quotient for "rest of body" and for "musculature" by  $\bar{m}$  and  $\bar{n}$ , respectively. Then, for the mature animal,  $\bar{m} \approx \bar{n}$ . The basal metabolic rate of a rat of 270 gm. is about 250 cc.  $O_2$ /hr. This, according to the considerations given above, can be expressed by  $250 \approx 270 \frac{\bar{m}}{2} + \frac{\bar{n}}{2} = 270 \bar{m}$ .

b) In a rat of 10 gm., about 25% is musculature, 75% rest of body (Donaldson, 1924). Since, according to hypothesis, the main role in the decline of basal metabolism is attributed to variations of the  $Q_{O_2}$  of musculature and empirically little change in the  $Q_{O_2}$  of other organs is observed, it may be assumed that  $\bar{m}$  (average  $Q_{O_2}$  for rest of body) is roughly equal in the 10 gm. and the 270 gm. animal. If  $\bar{n}$  (average  $Q_{O_2}$  of musculature) follows the surface rule ( $\alpha = -1/3$ ) it will, in space of weight 1 : 27, decline in a ratio 1 :  $27^{-1/3} = 1 : 0.33$ . According to the  $3/4$  power formula ( $\alpha = -1/4$ ),  $\bar{n}$  will decline in a ratio 1 :  $27^{-1/4} = 1 : 0.44$ . If, therefore,  $\bar{n}$  is the metabolic quotient of muscle for large animal,  $\bar{n}$  that for small animal,  $\bar{n} = 3 \bar{n} = 3 \bar{m}$ , or  $\bar{n} = 2.29 \bar{m} = 2.29 \bar{m}$ , respectively and, according to the two assumptions mentioned, total respiration for an animal of 10 gm. would be:

$$10. \frac{3}{4} \bar{m} + 10. \frac{1}{4} \cdot 3 \bar{m} = 15 \bar{m}, \text{ or}$$

$$10. \frac{3}{4} \bar{m} + 10. \frac{1}{4} \cdot 2.29 \bar{m} = 13.225 \bar{m}.$$

The ratio of the metabolic rates of the 10 gm. animal and the 270 gm. animal is therefore  $15 \bar{m} : 270 \bar{m} = 1 : 18$ , or  $13.225 \bar{m} : 270 \bar{m} = 20.4$ .

Actually, basal metabolic rate of a rat of 10 gm. is about 25 cc.  $O_2$ /hr., so that the ratio is:

25 : 250 = 1 : 10.

Naturally, this estimate is based upon crude and oversimplified assumptions. It shows, however, that, as far as intraspecific comparison is concerned, a decrease of muscular  $Q_{O_2}$  according to the surface or  $3/4$  power rule would not account for the decrease in weight-specific metabolic rate.

For the reasons mentioned, Bertalanffy and Pirozynski came to the conclusion that, "there seems to be no indication that the decrease of weight specific metabolic rate is to be explained by variations of  $Q_{O_2}$  of musculature."

Krebs (1950) emphasizes that data obtained on isolated muscle bear no simple relation to the basal respiratory rate of muscle in situ, which is regulated by the exigencies of thermoregulation, and for the determination of which there is as yet no satisfactory experimental procedure. It is to be borne in mind, however, that thermoregulation, and muscular activity in its service, although playing an important role in the energy economy of homeotherms, cannot be the basic principle in the dependence of metabolic rate on body size, for the surface rule also applying to poikilothermic vertebrates and even to certain invertebrate classes. Since there is hardly any evidence that factors like muscle tone are essentially different in small as compared to large specimens, it appears that, notwithstanding Krebs's proviso, investigation of isolated muscle should contribute to decide whether variation in respiration of the musculature can be considered the main factor for the

decrease of metabolic rate with increasing body size.

The present results add experimental evidence as to the dependence of the  $Q_{O_2}$  of skeletal muscle on body size. As far as the rat is concerned, the regression of  $Q_{O_2}$  of skeletal muscle is small and actually smaller than assumed in the previously indicated calculation, being  $\alpha = 0.07$  for skeletal muscle. Apparently, it does not account for the difference in weight-specific metabolic rate of the entire animals. This confirms the conclusions drawn by Bertalanffy and Pirozynski.

The present results are somewhat different with respect to the mouse, the two size classes investigated (ca. 7 & 28 gm. and 8 & 18 gm.) showing a steeper slope of the regression, with  $\alpha = 0.245$  and  $0.345$  respectively. The latter values correspond to the  $3/4$  power rule. These results show that one has to be very careful in extrapolations from one to another species, even if they are rather closely related. It appears that there are important physiological differences between rat and mouse. For instance, there is in the rat a continuous increase in body weight over the whole life, while in the mouse growth comes to a stop. Another well known difference seems to exist with respect to thyroid function, as emphasized by Zarrow and co-workers (1951), and mentioned in Chapter I under "Metabolism and the Thyroid Gland." To such species-specific physiological differences, is to be added the difference in the relationship of tissue respiration of musculature and body size. It will be worthwhile, to study these species-specific differences and their inter-relationship.

With respect to the diaphragm, the present experiments show that the characteristic dependence of  $Q_{O_2}$  on body size, as expressed by the slope of the regression line, is not consistently changed with alteration of the medium.

Interspecifically, the  $Q_{O_2}$  of diaphragm of adult mice obtained in the Medium mentioned is  $9.04 \pm 0.27$  (15 experiments). This corresponds to the  $Q_{O_2}$  of diaphragm of rats weighing ca 30 gm., and is in the range of the value for adult rats (over 300 gm.), as has been stated previously by Meyerhof and Himelich (1924).

Thus, as far as interspecific comparison is concerned, the results differ with respect to diaphragm and skeletal muscle. In the two species investigated, the  $Q_{O_2}$  of the diaphragm appears to be dependent on the size of adult animals, being much higher in adult mice than in adult rats. This, however, is not the case with the  $Q_{O_2}$  of skeletal muscle, this being similar in adult mice and rats. Also this consideration gives no indication that differences in muscular respiration of rats play a decisive role in the regulation of basal metabolic rate according to body size.

Further, interspecific correlation between  $Q_{O_2}$  and body weight is similar to intraspecific correlation in the case of the diaphragm, but different in the case of skeletal muscle. This is further evidence for the fact that intraspecific and interspecific size dependence do not necessarily correspond, as it was found in the variation of  $Q_{O_2}$  of various organs and also in morphological phenomena, (Bertalanffy and Pirozynski, 1952, 1953).

## 2. Influence of Thyroxine and Thyroglobulin on Tissue Respiration

The present results of the application of thyroid hormone in vitro on tissue respiration can be summarized as follows:

1) Thyroxine does not increase the  $Q_{O_2}$  values of diaphragm, heart, and skeletal musculature.

2) Application of thyroxine in vitro is also ineffective with tissues (skeletal muscle) of hypophysectomized animals which show lowered basal metabolism and which, as a consequence of the operation, may be supposed to be in a state of hypothyroidism.

3) In contrast to the action of thyroxine, the application of thyroglobulin in vitro leads to a significant increase in tissue respiration of cardiac musculature. These results essentially confirm the work of Canzanelli et al. (1937, 1939), these authors having worked with testis, brain, kidney, heart and liver of guinea pig.

The present results offer some more data for drawing conclusions. Nevertheless, these results rather pose than solve a number of problems:

1) Administration of thyroxine in vivo by way of injection, generally, although with some qualifications leads to an increase of total (basal) metabolic rate in the entire animal. It also leads to an increase of  $Q_{O_2}$  of isolated tissues. If, in contrast, thyroxine applied in vitro fails to show an influence on the oxygen consumption of isolated tissues, it would be reasonable to assume that the thyroid hormone does not act directly on tissue metabolism but primarily acts on the organism as a whole, or, in other terms, that thyroid action is dependent on some

factor or factors which are present only in the entire organism and not reproducible in vitro. Such interpretation was suggested by Gerrard and McIntyre (1933).

The concept of a restricted thyroxine action with respect to some central system as the nervous system, as suggested by Passch and Reinwein (1929), cannot be fully endorsed, in spite of the failure of thyroxine to influence oxygen consumption of isolated tissue. It has been demonstrated by Ring, Dworkin and Basq (1931), that the response to thyroxine injection was unchanged when sympathetic chains in cats were removed, showing that the sympathetic chains had no influence on thyroxine action.

The "organismic" interpretation mentioned would cover the positive effect of application in vivo of thyroxine on basal and tissue metabolism, and the lack of such effect in in vitro application. It does not, however, cover the positive effect of thyroglobulin in vitro.

2) The effect of thyroglobulin seems paradoxical. Thyroxine is a low molecular substance (molecular weight 776.8), while thyroglobulin is a protein of a high molecular weight of about 700,000. (Slaten, 1940). It would be reasonable to assume that thyroxine easily penetrates into the cells and exerts some influence on cellular metabolism. On the other hand, it is much more difficult to conceive the large molecules of thyroglobulin as penetrating into the cells. The present experimental findings, however, confirm that thyroxine is ineffective and thyroglobulin effective in vitro with respect to cardiac musculature.

3) At present more emphasis seems to be placed on thyroglobulin than thyroxine as being the active principle. Selye (1947 (c)) mentions : "The view that thyroxine is not the native hormone is based upon the observation that, compared on the basis of iodine content, thyroxine is less active than thyroglobulin or the glandular tissue itself." If thyroglobulin, rather than thyroxine is the active principle of the thyroid action, its activity in vitro as compared to lack of such action of thyroxine would be understandable. However, this does not solve the problem posed under 2).

4) It is generally understood that thyroglobulin as such does not leave the follicles. Leblond and Gross (1948), conducted experiments to determine whether the thyroid cell secretes thyroglobulin only in the direction of the colloid or also releases some directly into circulation. Their experiments revealed that the secretory activity of the cells was exclusively orientated toward the colloid. An hypothesis has been offered, therefore, that possibly thyroglobulin is broken down, by proteolytic enzymes, into its component polypeptides, peptones, or amino acids and is thus extruded with thyroxine, into the blood stream to be synthesized again by the tissues themselves.

Thyroglobulin has been found in the general circulation of dogs, but Lerman (1940), emphasized that this has to be attributed to trauma of the gland since the general belief is that under normal conditions thyroglobulin does not leave the follicles, normal or hyperplastic, as such. This has also been supported by Leblond and Gross (1948).

There are two schools of thought in regard to the mechanism of the release of colloid. According to one school, the release is intracellular. The colloid is reabsorbed by the acinous epithelium, which in turn discharges into the circulation. The other school states that the colloid passes between epithelial cells and in this way reaches the circulation. Evidence in support of the intercellular release of colloid was furnished by Zefya Hirschlerowa (1927). In her opinion the colloid is directed into circulation through canaliculi which she calls "Ausführungskanalchen", (conductive canal). This opinion is, however, contraindicated according to more recent work by Leblond and Gross, (1948), Leblond (1950).

Thus, it could be assumed that thyroglobulin is broken down to reach the tissues, and in the cells re-united with some protein fraction to give the active principle. This would account for the fact that the thyroxine-protein compound, namely thyroglobulin, is active on cellular metabolism if administered directly to the tissue. On the other hand, isolated cells would not be capable, at least during the experimental period, of synthesizing thyroxine into the active hormone; for this reason, thyroxine would fail to show an in vitro effect under the same conditions. Although this concept seems not to contradict current opinion, it is difficult to conceive how thyroglobulin, a high molecular substance, is able to overcome the cellular barrier.

As this discussion shows, the present experiments do not lead to any easy answer to the problem in question. They seem to offer, however,

some material to be considered in subsequent study of the problem.

### 3. Pituitary and Metabolism

#### a) The influence of Hypophysectomy

The pituitary-thyroid relationship has been fairly well established and there are numerous experimental evidence showing a decrease in B.M.R. and related function following hypophysectomy as well as thyroidectomy, (Rappert et al. (1946), Gordon et al. (1946), Diaz-Guerrero, Thompson and Hines (1947), Marvin, Wingo and Anderson (1950), Zarrow et al. (1951). So far as isolated tissues are concerned, extracts from the pituitary seem to have varying effects. Campbell and Davidson (1950), pointed out that anterior pituitary extract when added directly to the medium did not influence significantly the oxygen uptake of liver slices, while administration by way of subcutaneous injection of an anterior pituitary preparation (APP) to fasting rats increased the uptake of oxygen. The latter finding indicates that endogenous substrates were utilized at an increased rate. The APP used was found to contain about 4 percent of growth hormone and about 1.7 percent adrenocorticotrophic hormone. This result is in contrast to the result of Kleiber and Cole (1939), who found that chronic doses of pituitary extract result in a depression of basal metabolic rate and oxygen consumption in the isolated diaphragm.

Most of the experiments directly on tissue level seem to have been conducted with hypothyroid animals. This author has been unable to

find any recent experimental work on muscular metabolism (oxygen consumption) following hypophysectomy. A complete survey of older literature by Krebs and Johnson (1948), indicates experiments with testis from rats (infantile) showing decrease in oxygen consumption 14 days after hypophysectomy and a further decrease 44 days after hypophysectomy. A decline in oxygen consumption from 10.6 to 6.1 has been observed.

It is reasonable to assume, however, that metabolic changes following hypophysectomy will be similar to those observed in thyroidectomized animals because of the well-known pituitary thyroid relationship. The present work has indicated some of these changes.

A marked decrease in  $Q_{O_2}$  of cardiac and skeletal musculature following hypophysectomy was observed, which can be compared to the decrease in B.M.R. of the entire animal. Of course it is difficult to ascertain whether these metabolic effects are to be attributed directly to hormonal control of metabolism as influenced by hypophysis, or to the general (and in the long run detrimental) effects of hypophysectomy, which leads to general weakness, weight loss and inhibition of growth, and many other effects such as, loss of hair, trophic changes in nails and skin, atrophy of genital organs, sterility, muscular weakness, decreased blood pressure and body temperature (Hypothermia), anemia, etc. The latter was recently discussed by Silbergleit (1951). The color index is usually less than one, and the hemoglobin averages 50% (Selye, 1947 (d)). There is also atrophy of the adrenals as indicated by Long

(1943). Irrespective of whether the effects manifested after hypophysectomy are a direct result of a disturbance of hormonal balance, or merely an indirect effect of the operation, we would expect the depression more marked on metabolic functions the longer the time elapse between hypophysectomy and experiment. Consequently, in the present experiment a decrease in  $QO_2$  value is seen 11 days, and still more marked at 18 days after hypophysectomy.

Bearing in mind the qualifications just stated, it seems reasonable to assume that the changes on the cellular level as manifested by the marked decrease of  $QO_2$ , are rather a factor in, than a mere consequence of the general effect of hypophysectomy as manifested by weakness, weight, loss, etc.

#### b) Pituitary Dwarf Mice

The results obtained with hypophysectomized animals can be compared to those obtained with pituitary dwarf mice. In the latter case, we do not have an experimental injury the effects of which are detrimental in the long run, but rather a genetical condition leading to a cessation of growth (and to sterility) in the dwarfs, and leaving the individuals normal in other respects.

The comparison is to be made between pituitary dwarf (homozygous in the dw gene), phenotypically normal littermates and normal mice of normal strain.

The interpretation of the results, as given in Chapter III under "Tissue Respiration in Pituitary Dwarf Mice", will depend on whether we

compare "adult" animals, or rather animals of the same body weight, irrespective of whether this is reached as the final developmental condition (in the dwarfs) or as a transitory condition within the growth process (in the normal animal). Applying the first standard,  $Q_{O_2}$  of skeletal musculature from adult dwarf mice (6 to 9 gm.) corresponds favourably to that obtained from phenotypically normal adult littermates (16 to 20 gm.). Previous experimental results from normal adult mice (25 to 30 gm.) and under similar conditions have given slightly lower mean  $Q_{O_2}$  ( $3.48 \pm 0.17$ ). This, however, is understandable in view of the difference of weight, and genetic constitution.

It seems more correct, however, to make the comparison between animals of same weight irrespective of whether they are adult (dwarfs) or still growing (normal). In such comparison, we find the  $Q_{O_2}$  of musculature significantly smaller in dwarfs than in normal animals, as indicated in Table IX. Correspondingly, basal metabolism of the dwarf animals is much smaller than of normal animals of same strain and same body weight as shown in Table 8. From this comparison it may be concluded that the hereditary disfunction of the pituitary as shown in the dwarfs, which leads to a decrease in total as well as tissue metabolism, may be at the base of their inability to grow to normal final weight. In this way, the results as obtained with pituitary dwarf mice correspond to those with hypophysectomized rats, although it must be emphasized that the depression of  $Q_{O_2}$  is much more marked in the latter case. This, however, is understandable because hypophysectomy, as mentioned previously

seriously influences the general condition of the animal while hereditary dysfunction of the hypophysis in dwarf mice leaves other functions more or less normal.

These differences should obviously be interpreted in terms of the different pituitary hormones. Dwarfism probably concerns only the growth hormone produced by the eosinophilic cells which are degenerated in the dwarf animals. Hypophysectomy on the other hand removes the entire gland and thus obliterates the production of pituitary hormones in general. Such interpretation would, however, presupposes a much more detailed study of the influence of the various pituitary hormones than is given in the previous experiments.

Although the interpretation just given seems to be consistent as far as it goes, it is to be emphasized again that a number of the experiments concerning the influence of pituitary hormones on metabolism seems conflicting. As mentioned previously, Kleiber and Cole (1939) found a depression of total metabolism as well as tissue metabolism of diaphragm, after chronic injection of growth hormone leading to gigantism. On the other hand, the present experiments show that deficiency of growth hormone as effected by hypophysectomy or genetical factors, also leads to such a depression. To this would correspond that, according to Campbell Davidson (1950), administration of APP in vivo leads to increase of total as well as tissue metabolism. It may well be that these differences result from different preparations and techniques used, so that the contrasting results are to be attributed to different pituitary hormones. This

obviously requires further experimentation and is indicative of the complexity of pituitary action.

SUMMARY

- 1) Some influences on the respiratory metabolism ( $Q_{O_2}$ ) of isolated tissues in vitro have been investigated.
- 2) The  $Q_{O_2}$  of skeletal musculature of rats, ranging from 10 gm. to 384 gm. body weight, shows but a slight decrease with increasing body size, the correlation between body weight and  $Q_{O_2}$  being rather low.
- 3) Previous results showing a definite dependence of the  $Q_{O_2}$  of diaphragm in rats are confirmed. Applying different media, the  $Q_{O_2}$  values are increased in the sequence: saline alone, addition of glucose, and glucose and metabolites of the Krebs cycle. There is no consistent change of the slope of the regression line with increasingly favourable nutritional conditions of the medium. In mice, the  $Q_{O_2}$  of skeletal muscle of two different strains and taken in two groups ca 8 & 18 gm. and 7 & 28 gm. body weight show a more marked dependence on body weight (steeper slope of the regression line) than is found in rats.
- 4) In interspecific comparison, the  $Q_{O_2}$  of skeletal musculature of adult mice is similar to that of adult rats, while the  $Q_{O_2}$  of small mice (10 gm.) is similar to that of rats of corresponding weight. Thus, comparing interspecifically mature specimens of both species, no marked dependence on body size of musculature  $Q_{O_2}$  is found.
- 5) Interspecific comparison of the  $Q_{O_2}$  of diaphragm in mice and rats show that the  $Q_{O_2}$  of adult mice corresponds to that of rats

weighing ca 30 gm., and is thus about twice the value for adult rats.

6) Interspecific correlation of  $Q_{O_2}$  and body weight thus is similar to intraspecific correlation in the case of the diaphragm, but is different in the case of skeletal musculature.

7) The hypothesis that the decrease of weight specific basal metabolic rate is due to variation in  $Q_{O_2}$  of musculature is rejected.

8) No influence of thyroxine applied in vitro on tissue of rat diaphragm was found.

9) Also with tissues of hypophysectomized rats, administration of thyroxine in vitro fails to show an effect on tissue respiration of skeletal muscle.

10) Thyroglobulin administration in vitro leads to a significant increase of  $Q_{O_2}$  of cardiac muscle of the rat.

11) Hypophysectomized animals have a much lower basal metabolism than normal animals. Correspondingly, tissue respiration ( $Q_{O_2}$ ) of cardiac and skeletal musculature is about one half that of normal individuals.

12) Basal metabolism of pituitary dwarf mice is only about one half of that of normal individuals of the same size.

13) Tissue respiration of skeletal muscle of pituitary dwarfs is significantly smaller than that of their phenotypically normal littermates of the same body weight, although it is similar to that of mature animals.

- 14) No effect of insulin administration in vitro on  $Q_{O_2}$  was found.
- 15) Normal and regenerating rat liver show the same  $Q_{O_2}$  value.
- 16) Hydergine, an ergot preparation, seems to lead to a depression of  $Q_{O_2}$  which is possibly connected with an interference in the Krebs cycle of oxidation.
- 17) The results are discussed in their various theoretical aspects.

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