An Experimental Approach to the Endocrine Glands I

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The history of experimental work with the endocrine glands is long and colorful, albeit there is not yet a single explanation of the specific mechanisms by which the hormones operate at the cellular level. Much of the substantive content of this paper will not be new to the reader. The content is however encased in a methodological framework that may prove fruitful in endocrine gland experimentation. It is abundantly clear in the history of science that discovery emanates from the most unexpected places, but that more often than not the genius of the investigator is revealed by the particular experimental design at hand.

References to the relationships of glands to body functions can be traced at least as far back as Aristotle. In 1775 Bordeu described the function of each organ of the body as "the workshop of a specific substance which passes into the blood." He ascribed the physiological integration of the body as a whole to such substances.

Berthold in 1849 made the first characteristic attack on the function of certain endocrine structures in the body by the removal of the testes in fowl and subsequent replacement of the function by implantation of testicular material. Schiff in 1858 removed the thyroid gland from certain animals which soon died due to this loss. The association of thyroid gland with "the life principle" was inevitable from such necessarily crude extirpations. It was only later that it was discovered that death ensued not because of the loss of the thyroid but loss of the parathyroids which were often imbedded within the thyroid or closely attached anatomically in carnivorous forms. In the herbivorous animals, the close association of the two types of glands was absent, and observations of events attributed to thyroid removal were more accurately correlated with the "true" function. Knowledge in endocrinology can be extremely inaccurate because of the wide variety of anatomical or time relationships.

A system of simple postulates was soon developed which increased in number as science advanced. These postulates somewhat resemble Koch's postulates for disease organisms but were not laid down in formal style. They emerged spontaneously and erratically and are as follows:

1. The removal of the gland must produce specific (?) results not attributable to the operation per se. These results must be due to the loss of the structure.

2. Implantation of the whole gland within the body of the individual from which it was removed ought to restore the original "function."

3. A chemically isolated and purified component from the homogenized gland ought to "substitute" qualitatively and quantitatively for the gland itself.

4. A chemically known active portion of the pure isolated compound ought to "substitute" qualitatively and quantitatively for the gland itself.

5. A chemically synthesized molecule ought to "substitute" qualitatively and quantitatively for the natural active component or for the gland itself.

The author is not unaware of the intrinsic difficulties in following any of the postulates especially No. 5 when complex protein hormones are concerned. Nonetheless the five postulates served as a model which was useful in developing an experimental design or in setting limits in endocrine experimentation. This model aided the scientists in tracking down the specific molecule which was associated with a specific function. Before one can deal adequately with a hormone-function relationship, it is necessary as a first step to have a one to one relationship so that, for example, the injected hormone does not have two components only one of which is effective. Even if this is a crystallized component it may

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carry an impurity which may be the functional component.

An insight into the experimental operations carried out by scientists working with the endocrine glands and the deductions obtained therefrom can emerge from a brief excursion into the area of thyroid-hormone endocrinology. It is now 102 years since the first experimental contribution to thyroid physiology was made by Schiff, although Parry as early as 1786 had come to recognize the chief features of exophthalmic goiter. He knew nothing of its connection with the pituitary. The relationship between the thyroid gland and metabolism was noted on two occasions, the first in 1893 when Frederick Müller (1) discovered an increased outpouring of nitrogenous wastes in the urine of patients suffering from hypothyroidism, and two years later in 1895 when Magnus-Levy (2) noticed a lowering of heat production in clinical cases of thyroid deficiency. These latter results corresponded with the results of extirpation of the thyroid in animals. The ambiguity of the word metabolism is often overlooked because one mentions it, then slips over it quickly and quietly. It must be clear that the action of thyroid on metabolism is only one of a number of functions of the thyroid and that other glands also affect the metabolism of the body.

Observations and experiments were made early in thyroid history on the relationship between the size of the thyroid and the state of metabolism. It was soon found that children with subnormal growth and mental development were associated with the glandular atrophy. It was also discovered that glandular atrophy in the adult occurred (myxedema), but here metabolism as a function of time reveals considerably different symptoms inasmuch as full stature and sexual maturity had been obtained and the effect is on the already developed body.

The above relationship of atrophy or underdevelopment of the gland to metabolic malfunctioning was not simple but it was rather straightforward. Similar results could be obtained from experimental extirpation of the gland in animals. The opposite end of the size spectrum, that is, when the gland was abnormally large, gave two entirely different sets of symptoms. In simple goiter the thyroid gland enlarges in a compensatory fashion for the lack of certain necessary components (most likely iodine). The whole organ is hypofunctioning in spite of its larger size, and the patient may have a B.M.R. of some minus 40. On the other hand other patients with enlarged glands have opposite symptoms; they reveal a hyperfunctioning of the thyroid (exophthalmic goiter) via the pituitary and may have a B.M.R. of plus 80.

The thyroid does not have complete control of the rate of combustion in the body inasmuch as complete thyroidectomy lowers the rate by only 45 percent. If following thyroidectomy, the adrenals are removed and the animals put on adrenal extract to counteract shock affects and then injections of extracts stopped, the B.M.R. may drop by 75 percent.

The various ramifications of thyroid research as partly indicated above can be tentatively resolved into about nine patterns of results. Each of the patterns becomes a parameter of any thyroid hormone research; in short, they either serve as controls or post facto becomes areas of confusion of data. In biological research, as most researchers know, the system under investigation is never a simple one like those often established in mathematics and physics. In endocrinological research specifically, the various glandular interrelationships are so numerous as to discourage the mature scientist and completely frustrate the exact scientist. What one must do is to narrow down the system within which one operates so that more reliable results emerge. This often narrows down the scope and importance of the research, at least temporarily. It is a challenge, however, and with sufficient numbers of pertinent observations and experiments, fruitful generalizations can be made. The main parameters and some empirical evidence of their veracity follows.

Metabolism as a function of:

1) Size of the thyroid gland
2) Dosage
3) Time
4) Sex
5) External temperature
6) Diet
7) Species
8) Glandular relationships
9) Individuality

The relationship of size of gland to metabolism has been discussed. Two glands of the same size may have widely divergent secretion rates.

Dose rates, even when calculated and regulated according to body weight, produce metabolic rates which are not a straight line function. They often resemble a sigmoid curve. In treatment of myxedema with daily thyroglobulin or thyroxine there is an overshoot of metabolic rate beyond normal, and it then drops back.

Metabolism as a function of time has at least two components; one is related to age and the other related to a period and the events within that period. In the first case, metabolism is generally higher in young forms and lower in adults. In the second case, e.g., metabolism is higher during estrus than at other times. Also in the case of hormone injections, after a period of time, the organism may become unresponsive to the dose, or conversely, a characteristic lag may occur before the hormone becomes effective.

Sex differences make themselves manifest in metabolism. In guinea pigs, for example, the response to thyroxin is more marked in females than in males. In the U. S. there is a preponderance of simple goiter among human females. In the temperature range of 23-35° C. women have lower B.M.R.'s than do men.

It is well known that men's metabolism is higher in cold climates than in warm, and higher in winter than in summer. At 1° C rats produce hormone at the rate of about 10 gammas per day whereas at 35° C the rate is only 1.7 gammas per day. The thyroid of flies (see Figure 2) and dogs (Figure 1) follows the same pattern. Estivation and hibernation provide further examples.

T (°C)	Calories/Kg
7.6	86
15.0	63
20.0	56
25.0	54
30.0	56

FIGURE 1. Heat production in a short-haired dog at different external temperatures. (Rubner)

Lack of iodine and the concomitant lowered thyroid secretion rate is a well known example of dietary components affecting metabolism. In addition chestnuts, walnuts, cabbage, and rutabagas are known to have an inhibitory effect on metabolism. Also, sulpha drugs like sulphanilimide, sulphaguanadine, and others

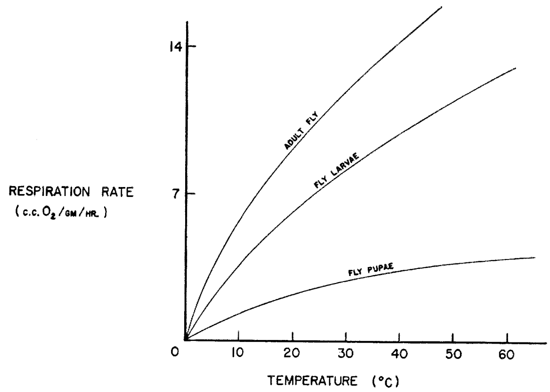

FIGURE 2. Effect of temperature on rate of respiration in a fly.

have suppressive actions (3). Thiouracil is highly antagonistic. High calcium in the diet depresses the thyroid activity while high protein, with its high specific dynamic effect, increases the metabolic rate (at least higher temperatures result).

Metabolism is also a function of the species. Mice (*Mus musculus*) for example, are relatively hypothyroidal while rats (*Rattus norvegicus*) are hyperthyroidal. Birds are hyperthyroidal generally. Chickens are less so while sparrows are more so. Chicks treated with thiouracil show an enormous thyroid enlargement.

Other glands in the body affect metabolism. The pituitary has well known growth effects. It also directly "stimulates" the thyroid by way of thyrotropic hormone. Adrenalectomy results in a 20 percent drop in B.M.R. as indicated elsewhere in this paper. Fifty milligrams of epinephrine administered to a rabbit can produce a 50 percent increase in B.M.R. in three hours. Male hormone is said to have a protein anabolic effect although the author (4) has never found any substantiating evidence for this.

The ninth parameter is that of the relationships of individuality to metabolism. Each animal has an individual metabolic rate, so that if, for example, there are five mice in a particular group, there may be five different metabolic rates involved and the range may be quite large. This aspect of the problem can be brought into sharp experimental focus by the use of closely inbred strains and the use of statistically significant numbers of animals.

The above parameters of thyroid research lend themselves to a more systematic and more concentrated attack on problems of thyroid function. When carefully worked into the experimental design, they produce results of higher reliability and lend greater probability for discovering specific roles for the thyroid or any of the various hormones.

In Part II of this paper the author will use a particular piece of his own thyroid-hormone research, as yet unpublished, to demonstrate those aspects of the parameters above which were met in addition to those not met. The experiment was one attempting to determine "the effect of hypermetabolism on the rate of incorporation of radioactive glycine into heart proteins and other viscera proteins in mice."

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