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Hormone Regulation

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IN higher organisms, whether animal or vegetable, there exist channels through which fluids are transmitted from one tissue to another. Into these fluids or humors are delivered many compounds elaborated in one or another tissue. It is to be expected that, in many cases, these compounds will exert recognizable physiological effects in other tissues to which they may be hydraulically transmitted. Among the host of compounds which meet these criteria, a limited number have been set apart under the special category of hormone or "chemical messenger." It is the purpose here to review briefly what these hormones are, whence they arise, whither they go, and how they accomplish their missions. Although hormonal mechanisms are well described in invertebrates and indeed in plants, these remarks are restricted to mammalian endocrinology.

To speak of the mission of a compound is to lapse into teleological jargon. Teleology, however, has been defined as a woman with whom no scientist cares to be seen in public, but with whom all scientists consort in private. It will simplify my task if you will forgive my teleological indiscretions in this paper which permits me to dodge the otherwise necessary cumbersome circumlocutions.

What then is a hormone? It is a compound devoid of intelligence but containing nonetheless in its structure certain information. While there are no Koch's postulates by which one may judge whether a compound is or is not a hormone, there are certain accepted standards to which most respectable hormones conform.

1. In many but not all cases, hormones arise in tissues which appear to be specialized for their production. In the mammal, these organs include the neurohypophysis, the adenohypophysis, the thyroid, the parathyroids, the islets of Langerhans of the pancreas, the adrenal medullae, the adrenal cortices, the testes, the ovarian follicles, and the ovarian corpora lutea. Other organs, such as the pineal body, the hypothalamus, the thymus, the carotid bodies, etc., are not included in the present list of so-called endocrine organs in view of their relatively uncertain status and lack of specific and convincing information.

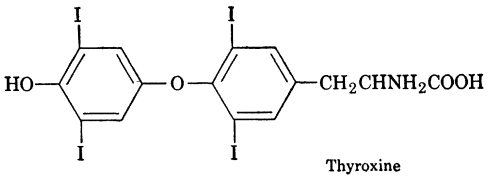
It should not be supposed, however, that all known hormones arise in tissues anatomically specialized for their production. The word "hormone" was coined actually to describe an as yet unidentified principle termed secretin, discovered by Bayliss and Starling, which appears to arise in the intestinal mucosa, to enter the blood stream, and to stimulate, upon its arrival there, the secretion of juices by the pancreas. A second but distinct material, arising from the same source, cholecystokinin, stimulates the contracture of the gall bladder, and the injection of bile into the duodenal lumen. Similarly, there is partial evidence for the existence of renal hormones affecting vascular tone, hepatic hormones affecting uptake of blood glucose by muscle, and indeed a hormone liberated by working skeletal muscle which affects the metabolism of resting muscle in the same animal.

Whereas a number of the hormones which arise in the specialized endocrine glands, e.g., the pituitary, the adrenal, etc., have been purified and characterized structurally, this has not been accomplished for hormones arising from such tissues as liver or intestinal mucosa. Consequently, the structural information presented is confined to products of the so-called glands of internal secretion.

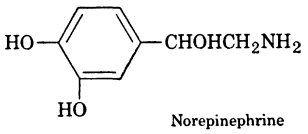
2. A second characteristic of hormones is that they normally circulate and exert their regulatory roles at low molar concentrations. On the basis of published estimates of the normal concentration of insulin in blood, it would appear that this agent is effective at concentrations of about 10^{-11} to 10^{-12} molar, and epinephrine, glucagon, and thyroxin all appear to exert recognizable effects at similar levels of concentration.

3. Some, but not all, hormones exert their effects upon highly specific target organs. Thus, adrenocorticotropic hormone of the pituitary operates essentially uniquely upon the adrenal cortex, while the activity of thyrotropic hormone is restricted largely to the thyroid. The sole convincingly demonstrated action of glucagon is upon the liver. On the other hand, epinephrine exerts actions upon liver and muscle, insulin has been shown to operate in many tissues including skeletal muscle, adipose tissue, and mammary gland, whereas the effects of adrenocortical steroids and thyroxin may be demonstrated in a wide variety of tissues.

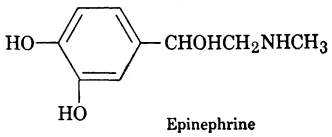
4. If a hormone is to exert a regulatory effect, clearly the rate of its release into the blood stream must be variable and should be determined by some function of the mechanism which it regulates. Such variation in rate of discharge has been demonstrated for certain but not for all hormones. For example, one may cite insulin, which regulates the level of glucose in the blood and the release of which by the islet cells of the pancreas is determined directly by the concentration of glucose in the pancreatic blood supply. Analogously, ACTH, released by the pituitary, specifically stimulates the adrenal cortex to discharge steroid hormones, which, if injected, cause a prompt suppression of ACTH produc-



Thyroxine



Norepinephrine

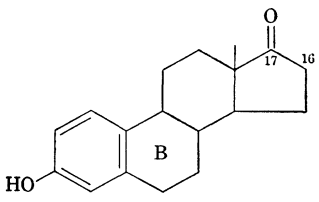


Epinephrine

FIG. 1. Structures of certain phenolic hormones.

tion. Many other such examples of negative feedback, more or less well-documented, might be cited.

It may be profitable to consider briefly some of the structural types which are represented among the hormones. These include a variety of substances, among the simplest of which are certain phenols (Fig. 1). Under the regulation of thyrotropic hormone derived from the anterior pituitary gland, a protein, thyroglobulin, is elaborated in the thyroid gland, which contains the unique amino acid thyroxin, 3,5,3′,5′-tetraiodothyronine. This amino acid, accompanied by triiodothyronine, is discharged into the bloodstream where it circulates, largely protein-bound, in the plasma. Epinephrine and norepinephrine derived from adrenal medulla are related closely both metabolically and structurally. There is a considerable amount of information



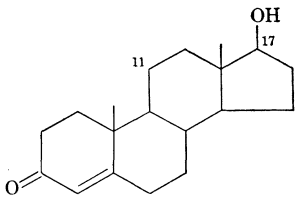
Estrone

Substituent at C Numbers		
Compound	17	16
α or β Estradiol	—OH	—H
Estriol	—OH	—OH

FIG. 2. Structures of certain C_{18} steroid hormones (N. B. Equilin and equilenin represent successive states of oxidation of B ring of estrone).

at hand defining the biosynthetic origins of these compounds. Tyrosine contributes the phenolic portions, in each case. With these, as well as with certain other hormones, secondarysites of origin exist. The synthesis of thyroxin-like materials in the totally thyroidectomized animal[1] is perhaps not surprising in view of the fact that tyrosine-containing proteins, after incubation with iodine in alkali, in the absence of enzymes, exhibit thyroxin activity.

Steroidal hormones comprise a large and much-studied group. They often are classified structurally according to the number of carbon atoms contained. Thus, the C_{18} steroids include the estrogens (Fig. 2), estrone, α- and β-estradiol, estriol, as well as the less familiar equilin and equilenin. All of these compounds have phenolic—i.e., acidic—properties. The methyl group born on carbon atom 10 of most other steroids is lacking in these compounds, as a consequence of the unsaturation. Arising predominantly in the Graafian



Testosterone

Substituent at C Numbers			
Compound	3	11	17
Adrenosterone	=O	=O	=O
Androstenedione	=O	—H	=O
Androstanediol*	—OH	—OH	=O

*Double bond at Δ^4 is reduced

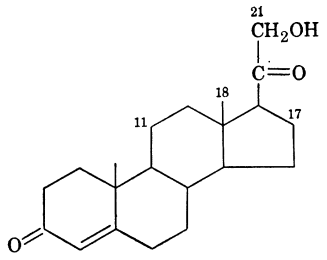
FIG. 3. Structures of certain C_{19} steroid hormones.

follicle of the ovary, under regulation by pituitary gonadotropin, estrogens are generated also by testis and possibly also by adrenal cortex. They are detoxified normally in the liver by conjugation with glucuronic or sulfuric acids, and the loss of this function in hepatic failure results in feminizing symptoms and signs.

The C_{19} steroids include (Fig. 3) the androgens, testosterone and its congeners. There are a number of steroids with androgenic activity and these may arise in the adrenal cortex as well as in the testis. The C_{21} steroids (Fig. 4) include not only the several adrenocortical steroids but also progesterone, the hormone of the ovarian corpus luteum. The generation of these hormones is under direct control of specific anterior pituitary hormones. Testicular production of androgens is regulated by pituitary gonadotropin, adrenocortical activity is determined by pituitary adrenocorticotropin, and progesterone production depends upon a luteinizing hormone of the pituitary gland.

The structural similarities of the several steroid hormones are explained by the fact that they are all biogenetically closely related. Cholesterol appears to be a parent compound to all of them, and many of the individual enzyme-catalyzed steps whereby they arise have been elucidated. It is, of course, of great interest that specific and important biological activities of a wide variety are associated with steroids. In addition to the steroid hormones, other biologically important steroids are vitamin D, the cardiac aglycones of the digitalis group of drugs, the toad poisons, and certain carcinogens. What it is in the steroid structure which imparts to the members of this class these various activities is not known.

The remaining groups of hormones about which there is structural information are polypeptides. Of these, insulin (Fig. 5) is probably the best-studied. Beef insulin, whose chemical structure was given by Sanger,[2] is made up of two polypeptides, an *A*- or acidic chain comprising 21 amino acids and a *B*- or basic chain containing 29 amino acids. The intramolecular disulfide bridges have been described previously by Oncley (p. 30), and their integrity is essential to the physiological activity. The C-terminal alanine in the *B*-chain is apparently not essential, however, and indeed the eight C-terminal amino acids of this chain may be eliminated without complete inactivation. The question of what constitutes the essential center of physiological activity of insulin, if indeed there is such, remains unanswered. When this structure first was published, attention was attracted to the pentapeptide closed ring in the *A*-chain, particularly since rings of similar size were found by du Vigneaud[3,4] to be present in both of the hormones of the posterior pituitary gland, vasopressin and oxytocin. The curious run of three adjacent aromatic amino acids in the *B*-chain also was pointed out.



Deoxycorticosterone

Compound	Substituent at C Numbers 11	17	18
Corticosterone	—OH	—H	—CH_3
11-Dehydrocorticosterone	=O	—H	—CH_3
17-Hydroxycorticosterone	—OH	—OH	—CH_3
Cortisone	=O	—OH	—CH_3
Aldosterone	—OH	—H	—CHO

FIG. 4. Structures of certain C_{21} steroid hormones (N. B. Progesterone differs from deoxycorticosterone only in that $-CH_2OH(21)$ is reduced to $-CH_3$.

No evidence has been presented implicating either of these sites specifically in insulin function.

The posterior pituitary hormones present striking similarities and, as might be expected, some degree of cross-reactivity physiologically. There is some reason to believe that these compounds may be parts of a larger polypeptide arising in the hypothalamus and transmitted down the pituitary stalk, to be stored in and released from the posterior lobe of the pituitary gland.

Attention is directed now to amino acids 8, 9, and 10 in the *A*-chain of insulin. Insulins of several species have been prepared; all are very similar in physical properties and are essentially identical in physiological assay, about 27 units per mg. Chemical differences do exist,

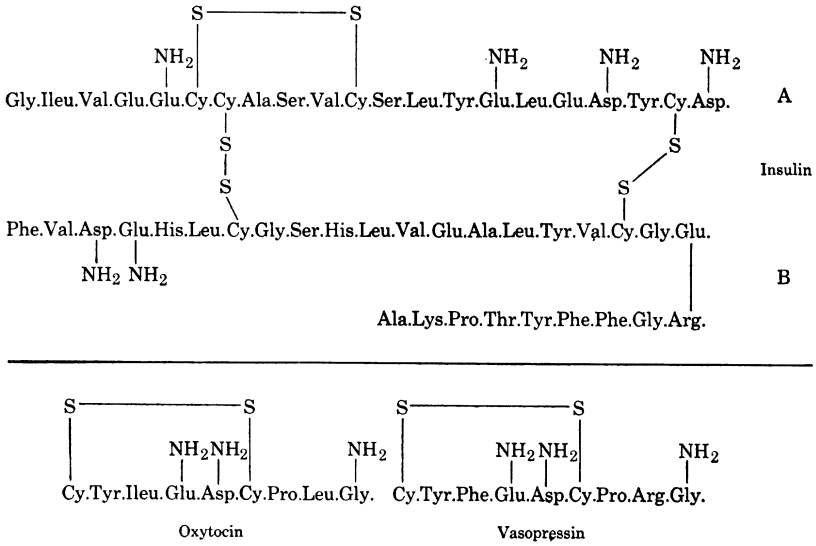


FIG. 5. Structures of bovine insulin and the hormones of the posterior pituitary.

Insulin–A–Chain*

1 — — 7 8 9 10 — — — — 20

...Cys.Ala.Ser.Val...	Beef
...Cys.Thr.Ser.Ileu..	Pig †
...Cys.Ala.Gly.Val...	Sheep
...Cys.Thr.Gly.Ileu..	Horse
...Cys.Thr.Ser.Ileu..	Whale †

*Remainder of A chain and entire B chain (1 through 29) are invariant

FIG. 6. Species variations in insulin structure.

however, but thus far the sole differences that have been detected reside within these 3 amino acids (Fig. 6). Although the sample studied is confessedly too small to permit firm conclusions to be drawn, a few suggestive findings have turned up. In each position, 8, 9, or 10, only one of 2 amino acids has been found. Thus, in position 8, there is either alanine or threonine; in position 9, serine or glycine; in position 10, valine or isoleucine. If this binary replacement proves general, and no other alterations are found, only 8 insulins are possible. There may be, however, an additional restriction. On comparison of positions 8 and 10, it appears that, whenever the former is alanine, the latter is valine; whenever the former is threonine, the latter is isoleucine. Should this represent a firm link in the coding to these 2 positions, only 4 insulins would occur, and indeed all types now are known. It is not surprising, then, that pig and whale insulins prove to be identical (Fig. 6†).

Insulin is generated in the β cells of the islets of Langerhans. Its function, as is discussed later, is to favor the utilization of blood glucose by muscle, to restrict glucose formation by liver. It is noteworthy, therefore, that the release of insulin by β cells is favored by a high concentration of blood glucose.

Intimately associated anatomically with the β cells are the α cells of the islets which are the source of glucagon. This polypeptide (Fig. 7) was for many years an unsuspected contaminant of the best available insulin from American commercial sources although, because of differences in manufacturing procedure, it was lacking in Danish insulin. Chemically and physiologically it is

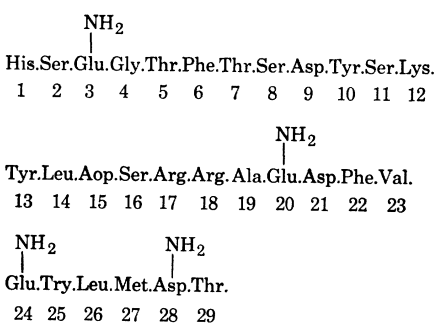


FIG. 7. Structure of glucagon.

unrelated to insulin.[5] The sole sequential similarity between insulin and glucagon that has been pointed out is the series 22–24 in glucagon (Phe. Val. Glu NH_2) which resembles Phe. Val. Asp NH_2 (1–3 chain B) of insulin. Whereas both glucagon and insulin affect the level of blood glucose, they do so by influencing quite different processes, and appear to be antagonists only on very superficial inspection.

The last group of hormones about which there is useful structural information are the polypeptides of the anterior pituitary gland. There are at least six of these, thyrotropin, adrenocorticotropin, somatotropin (growth hormone), follicle stimulating, luteinizing, and melanophore stimulating hormones. Other activities have been suspected in the past and may be revealed in the future. Complete structural information is available, for certain species, in regard to corticotropin and melanotropins. The formulas for certain of these have been given by Oncley (p. 30). Here, attention is directed only to certain salient points of similarity and dissimilarity (Fig. 8). Amino acids 4 through 10 of all corticotropins studied are identical with amino acids 7 through 13 of all known melanotropins. This identity of a series of seven amino acids is sufficiently startling

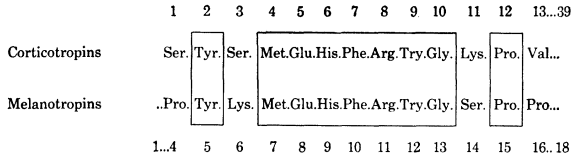


FIG. 8. Structural relationships of ACTH and melanophore stimulating hormone.

to attract attention. Li[6] has pointed out that the identical sequences would have been eleven rather than seven, but for a curious confusion about positions 3 and 11 of corticotropins, or 6 and 14 in melanotropins. An interchange between the serine and lysine in the appropriate positions of either of these peptides would prolong the identical sequences from seven to eleven.

This observation, as well as the previously mentioned species variations in insulin, suggests the occurrence of certain blunders in the transmission of the coded information pertaining to amino-acid sequences. Such blunders, if indeed so they be, may themselves be informative. Thus, the present figure suggests that the code is such as to permit interchange of information about 2 amino acids 8 positions remote in a polypeptide.

To date, accumulating knowledge of hormone structure has, for the most part, revealed little about how these compounds function. Only within the past few years have reasonable hormone effects been observed in biological preparations disorganized below the cellular level. Several of these now may be mentioned.

Thyroxin has been suspected for some time of exerting an effect similar to that of 2,5-dinitrophenol, a suspicion dating back to the early clinical observations of the

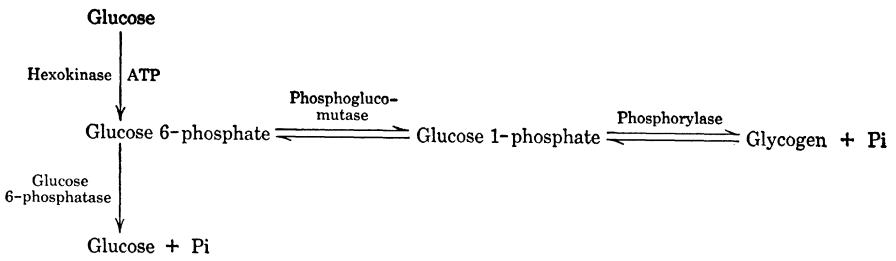


FIG. 9. Certain phases of the metabolism of glucose.

toxicology of the latter drug during World War I. When it was ascertained that dinitrophenol effected an uncoupling of oxidative phosphorylation, permitting respiration to proceed without obligatory and coincident phosphorylation, a similar effect was attributed to, and soon found with, thyroxin. This effect has been demonstrated with isolated mitochondria by Lehninger.[7] Such uncoupling, and consequent escape of glycolysis from the restraining influence of the Pasteur effect, would explain many of the clinical manifestations of thyrotoxicosis.

Estrogens have been shown by Villee[8] to activate the isocitric-acid dehydrogenase of placenta and this effect has been demonstrated in homogenized preparations (cell-free). More recently, Talalay[9] has indicated that the enzyme of placenta directly affected is a transhydrogenase which regenerates the TPNH required by the dehydrogenase in question. It is possible that, in this transhydrogenase reaction, the estrogen serves as a self-regenerating co-substrate.

Two hormones about which there is partial mechanistic information are epinephrine and glucagon. It is perhaps remarkable that these two substances, wholly unrelated structurally and biogenetically, should both exert, at equimolar concentrations, virtually identical effects. These effects, elucidated by Sutherland,[10] relate to the activation of hepatic phosphorylase. This enzyme, required for glycogen breakdown and probably also for its synthesis, is itself enzymatically inactivated with the loss of inorganic phosphate. Its reactivation also may be accomplished enzymatically, but this requires a novel cofactor, an anhydride of AMP. The synthesis of this cofactor, by yet another enzyme, proceeds only in the presence of epinephrine and/or glucagon. The two hormones, though acting identically in liver, are quite different in their effects upon muscle glycogen. Here, whereas epinephrine is highly active, glucagon is without effect.

Insulin, which has received the attention of many excellent investigators since its isolation, is in a more confused state than some of the other hormones which have been considered. The dramatic capacity to lower blood sugar apparently is the result of effects upon two processes. To evaluate these two effects, one should consider briefly some of the sources and fates of blood glucose (Fig. 9). Here are represented in abbreviated form some of the reactions of concern. In the absence of insulin, as in the severely diabetic human or as in the pancreatectomized animal, the conversion of extracellular glucose into intracellular glucose 6-phosphate, an essential first step in glucose utilization, is impaired. This impairment is especially demonstrable in muscle tissue and characteristically is seen in the isolated diaphragm. The basis for the impairment is still somewhat obscure but the bulk of current evidence favors the view, first propounded by Levine,[11] that insulin favors the entry of glucose into the intracellular compartment of muscle. Insulin demonstrably performs this function in relation to a number of other sugars. Possibly significant is the fact that the sugars most responsive to insulin in this regard are configurationally identical with glucose about carbon atoms 1, 2, and 3.

A second pertinent insulin effect is defined best by the fact that the activity of glucose 6-phosphatase is greatly enhanced in the liver of the diabetic animal, an effect reversible by administration of insulin. The importance of this enzyme is revealed by the fact that it catalyzes the reaction responsible for the majority of glucose contributed to the blood by animal tissues.

Consider the dilemma of the diabetic animal in the light of these two defects. With the conversion of glucose to glucose 6-phosphate restricted, and with the hydrolysis of this ester exaggerated, there results a curtailment of glucose phosphate needed for a variety of important processes, including glycolysis, oxidation, generation of reduced coenzymes, preparation of essential intermediates. Many, but certainly not all, of the late consequences of untreated diabetes are referable directly to this combination of biochemical defects. The question of which of the two defects is most significant is in a state of flux at the present time. Data from Hastings[12] and others suggest that the increase in utilization of glucose by muscle occurs much more promptly in response to insulin than does a decrease in glucose 6-phosphatase activity. On the other hand, Weinhouse and his collaborators[13] have data indicating that the reverse is true.

In relation to this figure, one can consider also the purported "antagonism" between insulin and glucagon. Glucagon, as mentioned previously, effects favorably the activity of hepatic phosphorylase, promotes breakdown of liver glycogen, and produces a resulting rise in blood glucose. Insulin, by its actions, produces a fall in blood glucose. If one's vision is restricted to the glucose of the blood, these two hormones act antagonistically. The blood glucose, however, is really relatively un-

$$HT \rightleftharpoons H + T$$

$$K = \frac{[H]\,[T]}{[HT]}$$

$$Q = [T] + [HT]$$

$$Q = K\frac{[HT]}{[H]} + [HT]$$

$$\frac{[H]}{[HT]} = \frac{K}{Q} + \frac{[H]}{Q}$$

or

$$\frac{1}{[HT]} = \frac{1}{[H]} \cdot \frac{K}{Q} + \frac{1}{Q}$$

FIG. 10. Expected arithmetical relationship between concentrations of free hormone, H, of bound hormone, HT, and of acceptor sites on target organ, Q.

important, except insofar as it nourishes the several tissues of the body, and the major consuming tissue is skeletal muscle. With respect to the nutrition of skeletal muscle, glucagon and insulin, far from being antagonists, are synergists. Glucagon supplies to the blood the glucose which muscle requires, and insulin facilitates the entry of this glucose into the cells so that it may be utilized.

It was mentioned at the outset that many endocrines, insulin among these, are physiologically active at remarkably low concentrations. The finding of Stadie[14] that several tissues responding to insulin *in vitro* are endowed with the capacity to bind insulin to their surfaces, and that the response to insulin is, under appropriate circumstances, proportional to the amount of insulin bound, is perhaps relevant. If the target organ has the capacity to bind the hormone to itself, local concentrations of hormone may be built up far in excess of the concentrations occurring in the blood. The dose-response relationship for several hormones is compatible with this idea in that, whereas the response is approximately proportional to dosage at low dosage levels, for many hormones a maximal response is attained asymptotically at high dosage levels. This suggests that something, possibly the acceptor site on the target organ, is being saturated with hormone. This situation may be treated arithmetically[15] by assuming a reversible association between hormone (H) and target-organ acceptor site (T). If K is the dissociation constant and Q is the total concentration of acceptor sites (Fig. 10), the problem may be treated as Lineweaver and Burk treated the Michaelis-Menten problem, to yield the final expression on the figure. If now it be assumed tentatively that the concentration of hormone H is proportional to dosage and that the response is proportional to the concentration of bound hormone HT, the reciprocals of these two quantities should be related linearly. In a limited number of cases where this relationship has been tested, the expected linear relationship indeed has been found. Values for K and Q can be estimated from the slopes and intercepts of such plots. The usefulness of the relationship diminishes insofar as some quantity other than the concentration of bound hormone limits the observed response.

It is perhaps unfortunate to terminate this distinguished series upon such an unsatisfactory note. In earlier papers on macromolecular structure and interaction, biological fine structure, information storage and retrieval, etc., one was left with the idea that there is a narrowing gap between the biological description and the physical model, or even explanation. In the matters presently under consideration, the gap is so wide that I, for one, am unable to see across it. Of such a gap, one may hope, but one can not know, that it is being narrowed. Certainly many experiments will have to be performed before the hormones, their origins, their modes of action, and their interrelationships can be defined in physical terms.

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