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The hormones of invertebrates

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Boston University
THE HORMONES OF INVERTEBRATES

by

Irma H. Kling
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by

First Reader. Brendan R. Lutz... Professor of Biology.

Second Reader. Stuart X. Harris... Professor of Biology.
Outline of Thesis:
THE HORMONES OF INVERTEBRATES

A. INTRODUCTION

I The physiological functions of invertebrates are controlled by hormones. page 1
II Early evidence for the existence of hormones in invertebrates. 2
III A definition of the term hormone, and a classification of the hormones found in invertebrates. 6

B. HORMONES

I Cell hormones 7
   1. Hormones of protozoa 8
      2. Gene hormones 8
   II Aglandular hormones 11
      1. Nerve hormones 12
         2. Heart hormones 14
   III Glandular hormones 16
      1. Color-change hormones 16
         2. Gonadal hormones 20
            3. Hormones of metamorphosis 23
   IV Glands homologous to the vertebrate glands 26
   V The effect of vertebrate hormones on invertebrates. 28

C. SUMMARY

Bibliography 34
THE HORMONES OF INVERTEBRATES

A. Introduction

I The physiological functions of invertebrates are controlled by hormones.

The idea of hormones regulating the physiological activities of invertebrates is comparatively new. Only recently have morphological studies been made of these lower forms which have revealed the presence of structures with a humoral function. Therefore, the lack of morphological facts concerning invertebrates and their physiological relation to the higher forms, vertebrates, makes the problem a difficult one. Not enough data have been accumulated to state with finality that all the physiological functions of invertebrates are dependent on, or determined by "hormones" as found in vertebrates. However, enough data have been amassed to lead one to conclude that some of the physiological functions of invertebrates are controlled, partially at least, by endocrine principles.

II Early evidence for the existence of hormones in invertebrates.

During the last fifty years great progress has been made in the field of comparative physiology which has helped explain many of the problems which before seemed unanswerable. Henle in 1865 discovered the staining reactions of the cells of the adrenal medulla with chromic acid (Cameron, 1936). "Adrenalin", the chemical substance secreted by the adrenal
medullary cells, was discovered in 1834 (Huxley, 1935). Poll and Summer, in 1906 (Taskell, 1919), were the first to discover chromaffine cells in the earthworm.

Plant hormones have been known for some time. The growth-regulator of plants is formed in the plant tissues at the tip of the coleoptile and can stimulate growth several millimeters below (Went, 1935). The same, or a similar substance is formed in root-tips, but exerts a retarding influence on the elongation of the root. It is transported by diffusion, at the rate of ten to fifteen millimeters per hour (Huxley, 1935). Other plant hormones are also known, such as the wound hormone of Haberlandt (Huxley, 1935).

The existence of secretions in plants and vertebrates which control the physiological functions of them has lead some to believe that all organisms, even invertebrates, are physiologically regulated by internal secretions. The attention that has been given to the vertebrate endocrine system has revealed a variety of humoral effects, but it is by no means certain that this important coordinating mechanism is confined to vertebrates alone. It is possible that every tissue possesses the ability to affect other tissues by means of a substance liberated into the circulating media not only in vertebrates but in invertebrates as well (Parker, 1934). To date only a few endocrine processes have been found among invertebrates; the crustacean eye hormone being perhaps the most spectacular. The occurrence of such a substance in
crustaceans which is effective in fishes and amphibians may indicate an evolutionary precursor of the more highly developed vertebrate endocrine system, and hence serve as a basis for the conclusion that all invertebrates have hormones.

The work in this field has extended enormously our scope of the problem. With the discovery of hormone-like substances in invertebrates it is now possible to explain, in part at least, such things as growth in general, dimorphism among animals, color changes in crustaceans and insects, the metamorphosis and imagery in insects, mosaicism and gynandro-morphism, and possibly even the development of differentiated tissues from a single fertilized germ cell.

The first homology of the thyroid gland of vertebrates appears in the endostyle of the tunicates (Rogers, 1927). By various standard tests, Butcher (1930) proved that the neural gland in the tunicates is homologous to the pituitary gland of vertebrates. Rogers (1927) points out that among the invertebrates, the substance of the adrenal gland of vertebrates appears to be represented by cells of the nervous system. Evidence, therefore, is not lacking that hormones are present in invertebrates as well as in vertebrates and plants.

III A definition of the term hormone, and a classification of the hormones in invertebrates.

An extension of the term "hormone", as used in its narrowest sense, is needed to explain the statement that all
organisms, vertebrates, plants and invertebrates are physiologically regulated by internal secretions of one form or another. The terminology is faulty perhaps, but for lack of a better one it is used by investigators to apply to the physiological regulators of these organisms.

The name is misleading. Bayliss and Starling in 1906 (Cameron, 1936) gave the term "hormone" (from the Greek hormôn, I excite) to the substances which have the property of exciting organs to activity when introduced into the blood but included in this group such a normal catalobite as carbon dioxide because the concentration of carbonic acid in tissues excites the action of the respiratory centre. The term has been so widely extended that it has lost its meaning entirely in many cases; for example, Bethe (Koller, 1938) exaggerated the idea by including any and all substances secreted, such as "ardour" which is a secretion specific for various species before mating. These secretions cast off to the outside world Bethe called "ectohormones" (Koller, 1938).

Other terms have been invented. Sharpey-Schafer (1927) suggested "autocoid" (Gk. autos, self; akos, a remedy). An "autocoid" which excites a tissue would be a hormone; and one which restrains or inhibits activity was termed in contradistinction a "chalone" (Gk. chaloa, I restrain). Cameron (1936) rejects the term as being even less specific than "hormone", since it suggests an unusual agent "whereas the 'endocrine 'secretions' are normal products, and in no sense...
remedial agents. Cameron (1936) does not approve of the term hormone because it is misleading, instead he prefers to call the chemical compounds or substances produced in the glands of internal secretions "endocrine secretions". The two terms, "hormone" and "endocrine secretions", are used synonymously by most investigators (Huxley, 1935).

Hormone, the term generally accepted, and used herein, is considered to be the chemical compound or substance produced in tissues or glands of internal secretions. Recent investigations, however, show that there are other substances which are comparable, though not products of glands, for example, heart hormones and nerve hormones. Hormones are physiologically active substances secreted not by glands alone, but other organs and tissues may normally produce or initiate, when in an abnormal state, the production of substances whose purpose it is to maintain or re-establish the normal state of the organism (Kropp, 1933). The production of internal secretions is not dependent upon any abnormality, however, since normal organisms are known to secrete such substances.

In speaking of hormones in invertebrates one can not view them in exactly the same way in which one views vertebrate endocrine secretions for many of the lower forms do not contain "organs", but are organisms. Therefore a definition and a classification of the term hormone, as found in invertebrates, is needed to clarify the title and contents of this
Koller's definition and classification best satisfies the need, without devaluing or excluding the findings of the other investigators. The definition, as will be seen, can likewise be applied to vertebrate endocrine secretions. Huxley (1935) defines vertebrate hormones as highly specialized agents of chemical correlation. "They are specialized in two ways: first, in being produced by special tissues for the special function of influencing other tissues; and secondly, in being carried by the blood stream". Koller (1938) defines invertebrate hormones as follows. "Hormones are organic substances which an organism produces for its own need, and which operate regulatory for the organism in a specific way". To this he ascribes the following characteristics: 1) hormones are produced in small amounts; 2) are transferred by humor; 3) are specific for neither species nor genus; 4) have a specific and regulatory action; 5) have a resistance to boiling; and 6) are effective at high dilution. Koller uses the place of production and the place of action specially for classification:

<table>
<thead>
<tr>
<th>TYPE</th>
<th>PLACE OF PRODUCTION</th>
<th>PLACE OF ACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Cell</td>
<td>hormones</td>
<td>In one cell, and transported by diffusion. (Represented by gene hormones and hormones found in Protozoa).</td>
</tr>
</tbody>
</table>
II. Place of Production

A. Hormones

<table>
<thead>
<tr>
<th>Type</th>
<th>Place of Production</th>
<th>Place of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>II. Aglandular</td>
<td>Cells which are not</td>
<td>In some other cell or tissue, and</td>
</tr>
<tr>
<td>tissue hormones</td>
<td>glandular in nature.</td>
<td>transported there by diffusion. (Represented by heart and nerve hormones).</td>
</tr>
<tr>
<td>III. Glandular</td>
<td>Internal secretory</td>
<td>The place of action is far from the place of production. (Comparable to the vertebrate hormones).</td>
</tr>
<tr>
<td>tissue hormones</td>
<td>glands.</td>
<td></td>
</tr>
</tbody>
</table>

B. Hormones

I. Cell hormones

As has already been pointed out, the problem of proving that unicellular organisms are regulated physiologically by hormones is difficult, since the seat of production and the place of action are in one and the same cell. Here speculation and theory tend to bridge the gaps of absent morphological and physiological facts.

Huxley (1935) points out the existence of a cell hormone in Acetabularia mediterranea, a large unicellular and uninuclear alga which shows marked regional differentiation of form and function. Its stalk may be over ten millimeters long. The reproductive portion or "hat" resembles the pileus of a mushroom, even to size. The single nucleus is confined to one of the rhizoids. Experiments show that the substances responsible for the morphogenesis of the "hat" and of the rhizoid are produced by the nucleus and then migrate. The former is
transported by diffusion to the reproductive portion, and the latter to the rhizoid.

1. The hormones in protozoa.

Koller (1938) considers the work done by Bayer and Wense in 1936, the best proof that hormones appear in protozoa. These two men centrifuged carefully cleaned cultures of paramecia and succeeded in getting a sediment of paramecia that was free of bacteria, so there was no possible concommitant material present. By standard methods an extract was obtained which, when injected in a denervated leech, caused contraction of muscle. This extract also had a stimulative effect on the heart of the frog, in which case it acted in an inhibitory manner, causing a decrease in the palpitations of the heart. The heart of the frog reacted to the extract from paramecia in the same manner in which it reacts to choline. The conclusions of these and other experiments lead some to believe that acetylcholine and choline are present in paramecia, and are probably universal in distribution.

2. Gene hormones.

In the field of experimental embryology is added evidence for the existence of cell hormones. Koller (1938) records the works of Kuhn and his co-worker Caspari, who have made significant discoveries in the field of comparative embryology with their research on gene hormones. They succeeded in getting gene action exactly and proving its hormonal action. Usually
it is impossible to get these efficient gene hormones in a substantial form because the place of production and the place of action are too near together. They worked with the meal-moth, Ephestia, using the method of transplantation. A mutant type given the genetic formula of (aa), possesses red eyes and nearly or quite unpigmented testes, as against the black-eyed and brownish colored testes of the wild type (AA). They transplanted a testis of the mutant aa into the host AA and found that the unpigmented testis of aa became pigmented. When an AA testis was transplanted into an aa host it remained pigmented, but the aa testis of the host became moderately pigmented, and the eyes of the host became darker, even black in some instances, instead of red. These effects can occur without contact, being exerted sometimes even when the graft had been wholly absorbed. This is proof, then, that AA tissues give off into the body fluids a substance which causes unpigmented eyes and testes to form pigment. The gene hormones of AA operated in aa, and was also noticable in the offspring of the host which was genetically an aa animal. Thus the hormones were transferred through the blood of the mother (in the case of transplanted ovaries). This case illustrates a procedure very similar to gland secretion, since the substance effective on the gene was humorally transported and had a specific and regulatory action.

The existence of gene hormones is used by Koller (1938) to explain the sex determination substance secreted by the
proboscis of Bonellia. The sexually undetermined, or partially
determined, larvae become potential males when they have contact
with the trunk of a female Bonellia. Larvae which develop with­
out this contact become females. He (Koller) states that this
secretion is a cell hormone dependent on outside characters of
the gene. It is resistant to boiling and can stand great
dilution. (See page 6).

Crew (1927) explains the significance of this substance
which Koller calls "gene hormones" in the following manner.

"All the results of experimental embryology indicate that
in the differentiation of the sex-equipment two phenomena
are to be distinguished, (1) the development of the em­
bryonic architecture, (2) the differentiation of the com­
ponent structures during further growth, and the attain­
ment of specific form under the direction of the physi­
ological action of the 'hormones'. The timely production
of these hormones is to be regarded as the function of
the genes resident in the chromosomes. In the insect
(and physiologically similar forms) the products of
metabolism that guide the development of tissues towards
definite form and structure are present within the indi­
vidual cell and are elaborated there almost if not
completely independently of the rest of the body."

Calkins (1919) in reviewing the life history of the hypo­
trichous ciliate, Uroleptus mobilis, hints that a physio-chemi­
cal regulator is released by the nucleus which is important in
the cell division and the vitality of these ciliates. Yocom
(1923) experimenting with Oxytricha, bears out the conclusions
of Robertson (Jahn, 1929), which are, namely, that a substance
is produced in infusoria which reacts on the organism to bring
about division, and that the concentration of this substance
is reached in sufficient quantities to bring about an increase
in cell division when the substance is excreted into the
surrounding media. This substance excreted determines the number of cell divisions that take place. Thus the cultures of ciliates with the greater number of cells would have a greater concentration of the substance, and the division rate in these cultures would be correspondingly greater. Allee and Evans (1937), Just (1929), and Peebles (1929) found that a substance is freed at each nuclear division. The latter, experimenting with the eggs of sea urchins and starfish, found some experimental evidence favoring the conclusion that the

"Inhibiting substances are associated with lipoid constituents, and the accelerating factor is contained in the protein molecule. After the removal of the fats from the extracts of gastrulae and plutei, a solution of the residue in sea water exerts a slightly stimulating effect on growth; when the alcoholic extracts are filtered, and the precipitate removed the filtrate has the same accelerating effect. Acetone extracts of eggs and of larvae, as well as alcoholic extracts, if used in pure form, are inhibitive in their influence. The retarding effect of secretions of growing embryos is removed in the presence of animal charcoal and fuller's earth. The percentage of normal larvae resulting from eggs grown in the presence of these absorbents is greatly increased while mortality is decreased" (Peebles, 1929).

The work done to establish the existence of cell hormones points out many problematic possibilities. The results show, with a good deal of certainty, that cell hormones exist. They are specific for neither species nor genus, and are regulatory in their action. Koller (1938) considers the hormones found in protozoa and genes as the best examples of cell hormones.

II A glandular tissue hormones.

The testing for aglandular tissue hormones does not present such complexities as in the case of cell hormones, because
the field of action is larger. Aglandular hormones are transported by diffusion from the place of production to the place of action. Gilchrist's (1937) work on the budding in scyphozoan polyps of Aurelia shows the way in which aglandular tissue hormones are transported. He suggests that the formation of the ectodermal tip of the stolon is the result of "an induction originating in underlying entoderm; and that the tip having thus originated acts as an organ of internal secretion in producing a 'growth hormone'. The hormone diffusing into the entoderm causes the entodermal cells to arrange themselves as the solid core of a stolon."

1. Neurohormones.

In speaking of aglandular hormones, Huxley (1935) reasons that "the simplest animals when adult and all animals in their early stages possess no circulatory system", and suggests that hence there must be chemical substances secreted by the cells and tissues of growing organisms which are diffused throughout the developing body. Recently it has been shown that these substances co-exist with the endocrine secretions in the adult vertebrates. The best known are produced at nerve endings, and have been given the name "neurohormones" or "neurohumoral substances" by Parker (1934), because they are produced in the nerves. It seems that in higher vertebrates, acetylcholine is the active substance produced at parasympathetic nerve endings, and an adrenalin-like substance is found at the sympathetic nerve endings. These Koller (1938) classes as aglandular
hormones of the vertebrates.

Sereni (1930) in his work on molluscs points out that these substances (adrenaline, choline and acetylcholine) are not found in the cephalopods. "There are, however, at least three substances, tyramine and histamine on one hand, betaine on the other, whose action in the cephalopods is very similar to that of adrenalin and of choline respectively. These substances have been shown to exist in the body of the cephalopods, and the two first to circulate in the blood. We have, therefore, a very close correspondence with the facts in the vertebrates" (Sereni, 1930).

Welsh (1939) in his work on the European spider crab, Maia squinado, found that the accelerating principle for the intact or isolated heart was acetylcholine extractable from nervous tissue. Extracts from the ventral ganglionic mass showed a considerable amount of acetylcholine; extracts from the eye stalk showed a little less; and the extracts from nerve fibers in the legs gave practically none. The stimulative effect from the eye extract was not due, however, to the chromatophorotropic hormone, but to the extracts from the four large ganglionic masses and their connectives. Crude aqueous muscle extracts were shown to contain one or more substances, which have an action on the heart opposed to that of the eye-stalk extract.

Koller (1929) regards the sympathetic nerves and the corpora allata as the places of formation of the substances concerned in the color change of Dixippus.
Scharrer (1937) discusses a new type of cell called the "gland-nerve cell" which are nerve cells having the appearance of secretory cells.

"Gland-nerve cells are either true nerve cells or are derivatives of nerve cells. The large gland cells in the terminal region of the spinal cord of the skate develop from the same neuroblasts as the cells in the anterior horn. In bony fishes, all stages can be found from typical nerve cells without histologically visible secretory products..., through cells with varying degrees of colloid formation and storage..., to cells transformed into gland cells lacking any nervous character... Gland-nerve cells have a wide distribution. Among the invertebrates, they have been found in annelids, molluscs, crustaceans and insects... All gland-nerve cells, both of invertebrates and vertebrates, produce granules and drops of colloid."

From Scharrer's account there can be little doubt that these "gland-nerve cells" are derived from nerve cells and are secretory in nature. It is yet unknown what substances are secreted by them and what is their physiological role.

There can be little doubt over the possibilities that neurohormones exist.

2. Heart Hormones.

The available literature on invertebrate hearts concerns itself with the reactions of the invertebrate heart to vertebrate hormones or drugs. Koller (1938, page 10) records the experiments that have lead some to conclude that heart hormones exist.

Haberlandt in 1913 (Koller, 1938) obtained a watery extract that showed hormonal properties. This extract, which Haberlandt extracted from certain parts of the hearts of the frog and the
dog, he called "heart hormone". He proved that the place of production of the hormone was limited and was not glandular. The secretion was humorally transferrable and was not the same as the extract which Loewi obtained and called "heart-nerve substance".

In 1930, Haberlandt (Koller, 1938) proved that heart hormones exist in the gastropods Helix and Aplysia. Excised hearts of Helix were kept in a Ringer solution until the hearts stopped beating and did not respond to any mechanical stimulus. This took from two hours to two days. Control hearts were put in extracts of the foot muscles. If they did not start beating they were transferred to a solution of heart extract, which had been made from four Helix' hearts. Twenty-seven out of the thirty-eight "dead" hearts started beating after a certain latency period. Haberlandt believes the whole wall of the heart is able to produce the heart hormone. Isolated hearts of Helix can be kept beating for nine days if they are kept in a proper solution, which would indicate that it was not a nutritive substance that was in the blood, but rather the stimulative substance in the heart tissue.

Dieder in 1935 (Koller, 1938), experimented on hearts of Mytilus and found that injections of Mytilus blood into excised hearts accelerated pulsation. Therefore, he concluded that the substance effective on the heart is something that is in the blood rather than a product of the heart.

More work needs to be done on heart hormones before a
definite statement can be made. It seems that the problem is difficult. Either the nerves of the heart or the blood could be responsible for the physiological functioning of the heart. However, the results obtained by Haberlandt favor the existence of heart hormones among invertebrates (Koller, 1938).

III Glandular Hormones.

1. The Color-Change Hormone.

The color change hormones of invertebrates have a chromatic behavior similar to that described for the catfish (Osborn, 1933; and Abramowitz, 1936), where the secretions of the pars intermedia of the pituitary is the factor controlling the production of the dark phase of body coloration in this animal. It has been tested for all six of the characteristics Koller (1938) ascribes to hormones (see page 6). It is effective at the great dilution of 1:1,000,000 (Hosoi, 1934). While its place of production varies its field of action and its distribution are similar in most cases.

Keeble and Gamble (1904) describe the contraction of the chromatophores of crustaceans when the eye-stalk extract is released into the blood stream of the crustaceans. The same extract causes the contraction of the chromatophores of fish (Perkins and Kropp, 1932), and the expansion of the melanophores of tadpoles (Kropp and Perkins, 1933a, 1933b). These and other reactions are suggestive of the actions of pituitary secretions (Osborn, 1936). But despite its pituitrin-like properties in its action on chromatophores, the crustacean eye-
stalk extract does not have any effect on the gonads of either male or female rats (Kropp, 1932).

Navez and Kropp (1934) tested the eye-stalk extract from the crustacean, Palaeomonetes, on the decapitated coleoptiles of oats and the roots of Lupinus. The reactions were identical to the ones obtained with "growth-substances" extracted from vegetal tissue and the substance "auxin" extracted from human pregnancy urine.

In the decapod, Grangon, the color-change hormone is produced in the cephalothorax (Koller, 1938). The place of production of the hormone causing expansion of the pigment granules in the chromatophores is, according to Koller, the lymph gland found in the rostral region. This substance he calls "melanin expantin". The substance having an opposite effect, that is, contraction of the pigments, he terms "melanin contractin" and places its source in the eye stalk. The blood organ found in the eye stalk, supposedly derived from the nauplius eye (Koller, 1938), is generally considered to be the specific place of production of the latter (Manstom, 1935; Abramowitz, 1936b; and Koller, 1938). Injected extracts of the organ cause color change in fish and amphibians identical to the response of the melanophore pigment in elasmobranchs, amphibia and some reptiles after the removal of the hypophysis (Abramowitz, 1936b). Injections of the extract into the hypophysectomized pups of the dog fish, Mustelus, was followed by melanophore expansion, a reaction which persisted for five
hours. The dispersion of melanophore pigment following treatment with the crustacean eye-stalk hormone has now been shown to occur in each of four groups of vertebrates (Abramowitz, 1936b).

Hansström (1935) was the first to show by histological examination that the middle third of the stalk contains the well developed blood gland, whereas the X-organ is either small or absent. He describes the blood gland of Pagurus as extending "through both the proximal and the middle thirds of the eye-stalk. The X-organ...is situated in the proximal third, which thus contains part of the blood gland and the whole X-organ. Since the middle third of the eye-stalk of Pagurus, that contains only part (the larger part, however) of the blood gland, concentrates as (at) least as strongly the red and yellow pigment as the proximal third does, this fact in connection with those earlier mentioned tells in favor of the theory that the blood gland is the real source of the pigment concentrating substance in the decapod crustaceans."

In studying Uca pugilator, Carlson (1935) found that removal of the distal two-thirds of both eye stalks had no effect on the pigmentedary system, whereas total removal of both stalks (the blood gland is located in the middle one-third of the eye stalk) caused a permanent concentration of the melanophores. The proximal retinal pigment, on histological examination, does not show any migration in the eyes of experimentally injected Palaeamonetes while the reflecting pigment and the distal pig-
ment migrates into a position typical for a light-adapted eye (Perkins, 1928; and Kleinholtz, 1936 and 1937).

Investigations have not settled the question as to whether these different rhythms of retinal migration of pigment are attributable to several different hormones, or whether there is only one to which these various pigment cells respond differentially. It is difficult to say whether or not the nervous system is directly involved. Perhaps the glands themselves function in a rhythmical fashion. Welsh (1936) suggests, after working with Anchisticides, a Bermuda shrimp, that the diurnal rhythm is an internal mechanism controlled by either the blood gland or the X-organ, or possibly both. Parker (1935) believes both the gland and the nervous system are responsible, the eyes acting as the receptors of light which stimulates (through the nervous system) the secretory process in the blood gland.

Various investigators have attempted to explain the manner in which these color-change hormones work. The following are the theories postulated. Kropp and Crozier (1934) and Brown (1935) believe that the eyes are the sole receptors involved in the response of the chromatophore system of Palaemonetes and other decapods. Gamble and Keeble (1900) noted in Hippolyte varians that the diurnal rhythm of concentration and dispersion of the pigments still persisted even after the eye stalks were removed. Nisoi (1934) experimented with the common Japanese pona shrimp, Paratya compressa, and claimed he found a chromatophore activator not only in the eye stalk but also in the
ventral nerve cord. He and Brown (1934) are the only two investigators that have reported such findings. The latter maintains that there are four different humoral substances present in the animal, one governing each type of pigment.

Some of these ideas seem too theoretical and speculative to be of any great value. Whatever the interpretations the above workers have used, it is established that color-change hormones exist in crustaceans; and that they are produced in the rostral region and in the eye stalk, which contains the blood gland (most likely the specific seat of production of the secretion) (Manstros, 1935; and Abramowitz, 1937).

2. Gonadal Hormones.

Considerable work has been done in trying to find a gonadal hormone. The experiments have been concerned with the effect of parasitizing the gonads, the implantation of gonads, and castration. The principle activity of such a hormone can be assumed in cases where parasitic or experimental castration brings about specific changes in the secondary sexual characters (Koller, 1929). One of the principle instances of this is the parasitic castration of decapod crustacea by Rhizocephala, which results in male crabs acquiring female characteristics. Parasitism of the females seems to bring only minor changes; but in the males parasitism changes the pincers, the copulatory organs, the male sex opening, the shape and size of the abdomen, a change in the hairs, and even causes the growth of an additional pair of legs which is definitely a female characteristic (Koller, 1929).
Interior signs show a deterioration of the gonads, a different position of the fat, especially in the liver. In some cases ovaries developed (Koller, 1938), and in two cases hermaphroditism resulted. This last, however, is exceptional, for spermatogenesis usually goes on, though at a reduced rate.

"Stylopisation" in the hymenopteran Adrena causes alterations in the sex organs, in the color of the clypeus, and the size and shape of the tibia resulting in an unmistakable approach to the opposite sex. In earthworms, in which the testes were destroyed by parasites, the clitellum which is a secondary sexual organ was changed (Koller, 1938).

Koller (1938) cites several possible explanations for this phenomenon. Perhaps the change comes through the hormonal influence of the parasites on the host. Thus, besides the destructive influence of the parasite parasitism of the gonade acts as an implanted female gonad. Perhaps the metabolism is changed through the invasion and that the "sexual formative substance" is likewise changed. Koller shows preference for the idea that this sexual formative substance is something like an aglandular hormone, produced by the invaders, or by the host through the influence of the parasites. In the instances where testes have been shown to produce oocytes Koller believes that these testes might have definite inclinations to produce oocytes, and with castration maleness is depressed with the result that oocytes appear. Crew (1937) prefers this explanation also.

Coe (1938) likewise favors this idea after experimenting with molluscs exhibiting the changes of maleness into
femaleness. While Shull (1931 and 1937), Lillie (1932), Cunningham (1903) and Domm (1927) agree that there is a hormone for each sex. "Development of the individual began with one of these hormones in the ascendancy, but somewhere in the course of differentiation it was overtaken by the other hormone, the moment of passing being the 'turning point'. Structures determined before that point were like those of one sex, characters determined after that point were like those of the other sex", is the way Shull (1937) explained the production of intermediate-winged aphids. Then intermediacy or mosaicism results from various combinations of the hormone at different times or periods of determination. "Intermediacy, as contrasted with mosaicism, is favored by extended periods of determination and ranges of stimulation, especially if there is overlapping of these periods and ranges for the several structures" (Shull, 1937). The same conclusion was arrived at by Domm (1927) in his experiments with fowl. He showed that intermittent hormone action may produce very precisely localized mosaics of male and female patterns in growing feathers.

Seemingly most differentiation in insects occur locally, without the intermediation of a morphogenetic hormone such as is found in vertebrates. In insect mosaicism these patches are usually sharply delimited, even when they differ in regard to sex (Muxley, 1935). Goldschmidt (Muxley, 1935) calls these masculinizing and feminizing substances "intracellular hormones".

The results of experimental castration indicate that there
is a fundamental difference between crustacea and insects. It has been proved in castrating Asellus aquaticus with radium that the development of the brood pouch is dependent upon the presence of functional ovaries (Koller, 1938). Other experiments of extirpations and transplantations do not permit the existence of such a hormone. Koller (1938) doubts the methods and tests of such experimenters, and states that it is possible that the endocrine organ is separate from the gonads and thus it was missed.

Gould (1917) found a sex determining secretion in the gastropod, Crepidula plana, that is furnished by larger individuals of the species, which is needed to develop and maintain the male phase.

From the results of experiments on castration, implantation, and parasitization there can be little doubt about the existence of a sex hormone in the molluscs, the crustaceans and the insects.

3. Hormones of Metamorphosis.

For some time it has been known that the metamorphosis of insects is dependent upon a hormone, and that each stage of development, larval and pupal, is governed by such a secretion.

The oenocytes of larval and adult insects are considered to be unicellular endocrine glands, with the nucleus acting as the secretory agent (Koller, 1938). These oenocyte endocrines are thought to be important in the metabolism as well as in the developmental processes, such as ecdysis. With growth and
and development three distinct hormones are involved, namely, the "change hormone", "the casting-off hormone", and the "image hormone" (Koller, 1933).

By blood transfusions Koller (1929) showed that internal secretions were concerned in ecdysis and pupation of caterpillars, but declined to give any place of formation for them. Wigglesworth (1934) investigating the ecdysis in nymphs of the bed bug, Rhodnius prolixus, established the function of various cells and glands that take part in the process. The "moulting hormone", the term used by Wigglesworth, is the same as that which Koller calls the casting-off hormone. Wigglesworth (1934) has found that it is secreted by the corpus allatum. The stretching of the abdomen after the animal feeds in the fourth and fifth instar provides the stimulus for its secretion. Molting occurs on the average fifteen days after the meal during the fourth instar and twenty-eight days after feeding during the fifth instar. In both cases, shortly after the meal, the epidermal cells become active and increase in number. A new epicuticle can be found within a few days, as an exceedingly thin membrane between the epidermal cells and the endocuticle, which forms in numerous folds allowing for the growth that follows. About the same time Verson's glands, the dermal glands, produce the hormone which makes the molting possible (Hoskins and Craig, 1935). The molting hormone dissolves the endocuticle while the cuticulin makes up all of the epicuticle and part of the exocuticle. Most of the old cuticle is
dissolved and reabsorbed along with the molting hormone. It is then that the oenocytes of the epithelial layer start production. It is thought that they are responsible for the secretions of non-chitinous portions of the cuticle, since they are also active during maturation of ova in the female when the egg shells are being formed (Hoskins and Craig, 1935). This substance would be called the "image hormone" by Koller, while Wigglesworth calls it the "change hormone" (Wigglesworth, 1934).

Decapitation of Rhodnius nympha before a "critical period", which occurs several days after feeding, prevents molting (Wigglesworth, 1934). The blood of the insect after the critical period will induce molting in insects which have been decapitated before that period, if the blood is injected. Wigglesworth (1934) noted that metamorphosis, the appearance of adult characters at the molting of the fifth nymph, is brought about by a chemical difference in the blood. Decapitated early nympha showed premature metamorphosis if they received blood from molting fifth nymphs. This he interpreted to be due to an inhibitory hormone secreted in the head of the early nymphs, which prevents metamorphosis. Fraenkel (1934) worked with the blow fly, Calliphora erythrocephala, and arrived at the same conclusion as did Wigglesworth.

To the writer's knowledge there is no literature opposing the idea that metamorphosis in insects is directly dependent on hormones. The place of production, the field of action, and the specific and regulatory manner in which the hormones work
has been proved. However, nothing is known about the processes that initiate or control the development of other animals exhibiting similar stages in development. Possibly research in this field will discover other hormones of invertebrates.

**IV Glands homologous to the vertebrate glands.**

Recent discoveries show definite endocrine glands among the Physosoma, the molluscs, and the tunicates, which seem to place them nearer vertebrates than they have previously been considered to be. Such findings have a value in the field of evolution and comparative physiology.

An organ, called the internephridial organ, has been found to be superimposed on the nephridial tubes of Physosoma. The nuclei of the older cells of the organ secrete granular substances which fill the cell plasm (Koller, 1938). Later this passes from the cell into the body cavity. Total extirpation kills the animal, but if part of the organ remains intact the animal can live. This shows that it does have a vital physiological function in the organism. However, not enough research has been done on this structure to tell its exact relation to the other invertebrate or vertebrate endocrine organs. Extracts of the organ have hormone-like properties, and since it comes from a known glandular structure the secretions of it are considered of hormone nature (Koller, 1938).

Another glandular hormone is extractable from the branchial gland in all dibranghiate cephalopods. This gland is found lying beneath the gill on each side and is visible from the
mantle cavity (Hutchinson, 1928). The organ consists of connective tissue with a rich blood supply. No lumen or ducts exist in it. Histologically, Hutchinson (1928) found that it is comparable to the gland found in Octopus. Sections fixed and stained showed irregular polygonal cells with vesicular nuclei. Most of the peripheral cytoplasm was found to be filled with a fine basophil suffusion or granulation. Eosinophil bodies, which apparently had been extruded from the cells, were found in the inter-cellular spaces. From these findings Hutchinson seems justified in concluding that it is a secretory organ. Extirpation of the branchial gland brings on hypertrophy of the rest of the organs. It is generally conceded to be a gland of internal secretion in the cephalopods (Koller, 1929).

Butcher (1930) proved that the neural gland in the ascidians is the "hypophysis of the tunicates". It is an organ embedded between the siphons, and originates from the wall of the larval neural tube. In the development of Ciona intestinalis, the neural gland or "hypophysial canal becomes grooved off from the brain vesicle...retains its connection with the neuropore, and later from its posterior end are formed glandular pockets which become the neural gland of the adult." It contains the active oxytocic principles of the pituitary. Since it is derived from the neural tube, as is the infundibular gland of vertebrates and contains the same principles there is little doubt as to it being homologous to the pituitary. Extracts of the gland have the same influence on the blood pressure of frogs, cats, and
guinea pigs as the extract of the posterior lobe of the hypophysis of vertebrates (Koller, 1938). By the usual method of extracting the active substance of the posterior pituitary, Butcher (1930) obtained an extract from the neural gland. Testing the solution it was found to be approximately twenty per cent of the strength of the U.S. standard powder, which is equivalent to the same potency as the extract from the pituitary glands of cattle. However, no experiments have been performed to demonstrate its effects on the metabolism and the development of the sexual organs of the tunicates.

As already suggested, there seems to be no doubt that there exists in the higher forms of the invertebrates structures which are comparable to some of the endocrine glands of vertebrates.

V The effect of vertebrate hormones on invertebrates.

Considerable experimentation has been done on testing the reactions of invertebrates to vertebrate hormones. The findings, however, do not necessarily contribute anything to the problem at hand, since there is too great a source for error, and the conclusions are of more importance pharmacologically than physiologically. The following are extracts that have been tested, and the records of such tests.

Adrenal extracts of vertebrates when tested on the cloaca of the echinoderm gave actions similar to those of mammalian intestines when treated with the same substance (Wyman and Lutz, 1930). "Adrenaline has both inhibitory and excitatory effects on invertebrate tissues" (Wyman and Lutz, 1930). The
same conclusion was arrived at by Wu (1939) in working with earthworms.

"Insulin", the extract from the secretions of the islands of Langerhans, was injected into Lepidoptera and crayfish. Contrary to what happens in vertebrates, it had no effect on the rate of sugar metabolism (Maluf and Rustum, 1939). From this Maluf and Rustum concluded that there must be other structures in invertebrates that take care of the storage and release of glycogen that is similar to the adrenal and islands of Langerhans found in vertebrates.

The action of the vertebrate thyroid hormone was tested by various investigators, with varying results and conclusions. Torrey, Riddle, and Brodie (1926) stated after experimentation that thyroid extracts acted as a depressant of division rate in paramecia, while it acted as an accelerator of certain metabolic processes.

Ashbel (1935) experimented with the thyroid extract "Elyteran" (Bayer), and found that its influence on oxygen consumption was instantaneous and lasted up to twenty minutes. On the hibernating eggs of the silk worm, Bombyx mori, the oxygen consumption increased 3,700 per cent.

The effect of thyroxin on the dissected heart of Limulus showed that in the presence of thyroxin it continued beating even though the median nerve was absent (Davis, 1936).

On arthropods Koller (1938) records that usually no influence was noticed, except in the case of certain butterflies
where it inhibited development. Koller noted a greater influence on molluscs where the metabolism was increased considerably. In the eggs of molluscs and echinoderms the oxygen metabolism was increased up to 300 percent (Ashbel, 1935).

The fertilized eggs of crustaceans, in all stages of development, showed an increased oxygen consumption up to 220 per cent (Ashbel, 1935).

In the tunicate, Ciona intestinalis, the ovary consumed up to 360 per cent more oxygen than it did without thyroxin (Ashbel, 1935).

C. Summary

The results of the experiments performed by the various investigators have contributed much to the general conception of invertebrates and their physiological functions and reactions. The studies have added more contributions to morphology and comparative physiology than to any other field. They indicate an evolutionary precursor of the more highly developed vertebrate endocrine system. The invertebrate hormones that have been verified have placed the invertebrates nearer the vertebrates than was previously believed possible. The glandular hormones found in invertebrates are perhaps the most easily understood because they are so nearly like those of the vertebrate endocrine system. The color-change hormones of crustaceans, the hormones regulating metamorphosis in insects, and the secretions from the glands that are homologous to the vertebrate glands are the best known.
Many of the conclusions have been too hypothetical. More physiological research on invertebrates is needed. Especially is this true in speaking of the cellular hormones. Here, as already pointed out, the seat of production and the place of action are too near together to make experimentation easy. In many of the cases the experiments and results have been indefinite, and hence speculation and theories have colored the conclusions of the investigators.

More experiment on aglandular hormones is needed, for instance on the heart hormone. Present findings have not given too clear a view. It is possible that these stimulative substances which have an effect on the heart are part of the blood itself. The place of production has been hinted at, but not conclusively demonstrated in the aglandular hormones.

There is justification for believing that the results stated are perhaps due to some other system than an hormonal one.

Extracts from the organs that appear to be homologous to vertebrate endocrine glands have been tested on other animals with seemingly positive results; but investigations are needed to find what are the specific functions these homologous glands control in the animals in which such organs are found.

To date no study has been made on other animals which exhibit a life history similar to that of insects which are accepted to have a definite hormone controlling their development.

The source of the so-called sex determining hormones is
not definitely known. A good deal of speculation seems to have entered into this particular problem. In the castration of insects the question is still unsettled as to whether the change from femaleness to maleness, or vice versa, is due to hormones. The explanations for such changes are mainly hypothetical, as nothing definite has been proved. Too many changes must take place before a testis can be made to form oöcytes to be attributed wholly to hormonal effects. While the hormone action is as logical an explanation as any, perhaps, it is by no means satisfactory.

The literature cited indicates the presence of hormones in invertebrates from which the following conclusions can be derived.

1. Cellular hormones exist, the nucleus being the possible seat of production. Such hormones have been found to exist in protozoa and genes.

2. Aglandular tissue hormones are to be found in invertebrates. The best known are the nerve hormones. There is evidence for the existence of a heart hormone.

3. Glandular hormones are present. The color-change hormones have been established.

4. Experimentation shows that there is a sex hormone for each sex.

5. The metamorphosis of insects is dependent upon three hormones; one regulates the larval stage, another governs pupation, and the third makes possible the change from pupa to adult.

6. Structures in invertebrates have been found which are homologous or similar to certain endocrine glands of vertebrates. The internephridial organ of Physosoma, the
hypobranchial gland of molluscs, the branchial gland of cephalopods, and the neural gland of the tunicates have been established as the endocrine glands of invertebrates.

7. The effect of vertebrate hormones on invertebrates has led to no definite conclusions. The experiments are more pharmacological than physiological.
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