delicate feeling which correctly foresees the laws of natural phenomena; but this we must never forget, that correctness of feeling and fertility of idea can be established and proved only by experiment."[7] "Genius is patience? No, it is not quite that, or rather it is much more than that; but genius without patience is like fire without fuel—it will soon burn itself out."[8] Loeb possessed the patience and he had the industry; alas, his industry was in excess of his physical constitution, so that he burned out his life before he consumed his talents.

In 1910 Loeb exchanged a professorship at the University of California for membership in The Rockefeller Institute for Medical Research. He organized at the Rockefeller Institute the Division of General Physiology, the first department of the kind to be created in the United States. It was fitting that Loeb, whose discoveries had so enriched general biological science, should have been the pioneer of general physiology in this country. The growth of the new establishment was such that in 1918 a *Journal of General Physiology* was called for and Loeb undertook the task of founding and editing such a journal. His removal to New York called for modification of the research program. As may be observed from his discoveries in the field of colloid chemistry, his fertile mind met the new conditions. By dividing the year between New York and Woods Hole, Loeb's working facilities were enlarged; and this happy arrangement filled his last years with scientific opportunity commensurate with his needs, sympathetic scientific association, and although grudgingly given with those recreative enjoyments which his intense nature required.

Loeb was of the type of the intensive individual investigator; hence his immediate pupils are not numerous. But if Loeb's direct influence was reserved for a favored few, his wider influence has been shared by a large body of students and investigators and even by the educated lay public. His personal contact with successive groups of scientific workers at Woods Hole, the Rockefeller Institute, and elsewhere has been of incalculable value. Loeb's profound scientific learning and experience, wide reading, liberal views often warmly expressed, vivid imagination widely dispensed, and his fund of sparkling wit made him at all times a stirring and delightful companion. No one could have been kinder than Loeb in his human relations; and fortunate were those who came under the reign of his genial, many-sided personality. It is unhappily too true of him "that he may be succeeded, but can not be replaced."

[7] Bernard, Claude, "An Introduction to the Study of Experimental Medicine," The Macmillan Co., 1927, p. 43.

[8] Lodge, Sir Oliver, "Pioneers of Science," London, The Macmillan Co., 1893, p. 202.

Jacques Loeb's life was spent in an ardent desire to interpret nature. It was peculiarly true of him, as has been said, that knowledge is at once the sole torment and the sole happiness. He knew as few come to know the joy of discovery, which is one of the liveliest the mind of man can feel. He knew also, almost too well, that this joy of discovery, to which his inner demon impelled him, is no sooner found than lost; that it is but a flash, whose gleam discovers fresh horizons, toward which our insatiate curiosity repairs with still more ardor. This is research: the search for truth which, if never found in its wholeness, is yet secured in significant fragments; and these fragments of universal truth are precisely what constitutes science.[9] To this search for fragments of universal truth in living matter Jacques Loeb devoted his great talents and his rich life.

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CHEMISTRY IN RELATION TO BIOLOGY AND MEDICINE WITH ESPECIAL REFERENCE TO INSULIN AND OTHER HORMONES

II

ORGANS OF INTERNAL SECRETION

I may now be permitted to call your attention to a field of knowledge that has occupied experimenters for more than half a century—a field of great importance not only to the biologist in general, but to those who are associated in any way with medicine, as the bio-chemist, the physiologist, pharmacologist, the numerous representatives of the medical and surgical professions—a field that is concerned with a study of the functions, both normal and abnormal, of the organs of internal secretion. To the chemist is given a unique opportunity in this field and here, as is so often the case in biology, the last word is his. In saying this I have particularly in mind the obvious chemical aspects of the problems here presented, as, once they are cleared up, the physiologist and the physician are enabled to outline their own problems relating to the function of these organs with greater precision.

The significance of the interdependence of the various mechanisms of the animal body, of their admirably regulated activity and the harmonious manner in which these mechanisms cooperate in the development and growth of the individual from the moment when they first become apparent in early embryonic life to

[9] Bernard, Claude, "An Introduction to the Study of Experimental Medicine," The Macmillan Co., 1927, p. 222.

the time when we are returned to the dust from which we sprang has no doubt always been apparent to the mind of man, as is so well illustrated in Aesop's fable that it will not do for the various members of the body to fall out with each other. The medicine of an older time has long used the words *consensus partium,* the mutual dependence on each other, in other words, this interrelationship, of the various organs. Generally speaking, until about a century ago the *consensus partium* was supposed to be effected solely through the intermediation of the nervous system—a point of view tersely expressed in 1752 by the French anatomist Cuvier when he said: "Le système nerveux est, au fond, tout l'animal, les autres systèmes ne sont là que pour le servir." According to this view, then, to put the matter in popular language, certain control stations in the brain and spinal cord and of the peripheral autonomic nervous system coordinate all the functional activities solely by means of telegraphic communication (though a nervous impulse is quite a different thing from an electric current). During the past seventy years, however, conjoined chemical and physiological discoveries have brought to light a new method of communication between organs and a coordination of their functional activities, closely linked, to be sure, with the longer known type of control but yet quite distinctly different. To-day we also have, as Professor Starling so aptly put it, an efficient postal service at the disposal of the various mechanisms of our body. The little packets of chemicals sent out by the organs of internal secretion are carried by the blood stream to their appointed destinations and are generally called hormones, the name given to them by Starling twenty years ago. The term is derived from the Greek verb ὁρμαω (I stir up or excite), and is perhaps less appropriate than the designation *chemical messenger,* earlier applied to this class of substances by the same physiologist.

These chemical messengers may initiate changes in the functional state of a distant nerve cell or nerve terminal, or a series of chemical changes, or take part in some intermediary chemical reactions in a distant organ—reactions that may or may not have been initiated primarily by a preliminary nervous impulse or telegraphic message.

The organs in which these chemical messengers are elaborated and from which they are despatched into the blood stream are known as organs of internal secretion, or endocrine organs. Some of these are called ductless glands—structures that are entirely devoid of secretory ducts, as their name implies, and can only perform their functions by sending their chemical messengers into the blood and lymph, by which they are transmitted to the various organs of the body. Examples of such ductless glands are the thyroid, the parathyroids, the pituitary organ and the intestinal mucosa. Examples of endocrine organs that play the double rôle of producing ferments or other chemical agents that are eliminated by special ducts or passages as well as chemical messengers that pass into the blood stream are the pancreas and the sex glands.

The entire list of organs possessing an endocrine function need not be given here. Let it suffice to say that additions to the list of chemical messengers are constantly being made as the conceptions in this department of physiology have broadened in consequence of more intensive experimentation. Organs such as the heart, for example, have been shown to produce substances that influence the rate of its contractions by an action on its regulatory nervous mechanism. The carbon dioxide that is excreted by our tissues as an end product of their metabolic activity acts as a hormone or regulator of the external respiratory apparatus. In the widest and perhaps truest sense of the word, every tissue, as Schäfer remarks, is an internally secreting structure.

Juice expressed from the embryonic heart of the chick contains a chemical substance, or perhaps more than one, that is indispensable for the continued growth, in the warm chamber, of an isolated fragment of an embryonic heart. This continued maintenance of life and growth in the thermostat of fragments of tissues excised from cold or warm blooded animals or from tumors was initiated by Ross G. Harrison and is known as tissue culture. Carrel has suggested the term "Trephones" (from γρέφω—I feed) for the growth-promoting substances of embryonic tissue juice and of leucocytic extracts. I have called to your mind the work of Carrel and his associates on the artificial propagation of the fibroblasts taken from a fragment of the embryonic heart of the chick because the indispensable trephones or growth-producing substances that must constantly be added to the culture medium may properly be called hormones. They are produced in the cell laboratories of the chick's heart and function in some as yet unexplained manner as regulators of the complex intermediate chemical processes that are necessary to the orderly life of the cell. It will interest you in this connection to learn that Carrel and his collaborators have, from January, 1912, to June, 1927, carried a fragment of embryonic heart tissue through 2,987 generations or "passages of cultivation." There is no reason to doubt that this fragment of tissue would continue to develop indefinitely, provided only the temperature of the thermostat be maintained at 37.5° and that the composition of the nutritive medium remains constant. These extraordinary growth-promoting substances or cata-

lysts of the embryonic tissue (and of leucocytes) may for the present be classed with the hormones.

You are all aware that modern research has revealed the astonishing fact that we require for the complete nourishment of the body, in addition to the energy-yielding and mineral constituents of our food, minute quantities of other substances which are called vitamins. There are many analogies between the physiological properties of these vitamins, which may be called hormones of plant origin, and those that are produced in the animal organism.

According to many plant physiologists, chemical messengers appear to play an important, if not occasionally a greater, rôle in the life of plants than they do in that of animals, but as this phase of the subject lies outside the field of my own experimentation I shall content myself with having called it to your attention.

I can not enter into the details of the story of the discovery of the functions of the ductless glands proper or of the endocrine functions of the organs that have both an external and an internal secretion. The whole subject constitutes one of the greatest contributions of the nineteenth century to scientific medicine. Osler in his "Evolution of Modern Medicine" gives it as his opinion that

> there is perhaps no more fascinating story in the history of science than that of the discovery of the so-called ductless glands. . . . No such miracles have ever been wrought by physicians as those which we see in connection with the internal secretion of the thyroid gland. The myth of bringing the dead back to life has been associated with the names of many great healers since the incident of Empedocles and Pantheia, but nowadays the dead in mind and the deformed in body may be restored by the touch of the magic wand of science. The study of the interaction of these internal secretions, their influence upon development, upon mental processes and upon disorders of metabolism is likely to prove in the future of a benefit scarcely less remarkable than that which we have traced in the infectious diseases.

We are but at the beginnings of knowledge, especially as concerns the chemical and physiological problems that are presented in this great field. Consider, for example, that remarkable and still unknown hormone elaborated in the anterior lobe of the hypophysis and passing from thence into the blood. In it we have a chemical agent that can affect the growth of bone and other structures to an extraordinary degree. The unchecked action of this growth-stimulating principle, extending over a period of years, leads to acromegaly or gigantism. Evans and Long, by injecting potent extracts of the anterior lobe of the hypophysis daily into young rats, have succeeded in producing veritable giants of their species. One typical experiment may be cited: a rat received intraperitoneally anterior hypophyseal substance for a period of 333 days. At the end of that time the animal weighed 596 grams, while its healthy litter mate control weighed only 248 grams.

Studies of this nature have succeeded for the first time in throwing a bridge across the chasm that has hitherto separated bio-chemistry from morphology. Distinguished anatomists, indeed, as Sir Arthur Keith and Professor Bulk, of Amsterdam, have expressed their conviction that the differentiation of mankind into racial types is due to the differential interaction of these endocrine organs. The cumulative experience of a host of medical observers during the past seventy-five years has demonstrated beyond a doubt that innumerable departures from the normal in respect to bodily stature, facial configuration, sexuality, general metabolism and even the mentality find their explanation in the over- or under-activity of these, anatomically speaking often quite insignificant, structures, or in a lack of harmony in their cooperation. It is one of the tragedies of life, a decree of fate, that we should be both "the beneficiaries and the victims of the chemical activities and correlations of our endocrine organs."

Our chemical knowledge of the elusive principles, elaborated in minute quantities only by these indispensable organs, is in its infancy, as I have already intimated, and still lags far behind our acquaintance with their physiological actions. Only two of them, substances of low molecular weight and relatively simple structure, have been prepared synthetically. In respect to these two, then, the organic chemist has again come to our rescue and both the experimentalist and the manufacturer are now free to accept their deliverance from the products of the slaughter house.

The first of these hormones to be conquered is the one that is elaborated in the so-called medulla of the suprarenal capsules, small yellowish structures, each shaped like a cocked hat and fitting snugly on top of its corresponding kidney. The two organs together, in a fully developed man, weigh less than a third of an ounce. Life is impossible without them. Like some others of the endocrine organs, notably the hypophysis, they are double structures, fused in the higher animals into what is apparently only one organ consisting of a medullary or inner portion and a cortical or outer portion. This cortical portion contains a hormone, or hormones, more immediately necessary to life than that produced in the medulla. Investigators are now occupying themselves with it and we look forward, I confidently believe, to a successful outcome of their researches.

The medullary hormone is called by various names, as adrenin, suprarenalin, suprarenin, adrenalin and

epinephrine, the latter having been adopted by the United States Pharmacopoeia as the official designation. This name was coined by me thirty years ago at a time when I supposed that the form in which I had succeeded in isolating it represented the base as it actually exists in the capsules.

Without going into the details of earlier attempts to isolate the principle, all of which I have described in a historical paper published in 1903 (*Amer. Jour. Pharm.* 1903 *75,* p. 301), I hope that you will permit me to say a few words about my own investigations towards its isolation. With the assistance of the late A. C. Crawford I succeeded in separating the hormone from its numerous tissue concomitants in the form of a benzoyl derivative. On decomposing this benzoyl derivative with hot dilute sulphuric acid in an autoclave we obtained the active principle in the form of a sulphate which possessed the characteristic physiological activities of suprarenal extracts and reacted, furthermore, with a series of chemical reagents in a manner that is quite specific for such extracts and limited to them. The principle as obtained by saponification of the benzoyl derivative was thrown out of its solution by means of ammonia in the amorphous state and was shown to be a weak base. A picrate, a bisulphate and other salts of it were prepared, all of which were shown to possess a high degree of physiological activity. An acetyl derivative, a phenylcarbamic ester and other derivatives were also prepared and certain degradation products of the base were isolated and studied. Without giving any further details of this earlier work, which occupied my time for a number of years, I will merely state that the elementary composition of the base was established by analysis of several derivatives, including a sulphate, and was stated to be represented by the formula $C_{17}H_{15}NO_4$ (*Zeit. f. physiol. Chem.,* 1899, xxviii, 318).

After I had completed the above described investigations and while I was still endeavoring to improve my processes I was visited one day in the fall of 1900 (as I recall it) by the Japanese chemist, J. Takamine, who examined with great interest the various compounds and salts of epinephrine that were placed before him. He inquired particularly whether I did not think it possible that my salts of epinephrine could be prepared by a simpler process than mine, more especially without the troublesome and in this case wasteful process of benzoylating extracts of an animal tissue. He remarked in this connection that he loved to plant a seed and see it grow in the technical field. I told Takamine that I was quite of his opinion that the process could no doubt be improved and simplified. At this very time also, v. Fürth had just prepared an amorphous, highly active, indigo-colored compound of the active principle, which he named suprarenin, but no analytical data were given and no empirical formula for his principle was established.

Takamine prepared suprarenal extracts more concentrated than mine, and without first attempting to separate the hormone from its numerous concomitants by benzoylating or otherwise, simply added ammonia —the reagent that I had so long employed—to his concentrated extracts, whereupon he immediately obtained the native base in the form of burr-like clusters of minute prisms in place of my amorphous base. I have often been asked why I had not myself attempted to solve the problem in this very simple fashion. The truth is that I had tried to do so but always found that the dilute extracts tested simply turned pink in a short time on the addition of ammonia without depositing a base, either crystalline or amorphous. Inasmuch as even very dilute solutions of the salts obtained by me on saponifying the benzoyl derivatives always gave a precipitate with ammonia, I fell back on the hypothesis that other constituents of the impure extracts prevented its precipitation by ammonia from my dilute native extracts—an erroneous assumption. Takamine's success was due to the employment of ammonia on very highly concentrated, though impure, extracts. The fact that my amorphous base could be precipitated from even highly dilute solutions was, as I soon found, due to the fact that one benzoyl radical had not been removed during the saponification. Takamine adopted the empirical formula $C_{10}H_{15}NO_3$ as the "probable empirical formula" of his substance, which was immediately patented in this country and manufactured, greatly to the advantage of medicine. I was soon able to demonstrate that my epinephrine—$C_{17}H_{15}NO_4$ —had retained a single benzoyl radical, C_6H_5CO, that had resisted saponification and could only be removed from the base by drastic treatment with strong acids and heat, a treatment which at the same time obliterated every trace of the characteristic physiological action of the hormone (Johns Hopkins Hospital Bulletin, 1901, vol. xii, p. 337). I suspect that the retained radical was attached to the imide nitrogen of the side chain of the molecule—an unusual circumstance in any event. Subtracting the molecular weight of the retained radical from my original formula, $C_{17}H_{15}NO_4$, leaves $C_{10}H_{10}NO_3$, which formula is very close indeed to that assigned by Takamine to his crystalline base, and Aldrich, who had been my assistant and who, coincidently with Takamine and quite independently of him, also discovered that the base is obtainable in crystalline form when ammonia is added in sufficient quantity to a highly concentrated suprarenal extract, wrote, with-

out knowing at the time that I had already discovered the concealed radical: "It is interesting to note in this connection that if we subtract a benzoyl residue from Abel's formula for epinephrin—$C_{17}H_{15}NO_4$—we obtain a formula $C_{10}H_{10}NO_3$ which is not far removed from that of adrenalin." (*Amer. Journ, Physiol.*, vol. V, p. 461).

I venture to say in extenuation of the blunders of a pioneer in this field that the results obtained by me and my close approximation to the true elementary composition of the hormone were not due to chance, but could have been obtained only from the study and analysis of a series of fairly pure chemical individuals. Every one of these derivatives had, however, as already stated, retained a single benzoyl radical. The efforts of years on my part in this once mysterious field of suprarenal medullary bio-chemistry, marred by blunders as they were, eventuated, then, in the isolation of the hormone, not in the form of the free base but in that of its monobenzoyl derivative. Aldrich finally established the true empirical formula, $C_9H_{13}NO_3$, the correctness of which was afterwards conclusively verified by others abroad.

I can not look back on my own poor efforts to elucidate the chemical constitution of the compound with pleasure. After the preparatory pioneer work thus far outlined there followed the brilliant researches, in respect to the chemical constitution of the hormone, of the chemists, Dakin, Jowett, Pauly, Friedmann, Stolz and Flächer, which have finally culminated in the synthetic production, first of the racemic and later of the laevo-rotatory form, as produced in the animal organism itself. The work of these chemists has shown that the suprarenal medullary hormone is an aromatic amino-alcohol, dihydroxymethyl-amino-ethylol-benzene,

$$C_6H_3(OH)_2 \cdot CHOH \cdot CH_2 \cdot NH \cdot CH_3.$$

The cells of the medullary portion of the suprarenal gland are intimately related in their origin to the sympathetic nervous system, and we are not surprised therefore to find their secretory product, adrenalin or epinephrine (U. S. P.), has a pharmacological and quite specific affinity for the sympathetic nervous system—the thoracic-abdominal part of the autonomic nervous apparatus. The changes induced by epinephrine in the activity of the various organs innervated by this system are in all respects like those that are brought about when the sympathetic fibers controlling these organs are stimulated by an electric current. A small quantity of the hormone administered intravenously will cause vaso-constriction, increased rapidity of heart action, dilatation of the pupil, relaxation of constricted bronchioles, inhibition of the peristaltic movements in the alimentary canal, contraction of the pyloric and ileo-coecal sphincters, increased motility of the pregnant uterus and mobilization of the glycogen of the liver with a resultant glycosuria. We have here then an example of a definite chemical principle of known structure which, when carried by the blood to a terminal point of the sympathetic system, induces exactly those alterations that follow electrical stimulation of the sympathetic fibers that pass to these terminal points. In more technical pharmacological terms, the hormone stimulates, sensitizes or acts in an inhibitory manner on sympathetic myoneural or adenoneural, junctions of the sympathetic nervous system. Because of this highly specific action this hormone and the many others of its class that have been isolated in recent years from various animal, plant and bacterial products have been given the name of sympathomimetic amines.

I can not pause to give an account of the various beneficial uses which several of these sympathomimetic amines have found in medical practice. Nor can I enter upon a chemical or pharmacological analysis of these numerous, naturally occurring or synthetically prepared, biologically very important substances that are classified with epinephrine as phenylalkyl- or phenylalkanolamines. This group is only one of many groups of physiologically more or less active substances grouped together as a class of biogenous amines.

It is worth while pointing out in this connection that from the structural point of view a whole series of alkaloids, as hydrocotarnine, anhalamine, anhalonidine, papaverine, laudanine, bulbocapnine, corydine, berberine, canadine, cryptopine, protopine and many others can be brought into genetic relationship with the phenylalkylamines above referred to, as has been outlined by Elger.

I may conclude this section of my lecture by a brief description of a plant principle, ephedrine, closely related in structure and physiological action to the hormone of the suprarenal medulla. For more than 5,000 years the Chinese have used the stems of *Ephedra vulgaris*, under the name of Ma huang, as a medicine famed among them as a diaphoretic, a circulatory stimulant, a sedative in cough and an antipyretic. The Japanese chemists Yamanashi and Nagai first isolated from Ma huang a derivative of phenylethylamine and named it ephedrine. The chemical structure of this laevo-gyrous plant base has been determined and is evident from its formula,

$$C_6H_5 \cdot CH \cdot OH \cdot CH \cdot CH_3$$
$$\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad NH \cdot CH_3$$

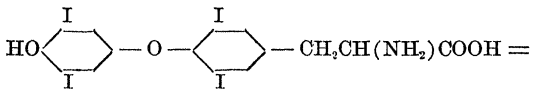
1-phenyl-1-hydroxy-2-methylaminopropane. It will

be noted, on comparing this structural formula with that of epinephrine, that ephedrine contains two asymmetric carbon atoms as compared with the one of epinephrine, which latter also differs in containing two phenolic hydroxyl groups in the ortho-position to each other, making it much more susceptible to oxidation than ephedrine. Ladenburg and Oelschlägel, who determined the chemical structure of ephedrine, have succeeded in isolating from the *Ephedra vulgaris* of Europe a stereoisomer, pseudo-ephedrine, which is not, strictly speaking, an optical antimere in its configuration. Its dextro-rotation is due to a difference in the spatial arrangement of the alcoholic hydroxyl group of the above structural formula. The four possible isomers of ephedrine have been prepared synthetically and isolated and an extensive literature has already appeared in connection with their chemical and pharmacological properties and with the medical uses of the laevo-gyrous isomer, ephedrine. The pharmacological action of this plant phenylalkanolamine is qualitatively identical with that of the suprarenal base, epinephrine; in other words, it is a typically acting sympathomimetic amine. It is less active physiologically than the latter, but its effects are more prolonged. In the form of ephedrine sulphate it has already been found to be of great value in nasal operations and in ophthalmology. Its ultimate value in serious diseases of the heart, in shock, hypotension and other pathological states is now being made the subject of numerous investigations.

THE HORMONE OF THE THYROID GLAND

A second hormone, the chemical constitution of which has only recently been unravelled, is that produced by the thyroid gland, a small trilobed organ set astride the windpipe, below the larynx, and weighing in man 35 grams or 1¼ ounces. In this connection I may add that we are also endowed with a number of very small endocrine organs, the parathyroids, usually four in number, two on each side of the neck, closely adherent to the dorsal surface of each lateral lobe of the thyroid gland. While the thyroid gland of man weighs about 35 grams or 1¼ ounces, the four parathyroids, each about the size of a hempseed, weigh together only half a gram or 7 to 8 grains. These minute glands, like the thyroid itself and most of the other organs of internal secretion, have an unusually good blood supply and are essential to life, one of their known functions being that of controlling in some as yet undefined manner the chemical combinations of the calcium in the tissues. Their hormone has not yet been isolated.

The hormone of the thyroid gland was first isolated in crystalline form by E. C. Kendall in 1914, and was named thyroxine by him. A year ago C. H. Harington, of University College Hospital, London, described an improved method for the separation of thyroxine from the thyroid tissue and by the brilliant application of well-known methods of break-down and synthesis obtained an iodine-free degradation product, desiodothyroxine, differing only from the natural hormone in being devoid of the four iodine atoms present in the latter. Harington next succeeded in working out the constitution of this desiodothyroxine and found it to be the p-hydroxyphenyl ether of tyrosine. Next, Professor Barger and he conjointly prepared in a masterly manner a series of organic derivatives which were utilized in finally effecting the synthesis of the hormone in its racemic form. The brilliant work of these investigators, carried out along classical chemical lines, has now incontestably established the constitution of the hormone as being β-[3, 5-diiodo-4-(3′, 5′-4′-hydroxyphenoxy) phenyl]-α-aminopropionic acid, as represented by the following formula:



$C_{15}H_{11}O_4NI_4$

Synthetic thyroxine, as prepared by Harington and Barger, is obtained in the form of a crystalline precipitate consisting of rosettes and sheaves of colorless needles. The compound is insoluble in water and the usual organic solvents, soluble at room temperature in solutions of the alkali hydroxides, provided their concentration be not too high. It dissolves in sodium carbonate solution only on boiling and is soluble in 90 per cent. alcohol containing either an alkali hydroxide or a mineral acid. Sodium, ammonium and barium salts have been prepared which agree in their properties with the corresponding salts of the natural product. The identity of the synthetic product with the natural hormone has thus been conclusively established by the brilliant researches of Harington and Barger.

Professor D. Murray Lyon, of Edinburgh, has studied the effects of synthetic thyroxine on two myxoedematous patients whose basal metabolism rates were respectively 32 and 45 per cent. below normal. After intravenous administration to each of the two of a total dose of 14 mgs. of the hormone, given in divided doses over a period of six days, the basal metabolism of the one rose to within 6 per cent. of normal and that of the other to 3 per cent. above normal. It is concluded, therefore, that the effect of synthetic thyroxine in raising the basal metabolic rate of these two patients is quantitatively similar to that reported by Boothby and Sandiford, of the Mayo Clinic, in 1924, for natural thyroxine.

THE PANCREAS AND ITS HORMONE, INSULIN

Of the endocrine glands which are now being so actively investigated, I have selected for further discussion the pancreas and its internal secretory product, insulin. Needless to say that within the brief time left I can do no more than just touch on a few of the important researches that constitute the historical background for our present conception of the indispensable rôle of this gland in carbohydrate metabolism. There are four important milestones in the history of carbohydrate metabolism which can receive but the barest mention here.

1. The epoch-making researches of Claude Bernard in relation to the functions of the liver and pancreas and more especially with reference to their rôle in carbohydrate metabolism constitute the first of the four milestones. Bernard, in his masterful and logical way, worked out the fact that the liver is capable of polymerizing dextrose into a starch—or dextrin-like substance which he named glycogen because the hepatic tissue is able, through the agency of a ferment, to reconvert it into sugar. His findings caused Bernard to view the liver as an organ of internal secretion (and he was one of the first to use this term), that is, an organ which manufactures in its cells a product which can be converted into a substance transportable by the blood to all parts of the body and utilizable by the tissues. Bernard, not content merely with proving the existence of such a product in the liver, set out to extract and to isolate it from this organ in pure form, a feat which he accomplished in 1857 after many years of preliminary work. Having now isolated glycogen, Bernard determined the conditions under which it is formed and is reconverted into sugar, and before long he was able to show that the reciprocal relationship between glycogen and glucose in the animal organism is entirely analogous to that existing between starch and sugar in the metabolism of plants. The name animal starch, by which glycogen is frequently called, is therefore well taken.

II. The second important development in this field came when the pathologist Langerhans, then a young investigator in Virchow's laboratory in Berlin, discovered (1867–69) that there are contained in the pancreas groups of cells situated between the acini and markedly different from those of the ordinary glandular type. These groups, usually round, are composed of small, irregular, polygonal cells with a round nucleus and a homogeneous refractive cell body. Numerous observers have verified this discovery and they have been designated ever since as the islands of Langerhans or the islet tissue. These islands of Langerhans are the seat of formation of the pancreatic hormone, which has been appropriately named insulin in accordance with the suggestion first made by the Belgian, de Meyer, in 1909 and independently by the distinguished physiologist, Schäfer, in 1916 and adopted at the latter's suggestion by the Toronto workers. But the proof that the islet tissue of the pancreas produces the hormone was not established until near the close of the last century, and it required the joint labors of many investigators to establish this point with certainty.

In certain teleostean fishes, the islets, homologous in structure and in their function with those of Langerhans, exist as organs quite separate from the pancreas, and an additional link in the chain of evidence in support of the theory that the islet cells alone can produce insulin was furnished by the Toronto investigators, Macleod and his associates, who extracted the hormone from these specialized teleostean organs and found that it possessed the physiological properties of the insulin prepared from the pancreas of beeves. The islets are more abundant in the pancreas of the higher animals than was formerly believed to be the case. In the entire pancreas of the guinea pig, according to Bensley, as many as 56,000 islets have been counted, "so that the endocrine tissue, instead of being rather scant, as it has usually been thought to be, is rather abundant" (Macleod).

III. A third great step forward was taken in 1889–91. In those years the clinicians v. Mering and Minkowski discovered that complete removal of the pancreas from dogs is followed by a diseased state which is practically in all respects like that seen in human diabetes mellitus. This clean-cut piece of experimental work, with the consequences that are logically deducible from it, constitutes one of the greatest achievements in this field since the discovery of glycogen and the demonstration of its physiological properties by Claude Bernard fifty years before. These consequences, indeed, played the chief rôle in the establishment of the proof outlined above under the heading (II) that the islets of Langerhans are the seat of formation of insulin, and furthermore, when taken in conjunction with the subsequent findings of pathologists, as Opie and others, they forced on men's minds the conviction that the cause of that frequently occurring and serious disease, diabetes mellitus, must be referred to an inadequate functioning of the islets.

The crucial demonstration by v. Mering and Minkowski that ablation of the pancreas leads inevitably to diabetes naturally served as a great impetus to further research which confirmed their work and paved the way for the fourth great historical event in this field, presently to be described. Leaving out here all reference to the investigations of about twenty workers in the decade or two following the discovery of v. Mering and Minkowski, let me speak very briefly

of the results obtained by workers in this field closer to our own time, results that almost succeeded in giving us insulin 15 years ago. The Toronto investigators have, in a very generous spirit, given full credit to the unsuccessful attempts of their predecessors to prepare serviceable extracts of the pancreas. In 1908 Zuelzer prepared an alcoholic extract from the pancreas of recently fed animals and obtained rather striking results from its use on a pancreatectomized dog and on eight diabetic patients. It is interesting to note, in studying Zuelzer's protocols, how greatly both the output of urinary sugar and of the acetone bodies was reduced in his patients as the result of the intravenous injection of his preparation. Untoward symptoms, such as a rise of temperature and chills, appeared both in Zuelzer's patients and in those of Forschbach, another clinician who tried Zuelzer's extract. In the opinion of the latter, the alleviation of the symptoms in diabetic patients was due rather to the febrile reaction induced by the injections than to a specific action of the hormone assumed by Zuelzer to be present in his extracts, and so it fell out that the successful use of pancreatic extracts for the treatment of diabetes has had to wait until our day for men equipped with newer methods and who, above all else, based their conclusions both on evidence obtained from well controlled animal experimentation and on clinical experience with diabetic patients.

E. L. Scott, who worked in this city in 1911–12 in Professor Carlson's laboratory, also came very close to obtaining a pancreatic extract that might have been serviceable, had it been tried out on human beings, since, by his method of preparation, blood-pressure lowering substances were removed. Scott's extracts, when injected intravenously into completely pancreatectomized dogs, diminished temporarily the sugar excretion and lowered the D:N ratio of the urine. Unfortunately, however, this investigator did not properly interpret his findings, inasmuch as he concluded that it does not follow that the effects induced by his extracts were due to the presence in them of the internal secretion of the pancreas. In 1913 Murlin and Kramer, in their study of the effects of pancreatic extracts on glycosuria, were led to the conclusion that neither their extracts nor the transfusion of normal blood "is as yet of any practical importance in restoring to the depancreatized dog the ability to burn sugar." Kleiner, using emulsions of the dog's pancreas infused slowly into a vein of a depancreatized dog, observed a temporary but marked decrease in the blood and urinary sugars and regarded his findings as furnishing evidence in support of the endocrine theory of experimental diabetes.

Other attempts to prepare an extract of the pancreas that would be serviceable in lowering the blood and urinary sugars and in ameliorating the symptoms of depancreatized animals and of human diabetics can not be detailed here.

IV. The numerous discoveries since Claude Bernard's day, grouped together under three periods as above, constituted the indispensable foundation for a fourth step—the preparation of an effective and stable extract that would unfailingly, or with rare exceptions, restore completely to health persons, old and young, sufferers from and often the early victims of that hitherto unconquerable malady, diabetes mellitus.

All the world knows of the brilliant achievements of Banting, Best, Macleod and Collip and their collaborators, acting on an original suggestion of Banting, that have led to the fourth great epoch in the combined fields of bio-chemical, physiological, pharmacological and clinical investigation. The results of these talented investigators have been and will continue to be of incalculable value to mankind, and have opened up many new possibilities for the study and better comprehension of the difficult field of carbohydrate metabolism.

This brings me now to a brief account of my own endeavors and those of my collaborators to isolate and separate the true insulin hormone from its numerous concomitants in the therapeutic preparations now employed, serviceable as they are, in the treatment of diabetics. It is a proper aim of the scientist, a mandate even, if I may say so, is laid upon him, wherever it is humanly possible, to isolate and to identify the elusive and indispensable hormones from their complicated mixtures (messes, the chemist would say) in which nature presents them. Once this aim has been realized and the hormone has been separated as a well defined chemical individual, the next steps, such as the study of the constitution of the hormone and its eventual synthesis, if ultimately possible, fall to the chemist. As in all previous instances, the isolation of a hormone as a chemical individual gives to the biochemist and physiologist a cleaner approach for the solution of their problems than when they are compelled to use mixtures of unknown composition.

These studies were made possible through a generous grant from the Carnegie Corporation of New York. The earlier ones are set forth in the following publications from my laboratory: Abel and Geiling, *Journ. Pharmacol. and Exper. Therap.*, 1925, xxv, 423; Abel, Geiling, Alles and Raymond, SCIENCE, 1925, xlvii, 169. They deal chiefly with establishing the fact that sulphur in a labile form is an integral

part of the insulin molecule and that the physiological activity goes hand in hand with the labile sulphur content of the molecule. I next succeeded in obtaining insulin in crystalline form and during the past year its preparation has been so simplified that, starting with commercial preparations such as the dry powder manufactured by the Connaught Laboratories of Toronto (13 units per milligram) or the concentrated liquid extracts furnished by Eli Lilly and Co. and E. H. Squibb and Sons (250–450 units per cc.), the crystals can be obtained in any desired quantity within a very few days. The salient features of the new method, the full details of which are given in *The Journal of Pharmacology and Experimental Therapeutics,* 1927, xxxi, 65, can be seen from the following brief description of a typical experiment in which, starting with 2.001 grams of a Toronto powder evaluated at 13 units per milligram, there was obtained a total of 0.5284 gram of crystalline insulin. Various samples of these crystals have been submitted to the insulin committee at Toronto for standardization, but owing to the press of other work the committee has not as yet been able to make a report on them. Our own standardizations of a recrystallized preparation against the International Standard Powder gave us a conservative value of 40 international units per milligram.

To the powder, dissolved in 20 cc. of 10 per cent. acetic acid, was added 80 cc. of brucine acetate solution (1 gram of base in 18 cc. of N/6 acetic acid) and then 40 cc. of 13.5 per cent. aqueous pyridine; the resulting "pyridine precipitate" was centrifuged off and the clear fluid treated with 40 cc. of 0.65 per cent. aqueous ammonia. The "ammonia precipitate" so obtained was likewise centrifuged off and the fluid set aside to crystallize in an Erlenmeyer flask. Next morning the walls and bottom of the flask were found lined with crystals which, after washing and drying, weighed 0.2776 gram. The "pyridine" and "ammonia" precipitates treated in the same way gave further crops of 0.1458 and 0.0614 gram, respectively, and a final crop of 0.0436 gram was obtained from the residue left by evaporating in a current of air at room temperature the mother liquors from the preceding fractions. The total yield of crystals was thus 0.5284 gram.

Starting with liquid preparations, the procedure is the same except that the crude insulin is first precipitated with insulin as described in earlier papers.

Measurements with the Michaelis' nitrophenol indi-

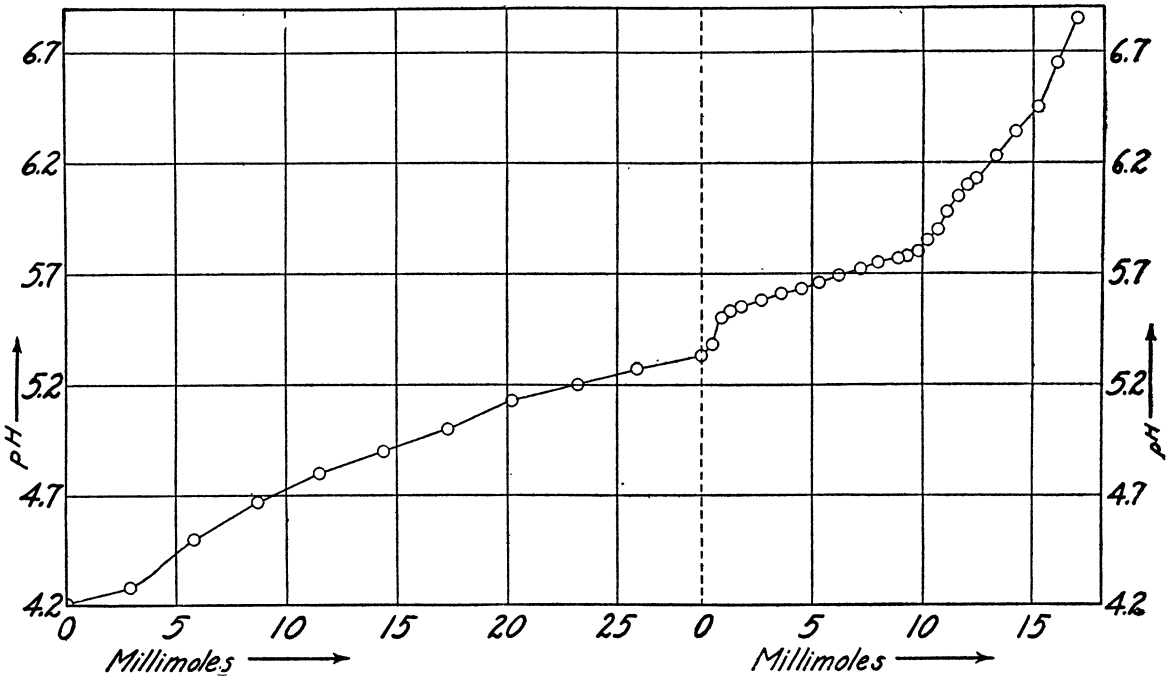


FIG. 1. TITRATION CURVE SHOWING pH VALUES OF A MIXTURE OF 40 CC. OF BRUCINE ACETATE SOLUTION (1 GRAM OF BRUCINE ALKALOID DISSOLVED IN EACH 18 CC. OF 0.132 N ACETIC ACID) AND 10 CC. OF 1.317 N ACETIC ACID UPON THE ADDITION OF PYRIDINE AND AMMONIA.

A total of 20 cc. of 1.441 N pyridine was added (left portion of curve), followed by 38 cc. of 0.444 N ammonium hydroxide (right side of curve). The amounts of the bases added are plotted in millimoles. The hydrogen-ion concentrations were estimated colorimetrically with Michaelis' nitrophenol indicators, using 0.1 cc. of buffer mixture for each determination.

cators as well as with the quinhydrone electrode showed that the pH of the solution from which the insulin separated in crystalline form was 5.55–5.65. After centrifuging off the "ammonia precipitate" it may be necessary to add a little more ammonia to the fluid to bring it to the proper hydrogen-ion concentration before setting it aside to crystallize. The accompanying curve shows how the pH of a mixture of acetic acid and brucine, made up in the proportions employed in this method, varies with the gradual addition of the usual amounts of pyridine and ammonia.

The crystals are apparently dimorphous and fall into two general groups: (1) Crystals with well-defined double refraction, of negative character, with several habits, in the rhombohedral class; (2) crystals of a more equant habit, often with clearly defined crystal edges and no double refraction.

They give the Pauly, Millon, biuret and ninhydrin reactions but not the Voisonet, Hopkins-Cole or Acree tests for tryptophan or the Sullivan test for free cystine and cysteine.

The many solutions (in acetic acid, hydrochloric acid and ammonia) examined polarimetrically were always found to be laevo-rotatory, the magnitude of the rotation varying widely with the concentration and pH of the solution and with the nature of the solvent. For example, one preparation in hydrochloric acid showed a specific rotation of $-40°$; another, twice recrystallized, gave $-30°$ in N/6 acetic acid and $-17°$ in 0.011 N hydrochloric acid; with another in 0.65 per cent. ammonia the rotation was $-48°$ and changed in the course of several days through a maximum at $-63°$.

Numerous microanalyses on various preparations gave very concordant results agreeing closely with the empirical formula $C_{45}H_{69}O_{14}N_{11}S$ in the case of material dried at 105–20° in nitrogen under low pressure and $C_{45}H_{75}O_{17}N_{11}S$ (or $C_{45}H_{69}O_{14}N_{11}S \cdot 3H_2O$) for air-dried preparations; the labile or so-called "carbonate" sulphur content of the latter is about 1.10 per cent. or approximately 37.5 per cent. of the total sulphur. No satisfactory solvent for molecular weight determinations has yet been found.

No evidence has ever been obtained which would indicate that the crystals are not a homogeneous substance crystallizing in different types but a mixture of two substances, only one of which is physiologically active but both having the same solubilities and identical or nearly identical empirical compositions.

JOHN J. ABEL

THE JOHNS HOPKINS UNIVERSITY

SCIENTIFIC EVENTS

A STUDY OF ASCARIASIS

THE American Child Health Association has arranged to furnish support for an extended investigation of ascariasis, an infestation widely prevalent especially in children. Through the courtesy of the Johns Hopkins University, the work will be conducted under the direction of Professor W. W. Cort, of the department of helminthology of the School of Hygiene and Public Health, under the auspices of the division of medical sciences, National Research Council, through its Committee on Medical Problems of Animal Parasitology.

Professor Cort and his selected staff will investigate the life history of the parasite, its mode of transmission, the incidence of infestation, the effects upon infested animals and man and the methods of treatment and control. The central feature of the program will be the relation of this parasite to the health and development of children, since it is in young children that the infestation is the heaviest and the injury produced the greatest. Studies are to be undertaken in the School of Hygiene and Public Health, with the admirable facilities there available. Most of the investigations, however, will be in the field for which stations will be established at strategic points in the United States and their territories and insular possessions. The information and material yielded by the field work will be further studied, amplified and extended by, and correlated with, the investigations in Baltimore.

HOWARD T. KARSNER, *Chairman,*
Division of Medical Sciences,
National Research Council

GIFT OF WARD'S NATURAL SCIENCE ESTABLISHMENT TO THE UNIVERSITY OF ROCHESTER

THROUGH the gift of members of the Ward family, ownership of Ward's Natural Science Establishment, Rochester, N. Y., passes to the University of Rochester under conditions enabling its museum features to be preserved and its scientific work carried on.

Founded in 1862 by Professor Henry A. Ward, then holding the chair of geology in the University of Rochester, the establishment was carried on from the early eighties by the late Frank A. Ward, son of Levi A. Ward, who had largely financed the undertaking. Professor Henry A. Ward spent a large part of the year in travel in all parts of the world in search of specimens which were assembled and arranged at the workshops.

The following paragraphs are taken from a statement on the gift made by Raymond N. Ball, treasurer of the University of Rochester:

> The University of Rochester feels greatly honored in being asked to accept the splendid foundation which the Ward family proposes to found in memory of Frank A. Ward.
>
> It was the energies and business ability of Frank A.