ENDOCRINES IN THEORY AND PRACTICE

This article is one of a series on Endocrinology contributed by invitation

THE CHEMISTRY OF OESTROGENIC COMPOUNDS AND METHODS OF ASSAY

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It is difficult to state with certainty which worker obtained the first potent extract of the ovary and of the testis, but there is little difficulty in recognizing the pioneer work from which our present-day exact knowledge has developed.

In the case of the ovary the work of Allen and Doisy (1923) can be unhesitatingly quoted as laying the foundation for all the successful work that has been accomplished on this subject. It will be remembered that these workers demonstrated that an active stable substance could be extracted from the ovary which, when injected into castrated rodents, was capable of re-establishing the reproductive or oestrous cycle. Once a stable and uniformly active preparation had been obtained it followed that chemists and biochemists would attempt to discover the actual structure of the active principle. No progress was made, however, in this direction other than to show that it consisted of an organic compound soluble in organic solvents and containing carbon, hydrogen, and oxygen.

Attempts at purification failed owing to the large number of substances of a similar constitution which were extracted together with the hormone. The next step was made by Aschheim and Zondek (1927), when they discovered that the oestrus-producing hormone was excreted in the urine of pregnant animals. It is excreted in a form very suitable for purification, and since relatively enormous quantities are excreted a large supply of starting material was thus provided for the chemist. The usual chain of events in the establishment of the identity of a compound rapidly followed. The substance was crystallized by Doisy (1929) in America and by Butenandt (1929) in Germany independently, and considerable advance was made with regard to its probable structure. It is not proposed to attempt even a summary of the experimental evidence that can be advanced for the constitution of the oestrus-producing hormone, since this is a matter of pure organic chemistry. It was shown by a number of workers that the oestrus-producing hormone is related to the sterols, and it is interesting to note that the constitution of this group of substances had been worked out only a short time before the purification and crystallization of the sex hormones. Unless the structure of the sterols had been discovered no progress would have been made in the work on the constitution of the sex hormones.

Up to the present five oestrus-producing substances have been isolated from either the ovary, the urine of pregnancy, or the placenta. These substances are:

Of these compounds oestrone was the first one to be isolated in crystalline form and was for a considerable time looked upon as being the true hormone of the ovary. By a process of partial hydrogenation it was found possible to convert the ketone group into a hydroxyl group, thereby giving rise to the compound now known as oestradiol. It has been shown that this is some five times as active as oestrone itself, and workers in this field were faced with the rather unusual state of affairs that an artificial substance was apparently more active than the natural one. Later, however, oestradiol itself was isolated from follicular fluid and from the urine of pregnancy, and it is now regarded as being the true hormone of the ovary.

From the clinical standpoint the only compounds which find extensive use to-day are oestrone and oestradiol, and it is interesting to note that oestradiol appears to be assuming major importance in therapeutic use. Equilene and equilenin, though capable of producing oestrus, are not so active and are not available to the clinician.

Standardization

Standardization has presented a great many problems, and despite the successful application by Allen and Doisy (1923) of the vaginal smear method, no thoroughly comparable results were obtained until the crystalline material had been prepared. The process of the method of standardization is to inject subcutaneously a series of castrated rats or mice with the material, and by following the vaginal smear to determine whether oestrus supervenes. Every type of unit has been defined, and until recently complete chaos reigned.

To indicate the difficulties of standardization it must be pointed out that the activity of a given sample, even when in crystalline form, depends upon the vehicle in which it is dissolved and the number of spaced injections which are administered to the animal. By giving an aqueous solution (obtained by diluting an alcoholic solution) in a large number of small doses spread over forty-eight hours it is possible to enhance the activity to a very remarkable extent. Thus a solution which is inactive when injected in one or two doses will be highly active when administered in, say, six smaller doses. Single injections of oily solutions, again, yield quite different results. From this it can be seen that the old terms of "rat unit" and "mouse unit" really mean nothing except to the actual person who has conducted the test. Further, even though the same method of injecting is used, unless a large number of animals are employed for each test the results will not be reproducible.

An attempt was made to clear up this situation by the Committee on Hormones of the League of Nations Permanent Health Commission. The committee suggested the adoption of a process well established in

biological assay—namely, that of defining the activity in terms of a standard preparation. This method has worked excellently in the past in the case of hormones such as insulin, oxytocin, etc., and its application to the sex hormones has certainly clarified the issue considerably. At the last meeting of the committee a number of standards were laid down. The international unit for oestrin was given as 0.1 γ . In its use the investigator takes the international standard and finds out the quantity required to produce oestrus in 50 per cent. of ovariectomized rats or mice: he then determines the amount of the unknown preparation required to produce the same response, and by this means a standardization is effected. By this technique the variability of individual animals is overcome, and at the same time a control is obtained on the use of solvents, spacing of injections, etc.

The committee pointed out that since the crystalline pure preparations were becoming more and more easily available the necessity for biological standardization became less and less, and that in time, with accurate chemical characterization, weights of crystalline material and not units should be prescribed.

By far the most important preparations on the market to-day which are employed clinically are the benzoylated derivatives of the hormones. The action of these compounds is much more prolonged and gives rise to more lasting effect. The greater part of the clinical work has been performed with the benzoylated oestradiol, the esterification occurring at the phenolic hydroxyl group. The international standard of this compound is recognized at 0.1 γ . This unit, although suitable for experimental work on rats and mice, requires very great multiplication before it can be used in human therapeutics. Since these hormones act quantitatively in relation to the body weight it necessarily follows that a castrated woman will require many hundreds of thousands of units of oestrone and correspondingly less of oestradiol benzoate in order to effect the menstrual response. This gives the impression that very large quantities of hormone are being administered when in actual fact the quantities in terms of weight are only very small. It is to be hoped that in the near future agreement will be reached by the commercial firms supplying this material, and that the labels on the preparations and the commercial literature will indicate the milligrammes of crystalline material to be prescribed, thus avoiding the confusion of the biological unit.

The fact that there are five naturally occurring oestrogenic compounds all differing slightly in molecular structure leads to the question as to what chemical groupings are actually necessary for the production of oestrus. It is interesting to note that a series of synthetic oestrogenic compounds of relatively simple molecular structure have been described (Cook, Dodds, Hewett, and Lawson, 1934; Cook, Dodds, and Lawson, 1936). Molecules as simple as 1-keto-1:2:3:4-tetrahydrophenanthrene, and alkyl derivatives of 9:10-dihydroxy-9:10-dihydro-1:2:5:6-dibenzanthracene are found to be capable of replacing

pounds, in common with oestrone and oestradiol, all possess the phenanthrene nucleus, and until comparatively recently it was thought that this ring system was essential for the production of oestrus-producing properties. It has been shown, however, that this is not the case (Dodds and Lawson, 1936; Dodds, 1936), and a surprisingly simple series of compounds have been synthesized capable of producing oestrus. The most simple of these is 4:4'-dihydroxydiphenyl.

It is not possible at present to give an explanation as to why these simple compounds are active, nor is it possible to predict the essential groupings for oestrogenic activity. There still remains a great deal of work to be done on the simpler oestrogenic compounds before any adequate theories concerning the relation between molecular structure and oestrogenic activity can be formed.

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INTERNATIONAL CO-OPERATION ON HEALTH QUESTIONS

The report of the medical director of the International Health Office on the work of the session of the Permanent Committee of that body held in Paris last October has now been issued.¹ Among the matters discussed was the role of the Office under the projected scheme of reform of the Health Organization of the League of Nations. The terms of the new statute provide that the Permanent Committee shall continue to act as general advisory health council of the League and hold an annual assembly of the council at the end of one of its sessions, to which assembly representatives of States which do not participate in the work of the Office will be invited and at which members of the Health Committee of the League will have the right to attend.

Venereal Disease in Seamen

The efforts made to ensure continuity and uniformity of treatment in the different ports of seamen suffering from venereal disease seem to be bearing fruit. Australia, Belgium, Canada, and the Dutch colonies of Curaçao and Surinam have now been added to the countries which have notified the Office that the treatment recommended in the recent pamphlet distributed by the Office will be applied in future in their treatment centres.

Sanitary Convention for Aerial Navigation

Progress was reported in the designation of aerodromes according to the various categories specified in the International Convention of 1933, and particularly of anti-amaril aerodromes. It was agreed that the sanitary authority of the aerodrome is competent to decide which of the passengers of an aircraft have been exposed to yellow fever infection.

Discussion took place on the recent action of the Government of India in refusing access to its territory to (1) any aircraft within nine days of its leaving a country where the existence of yellow fever has been demonstrated either clinically or by the mouse protec-

¹ Paris: Office International d'Hygiène Publique, 195, Boulevard Saint-Germain.

1-keto-1:2:3:4-tetrahydro-phenanthrene.

9:10-dihydroxy-9:10-dihydro-1:2:5:6-dibenzanthracene.

oestrone in all its known actions. Maximum activity is obtained in the latter compound when the alkyl substituent is *n*-propyl. It is interesting to note that these com-