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EFFECTIVE ABSORPTION OF HORMONES

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In the preparation of active extracts of the endocrine organs it has usually been found that very little of the active substance is contained in the gland compared with the amount which has to be injected to produce an effect in the test animal. This discrepancy is no doubt due largely to the fact that there is continuous secretion by the gland and little storage, so that the content at any given time may represent but a small fraction of a day's output. The amounts which it is necessary to administer are, however, often unnaturally high because of low efficiency resulting from the method of administration. The normal gland must be supposed to keep up a slow, steady secretion which maintains a threshold concentration in the circulation but avoids wasteful or harmful excess. The exogenous injected hormone, on the other hand, is usually absorbed rapidly and utilized, destroyed, or excreted. In these circumstances the concentration in the blood will be highest shortly after injection, and will then fall away till a sub-effective concentration is reached. In many cases there can be little doubt that this cycle occupies only a few hours, leaving the organism deficient of the hormone until the next injection. If the dose is raised so as to prolong the period during which effective amounts of the hormone are present, the wastage at the time of maximum concentration will be correspondingly greater. The effectiveness of a method of administration thus depends on the degree to which the necessary concentration in the blood can be maintained at a steady even level slightly above the threshold value. The problem of how to attain this end is one of the most important in endocrine therapy.

Oestrogenic Substances

The influence of method of administration on effectiveness was seen early in the work on ovarian extracts when Allen and Doisy and their co-workers (1924) found that a certain amount of active substance was more effective when given as a series of small doses than it was in one large dose. As preparations became more purified and semi-aqueous solutions were prepared, the advantage of divided dosage became very clear. Laqueur and de Jongh (1929) found that less than one-thirtieth of the active substance required to give a response as one injection was effective when given as six injections over thirty-six hours. Similar results were obtained by Allan *et al.* (1928), and Marrian and Parkes (1929). These facts are, of course, of importance from the points of view of both clinical dosage and biological assay.

The problem is further complicated by the fact that the various naturally occurring oestrogens are differently

affected by alteration of the method of administration. When oestrone and oestriol were first isolated from pregnancy urine no two observers agreed as to their relative activity. It was soon realized that this was due to the difference in the methods used for comparison and to the more rapid and less effective absorption of oestriol. At the first International Conference on the Standardization of Sex Hormones, held in 1932, a preparation of oestrone was established as an international standard for oestrus-producing hormones, but it was realized at the time that attempts to assay oestriol in terms of the international unit, defined as the specific activity of 0.0001 mg. of the oestrone standard, would lead to difficulties. The importance of the absorption problem was also recognized by a stipulation that, in assay work, absorption should be retarded either by the use of oily media or by multiple injections. The third of the important natural oestrogens—oestradiol—originally produced by reduction of oestrone but afterwards isolated from the ovary by Doisy, is more like oestrone in its behaviour than is oestriol; by the plumage test on brown leghorn capons it was not possible to demonstrate that it has a less prolonged action following a single injection than has oestrone. There is little doubt, however, that the activity relative to oestrone by the ordinary test on the ovariectomized rat or mouse may be influenced by the method of administration.

The necessity for multiple injections is shown to an increasing extent by oestrone, oestradiol, and oestriol, in that order. This is almost certainly due to increasing solubility in body fluids, and is in close relation to the number of hydroxyl groups present. The comparative inactivity of these compounds by mouth is probably caused by lack of absorption from the gut, since there is little evidence of destruction by the digestive enzymes. It might be expected that oestriol, the most water-soluble, would be the most active by mouth. The evidence available is concordant with this supposition (Rowe and Simond, 1936). Another method of administration, union of an oily or fatty solution, tends to increase the duration of action of the hormone (Deanesly and Parkes, 1937), but the method is not efficient except where the responding structure is superficial and can be treated directly (as the mammary gland, comb, plumage, etc.). Even so, in the absence of detailed information, one must deplore the increasing tendency to add oestrogenic substances to face creams.

A method of obtaining optimum efficiency other than by multiple injections was demonstrated by Butenandt and Störmer (1932) when they showed that the benzoate of oestrone had a prolonged action. Oestradiol mono-

benzoate behaves in much the same way. This prolongation of action is not seen if the compound is administered intravenously (Deanesly and Parkes, 1937), and it must therefore be due primarily to the esterification having decreased the solubility of the hormone in body fluids, and having thereby increased the time taken for absorption. Frequent injection is thus no longer necessary to achieve high efficiency with oestrogenic compounds.

A large number of esters of oestrone and oestradiol have been examined in a search for greater potency. David *et al.* (1935b) found that oestradiol diacetate has a more prolonged action than the free hormone, while oestradiol dibenzoate has a very prolonged action but requires a high minimal effective dose. Working with the feminization of plumage test, I (Parkes, 1937) found that oestrone acetate, oestradiol diacetate, oestradiol monobenzoate, oestrone benzoate, oestradiol-3-benzoate-17-acetate, and oestradiol dibenzoate showed increasing duration of action. Miescher, Scholz, and Tschopp (1937) have recently shown that a series of aliphatic esters of oestrone have durations of action proportional to the length of the acid chain. The higher esters, showing very prolonged action, have a high threshold dose. Deanesly and Parkes (1937) found that, so far as the plumage test is concerned, oestrone has the most prolonged action when administered by a single intramuscular implantation of a solid crystal of the free hormone. Given so, doses of 2 to 3 mg. continued to feminize plumage for about three months, a result far beyond those obtained with even the most effective esters in solution.

This body of facts makes it clear that it is not practicable to assay an ester in terms of the free hormone, since the result will depend entirely on the comparative test methods. In view of this fact, and of the increasing use of oestrone benzoate and oestradiol monobenzoate for clinical and experimental purposes, the second International Conference on the Standardization of Sex Hormones, held in 1935, established a preparation of oestradiol benzoate as an international standard for benzoylated preparations. The unit was again defined as the specific activity contained in 0.0001 mg. of the standard, but it was expressly stated that no equivalence of biological activity with the unit of free hormone was implied. The relation between 1 I.U. (international unit of free hormone) and 1 I.B.U. (international benzoate unit), whether clinically or experimentally determined, must depend on the method of administration and the test object employed, and can have no general significance. Further, it is doubtful whether it is practicable to standardize one ester in terms of another. As the number of market preparations of oestrone and oestradiol and their esters is likely to increase, and it is obviously impracticable to set up standards for every oestrogenic compound, it is highly desirable that the recommendation of the 1935 Conference should be followed by manufacturers, and that the precise nature and amount of the substance present should be marked on each ampoule. Only thus can the clinician have adequate data upon which to base an opinion as to the relative merits for clinical use of the various compounds available.

Male Hormones

Many of the facts described above for the oestrogenic substances apply with equal force to the various male hormone compounds. The first crystalline substance with androgenic activity was obtained from men's urine by Butenandt (1931). This had a fair activity on the capon comb but a low activity on rats, and it was noted by

Freud (1933) that, in relation to the activity on capons, the capacity of urine extracts to stimulate the accessory glands of the castrated rat decreased as the extracts were purified. A similar odd phenomenon was observed in the case of testis extracts. In comparison with androsterone or urine extracts, testis extracts were highly active on rats in relation to their activity on capons. When testosterone was isolated from testes it was found, as ordinarily injected in concentrated oil solution, to be less effective on rats than had been expected. David *et al.* (1935a) showed that recombination of the pure testosterone with discarded inert fractions of the testis extract led to greatly enhanced activity of the testosterone. Subsequently Miescher and his co-workers (1936a) showed that addition of any one of a large number of fatty acids to the testosterone solution led to the same effect, while Deanesly and Parkes (1936) found that increase of the volume of oily medium led to increased effectiveness of both androsterone and testosterone. It seemed clear, therefore, that the loss of effectiveness on rats of purified urine extracts, and the unexpectedly low effectiveness of crystalline testis hormone in concentrated oil solution, were caused by too rapid absorption from the site of injection, and that absorption could be delayed and the hormone made more effective by the re-addition of discarded fractions or fatty acids, or by increasing the volume of oil used for injection. The disturbance of the ratio of activity on capons to that on rats was due to the fact that effectiveness on capons is much less influenced by the presence of inert substances than is effectiveness on rats.

By analogy with the oestrogenic substances it seemed likely that a useful advance in the technique of administration might be made by esterification of the hormone. Miescher and his co-workers (1936b) made a series of aliphatic esters which showed an increasing duration of effect with increasing length of the acid chain. They were able to choose testosterone propionate as having the optimum combination of intensity and duration of activity. This compound, as examined by Parkes (1936), was more effective when a certain dose was given as two injections over ten days than when a corresponding amount of the free hormone was given as twenty injections over ten days. Since daily injection may be highly inconvenient for the clinician, testosterone propionate would seem to be a very suitable preparation in cases where male hormone therapy is indicated. Testosterone propionate, like oestradiol benzoate, shows no prolongation of effect if administered by intraperitoneal or intravenous injection, which ensures rapid or instantaneous entry into the circulation (Deanesly and Parkes, 1937), and it is evident that, as with the oestrogenic ester, the prolonged action is due primarily to slow absorption from the site of subcutaneous injection. Significant experiments are recorded by Deanesly and Parkes, who have shown that the effectiveness of free testosterone is greatly increased when it is administered by a single subcutaneous implantation of a compressed tablet of pure dry hormone. Under these conditions free testosterone becomes as effective as the propionate in oil solution. The propionate also shows a greater duration of action when given in tablet form. Even a small tablet of testosterone is only partly absorbed by the castrated rat in the course of a ten-day experiment, though great development of the atrophic accessory organs takes place during this time. The tablet can be recovered from the subcutaneous fascia and reweighed, and the dose absorbed by the rat calculated. The residual tablet can then be used for implantation a second time. With free testosterone this process has been repeated three times with a 2-mg. tablet. With androstenediol, a highly active

reduction product of androsterone, which is very insoluble in body fluids, no fewer than thirteen successive implantations of the same set of 6-mg. tablets were made into successive sets of castrated rats for ten-day periods. The total time of each tablet in the animals was thus 130 days—about four calendar months. At the end of this time the tablets were reduced almost to nothing, but the remaining material was still physiologically active. The total weight of accessory organs produced in these experiments was incomparably greater than can be obtained by the injection of 10 mg. of the hormone in oil solution.

Tablet administration has the obvious advantage that there is no necessary fluctuation in the amount of material absorbed, such as must result from even frequent injection. Once absorption has begun it will continue week after week, or even month after month with substances of suitable solubility, declining steadily as the tablet decreases in surface area, but free from minor irregularities. There is no obvious local reaction in experimental animals following the subcutaneous implantation of tablets of oestrone, testosterone, or testosterone propionate. The evidence that neither testosterone nor oestrone is inactivated by long incubation at body temperature in the subcutaneous fascia is conclusive, and it would seem that where very prolonged effects are required a clinical trial of the technique is warranted.

Progesterone

The effectiveness of progesterone does not seem to be so much altered by conditions of administration, though possibly this is because the rabbit, the usual test animal, seems to make more economical use of injected gonadal hormones than does the rat (Deanesly and Parkes, 1937). So far as our preliminary experiments show, tablet administration of progesterone gives no increase of response in short duration experiments as compared with daily injections, possibly because absorption is extremely slow, but it is already obvious that a very prolonged effect from a single administration could be obtained by the tablet technique. In view of the difficulty of esterifying progesterone the technique may be of practical value.

Other Substances

Problems of absorption are not peculiar to gonadal hormones. It is well known that the effectiveness of gonadotropic extracts is raised by increasing the subdivision of the dose. Addition of a precipitant such as zinc sulphate to the solution also greatly increases effectiveness, presumably by retarding absorption (Maxwell, 1934). Unfortunately their high water solubility and their lability in contact with water at body temperature make gonadotropic preparations unsuitable for tablet administration. The observations of Bates and Riddle (1936) on the influence of route of administration on the effectiveness of prolactin, and those of Ch'en and van Dyke (1936) on increasing the action of thyrotropic extracts by the addition of merthiolate, are also relevant.

There are a number of well-known examples of the same principles applying to other hormones, notably the work leading up to the production of protamine insulin. In general it may be said of many of the hormones concerned in producing chronic effects that purification and isolation make the active substance so readily absorbed by the animal that in practical use high effectiveness can only be obtained by frequent injections or by retarding absorption in one of the ways described above.

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OTITIS EXTERNA: "HOT-WEATHER EAR"

AN INVESTIGATION OF 100 CASES AND A METHOD OF TREATMENT

BY

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The scope of this contribution is limited to inflammation of the external auditory meatus apparently caused by hot humid climatic conditions and associated with an infection with *B. pyocyaneus* in pure culture. At the aural clinic of the Royal Air Force Hospital, Aden, a series of 100 cases was investigated, a line of treatment evolved on a simple practical basis, and search made for a common causative factor.

Occurrence

Infections of the external auditory meatus with *B. pyocyaneus* (*Pseudomonas pyocyanea*) among the troops in Egypt were described during the great war, and more recently have been reported from the Seychelles, Canton, Ascension, and East Africa (Hermitte, 1932), America (Greaves, 1936), and Australia (Bettington, 1934). Inflammations of the meatus are common in the troops generally,* and are often ascribed to a fungus infection. The conditions appear to be very similar and equally resistant to treatment, but as none of the cases which were bacteriologically examined in this series was found to have a fungus infection or any organism other than *B. pyocyaneus* and *Corynebacterium ceruminis diphtheriae*, no attempt is being made here to discuss any other infection.

It has not been possible to assess the incidence of the trouble in Aden. Many cases are treated as out-patients elsewhere than at the Royal Air Force Hospital, but all types seem to be affected, and it is by no means confined to the troops. A record of the monthly total of new cases was kept (see Chart), and it was noted that the majority were affected during the May-June and August-September

* This subject is dealt with in a joint discussion on the effect of aural conditions on fitness for active service, *Proc. roy. Soc. Med.*, 1937, **30**, 1523.