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PELLET FOR ADMINISTERING GONADOTROPIC PITUITARY HORMONES

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This invention relates to a method of administering the gonadotropic fractions of the hormones of the anterior pituitary gland, more particularly to a method which causes a very slow constant absorption of the active hormone material by the tissues, and to a solid, relatively non-absorbable preparation of these fractions, suitable for subcutaneous implantation, for use in this method of administration. The present application is a continuation-in-part of our co-pending application, Serial No. 391,456, filed May 2, 1941, and of an earlier application, Serial No. 361,950, filed October 19, 1940, of which the first-mentioned application was a continuation-in-part.

It is well known that most ductless glands function in a substantially continuous manner, secreting their hormones into the blood gradually, as needed by the body. The rate of secretion may vary widely with circumstances, but in no case does a process of natural secretion even remotely resemble the common method of therapeutic administration which is to present relatively massive doses of the hormone, in relatively readily absorbable form, and at relatively distant intervals. Such a method of administration results in the temporary presence of excessive amounts of hormone, followed by longer periods when the hormone is present in insufficient amount or may be entirely absent. It is one object of this invention to provide a means of administration of the anterior pituitary gonadotropic hormones which resembles natural secretion in that there is a slow, more or less uniform and prolonged release of the active material, from depots which may be established at relatively infrequent intervals. This leads to the further practical result that clinical use of these hormone fractions is simplified and rendered more satisfactory by requiring less frequent treatment at the hands of the practitioner.

It is recognized, further, that the common method of administration of the anterior pituitary gonadotropic hormone, as referred to above, is wasteful of hormone substance in that relatively large doses must ordinarily be administered in order to prolong the effect for more than a very short interval, much of the excess being quickly destroyed due to the labile nature of this hormone substance. It is another object of this invention to provide a method of administration of this hormone which requires, in many instances, distinctly smaller doses than methods heretofore in use to achieve the desired effects.

Closely related to this object is a further one,

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based upon the established property of this hormone that whereas small, repeated injections of this hormone lead to a significant gonadotropic response, single injections of even massive doses are of negligible effect. For example, in one method of conducting the routine assay of this hormone, it is specified that the test dose be administered in six injections given twice daily for three days. The same total dose, administered in only three daily injections, produces a radically diminished response, while doses up to five hundred times the amount required to produce the end point when administered according to the assay directions, have been totally ineffective in producing this end point when given in a single dose. Thus, it is seen that response to a given amount of this hormone is increased by administration of the total dose of hormone in an increasing number of injections each of decreasing size. A further object of this invention is to present this hormone in a form which is maximally effective, that is, where the total dose may be considered as being divided into an infinite number of infinitesimal doses.

Another closely allied object of this invention is to increase the maximum response obtainable in the use of these hormone fractions, regardless of dose. It is a widely recognized principle in pharmacology that, while increasing doses of a medicament may elicit more or less proportionate increases in response from the organism being treated, yet this proportionation ordinarily holds only over a limited range of doses. When the upper limit of this range has been exceeded, the increase in response becomes less and less, until finally there is obtained a maximal response, which cannot be increased by any dosage. This phenomenon of maximal response is observed in the administration of the anterior pituitary gonadotropic hormones in experimental animals. However, by the use of the invention described herein, we have been able consistently to produce responses in experimental animals exceeding the maximum which we have heretofore been able to obtain by standard injection techniques.

A further object of this invention is to provide a practical, convenient method of marketing these hormone fractions in a completely stable condition. It is acknowledged that aqueous solutions of anterior pituitary gonadotropic hormones are unstable with respect to time, even when stored in refrigerators, while desiccated powder preparations of such hormones are indefinitely stable. Since the only heretofore known, effective method of administration of these hor-

mones is by injection of the aqueous solution, a manufacturer desiring to offer a stable product must prepare and market a dry powder in ampoules suitable for the extemporaneous preparation by the physician of a solution for injection. This is frequently a difficult and expensive procedure for the manufacturer, and the preparation of the solution is ordinarily a source of annoyance to the physician. By the use of the invention herein described, these objections are largely overcome and a convenient stable product is made available.

We have discovered that all of the above objects, as well as others which may become apparent elsewhere in this disclosure, may be attained by the administration of the gonadotropic fraction of the hormones of the anterior pituitary gland in the form of a pellet, which pellet consists of a dispersion of the desiccated, powdered, water-soluble hormone substance in a waxy medium which is solid at body temperature and relatively non-absorbable by body tissues. This pellet is implanted under the skin of the individual under treatment, resulting, in spite of the relative non-absorbability of the medium in which the hormone is dispersed, in a striking manifestation of the hormone effects, as can be quantitatively demonstrated in experimental animals. This manifestation of effect is striking, unusual, and unexpected, not only in its duration (for it is commonly accepted that individual doses of anterior pituitary gonadotropic hormones are comparatively evanescent and must be frequently repeated to become effective), but also in its magnitude of response, as will be shown in the table cited below.

We have found that the nature of this waxy dispersing medium exerts considerable influence upon the results which may be obtained by this method of administration. It is important that the medium be not fluid at body temperature, for we have found that fluid media permit too ready absorption of the hormone substance, thereby preventing the attainment of the above-stated objects of this invention.

The terms "wax" and "waxy" as used in this specification and in the appended claims are not to be construed as referring only to substances of the chemical class ordinarily defined as the esters of long-chain alcohols with long-chain fatty acids, but it is intended to include substances of similar physical properties of whatever chemical composition, such as the hard fats, paraffin (and other mineral waxes derived from petroleum) and the so-called synthetic waxes of various chemical types. The waxy substances which we have found useful as dispersion media may be recognized by these common properties: they are insoluble in water (though generally soluble in such organic solvents as ether and chloroform); they soften appreciably before melting when warmed; they are, in general, greasy to the touch; they tend to spread or flow rather than disintegrate during such an operation as grinding; their melting point lies above 40° centigrade; they are substantially amorphous in nature. With respect to the latter limitation, it is recognized that certain of the waxes (particularly the "synthetic waxes") contemplated within this invention may, in fact, present a microcrystalline structure, especially when carefully purified, though their gross appearance and general properties resemble those of the more truly amorphous waxes. Though certain substances of the classes known as resins, plastics, and synthetic

rubbers may possess some of the properties enumerated above, they are not to be considered as coming within the scope of this definition. The following substances are illustrative of the waxes contemplated as dispersion media in the practice of this invention, though the invention is not to be construed as limited thereto: beeswax, bayberry wax, Chinese wax, paraffin wax, ceresin, stearin, palmitin, tallow, myristin, spermaceti, Japan wax, cetyl alcohol, stearic acid, propylene glycol monostearate, glycerol monostearate, oleostearin, hydrogenated coconut oil, and a variety of synthetic substitutes now offered on the market and in use at the present time. Among the latter may be mentioned the waxes currently sold under the following trade names: "Gellowax," "Flexowax C," "Glycowax A," "Nipocer," "BZ Wax A," "Ceraflux." For illustration of the chemical composition of these waxes, "Flexowax C" is a hydrocarbon wax made up of approximately 85½ per cent by weight of carbon and approximately 14½ per cent by weight of hydrogen, while "BZ Wax A" is a modified hydrocarbon wax made up of approximately 84 per cent by weight of carbon and approximately 14 per cent by weight of hydrogen.

These substances may be further modified by admixture with each other or with other compatible fats or oils for the purpose of modifying their physical nature. An example of such admixtures is the addition of coconut oil to a hard wax to soften it.

As will be expected, the various substances contemplated as dispersion media will vary among themselves in the efficiency with which they achieve the objects of this invention. This efficiency can be further modified by varying (1) the ratio of the weight of the medium to the weight of the water-soluble hormone material dispersed therein and (2) the size and shape of the resultant pellet. For most substances, we have found it desirable to use equal weights of wax and water-soluble hormone material. However, in the case of a solid fat such as oleostearin which is relatively easily penetrated by body fluids, we have found it effective and desirable to use up to nine parts of medium to one of hormone. On the other hand, in employing some of the harder waxes unmixed with a softening agent, we have found ratios of four or even five parts of hormone to one of wax to be desirable.

Using a hormone preparation of a given degree of purity (i. e., a given number of rat units of activity per milligram), fixing the dose and the ratio of wax to hormone will automatically fix the size of the pellet. The size may be varied independently of both dose and ratio, however, by the expedient of diluting the powdered hormone substance with a suitable inert, non-toxic, water-soluble filler such as lactose or sodium chloride, and considering the total diluted material as the water-soluble hormone substance when adding wax to the desired ratio. In general, it may be said that the greater the ratio of water-soluble hormone substance to waxy dispersion medium, and the smaller the total size of the pellet, the more rapid will be absorption of the hormone activity by the host animal. In order best to attain the objects of this invention, this absorption should be neither too rapid nor too slow, but should, in the ordinary case, be largely accomplished within a period of about four to twenty days. This rate of absorption may be varied widely by the means discussed above to suit indi-

vidual conditions, such variations changing only the degree rather than the kind of response.

These pellets may be prepared in any convenient manner with the limitation that heat must be cautiously applied since the hormone is readily denatured and rendered inactive by excessive heating. In most cases, we have found it desirable to mix the hormone with the waxy medium while the latter is held in the molten state at the lowest possible temperature. When the resultant mass is cool, it is extruded through a die under pressure, forming a rod which can be cut into lengths of appropriate weight to contain the desired dosage. Cylindrical pellets of approximately 1.5 mm. in diameter have been found convenient to implant and to give a reasonable rate of absorption.

Example 1

The following is a specific example of how this invention may be carried out on an experimental animal. A sample of desiccated anterior pituitary gonadotropic hormone of known potency is mixed with sufficient lactose so that the mixture has one rat unit in five milligrams of mixture, and a convenient weight of this mixture is thoroughly incorporated into an equal weight of one of the media specified in the table, warming the mass just sufficiently to permit thorough mixing. The mass is cooled, and extruded through a 1.55 millimeter opening to form a rod. This rod is cut into lengths weighing 40 milligrams each, thus producing a convenient form of pellet for use in this invention. One such pellet is implanted under the skin of each of a group of 24-day-old white rats. Ninety-six hours later, the animals are autopsied and ovarian and uterine weights determined. Another group of similar rats is given the same total dose of hormone divided into six injections of aqueous solution during three days, and autopsied 96 hours after the first injection (standard assay technique), while a third group is kept as a control group. The table shows the effect of hormone dispersed in pellets of various media, as compared with control and standard assay groups. The figures represent the average weight in milligrams of the organ.

Wax	Per cent wax in pellet	Ovarian weight	Uterine weight
		Mgs.	*Mgs.*
None (control, no hormone)		12.7	16.0
None (6 injections of aqueous solution in 3 days)		18.8	51.7
Beeswax	50	41.0	112.5
Do	90	18.0	102
Beeswax 90%, coconut oil 10%	50	52.2	58.5
Beeswax 75%, coconut oil 25%	50	62.7	92.3
Beeswax 90%, lecithin 10%	50	19.7	85.0
Beeswax 50%, cholesterol 50%	50	37.5	55.0
Oleostearin	90	38.0	76.0
Paraffin	50	15.0	72.0
"BZ Wax A" approximately 84% by weight of carbon and 14% by weight of hydrogen	50	32.0	48.4
Glyceryl monostearate, 90%	50	30.0	50.5
Propylene glycol monostearate, 10%-Stearic acid	50	21.0	42.0

In the above examples, coconut oil is used merely as a softening agent, and may be replaced for this purpose by any bland vegetable oil.

It is clear from this example that when the hormone is administered according to this invention, ovarian and uterine weight increases are obtained which are far greater than those greater than those produced by the standard

assay techniques. Effects of this magnitude are the more remarkable and surprising in the light of the fact that we have been unable to equal them with a many-fold increase of dose when administered by the standard assay method.

Example 2

The above demonstrations of unusual efficacy have been based on work done on experimental animals, since in that way a quantitative, objective measurement of results can be had to a degree that is impossible in clinical trial upon human subjects. We have nonetheless caused these pellets to be implanted in human beings. For this purpose pellets of the following different compositions have been used with equal satisfaction: (1) pellets containing 33% of beeswax and 67% of hormone substance, carrying 500 rat units of activity in a 50 milligram pellet; (2) pellets containing 50% of beeswax, 30% of hormone substance and 20% of lactose, carrying 200 rat units of activity in a 50 milligram pellet; (3) pellets containing 50% of "Flexowax C" (having the composition of approximately 85½% by weight of carbon and 14½% by weight of hydrogen), and 50% of hormone substance, carrying 500 rat units of activity in a 40 milligram pellet; (4) pellets containing 50% of "BZ Wax A" (having the composition of approximately 84% by weight of carbon and 14% by weight of hydrogen) and 50% of hormone substance, carrying 500 rat units of activity in a 40 milligram pellet; (5) pellets containing 60% "BZ Wax A" (having the composition of approximately 84% by weight of carbon and 14% by weight of hydrogen) and 40% hormone substance, carrying 500 rat units of activity in a 40 milligram pellet. These pellets were prepared as described above but using proper precautions to keep the preparation sterile. The finished pellets were approximately 1.5 millimeters in diameter and 16 millimeters long, and were preserved in sealed ampoules under 80% alcohol. They were placed under the skin of either the abdomen or the side of the thigh. To accomplish the implantation, it was frequently found convenient to insert a large-size hypodermic needle in the desired site and pass the cylindrical pellet through to the end of the needle.

When thus used in a series of over thirty non-ovulating and non-menstruating women, menstruation ensued after one (or occasionally two) implanation of two pellets (1000 rat units) in about 90 per cent of the cases and continued more or less regularly for periods of many months.

In another series of over thirty cases, these pellets have been used to control functional uterine bleeding. Following a single implanation of one 500 rat unit pellet, successful responses were obtained in about 65 per cent of the cases, successful cases being considered those in which both excessive and too frequent bleeding were inhibited and normal menses reported for at least several months following implanation of the hormone. In neither series were any deleterious effects obserbed during the treatment.

We are aware that it is not new to attempt to delay the absorption of a medicament to produce beneficial results. Such delay has been accomplished by others by rendering the medicament insoluble and thus poorly absorbable; by injecting the medicament in oily solutions and suspensions; by adding to the medicament an irritant of some sort in order to cause the sur-

rounding tissue to "wall off" the side of the injection. However, it will be obvious from a consideration of the preceding discussion that none of these methods can be effective in the present instance, as we have further shown experimentally. We are further aware that pellets of some of the pure steroid hormones have been successfully used, as reported by a number of investigators in recent years. In our early work we attempted to adapt such technique to the administration of the gonadotropic anterior pituitary hormone by implanting into rats pellets of this hormone, mixed only with lactose to provide sufficient bulk to handle, and found it to be completely without success, since no effect at all was observed even though the dose given was up to one hundred times that used in the pellets of Example 1. It thus appears that the use of a wax as an excipient is necessary for the successful administration of the gonadotropic pituitary hormones in solid form.

We are further aware that pellets impregnated with beeswax have been used to administer posterior pituitary hormones (Green and January, Proceedings of the Society for Experimental Biology and Medicine, vol. 44, page 217 (1940)). Such pellets were shown by those investigators to be ineffective and undesirable as means of administration of posterior pituitary hormone, for they report, "The reaction which developed at the site of implantation was so severe that the pellets had to be removed before complete absorption occurred," and later state that "this method is not applicable for treatment in man."

We have further attempted to extend the principle of this invention to the administration of the gonadotropic hormone derived from pregnant mare serum or human pregnancy urine. While not totally ineffective when applied to these hormones, we have found that the administration of wax pellets thereof presents none of the unusual advantages accruing from the use of wax pellets of the gonadotropic anterior pituitary hormones. It thus appears that the use of a wax dispersion of the gonadotropic anterior pituitary hormone, as described and claimed herein, presents a unique advantage when applied to this hormone only.

The gonadotropic fraction of the hormones of the anterior pituitary gland is known to contain more than one active principle, among which the so-called luteinizing fraction and the follicle-stimulating fraction are best recognized. The presence of these two principles is demonstrated by the increases in ovarian and uterine weight, respectively, and the table in Example 1 clearly shows that the response of each is beneficially

affected by administration according to the teachings of this invention.

While we have found that the cylindrical pellets referred to above are a convenient form to be used in this invention, with respect both to preparation and administration, such shape is not an essential feature of the invention. The pellets may be of any convenient shape, as, for instance, in the form of disks or ribbons or spheres, without departing from the essential features of this invention. The dose per pellet may be varied by changing either the weight of the pellet or the concentration of hormone in the pellet, or both, as illustrated in the examples. Further, while we have found the ratio of equal parts of water-soluble material and of non-absorbable medium to be convenient, and have done much of our work with pellets of such composition, we have also varied that ratio widely (as from about 10 per cent to about 70 per cent water-soluble material), and have still been able to achieve the objects of this invention. Further, the value of this invention is not dependent upon the purity of the gonadotropic pituitary hormone employed, since it may be equally well applied to comparatively crude or to highly refined hormone preparations. The nature of the response produced may, of course, be modified by the presence of such other impurities as may not have been removed in a crude preparation. It will be understood that this invention is not limited in scope by the examples quoted herein, but is to be construed as including such equivalents as may be obvious to one skilled in the art within the scope of the appended claims.

We claim:

1. A pellet for the administration of the gonadotropic fraction of the hormones of the anterior pituitary gland by parenteral implantation, which pellet consists of a dispersion of the desiccated hormone substance in a waxy medium.

2. A pellet as in claim 1 in which the waxy medium is a naturally occurring substance.

3. A pellet as in claim 1 in which the medium is beeswax.

4. A pellet as in claim 1 in which the medium is a synthetic wax.

5. A pellet as in claim 1 in which the medium is a synthetic hydrocarbon wax having substantially the composition of 85½ per cent by weight of carbon and 14½ per cent by weight of hydrogen.

6. A pellet as in claim 1 in which the medium is a synthetic hydrocarbon wax having substantially the composition of 84 per cent by weight of carbon and 14 per cent by weight of hydrogen.

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