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HORMONE PREPARATIONS AND METHOD FOR PRODUCING THE SAME

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This invention relates to hormone preparations, viz. to hormone preparations of such a kind the hormoneactive component whereof consists of so-called proteinaceous hormones. Such hormones are, e. g. insulin, the hormones of the pituitary gland, etc. Especially, the invention is aiming at insulin preparations. The purpose of the invention is to provide hormone preparations of such a kind as can be introduced in to the human organism in an effective manner enterally (i. e. by way of the intestinal canal).

It is well known that hormone-active substances having the character of proteins will, if introduced into the organism enterally, exert quite insignificant effects only or no effects at all on the organism. According to the view prevailing at present this fact is due to the circumstance that these hormones are decomposed by the proteolytic enzymes of the intestine, as e. g. by tripsin, before it would be possible for the hormone to be absorbed by the organism.

It should be remarked that the expression "hormone-active substance" should be interpreted in the widest sense. It is understood to cover natural hormones (products of endocrine glands) or synthetic products having similar effects, as well as hormone-containing organs and organ preparations, or extracts of hormone-containing organs.

We have found that it is possible to obtain hormone preparations capable of being introduced effectively enterally, by adding to substances containing hormone-active substances of protein character, by way of accompanying substances, compounds belonging to a group of compounds comprising the classes enumerated below.

One of the classes of compounds referred to above comprises heterocyclic nitrogen bases of the monocyclic and polycyclic types not possessing the character of dyestuffs, built up of rings of five and/or six members with one or more atoms of nitrogen in the ring, as well as the compounds which can be derived from these nitrogen bases, as e. g. products of lateral chain substitution of these bases, e. g. alkaloids capable of being derived from these bases. Preferably there are employed such nitrogen bases of the kind referred to above as are of more or less aromatic character, and/or compounds capable of being derived from such nitrogen bases. The nitrogen bases mentioned can be employed as such or in the form of their salts.

The second class of compounds referred to above comprises phenolic substances, i. e. compounds of aromatic character, possessing at least one free hydroxyl group, and/or substances from which under the conditions of their employment such compounds are split off.

As compounds belonging to the first class there should be mentioned in the first place, as highly advantageous accompanying substances enabling enterally introduced hormones to be effectively absorbed, the compounds presenting the structural skeleton of the cinchona alkaloids (which accordingly may be considered derivatives of quinoline). The substances presenting the structural skeleton of cinchona alkaloids are, generally comprising natural or synthetical cinchona alkaloids, their derivatives such as substitution products, isomeric conversion products, oxidation products, reduction products, or compounds formed by them with other substances, e. g. with organic or anorganic acids and the like. By way of examples of such compounds which have been found to be well suitable for employment as accompanying substances we mention quinine, quinidine, cinchonin, cinchonidin, optochin, vuzin, eucupin, quininone. These bases are preferably employed in the form of their water-soluble salts formed with anorganic acids, e. g. with hydrochloric acid. These compounds are distinguished not only by their eminent efficacy, but also by the fact that of all these compounds relatively high doses can be introduced permanently into the human system without any harmful effects being exercised by them.

The efficacy of the substances enumerated above can be shown very clearly by tests made with insulin preparations on rabbits, in course of which the blood sugar level of the rabbits is observed.

Thus, there was obtained in a group of rabbits kept on a hunger diet (the group being composed of 36 rabbits kept under the precautionary measures usual in the case of insulin calibration), after the rectal introduction of a mixture of 20 I. U. of insulin and 0,05 grams of quinine hydrochloride in 1,0 cubic centimetre of water, a blood sugar drop amounting on the average to 55%.

If 20 I. U. of insulin, in one cubic centimetre of water, was injected rectally to the same animals without the accompanying substance, it was only in about 25 per cent. of the total number of experimental animals that it has been possible to detect an insignificant amount, not exceeding 10%, of blood sugar drop, whereas in the case of the other animals the blood sugar content was either not altered at all or even increased 10 by to 30 per cent.

It has been possible to obtain similar results by

employing, instead of quinine hydrochloride, 0.05 grams of cinchonin hydrochloride, 0.05 grams of quinidine hydrochloride, 0.05 grams of optochin (ethyl-hydro-cuprein) hydrochloride, 0.05 grams of vuzin (iso-octyl-di-hydro-cuprein) hydrochloride, and in each case 20 I. U. of insulin, obtaining hereby an average of blood sugar drop of 48 per cent, with the first-named, of 40 per cent. with the second and of 45 per cent. with the third and fourth substance.

By means of control experiments it has been ascertained that, when introduced rectally neither the quantity of cinchona alkaloids indicated above alone, nor insulin, introduced rectally together with the quantity of hydrochloric acid required, for the formation of the alkaloid salts in the examples given above, produces any substantial alteration of the blood sugar content.

The figures of blood sugar content given above have been determined by means of the colorimetric method of Folin-Wu, in the form as modified by Kowarski.

Among the other heterocyclic nitrogen bases belonging to the first class mentioned above, and/or among their salts, we would by way of example mention pyridine and its derivatives, quinoline, iso-quinoline and its derivatives, e. g. papaverin which may be derived from iso-quinoline, and pyrazolon derivatives, e. g. di-methyl-phenyl-pyrazolon. These compounds also show a well-defined effect of the character referred to above in connection with proteinaceous hormones in case of being introduced together with them enterally, particularly rectally, into the organism.

Among the compounds belonging to the second class mentioned above, it is possible to employ monohydric phenols, as e. g. tricresol, as well as polyhydric phenols, as e. g. resorcinol. The phenols may be partly etherified, as for instance guaiacol, and may also be substituted in the lateral chain, as e. g. methyl resorcinol; it seems, however, that phenols having a long lateral chain (containing more than five carbon atoms) are presenting a slightly weaker effect, and are therefore less suitable for the purposes of the invention. In the course of tests, carried out in a manner similar to those enumerated above in connection with cinchona alkaloids, it was possible to obtain, by means of a preparation containing 20 I. U. of insulin, and 0.05 grams of resorcinol in 1.0 cubic centimetre of aqueous solution, an average blood sugar drop of 50 per cent. Among the phenols, resorcinol possesses the advantage that it may be employed in the case of the human organism in relatively large doses, up to about 0.5 grams, without any harmful secondary effects resulting.

Among the substances which in their admixture to hormones according to the invention render effective enteral introduction of proteinaceous hormones possible, it is preferable to select such substances as will not exercise any harmful secondary effect during a long—in the case of diabetic patients often almost permanent—treatment. This is of importance, because in order to obtain the desired effect, relatively large quantities of the compounds which according to the invention are added as accompanying substances to the hormone-containing substances have to be added to one dose of the hormone, i. e. to such a quantity of the hormone as is usually introduced into the organism at one occasion.

The method of adding various substances, among them also phenols or their derivatives, as preserving means to hormone preparations intended for administration by means of injection has already been known. These substances were in general added to the hormones in such quantities that the quantities of preserving preparations added to each dose of the hormone were of the order of magnitude of milligrams. In the case of the enteral introduction of the preparations such quantities do not show any effect. Distinctly differing therefrom, we employ according to our invention, in hormone preparations intended for enteral administration, such quantities of the accompanying substances (understanding thereby the total quantity of accompanying substances added) which amount for one dose of hormone to one centigram or more, in many cases to five centigrams or more, and in most cases to one decigram or more, and in general are of the order of magnitude of several centigrams or decigrams up to about one gram, and in some cases even more.

Now from this point of view it is of considerable importance that we have found that a large number of the accompanying substances present an increased efficacy in case they are employed in mutual mixtures. It is possible to mutually mix either compounds belonging to the same class or compounds belonging to different classes, as e. g. quinine hydrochloride and resorcinol. It was found that a certain synergism exists between these accompanying substances, i. e. that in case of the employment of mixtures relatively smaller quantities of the individual products may be able to exert the same effect. This may be described with reference to the following example. In order to obtain the same effects as obtained on rabbits with a certain quantity of insulin and 0.025 grams of quinine hydrochloride or 0.05 grams of resorcinol, it is not necessary, in case of the employment of a mixture of quinine hydrochloride and resorcinol, to employ—as might be expected—0.0125 grams of quinine hydrochloride and 0.025 grams of resorcinol, but only 0.01 grams of quinine hydrochloride and 0.02 grams of resorcinol. By utilising this increased effect of some mixtures of accompanying substances, it is possible to employ also such substances, of which, in order to obtain the desired effects, it would, in case these substances were employed alone, be necessary to employ such quantities as would already show undesirable secondary effects. Moreover, the permanent rectal employment of hormone preparations in the case of highly sensitive patients, is facilitated by the alternating employment of preparations prepared with different accompanying substances. In the case of existing idiosyncrasies, likewise, an opportunity is offered of employing such a mixture as may be convenient for the particular individual concerned.

As mentioned above, the effective enteral administration not only of insulin, but also of other hormones of proteinaceous character is rendered possible by the employment of the accompanying substances according to the invention. It is well known that extracts of the posterior lobe of the pituitary gland have the effect of reducing the excretion of water by the kidneys. This can, however, only be observed in case the extracts are administered by means of injection. By employing resorcinol as an accompanying substance, it was possible to observe the inhibition of the excretion of urine also in case of the rectal introduction of extracts of the posterior lobe of the pituitary gland in the case of rabbits on which previously an artificially increased diuresis had been produced by intrastomachal adminis-

tration of water. In connection herewith it has not been necessary to employ the purified preparations obtainable in commerce, it having been sufficient to triturate the posterior lobe of the pituitary gland of a calf with a small quantity of physiological sodium chloride solution, mix it with 0.1 grams of resorcinol and introduce the mixture into the rectum of rabbits.

It should be remarked here that for the preparation of products of the pituitary gland it is preferable not to employ cinchona alkaloids by way of accompanying substance, because the preparations of the pituitary gland are in any case very substantially stimulating the peristaltics of the intestine, which effect is intensified in such extent by the addition of cinchona alkaloids that in case of rectal administration it is hardly possible to prevent a premature emptying of the intestine.

It should be remarked that the invention is not dependent on any theory, and is not limited to any theory, either as to the question whether compounds may possibly become formed between the hormones and the accompanying substances or not, nor as regards the mechanism of the effect of the accompanying substances.

When preparing the hormne preparations according to the invention in such a form in which they are introduced into the human system, it is possible to proceed according to different methods. The method of procedure will depend on the one hand on the kind of starting materials employed, i. e. on whether liquid or solid substances are available and on the other hand on the nature of the preparations to be produced. For the introduction of hormone preparations through the alimentary canal it is, substantially, the following methods of administration that are available. The preparations can be administered, for instance, by mixing the dry constituent substances or by precipitating the solutions of the constituent substances on suitable carrier substances, in the shape of tablets or lozenges covered with a coating, e. g. of hardened gelatine, which will become dissolved in the intestine only. The most advantageous method of producing the preparations is, however, to produce them in such a shape as will enable them to be administered rectally. In this case it is also possible to start either from solutions, preferably from aqueous solutions of the constituent substances and possibly add to them agents increasing viscosity, as e. g. gelatine, or incorporate the said constituent substances in dry mixture or in solution into suitable fundamental materials as e. g. into oils or fats, e. g. into gelatine or cacao butter and thus produce paste-like preparations or solid suppositories.

The production of the hormone preparations according to the invention in a shape suitable for rectal introduction has shown itself highly advantageous for various reasons. It appeared that the efficacy of the preparations is all the higher, the higher the concentration of the active substances introduced, and any dilution by intestinal contents can be prevented most easily in the rectum which can be well emptied spontaneously or provokedly, and which can, following that, by a small addition of opium to the preparation (e. g. 0,01 to 0,04 g extractum opii for one dose) be quieted for about 12 hours or more. By these means it is possible to produce a so-called "deposit of medicine" in the rectum, which deposit is absorbed gradually.

Another advantage of the production of preparations for rectal introduction consist in that in this case it is not necessary to employ expensive hormone-containing substances purified in a complicated manner. Accordingly, it is possible, for instance, to employ products of the preliminary stages of the processes of purification and/or extraction, as for instance raw insulin. This circumstance is all the more advantageous, as in the case of the hormone preparations according to the invention a greater number of hormone units are required for assuring a certain effect than would be required for obtaining the same effect by the injection of the hormones. In the case of the tests on animals, this quantity of hormone was about 10–15 times as high as the quantity of hormone by means of which the same effect could be obtained by subcutaneous injection of the hormone. In the case of humans about 6–8 times of the quantity are in most cases sufficient with rectal introduction. This quantity is of course ineffective in the case of rectal administration without the accompanying substances according to the invention. As, however, it is not necessary to employ expensive purified hormone preparations, this circumstance will partly or entirely balance the increase of cost caused by the necessity of employing greater quantities of hormones. Even if by the employment of greater doses of hormones an increase of the cost of the preparations should nevertheless arise, the fact of dispensing with injection, which is inconvenient and frequently means sources of danger, particularly for diabetic patients, represents an advantage which must be valued very highly.

In the production of the hormone preparations according to the invention it will be preferable, when employing heterocyclic nitrogen bases by way of accompanying substances, to employ the water-soluble salts of these bases. Notably, some of these bases are insoluble in water, and can accordingly not be employed if the preparations are to be produced from solutions. On the other hand, many of these compounds are powerful bases which are able to exert a decomposing effect on proteinaceous hormones. The H-ion concentration of the system introduced into the intestine is—if necessary by the addition of a small quantity of acid—preferably adjusted to a pH value between about 4 and 5. In the case of such a hydrogen ion concentration the proteinaceous hormones employed are frequently situated in their iso-electric territory i. e. possibly in precipitated condition, which fact however does not represent any drawback for absorbability. On the contrary, the employment of more strongly acidified systems has in most cases not shown itself to be favourable, whereas more alkalinized systems appear to be objectionable as regards the durability of the preparations.

In the case of highly sensitive patients it is possible to admix to the preparations, moreover, small quantities of anasethetics, e. g. 0,01 to 0,02 grams of novocain (hydrochlorine of the p-amino-benzoic acid ester of N-diaethyl-amino-ethyl alcohol).

If the hormone preparations are produced in the form of viscous solutions or pastes, these can preferably be introduced into a container fitted with a conduit suitable for introduction into the rectum, the said container consisting of a resilient substance, e. g. rubber. The preparation can be emptied into the rectum by simple pressure exerted on the container. The quantity of preparation introduced into the container should

preferably be such as contains a quantity of hormone sufficient for a single administration.

In what follows we shall indicate a few examples for the production of enteral insulin preparations for employment on humans, adding that the invention is of course not limited to these examples.

(1) 20 to 100 I. U. of insulin are worked up (in dry condition or in solution) with 0.25 grams of quinine hydrochlorine, 0.25 gram of quinidine hydrochloride, 0.03 gram of Extr. Opii and 2.0 to 3.0 grams of cacao butter so as to form suppositories (rectal inserts).

(2) 20 to 100 I. U. of insulin, 0.5 gram of quinine hydrochloride, 0.03 gram of Extr. Opii are thoroughly mixed in 25 cubic centimetres of a 5 per cent gelatine solution. After having been heated to a temperature of 37° C the preparation can be introduced into the rectum by means of a syringe or of a bulb.

(3) 20 to 100 I. U. of insulin, 0.5 gram of resorcinol and 25 cubic centimetres of a 1 per cent. starch solution are mixed with each other. The solution obtained can be administered rectally as indicated in Example 2.

(4) 20 to 100 I. U. of insulin, 1.0 gram of dimethyl-phenyl-pyrazolon, 0.08 grams of papaverin hydrochloride, 0.02 grams of novacain and 2.0 to 3.0 grams of cacao butter are worked-up into a suppository.

(5) 20 to 100 I. U. of insulin, 0.5 gram of resorcinol, 0.02 gram of Extractum Opii, 0.02 gram of novocain and 2.0 to 3.0 grams of cacao butter are worked-up into a suppository.

(6) 20 to 100 I. U. of insulin, 0.15 gram of quinine hydrochloride, 0.25 grams of resorcinol, 0.03 grams of Extr. Opii and 2.0 to 3.0 grams of cacao butter are mixed with each other so as to form a suppository.

It has already been proposed to add to preparations of the posterior lobe of the pituitary gland intended for being administered by injection, quinine compounds for the purpose of stabilizing the preparations i. e. for preventing decomposition during storage. In connection herewith an increase of the effect of the preparations has also been observed, which fact may undoubtedly be ascribed to the circumstance that quinine compounds possess a physiological effect similar to that of the pituitary gland preparation in question (viz. the production of contractions of the uterus and of the intestine). Certainly this proposal did not contain any indications whatever regarding the behaviour of quinine compounds in the case of the introduction through the alimentary canal of the hormone concerned or of other hormones. Apart herefrom we do not, as already mentioned, intend—for the reasons referred to—to employ rectally the hormones of the posterior lobe of the pituitary gland in connection with quinine compounds.

Experiments have also been published regarding the employment of hexylresorcinol with insulin in the case of dogs, the insulin together with the hexylresorcinol having been introduced directly into the stomach of the dogs in the course of these experiments. From these experiments, likewise, it has not been possible to foresee the possibility of effectively administering hormones with phenolic substances through the intestinal canal, as it is expressedly stated in the publication referred to that the hexylresorcinol does not protect the insulin against destruction by trypsin (which is present in the intestine only). In addition hereto, as mentioned, our experience has shown that phenols possessing a long lateral chain have proved less advantageous than non-substituted phenols or phenols only possessing a short lateral chain.

It has also been proposed to employ various dyestuffs for protecting medicaments against any premature decomposition in the stomach and in the intestine. We do not claim the employment of dyestuffs. Dyestuffs are saddled with the substantial drawback from the practical point of view that they are all too easily liable to make colour marks on clothing and sometimes even on body tissues.

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