XCII. OBSERVATIONS ON THE PREPARATION, PROPERTIES AND SOURCE OF THE PARATHYROID HORMONE. PART I.

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THE isolation of a parathyroid hormone has been described by Collip and his co-workers [Collip, 1925, 1, 2; Collip, Clark and Scott, 1925; Collip and Clark, 1925, 2, 3, 4]. Their method of extracting the active principle consists in digesting the glands with 5 % hydrochloric acid at 100° for a time not exceeding 1 hour, and the final product is obtained by a series of isoelectric precipitations from the digest fluid. When injected subcutaneously into dogs, it was found that the serum calcium content could be raised. To obtain the maximum effect, Collip recommended injection at four-hourly intervals for a period of at least 24 hours. He also stated that herbivorous animals were resistant to the action of the hormone, a statement supported by the recent observations of Macleod and Taylor [1925].

EXPERIMENTAL.

Variation in the serum calcium content of rabbits.

Owing to the difficulties, such as accommodation, etc., in using dogs for these experiments, it was decided to re-investigate the response of rabbits to the hormone. Large animals of 3 kg. weight or over were employed, and they were fed on cabbage and bran mash throughout the experiments. In the first place it was necessary to investigate the accuracy of methods of determining the serum calcium, and in the second place to work out the normal variations in the serum calcium content of rabbits throughout the 24 hours. By means of Collip and Clark's [1925, 1] modification of Kramer and Tisdall's process, it was found that the serum calcium content could be easily estimated to an accuracy of approximately $\pm 2\%$. To determine the second point, a series of rabbits were bled at four-hourly intervals throughout the 24 hours, and the changes in their serum calcium contents were followed. In addition, the effects of injecting normal saline and distilled water at four-hourly intervals were controlled. In Fig. 1 five typical curves are reproduced, and it can be seen that the serum calcium content does not vary more than about 1 mg. per

100 cc. during the whole time of the experiment. In all, 15 control animals have been studied, and the rise has never been over 1.5 mg. per 100 cc., but in a few instances the figures have fallen more or less steadily as much as 1.5 mg. per 100 cc.

Fig. 1. Variations in normal rabbits.

It was therefore decided that any increase over 1.5 mg. per 100 cc. in the serum calcium content must be regarded as being due to external factors, such as the injection of tissue extracts, and that the conditions of the animal experiment were sufficiently standardised to commence tests upon the activity of various preparations. All solutions employed for this purpose were first tested and found to contain no calcium.

Effect of Collip and Clark's extract upon the serum calcium content.

Despite the statements of previous workers it was felt that, provided sufficient quantities of the hormone were administered to rabbits, there should be an elevation in the serum calcium content. To test this point, a very con-

Fig. 2. Parathyroid extract (Collip and Clark).
The arrows indicate time of injection.

centrated extract was made from parathyroid glands by the method of Collip and Clark [1925, 3]. The strength of the final solution was so adjusted that

each injection corresponded to 15 g. of fresh glands. The effects of four-hourly injections for 24 hours on the serum calcium content of two rabbits can be seen in Fig. 2. Although the dose was greatly in excess of that employed by Collip, the observation succeeded in showing that under these conditions rabbits responded to injections of the hormone in a manner exactly similar to that of the dog. An attempt to calibrate these results in terms of dog-units was made by injecting diluted and undiluted "parathormone" (Lilly). This material is standardised according to the method recommended by Collip: each cc. contains 10 dog-units—a dog-unit being one-hundredth part of the amount of hormone required to raise the blood calcium content of a 20 kg. dog by 5 mg. per 100 cc. Injection of this solution into rabbits failed to produce any increase in the serum calcium content, even when 1 cc. was given at four-hourly intervals for 24 hours.

A few experiments were made to determine the minimum quantity of hormone necessary to produce a rise in serum calcium, and, although injections corresponding to 7 g. of gland were found to be effective, it became obvious that a great deal of work would have to be done before any quantitative relationship between the dog- and rabbit-units could be established. The rabbit is therefore much more resistant to the action of the hormone than is the dog, yet, since a uniform rise in serum calcium could always be provoked by the injection of suitable quantities, it was decided to continue the investigation employing rabbits for the test. As already stated, the control experiments had shown that the normal serum calcium content of rabbits never showed a rise of more than 1.5 mg. per 100 cc. throughout the 24 hours, and it was decided to investigate, on a qualitative basis, the nature of substances capable of causing a rise of more than 1.5 mg.

Preparation of the picrate and its properties.

A study of the properties of the hormone suggested that it resembled insulin in constitution: thus its activity is destroyed by pepsin and trypsin, and it gives the common protein reactions [Collip and Clark, 1925, 3]. It was also found to give a strong Pauly reaction. The following experiment also emphasised the similarity to insulin. 49 mg. of the material obtained by Collip and Clark's process were dissolved in 3 cc. of slightly alkaline water, giving a clear straw-coloured solution. 9 cc. of saturated aqueous picric acid solution were added, and the resulting precipitate was collected by centrifugation. The yellow residue was treated with 5 cc. of 70 % acetone, and on stirring it dissolved completely. The acetone was evaporated off and the residue was stirred with 3 cc. of acid alcohol (made by mixing 25 cc. of 3 *N* hydrochloric acid with 75 cc. of absolute alcohol). Ten volumes of acetone were added to the resulting slightly turbid solution, and the white, insulin-like precipitate was allowed to settle. It was then collected and washed with acetone until free from picric acid, and finally with dry ether. The resulting white powder was dried *in vacuo* and was then found to weigh 27 mg. It dissolved

readily in water, giving a clear solution. The potency was tested by injecting three doses of 7 mg. at hourly intervals into rabbits. In Fig. 3 the changes in the blood calcium contents can be followed. This experiment demonstrated that the hormone yielded a picrate which was insoluble in water, but soluble in 70 % acetone, and which could be converted into the hydrochloride by Dudley's method [Dudley and Starling, 1924].

Fig. 3. *Upper curve.* Collip and Clark extract precipitated as picrate and picrate converted into hydrochloride. Injections shown by small arrows.
Lower curve. Extract of parathyroid glands by acetone-picric acid method.

The question at once arose as to whether the hormone could be extracted from the fresh glands by the acetone-picric acid process [Dodds and Dickens, 1924]. 300 g. of glands were extracted by the acetone-picric acid process in exactly the same manner as in the preparation of insulin, and a yield of 405 mg. of hydrochloride was obtained. This was dissolved in water so that 3 cc. of the solution corresponded to 10 g. of glands. The results of administering this quantity at four-hourly intervals can be seen from Fig. 3. The rise in the serum calcium content shows that the hormone can be extracted by this process, without a preliminary acid digestion as in Collip's process. Whether previous treatment with acid would improve the yield by the acetone-picric acid method is at present under investigation.

Effect of insulin and pituitrin upon the blood calcium content.

Since these observations emphasise still more the similarity in the properties of insulin and the parathyroid hormone, it was decided to investigate the action of insulin upon the blood calcium when administered to rabbits under the conditions of the above experiments. Commercial insulin solution, containing 20 units per cc., was diluted 1 in 30 with distilled water and 2 cc. were injected into rabbits in the manner described for the parathyroid hormone. Curve A, Fig. 4, shows the effect upon the serum calcium content, and it can be seen that there is a marked rise. Although the animal was fed throughout the experiment, convulsions occurred twice and their time of appearance is

marked on the chart. The animal was revived with glucose, 5 cc. of a 10 % solution in normal saline being injected intravenously. In order to control the possible effect of glucose injection upon the serum calcium, a rabbit was bled, and 10 cc. of 10 % glucose in normal saline solution were injected intravenously. The rabbit was bled at the end of 4 hours, and the calcium contents of this and of the first specimen were estimated, giving values of 12.1 and 11.9 mg. per 100 cc. respectively. It is safe to conclude, therefore, that this factor could not have influenced the serum calcium curves. 100 g. of fresh pancreas were extracted by the Collip and Clark [1925, 3] process and the resulting material was dissolved in 20 cc. of water to give an opalescent solution. The effect of injecting quantities of 3 cc. of this solution into a rabbit at four-hourly intervals is shown in Curve B, Fig. 4. Insulin, whether made by

Fig. 4.

Curve A. Commercial insulin.

Curve B. Extract of pancreas made by Collip and Clark's method.

Curve C. Insulin prepared from pancreas by the acetone-picric acid method.

the alcohol or by the acetone-picric acid process is capable of elevating the blood calcium, and the same applies to an extract made from the pancreas by Collip and Clark's process.

The next problem to arise was whether the calcium raising property of insulin was in any way associated with its blood-sugar lowering power, or whether the former property was due merely to a component of the mixture of substances composing insulin. The following experiments were performed with a view to abolishing the blood-sugar lowering action of insulin, and at the same time leaving the calcium raising action intact. Concentrated hydrochloric acid was added to a commercial insulin solution until the concentration of acid was 5 %. The solution was then heated in a boiling water-bath for 1 hour, and after cooling was neutralised. After animal tests, it was found that the blood-sugar lowering activity had been reduced to one-seventh of its original value, and that its calcium elevating power had also been reduced considerably (Curve B, Fig. 5). This experiment unfortunately does not make

it possible to arrive at any decision, since it must be remembered that although the parathyroid hormone can withstand boiling with 5 % hydrochloric acid in the extraction process, the quantity of protein present is sufficient to reduce the hydrogen ion concentration of the mixture very considerably. This factor is not present when purified insulin is treated with 5 % acid, and the conditions are therefore much more severe. Collip has shown that an hour at 100° in 10 % hydrochloric acid is sufficient to destroy the activity of the parathyroid hormone completely.

Fig. 5.

Curve A. Pituitrin diluted 1 in 4. Dose: 2 cc.

Curve B. Insulin after treatment with hydrochloric acid.

Curve C. Insulin and pituitrin injected together.

An attempt was made to approach the problem from a pharmacological aspect. It has been shown by Burn [1923] that pituitrin inhibits the blood-sugar lowering action of insulin, and it was thought that if simultaneous injections of insulin and pituitrin were given, interesting effects might be observed on the serum calcium. A commercial solution of pituitrin was diluted 1 in 10. 2 cc. each of these solutions were injected simultaneously into rabbits at four-hourly intervals in the usual manner. The effect on the serum calcium content can be seen in Curve C, Fig. 5. The effect of pituitrin alone is shown in Curve A, Fig. 5.

Fig. 6. Effect of insulin on the blood-phosphate of rabbits.

Dose as in fig. 4, curve A.

Pituitrin, therefore, not only inhibits the blood-sugar lowering action of insulin, but also the calcium raising action. The effect of pituitrin on the hormone extracted from the parathyroid gland by Collip's method is at present under investigation.

The effect of injecting insulin at 4-hourly intervals on the blood phosphorus content of rabbits was also investigated in the course of these observations. It can be seen from Fig. 6 that there was no change in the organic phosphorus content of the blood, thus differing from the effect of insulin when studied over short periods. It must also be borne in mind that these animals were fed throughout the experiments.

DISCUSSION.

It would seem that the specificity of the parathyroid hormone remains to be proved, since an extract made from pancreas by Collip and Clark's method gave marked rises in the serum calcium content. Insulin, and to a less extent pituitrin, also raised the serum calcium content very considerably. The question naturally arises as to whether these extracts made from other tissues contain the same calcium elevating substance as do the parathyroid glands, or whether their pharmacological activity is due to an entirely different substance. The experiments detailed in this communication do not give a decisive answer to this question, but it would be reasonable to assume that the calcium raising factor is the same in all cases. Thus neither the extract made by the Collip and Clark process from parathyroid glands, nor that prepared by the same method from pancreas had any action upon the blood-sugar content of the animals. As already pointed out, little can be deduced from the inactivation experiments described earlier. The similarity in the properties of the substances extracted from the parathyroid and other tissues, such as the solubilities of the picrates, etc., suggest that the active substances are either the same or very closely related. If further experiments support this view, then the parathyroid hormone will occupy a position very similar to that of insulin. For instance, it has been shown by various workers that insulin can be recovered from tissues other than the pancreas. Large yields were obtained from salivary glands, kidneys and spleen [Baker, Dickens and Dodds, 1924]. Considerable quantities were also extracted from the tissues of a person who had died in diabetic coma. This observation corresponds to that of Best, Smith and Scott [1924], who showed that the muscles of a depancreatized dog contained large quantities of insulin. A calcium elevating substance can be extracted from the pancreas and from the pituitary body, but up to the present no observations have been made on the tissues of thyro-parathyroidectomized animals, nor has the potency of these extracts been tested upon such animals. The chemical and pharmacological similarity between the two hormones is very striking, and it is hoped that further observations upon the lines indicated will emphasise their relationship.

SUMMARY.

1. The serum calcium content of rabbits was found to vary within about 1 mg. per 100 cc. throughout the 24 hours.
2. When injected at four-hourly intervals with large doses of a parathyroid preparation made by Collip and Clark's process the serum calcium content of rabbits' blood rose during the period of the experiment.
3. The hormone yields a picrate which is insoluble in water, but is soluble in 70 % acetone, and which can be converted into a hydrochloride by Dudley's method.
4. Active material can be extracted from the parathyroid glands by means of the acetone-picric acid process.
5. Insulin, when administered in the same manner to rabbits as the parathyroid extract, was found to cause an increase in the serum calcium content. Pituitrin also gave a smaller rise, but when these two substances were administered simultaneously, both the calcium elevating powers and the blood-sugar lowering activity of insulin were inhibited. The significance of these observations is discussed.

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