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PROLONGATION OF ACTION OF CHORIONIC GONADOTROPHIN

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It is frequently of advantage to prolong the duration of action of a drug and thus to decrease the number of administrations necessary to produce the required therapeutic effect. This has been successfully accomplished with steroid hormones, e.g., by the administration of suitable esters or by the implantation of tablets. Comparatively limited progress has attended the attempt to extend the period of action of protein-like substances. Combination of insulin with protamine or other proteins will prolong its action to some 24 hours, but attempts to extend this period still further by the use of implants have not been particularly successful (Gilliland and Martin, 1951). Posterior pituitary hormones have been administered in oil and their period of action thus somewhat extended, but to the best of our knowledge no method of prolonging the action of pituitary hormones or of gonadotrophin from other sources by the use of implants has so far been devised.

In the present paper a method is described of making implants of urinary gonadotrophin which have a prolonged effect.

METHODS

A preparation of chorionic gonadotrophin with an activity of 446 i.u. per mg. was used in all the experiments. The implants were prepared as follows: The gonadotrophin was mixed intimately with three times its weight of magnesium monostearate* and the mixture compressed to an implant in a tableting machine.

The duration of action of such implants was investigated by determining their effect in immature mice. Female albino mice, from a strain obtained from one dealer and bred in the laboratory, were used. These mice become sexually mature in about seven weeks. In all experiments the effects of implants and of injections containing the same amount of hormone were compared on separate groups of five mice, obtained by suitably dividing litter mates according to weight. Treatment was started when the mice were about three weeks old and weighed around 10 g.

The implants (weighing 1 mg.) were inserted subcutaneously in the upper dorsal region, under ether anaesthesia. For injection, the gonadotrophin was dissolved in saline and given either as a single injection of 0.2 ml. or in six injections, each of 0.1 ml., on the morning and evening of three consecutive days. The total dose of gonadotrophin per mouse was always 0.25 mg. of the preparation ($\equiv$111.5 i.u.). Vaginal smears were taken daily in some groups and the intensity of the vaginal effect evaluated in a semi-quantitative manner, according to the method of Robson (1938). The mice were killed after intervals of 5, 10, 15 or 20 days from the beginning of the experiment. *Post mortem* the ovaries and uterus were dissected out and weighed on

* Obtained from Morson Thomas and Son, Ltd., London.

a torsion balance after being roughly dried on filter paper. In addition the number of blood spots in the ovaries was noted, as was the degree of follicular development.

The implants were recovered from all the animals *post mortem*, and implants from any particular group of five animals were pooled, dried overnight in a vacuum desiccator over phosphorus pentoxide, and the gonadotrophin extracted by grinding several times with about 1 ml. amounts of cold water which were pooled. The assay was performed on immature female rats in comparison with the actual preparation used to make up the implants. The rats were injected on the morning of five consecutive days, and killed on the sixth day; the assay was based on the ovarian weight.

RESULTS

The results of all the experiments on immature mice are shown in Table I. Each figure in the Table represents the average for five mice. The figures under "Blood Spots" are the average numbers per ovary.

TABLE I

Showing the effects of chorionic gonadotrophin, administered as a single injection, in six injections, or as an implant, on the uterus and ovaries of immature mice at various periods after the administration. Each mouse received 0.25 mg. of a preparation containing 446 i.u. per mg. and every figure represents the average result for five mice. Results bracketed together were obtained on balanced groups of litter mates. The numbers under Ut. and Ov. represent the average weights of the uterus and ovaries in mg. The results under BS represent the average number of blood spots per ovary

Method of administration	Interval between first administration and *post mortem* (days)											
	5			10			15			20		
	Ut.	Ov.	BS	Ut.	Ov.	BS	Ut.	Ov.	BS	Ut.	Ov.	BS
Single injection	22.6	8.25	0.75	35.1	15.3	0.3						
Implantation	88.0	12.5	1.6	49.2	16.9	2.75						
	70.0	18.9	2.5	50.0	15.1	2.4	35.6	10.2	1.6	55,6	15.9	0.0
Six injections	57.3	14.0	1.0	24.7	13.2	0.5	13.2	6.1	0.0	11.2	6.6	0.0

It will be seen from both the uterine and ovarian weights that effects from the implants were still evident 20 days after their insertion. The morphological effects on the ovaries were in general agreement with this conclusion: up to 15 days there is a striking difference between the number of blood spots in the animals receiving the implants and in those receiving the same dose of gonadotrophin by injection over three days. The effects on follicular growth, not shown in the Table, were also similar, and there was, indeed, a difference between the two groups 20 days after the beginning of treatment.

The effect on the vaginal smear also showed the more prolonged effect of the implants. In the group injected over three days the smears had become negative by the eighth day, whereas in the implanted group some positive effect on the vagina was still to be seen after 20 days.

These results on the effects of the implanted gonadotrophin are confirmed by the assays of the recovered implants, the results of which are shown in Fig. 1. After

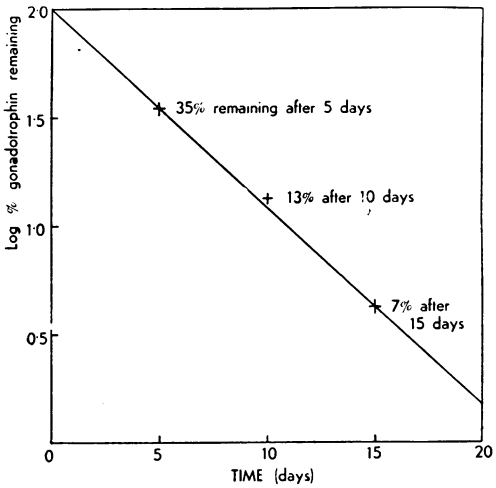

FIG. 1.—The percentage of active material (chorionic gonadotrophin) remaining in the subcutaneous implants at various periods after implantation.

five, ten, and fifteen days the implants had lost 20, 30, and 50 per cent of their weight, and 65, 87, and 93 per cent of their activity. The gonadotrophin content of the implants recovered at 20 days was not assayed, as extrapolation suggested that the amount available would be too small to estimate with any accuracy.

DISCUSSION

The results show that the period of action of chorionic gonadotrophin is considerably extended when the hormone is administered as a compressed implant made with magnesium monostearate, a material readily available. In the particular experiments described above the implants contained one part of the active material with three parts of the excipient, and the active material was absorbed from the implant in about 20 days. Other experiments (not recorded here) suggest that the period of absorption depends on the relative proportion of active material and excipient, and that periods of action suitable for the clinical use of the material could readily be obtained by varying the relative proportions of gonadotrophin and magnesium monostearate.

Careful macroscopic examination has not revealed any reaction around the implantation sites, which have, however, not been examined histologically.

The method described here might also be suitable for extending the period of action of other protein-like substances and perhaps even of other drugs ; experiments at present in progress suggest that it may be of value in the administration of ACTH and of insulin.

SUMMARY

A method of making implants containing chorionic gonadotrophin, by compressing a mixture of the hormone and magnesium monostearate into tablets, is described. When implanted subcutaneously into immature mice, the tablets produced an effect lasting for about 20 days. Other possible uses of the method are discussed.

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REFERENCES

Gilliland, I. C., and Martin, M. M. (1951). *Lancet*, **1**, 143.
Robson, J. M. (1938). *Quart. J. exp. Physiol.*, **28**, 195.