pituitary growth hormone, a principle which is generally assumed to control somatic growth. In agreement with this finding are observations by various authors that mineral exchange in the skeletal system may continue even after practical cessation of growth, such as is the case in old age, or, in young animals, after ablation of the pituitary.

Summary. Hypophysectomized rats treated with growth hormone and consequently in a state of rapid growth, deposited in femur and mandible, when injected with radioactive strontium, essentially the same amounts of strontium, as did untreated hypophysectomized control rats injected simultaneously with the same amount of radioactive strontium.

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Purification of Antigonadotropic Sera by Enzymatic Digestion.*

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It has been shown by Coghill *et al.*[1] that antitoxin in horse serum can be purified to a considerable extent without much loss of activity by digestion with the enzyme Taka-Diastase. Since the antitoxins as well as the antigonadotropic substances produced in the blood of animals over a long period with gonadotropic extracts are found in the globulin fraction of the serum, it was considered probable that the antigonadotropic serum could be purified by the same method. The results of our preliminary work support this view.

Antigonadotropic serum of the horse and the goat were used in this study. The horse serum was obtained from a mare pony which had been injected 3 to 6 times a week for 3 years with a crude sheep pituitary extract (SAP). The goat serum was obtained from an adult female goat which had been injected 5 to 6 times a week for 2 years with a purified pregnancy urine preparation (PU).

Both sera were digested with Taka-Diastase (obtained from Parke, Davis and Company, Detroit, Michigan) in a manner similar to that employed by Coghill *et al.*[1] One volume of serum was diluted with 2 volumes of distilled water and the pH adjusted to 4 by the addition of crystalline citric acid. Taka-Diastase in the proportion of 5 mg to each ml of undiluted serum was added together with a few drops of toluene. The mixture was shaken well and incubated for 96 hr at 37°C. During the digestion a large, flocculent precipitate was formed. At the end of 96 hr the mixture was filtered. The precipitate was removed from the filter paper, taken up in a volume of distilled water equal to that of the undiluted serum, and shaken well with glass beads to make a homogeneous suspension. This was filtered as before. The combined filtrates were dialyzed first against running tap water and then against several changes of distilled water.

This practically salt-free solution was made 40% saturated with ammonium sulfate and allowed to stand in the refrigerator for several hours. The precipitate (Fraction A) was removed by filtration through a hard filter paper (S&S 575). The filtrate was made 50% saturated with ammonium sulfate and, after standing, the precipitate (Fraction B) was removed as before. Assays showed that the antigonadotropic substance was present in fraction A of the goat serum, and

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1 Coghill, R. D., Fell, N., Creighton, M., and Brown, G., *J. Immun.*, 1939, **39**, 207.

TABLE I.
Solids Content and Antigonadotropic Activity of Digested Horse and Goat Sera.

Serum preparation	Solids mg per ml	Solids %	Serum ml eq	Dose gonadotropin	Ovarian weights, mg	No. rats
Horse antiserum untreated	82.7	100	1.0	100 mg eq SAP	13	6
Precipitate*	21.3	25.7	1.0	" " " "	61	4
Filtrate*	38.5	46.5	1.0	" " " "	16	6
Fraction A	12.2	14.7	1.0	" " " "	59	6
Fraction B	19.9	24.0	1.0	" " " "	15	6
Sub-fraction B	9.4	11.3	1.0	" " " "	13	6
Goat antiserum untreated	107.2	100	0.75	0.5 mg PU	17	3
Precipitate*	39.0	35.6	1.0	" " "	62	3
Filtrate*	40.0	37.3	1.0	" " "	13	5
Fraction A	3.4	3.1	0.25	" " "	17	9
Fraction B	6.4	5.9	0.75	" " "	59	3

*After digestion.

in fraction B of the horse serum. Further fractionation of the latter was accomplished by chilling to 0°C (after dialysis to remove excess salt) and adding acetone at —5°C to make a 40% acetone solution. After several hours standing in the cold the mixture was centrifuged. The supernatant liquid was poured off and the bottles were inverted to drain off the acetone. The acetone adhering to the precipitate was removed by inserting a 2-hole rubber stopper into the centrifuge bottle and by removing the air by way of one opening while dust-free air was admitted by way of the other. Thus the precipitate was largely freed of acetone and at the same time it was dissolved in the residual water (Sub-fraction B).

For purposes of determining the antigonadotropic activity of the serum fractions, 21-day-old female rats were used as assay animals. Appropriate doses of the horse serum fractions and the sheep pituitary extracts were injected subcutaneously and separately on opposite sides of the body. Each goat serum fraction was mixed with PU and administered in one injection. Both groups of rats were injected twice a day for 4.5 days. They were killed on the morning of the 6th day and the ovaries were removed and weighed. A representative sample of the results is given in Table I.

The data presented indicate that digestion of antigonadotropic serum with Taka-Diastase for 96 hr removed approximately one-half the solids and little, if any, of the antigonadotropic activity. Fractionation with precipitating agents resulted in the removal of more solids. The antigonadotropic substance of the horse serum was concentrated in a fraction which represents about 12% of the original solids, while that of the goat serum was concentrated in a fraction which represents 3.1% of the original solids.