ence of hyperparathyroidism the simple determination of the total calcium and phosphorus is not entirely reliable.

McLean and Hastings[2] have recently proposed a simple method for the determination of the ionized calcium in the serum. This they believe is the most sensitive test available for detecting the presence of hyperparathyroidism.

Twenty-four patients with proven calcium stones were referred to the Metabolic Clinic by the Second Surgical Service (Dr. Edwin Beer). The Collip modification of the Tisdall method was used for the determination of the total serum calcium, and the Fiske-Subbarow method for the inorganic phosphorus. The serum proteins, after removal of non-protein nitrogenous substances, were determined by a modification of the Pregl micro-Kjeldahl procedure. The results are shown in Table I with the addition of data from control cases. These controls are used in addition to the normal figures published by McLean and Hastings.

There is no evidence of increased calcium ion concentration in any of the stone cases.

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A Method of Purification of Gonad Stimulating Principle from Pregnant Mare Serum.

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The preparation of extracts from pregnant mare serum presents a problem different from that found in the extraction of pituitary gland material. The main difficulty is found in the solubility of serum proteins which form colloidal solutions and cannot easily be separated from the gonad stimulating factor itself. Such solutions appear opalescent or turbid and pass through a bacteria-proof filter only at a very slow rate. The method as described by Evans, Gustus and Simpson[1] overcomes this difficulty by adsorbing the active principle on aluminum hydroxide. This method yields a highly purified product, but requires special equipment not available in every laboratory.

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[2] McLean, F. C., Hastings, A. B., *Am. J. Med. Sci.*, 1935, **189**, 601.

[1] Evans, Gustus and Simpson, *J. Exp. Med.*, 1933, **58**, 569.

Following is a rather simple method which gives a product suitable for biological and clinical use.

Serum from pregnant mares was precipitated in the usual way with 2 volumes of acetone, washed with acetone and dried. This precipitate formed the raw material used in the preparation of the extract. The powder was treated with a 6% solution of butyl alcohol in water, as described for pituitary gland extraction.[2] It is doubtful whether the addition of butanol, advantageous in the extraction of the pituitary gland, is of any particular value in this case, since the serum quickly swells up in water and does not present any problem as far as extraction is concerned. However, the slightly antiseptic properties of the butyl alcohol, which prevent putrefaction during the process of extraction at room temperature, made it desirable to apply this method to serum also.

One hundred grams of dry powder was extracted for at least 12 hours each time with 700, 700, 600, and 600 cc. of butanol solution. The combined extracting fluids were filtered and evaporated *in vacuo* to 500 cc. at a temperature below 40°. Four volumes of acetone were added, which caused a flocculent precipitate. After standing for 15 hours, the clear liquid was decanted from the precipitate. The latter was then extracted for 3 hours with 1 liter of water and 500 cc. of acetone, filtered and re-extracted with half the amount of the same mixture. The combined extracts were evaporated to 500 cc., resulting in a turbid aqueous solution (called crude extract) 5 cc. representing one gram of dry serum powder.

A concentrate solution of 5 gm. of crystallized aluminum sulfate was added under constant stirring, causing the formation of a heavy precipitate. The reaction of the supernatant liquid was pH 4.0 to 5. The material was difficult to filter, but this was overcome by adding 200 cc. of acetone. The precipitate was washed with 20% acetone, and the combined filtrate (about 700 cc.) was precipitated with 2 liters of acetone. After standing for 12 hours, the liquid could readily be poured off from the sediment. The latter was dissolved in 80 cc. of water, containing about ½ gm. of disodium phosphate. Practically all of the aluminum could be removed by careful addition of sodium hydroxide, adjusting the reaction closely to pH 7.0. A translucent solution was obtained by filtration. This was brought up to 100 cc., so that each cc. corresponded to 1 gm. of dry serum powder. The crude extract as well as the purified solution, was assayed on female rats 22 days old, as described (l. c.).

[2] Meyer and Fevold, PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 570.

TABLE I.

	cc. injected	Gm. of serum powder contained	Wt. of ovaries* mg.
Crude	.05	.01	15
	.085	.017	20
Purified	.02	.02	17
	.029	.029	22
	.03	.03	28

*Average of 4 rats.
Ovarian weight of the controls was 10 to 12 mg.

It can be estimated that about one-third of the potency of the material was lost, probably due to the quantity of liquid retained in the voluminous precipitate.

The residue from 1 cc. of the purified extract dried at 105° amounted to 10.7 mg. It consisted largely of sodium sulfate and phosphate. The loss by incineration was 2.6 mg., which figure represented the maximum of organic matter which might be present. As the above figures give an estimation of approximately 40 units per cc., it might be said that 1 mg. of organic substance represents the activity of 15 units.

Slow reprecipitation of the solution with acetone caused first the formation of a heavy precipitate containing practically all the inorganic material, but including most of the active principle. A fine layer of very light precipitate which did not settle out readily, could be separated. Only about 8 mg. of the latter could be collected from 200 gm. of serum powder. In the assay, an injection of 0.023 mg. produced ovaries of 16.3 mg. weight, opening of the vagina and stimulation of the uterus. The more impure and copious layer of inorganic salts included the larger part of the activity: 0.4 mg. increased the ovarian weight to 25.3 mg.

Removal of inorganic matter to a certain degree could be effected by transforming the sulfates into chlorides. While alkali sulfates are readily precipitated by adding 2 volumes of acetone to an aqueous solution, the chlorides remain dissolved. When serum powder of lower activity was used, as happens in slightly advanced stages of pregnancy, a larger quantity of aluminum sulfate was necessary. The sulfate content in the final product was increased accordingly. Transformation into chlorides was effected by addition of barium chloride, avoiding an excess of the latter; for that reason a trace of sulfate was allowed to remain.

The precipitate of barium sulfate was filtered off and the clear

liquid precipitated with 2 volumes of acetone. This precipitate was redissolved to the same volume as the previous solution. While the latter contained 5.26% solids with 1.52% apparent organic matter, the purified solution contained 1.40% solids and 0.63% organic matter. There was probably a certain loss through absorption, although the difference in organic matter must be explained in part by the loss of crystal water from the inorganic constituents. The assay showed 30 units per cc. in the unpurified, and 25 units in the purified product.

An attempt was made to apply the method to pituitary material, but results were not very satisfactory. By using isoelectric or benzoic acid precipitation for removal of proteins, horse pituitaries will give a solution containing from 70 to 100 units per gm. of dry gland material. When the aluminum sulfate method was used, only 35 units were found in the final product. Nevertheless, the quantity of organic material in the end product by the aluminum method was higher than in the extracts obtained by the other methods.

Sections from the ovaries* of the rats showed that luteinization was obtained with the pituitary as well as the serum extracts, as soon as quantities were given which caused an increase of at least 50% in ovarian weight. Luteinization was heavier with the pituitary material than with the serum extracts. When the serum extract was given at a level to produce less than a 50% increase in the weight of the ovaries, only follicle formation was observed. A separation of both gonad stimulating factors was obviously not obtained by this method.

* I am indebted to Dr. A. A. Hellbaum of the University of Wisconsin for the preparation of the sections from the ovaries.