estradiol given to castrates restored the rate of formation of the hydro-uterus.

Histological examination of uteri after cervical ligation showed that the wall of the uterus, the endometrium and the endometrial glands react normally to stimulation by estrogens (Fig. 1). Testosterone produced atrophy of the glandular elements of the uterus and edema of the uterine wall with rarification of its cellular elements (Fig. 1). In all instances studied histologically there was complete absence of any inflammatory reaction in the uterine wall.

Discussion. Ligation of the cervix of mice, rats and hamsters leads to accumulation of a fluid in the uterus which can be harvested by uterine puncture and which keeps accumulating after each consecutive tap. As much as 100 ml of hydro-uterine fluid has been obtained from a single mouse during a year's time . The hydro-uteri provide an ideal site for tumor growth(7).

Endocrine imbalance produced by castration retards formation of hydro-uteri but does not prevent it, and administration of estrogen restores the rate of accumulation of intrauterine fluid to normal. In immature mice the incidence of formation of hydro-uteri is reduced to about one-half of that seen in normal mature animals of the same strain.

Characteristics of hydrouterine fluid are different from those found in ascitic fluid, since the protein content is low and carbohydrate is high. This feature of hydrouterine fluid is compatible with the characteristics one would expect from a collection of endometrial secretions. If the collecting fluid is allowed to accumulate for several months, the relative proportions of its main components do not change but their concentrations are lowered, indicating some dilution taking place with time. It is believed that the experimental production of hydro-uteri in rodents constitutes a new method for the collection of endometrial secretions.

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Estimation of Bromide on Fingertip Blood in Bromide Intoxication. (22149)

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On several occasions it has been necessary to follow bromide levels of serum of small infants after bromide administration either therapeutically or accidentally. The method usually employed (bromide as gold bromide) requires amounts of serum greater than is conveniently obtainable from capillary blood. This paper describes a procedure practical for fingertip blood. The gold bromide procedure is used except that readings are taken in ultraviolet where a higher absorption peak exists for gold bromide.

Method. Reagents and materials. The contents of a one g vial of gold chloride ($AuCl_3 \cdot HCl \cdot 3H_2O$) 0.25%, is washed into distilled water and made up to 400 ml. Trichloracetic Acid: 20 g is made up to 100 ml with distilled water. Carbon Tetrachloride: Analytical reagent grade. Bromide Standard (100 mg/100 ml): 641 mg of sodium bromide (dried overnight in vacuum desiccator) is made up to 500 ml with distilled water. Screw Cap Tubes: Kimble #45066, 16 x 125 mm, fitted with screw caps, teflon lined, are rinsed with dilute nitric acid, distilled water, and dried.

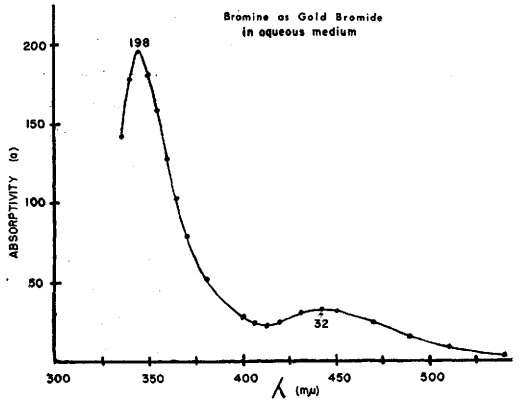

FIG. 1. Absorption spectrum of gold bromide in trichloracetic acid, under conditions of the procedure described. Beckman spectrophotometer.

Procedure: Add 0.2 ml distilled water to screw cap tube. Wash in 0.05 ml of unknown serum. Add 1.3 ml of 20% trichloracetic acid, mix. Follow with 1 ml carbon tetrachloride. Cap the tube and shake vigorously by hand or shaking machine for a few minutes and centrifuge 5 minutes at 2000 r.p.m. in a #1 International centrifuge or its equivalent. Transfer 1 ml aliquot from top layer to the quartz cuvet (1.5 ml capacity, 1 cm light path)*. Add 0.2 ml of 0.25% gold chloride, mix and read on Beckman Spectrophotometer at 350, or 440 mμ if optical density is greater than 0.8. For the standard, use 0.05 ml of standard instead of serum. For the blank, use 0.05 ml distilled water instead of serum. Calculations: Read bromide concentration off standard curve. For clinical purposes the formula below will yield sufficiently accurate results:

$$\frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \times 100 = \text{mg/100 ml bromide in unknown serum}$$

Results. Fig. 1 illustrates the absorption curve of gold bromide which exhibits a peak at 350 mμ and at 440 mμ. The latter is the peak customarily employed(1,2). By reading in the ultraviolet an increase in sensitivity by a factor of approximately 6 is obtained.

The concentration of gold affects the shape of the absorption spectrum. At higher gold concentrations the peak shifts toward the higher wave lengths. If the gold concentration is doubled the ultraviolet peak moves to 355 mμ. The peak at 440 mμ is not affected by changes in gold concentration.

Location of the lines in Fig. 2 were obtained by applying the method of least squares(3) to 6 determinations, at each level shown, to find the line of best fit. It is to be noted that both at 350 mμ and at 440 mμ the lines do not go through the origin. Thus the method may not be used for normal serum where concentrations are of the order of 0.1 mg bromide per 100 ml(4).

A line may be drawn through the origin passing near the points. Thus the Beer-Lambert law may be applied, approximately, when dealing with bromide intoxication, since the toxic level is above 80 mg bromide/100 ml serum(5).

Gold chloride and bromide complex is extractable into higher alcohols and ketones at strongly acid pH but not into other solvents such as chloroform, benzene or heptane. Fig. 3 shows absorption spectrum of gold bromide

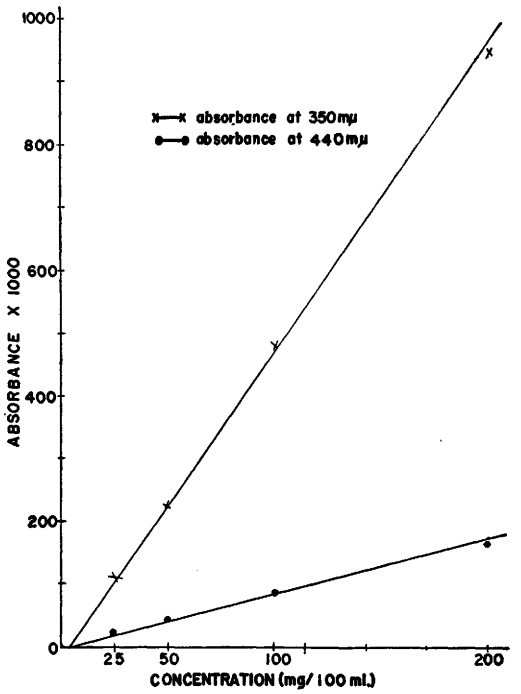

FIG. 2. Calibration curve plotting concentration vs. absorbance. Each point is the mean of 6 determinations. Position of line calculated by method of least squares. Concentration refers to concentration of the serum.

* Pyrocell Mfg. Co., N. Y. City.

extracted into octyl alcohol from a 10% sulfuric acid solution. Attempts to extract the bromide quantitatively, without first precipitating proteins, were not successful, and this method, then, had no advantage over the aqueous method for serum or plasma analysis. This procedure would find application when small amounts of bromide are present in large aqueous volumes, the volume of octyl alcohol layer is then limited to a small volume.

Filtrates with trichloracetic acid tend to be cloudy, occasionally. This is true especially when serum is first diluted with water before adding trichloracetic acid. This is avoided by adding carbon tetrachloride when precipitating the proteins as described in the procedure.

Table I shows recoveries of added bromide to a pooled sample of serum at different levels. High recoveries for the lower quantities are probably due to substances in the centrifugate other than bromine reacting with the gold chloride. In the high range, disappearance of appreciable amounts of gold lowers recovery values. In these high ranges it is suggested that smaller amounts of serum (0.02 ml) be used. The results at 350 mμ compare with those at 440 mμ, indicating that readings at 350 mμ permit estimation of smaller quantities of bromide without loss in precision. When absorbance at 350 mμ is higher than 0.800, then one may read at the less sensitive peak, 440 mμ. This permits a wide range of concentrations to be read without the need of repeating the analysis with smaller quantities.

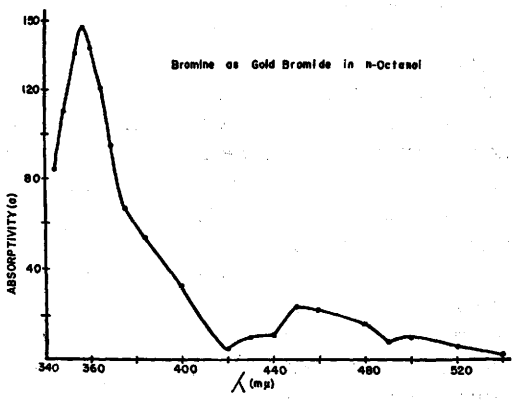

FIG. 3. Absorption spectrum of gold bromide extracted from 10% sulfuric acid into n-octanol. Beckman spectrophotometer.

TABLE I. Recovery of Bromide Added to Serum.

Wave length (mμ)	μg added	μg found*	% recovery
350	12.5	13.1 ± 1.8†	104
"	25	26.6 ± 2.3	104
"	50	51.2 ± 4.4	102
"	100	98.5 ± 6.5	98.5
440	25	26.1 ± 3.4	104
"	50	51.1 ± 4.9	102
"	100	97.0 ± 5.8	97

* Six determinations at each level.
† Mean ± stand. dev.

Summary. A procedure is described for bromide analysis in serum of patients with bromide intoxication which uses the ultraviolet absorption peak for gold bromide to increase sensitivity. This permits the analysis of serum or plasma, from fingertip blood, for bromide analysis.

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