

1. $(A-B) \times \text{glucose factor} = \text{glucose in the original solution.}$
2. $(C-D) \times 2 \times \text{glucose factor} = \text{original glucose} + \text{glucose from the hydrolysis of lactose.}$
3. $D \times 2 \times \text{galactose factor} = \text{original galactose} + \text{galactose from lactose hydrolysis.}$
4. $(2-1) = \text{glucose from lactose. This value} \div 0.53 = \text{lactose.}$
Glucose from lactose is also equal to galactose from lactose.
5. $(3-4) = \text{galactose in original solution.}$

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Rapid Method of Preparing Solutions of Gonadotropic Substance of Pregnant Mares' Blood.

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The method of purifying the gonadotropic substance in pregnant mares' blood by adsorption of the active material to specially prepared aluminum hydroxide followed by elution¹ is a difficult and time-consuming procedure. The method yields preparations directed to the requirements of clinical investigation but for studies on laboratory animals somewhat less purified solutions of the hormone are entirely satisfactory. For this purpose it would be desirable to have a rapid method of eliminating the bulk of the inert material from the serum or plasma of the mare and of concentrating the activity in a smaller volume.

Although there are many chemical and biological differences between the gonadotropic material present in pregnant mares' blood and that of the urine of pregnant women, we have found that the method of Katzman and Doisy² for the preparation of the gonadotropic substance of the latter by adsorption of the active material to benzoic acid may be successfully employed to effect a partial purification of the gonadotropic material in pregnant mares' blood. Solutions of the hormone prepared by this method contain much larger amounts of horse serum proteins than solutions prepared by adsorption to aluminum hydroxide.

One liter of citrated plasma from a pregnant mare³ was diluted

¹ Evans, H. M., Gustus, E. L., and Simpson, M. E., *J. Exp. Med.*, 1933, **58**, 569.

² Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 745.

³ Cole, H. H., and Hart, G. H., *Am. J. Physiol.*, 1930, **93**, 57.

with 9 liters of water and acidified with 10% HCl (pH 3.0 to 2.5). When necessary the acidified solution was clarified in the Sharples supercentrifuge. After chilling, the solution was vigorously stirred while 750 ml. of an acetone solution of benzoic acid, saturated at room temperature, was slowly added, following which the suspension was vigorously stirred for half an hour. The suspended benzoic acid was allowed to settle, the supernatant decanted and the precipitate collected on a Büchner funnel. The filtrate and supernatant from the benzoic acid precipitate were combined and the process of adsorption repeated since a single adsorption rarely recovered all of the active material.

The combined benzoic acid precipitates were suspended in a small amount of water and the benzoic acid was extracted by shaking with chloroform. The amount of water used depended on the desired concentration of the final hormone solution. Frequently emulsions were encountered due to the separation of protein material. When this occurred the suspensions were separated and clarified in the supercentrifuge. After removing the dissolved chloroform from the aqueous solution by blowing through it a stream of nitrogen, the solution was buffered to pH 6.5 with phosphate buffer and preserved with a trace of toluene. When kept at 0°-5° such hormone solutions have been found undiminished in potency after several months.

Summary. The preparation of solutions of the gonadotropic hormone in pregnant mares' blood by adsorption to benzoic acid has been studied. As in the case of the gonadotropic substance of human pregnancy urine, the recovery of the active material of pregnant mares' blood by adsorption to benzoic acid is not quantitative. Satisfactory yields of the hormone are obtained, however, when the process of adsorption is repeated.