

lected in (J). Ice water-baths were then placed around (B) and (C) and a hot water-bath around (A). Distillation of the bromide began accompanied by darkening and film formation in (A). The distillate collected mostly in (B). When distillation was finished, the bath around (B) was heated almost to boiling and an ice water-bath placed around (G). When this distillation was over, the bath around (C) was raised to boiling and an ice-bath placed under (H). This was the final distillation, the colorless bromide distilling into (G) while the temperature remained at 32.5 to 34.5°. A very small amount of distillate passed into the bulb (H). When the distillation was complete, the apparatus was brought back to atmospheric pressure by slowly letting air through (J). The side-tube capillary was then broken and the liquid siphoned off into a small flask by gently sucking out the air from the flask.

The residues in (A), (B) and (C) were black and tarry but the distillate in (G) was colorless throughout the distillation, though shortly after it began to color. Five runs were made, each with a new apparatus as it was found easier to discard the one used than to attempt to remove the graphitic films formed in it from the bromide decomposition.

The yields were about 50% of the theoretical.

**Properties of the Compound.**—As mentioned, the compound is a colorless liquid which decomposes rapidly, turning yellow then brown and ending in a tarry mass which adheres tightly to the container. Attempts to keep the compound for more than a few hours proved futile. Moisture doubtless plays an important role in initiating the decomposition. Phosphorus pentoxide or anhydrous sodium sulfate, however, proved useless in stabilizing the compound. Solid potassium hydroxide, though unable to prevent blackening, delays total decomposition for a much longer time. This points to the possibility that the hydrobromic acid formed by the decomposition may act as an accelerator. The compound thus prepared and dissolved in ether remains undecomposed for days.

**Identification.**—Analyses gave for bromine 50.3 and 49.0%, calculated, 49.66%.

Four and one-half grams of the bromide was condensed with excess of furfuryl alcohol and potassium hydroxide and the product distilled at 2 mm. The di- $\alpha$ -furfuryl ether came over at 100–102°; yield 85%.

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RECEIVED MAY 5, 1939

## COMMUNICATIONS TO THE EDITOR

### HOMOGENEITY OF GONADOTROPIC HORMONE PREPARATIONS ISOLATED FROM PREGNANCY URINE

Sir:

We have reported the preparation from pregnancy urine of gonadotropic fractions containing 4000 minimal ovulating doses per milligram when assayed in the post-partum rabbit [*J. Biol. Chem.*, **128**, 525 (1939)]. Two such samples have recently been examined on the ultracentrifuge,<sup>1</sup> and appear to be homogeneous with respect to sedimentation. In each case, in the concentrations employed, a single sharply defined band was observed at approximately 250,000 times gravity.

In addition, electrochemical homogeneity of one of these preparations was demonstrated as a result of studies with the electrophoresis apparatus of Tiselius.<sup>2</sup> Evidence for the presence of only a single component (which previous analysis had shown to be a polypeptide-poly-

saccharide complex), was indicated by the appearance of a single migrating band having sharp boundaries in a phosphate buffer of ionic strength 0.1 (pH 7.0). The mobility was  $4.85 \times 10^{-5}$  cm.<sup>2</sup>, sec.<sup>-1</sup>, volt<sup>-1</sup>.

Isoelectric point determinations, made by adsorbing the hormone upon collodion particles, and determining migration in a microelectrophoresis cell, showed an isoelectric point of pH 3.2–3.3.

Since such preparations appear to be identical with regard to biological activity and chemical composition, we feel that the evidence obtained with the ultracentrifuge and Tiselius apparatus demonstrates the homogeneity of our preparations.

Further detailed data bearing upon the physical and chemical properties of these and similar preparations of the hormone will be reported shortly.

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SAMUEL GURIN  
CARL BACHMAN  
D. WRIGHT WILSON

RECEIVED JULY 20, 1939

(1) Through the courtesy of Drs. A. E. Severinghaus and J. A. Chiles, Jr.

(2) Through the courtesy of Dr. Florence B. Seibert.