

of the effectiveness of the separation resided in the determination of the biological activities of the preparations. Before separation, the preparations of mixtures at a concentration of  $10^{-5}$  g/cc protein in 0.1 M phosphate at pH 7.1 caused both tobacco mosaic and tobacco necrosis to develop in Turkish tobacco. After the procedure described above, the same protein concentration produced only tobacco necrosis in Turkish tobacco plants.

*Summary.* Preparations of mixtures of TMV and TNV were obtained from plants having both diseases. These viruses were then separated by removal of TMV by isoelectric precipitation and by means of absorption with rabbit antiserum to TMV. The separation was followed by means of the analytical ultracentrifuge, the Tiselius electrophoresis apparatus and the biological activities of the preparations.

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#### Assay of the Estrogenic Hormone in Hemophilic and Normal Male Urine.

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Because hemophilia occurs only in males and is transmitted directly only by females, an assay of the urinary estrogenic hormone was undertaken.

The technic used was identical with that of Dr. Petersen of this institution in order that we might compare the results of our normal control group with his.

White female rats 2 months of age were used. Vaginal smears were made every 12 hours for 3 weeks to ascertain that estrous cycles were normal. Then a laparotomy was performed on each rat under ether anesthesia, the ovaries, broad ligaments and upper part of the cornua of the uterus being removed. The rats were then allowed 10 days to recover from the operation, following which

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vaginal smears were made at 12-hour intervals for 3 weeks to determine whether the estrous cycles had ceased.

One liter of urine from untreated hemophilic patients was used in each series of experiments. The urine, rendered acid to congo red, was extracted 3 times with benzene in a reflux condenser and evaporated to a syrup. Corn oil was then added and the remainder of the benzene evaporated. This leaves the hormone in the corn oil.

The rats were then divided into groups of 4. Three injections of urine extract of 1/6 cc each were made at 4-hour intervals into each of the 4 rats. Vaginal smears were made at 6-hour intervals for at least 48 hours or until activity ceased. If estrus was induced in 3 out of 4 rats, the test was considered positive.

The number of estrous cycles and the total hours of estrus were determined for the normal and hemophilic groups.

The urine of the first 5 hemophiliacs induced no estrual cycles. These results were recorded in a preliminary report in the *J. Am. Med. Assn.*, Vol. 97, page 244. From this it was suggested that hemophiliacs might be deficient in urinary estrogenic hormone.

A summary of the results of 252 assays on the urine of 52 hemophiliacs and their controls is:

17% of the determinations on hemophilic urines were positive.

28% of the control urines were positive.

The average hours of estrus per positive rat in both groups was 14.

In the control group there was so much variation, not only in different individuals but also in the same individual at different times, that it was decided to assay the urine of 4 individuals for 30 or more consecutive days. This was done to see if there was an orderly sequence in the amount of hormone present. The 4 curves varied widely. Within the time limits of the experiment there was no indication of a regular cycle.

Daily urinary assays were also made for 30 days on 2 hemophiliacs. There was no indication of a regular cycle.

Although the hemophilic group is below that of the normal, the variations in both were so great that the difference is probably not significant.