peratures of the two sides are extremely close together. To be sure, humoral mechanisms are present here but they must be acting upon an anatomical substrate that is closely alike on the two sides.

Summary. 1. Deafferentation of a cat's forelimb does not lower the skin temperature of the footpad which may remain similar to the contralateral normally innervated one or may be warmer. 2. Deafferentation of a hindlimb may not affect the pad temperature, or it may be lowered or raised. 3. Any differences present in the forelimbs or in the hindlimbs disappear after bilateral sympathectomy. 4. Section of the L_6-L_7 and S_1-S_2 ventral roots of a cat may not significantly affect the pad temperatures.

13257

Separation of Tobacco Necrosis Virus and Tobacco Mosaic Virus.

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Introduction. During a study of the properties of tobacco necrosis virus, occasional batches of Turkish tobacco plants were encountered which developed not only the non-systemic tobacco necrosis on the lower inoculated leaves, but also through contamination the systemic tobacco mosaic disease. The yield of tobacco mosaic virus (TMV) may be between 2-3 mg per cc of juice,[1] or 100 times that of tobacco necrosis virus (TNV), the average yield of which is about 0.02 mg per cc.[2] Therefore, a single plant carrying a mixed infection in a batch of 100 plants diseased with tobacco necrosis will eventually yield a preparation of TNV containing approximately an equal amount of TMV. In order to avoid the complete loss of TNV in such contaminated preparations, it was considered desirable to attempt the removal of TMV.

TNV is completely soluble over the entire pH range in the absence of large amounts of salt. Since TMV is almost completely insoluble in the region of the isoelectric range, preparations containing very large amounts of TMV were first isoelectrically precipitated. Pirie,

* Fellow in the Medical Sciences of the National Research Council.

1 Stanley, W. M., *J. Biol. Chem.*, 1937, **121**, 205.

2 Cohen, S. S., and Stanley, W. M., unpublished data.

et al.,[3] have demonstrated the absence of cross reaction between TNV and rabbit TMV antiserum. TMV antiserum may, therefore, be used in the quantitative removal of amounts of TMV within the limits of the titer and amount of available antiserum. TNV was finally separated from the lighter serum proteins by sedimentation in the ultracentrifuge.

Although isoelectric precipitation of TMV apparently removed considerable amounts of TNV from solution, the separation by means of antiserum appeared to entail no loss of TNV.

Experimental. Leaves of Turkish tobacco plants having large numbers of necrotic lesions characteristic of tobacco necrosis were harvested 5-7 days after inoculation. The leaves were frozen and stored at −14°C till used. The frozen leaves were ground and allowed to thaw. The juice was expressed at 4°C and a purified virus solution was obtained in the usual manner after at least 4 differential centrifugation cycles, involving a hundredfold reduction in volume. A cycle consisted of ultracentrifugation in the quantity ultracentrifuge at 30,000 rpm for 75 minutes, emulsification of the pellet in water or buffer and centrifugation in an angle centrifuge for 30 minutes at 3000 rpm. The supernatant was then ready for another cycle. All operations were carried out at about 4°C.

Preparations contaminated with TMV were found to have a much greater opalescence than those containing TNV alone. Such preparations were examined in an analytical ultracentrifuge[4, 5] equipped with a Svensson-Philpot[6] optical system. In one case the virus solution before and after treatment with antiserum was examined at 0°C in the Tiselius electrophoresis apparatus equipped with the modification of the schlieren optical system described by Longsworth.[7]

A virus solution containing 1.3 mg per cc TNV and approximately the same amount of TMV was subjected to electrophoresis at pH 6.72 in veronal buffer (0.02 N Na veronal and 0.08 N NaCl). The mobility of TNV (in $cm.^2\ volt^{-1}\ sec^{-1}$) was -1.85×10^{-5}; that of TMV was -5.50×10^{-5}. The current was reversed and the boundaries returned to their original positions. To 12 cc of the recovered virus mixture, 2 cc of rabbit TMV antiserum was added. After

[3] Pirie, N. W., Smith, K. M., Spooner, E. T. C., and MacClement, W. D., *Parasitology*, 1938, **30**, 543.

[4] Bauer, J. H., and Pickels, E. G., *J. Exp. Med.*, 1937, **65**, 565.

[5] Pickels, E. G., *Rev. Sci. Instruments*, 1938, **9**, 358.

[6] Svensson, H., *Kolloid Z.*, 1939, **87**, 181.

[7] Longsworth, L. G., *J. A. C. S.*, 1939, **61**, 529.

standing in the cold room overnight, the precipitate was centrifuged off and the solution dialyzed 24 hours against veronal buffer. The solution which was at pH 6.76 was again subjected to electrophoresis. The peak comparable to TNV possessed a mobility of -2.25×10^{-5}. After 3 hours no boundaries other than those of TNV and serum proteins could be detected. It is noteworthy that there is practically no loss of TNV as indicated by the size of the peaks. (Fig. 1.)

In a second experiment, TMV was first isoelectrically precipitated and small residual amounts subsequently removed with TMV antiserum. Fig. 2 represents the sedimentation of the virus boundaries before and after separation. In this case, the sedimentation constant of TMV was 157×10^{-13} cm/sec/unit field, indicating a concentration of approximately 10 mg/cc.[8] To 10 cc of this virus solution, 0.2 N HCl was added till the pH was 3.5. The solution was placed in the icebox for 1 hour. The supernatant liquid after centrifugation at 3000 rpm for 30 minutes was adjusted to pH 7.4

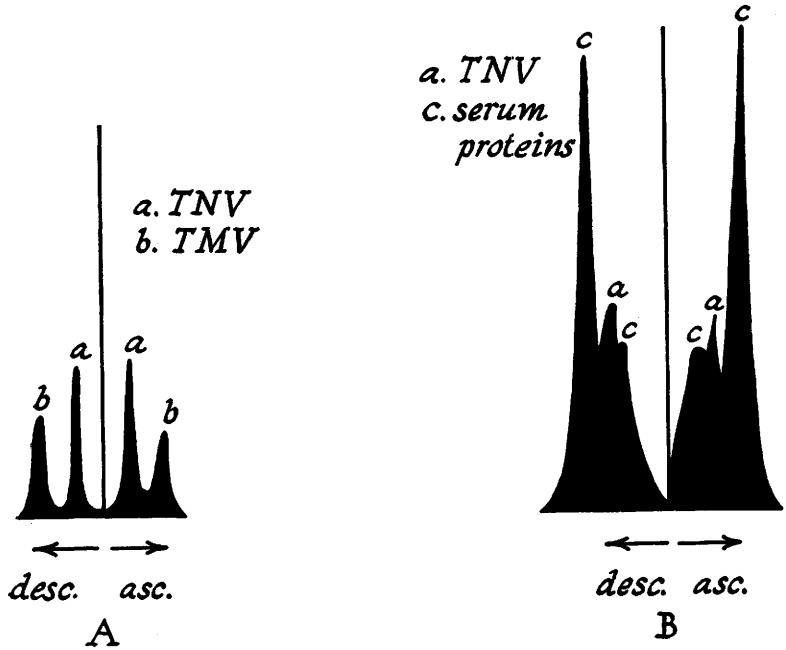

FIG. 1.
Separation of tobacco necrosis virus (TNV) and tobacco mosaic virus (TMV) followed in Tiselius electrophoresis apparatus. A represents boundaries of mixture before separation. B represents boundaries after addition of antiserum to TMV. Boundaries were observed 1 hour after beginning of electrophoresis.

[8] Lauffer, M. A., *J. Phys. Chem.*, 1940, 44, 1137.

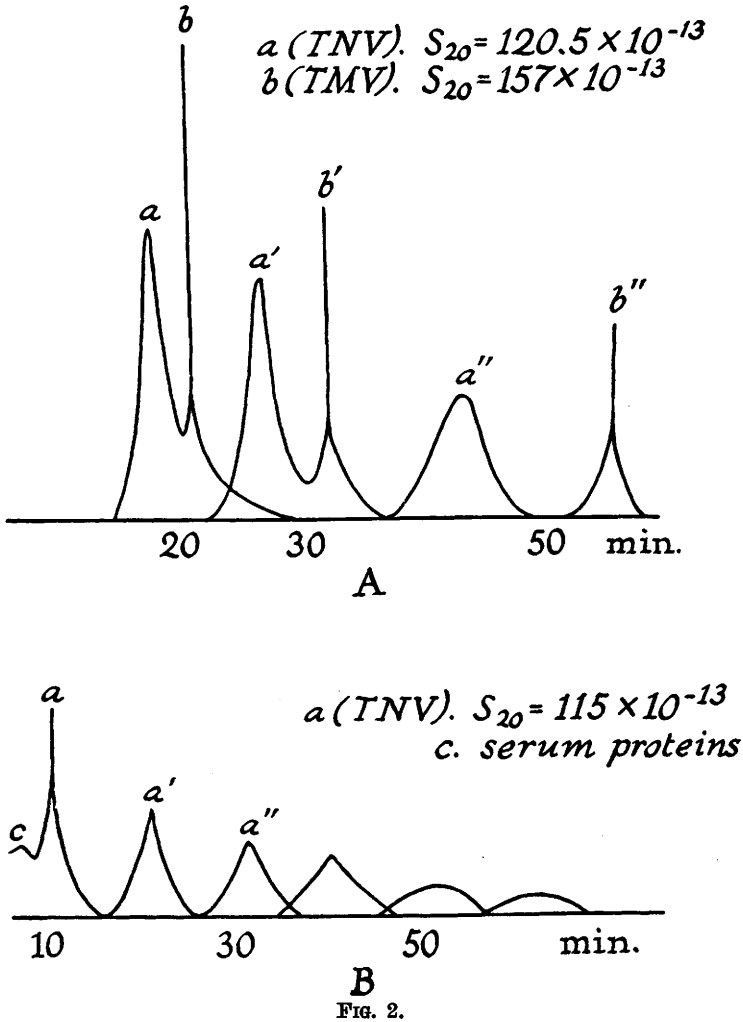

FIG. 2.

Separation of tobacco necrosis virus and tobacco mosaic virus followed in analytical ultracentrifuge. A represents boundaries of mixture before separation. B shows separation of TNV from serum proteins after removal of TMV.

with 0.2 N NaOH. This solution after addition of 0.5 cc TMV antiserum was placed in the icebox overnight. After centrifugation at 3000 rpm for 15 minutes, the supernatant liquid was examined in the analytical ultracentrifuge. The boundary due to TMV had been eliminated.

While the analytical ultracentrifuge and Tiselius apparatus are quite sensitive, it is impossible to detect by means of these instruments concentrations of TMV 100 times greater than is necessary to produce tobacco mosaic in Turkish tobacco. Therefore, the final test

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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