

inulin. The average creatinine/inulin clearance ratio of all collections was 0.996, with a standard deviation of 0.0643.

The clearance of both inulin and creatinine is not constant for any one animal, but varies with the urine flow. The average clearance at a urine flow of 1 cc. per kg. per hour is 3.5 cc. per kg. per hour, and this rises to an average of 20 at a urine flow of 15. When collection periods are selected within a limited range of urine flow, and plasma concentration of inulin and creatinine then plotted against the amount excreted per unit time the data describes a straight line, extrapolating to the zero axis, as illustrated in Fig. 1. This direct proportion to plasma level of inulin and creatinine, and the identical clearances of these substances under all circumstances investigated, is interpreted as indicating that they are excreted solely by glomerular filtration, and that tubular secretion is not a factor in their elimination.

9813 P

Partial Reactivation of Formolized Tobacco Mosaic Virus Protein.*

A. FRANK ROSS AND W. M. STANLEY. (Introduced by Richard E. Shope.)

From the Rockefeller Institute for Medical Research, Princeton, N. J.

A marked reactivation of tobacco mosaic virus protein that has been partially or completely inactivated by formaldehyde¹ has been obtained by dialysis at pH 3. In the experiments to be reported, inactivation was allowed to proceed at room temperature in reaction mixtures containing 2% purified virus protein and 2% formaldehyde in M/10 phosphate at pH 7. Samples were removed after different periods of time and immediately dialyzed against cold distilled water for about 6 hours in order to stop the reaction by the removal of excess formaldehyde. Preliminary experiments indicated that such inactivated virus protein could be partially reactivated by incubation with dimethyldihydroresorcinol² or with his-

* The details of the work are contained in a paper to be submitted to *The Journal of Biological Chemistry*.

¹ Stanley, W. M., *Science*, 1936, **83**, 626.

² Wadsworth, A., and Pangborn, M. C., *J. Biol. Chem.*, 1936, **116**, 423.

tidine,³ or by dialysis at pH 3 or at pH 7. Dialysis at pH 3 gave the best results and, since very little change occurred after the third day, a 3-day period of dialysis was adopted as the reactivation procedure.

The dialyzed solutions of inactivated and of reactivated virus protein were adjusted to M/10 phosphate (pH 7), analyzed for total nitrogen, and diluted with M/10 phosphate to concentrations suitable for inoculating against controls containing 10^{-5} or 10^{-6} gm. of active virus protein per cc.

Nicotiana glutinosa L. was chosen as test plant and the half-leaf method of inoculation was used,⁴ with the exception that whole leaves were inoculated in tests for complete or nearly complete inactivation. The relative activities were interpreted after comparison with dilution curves of active virus and of mixtures of active and of inactive virus proteins. Inactive protein was found to reduce the lesion count by 40 to 50% when present at a concentration of 10^{-2} gm./cc., but had very little effect at 10^{-3} gm./cc. This reduction was no greater than that caused by comparable amounts of hydrogen peroxide-inactivated virus protein or of egg albumin. The preparations used for inoculation contained less than 10^{-5} gm./cc. of free formaldehyde, and several tests showed that this amount of free formaldehyde has no effect on activity and that concentrations as high as 10^{-3} gm./cc. have very little if any effect. The inactivation by formaldehyde can not be due to an aggregation of particles, for the formolized virus protein has about the same sedimentation constant⁵ and exhibits approximately the same amount of stream double refraction as does active virus protein. Inactivation by means of formaldehyde causes a decrease in amino-nitrogen, as evidenced by Van Slyke gasometric determinations and by colorimetric estimations using ninhydrin. Inactivation is also accompanied by a decrease in the number of the groups that react with Folin's phenol reagent. It is probable that the latter are the indole nuclei of tryptophane. That formaldehyde is actually bound to the protein is indicated by the fact that, even after many days of dialysis, the formolized proteins give a strong Rosenheim-Acree test upon addition of H_2SO_4 and an oxidizing agent. These results, together with the fact that during the inactivation of about 99% of the virus protein the rate approximates that of a monomolecular reaction, demonstrate that the inactivation is not due to the toxicity of free

³ Holden, H. F., and Freeman, M., *Austral. J. Biol. and Med. Sci.*, 1931, **8**, 189.

⁴ Loring, H. S., *J. Biol. Chem.*, 1937, **121**, 637.

⁵ Wyckoff, R. W. G., Biscoe, J., and Stanley, W. M., *J. Biol. Chem.*, 1937, **117**, 57.

formaldehyde or of the protein-formaldehyde complex and that it probably results from the blocking of amino groups, indole nuclei, or both.

When the reaction was stopped after suitable periods of time, preparations retaining approximately 10%, 1%, and 0.1% of their original activity were secured. These preparations following reactivation were found to possess approximately 20%, 10%, and 1%, respectively, of their original activity. Preparations that were inactive when inoculated at a concentration of 10^{-8} gm./cc. were appreciably infectious at 10^{-3} gm./cc. after reactivation. Varying the concentration of formaldehyde or lowering the pH did not significantly alter the amount of reactivation obtainable. The data indicate that two simultaneous reactions occur, one reversible and the other irreversible. The ten-fold increase in activity that may be obtained by the reactivation process is definite and is reproducible at will. The reactivation can be demonstrated by a comparison of the activity of an inactivated sample with that of the corresponding reactivated sample of the same protein concentration and can be measured accurately by a comparison of each at suitable dilutions against a common control. The results of several experiments agree quite closely. The amount of reactivation is many times greater than can be attributed to experimental error, to a toxic action of inactive protein, or to the removal of any remaining traces of free formaldehyde by the prolonged dialysis. Evidence that reactivation is accompanied by an increase in amino-nitrogen and in groups that react with the phenol reagent was obtained by colorimetric estimation.

The demonstration that the addition of formaldehyde to the virus protein results in a simultaneous decrease of activity, of amino groups and of reducing groups, and that under conditions favorable for the removal of formaldehyde the virus activity is regained and the number of such groups increases indicates that certain of these groups play at least a partial rôle in the structure necessary for virus activity. It should be noted that the reactivation of formolized virus differs from that which may be secured following inactivation with safranin^{6, 7} or with the salts of heavy metals,^{8, 9} for in the latter instances the complexes are insoluble and the possibility that they are non-infectious because of toxicity has not been excluded. In con-

⁶ Vinson, C. G., and Petre, A. W., *Bot. Gaz.*, 1929, **87**, 14.

⁷ Krueger, A. P., and Baldwin, D. M., *J. Infect. Dis.*, 1935, **57**, 207.

⁸ Stanley, W. M., *Phytopath.*, 1935, **25**, 899.

⁹ Went, J. C., *Phytopath. Z.*, 1937, **10**, 480.

trast, the formolized virus protein is soluble, and it has been proved that the inactivation is not due to toxicity. The inactivation by means of formaldehyde, the subsequent reactivation, and the condition of certain groups in each case can best be interpreted by means of familiar chemical reactions and are direct experimental evidence that virus activity is a specific property of the protein.

9814

Sulfanilamide and Prontosil in the Treatment of Canine Distemper.*

VIRGINIA C. DICKERSON AND LEON F. WHITNEY. (Introduced by H. G. Barbour.)

From the Laboratory of Pharmacology and Toxicology, Yale University School of Medicine.

Sulfanilamide and prontosil have been found effective in otherwise apparently fatal cases of infection with meningococci¹ and hemolytic streptococci,^{2, 3, 4} as well as in gonococci⁵ infections, but curative value has not been shown in cases of infection with filtrable viri or with bacilli. It is now well established that canine distemper is caused by a filtrable virus.⁶ The naturally occurring disease is, however, invariably complicated by infection with *B. bronchisepticus* and frequently with other secondary invaders. These greatly increase the mortality in distemper, and might be susceptible to the influence of sulfanilamide and its derivatives. We undertook to test this hypothesis in a naturally occurring epizootic of distemper at the Whitney Kennels, Orange, Connecticut.

All the dogs exhibited typical distemper symptoms, including temperature curves. Moreover, two ferrets, injected with a suspension of the spleens of dogs which died during the epizootic, developed severe distemper in 9 days; while 7 young dogs of known distemper-

* This investigation was made possible through support given by the W. E. Weiss Fellowship Fund of the Winthrop Chemical Company.

¹ Schwentker, F. F., Gelman, S., and Long, P. H., *J. Am. Med. Assn.*, 1937, **108**, 1407.

² Mellon, R. R., Cross, P., and Cooper, F. B., *J. Am. Med. Assn.*, 1937, **108**, 1858.

³ Colebrook, L., and Kenny, M., *Lancet*, 1936, **2**, 1319.

⁴ Long, P. H., and Bliss, E. A., *Arch. Surg.*, 1937, **34**, 351.

⁵ Dees, J. E., and Colston, J. A. C., *J. Am. Med. Assn.*, 1937, **108**, 1855.

⁶ Laidlaw, P. P., and Dunkin, G. W., *J. Comp. Path. and Therap.*, 1926, **39**, 222.