promptly and were almost as extensive as those produced by the control injections of fresh vaccine virus.

Virus III after drying in the frozen state proved almost as potent as the fresh virus in the production of the typical testicular lesions in rabbits.

*Herpes febrilis* vaccine subjected to the freezing and drying produced the typical *Herpes febrilis* reaction in rabbits, killing the animals in 5 days. The brains showed very many typical eosin-staining nuclear inclusions.

Viruses dried by this method and kept in sealed tubes in the icebox lose their virulence very slowly. There was no appreciable loss after one year under these conditions. This offers a convenient and economical method of preserving strains of viruses.

## 4356

## Resistance of Causative Agent of Chicken Tumor to Certain Organic Solvents Compared with Vaccine Virus and Herpes Febrilis Virus.*

JAMES A. HAWKINS. (Introduced by James B. Murphy.)

*From the Laboratories of the Rockefeller Institute for Medical Research.*

Because of the theoretical importance of the chicken tumors, it seems desirable to continue the study of the properties of their causative agent from a number of angles with the hope that this may lead to an understanding of their nature. The present study deals with the resistance of the agent of Chicken Tumor I to the action of certain solvents and a comparison of this resistance with that of some of the filterable virus group.†

*Experimental Procedure.* The various tissues containing the active agents were treated as described in the previous paper to obtain dried materials. One half gram portions were transferred to long glass tubes containing 50 cc. of various organic solvents. The tubes were sealed by fusing the open end in a flame and were shaken for 6 days by a mechanical shaker. They were then opened and the emulsion of solvent and tissue was filtered through filter paper. The

* This investigation was carried out by means of funds from the Rutherford Donation.

† We wish to acknowledge the assistance of Dr. Thomas Rivers, who supplied the viruses used and tested their infectivity after the treatment with the various solvents.

residue was washed with solvent twice, transferred to a petri dish and freed from solvent by evaporation. The clear filtrate was also placed in a petri dish and evaporated to dryness by passing a stream of cold, bacteria-free air over it. In each case a small amount of fatty material was left after evaporation. The residue after the extraction of the dried materials and the fatty substances extracted were taken up in Ringer's solution and tested for the potency of the agent.

*Chicken Tumor I.* The tumor producing activity of the residues after extraction with dry alcohol, toluol, or acetone was unimpaired. On the other hand, the material was inactivated by treatment with chloroform, 60 and 95% alcohol, or 60 and 95% acetone. The fatty fractions removed by the solvents all proved completely inactive.

*Vaccine Virus.* The testicles of rabbits which had been inoculated 4 days previously with vaccine virus were removed, dried, and prepared as described above. This material, after shaking for 6 days with alcohol, acetone, chloroform or toluol, gave typical vaccinal lesions in rabbits when injected intradermally. The virus treated with chloroform and alcohol was less active than that treated with acetone or toluol. The fatty fractions removed by the solvents were inactive.

*Herpes Febrilis Virus.* Fresh rabbit brain containing active *Herpes febrilis* virus was treated in the manner described. Some of the fresh brain was put in 40% glycerol as a control and some of the untreated desiccated brain was also kept as a control. After extraction with dry acetone, toluol, chloroform, or alcohol, the various extracts and residues were taken up in Locke's solution and 0.25 cc. of each was injected into the brains of young rabbits. The rabbit inoculated with the glycerol control material died in 3 days, but its brain had undergone too much post-mortem change for a study of the nuclear changes due to the virus. The desiccated material produced typical *Herpes febrilis* reaction, killing the animal in 5 days. The brain showed very many typical eosin-staining nuclear inclusions. The other rabbits injected with extracts or residues from the various solvents were not affected in any way. All surviving rabbits were tested 6 weeks later by reinoculation of active herpes virus with proper controls and all died very promptly, showing that no immunity had been produced by the previous inoculation of desiccated virus treated with the various organic solvents.

*Summary:* The causative agent of Chicken Tumor I resists freezing and desiccation, and this dried material retains its activity after being shaken for 6 days in dry acetone, alcohol, and toluol. The

same is true of vaccine virus. The dried material of Chicken Tumor I is rendered inactive by shaking with chloroform while vaccine virus in the dried state withstands such treatment. *Herpes febrilis* in brain tissue resists freezing and desiccation but is destroyed when the dried material containing it is shaken with alcohol, acetone, toluol, or chloroform. None of the agents studied are extracted in their active form from the dried material by the solvents.

From these results it would appear to be impossible to distinguish between the agent of Chicken Tumor I and viruses by desiccation and subsequent treatment with the ordinary organic fat solvents.

4357

## Reaction Between Proteins and Diazotized Aromatic Amines in Neutral Solution.*

MICHAEL HEIDELBERGER AND FORREST E. KENDALL.

*From the Laboratories of the Department of Practice of Medicine, Presbyterian Hospital and College of Physicians and Surgeons, New York City.*

In a series of 3 challenging papers entitled "Chemospecific Antigens" Klopstock and Selter[1] have raised a number of important questions dealing with the chemical basis of certain immune reactions. While several of their minor points will be dealt with in connection with other work now in progress, the present note concerns the underlying thought and principal conclusion of their work, namely, that no chemical interaction takes place in neutral solutions of diazotized aromatic amines and proteins or lipoids. It is held that such solutions are merely simple mixtures, and that "eine chemisch zu verstehende Substitution des Chemikals im Eiweissmolekül bei der chemospezifischen Komplexantigenbildung keine Rolle spielt." Klopstock and Selter have failed entirely to report chemical control experiments in testing this conclusion, and the following are therefore submitted:

I. Conditions the same as in Paper II, p. 455, showing appearance of chemospecific antigen only after mixtures had stood in the cold 1 to 24 hours.

1% diazotized arsanilic acid, "neutralized to litmus."

* The work reported in this paper was made possible by the Harkness Research Fund of the Presbyterian Hospital.

[1] *Z. f. Immunitätsforschung*, 1928, lv, 118, 450; lvii, 174.