Inasmuch as decided immunological differences have been distinguished between separate strains of other viruses such as those of equine encephalomyelitis[12] and of human encephalitis[13,14] wherein the same clinical manifestations are given by the respective strains within each group of viruses, it may well be worth considering such a possibility for the virus of poliomyelitis. Regional differences in strains, not only in respect to invasive power or potency but in respect to qualitative dissimilarity of the antigenic structure, might help to account for the mildness of an outbreak in a certain section as compared to the severity in another. While undoubtedly the high immunity rate of the community as a whole, regardless of how accomplished, largely accounts for the comparatively low morbidity in poliomyelitis, yet sudden outbreaks with an unexpectedly high mortality rate do occur and might well be ascribed to a virus of slightly different immunological makeup combined with high infectiousness. In judging the results of serum therapy, therefore, account should be taken of possible differences in virulence of the virus in different regions combined with a possible difference in antigenic structure. A population ordinarily exposed to a milder strain of virus might not be resistant to one of greater potency and consequently would not respond as well to treatment with serum from those immune to the former strain.

In conclusion, a recently isolated strain of poliomyelitis virus has been found to possess certain immunological properties combined with a slight difference in tissue reactions that suggest the possibility of finding both a qualitative as well as a quantitative difference in the strains of virus causing poliomyelitis.[15]

B. F. HOWITT

THE GEORGE WILLIAMS HOOPER FOUNDATION
UNIVERSITY OF CALIFORNIA
SAN FRANCISCO

RELATION OF CERTAIN VIRUSES TO THE ACTIVE AGENT OF THE ROUS CHICKEN SARCOMA[1]

THE belief held by some investigators that mammalian tumors are caused by viruses is due largely to the demonstration by Rous[2] that the tumor-producing agents of some chicken sarcomas do not lose their activity when passed through a Berkefeld filter. The evidence in support of this hypothesis has been set forth in detail recently by Andrewes[3] and Rous,[4] and will not be discussed here. Instead, we wish to report some observations distinguishing the active agent of the Rous chicken sarcoma No. 1 from two well-recognized virus diseases: vaccinia, an animal infection, and tobacco mosaic, a disease of plants.

The lipid fraction of the Rous chicken sarcoma is capable of reproducing the tumor in a high percentage of inoculated animals.[5] Allard[6] tested the effect of various lipid solvents on the dried virus of tobacco mosaic. Very few of them affected its activity, and the lipid extracts were always inactive. We have been unable to find reports of similar experiments with the virus of vaccinia, though many attempts to use these solvents as disinfecting agents have been made.[7]

The work of Stanley,[8] with the virus of tobacco mosaic disease, and of Northrup,[9] with bacteriophage, indicates that the infective agents in these diseases are protein in nature, and therefore we should not expect to recover them in the lipid extracts by the technique we are using. The experiments to be described were conducted with two possibilities in mind. It is conceivable that the active agent of the tobacco mosaic disease might be merely adsorbed by the protein crystals, though this would seem improbable in view of Stanley's repeated recrystallization of the proteins. In addition, they will serve as a check on the work done in this department with the Rous chicken sarcoma, as it is possible that a protein, representing the active principle, has been carried along in the lipid extract. The solvents used would seem to obviate this possibility, and chemical and biological tests have failed to reveal its presence.

The material used in the vaccine virus experiments consisted of three lots:[10] first, calf skin pulp, frozen promptly and kept in this condition until the time of the experiments; second, calf pulp dried immediately

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[15] Aided by grants from the anonymous Poliomyelitis Donation of the Hooper Foundation and from the President's Birthday Ball Commission for Infantile Paralysis Research.

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[10] We wish to express our appreciation to Dr. Clowes, of Mulford and Company, to Dr. Reichel, of Sharpe and Dohme, and to Dr. Beard, of Lederle and Company, for the large amounts of vaccine virus which we found necessary in these experiments.

in the Lyophile apparatus; and third, chick embryo virus, which was frozen while fresh and dried *in vacuo*. In no instance had preservatives been added. The frozen material was thawed and ground to a fine pulp by passage through a meat grinder. It was then placed in tubes, frozen with carbon dioxide snow and desiccated in the Flosdorf-Mudd Lyophile apparatus. In the first experiment carbon tetrachloride was used as a solvent; in the second, petroleum ether; and in the third, the solvent consisted of equal parts of petroleum ether and chloroform, as experience with chicken sarcoma has shown this mixture to be a better extractive. However, with the technique described, all three solvents have extracted the active principle from the sarcoma. In each instance the dried, finely ground material was put in a flask and extracted 3½ hours with four changes of the solvent in a water bath kept at 37° C. During extraction nitrogen was bubbled through the mixture with the double purpose of keeping it agitated and driving out the air. The solvent was filtered until clear and evaporated to dryness in a stream of nitrogen at 37° C. under negative pressure. The dried lipid extract was divided into three portions, one of which was emulsified in distilled water; the second, in a 10 per cent. saline extract of rabbit skin, as this was to be the test animal; and the third, in a 0.5 per cent. casein solution. The casein solution was added because it has proved capable of preserving or augmenting the activity of the lipid from the Rous sarcoma, which was inactive when injected alone, and the skin extract because of the frequency with which lesions occur in this tissue. Each preparation was tested on the skin and cornea of rabbits.

To test the activity of the original material, some of the dried but unextracted tissue was suspended in water at 37° C. for two hours. It was then centrifuged and the supernatant fluid tested on the cornea and skin of rabbits. After removal of the solvent, an extract of the treated pulp was prepared in a similar manner to learn if the virus had been destroyed by the solvent.

Briefly, the extract of the untreated virus was found to be quite active on both the cornea and the skin of rabbits, while the lipid extracts failed to produce any lesion. The water extract prepared from the lipid extracted residue was also active. Guarnieri bodies were present in the corneas of the rabbits inoculated with extracts of the untreated dried virus, and of the extracted tissue residue, but not in those inoculated with the lipid preparations. Identical results were obtained in three experiments.

The air-dried tobacco mosaic virus No. 1[11] and the plants inoculated in our tests, the *Nicotiana glutinosa L.*, were obtained from the Boyce Thompson Institute through the kindness of Dr. Helen Beale.[12] The local-lesion method[13] was used in inoculating the plants. In these experiments, the technique of preparing the extracts was similar to that used with vaccine virus and Rous chicken sarcoma. In the first experiment the solvent used was carbon tetrachloride and in the second, petroleum ether. In both experiments the disease was produced with water extracts of the unextracted and the extracted dried leaves, but there was no evidence of it in the plants inoculated with the lipid extracts.

If we accept the rather generally held view that a disease-producing agent which retains its activity after being passed through a Berkefeld filter should be termed a virus, then we must believe that viruses should be classified according to their chemical properties—as proteins, lipids, etc.—and in the lipid group place the Rous chicken sarcoma. However, it would seem more logical to look upon the agent causing chicken sarcoma not as a virus but as a product of abnormal cell metabolism. It is most unlikely that the lipid can reproduce itself, and therefore it seems probable that it possesses the ability when injected into normal animals under proper conditions to stimulate normal cells to produce a similar substance and thus perpetuate the disease. Certainly our failure to produce disease with the lipid extracts of the two viruses examined indicates that they are of a different chemical nature.

J. W. JOBLING
E. E. SPROUL

INFLUENCE OF DEUTERIUM OXIDE ON PHOTOCHEMICAL AND DARK REACTIONS OF PHOTOSYNTHESIS

IN experiments previously reported,[1] cells of the alga *Chlorella* liberated O_2 about 0.41 as rapidly in buffers prepared with 99.9 per cent. D_2O (deuterium oxide or heavy water) as in those prepared with H_2O (0.02 per cent. D_2O); CO_2 was supplied in excess and illumination intensity was 6,500 lux (correction from 2,000). To study the influence of D_2O on different stages of the photosynthetic process, additional experiments have been made with the manometric technique previously described. Warburg's[2] No. 9 buffer, prepared with potassium salts, was used in the light intensity experiments, and 0.1 M $KHCO_3$ alone was used in the experiments with intermittent light.

[11] James Johnson, *Wisconsin Agric. Exp. Sta. Res. Bull.*, 76, 1927.

[12] We wish to express our great appreciation to Dr. Helen Beale, not only for supplying us with material, but also for teaching us the technique used in this type of experiment. Without her assistance the work would have been much more difficult.

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