This observation is of interest in view of the recent reports of Haas[16] showing that hyaluronidase from different sources shows different sensitivity to antiinvasin, the hyaluronidase inhibitor present in normal blood. He has attributed these differences to the presence of other substances which he has termed proinvasin I and antiinvasin II. It is possible that our results depend on differences in purity of the various enzymes or merely chemical differences in the hyaluronidases from different sources. That such chemical differences exist is indicated by the work of Hahn.[10,15]

It is apparent from these data that sodium salicylate does exert an inhibitory effect on the activity of partially purified testicular and perfringens hyaluronidase. The specificity of this effect may be open to doubt in that the concentrations necessary are in the range in which sodium salicylate produces denaturation of other biologically active proteins. This concentration (0.01 *M*-0.1 *M*) is considerably above that obtained therapeutically (0.001 *M*-0.005 *M*). Our work and that of Guerra indicates that sodium salicylate at therapeutic levels does however affect the spreading activity of hyaluronidase. The direct inhibition of hyaluronidase at these concentrations would seem unlikely on the basis of the data presented above but cannot be ruled out in view of the impurity of the preparations used and the variability of sensitivity of hyaluronidases from different sources. The complex mechanism proposed by Haas[12] as well as the demonstration of the role of salicylates in inhibiting antibody formation[16] raise other possibilities as to the locus of action of salicylates on the hyaluronidase system.

Summary. Sodium salicylate has been found to inhibit the spreading effect of hyaluronidase *in vivo*. Hyaluronidase derived from *Cl. perfringens* and bull testis was also inhibited by sodium salicylate *in vitro*. The concentrations necessary for *in vitro* inhibition are considerably higher than those obtained *in vivo*.

15 Hahn, L., *Arkiv. Kemi, Mineral. Geol.*, 1945, **19A**, No. 33.

16 Homburger, F., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 101.

15793

Recovery of Herpes Simplex Virus from Rabbit Brain Nine Months After Inoculation.*

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We reported the precipitating effect of anaphylactic shock on latent Herpetic encephalomyelitis.[1] Further progress has been made in substantiating our original observations.[2] Although at that time we did not demonstrate the existence of the Herpes simplex virus in the central nervous tissues of the rabbits showing the encephalitic symptoms following anaphylactic shock, we pointed out the symptomatological and pathological identity of the disease produced with that of Herpes encephalomyelitis. We further reported the finding of intranuclear inclusion bodies in the brain of the rabbit. Experiments pointing out the production of encephalitic symptomatology and central nervous system pathology by various noninfective mechanisms[3-6] made it of considera-

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1 Good, R. A., and Campbell, B., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 305.

2 Campbell, B., and Good, R. A., unpublished MS.

3 Rivers, T. M., and Schwenkter, F. F., *J. Exp. Med.*, 1935, **61**, 689.

4 Ferraro, A., *Arch. Neurol, and Psychiat.*, 1944, **52**, 443.

Protocol—Rabbit G 370.

11/8 to 11/12/45	One young adult albino rabbit was sensitized to egg white by administration of the following dosages on alternate days: 1 cc I.V.; .5 cc I.V.; and 1 cc I.M.
12/27	The rabbit was inoculated in the quadriceps muscle with 1 cc of a 10^{-1} suspension of H. F.$_{21}$ virus.
1/6/46	Definite evidence of encephalomyelitis, elevated temperature, paralysis of the right hind leg and encephalitic manifestations.
1/18	Temperature normal, no evidence of encephalitis, no paralysis. Apparent recovery from a mild Herpetic encephalomyelitis.
1/29	After temperature had been normal for 11 days and there was no evidence of encephalitis or myelitis the animal exhibited moderate anaphylactic shock following intravenous administration of .15 cc egg white.
2/7	Slight elevation of rectal temperature.
2/7 to 2/27	Development of full blown encephalomyelitis. The symptoms and signs included elevated temperature to 105.5, paralysis of both hind legs, weakness of front legs, excessive salivation, opisthotony, nystagmus, and facial twitching.
2/27	Temperature returned to normal and except for a few sequelae, animal essentially normal.
2/27 to 4/15	Temperature remained normal.
4/15	Mild anaphylactic shock.
4/22	Temperature elevated, nystagmus, increased salivation, gnashing of teeth and disorientation.
4/22 to 5/1	Elevated temperature and evidence of mild but definite encephalitis.
5/1 to 7/10	Essentially normal except for slight disposture of the head.
7/10	Severe anaphylactic shock produced by the intravenous administration of .3 cc egg white I.V.
7/18	Temperature elevation noted, animal began to appear ill.
7/18 to 8/30	Gradual progressive encephalomyelitis with very high fever and severe symptomatology.
8/30	Dead.

ble importance to know whether or not an active virus agent is present in the nervous tissues of the animals suffering acute exacerbations of encephalitis. Hurst, Cooke, and Melvin[7] reported the isolation of canine distemper virus from the central nervous system of dogs 6 weeks after a systemic distemper infection at a time when the dogs showed symptoms of central nervous system involvement. Brodie[8] and Bodian and Cumberland[9] have reported the recovery of poliomyelitis virus from the central nervous system of monkeys 16 and 14 days respectively after disappearance of the paralysis. The persistent excretion of polio virus in the stools of convalescents, Horstman and Melnick,[10] has received considerable attention.

This protocol illustrates the capacity of anaphylactic shock to precipitate latent virus encephalitis since on all 3 occasions when the animal was shocked symptoms and signs of encephalitis followed after a latent period of one week.

Recovery and Identification of the Virus. Under aseptic precautions samples of central nervous tissue were removed from the lumbar spinal cord, medulla oblongata, cerebellum, cerebral cortex and thalamus of Rabbit 370 and placed in separate test tubes containing 50% buffered glycerine solution (pH 7.4). After 2 days' storage at ice box temperature each sample of tissue was ground in a sterile mortar and suspended in saline to form a 1:10 dilution of rabbit brain.†

Three mice were inoculated with .03 cc of the tissue suspension from each of the above described areas of the rabbit's central nervous system. All the mice died within 3 days except those inoculated with the medulla oblongata tissue. On the third intracranial passage in mice a 10^{-1} suspension of the virus killed all mice inoculated on the second day after inoculation. A determination of the M.L.D.$_{100}$ showed it be .03 cc of a 1/10,000 dilution of mouse brain in normal saline.‡

5 Kabat, E. A., Wolf, A., and Bezer, A. E., *Science*, 1946, **104**, 362.

6 Morgan, I., *J. Exp. Med.*, 1946, **85**, 131.

7 Hurst, E. W., Cooke, B. T., and Melvin, P., *Aust. J. Exp. Biol. and Med. Science*, 1943, **21**, 115.

8 Brodie, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1238.

9 Bodian, D., and Cumberland, *Am. J. Hyg.*, 1947, in press.

10 Horstman and Melnick, *J. A. M. A.*, 1944, **126**, 1061.

† These suspensions showed no evidence of bacterial growth on blood agar streak plates or Brewer's medium deep cultures.

‡ This was the same M.L.D.$_{100}$ as the H.F.$_{21}$ virus possessed which was inoculated into G 370 nine months previously.

The virus, after a subsequent passage in mice, was inoculated onto the cornea of a young adult rabbit. This rabbit developed a kerrato-conjunctivitis typical of Herpes infection.§ After a period of 8 days this rabbit developed a fulminating encephalitis and died. Pathological study of the brain revealed findings typical of Herpetic encephalitis.

In another rabbit, No. 548, following the precipitation of active encephalomyelitis from the latent state by anaphylactic shock 120 days after inoculation of Herpes simplex virus into the quadriceps femoris muscle group, a virus agent was again recovered from the central nervous tissues. The technics of recovery of the virus and its identification by biological characteristics were the same as were used in Rabbit No. 370. The virus after 3 mouse passages was identical in all respects to the virus inoculated 4 months previously.

Summary. 1. An infectious agent was recovered from the central nervous system of a rabbit which had been inoculated 9 months previously with Herpes simplex virus and which had suffered 3 acute relapses of the disease. The virus recovered exhibited characteristics identical with those of the original virus. 2. A second instance of virus recovery 4 months after inoculation is reported.

§ Corneal scrapings were obtained from the infected eye three days after inoculation and showed typical intranuclear inclusion bodies in the epithelial cells.

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Artifacts in Gold Shadowed Electron Micrographs Due to Electrons of High Intensity.

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The electron microscope has been used widely for the examination of virus preparations, for the sizes of many viruses are below the range in which the light microscope can be used to good advantage.[1] However, because of the low contrast which is afforded by small viruses, difficulties in securing good electron micrographs have been encountered. Electron microscope studies on small viruses were aided immensely by the development of the metal shadow-casting technic by Williams and Wyckoff.[2,3] This technic has also proved of value in securing good definition of virus particles in the presence of a background of material of smaller particle size. By means of this technic Sigurgeirsson and Stanley[4] and Oster and Stanley[5] have secured good delineation of the particles of tobacco mosaic virus in the unpurified juice and contents of hair cells, respectively, of plants diseased with tobacco mosaic. Although there was no difficulty in these studies in showing that most of the rods present in fresh virus preparations possessed a length of 280 mμ, occasional pictures were obtained which showed segmentation of the virus rods similar to that described by Williams and Wyckoff.[6] Further study has shown that this segmentation into shorter pieces of variable

1 Stanley, W. M., *Chronica Botanica*, 1943, **7**, 291.

2 Williams, R. C., and Wyckoff, R. W. G., *J. Applied Physics*, 1944, **15**, 712.

3 Williams, R. C., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 265.

4 Sigurgeirsson, T., and Stanley, W. M., *Phytopathology*, 1947, **37**, 26.

5 Oster, G., and Stanley, W. M., *Brit. J. Exp. Path.*, 1946, **27**, 261.

6 Williams, R. C., and Wyckoff, R. W. G., *Science*, 1945, **101**, 594.