when large doses were given and showed a more abrupt temperature curve in guinea pigs. The new S strain was infective to mice intracerebrally in a dilution of 1–100,000 and occasionally 1 to 1,000,000 and was also fatal by intranasal, subcutaneous and intracutaneous routes of injection.

Wild mice, *Peromyscus maniculatus,* could be infected intracerebrally with 0.03 cc of a 1–100,000 dilution of the virus. Young puppies also succumbed after injection by the same route. The virus could easily be grown on the chorioallantoic membranes of the developing chick, and is now in the 40th passage. The embryo dies in about 15 to 18 hours and the virus may be recovered from the membranes, the amniotic fluid and the tissues of the chick. The Br strain could likewise be grown in the developing egg.

Immune serums of the S strain gave positive neutralization and complement fixation tests against the Br and the western equine types of virus, but occasionally there was a slight crossing with the eastern variety by the first method. Eastern immune serums, however, failed to neutralize the S virus, as did also the immune serums of the Moscow 2 equine type and the Japanese B virus. Both tests were negative against the St. Louis encephalitic strain. There was no crossing with the eastern variety in the complement fixation test, although occasionally the S antigens were weak when used against the Br and the western equine serums. The S serums usually were positive against the other two western antigens. It was also found feasible to use as antigen the supernatant fluid after centrifugation of the ground membranes of eggs infected with either of the two strains.

In cross tissue immunity experiments, 3 out of 4 guinea pigs immune to the S strain succumbed after intracerebral injection of the eastern type, while the fourth became sick and weak but recovered. One old guinea pig immune to the latter virus, after inoculation with the S strain, ran a temperature and became ill but recovered.

From the general characteristics of this S strain, the incubation period, temperature curve, clinical picture in animals and from the serological and immunological reactions, it is apparent that the western variety of equine encephalomyelitic virus may be recovered from adult human blood serum even after prolonged storage in the refrigerator.

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ENCEPHALOMYELITIS IN MONKEYS

DURING the last few years equine encephalomyelitis has been spreading widely among the horses of the United States. Though the possibility of its infectiousness for man had been realized,[1] it was first recognized[2] last year as the cause of serious disease in man. Innumerable people are exposed during an epidemic in horses; and now many laboratory workers have intimate contact with the disease and its causative viruses while making the embryo vaccine[3] being used to control the disease. We have been carrying out experiments with monkeys to throw light on probable modes of human infection and to indicate what might be done to control or check the disease in man. These experiments have been designed to determine (1) the sensitivity of monkeys to infection by different routes; (2) the influence of hyperimmune serum on the course of the disease; and (3) the possibility of protecting by vaccination.

It has been known[4] that monkeys are susceptible to encephalomyelitis virus injected into the brain and that they can in some instances be infected by virus introduced into the peripheral circulation. We have found that when a massive dose of either eastern or western virus is instilled intranasally into a young rhesus monkey, it will in most instances succumb to a fatal infection. The first symptom of this infection has been fever. The animal has been mildly excitable during this febrile stage, after which it has become paralyzed, has sunk into coma and has died. The symptoms of the two American diseases have been similar, except that as in other animals, the eastern has run a shorter course. We have never observed the recovery of any young monkey infected with either strain if it became paralyzed. A few of the animals receiving intranasal virus have remained healthy; we have found that at least some of these monkeys developed a high content of neutralizing antibodies after exposure. They have therefore suffered non-clinical infections. In our experiments disease has not been produced by very large doses of virus injected subcutaneously or intravenously, though these inoculations have been followed by the appearance of circulating antibodies. Dropping virus into the eye has not resulted in either disease or measurable antibodies. Eastern virus injected intralingually and western virus introduced by stomach tube have proved fatal, but we have not diseased healthy animals by keeping them caged with sick ones.

Hyperimmune horse serum has provided passive protection against nasally instilled virus. Incomplete protection has been furnished by serum administered within three hours of infection. In numerous trials we have never seen any beneficial effect from such a serum given at and after the time of first temperature

[1] K. F. Meyer, *Ann. Int. Med.,* 6: 645, 1932.
[2] L. D. Fothergill, J. H. Dingle, S. Farber and M. L. Connerly, New England Jour. Med., 219: 411, 1938; L. T. Webster and F. H. Wright, SCIENCE, 88: 305, 1938; B. F. Howitt, SCIENCE, 88: 455, 1938.
[3] J. W. Beard, H. Finkelstein, W. C. Sealy and R. W. G. Wyckoff, SCIENCE, 87: 89, 490, 1938.
[4] E. W. Hurst, *Jour. Path. Bact.,* 42: 271, 1936.

rise, even when this serum was equivalent in amount to the injection of 500 cc into a 150-pound man.

Animals twice vaccinated with crude chick embryo vaccine have in all but one instance been completely protected against massive doses of intranasal virus. This vaccination has produced antibodies which were present in high titre after eastern, in lower titre after western vaccine injection. Such titres were not appreciably enhanced by the subsequent test dose of virus. Clarified and deformalinized embryo vaccines have now been made which have been effective in guinea pigs and which should be more suitable for any human use that may in the future be needed.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A HIGH RESOLVING POWER ULTRACENTRIFUGE

IN the case of most large molecular weight substances the molecular weights and sizes are determined by measuring the rates with which they settle out in an ultracentrifuge, together with their diffusion constants.[1] Usually the material is enclosed in a sector-shaped cell with transparent windows so that the rate of sedimentation can be observed optically. Since the diffusion constants are very small for large molecular weight substances, the sedimentation boundaries remain comparatively sharp for long periods of time provided the substance is homogeneous. If the substance contains two or more molecular species, each forms a separate sedimenting boundary. The ability of an ultracentrifuge to separate a mixture of molecular species is proportional to $\omega^2 r h$, where ω is the angular velocity of the centrifuge, r the distance from the axis of rotation and h the length of the column of solution which may be observed.[1] In the modern ultracentrifuges the strength of the rotor materials sets an upper limit to ω, r and the height of the cell, h, that can be used.[1,2] The purpose of this note is to describe briefly a method which, in effect, increases the length of the column of solution under observation without increasing the length of the cell. The method consists in forcing the solution through the transparent cell along the radius in the opposite direction to the motion of the sedimentation boundary. Consequently, if the rate of flow of the solution is equal to the rate of sedimentation, the sedimentation boundary remains stationary in the cell for long periods of time. This not only allows the sedimentation constants to be measured with precision but also makes it possible to determine the sedimentation constants for two or more molecular species in a mixture with very small differences in molecular weight.

Fig. 1 shows a cross-section of the ultracentrifuge rotor which was used to make a preliminary test of the method. This rotor was spun inside an evacuated chamber by an air-supported air-driven turbine (not shown but described previously[2,3]) situated above the vacuum chamber. The shaft A is a stainless steel tube which connects with the tubes B. B connects with a ring-shaped tube D, which in turn communicates with the periphery of the cell C. The cell C is sector-shaped with crystal quartz windows for viewing the sedimentation; it is 12 mm long and its center is 45 mm from the axis of rotation. Short channels connect the ends of the cell C and the cylindrical collecting chamber E. To operate the apparatus the rotor is accelerated to the desired speed, and the material to be centrifuged is injected into A with a hypodermic syringe. The material flows through B, D, and into the cell C. In this way the cell is filled about two thirds. Then as the sedimenting boundaries move outward toward the periphery, more material is injected into A at the proper rate to maintain the boundaries near the center of the cell. The solvent is collected in the chamber E. In most of these preliminary tests hemoglobin (kindly furnished by Professor A. Chanu-

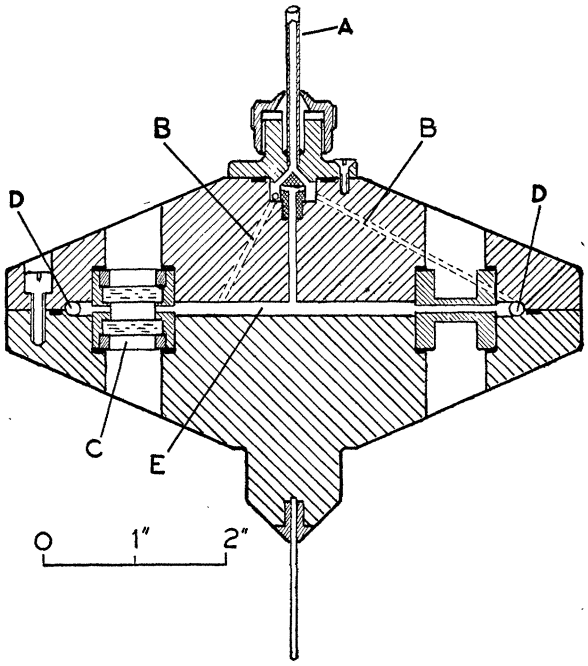

FIG. 1

[1] Svedberg, *Ind. Eng. Chem., Analytical Ed.*, 10: 113, 1938; *Proc. Roy. Soc.*, A 170: 40, 1939.

[2] Beams, *Rev. Mod. Phys.*, 10: 245, 1938.

[3] Beams, Linke and Sommer, *Rev. Sci. Inst.*, 9: 248, 1938.