to a new and better orientation in dealing with mental diseases and disorders, both as to treatment and prevention, in their personal and more general aspects. Out of this undertaking, we hope, will come a publication of the highest scientific and practical value, one that will provide a secure base for future operations against this scourge of mankind, and will influence the development of mental hygiene for years to come.

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SPECIAL ARTICLES

RECOVERY OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS FROM BRAIN TISSUE OF HUMAN CASES OF ENCEPHALITIS IN MASSACHUSETTS

DURING late August and early September, 1938, there occurred an unprecedented outbreak of Eastern equine encephalomyelitis in southwestern Massachusetts. This was accompanied by cases of fatal encephalitis in children nearby. Fothergill, Dingle, Farber and Connerley[1] have just reported the isolation of Eastern equine virus from one of these fatal human cases, and our report confirms their results and describes positive findings in four additional cases.

Brain tissue from seven cases has been sent to us for study by Drs. Pope and Feemster, of the Massachusetts State Department of Health. Five of them yielded the Eastern equine virus as follows.

Brain tissue from each case received in sterile glycerine was triturated in a mortar and diluted in hormone broth to make a 10 per cent. suspension. 0.03 cc of the supernatant was injected intracerebrally into 3-weeks-old Swiss mice.

Forty-eight hours later most of the mice from four cases showed ruffled fur, slowing of activity, alternating with convulsive twitchings. They rapidly became prostrate with occasional accompanying convulsions and succumbed in 48 to 72 hours. Cultures from brain and organs proved sterile. Certain prostrate mice were sacrificed and their brains removed, prepared as above, and injected intracerebrally into further mice.

The mice receiving the second passage material became ill in 48 hours and died or were sacrificed within 72 hours. Further passages have given similar results.

Third and fourth passage brain suspensions were filtered through a Seitz pad and injected intracerebrally into mice. The animals succumbed promptly after 2 to 3 days, indicating that the transmissible agent was a virus of relatively small dimensions.

Similar material was titred intracerebrally in 30-day-old mice and subcutaneously and intraperitoneally in 12- to 16-day-old mice. All mice died through the 10^{-8} dilution. The high virulence of the agent in young mice by the subcutaneous and intraperitoneal routes is characteristic of the Eastern equine encephalomyelitis virus.[2] Moreover, the extremely short incubation period of 48 hours is likewise suggestive of the equine virus.

TABLE I

DIFFERENTIAL VIRULENCE (INTRACEREBRAL) IN LABORATORY ANIMALS OF VIRUSES ASSOCIATED WITH PRIMARY ENCEPHALOMYELITIS IN MAN

Virus	Rabbit	Guinea pig	Mouse	*M. rhesus* monkey	Young sheep
Rabies	++	++	++	++	++
Eastern equine encephalomyelitis	±	++	++*	++	++
Louping-ill	0	0	++	++	++
Japanese B encephalitis	0	0	++	++	++
St. Louis encephalitis	0	0	++	0–±	0
Poliomyelitis	0	0	0	+	0

* Virulent peripherally in high dilution for 16-day-old mice.

The virus was highly infectious for mice by the nasal route. Third passage mouse brain virus was fatal to monkeys and guinea pigs when injected intracerebrally in large doses. Temperatures rose to 105°

[1] L. D. Fothergill, J. H. Dingle, S. Farber and M. L. Connerley, *New England Jour. Med.*, September 22, 1938.

[2] A. B. Sabin and P. K. Olitsky, *Proc. Soc. Exp. Biol. and Med.*, 38: 595, 1938.

TABLE II

NEUTRALIZATION OF MASSACHUSETTS VIRUS IN EASTERN EQUINE ENCEPHALOMYELITIS IMMUNE SERUM

Serum rabbit	Age of mouse and route of injection of serum-virus mixtures	Fate of mice injected with mixtures containing virus diluted as follows							No. M.L.D. protection
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
Normal	4 weeks, intracerebral	....	2*,3,3,3,	2,2,3,4	2,3,4,4	3,3,4,4	3,3,3,4	3,4,S,S	
Immune	" " "	....	5,5,6,6	5,5,7,8	6,7,8,S	7,S,S,S	S,S,S,S	S,S,S,S	10^3
Normal	2 " intraperitoneal	2,2,3	2,3,3,	2,3,3	3,3,3	3,4,S	3,3,3	3,4,S	
Immune	" " "	S,S,S,	S,S,S,	S,S,S,	S,S,S,	S,S,S,	S,S,S,	S,S,S,	10^{6}+

* Day of death following injection.
S = Remained well.

at 24 hours and dropped to 104° or less after 48 hours. At 48 hours the animals appeared dazed, showed muscle weakness and then became prostrate, succumbing in 72 to 96 hours. Blood and spinal fluid taken at 48 hours contained virus. Brains from the animals removed just before or after death were free of bacteria and positive for virus. Rabbits similarly injected with the massive dose succumbed within 72 hours and their brain tissue injected intracerebrally into mice yielded virus after an irregular incubation period. Guinea pigs given virus in the pad developed a fatal encephalitis after 5 days. These reactions in animal species are different from those of other known viruses causing central nervous system virus infection in man and are characteristic of the equine strain (Table I).

Finally, specific neutralization tests were run on the four strains with hyperimmune equine encephalomyelitis rabbit sera kindly furnished by Dr. P. K. Olitsky. 0.3 cc of each brain emulsion dilution of virus was mixed with an equal quantity of undiluted immune serum and similarly with undiluted normal rabbit serum. The mixtures were shaken and then injected without delay intracerebrally into Swiss mice according to the standard technique, and in duplicate intraperitoneally into 16-day-old mice according to Dr. Olitsky's technique.[3] The immune sera showed a protective effect in the intracerebrally injected mice of at least 10^3 lethal doses and in the young mice of 10^6+ lethal doses (Table II), thus completing the identification of the unknown virus strains as Eastern equine encephalomyelitis. Western equine encephalomyelitis immune serum did not neutralize the virus.

This is the first instance in which the horse virus has been definitely implicated as causing encephalitis in man.

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THE PRODUCTION OF A GONADOTROPHIC SUBSTANCE (PROLAN) BY PLACENTAL CELLS IN TISSUE CULTURE

IT has been generally accepted that the placenta produces the anterior pituitary-like hormone (prolan) excreted in the urine during pregnancy. The weight of evidence supports such a theory, but there has been no direct proof. It seemed that the culture of placental cells *in vitro* might provide a method of demonstration. With this in view continuous culture of human placental tissue was undertaken.[1]

In the present investigations the specimens cultured were a placenta of three months, obtained by hysterotomy, and a hydatidiform mole. The mole specimen was sent us from the Boston Lying-In Hospital through the courtesy of Dr. Arthur Hertig. At the time of the first assays the placental tissue had been in culture for two months and the mole for nine days. The culture medium used was human cord serum 40 per cent., beef embryo extract 10 per cent., balanced salt solution 10 per cent., and chicken plasma 40 per cent. For microscopic observations some fragments were explanted into large lying drop preparations on No. 1 cover slips (40 × 45 mm). Most of the tissue fragments were explanted in the same medium in roller tubes. The roller tubes with added supernatant fluid were rotated at a constant speed of twelve revolutions per hour by the Gey[2] method. The supernatant fluid was changed every three and four days, and the cultures were patched and transferred as necessary.

In the mole cultures three morphologically different types of cells migrated from the explants: (1) large epithelial cells with clear cytoplasm and round delicate nucleus with several nucleoli. Some of the large cells showed as many as four nuclei and by their general appearance were considered as possibly the precursors of the syncytium. (2) Fairly large and densely granular cells. These occurred usually in closely packed masses with the single migrating cells in the medium showing many short processes and giving to them an asteroid or chestnut burr appearance. These cells had small delicate round nuclei. (3) Large spindle cells, usually vacuolated, with prominent slightly oval nuclei and with a single large nucleolus. These cells showed evidence of mitotic and of amitotic division.

The placental cultures showed chiefly the large vacuolated spindle cells, but with some transitional types to ones of epithelioid appearance. In these cultures the syncytium exhibited no growth. The myxomatous connective tissue grew very poorly and was soon outstripped by the growth of Langhans' cells. After about two weeks of cultivation in the roller tubes only cells of the Langhans' type were found. A cytological description of the cells and their cultural characteristics will appear in a later publication.

The production of prolan by the placental cells was tested by performing an Aschheim-Zondek test on 21-day-old rats with the cell free supernatant fluid which was removed after having been in contact with the living cultures for three days.

[3] P. K. Olitsky and C. G. Harford, *Jour. Exp. Med.*, 68: 173, 1938.

[1] According to published reports placental tissue has been cultured by Heim, Neuweiler and Guggisberg, Friedheim, Sengupta and Taszkan but was not maintained longer than three weeks. In unpublished work done about the same time (G.O.G.—1927) hydatidiform mole was cultured for a period of sixteen days and human placenta for ninety-four days.

[2] G. O. Gey, *Am. Jour. Cancer*, 17: 752, 1933; G. O. Gey and Margaret K. Gey, *Am. Jour. Cancer*, 27: No. 1, May, 1936.