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.

The first assay was performed on the combined fluid from the tissue cultures of the three-month placenta and the hydatidiform mole. There were five tubes of placental tissue containing approximately twenty fragments each and four tubes of hydatidiform mole containing about ten fragments each. The total amount of fluid injected was 4 ml; 0.6 ml was injected twice daily for three days. The rats were opened and the ovaries examined on the fifth day. The test was strongly positive for prolan, each ovary having several blood points. One ovary was fixed and examined microscopically in each assay. As controls 4 ml of human cord serum and 8 ml of beef embryo extract were injected separately into two rats. These substances were the main constituents of the nutritive medium used in the cultivation of the tissue. Both gave negative assays.

The following week, using the same technique, fluid was removed from the tissue cultures of both the three-month placenta and the hydatidiform mole and tested separately for prolan. 1.75 ml of fluid from the placental cultures proved positive for prolan. The fluid from the mole gave a positive Friedman test. As a control 1.75 ml of fluid removed from roller tube cultures of normal human muscle fibroblasts which had been growing in continuous culture for 20 months was used. This gave a negative reaction.

The next series of assays, using 1.5 ml of supernatant fluid from the three-month placenta, 1.25 ml of fluid from mole and 1.75 ml from normal human muscle fibroblasts were all negative for prolan. The fluid from the mole, however, produced several large follicles in each ovary and a swelling of the uterus. Microscopic examination of the ovaries showed an increase in the size of the interstitial cells. This series of assays was performed irregularly, two of the injections being given only three hours apart instead of the desired eight hours and the rats used, although chronologically 21 days old, were noted to be smaller than usual.

The assays were repeated at a later date, using 4 ml of fluid from the mole cultures, 2 ml from the placental cultures and 3.5 ml of fresh unincubated culture fluid as a control. The culture techniques had been slightly changed before this fluid was removed, and the fragments were not very active. The hydatidiform mole fluid again proved positive for prolan. The placenta and the control gave negative prolan reactions.

1.5 ml of fluid from a culture of cells of the anterior pituitary gland of an eight-month human foetus was also assayed for prolan. The ovaries of the test rat showed no blood points grossly. Microscopically some increase in the size of the interstitial cells and an apparent increase in the amount of interstitial tissue was noted. Although this could not be interpreted as a positive assay for prolan it suggested some hormonal activity.

In summary: (1) evidence is presented which shows that the placental cells produce a substance similar to the prolan-like substance found in the urine of pregnant women. (2) Placental cells growing in tissue culture for a period of over two months retain the ability to produce the hormone *in vitro*. (3) The cells responsible for this hormonal activity are possibly the Langhans' cells. (4) A hydatidiform mole growing in tissue culture for one month also produced the hormone. (5) The supernatant fluid bathing cultures of anterior pituitary cells of a human foetus gave negative prolan assays, but the microscopic appearance of the ovaries in the test rat suggested some hormonal activity. (6) We desire to extend these observations to additional material, and inasmuch as mole and chorionepithelioma are of rare occurrence we would greatly appreciate the help of others in supplying us with fresh, sterile specimens of these tumors.

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THE COLD WATER LAYER OF THE SCOTIAN SHELF

PROBABLY the most important feature of the waters of the continental shelf, to the south of Nova Scotia, is the cold water layer of a temperature less than 5.0° C.—as low or lower than 0.0° C. This layer is found in summer at depths between 17 and 66 fathoms offshore, and between 33 and 80 fathoms inshore.[1] Considering the salinity of the waters of such temperatures, on the Scotian shelf, they may be classified as "bank" or "slope" water.[2] There have been two opposing views as to the origin of this water, one of which favors "winter chilling in situ" while the other favors "water movements from the east." A cooperative effort between the Fisheries Research Board of Canada and the Woods Hole Oceanographic Institution, during the winter of 1936, furnished the first extensive body of data for the assessment of the relative importance of these two points of view.[3]

The temperature and salinity data from a February section, extending outwards from Halifax to beyond the edge of the continental shelf, furnish the T-S diagram of Fig. 1. The volume transports in the

[1] H. B. Hachey, *Trans. Amer. Fish.*, 66: 237–241, 1937.
[2] Paul Bjerkan, Can. Fish. Exped. 1914–15, Department Naval Service, Ottawa, 1919.
[3] The writer is indebted to Mr. C. O'D. Iselin, of the Woods Hole Oceanographic Institution, who agreed to the interchange of data.