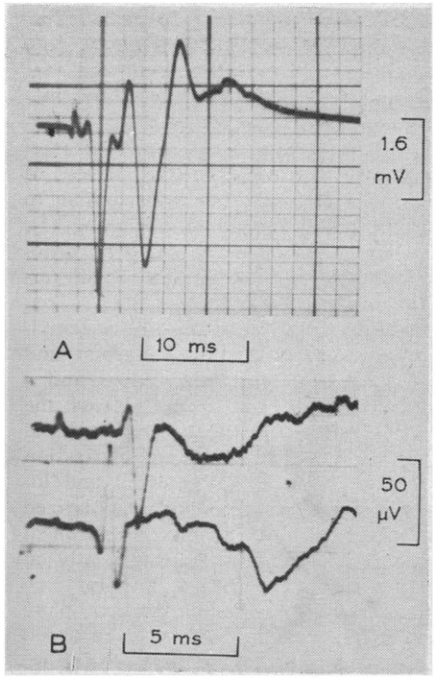

Fig. 1. Nerve action potentials recorded (*A*) in normal dog at sciatic-peroneal nerve, and (*B*) in moderately paralyzed dog at sciatic-peroneal (above) and peroneal nerve at fibula (below). Note difference in calibration between *A* and *B*.

(= choline acetylase activity), ± the standard error, for anterior motor roots and peroneal nerve, were 15.6 ± 0.7 and 1.3 ± 0.5, respectively. The respective values in nine paralyzed dogs were 13.6 ± 1.8 and 1.2 ± 0.2. The acetylcholine content (mean ± S.E.) in micrograms per gram of anterior motor roots and sciatic-peroneal nerve were 8.4 ± 0.9 and 2.0 ± 0.3, respectively, in four normal dogs, and 12.2 ± 3.1 and 2.5 ± 0.7, respectively, in three paralyzed dogs. No significant difference exists between normal and paralyzed animals in either case. In the estimation of choline acetylase activity, another enzyme system is included to produce acetyl coenzyme A, which supplies the active acetyl for the synthesis of acetylcholine from choline by choline acetylase. In order to exclude the possibility that deficiency of this cofactor and of the substrate are responsible for the paralysis, acetyl coenzyme A and choline were injected into a paralyzed dog, but they failed to counteract the paralysis (*8*). MacIntosh (*12*) has recently emphasized the importance of the calcium ion, carbon dioxide, and the chloroform-soluble dializable plasma factor for acetylcholine release. When calcium salts were injected and carbon dioxide increased on inducing asphyxia, and when part of the plasma of the tick-paralyzed dog was replaced by plasma from a normal dog, no lessening of the paralysis occurred. All this evidence indicated that tick paralysis is due neither to defective synthesis or storage of acetylcholine nor to absence of release factors, but apparently must be due to inability of the nerve impulse to traverse the terminal motor nerve fibers.

At this stage it was decided to reinvestigate conduction in motor nerve fibers, because in the initial study in 1956, although conduction had been demonstrated to be present, the measurements were not complete. In the reinvestigation (*13*) the sixth lumbar ventral root was stimulated and the nerve action potentials were recorded with silver-silver chloride surface electrodes at three sites. The sciatic-peroneal nerve in the thigh was laid on a triple pole electrode, the sciatic lying on the uppermost pole, and the peroneal on the lower two. Recording from the upper two poles was designated *S-P*; from the lower two, P_t. The peroneal nerve, where it winds round the neck of the fibula, was laid on a double pole electrode and this recording site was designated P_f. In six normal dogs the potentials recorded at any one of the three sites consisted of (i) an initial single biphasic potential of small amplitude (mean 0.7, 0.24, and 0.1 mv at *S-P*, P_t, and P_f, respectively), and (ii) subsequent multiple potentials of large amplitude (5 to 10 times that of the initial). In four moderately paralyzed dogs the mean amplitudes of the intial simple and subsequent multiple potentials were approximately ⅓ and 1/18, respectively, that of the normal, and the conduction velocity of the multiple potentials was significantly decreased (see Fig. 1). In four severely paralyzed dogs, only one biphasic potential of very small amplitude was recorded at each site.

These results indicate that tick paralysis is due to a defect in conduction of motor nerve fibers and that the tick "toxin" evidently exhibits a predilection for the slower conducting, smaller diameter fibers. Since direct stimulation of the perfused paralyzed muscle failed to liberate acetylcholine, a conduction block in the small-diameter terminal motor fibers must also be present (*14*).

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14. This investigation was aided by research grant EMR-54 from the Department of Agriculture, Ottawa, Canada.

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In vitro Culture of Ehrlich Ascites Tumor Cells

Abstract. The successful culturing and subculturing of ascites tumor cells for 80 odd days are described, as well as the growth pattern of these cells during this period. The cytological appearance of cells after 3 weeks in tissue culture and reinoculation into mice is also presented.

Following the successful culturing of both normal and abnormal cells by Puck and his associates, we have used the basic suggested medium of Marcus, Ciecura, and Puck (*1*), with a slight modification, to grow Ehrlich ascites tumor cells. The medium was modified by the substitution of horse serum for the calf serum and chick embryo extract employed by the above-named authors (*1*). It has not been necessary at any time to use spleen monocytes (*2*), explants of normal cells (*3*), or normal rat fibroblast cultures (*4*), or any adjunct in order to obtain a successful pure culture of Ehrlich ascites tumor cells.

To date, the Ehrlich ascites tumor cells have been maintained some 80 odd days and have been subcultured three times during that period; each culture is still active. The complete nutrient solution found to produce luxuriant growth on more than one occasion was made up of 45 percent Hanks' saline, 40 percent Puck's nutrient solution, and 15 percent horse serum. This is in contrast to Puck and Marcus' attachment solution of 40 percent nutrient solution, 5 percent calf serum and chick embryo extract, and 55 percent Hanks' saline (*1*).

The Ehrlich ascites tumor cells were aseptically harvested from a CF_1 male mouse bearing a 5-day-old intraperitoneal tumor and were transferred to a sterile 200-cm³ rubber-sealed, screw-capped milk dilution bottle gassed with 5 percent CO_2 and 95 percent air. The cultures were begun with a high population cell density, one culture with 5×10^7 cells and the other with 2.5×10^7 cells. The cells were maintained in a fluid culture without agita-

tion. The cultures were fed approximately once a week, at which time 5 ml was decanted, cells and all, and 5 ml of new medium was added. The bottles were then gassed with 5 percent CO_2 and 95 percent air and placed on their sides in an incubator maintained at 37°C. The cells removed from the culture bottle were centrifuged down, stained with aceto-orcein, and examined to determine cell size, number of mitotic figures, and types of cellular abnormalities.

At regular intervals (usually 24 hours), the culture bottles were examined microscopically. At 48 hours, the cells appeared normal in cell size and shape and had not adhered to the glass surface. In a few days, the majority of the cells could still be found as a free-floating cell population, although many of them began to stick to the glass surface. Those cells in suspension remained as single spherical cells, and even those on the glass retained their spherical shape. With time, the majority of the cells had adhered to the glass and could be seen to form groups which eventually met to form sheets of cells.

At 21 days post inoculation, cells were aspirated from the tissue culture and injected into the peritoneal cavity of four CF_1 mice. An ascites tumor developed in three of the mice inoculated. Cytological examination of the cells showed that there was 14 percent spontaneous abnormality present in addition to 10 percent polyploidy. The original tumor cells contained 4 percent spontaneous abnormality in the form of chromosome bridges or fragments, or both, and approximately 2 to 3 percent polyploidy. While the average ascites cell is about 15 μ in diameter, some of the polyploid tissue culture cells measured 27 to 36 μ. In addition to the polyploids present after 21 days in tissue culture, there were a number of abnormal ascites cell types. Some cells contained eight or more nuclei, possibly owing to inability of the cell to divide, while others were filled with micronuclei which may indicate blocked and degenerating metaphases. It is yet to be determined whether the polyploid cells or the other abnormal cell types are reproductively active, or merely sterile metabolizing cells.

Thirty-nine days after the initial date of the successful culturing of the Ehrlich ascites tumor cells, four mice were again inoculated. Two days later, one mouse was killed, and its peritoneal cavity was washed with saline to obtain any cells present. Approximately 100 cells, or one-tenth of the number inoculated, were intact and recognizable as ascites cells. One month after inoculation, the remaining three mice had not yet produced the visibly distended abdomen characteristic of ascites growth.

Again, 55 days after the initiation of the tissue culture, approximately 4000 cells were inoculated into each of eight mice. To date, it would appear that no tumor growth will take place, indicating that continued time in tissue culture enables fewer and fewer cells to retain the potency to produce an ascites tumor. That is, as Klein stated it (*5*), "the normal ancestor cell of a given tumor may exist in low frequency only" and then may exist no longer.

An attempt to trypsinize the cells attached to the glass top of the culture bottle was made by replacing the medium with 5 ml of 0.5-percent trypsin in Hanks' saline for a period of 35 minutes at 37°C. At the end of this period there was no evidence that the cells had been dislodged from the glass surface. A sterile spatula was finally employed to scrape the cells from the bottle into the saline. A subculture was then made by aspirating 2 ml of the "trypsinized" cells from the original culture and transferring them to another milk dilution bottle containing 8 ml of fresh medium. The only difficulty attached to the scraping procedure described is the enhanced possibility of contamination, although healthy, active subcultures were obtained by this method.

In addition to the above-mentioned subcultures, 60-mm petri dishes were prepared with 0.5 ml of fresh medium to which approximately 500 cells aspirated from the culture medium were added. Growth and division did not appear to occur, but it was found that the cells would do well if old medium —that is, medium in which other cells had been growing—was filtered and used in place of the new. Five days later, a cell count showed that approximately 10,000 cells were then present.

One of the most interesting aspects of our experience with the growth of the ascites cells in tissue culture is the cells' unconventional mode of attachment to a glass surface. When the cells are obtained from the animal, they are suspended in ascitic fluid, and they remain suspended for about 3 weeks in tissue culture medium. It is then that the culture appears to be dying out, since fewer cells are suspended in the medium. However, the cells are now found to be growing on the roof and sides of the culture bottle, completely out of the tissue culture medium, and only a few cells remain suspended in the medium. The cells clinging to the roof and sides were only in contact with the medium once every 24 hours, when the culture bottle was inverted for microscopic examination. Certainly, the growth is not characteristic of the majority of cells in tissue culture—for example, Hela, Mepi, amnion, and rat mammary adenocarcinoma cells. This growth pattern was duplicated in smaller test tubes with 2 ml of medium and approximately 2500 cells. Within a few days, it was seen that many cells growing in the medium became fibroblast-like in appearance, while this form was never seen in cells outside the medium. In time, the growth on the roof and sides of the test tube became greater than that in the medium. The cells on the roof appeared in groups which might be merely accumulations of cells or colonies derived from single cells.

From our study of the Ehrlich ascites tumor cells in tissue culture, it has become evident that the cells can be grown in an environment with a thin layer of nutrient renewed at intervals, high humidity, and an atmosphere of 5 percent CO_2. Further experiments are now in progress to study the peculiar growth pattern as well as the behavior of the ascites tumor cells on a completely synthetic medium—that is, one eliminating the use of horse serum (*6*).

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Acyl N → O Shift in Poly-DL-Serine

Abstract. It has been demonstrated that concentrated sulfuric acid causes the polypeptide, poly-DL-serine (MW≈5000), to rearrange to the polyester, to the extent of 70 percent of the original number of amide bonds. The remaining hydroxyls of the serine residues become sulfonated.

The effect of strong acids on the peptide link involving a hydroxyamino acid has been widely investigated since Bergman *et al.* (*1*) first demonstrated an N → O shift under such conditions.

Recently, Bock and Thakur (*2*) have reported evidence strongly supporting an N → O shift due to the action of concentrated sulfuric acid on proteins. Reitz *et al.* (*3*) had originally reported that when proteins were treated with sulfuric acid for a short time, all hy-