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-

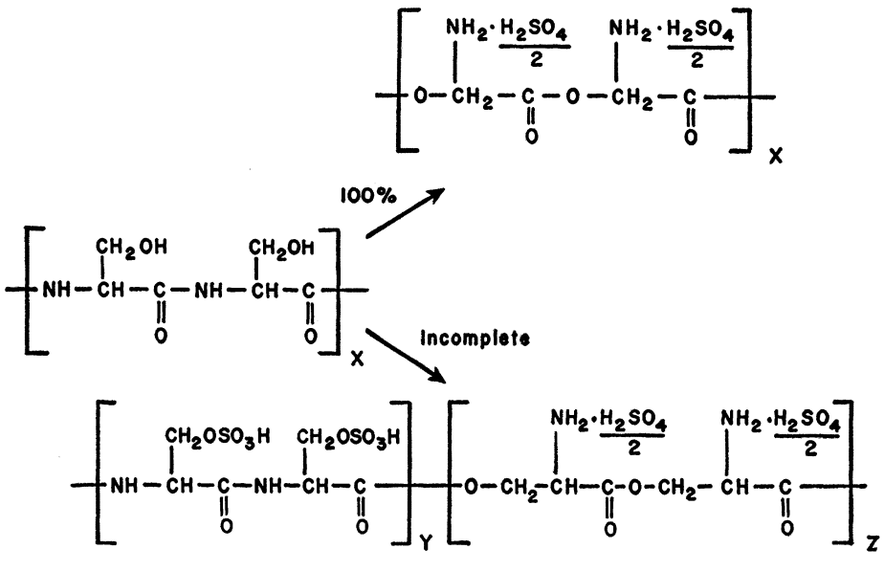

Fig. 1. Conversion of poly-DL-serine with 100-percent and with incomplete N → O shift.

droxyls were sulfonated, and on prolonged treatment a more pronounced change occurred with simultaneous sulfate elimination, which could be interpreted as an N → O shift (*4*). Elliott (*5*) demonstrated that concentrated sulfuric acid causes this shift about the serine and threonine residues in silk fibroin and lysozyme. This acyl shift was also shown to occur by the action of anhydrous phosphoric acid on silk fibroin (*6*). The effect of sulfuric acid on glutein and gliadin has also been studied (*7*). Insulin has been shown to retain its biological activity after H_2SO_4 treatment, and there was no indication that peptide cleavage occurs under such conditions (*8*).

Treatment with anhydrous formic acid, previously interpreted as causing an acyl shift, has recently been shown to involve exclusively O-formylation (*9*).

In this report, poly-DL-serine (*10*) was used as a model to examine the N → O shift in polypeptides and proteins. This polyamide would be converted to a polyester if a 100-percent shift occurred, or to a mixed polyester-polyamide if the shift was not complete, and the remaining hydroxyls would be expected to be sulfonated (see Fig. 1).

Poly-DL-serine (80 mg) was dissolved in 5 ml of cold (−30°C) concentrated sulfuric acid, and allowed to stand at room temperature for 3 days (*11*). The yellow solution darkened slightly on standing.

The polymer was precipitated in anhydrous ether at −30°C, centrifuged, washed with anhydrous ether until sulfate-free, suspended in dioxane, lyophilized, and dried at 70°C in high vacuum.

The infrared spectra of the product and model compounds are presented in Table 1. The infrared spectra show the appearance of an ester bond at 1740 cm^{-1}, a decrease in the amide II frequency at 1545 cm^{-1}, strong NH_3^+ absorption frequencies at 1610 cm^{-1} and 1520 cm^{-1} due to the ammonium salt of sulfuric acid, sulfate absorption frequency bands at 1440 cm^{-1} and 1040 cm^{-1}, and a covalent sulfate absorption frequency at 1200 cm^{-1}. The amide I absorption at 1655 cm^{-1} was not observed in the treated polymer.

These results indicate that the N → O shift has occurred, but the presence of O-acid sulfate shows that this rearrangement did not occur at every amide link.

The elementary analysis obtained was: C, 23.49; H, 4.53; N, 8.57; and S, 13.32, yielding an average residue weight of 153, lying between the residue weights of poly-DL-serine (87) and poly-O-acid sulfate-serine (183). The amount of ester in the product was found to be 70 percent by the hydroxamic acid test (*12*); DL-serine acid sulfate gave a negative result with this test. Using this figure to assess the amount of sulfate present as the amine salt, the amount of O-acid sulfate can be determined from the total sulfur content. A value of 30 percent sulfate was calculated by this procedure, indicating that all free hydroxyls were sulfonated.

After the hydroxamic acid test the material was chromatographed under conditions which hydrolyzed the hydroxamic acid (pyridine : MeOH : H_2O, 1 : 20 : 5). Three spots were obtained, one of which was identified as serine; the other two were probably di- and tri-serine. The presence of dimers and trimers would be expected, since 30 percent of the peptide bonds were not cleaved.

These results indicate that concentrated sulfuric acid caused an acyl shift of 70 percent of the peptide bonds to esters in poly-DL-serine.

Table 1. Infrared spectra (in cm^{-1}) of poly-DL-serine after H_2SO_4 treatment and model compounds.

Poly-DL-serine (before H_2SO_4)*	1655, ~ 1545, 1520, 1400, 1245, 1065
Poly-DL-serine (after H_2SO_4)*	1740, 1700, 1610, ~ 1545, 1520, 1440, 1200, 1040
DL-Serine-acid-sulfate†	1755, 1600, 1520, 1215, 1060, 1005
DL-Serine methyl ester HCl†	1745, 1595, 1505, 1450, 1390, 1200, 1005

*KBr discs. †Cast film from H_2O.

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