of 5 rabbits was fed 2% cholesterol and a second group was fed 2% cholesterol together with 20% rape oil for 125 days. At autopsy these groups showed no difference in number or size of plaques in the aorta‡ and there were no significant differences in adrenal, liver and blood cholesterols of the two groups.

Thus it appears that rather than having an enhanced effect on cholesterol levels, rape oil fails to produce any marked changes in species susceptible to experimental atherosclerosis. There is, in fact, some indication of a negative correlation between susceptibility to experimental atherosclerosis and ability to respond to rape oil with an increase in adrenal cholesterol levels. It is conceivable that both of these characteristics may be related to species differences in the metabolism of phospholipids like those observed by Pilgeram and Greenberg(17).

Summary. Rape oil caused a marked increase in adrenal cholesterol when fed to rats or mice but had little or no effect in rabbits, guinea pigs, chickens and dogs. This contrasts with results obtained by feeding cholesterol since dietary cholesterol tends to be deposited more readily in the latter four species. The differences in response to rape oil do not appear to be due to species differences in digestibility of rape oil.

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Changes in Serum Proteins During Growth of Malignant Cells *in vitro*.* (22901)

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During periods of progressive growth of cells maintained as strains in continuous culture there is usually an initial adaptive growth followed by growth at near maximum rates and then a slowing down of growth but with the individual cells still active in fluid exchange, transport and storage. The products of these cellular activities may be expected to be reflected in the composition of the serum proteins when used as culture medium. The protein components appear to have importance in cellular responses not only because of their nutritional value but also in immune phenomena. Little is known how-

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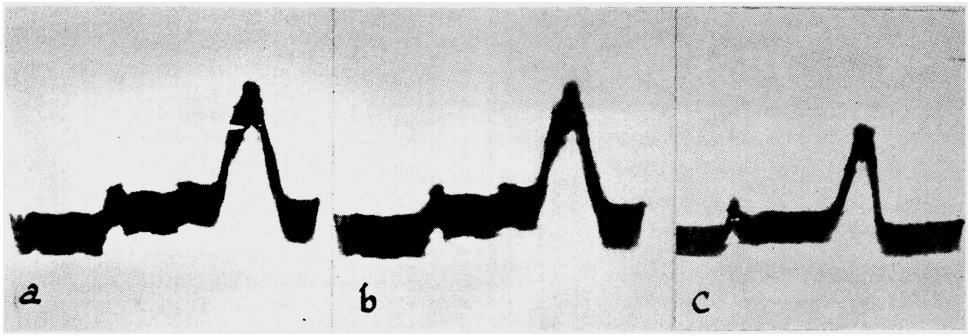

FIG. 1. *Schlieren diagrams* (electrophoresis): (a) *5050* fluid, (b) incubated *5050* fluid, (c) fluid from incubated cell cultures, TSAT-72.

ever about the cellular utilization of, or the contribution to, the serum proteins already present in tissue culture media during various periods of *in vitro* growth.

The role of the proteins in tissue culture has always been a difficult problem because of the complexity of these substances as they exist in biological fluids. We have carried out a series of preliminary studies in order to evaluate the changes in various serum proteins during continuous cell cultivation, especially in culture media derived wholly from such natural sources.

Materials and methods. The medium used in all the experiments was a mixture, designated *5050*, consisting of 50% human placental cord serum with 50% balanced salt solution (Gey). The continuously cultured cells tested were from the rat tumorous strains TSAT-72 and TSAT-333 which originated *in vitro* from normal rat mesenchyme, approximately 17 and 15 years ago, respectively. Also tested was the human fibrosarcoma strain A. Fi., explanted in 1938. Cultures of these strains were incubated for periods of 5 to 10 days without renewal of medium in standard 16 x 150 mm Pyrex roller tubes. The volumes of media used were carefully controlled. Three milliliters of culture fluid, *5050*, were used in these experiments with the cells grown directly on the tube wall. Colony sizes ranged from initial values of 5 mm with 4 colonies per tube to 8 mm at 5 days and 10 mm at 10 days. All experimental culture tubes were accompanied by 2 unincubated and 2 incubated *5050* fluid blanks without cells. These contained the same volumes of medium as their experimental partners and were run for the same periods of time. After 5 days, and as well after 10 days, the colonies and cells were observed to be healthy with many dividing cells present following this single fluid treatment.

Observations and results. At the end of the 5- and the 10-day periods, the culture fluids were centrifuged and the cell-free medium analyzed. Most of the electrophoretic studies were carried out on the fluids by the moving boundary technic, and as well by the paper zone microelectrorheophoresis technic. The Antweiler microelectrophoresis† and the Macheboeuf-Rebeyrotte‡ microelectrorheophoresis apparatuses were used. Electrophoresis with the Antweiler instrument was performed with a barbital buffer system at pH 8.6 with an ionic strength of 0.01. The Schlieren diagrams were photographed after 18 minutes migration under 70 volts potential and 1.8 milliamps. current (Fig. 1).

Significant depletion of the alpha and beta globulin fractions of the serum were noted in the fluids from all the cell cultures. The incubated *5050* fluid blanks did not show a variation in the protein pattern. Similar results were obtained with the technic of Macheboeuf(1). In this case the buffer was also Na-barbital-barbituric acid mixture at pH 8.6 with an ionic strength of 0.05. The duration of migration was 4 hours under 255 volts and 0.5 milliamp. current for one square centimeter of paper. The protein pat-

† From I. Sorvall, Inc., Norwalk, Conn.

‡ From Laboratoires Leres, Paris, France.

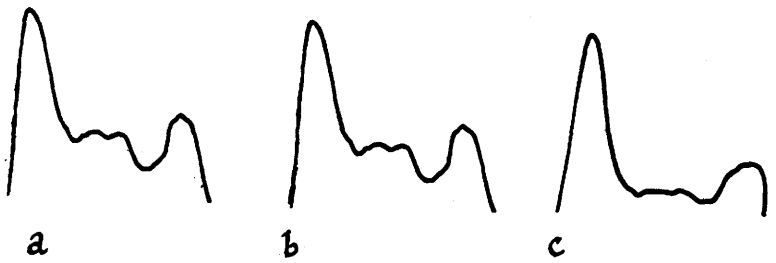

FIG. 2. *Paper electrophoresis recordings:* Same as in Fig. 1.

terns were photographed from the paper strips with an automatic recording photometer‡ (Fig. 2). Here again the decreases in the alpha and beta globulins were noticed in all the cell culture fluids. The albumin fraction seems to remain unchanged. As can be seen from these results, "the alpha-beta reduction phenomenon" appears to be a reproducible one and there is a general agreement in the patterns obtained by the two different procedures.

To support the electrophoretic studies on a more quantitative basis, the Folin-Ciocalteau-biuret procedure described by Lowry *et al.* (2) and the classical microanalytical procedure of Kjeldahl were used for the determination of the proteins and the total nitrogen consecutively. The diagrams (Fig. 3) show the decreases in proteins and also total nitrogen content of the media obtained under these experimental conditions. These microanalytical values support the electrophoretic observations that a significant reduction of the alpha and beta globulins accompanies *in vitro* cell growth during the 5- and 10-day periods.

In a limited number of preliminary tests with pseudo and euglobulins isolated from human placental cord serum and used as the only source of nitrogen in the culture medium, there was also evidence, in both cases, of a reduction of the alpha and beta globulins. Other direct culture tests with alpha globulins isolated by electroconvection showed a significant reduction of these proteins in the cell cultures of the TSAT-72 rat tumorous strain. Fig. 4 illustrates the results of this particular experiment.

Discussion. One might think that these proteins had undergone proteolysis under the influence of enzymes liberated by the growing cells in the culture fluid. However, the total nitrogen values do not support this possibility unless the peptides or the amino acids released by a postulated proteolysis were incorporated by the cells immediately. On the other hand, without cells present the incubated blanks of *5050* fluid showed no evidence of proteolysis. Microchromatographic studies for amino acids with such fluids incubated for 2, for 3, and for 4 days, even in the presence of growing cells, do not reveal any significant increase in the free amino acids already present, nor any appearance of other amino acids. There still remains the question of whether the protein molecules can be incorporated by the cells as such or whether they are first broken down into smaller frag-

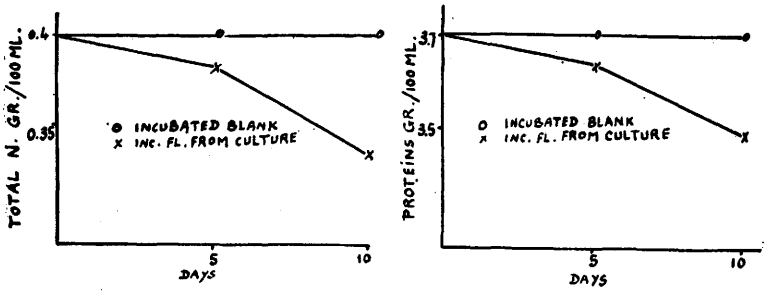

FIG. 3. Decrease in protein and nitrogen of TSAT-72 culture fluid.

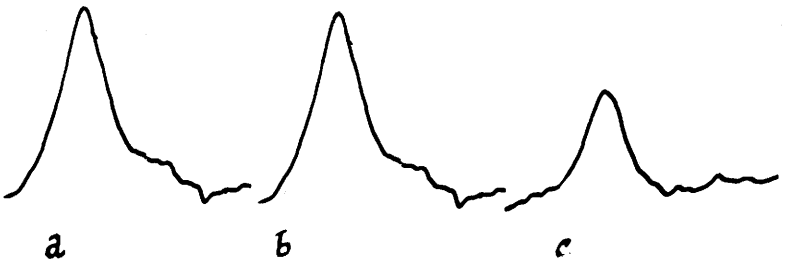

FIG. 4. *Paper electrophoresis recordings:* (a) Alpha globulin medium—no cells, (b) incubated alpha globulin medium—no cells, (c) decrease in alpha globulin when used as culture medium with cells.

ments extracellularly. Attractive experimental data exist concerning the incorporation of whole proteins as shown by Coons(3), as well by Gitlin and Whipple(4) and also by Winnick(5,6) in a series of interesting studies. Using human placental cord serum Jaquez and Barry(7) observed the growth promoting activity of the human placental cord serum which we have used in our laboratories for over 25 years, and reported that this activity is associated with the nondialyzable proteic fraction; the globulins in general had all the activity of the serum. Madden and Whipple's experiments(8) on the participation of serum proteins in the metabolism of cells show another example of the utilization of these substances by living cells *in vivo*. Concerning the method of incorporation of these macro-molecules, one has little difficulty in establishing protein incorporation by the pinocytosis phenomenon of Lewis(9, 10). Other workers claim to have demonstrated extracellular breakdown of proteins before or during incorporation(11,12).

According to our data, apparently entire molecules of protein may be taken up by cells in continuous cultures, yet one must assume that some structural modifications occur during the incorporation. These studies are by no means complete, yet the results appear to be consistent and therefore have considerable significance if we are to understand the method of incorporation of these components into cells and the cellular donation to the external milieu. Some of the future work will be directed toward the use of labelled components in these incorporation studies carried out with living cell systems and will be coupled with quantitative procedures already in use.

Summary. The changes that occur in the serum protein fractions of the tissue culture medium during growth of 2 rat and one human tumorous cell strains maintained in continuous tissue culture were studied. Electrophoretic studies on the culture fluids by the moving boundary, and as well the paper electrophoresis technics, revealed a significant decrease of the levels of alpha and beta globulins of the serum nutrient during specific periods of growth. These findings are supported by microanalytical data.

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