SOME FACTORS INFLUENCING MITOSIS*

The Effect of Chick Embryo, Calf Thymus, and Regenerating Rat Liver Extracts on a Hamster Ascites Tumor

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Introduction

Recently, a hamster ascites tumor has been described in which approximately 20 per cent of the cells are polynucleated** (2 or more nuclei per cell).

The present studies have confirmed that the multinucleated cells are formed by processes involving nuclear budding without the appearance of chromosomes and may be classified as amitotic cells (Kater, M., 1940). As a working hypothesis, it was considered that during cell division daughter cells are occasionally produced which have lost, or lose, their ability to form those enzymes or products necessary for the initiation and completion of mitosis, and may be regarded as mutants of the original ascites cells. The significant numbers of amitotic cells formed offered a suitable test system to investigate requirements essential for division by influencing amitotic cells to return to mitotic division.

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**Stevens, D. and Schwenk, E. In press - Experientia, Nov. (1959).

Methods and Results

The ascites cells were cultured for 12-17 days in the peritoneal cavities of adult female golden hamsters, prior to the intraperitoneal injection of the material under test. Twenty-four hours later, ascites fluid was removed from each animal and a sample taken for smear and Feulgen staining in order to determine the percentage of amitotic cells. One thousand tumor cells were counted on each slide. Total tumor cell counts were also made using a standard hemocytometer.

Influence of Sodium Chloride Extracts of Various Tissues

Saline extracts of chick embryos (11 days), or of the other tissues used, were prepared by homogenization in saline (1 gram wet weight tissue in 4 ml. sterile 0.9 per cent sodium chloride solution), followed by centrifugation at 3000 r.p.m. for 10 minutes.

The results shown in Fig. 1A clearly demonstrate that small amounts of chick embryo extract (equivalent to as little as 1/40th gram wet weight tissue) are capable of reducing amitosis from an average value of 20 per cent to 11 per cent. Saline or boiled extract and adult fowl muscle were without effect.

With a decrease in amitosis, there is a concomitant increase in the total number of tumor cells of approximately 40 per cent. That the decrease in appearance of amitotic cells is due to the capacity of some of them to divide in the presence of active added factors, and is not entirely due to increased division of the mitotic cells is strengthened by the following observations:

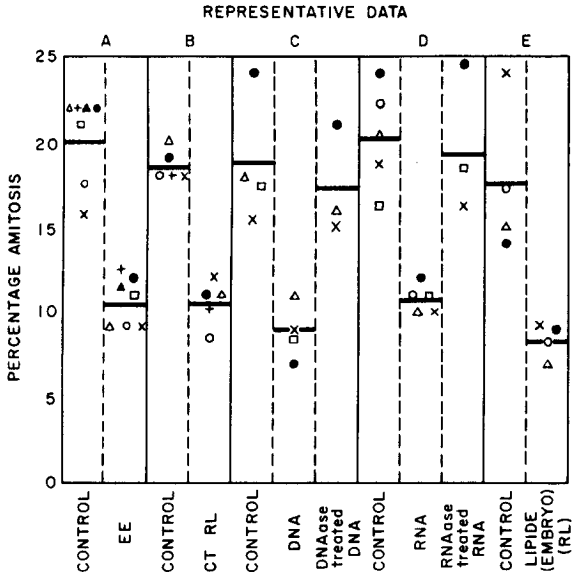

Fig. 1 Each pair of symbols represents a separate experiment using a minimum of eight animals. EE=chick embryo, CT=calf thymus, RL=rat liver.

a. Microscopic examination of the stained cells revealed that where amitosis is reduced, chromosomal figures can often be seen in the nuclear areas of the polynucleated cells, whereas such appearance is rarely, if at all, observed in the untreated or control cultures.

b. Occasionally, in the cultures treated with an active preparation, amitotic cells can be seen with the cytoplasm invaginated around the nuclear areas (cytokinesis). This is not observed in untreated or control cultures.

The data in Fig. 1B also show that similar saline extracts of calf thymus, or adult rat regenerating liver, are comparable to chick embryo extracts in their capacities to reduce amitosis and cause the appearance of chromosomal figures and cytokinesis in the amitotic cells. Saline extracts of normal rat liver are without these influences.

Influence of Nucleic Acid Preparations Obtained from Chick Embryo

Desoxyribonucleic Acid: DNA was obtained and deproteinized by a method similar to that employed for obtaining DNA transforming factors from microorganisms (Braun et al., 1957). The material was dissolved in saline and 350-500 μg injected per animal. The results in Fig. 1C show that the DNA preparations are active in reducing amitosis from a control value of 19 per cent to 9 per cent. Microscopic examination of the cells shows a similar picture to those obtained with the active saline extracts. Prior treatment of the DNA with DNAase inhibited this activity.

Ribonucleic Acid: RNA was extracted by procedures similar to those used to obtain the soluble RNA which has been found to be active in studies on protein synthesis (Hoagland et al., 1959). The RNA was dissolved in saline and each animal received approximately 350 μg intraperitoneally. The results, Fig. 1D, show that amitosis is considerably reduced from a control value of 20 per cent to 11 per cent in the presence of RNA, and a value of 19 per cent after prior treatment of the nucleic acid with RNAase.

It should be pointed out that the nucleases alone, used in the concentrations employed to hydrolyze the nucleic acid preparations, had no observable effect on the cells.

The Influence of Chick Embryo Lipide Extract

Chick embryos were homogenized in acetone (2x) and then ethyl acetate (3x). The combined extracts were taken to near dryness and partitioned between water and ethyl acetate. The water-washed ethyl acetate extract was then taken to dryness *in vacuo* after drying over anhydrous sodium sulphate. The lipide was suspended in propylene glycol, and each animal

received an intraperitoneal injection equivalent to 1/40th of the total lipides extractable from 1 gram of chick embryo. The data in Fig. 1E show that in the presence of the lipides obtained from the chick embryo, the cultures contain 8 per cent amitotic cells as compared to 18 per cent for control cultures or those treated with propylene glycol alone. In addition, preliminary investigations show that lipides extracted from regenerating but not from normal adult rat liver exhibit activity comparable to those obtained from the chick embryo.

Discussion

While there are various references in the literature regarding the influences of tissue extracts on overall cell growth and division (Swann, M. M., 1957, 1958), the cells employed in the present study appear to offer an opportunity to study a more limited aspect, i.e. mitosis.

The present investigations have shown that saline extracts, DNA, RNA, or lipide preparations obtained from certain tissues have the capacity to decrease the percentage of amitotic cells in cultures of an ascites tumor. The relationships between these substances are being studied. That these data are inconsistent with the hypothesis that the active preparations merely increase the rate of division of the mitotic cells without influencing the existing amitotic cells to divide, has been indicated by microscopic examination of the cultures. Also, it would be necessary to postulate that the active preparations cause the mitotic cells to divide without the formation of amitotic cells. Further, using an ascitic form of a renal carcinoma which exhibits 8-10 per cent amitosis, recent work has shown that in the presence of saline extracts, RNA or lipide preparations of chick embryo, amitosis is reduced to approximately 2 per cent. That such a decrease cannot be

entirely due to increased rates of division of the mitotic cells has been indicated, since the activity of the lipide on the culture is maximal within 2-4 hours after injection, a period much less than the division time of the tumor. Consistent with the view that the active preparations do indeed give the amitotic cells the capacity to divide mitotically is the observation that the cultures once treated with DNA (9 per cent amitosis) exhibit the same low amitotic cell count after retransplantation and reculture for a further 14 days with no further treatment (i.e. suggestive of transformation). That the loci of action of the DNA and lipide preparations are different on the amitotic cells has been indicated, since cultures treated with lipide (8 per cent amitosis) rapidly return to a high amitotic level (20 per cent) on similar retransplantation and reculture.

It should be pointed out that while active preparations have been obtained from various tissue sources, there is some specificity to the factors which influence the test cultures, since similar effects have not been observed with preparations obtained from normal rat liver or adult muscle. In this regard, calf thymus has been centrifugally fractionated* and three RNA-protein fractions (one cytoplasmic and two nuclear), separable on moving boundary electrophoresis, have been tested for their capacities to reduce amitosis and produce chromosomal figures in the amitotic cells. Only the cytoplasmic fraction has been shown active in the test system employed.

*A. Herranen (to be published).

Summary

Using an ascites tumor which contains a high percentage of amitotic cells, it has been shown that saline extracts of certain tissues appear capable of causing amitotic cells to undergo mitosis. Similar activities have been found with DNA, RNA, and lipide prepared from such tissue.

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