Trotzdem noch andere Erklärungsmöglichkeiten in Betracht kommen, zeigen die vorgelegten Befunde, dass von geschädigten Zellen unter bestimmten Schädigungsverhältnissen leukozytenemigrationsfördernde Effekte ausgelöst werden können. Damit wird es möglich, das Auftreten leukozytärer Reaktionen im Gewebe bei entzündungserregenden Einwirkungen, die selbst keine leukozytenemigrationsfördernde Wirkung besitzen, einer einheitlichen Deutung zuzuführen.

B. SCHÄR und R. MEIER

Forschungslaboratorien der CIBA Aktiengesellschaft, Pharmazeutische Abteilung, Basel, 7. Oktober 1959.

Summary

Leukocytic cells after slight damage produce stimulation of migration of other normal leucocytes; normal leukocytes inhibit to some extent migration of other normal leukocytes, killed leukocytes have no effect. The significance of this result is, that secondary accumulation of leukocytes with not primary chemotactic substances may be explained by the interaction of damaged leukocytes.

Amitosis in a New Ascites Tumor[1]

In attempting to produce an ascitic form of a methylcholanthrene-induced sarcoma in the hamster (*Mesocricetus auratus*), one resulted which displayed an unusual number of polynucleated cells. The cells of the original tumor employed in these experiments normally divide by mitosis as they grow subcutaneously, yet transformation to the ascitic form resulted in a high percentage of the tumor cells containing more than one nucleus. Microscopic examination showed these occur by budding of a daughter nucleus from the parent nucleus, a type of nuclear division which according to KATER[2] can be classified as amitotic division.

Methods and Results. The original methylcholanthrene-induced tumor was a spindle cell sarcoma[3] and had no amitotic figures present. Using sterile conditions, viable solid tumor was removed from the thigh region of the hamster. 1 g of this tumor was homogenized in 10 ml of sterile isotonic saline pH 7. Each of 20 normal adult female hamsters was injected intraperitoneally with $^1/_2$ ml of the total homogenate. Twenty-eight days later all the hamsters had greatly distended abdomens. They were sacrificed and the peritoneal fluid withdrawn using a sterile 16 gauge needle and syringe. This fluid was smeared, fixed, and then stained by the Feulgen reaction. In addition to the ascitic fluid, each animal had large masses of solid tumor nodules spread through the peritoneal cavity. This material was fixed in 10% formalin and stained with hematoxylin and eosin.

The new ascitic tumor was maintained by injecting 0·5 ml of fresh ascitic tumor intraperitoneally into normal adult female hamsters using sterile technique.

The volume of ascitic fluid which was obtained from each animal varied from 3 to 10 ml and was hemorraghic. Microscopic examination of the ascitic fluid showed that excluding red blood cells there were 95–98% tumor cells present, the remaining cells being leucocytes. Thirteen to 25% of the tumor cells were polynucleated, of these 60–75% had two nuclei present in each cell (Figure 3). The remaining cells had more than 2 nuclei (Figure 4) with 1–2% having as many as 10 nuclei (Table).

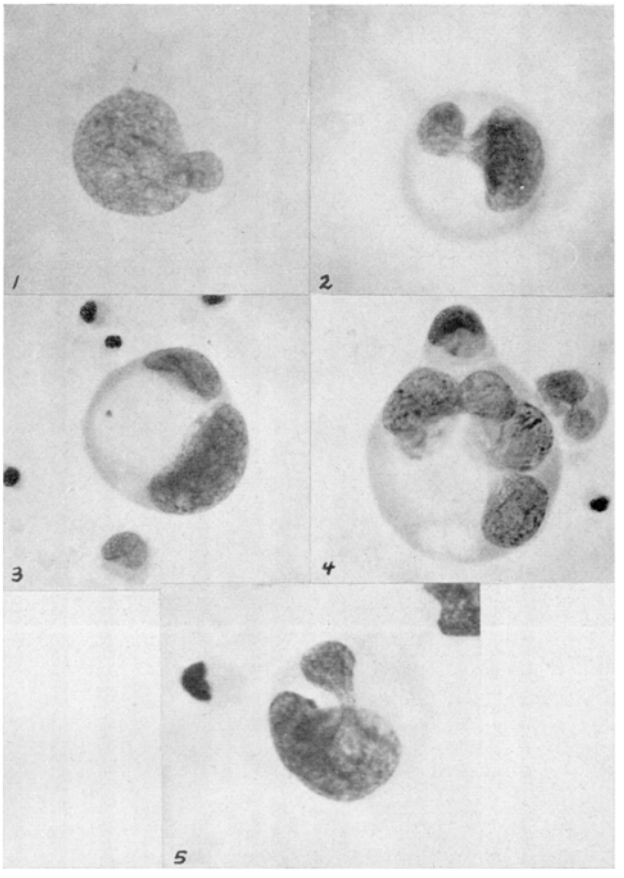

Fig. 1. – Nucleus with a well defined bud
Fig. 2. – Nucleus with the bud more discreet and demonstrating the less densely stained bridge
Fig. 3. – A binucleate ascites cell with a small granule of DNA in the cytoplasm
Fig. 4. – An ascites cell with 7 nuclei present
Fig. 5. – A binucleate ascites cell with a well defined bridge in which parallel filaments are present

The nuclei within one cell were either discreet from one another or attached by bridges which varied in thickness from single strands approximating the thickness of chromatin threads to widths slightly less than the diameter of the smaller nucleus. The nuclei within one cell were usually not equal in size and they tended to be irregular in shape; cells, however, were always oval in shape. Phase microscopy and microscopic observations on many stained cells in various degrees of nuclear budding indicated that the parent nucleus buds off equal size or smaller nuclear segments in much the same manner as an amoeba sending out pseudopods (Figure 1). A connection or bridge between the parent nucleus and the bud appears as the bud becomes more discreet (Fig. 2) and gradually stretches, narrows, and eventually parts completely or remains as a very thin strand. It was quite evident that these bridges stain less densely than the nuclei which they connect.

[1] This investigation has been aided by the U.S. Public Health Service (Grant No. C 321), and an institutional grant of the American Cancer Society.

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Percentage of Amitotic Cells Present in the Hamster Ascites Tumor

Experiment	Tumor development time in days	% Amitosis	Range %
1	28	17	(14-20)
2	16	17·5	(13-21)
3	13	21·3	(17-25)
4	15	17·0	(15-19)
5	12	20·0	(16-22)
6	15	18·4	(13-22)

All amitotic cell counts were made at the terminal stage of tumor development
A minimum of 12 animals were used for each experiment
Experiment 1 represents the transformation from solid to ascites tumor. Experiments 2–6 are subsequent transplant generations of the ascites tumor

This less dense area continues into the nucleus proper where the bridge attaches and gradually shades into the darker stain of the nucleus. The less densely stained bridge has parallel filaments which connect the nuclei and these filaments exhibit kinks, local enlargements, and some coiling along their length (Figure 4 and 5). The Feulgen stain which is specific for nuclear DNA shows small granules spread sparsely between the parallel strands of the bridges, whereas the DNA in the nucleus proper occurs as chunks, granules, and filaments.

The polynucleated cells all tend to be larger than the mononucleated cells although some mononucleated cells of equal size were evident as well as some small polynucleated cells. This observation agrees with ATSUMI's findings[4].

Extensive observations of stained cells and phase microscopy observations of living polynucleated cells of this new hamster ascitic tumor never showed cytokinesis occurring or mitotic figures present in the amitotic cells. Some polynucleated ghosts were present, however, and some polynucleated cells with highly vacuolated cytoplasm were seen.

Microscopic examination of the solid tumor nodules from the peritoneal cavity showed many polynucleated cells present but because of the nature of the closely packed cells it was not possible to obtain as clear a view of their minute structure as in the ascitic fluid smear.

Amitosis is a widely spread occurrence [2] and is common in cancerous tissue. However, other than a study of Yoshida ascites by ATSUMI[4] and brief references by STASNEY[5], KLEIN[6], and LETTRÉ[7], relatively little recent work on amitosis in cancerous tissue appears to have been reported. Usually the percentage of amitotic cells in cancerous tissue is small (1–3%)[4]. This ascites tumor however has 13–25% amitotic cells present.

Amitosis is clearly reproductive in the macronucleus of the ciliates[8] yet the status of amitosis in higher specialized tissues and cancerous tissue is still in question. Cytokinesis was never seen to occur in this ascitic tumor in spite of its high percentage of amitotic cells[9]. Also polynucleated cell ghosts were seen as well as polynucleated cells with highly vacuolated cytoplasm. These evidences would indicate that amitosis in this tumor does not contribute to cell multiplication and is instead a physiological phenomenon and non-reproductive.

D. STEVENS and E. SCHWENK

Worcester Foundation for experimental Biology, Shrewsbury (Mass.), May 19, 1959.

Zusammenfassung

Durch Methylcholanthen-Implantation in Hamster (*Mesocricetus auratus*) verursachtes Sarkom konnte in die Ascitesform verwandelt werden. Der so erzielte Ascites-Tumor weist eine auffallend grosse Zahl (bis zu 23%) amitotischer Zellen auf.

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[6] G. KLEIN, Exper. Cell Res. *2*, 518 (1951).

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[9] MEIER and ALLGÖWER[10] observed amitotic nuclear divisions in experiments on the action of quinones on chicken heart fibroblast cultures. From cinematic observations they also could not find any completed cytokinesis, but believe it is probable that it does occur.

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Immunological Analysis of Bovine Blood Serum and Milk

By means of diffusion-in-gel techniques, it was recently shown by HANSON and JOHANSSON[1] that bovine milk contains twelve antigenic factors. It was also shown that at least six of these substances were related to bovine blood serum proteins, a finding similar to that which has been demonstrated in human milk by the same authors[2–6]. This report concerns some further results in the analysis of these bovine milk proteins related to blood serum.

As antigens mature bovine milk and bovine blood serum were used. In some instances colostrum was used. Immune sera against the milk and the blood serum were obtained from hyperimmunized rabbits. The immune electrophoretic experiments were made according to GRABAR and WILLIAMS[6]. In some instances the micromodification described by SCHEIDEGGER[7] was used. Controls of the absorption experiments were performed with the microslide technique of WADSWORTH[8].

The analysis of blood serum was performed in order to use it as a reference in the immunological analysis of the proteins in bovine milk that are related to blood serum proteins. Agar electrophoresis of the blood serum showed after staining with Amidoschwarz seven fractions that are suggested to be: prealbumin, albumin, α_1-, α_2-, β_1-, β_2-, and γ-globulin (Fig. 1a). Immune electrophoretic analysis of bovine blood serum by means of its homologous immune serum showed a precipitation pattern of 21 separate precipitation lines (Fig. 1b and c). These lines were localized throughout the regions of the aforementioned fractions found in agar gel electrophoresis of the blood

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