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.

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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.

[4] A. ATSUMI, Gann *44*, 21 (1953).

[5] J. STASNEY, A. CANTAROW, and K. E. PASCHKIS, Cancer Res. *10*, 775 (1950).

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

[7] H. LETTRE, H. BALLWEG, H. ENDO, A. SCHLEICH, and W. SIEBS, Biochem. Pharm. *1*, 137 (1958).

[8] J. P. TURNER, Zool. *33*, 193 (1930).

[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.

[10] R. MEIER and M. ALLGÖWER, Exper. *1*, 57 (1945).

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[2–5]. 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

[1] L. Å. HANSON and B. JOHANSSON, Exper. *15*, 377 (1959).

[2] B. JOHANSSON, Nature *181*, 996 (1958).

[3] L. Å. HANSON and B. JOHANSSON, Int. Arch. Allergy *15*, 260 (1959).

[4] L. Å. HANSON, Int. Arch. Allergy *15*, 248 (1959).

[5] L. Å. HANSON, Exper. *15*, 377 (1959).

[6] P. GRABAR and C. A. WILLIAMS, Biochim. biophys. Acta *17*, 67 (1955).

[7] J. J. SCHEIDEGGER, Int. Arch. Allergy *7*, 103 (1955).

[8] C. WADSWORTH, Int. Arch. Allergy *10*, 355 (1957).

serum. Thus two precipitation lines were found in the prealbumin region. The one most toward the anode stained with lipoprotein stain (Sudan black). In the albumin region a dense precipitate was seen, that did not appear when bovine serum was analyzed with immune serum absorbed with a preparation of bovine blood serum albumin. In the same region one more precipitate was seen. Six precipitation lines were situated mainly in the α_1- and three in the α_2-globulin region. The line in the α_2-globulin area that was situated furthest from the immune serum basin stained with benzidin, the dye which has been used for staining the haptoglobin-hemoglobin complex in human blood serum. Three precipitates were found in the β_1- and one in the β_2-globulin region. One of these was a long line without a distinct maximum extending into the α_2-globulin region and situated furthest from the immune serum basin. Finally, in the γ-globulin locale, four lines were seen, one of which was a dense precipitate that reached into the α_1-globulin area. None of the four precipitates in the γ-globulin region were seen when blood serum was analyzed with immune serum absorbed with a preparation of bovine blood serum γ-globulin.

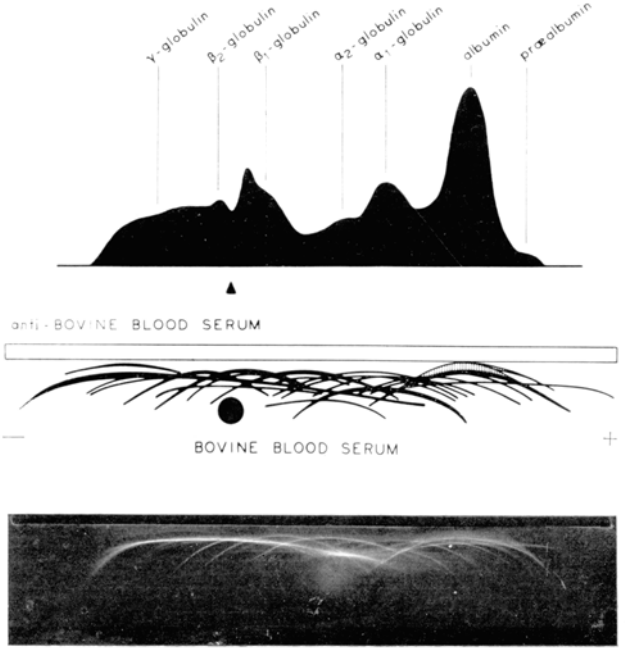

Fig. 1

(*a*) Agar gel electrophoresis of bovine blood serum. Note the seven fractions. (The prealbumin, however, stains more heavily with Sudan black.) The arrow indicates the starting basin.

(*b*) Diagram of immune electrophoretic analysis of bovine blood serum by means of anti-bovine blood serum immune serum.

(*c*) Photograph of the analysis drawn in Fig. 1b.

The relationship between antigenic factors in blood serum and in milk was shown by immune electrophoretic analysis of blood serum with its homologous immune serum absorbed with milk. Of the 21 aforementioned precipitates in the blood serum-anti-blood serum spectrum, only seven remained after this absorption of the immune serum (Fig. 2a). The lipoprotein-staining prealbumin line remained and so did the benzidin-staining line in the α_2-globulin region. The other remaining precipitates were found in the α_1-, α_2-, and β_1-globulin regions. The precipitate corresponding to serum albumin was removed by the absorbtion and so were several precipitates in the α_1- and

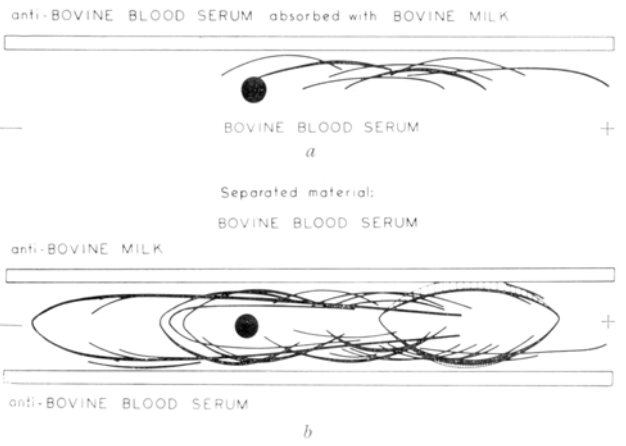

Fig. 2

(*a*) Diagram of the immune electrophoretic analysis of bovine blood serum by means of anti-bovine blood serum immune serum absorbed with milk.

(*b*) Diagram of the immune electrophoretic analysis of bovine blood serum by means of anti-bovine milk and anti-bovine blood serum immune sera.

α_2-globulin areas. All precipitates in the β_1-, β_2-, and γ-globulin regions, except one in the β_1-globulin area, were also removed. These results were further confirmed through immune electrophoretic analysis where anti-milk and anti-blood serum immune sera were put on each side of the electrophoretically separated blood serum. Identity reactions between some of the precipitation lines in the two spectra occurred as seen in Figure 2b. Thus identy was found for the precipitate corresponding to serum albumin, for three of the precipitates found in the β_1- and β_2-globulin regions and finally for the dense precipitate in the γ-globulin region.

Corresponding analysis of colostrum showed some quantitative and qualitative differences as compared to mature milk. These findings are similar to what has been seen in human milk[4].

The immunological analysis has revealed a large number of antigenic substances in bovine milk and bovine blood serum. Serological relationship has been found for several of these factors. Further analysis of these antigenic factors will be published in detail elsewhere.

The preparations of bovine blood serum albumin and γ-globulin were kindly supplied by Dr. B. JOHANSSON, Dept. of Medical Biochemistry, University of Gothenburg.

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Zusammenfassung

Antigene Faktoren in der Kuhmilch, die mit antigenen Faktoren im Blutserum immunologisch identifiziert werden können, sind mit Hilfe von Immunoelektrophorese analysiert worden. Als Vergleich wurde Kuhblutserum verwendet. Dieses zeigt mit homologem Immunserum, dass es wenigstens 21 antigene Faktoren enthält. 10 bis 15 davon können mit Faktoren in der Milch serologisch identifiziert werden.