13476 P

Characterization of Milk Influence in Spontaneous Mammary Carcinoma.

MAURICE B. VISSCHER,* ROBERT G. GREEN† AND JOHN J. BITTNER.‡
(With the assistance of Zelda B. Ball and Heinz A. Siedentopf.)
From the Departments of Physiology and Bacteriology, University of Minnesota, and the Jackson Memorial Laboratory, Bar Harbor, Maine.

Previous studies[1-6] indicate the presence, in at least certain tissues of high cancer strain mice, of a substance or substances whose presence is essential to spontaneous mammary carcinoma. In these studies lactating mammary tissues inclusive of their contained milk have been brought into suspension and partial solution by micro-homogenization and the resulting fluid separated into several fractions by the use of an ultracentrifuge.

Procedure. Fostered female C_3H mice 1 month old were employed as test animals. Lactating breast tissues were obtained immediately after death by etherization, from high cancer C_3H and A strain mice. The material was immediately homogenized with addition of between one and two times its volume of sterile distilled water. The resulting fluid was spun in the centrifuge at 15,000 rpm, giving a maximum accelerational force of 15,000 x g, for 30 minutes. Three layers resulted, a fatty top layer, designated as the fat fraction, a middle aqueous layer, the first supernatant fluid, and a bulky precipitate, the first sediment. The fat fraction was removed and employed as noted below. The aqueous layer was removed by suction without disturbing the sediment. The first supernatant fluid was then spun at 40,000 rpm, corresponding to 110,000 x g, for a period of 60 minutes. The final supernatant fluid was removed without disturbing the second sediment.

* Aided by grants from the Sivertsen Foundation for Cancer Research and by technical assistance from Sub-Project No. 363-65-1-71-140, Works Progress Administration.

† Aided by grants from the Carnegie Corporation and the Cancer Institute Committee of the University.

‡ Aided by grants from the National Cancer Institute and the Jane Coffin Childs Memorial Fund for Medical Research.

1 Bittner, J. J., *Science*, 1936, **84**, 162.

2 Bittner, J. J., *Public Health Reports*, 1939, **54**, 1827.

3 Bittner, J. J. PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 805.

4 Bittner, J. J. *Cancer Research*, 1941, **1**, 290.

5 Bittner, J. J., *Science*, 1941, **93**, 527.

6 Andervont, H. B., and McEleney, W. J., *J. Nat. Cancer Inst.*, 1941, **2**, 13.

The materials used for feeding or injection were (1) homogenized tissue, (2) fat fraction, (3) first sediment, (4) second sediment, and (5) final supernatant fluid. The amount of material of each fraction which was injected or fed was usually that which was obtained in that fraction from 1 g of original tissue. The materials were administered by one of two means: (1) by subcutaneous injection, or (2) by stomach tube. All mice were maintained on Purina Fox Chow, *ad libitum*. Lettuce was supplied at intervals. Each animal was allowed to deliver 5 litters and to carry them to weaning in most instances.

Results. Eighty-four mice were given one or another of the 5 materials listed above, either by subcutaneous injection or by stomach tube, and survived the administration. There was a heavy immediate mortality in animals injected with the homogenized tissue and the first sediment. The incidence of verified mammary carcinoma in these animals at age 12 months is given in Table I. It will be noted that the highest incidence occurs in the group given homogenized tissue, and that the second sediment animals showed a somewhat smaller, but still very large incidence. The first sediment, fat fraction, and supernatant fluid, produced progressively smaller cancer incidence rates.

Centrifugation at 15,000 rpm removed from the aqueous phase nearly all of the large particulate matter. The disperse phase in the first supernatant fluid consists essentially of colloids and crystalloids in solution. Centrifugation at 40,000 rpm for one hour sediments a large fraction of the higher molecular (particle) size dispersed material. Exactly what fraction, in the case of the materials here employed, has not been determined. The second sediment consists mainly of the larger particle size colloidal material, probably largely protein. If a virus protein were present in the material it is very likely that it would be sedimented in this fraction. The low cancer incidence in 22 mice given the fat fraction is believed to indicate that a little of the active material is occluded with the low density fatty material, rather than that the fat itself possesses carcinogenic activity. The lower incidence of cancer in animals given

TABLE I.

Material	No. of mice	No. with tumors	% with tumors	Avg tumor age, mo.
Homogenized tissue	14	10	71	9
Fat fraction	22	3	14	10
First sediment	17	7	41	9
Second "	20	11	55	10
Final supernatant	11	0	0	—

first sediment, than in those given second sediment or homogenized tissue may have one of several interpretations. It is possible that less of the active material is actually sedimented, because of ready solubility. On the other hand, it is equally possible that the large amount of inert material associated with active material exerts either directly or indirectly an inactivating effect.

The observations reported appear to show: (1) That the active carcinogenic agent or agents in the lactating breast from high cancer mice are present in a fraction obtained by ultracentrifugation of homogenized tissue, which fraction is virtually free of matter above colloidal dimensions, and contains a large share of the soluble material of high molecular weight. (2) That the active material in the above-mentioned fraction is either greatly concentrated by the procedures employed or that interfering substances are removed. (3) That the agent or agents in question appear in traces if at all in the fat fraction and in the final supernatant fluid. The exact nature of the active agent is not certain from these studies, but it becomes very probable that the agent is a colloid of high molecular weight and may be a virus. Further studies on a larger series of animals are in progress.

13477

Transfusion of Bovine Serum Albumin into Human Beings.

HARRY A. DAVIS, A. G. EATON AND J. WILLIAMSON.

From the Departments of Surgery and Physiology, Louisiana State University School of Medicine, New Orleans, La.

In a previous communication[1] it was reported that the intravenous administration of bovine serum albumin was effective in raising and maintaining the blood pressure of dogs subjected to severe hemorrhage. The present report is concerned with the effects of transfusion of bovine serum albumin into human beings.

Materials and Methods. The serum was obtained from bovine blood and centrifuged. The supernatant serum was removed, pooled and passed through a Seitz filter. The pooled serum was treated with an equal volume of a saturated solution of ammonium sulfate (54%). This mixture was now filtered through a Buchner filter which removed the precipitated globulin. The filtrate was fully sat-

[1] Davis, H. A., and Eaton, A. G., PROC. SOC. EXP. BIOL. AND MED., in press.