cytolysis by isoimmune serum and limited amounts of complement may provide a method for preparing populations of peritoneal macrophages free of other cell types and suitable for immunological studies(17).

Summary. The entire population of peritoneal cells maintained in suspension is susceptible to cytolysis by isoantibody and complement *in vitro*. In tissue culture, the macrophages spread on glass and become resistant to cytolysis by isoantibody and limited quantities of complement. This contrasts with the marked susceptibility of the other peritoneal elements (lymphocytes, eosinophils, neutrophils and mast cells). In the presence of isoimmune serum and additional complement, however, macrophages are also lysed. Peritoneal macrophages are capable of phagocytizing isogenic tumor cells *in vitro* in the presence of isoimmune serum directed against both macrophages and tumor cells.

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Flocculation of Unsensitized Bentonite Particles by Cancer Serum.* (29262)

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Unheated sera from healthy individuals and from those suffering from cancer flocculate bentonite particles to which no antigenic material has been adsorbed. If, however, both types of sera are heated at 50°C for 30 minutes flocculation is observed with the cancer sera while normal sera reacts negatively or with a much reduced titer. This report concerns the quantitative differences in the above reaction when cancer and other sera are heated to 50°C.

Materials and methods. A. Bentonite suspension was prepared according to the method described by Bozicevich *et al*(1) without use of a dye. The suspension was kept at 6°C and prepared fresh every 5 to 7 days.

B. Serum samples. Two hundred and thirty-eight serum samples from the same number of individuals suffering from various types of cancer were obtained from the Laboratory of Clinical Chemistry; Tumor Surgery

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Ward; from the Blood Bank of the Clinical Center, Nat. Inst. Health; and from the Tumor Clinic, Municipal Hospital, Santurce, P. R. These comprised cancer of the cervix, breast, larynx, nose, tongue, lip, kidney and choriocarcinomas. Sera were also obtained from individuals suffering from: lupus erythematosus, rheumatoid arthritis, syphilis, leprosy, tuberculosis, schistosomiasis *Mansoni*; and from children heavily infected with ascaris, hookworm and trichuris. Six hundred and ten serum samples were obtained from blood bank donors in apparently good health.

Small volumes of each sample were heated at 50 and 56°C for 30 minutes, then tested against the bentonite suspension. Serum dilutions beginning with 1:10 were made 2-fold in 0.05% normal rabbit serum in 0.85% sodium chloride. The rabbit serum was heated for 30 minutes at 56°C and was used to prevent non-specific flocculation. For the actual test 0.1 cc of the serum dilutions was mixed with 0.05 cc of the bentonite suspension. The mixtures in (10 × 75 mm) tubes were then shaken for 30 minutes using a rotary shaker and examined directly under the stereoscope at a magnification of × 20.

Sera from patients with cancer previously known to flocculate bentonite suspension at high titers were treated with bentonite. To 0.1 cc of sera, 0.05 cc of the bentonite suspension was added, shaken for 30 minutes and then centrifuged at 3,000 RPM. The supernatant, which no longer flocculated bentonite, was used as the absorbed serum. Electrophoretic studies were run on the serum samples before and after treatment with the bentonite(2). The standard paper electrophoresis technique was used, with Spinco equipment. Serum samples were run on Whatman 3 mm filter paper strips for 16 hours applying a current of 5 ma using a veronal buffer of pH 8.6, ionic strength of .075. Strips were stained in bromphenol blue (Spinco B-1) and scanned in a Spinco analytrol.

Results. Of the 238 cancer sera tested 236 (99.1%) flocculated the bentonite particles with titers varying from 40 to 2560 (Table I). The percent of positive reactions observed in sera from other diseases was much less and titers were lower than those observed with cancer sera. Among the 610 normal sera only 5.8% were positive (Table II).

TABLE I. Bentonite Flocculation Titers of 238 Cancer Sera.

No. of sera	Titer	No. of sera	Titer
2	0	33	320
4	40	45	640
7	80	87	1280
11	60	49	2560

TABLE II. Flocculation of Bentonite Particles by Various Types of Sera Heated at 50°C for 30 Min.

Source of sera, diseases	No. of individuals tested	Positive No.	Positive %	Titer range
Cancer	238	236	99.1	40–2560
Lupus erythematosus	23	3	13.1	10– 40
Rheumatoid arthritis	18	2	11.9	10– 40
Syphilis	69	1	1.4	10–
Leprosy	31	5	16.1	10– 80
Tuberculosis	46	5	10.8	10– 40
Schistosomiasis	51	2	3.9	10– 40
Ascaris, Hookworm, Trichuriasis	33	0	0	—
None (blood donors)	610	34	5.8	10– 20

All sera reacted negatively when heated at 56°C for 30 minutes.

Electrophoretic studies of 15 cancer sera revealed that treatment with bentonite reduced the a_2 globulins. In 12 of the 15 the reduction ranged from 1.4 to 4.8% and 0.5, 0.8 and 0.9 in the remaining 3. This effect on the a_2 globulins was not observed when bentonite was added to 5 negatively reacting sera. Increase of the remaining serum fractions to compensate for the reduction of the a_2 was not constant for any other fraction.

Discussion and summary. The high degree of flocculation of unsensitized particles of bentonite by cancer sera and not by normal or by sera from other disease conditions, may be of help in developing a test for cancer diagnosis. Heating of the sera at 50°C for 30 minutes is required to establish the difference in reactivity between cancer and other sera. The factors affected by such heat treatment are not yet clear.

Although the flocculation reaction occurs

in cancer sera in a gradient and at high titers, no other indication could be found to show that this may be an antigen-antibody reaction, as could have been expected if the bentonite particles became coated with either excess antigen or antibody present in the sera. Also, addition of guinea pig complement did not restore the reaction in sera heated to 56°C for 30 minutes. Since *in vivo* antigen-antibody interaction has been considered to take place in some acute or chronic diseases such as systemic lupus erythematosus and glomerulonephritis, this theory should not be discarded.

Absorption of the a_2 globulin by the bentonite suggests an ionic type of reaction with some component of this fraction. Immunoelectrophoretic studies using anti-a_2 globulin sera and bentonite may clarify this point.

The low percentage of reactivity of normal and other sera as compared with cancer sera suggests the possibility of a test based on the effect of bentonite on specific components of the a_2 globulin.

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Placental Transfer of Free Fatty Acids in the Pregnant Rat. (29263)

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The transfer of maternal plasma lipids to the fetus has been investigated with isotopic technics by Goldwater and Stetten(1) and by Popjak and collaborators(2), who demonstrated that the placenta is relatively impermeable to cholesterol and phospholipids (PL). These lipids, as well as triglyceride esters of fatty acids (TG) circulate as constituents of lipoproteins of large molecular size, which seems to preclude their direct passage through the placental barrier. Free fatty acids (FFA) are known to be available for the metabolism of body tissues, where they are assimilated by esterification into TG or PL. Albumin, the main carrier protein of FFA has been shown to enter the fetal circulation of man and rabbit to a small degree(3,4). The sheep and rabbit placental membranes were reported to be somewhat permeable to palmitate-1-C^{14}(5,6). The crossing through rat placenta from the maternal or fetal side, of labeled palmitic, stearic and linoleic acids, was presently studied.

Experimental. FFA labeled with C^{14} at 1-position were purchased from Amersham (Great Britain). After conversion to their respective sodium salts the FFA were complexed to a 1% solution of bovine albumin (Pentex, U.S.A.) in phosphate buffer pH 7.4, and the solution filtered before use. Albino rats of a local strain were kept on a stock diet and used during 17-19th day of pregnancy. The abdomen was opened under light ether anesthesia and either 0.5 ml of the FFA solution injected into the lower abdominal aorta, or doses of 0.1 ml injected into each of the amniotic sacs. Intrafetal injections were effected by entering through the uterine wall into the peritoneum of each fetus. On the average 8 fetuses were present (range 6-11) and total radioactivity introduced per animal was within 2-3 μc. The rats were then closed and sutured and at various times again anesthetized and bled through the abdominal aorta. The tissues were promptly excised, weighed, rinsed and extracted thoroughly by homogenization in a large volume of ethanol-ether 3:1. Aliquots of the extracts were counted after evaporation *in vacuo* in a Packard Tricarb Scintillation Spectrometer. Other aliquots were used for separation of PL by precipitation with cold