of which has also been demonstrated in the mouse L-cell strain and in tissues of certain inbred mouse strains(12). Others might well possess characteristics of isoantigens, *e.g.*, those which have not uniformly been found in every human cell strain(10,11). The latter possibility would have to be verified by comparatively analyzing these antigens in many individuals. Once the isoantigenic nature of some of these antigens is established, the door would be open for genetic studies and possibly even practical applications. On the other hand, connections to transplantation and transfusion problems might have to be taken into consideration.

Summary. Antisera were available which had been prepared in guinea pigs and other animal species against established human cell strains such as HeLa, etc. By means of agar-immunodiffusion tests numerous cellular antigens had been defined. Individual sera had been analyzed regarding the antibody specificities they contained. In the present study, selected reference sera were absorbed with pooled human erythrocytes and then subjected to immunodiffusion tests against cell antigen extracts of known composition. Disappearance or significant weakening of specific precipitation lines indicated successful absorption of a specific antibody and, hence, the presence of the corresponding antigen on the erythrocyte membrane. This was established for 8 out of 15 specific cellular antigens investigated. An attempt to correlate precipitating and species-specifically hemagglutinating antibodies was in part satisfactory. It was emphasized in the discussion that precipitable cellular antigens exist in soluble form whereas the corresponding antigens on the erythrocyte membrane are structurally bound.

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Differences in Serological Properties of Macroglobulins from Cancer And Normal Sera.* (31608)

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Previous investigations have revealed that sera from patients with cancer flocculate unsensitized particles of bentonite, while sera from normal humans give negative or low titered reactions. Both the cancer and normal sera had to be heated to 50°C for 30 minutes in order to establish this difference in reactivity. In addition, electrophoretic studies showed that when bentonite particles were added to cancer sera, alpha-2 globulins were reduced markedly, which was not the case when the particles were added to normal human sera(1).

Using Sephadex particles as molecular sieves, serum globulins were separated from

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normal and cancer sera in order to determine which components were responsible for the flocculation of bentonite particles. When these were separated, other studies were performed to compare the serological activities between globulins isolated from cancer and from normal sera.

Materials and methods. Separation of macroglobulins using gel filtration and ultracentrifugation. Three ml of serum were utilized for fractionation in a column of Sephadex G 200 using phosphate buffer of pH 7.3 and 0.15 M NaCl as eluant. Serum proteins are eluted in 3 main peaks. The first peak contained the alpha-2 macroglobulin, alpha and beta lipoproteins. Gamma globulin is eluted in the second peak and albumins and transferrin in the third peak.

The alpha-2 macroglobulin in the first peak was separated from the lipoproteins by mixing equal parts of the pooled fractions in the first peak with a solution of NaCl of 1.13 density, (203.7 g NaCl/l), after centrifugation for 18 hours in a Spinco Ultracentrifuge at 40,000 r.p.m. Lipoproteins remained in the supernatant and the macroglobulin was recovered as a jelly-like disk at the bottom of the tube (2).

Serological studies. Bentonite flocculation tests were performed as described previously (1). All serum fractions to be tested were diluted in serial double dilutions beginning with 1:2 and made in a medium consisting of 0.05% heated normal rabbit serum in 0.85% sodium chloride. To 0.1 cc of the diluted fraction, in 10 × 75 mm tubes, 0.05 cc of the bentonite suspension was added and mixed properly using a rotary shaker. After shaken for one hour at 37°C, the tubes were examined directly under the stereoscope at a magnification of × 20.

Serum fractions were tested for isoagglutinin content by adding human erythrocytes of Groups A, A_2 and B. One drop of a 1% suspension of erythrocyte in saline was added to 0.1 cc volumes of the diluted fraction. The mixtures were shaken and read under the stereoscope after 1 hour at room temperature.

Inhibition of isoagglutinins was attempted by adding various extracts from *Ascaris suum,* the common intestinal nematode from swine. A very active inhibitory substance was isolated from the coelomic fluid of these worms (3). The fluid was extracted using 1 cc syringe and 27 gauge needle and centrifuged at 3,000 r.p.m. for 30 minutes to remove undissolved particles. The supernatant was lyophilized and stored at −20°C. Portions of the lyophilized material were reconstituted in water and filtered through columns of Sephadex 200 using phosphate buffer pH 7.3 as eluant. The soluble proteins separated into 3 main peaks. The substance from the first peak (CFS) was shown to have A_2 isoagglutinogen-like properties, since it inhibited the a_2 agglutinins in human sera of Groups A and B. This substance was used to test serum globulins for inhibition of a_2 isoagglutinins. The substances from the other two peaks were inactive.

The inhibition tests were performed by adding known volumes of coelomic fluid substance (CFS) to an equal volume of serum fraction dilution, and the mixture incubated at 37°C for 60 minutes. The A_2 erythrocytes were then added and tubes examined for erythrocyte agglutination after standing 1 hour at room temperature.

The alpha-2 macroglobulins isolated from cancer and normal sera were tested against human anti-alpha-2 commercial serum prepared in rabbit (Lloyd Brothers Inc., Cincinnati, Ohio). The agar gel double diffusion slide technique of Yakulis and Heller was utilized(4). The anti-alpha-2 sera and the macroglobulin were diluted in saline from 1:2 to 1:64 in order to reach optimum titrations of both antibody and antigen. The slides were observed after 24 hours incubation at 37°C and 5 to 6 days after incubation at 6°C.

Results. Flocculation tests. A total of 83 blood samples were obtained from individuals suffering from cancer of cervix, breast, nose, tongue and lip. Flocculation of bentonite particles was observed in 76 or 91.5% of the alpha-2 macroglobulins; reactions with the alpha and beta lipoproteins, gamma globulin, albumin and transferrin from these same individuals were negative. The titers observed with the alpha-2 macroglobulin varied from 1:4 to 32, (Table I).

Eleven of 48 (22.9%) alpha-2 fractions

TABLE I. Flocculation of Bentonite Particles by Macroglobulins from Cancer and Normal Sera.

Cancer			Normal		
No. of macroglobulins	Titer range	No.—% positive	No. of macroglobulins	Titer range	No.—% positive
83	4-32	76—91.5	48	2-8	11—22.9

TABLE II. Inhibition of a_2 Isoagglutinins in Cancer and Normal Sera of Blood Groups O and B, by CFS.

Cancer			Normal		
No. of macroglobulins titers tested	Titer range	No.—% with complete inhibition	No. of macroglobulins titers tested	Titer range	No.—% with complete inhibition
25	4-32	4—16.0	25	4-32	21—84.0

from the sera of healthy individuals gave positive bentonite flocculation. The titers observed in the positive fractions varied from 1:2 to 1:8.

Reactions with coelomic fluid substance. The a_2 isoagglutinin titers of alpha-2 macroglobulins isolated from 40 cancer sera of Groups O and B, varied from 1:4 to 1:32. Twenty-five were selected with the highest a_2 agglutinin titer and were used to react with 5 mg per ml of the CFS material. In only 4 out of 25, or 16.0%, the a_2 agglutinins were completely inhibited with negative titers when tested against A_2 erythrocytes. In contrast to this, complete inhibition of a_2 agglutinins was observed in 21 out of 25 sera (84.0%) from normal individuals, (Table II.)

Precipitin reactions between alpha-2 macroglobulins and antisera. The alpha-2 macroglobulins from 30 cancer and 25 normal sera were tested against anti-alpha-2 sera. Of the 30 fractions from cancer sera, 27 or 90% reacted with 3 bands of precipitate; 2 with 2 bands, and 1 with 1 band. Of the 25 fractions from normal sera, 19 or 76.0% reacted with 1 band and 6 or 24.0% with two bands.

Discussion and summary. Studies on the alpha-2 macroglobulins isolated from the sera of patients with various types of cancer and from the sera of healthy individuals revealed definite serological differences between the two groups of fractions. These differences relate to flocculation of bentonite particles, reaction with coelomic fluid substance, and precipitin reactions with anti-alpha-2 sera.

The alpha-2 fraction from cancer sera was found to flocculate bentonite particles in 91.5% of the fraction tested, while the reaction was negative in 77.1% of the fractions from normal sera. This is in accord with previous investigation in which the addition of bentonite particles reduced the alpha-2 content of cancer, but not of normal sera. The increased affinity of macroglobulins from cancer sera to bentonite may be based on differences in total electric charge. This, however, should be investigated using ion exchangers to separate the fractions from both types of sera.

Treatment of cancer sera and normal sera of blood Groups O and B with CFS revealed that in cancer sera the a_2 agglutinins may be altered, since a large percentage of them were not inhibited by CFS, as was the case of agglutinins in normal sera. Changes of a_2 isoagglutinins present in cancer sera may be due to the effect of antigenic substances from blood and other tissues released during tissue destruction. These may not have the normal antigenic pattern and thus immunize the individual producing antibodies differently from the normal isoagglutinins. Cancer sera, unlike normal sera, may therefore have abnormal agglutinins not inhibited by CFS, but normal a_2 isoagglutinins may also be present, depending on the stage of the disease.

The fact that a large percentage of alpha-2 globulins from cancer sera reacted with 3 bands of precipitate when tested against anti-alpha-2 sera, while no more than 2 bands were observed with the fractions from normal sera, suggests that the macromolecules from cancer

sera are affected, and possibly split into smaller molecules. Since cancer processes alter serum globulins, this may be also the case with the alpha-2 macrogobulins.

The findings above suggest that the changes produced in alpha-2 macroglobulins by cancer processes should be further evaluated in relation to the development of a serological diagnostic test.

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Effects of Early Embryectomy and Hormonal Therapy on the Fate Of the Placenta in Pregnant Rhesus Monkeys. (31609)

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Early death of the embryo has been suggested as one of the possible etiological factors of hydatidiform mole, a complication of human pregnancy(1,2). That hypothesis was the basis for this study of the effects of embryectomy on the retained placenta in the rhesus monkey *(Macaca mulatta)*. This species was selected because of the similarity of its placenta to the human placenta and because the diagnosis of pregnancy can be established early by testing for chorionic gonadotropin activity in the serum or urine from the 14th to the 35th day of gestation(3, 4,5).

It has been demonstrated in rabbits(6,7), rats(8), and monkeys(9) that careful removal of the embryo results in persistent viability and function of the placenta for the normal duration of gestation in a large percentage of the animals so treated. Van Wagenen and Newton(9) have shown that removal of the embryo from the rhesus monkey at 70 to 157 days of gestation (normal gestation averages 164 days) does not interfere with the viability or growth of the placenta until it is spontaneously delivered near the expected due date. No abnormalities in placental shape, size, or histology were commented on in that study. The present study was carried out to determine whether early removal of the embryo (29th to 34th day of gestation) would cause any morphologic changes in the placenta.

Materials and methods. Seven adult female rhesus monkeys underwent embryectomy at 29 to 34 days of gestation. The onset of gestation was taken as the time the monkeys were housed with the male if serum drawn approximately 3 weeks later showed evidence of chorionic gonadotrophic activity in the mouse uterine weight assay of Klinefelter *et al* (10). Four of the monkeys received no medication and underwent hysterectomy 14 to 36 days following embryectomy. Three others received cortisone (25 mg s.c. daily) and 19-norethisterone (20 mg p.o. daily) and were sacrificed and autopsied 166 to 168 days following embryectomy. The latter time is at least 4 weeks later than the expected duration of gestation for those mating. An outline of the study protocol is included in Table I.

Removal of the embryo was carried out under pentothal anesthesia through a midline lower abdominal incision and a vertical incision in the fundus of the uterus. Care was taken to enter the chorionic cavity in the clear space between the two discs of placental tissue(11). The umbilical cord was cut and the embryo removed. The uterus was then closed in one layer with a continuous cat gut suture.

Results. Viable chorionic villi were found

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