mia was due to increases in all fractions though the major increase was still in the fraction of density 1.006-1.063. The rate of cholesterol influx was most closely related to the concentration of cholesterol in this fraction. Among animals with dietary hypercholesterolemia, there was a significant relation between influx rate and aortic cholesterol concentration; in nephrosis, aortic cholesterol was higher than in animals with comparable influx rates produced by dietary means.

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Placental Barrier to the Fetal Transfer of Maternal Chloroleukemia In Rats.* (30799)

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At least two types of virus transmissible rodent leukemia are capable of transplacental passage with resultant vertical transmission patterns(1,2). The object of this study was to determine whether or not the direct transplacental transmission of Shay chloroleukemia in the rat could be demonstrated. Injection of cellular suspensions from Shay chloroleukemic rats to normal newborn recipients less than 7 days old results in virtually 100% transmission of the leukemia(3). If maternal leukemic factors enter the fetal circulation the offspring of the pregnant leukemic rat either should have congenital leukemia or should develop the disease during the usual incubation period of 4 to 12 weeks. The results of this study indicate that either the placenta or some other factors associated with pregnancy are inhibitory to the transmission of this type of rodent leukemia.

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Experimental method. The initial experimental subjects for these studies were adult, albino, female Sprague-Dawley rats. Each was caged separately and placed on a diet of Purina Chow biscuits and water. Several groups of rats were identified as follows:

Group I was composed of 5 rats with the Shay chloroleukemia which had been kept in remission with thiotepa (N,N′,N″-triethylenethiophosphoramide) in order to reach maturity. Leukemia had been transferred initially to these rats during the first few days of life. At 4-5 months of age each was mated with a normal male Sprague-Dawley rat. The

TABLE I. Results of Chloroleukemia Transmission Studies in Rats.

Group	No. followed	Total months followed	No. developing leukemia
(I) Mother rats with leukemia in remission	4	3	4
F_1 generation	29	24	0
(II) Mother rats injected with leukemia cells	4	24	0
F_1 generation	29	24	0
F_2 "	22	20	0
(III) Mother rats cross-transfused with leukemic rats	5	24	1
F_1 generation	26	18–24	0
(IV) Non-pregnant female rats cross-transfused with leukemic rats	3	4	3

thiotepa then was discontinued so that they again might develop leukemia at about the time of parturition.

Group II consisted of 6 pregnant rats without leukemia which were injected intravenously about 2 weeks after conception with 0.1 ml of a suspension of spleen cells from a rat with overt leukemia. The spleen cell suspension contained 98,500 leukocytes/ml[3].

Group III contained 6 non-leukemic pregnant rats, each of which was cross-transfused 7 to 23 days antepartum with an adult rat with leukemia. About 40 ml of blood was transferred from one rat to another by the technique of Brodish and Long(4). This amount represented about 1½ to 2 complete circulatory mixings.

Group IV consisted of 3 adult, non-pregnant female rats each of which was cross-transfused with another rat with overt leukemia. The procedure was identical to that for the rats in *Group III*.

The pregnant, female rats were weighed 3 times a week, and on each a total and differential leukocyte count was performed at weekly intervals. During the first postpartum week the mothers were not disturbed, but thereafter weighings and blood counts were resumed as indicated. At 3 weeks of age, the infant rats were separated from their mothers. Animals which survived for a period of 3 months were followed with weekly weights and monthly blood counts, unless any sign of weight loss intervened at which time careful total and differential leukocyte counts were performed.

Results. Table I summarizes the outcome of the follow-up observations on all adult female rats and their progeny.

Group I: Four of the 5 rats in remission of their leukemia became pregnant. Two had overt leukemia at time of delivery and the other 2 developed leukemia 37 and 62 days postpartum. One animal developed leukemia shortly after initiation of the experiment, but did not respond to therapy and died without mating successfully. Twenty-nine of the 31 offspring of these rats have been followed for a period of 2 years, and none has developed leukemia. Two rats died when less than 2 weeks old while being cared for by a foster mother. Their blood counts just before death were within normal limits.

Group II: Four of the 6 female rats injected with leukemic spleen suspension became pregnant. None was overtly leukemic at time of parturition but 2 died without leukemia at 5 and 20 months following injection. None of the 29 offspring developed leukemia over a 2-year follow-up period. Three of the female offspring were mated with normal, albino, male Sprague-Dawley rats and none of the 22 second generation offspring developed leukemia.

Group III: Five of the 6 pregnant rats subjected to cross-transfusion with leukemic rats survived and delivered their offspring. Twenty-six of these offspring were followed for periods ranging between 1½ and 2 years. None developed leukemia. Of the 5 pregnant rats that survived cross-transfusion 1 died of leukemia and 3 others showed transient abnormalities of the total and differential leukocyte counts which disappeared within 1 and 2 months.

Group IV: All 3 of the non-pregnant, adult,

cross-transfused female rats developed leukemia between 14 days and 4 months following the cross-transfusions.

Discussion. None of the progeny of the mother rats with either active or inactive leukemia developed leukemia. A number of rats in the F_2 generation also was followed, and no leukemia was observed. Of additional interest was the fact that only 1 of the 5 pregnant female rats cross-transfused with a leukemic donor developed leukemia in comparison to 3 of 3 non-pregnant controls. In an earlier study 5 of 10 normal adult rats developed leukemia following cross-transfusion with Shay chloroleukemia rats(5). The complete absence of maternal-fetal leukemia transmission along with the apparent reduced susceptibility of pregnant rats to this leukemia suggests that some factor or factors associated with pregnancy inhibits transmission of this type of leukemia. Evidence for a common factor, protective to both maternal and fetal leukemia transmission, however, is purely speculative.

The presumed high degree of Shay chloroleukemia susceptibility for the developing fetus is based on the high incidence of leukemia transfer to neonatal rats, evidence for fetal enhanced immunologic tolerance, and the human occurrence of congenital leukemia (6,7). Successful maternal-fetal leukemia transmission, however, depends not only on fetal susceptibility, but the perfusion of fetal tissues with leukemogenic factors. The Shay chloroleukemia seems to require the actual transfer of cells for its transmission and the cell free filtrate capable of transmitting leukemia has not been demonstrated. Recently intercellular particles with the morphologic features of type C virus particles have been identified in chloroma tissue from rats with the Shay chloroleukemia(8). The distribution of the particles was similar to that reported for other murine leukemia viruses.

The mechanism of fetal and maternal chloroleukemia resistance during pregnancy is unclear. Placental leukotoxicity or mechanical leukocyte blockade might protect the developing fetus during intra-uterine life, but not the mother. One simple explanation for a mechanical effect might be small vessel leukocyte trapping(5) which could lead to eventual sequestration and destruction of the leukemic leukocytes in the placental capillaries. Several reports have emphasized the efficiency of the human placenta as a barrier to the fetal transmission of leukemic cells(9-11). Endometrial and intervillous but neither placental nor fetal infiltration has been noted. Bierman *et al*(12) found a leukocyte count of 3,300/mm^3 immediately after birth in a baby from a leukemic mother with a leukocyte count of 154,000/mm^3. In spite of this apparent efficient placental blockage, it is unlikely that some leukocytes, their degradation products, and non-formed blood elements do not enter the fetal circulation. A maternal or placental humoral factor would protect both the mother and fetus from development of leukemia during pregnancy but only for a limited period thereafter. This mechanism would be in accord with the concomitant postpartum development of leukemia in a mother and her newborn offspring(13). A filtrate with tumor inhibiting properties has been prepared from human placental lysates (14) and it seems likely that the "maternal resistance factor" or "milk factor" may be an example of a humoral leukemia antagonist (15). It is possible that the F_1 generation and their succeeding generations of rats do harbor a latent leukemia virus. Perhaps exposure to the proper exogenous stimulus sometime later in life would activate this virus, but this remains a matter of conjecture.

Summary. I. No leukemia developed in a total of 84 offspring of a group of female rats with either active or latent Shay chloroleukemia followed for periods up to 2 years. None of 22 F_2 generation offspring followed for a similar period developed leukemia. II. These data indicate efficient transplacental inhibition of Shay chloroleukemia transmission in rats. Although transplacental passage of inadequate numbers of leukemic cells may account for this result there is some evidence that either the placenta or some humoral placental factor may be responsible.

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Dissociation of Esterolytic and Clotting Activities of Thrombin by Trypsin-Binding Macroglobulin.* (30800)

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Recently, a protein from human plasma has been isolated which forms an enzymatically active complex with trypsin and is able to protect trypsin from inhibition by soybean trypsin inhibitor(1). Preparations of this material have demonstrated it to be an alpha-2 macroglobulin which may be identical with the component previously described as 19S-glycoprotein(2). This binding protein differs from serum trypsin inhibitors which appear to reversibly inactivate the proteolytic and esterolytic activities of the enzyme(3,4).

Trypsin-binding macroglobulin (TBM) is also capable of binding chymotrypsin as an enzymatically active complex(4). Because of the similarities in certain physical and kinetic properties of these estero-proteolytic enzymes with those of thrombin(5), it was of interest to determine if TBM might also bind thrombin and thereby be implicated in the blood coagulation mechanism.

Evidence is presented here for the binding of human thrombin by preparations of TBM. The nature of the binding is unusual, however, in that the complex formed is enzymatically inactive against fibrinogen but readily hydrolyzes synthetic esters such as N-p-tosyl-L-arginine methylester (TAMe) and N-carbobenzoxy-L-tyrosine p-nitrophenyl ester (CTN). This dissociation of the esterolytic and clotting activities of thrombin may have important implications concerning the "binding" and/or "active" site of this enzyme as well as the role this complex may have in blood clotting.

Materials and methods. Prothrombin was prepared from human plasma(6) and converted to thrombin in 25% (w/v) sodium citrate(7). Two human thrombin preparations were used in these studies; one had a specific activity of 1,280 N.I.H. clotting units and 256 TAMe units/mg protein, and the other, which had been partially purified by ion exchange chromatography(8), was about 2,500 clotting units and 410 TAMe units/mg. Bovine thrombin (specific activity = 2,900 clotting units/mg) was purified from a commercial source (Parke-Davis & Co.) by chromatography(8) and used in some experiments. Clotting assay procedures at 28°C for prothrombin (2-stage method) and thrombin (N.I.H. method) were as described pre-

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