duced by injecting at birth spleen cells from individuals of another inbred strain, the recipients accept skin grafts taken from mice of the strain donating the spleen cells as well as skin taken from F_1 hybrids resulting from the cross of the 2 strains involved. Similarly, when tolerance is induced in one of the parents by injection of spleen cells from F_1 hybrid, the pretreated mice accept not only skin grafts from the hybrid but also skin grafts from the other parent strain.

These observations indicate, first of all, that individuals of the F_1 hybrid of the 2 strains involved share all the histo-compatibility antigens present in each parent strain. Perhaps of greater significance are the experiments demonstrating that no new histo-compatibility antigens appear as a consequence of hybridization. These observations do not exclude completely the possibility that in mice, as in other species(4,5), new antigens not present in either parent strain may appear as a consequence of genic interaction. However, the investigations do demonstrate that, if such be the case, transplantation studies and employment of systems including immunological tolerance are inadequate for detection of such antigens and the responsible genic interaction. In spite of these results, it seems possible that other techniques, i.e., immunochemical methods or even study of the phenomenon of runt diseases, may be more fruitful in detecting evidence of the postulated genic interaction.

Summary and conclusions. 1. C3H mice made tolerant by injection of A spleen cells at birth are tolerant of skin homografts from A strain and (A x C3H) F_1 donors. 2. C3H mice made tolerant by injection of (A x C3H) F_1 spleen cells at birth are tolerant of skin homotransplants from both A strain and (A x C3H) F_1 donors. These observations are interpreted as evidence indicating that new histocompatibility antigens do not derive from genic interaction during hybridization in the 2 inbred strains of mice in these studies.

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Separation of Thrombin from Thrombokinase by Continuous Flow Paper Electrophoresis.* (25052)

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For several years, thrombokinase has been prepared in this laboratory from bovine plasma. It is heat-labile and associated with globulins. However, its identifying characteristic is its capacity to activate prothrombin. In this function it does not act like platelets, but rather is complemented by platelets(1). Recently, thrombokinase has been purified by a method which yields about 1.2 mg/liter of plasma(2). This material has now been subjected to continuous flow paper electrophoresis.

Methods and materials. Electrophoresis was performed in Spinco Model CP cell enclosed in refrigerator at 1.3 to 2.9°C. Buffer: veronal, pH 8.6, ionic strength, 0.02. Current: 50 ma; 807 volts. 77 ml thrombokinase, representing 77 liters of plasma, was diluted with 1463 ml of 0.02 M acetate, pH 5.2. The precipitate was dissolved in veronal buffer to make 25.7 ml of solution, which had the same

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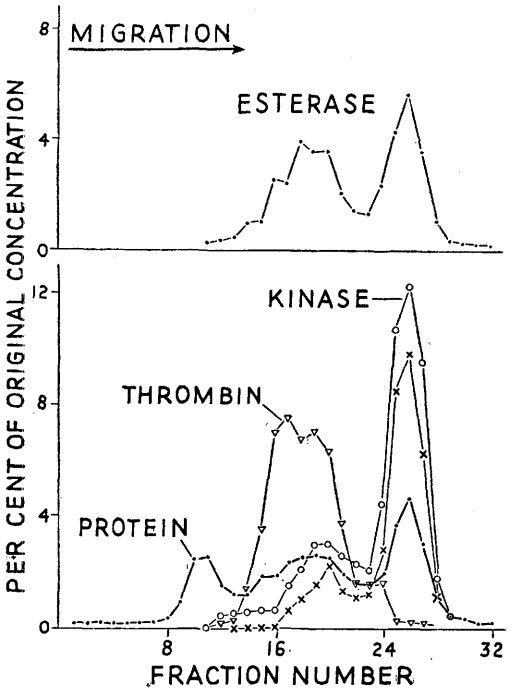

FIG. 1. Continuous flow paper electrophoresis of thrombokinase prepared from bovine plasma. There are 2 curves for kinase activity. The upper one presents assays performed in presence of oxalate; the lower one gives values obtained in 5-reagent system.

conductivity as the buffer. This "original solution" was applied at rate of 0.43 ml/hour to the extreme left tab of paper curtain. Thrombokinase was assayed by its capacity to activate prothrombin in presence of oxalate, and also in a 5-reagent system containing prothrombin, cephalin, calcium, bovine barium carbonate serum and sample to be tested(2). In each case relative activity was derived from a reference curve obtained with dilutions of original solution. Thrombin was estimated as described(2), with dry sample of NIH thrombin as the ultimate standard. Esterase activity on tosylarginine methyl ester (TAMe) was determined by method of Sherry and Troll(3). Protein was estimated by method of Lowry *et al.*(4) with crystallized bovine albumin as standard; and the values are subject to limitations noted by them.

Results. A large proportion of thrombokinase was separated in the rapidly moving peak shown in Fig. 1. In fraction 26 the kinase activity was 12.3% of the original, and the protein was 4.6%. Hence specific activity was 2.7 times that of the original solution.

A trail, or second smaller rise of kinase activity occurred in all 5 such analyses. For one of these, the kinase had been dialyzed overnight against the veronal buffer. The trailing effect might be due to association of proteins as complexes in the middle protein band. It is not necessary to assume that 2 different kinases were separated; although that possibility must be considered. The fact that the 2 measures of kinase activity gave essentially the same result for the peak and the trailing hump, indicates that the same factor could be responsible for the activity in both regions. The difference between the 2 kinase curves is within limits of error. For assay of kinase in presence of oxalate, fraction 26 was diluted 1/40, to protein concentration of 5.3 μg/ml. For assay of kinase in the 5-reagent system, fraction 26 was diluted 1/100,000 to a concentration of 0.0021 μg/ml.

The original solution had a high kinase and low thrombin activity. It contained only 68.3 thrombin units/ml or 14.8 units/mg protein. The moderately high thrombin peak in Fig. 1 represents a high percentage yield rather than a high activity.

The original solution had 966 esterase units/ml or 209 units/mg protein. The Figure suggests that esterase activity is associated with both thrombin and kinase. Much of esterase activity migrated with kinase in the leading peak where there was very little thrombin. In terms of percentage scale of Fig. 1, the ratio of esterase to kinase was 0.40, 0.46 and 0.37 for fractions 25, 26 and 27, respectively. Fraction 26 had 251 esterase units/mg.

Discussion. Gross separation of thrombin from thrombokinase was achieved by continuous flow electrophoresis. But such separation had been accomplished previously by repeated fractionation with ammonium sulfate(1); and it is not certain that electrophoresis is superior as a method of preparation.

From the analytic viewpoint, the electrophoretic procedure offered several opportunities. One of these was comparison of values obtained when kinase was assayed with and

without complementing reagents. It appears that thrombokinase can activate prothrombin, unaided by other factors(5). But thrombin is produced much faster if the system is complemented by platelets or cephalin, provided that ionic calcium is also added. Production of thrombin is further accelerated by the serum reagent. Together, these reagents enormously magnify the effect of kinase. When minute amounts of kinase are assayed in this system, serious uncertainties are involved(2). Therefore, it is of interest that assays in this complex system paralleled those with oxalated prothrombin; although the former system was 2500 times as sensitive.

In some respects, thrombokinase resembles trypsin, which also can activate prothrombin in presence of oxalate. TAMe is a good substrate for trypsin(6); and others have anticipated the possibility that TAMe might be a substrate for natural activator of prothrombin (7). The present results suggest that TAMe may well be a substrate for thrombokinase; but more detailed study of this point is desirable.

Summary. Thrombokinase prepared from bovine plasma was further purified by continuous flow paper electrophoresis. Thrombokinase was assayed by its capacity to activate prothrombin in the presence of oxalate, and also by production of thrombin in a system containing prothrombin, cephalin, calcium and bovine "barium carbonate serum." Curves of these 2 assays were similar and formed a peak ahead of the thrombin peak. TAMe esterase activity appeared in 2 peaks which corresponded respectively to thrombin and thrombokinase peaks.

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Genetics of Human Cell Lines I. 8-Azaguanine Resistance, a Selective "Single-Step" Marker.* (25053)

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From the methodological point of view, the mammalian cell grown *in vitro* can be regarded as a unicellular microorganism. Thus, methods developed for quantitative work with microbes can be adapted to the genetic study of human cells, provided suitable selective markers are available. Plating and colony-counting technic, so useful for assay of viable microbial cells, was introduced into tissue culture methodology by Puck *et al.*(1). Our purpose was to find a suitable mutational system for mammalian cells in which, under selective conditions, mutant cells survive and form well developed colonies while parental population is completely inhibited or eliminated. Mutation from 8-azaguanine (AG) sensitivity to resistance satisfied the foregoing criteria(2). Moreover, the property of 8-azaguanine resistance appears to be an excellent genetic marker, since it is not associated with modifications in morphological or cultural characteristics of cells either in presence or absence of the selective agent.

Materials and methods. Strain *Detroit-98* (D98), derived from human sternal marrow by Berman and Stulberg(3), was kindly supplied by Dr. H. Moser of Cold Spring Harbor Labs. A single-cell-derived clone D98S was used throughout these studies. *Media.* The basic medium was essentially that described by Eagle(4), containing 10% com-

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