

isms such as mollusks concentrate metallic ions at levels many times higher than the levels found in the surrounding water. The concentration of Ce^{144} in clams during April 1959 was about $7 \times 10^2 \mu\mu c$ per kilogram of clam muscle (wet weight). It had decreased to $7 \times 10 \mu\mu c$ by July 1959.

The gross radioactivity of surrounding water during the time from August 1958 to February 1959 was about $3 \pm 3 \mu\mu c$ per liter, but it increased to $21 \pm 3.2 \mu\mu c$ per liter in March 1959. The radioactivity of the water has not been measured since April 1959, but radioactivity in rain and fallout in this region increased from March 1959 and reached a maximum in April 1959. Since August 1959, there has been less radioactivity in rain and fallout.

It has been reported that the concentration of Ce^{144} in the sediment of the sea in the year 1960 was $1 \mu\mu c$ per gram of dried sample. This amount corresponded to that found in 5 liters of surrounding sea water. The concentration of Ce^{144} in sediment was about 100 times higher than that of Sr^{90} (6).

A clam is a marine organism that makes its home in brackish water, and thus can be found in marsh sediment. Since radioactive contamination in a marsh bed has its origin in fallout, it may safely be assumed that Ce^{144} was deposited in the marsh bed by fallout and then absorbed by the clams.

The concentration of Sr^{90} was generally at a higher level in bone than in other biological samples, whereas Ce^{144} was more concentrated in clams. Cattle and horse bone contained large quantities of both radionuclides. The presence of Ce^{144} in food was at a quite trivial level compared to the maximum permissible concentration of Ce^{144} ($1 \times 10^5 \mu\mu c$ per liter of water).

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Distribution of Coagulation

Proteins in Normal Mouse Plasma

Abstract. Clotting factors in pooled mouse plasma, segregated by continuous-flow electrophoresis, show a distribution similar to those in human plasma, but the separation is more discrete.

After the discovery of an acquired hemorrhagic disorder in certain inbred strains of mice, the mechanisms of normal and abnormal clotting were investigated. In normal mice, single-factor activities determined in titrated assays, by using known single-factor-deficient human plasma, were comparable, regardless of strain or sex. The relative activity of certain factors was greater in the mouse than in man (1).

A successful approach to the study of clotting factors and their hemostatic function was the assay of fractions from normal mouse serum obtained by continuous-flow curtain electrophoresis in various deficiency systems. Since in mice with hemorrhagic diathesis a deficiency of factor X appeared earliest in the course of the disease or greatly increased its severity, a Stuart factor fraction from mouse serum was first prepared (2). This fraction, corresponding to the leading edge of alpha-1 globulin of serum protein, was rich in factor X with very little contamination by plasma thromboplastin component. Although X-deficient human plasma does not correct for Quick time, prothrombin con-

version rate, thromboplastin generation time, or Russell venom time, the mouse serum fraction was all corrective.

In our investigation, we applied the technique of continuous flow curtain electrophoresis for studying the distribution of various coagulation factors in normal mouse plasma (3). Forty C57 BL/6J mice (20 male and 20 female), from 4 to 5 months of age, were bled, and plasma was obtained using 0.1M sodium oxalate. We fractionated the plasma in a Spinco continuous-flow electrophoresis apparatus, employing Veronal buffer T/2 0.020, pH 8.6, 54 ma, 720 volts for 18 hours at 11°C. Phenol red added to the plasma sample and migrating ahead of the albumin fraction served as a constant visual check of curtain stability (4). No variation in the position of the phenol red front occurred during the collection period. Protein was determined for the contents of each tube by trichloroacetic acid precipitation at low temperature. Turbidities were read at 535 m μ in a spectrophotometer against a Versatol standard. Protein was contained in tubes 5 through 26. Clotting factors were identified by standard and specialized assays [substitution analyses (5)], by employing known single-factor-deficient human plasma (6). The distribution and relative activities of various clotting factors are shown in Fig. 1. The relative factor activity contained in tubes is expressed in "units" as derived from

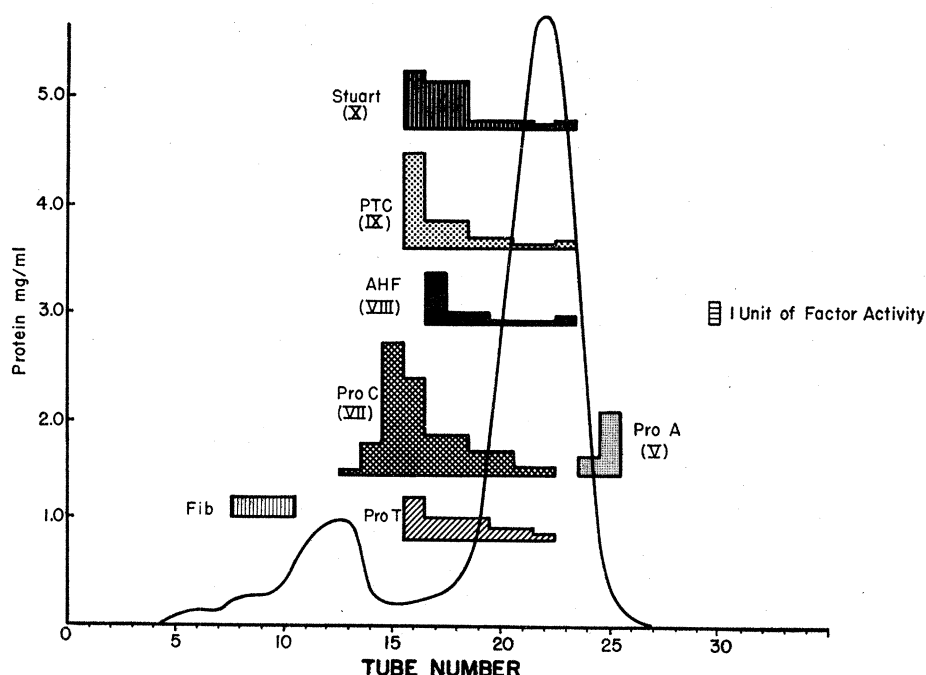


Fig. 1. Distribution of clotting factors in pooled C57BL/6J mouse plasma, expressed in units of relative activity. Fibrinogen is not expressed in units.

the reciprocal of the protein concentration substituted in each test multiplied by the time of clot appearance (in seconds). For example, 80 percent of the relative prothrombin activity was found in the alpha-1 globulin and 20 percent in the albumin; approximately 14, 79, and 7 percent of relative factor VII activity was found in the albumin and the alpha and beta globulins, respectively. In controls, saline was added to deficient human plasma (1:1) in place of curtain fractions. Presence of activity was indicated when either prothrombin time or thromboplastin generation time was improved, upon admixture of curtain fractions, over that of the controls. Although separation of coagulation proteins in human plasma is incomplete, complete separation being effected only by utilization of serum rather than plasma or of specific factor-deficient plasma (7), the results of analyses of mouse plasma fractionation indicate that factors VII (tubes 13 through 15) and V (tubes 24 and 25) may be obtained free from other factor contaminations. In tube ranges with overlapping factors, certain of them may be eliminated by BaSO₄-adsorption, with or without subsequent citrate elution (5).

The genetic homogeneity of this inbred strain of mice with respect to clotting factor mobilities provides for excellent reproducibility of the fractionation procedure, even when pooled plasma samples are used. In another separation employing a pool of 50 C57BL/6J mice, 6 weeks of age, a similar distribution pattern was obtained. The distribution of mouse coagulation factors was found to be similar to that of man in respect to the familiar gamma, beta, alpha, and albumin pattern. However, the zones of activity were narrower and the boundaries of activity more distinct in the mouse plasma. These observations suggest the possibility of using mouse plasma or serum for the preparation of test reagents and single- or multiple-factor-deficient plasmas (8).

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Composition of the Milk from *Zalophus californianus*, the California Sea Lion

Abstract. The milk of *Zalophus californianus* is similar to that of other marine mammals. The chief protein of the milk is casein, which has a lower phosphorus content than bovine casein. There appears to be a complete absence of lactose, and it is believed that this is the first unequivocal demonstration of the absence of lactose from the milk of any mammal.

Although the composition of the milk of over three dozen mammalian species has been reported in the literature, most of the investigators have been concerned only with terrestrial mammals. Without exception, the milks produced by marine mammals have been found to have high concentrations of total solids, protein, and fat and rather low concentrations of lactose, as compared with milk from terrestrial animals. Only two species have been reported to yield milk without lactose. Sivertsen (1) stated that the harp seal, *Phoca groenlandica* (two analyses) and the hooded seal, *Cystophora cristata* (one analysis) had no lactose in their milk. Sivertsen did not make the analyses himself, did not state the method of analysis, and expressed some doubt concerning the results. In any case, the methods available at that time were such that small amounts of lactose might have been missed. High concentrations of protein and fat in milk of marine mammals make analysis difficult. One report has been published concerning the composition of the milk from *Zalophus californianus* (2). This analysis was carried out on the stomach contents of a pup, and the reported values were as

follows: fat, 15.45 percent; protein, 18.86 percent; and ash, 1.07 percent. No carbohydrate analysis was reported.

In all the analyses reported here, the milk was collected by incision and drainage from the mammary glands of lactating females killed for other purposes. Total nitrogen was determined by the micro-Kjeldahl procedure, and protein was calculated with the factor 6.38 (3). Crude fat was determined by the Roese-Gottlieb method (3). Total solids were determined by drying 1 g of milk at 103°C for 3½ hours and weighing the residue; the ash was determined after ignition of the residue at 500°C for 4 hours. All these analyses were carried out in duplicate; the results are reported in Table 1. Corresponding percentages for the average composition of bovine milk are as follows: fat, 3.8; protein, 3.3; lactose, 4.8; ash, 0.71; and total solids, 12.8 (4).

The solutions remaining after extraction of the fats from sample 1 were combined and diluted to 150 ml. Acid (0.1N HCl) was added until the pH was 4.5, at which point flocs appeared. The precipitate (casein) was collected by centrifugation and washed twice with 40 ml of water. This precipitate was softer than the equivalent precipitate from bovine milk. The casein was dissolved in 85 ml of water with 0.1N KOH added, to a pH of 9.0, and the solution was filtered through Whatman No. 40 paper and carefully acidified with 0.1N HCl. Flocculation occurred at pH 5.2. The precipitate was collected by centrifugation and reprecipitated, flocculation again occurring at pH 5.2. The final precipitate was dried by successive washing, settling, and decantation with absolute ethanol and acetone. The final yield, after drying *in vacuo*, was 516 mg from about 6 ml of milk, indicating that the major protein in the milk is casein. The phosphorus and nitrogen contents of this casein were determined (5), and the nitrogen-phosphorus ratio was calculated to be 37.8, as compared to a ratio of 19.3 for bovine whole casein (6). Thus, if *Zalophus* casein has the same nitrogen content as bovine casein, the phosphorus content is 0.41 percent—much less than the 0.86-percent for bovine casein.

The lower phosphorus content is in agreement with the higher isoelectric point of *Zalophus* casein (as judged by the flocculation point). Paper electrophoresis of the *Zalophus* casein (Veronal buffer, $\mu = 0.05$, pH = 8.6) gave