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 $BaSO_4$-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*).

ROBERT C. ALLEN
HANS MEIER
WARREN G. HOAG
Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

References and Notes

1. H. Meier, R. C. Allen, W. G. Hoag, *Am. J. Physiol.* **201**, 375 (1961).
2. H. Meier, W. G. Hoag, R. C. Allen, *Federation Proc.* **20**, 54 (1961).
3. J. H. Lewis, D. Walters, P. Didisheim, W. R. Merchant, *J. Clin. Invest.* **37**, 1323 (1958).
4. R. C. Allen, *Am. J. Vet. Research* **22**, 558 (1961); J. B. Davis, R. C. Allen, R. M. Smibert, *ibid.* **22**, 736 (1961).
5. J. H. Ferguson, *Lipoids and Blood Platelets* (Univ. of North Carolina Press, Chapel Hill, 1960).
6. We gratefully acknowledge the gifts of single-factor-deficient human plasma and serum from the following persons: J. G. Lenahan, Warner-Lambert Research Institute, Morris Plains, N.J. (factor V, VII, and VIII deficient plasma); J. B. Graham, University of North Carolina, Chapel Hill (factor X deficient plasma); and R. Wadsworth, Eastern Maine General Hospital, Bangor (factor IX deficient serum).
7. C. L. Johnston, Jr., and F. A. O'Hanlon, *Federation Proc.* **18**, 77 (1959); J. H. Lewis and W. R. Merchant, in *Hemophilia and Other Hemorrhagic States*, International Symposium, Rome (Univ. of North Carolina Press, Chapel Hill, 1959).
8. This work was supported in part by grants C-4691 and CRT-5013 from the Public Health Service.

5 September 1961

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 (0.1*N* HCl) was added until the *p*H 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 0.1*N* KOH added, to a *p*H of 9.0, and the solution was filtered through Whatman No. 40 paper and carefully acidified with 0.1*N* HCl. Flocculation occurred at *p*H 5.2. The precipitate was collected by centrifugation and reprecipitated, flocculation again occurring at *p*H 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, $\mu = 0.05$, *p*H = 8.6) gave

Table 1. Results of analyses of sea lion milk.

Item	Sample		
	I	II	III
Collection date	16 Feb. 1959	3 June 1959	20 Dec. 1960
No. of animals	3	1	1
Total vol. collected (ml)	10	15	7
Total fat (%)	31.1	36.5	37.0
Protein (%)	13.3	13.8	—
Solids (%)	—	52.7	—
Ash (%)	—	0.64	—
Specific gravity (23°/4°)	—	1.0102	—
Lactose (%)	—	0	0

a pattern similar to that of bovine whole casein run at the same time. The mobility of the "alpha" band was exactly the same as that of bovine alpha casein, but the "beta" band moved somewhat faster than bovine beta casein.

Samples 2 and 3 were analyzed for lactose by paper chromatography (*7*). The methods of treatment were different for the two samples, but both gave negative results. The procedure used for analysis of sample 3 was as follows.

A 1-ml sample of commercial homogenized whole bovine milk, 1 ml of *Zalophus* milk from sample 3, and 1 ml of *Zalophus* milk to which had been added 1 mg of lactose in 0.1 ml of water were used. The *Zalophus* milk samples were centrifuged, and the top layers of fat and fat-protein mixture were extracted several times with 3-ml portions of diethyl ether. This permitted the protein to sink later, after the addition of trichloroacetic acid. Each of the three samples was then mixed with 4 ml of 10-percent trichloroacetic acid solution, stirred, and allowed to settle for 15 minutes; the precipitate was then removed by centrifugation. Each supernatant solution was extracted five times with 3 to 4 ml of diethyl ether to remove the trichloroacetic acid. Excess ether was removed by aeration. Each solution was adjusted to a volume of 4 ml, and aliquots were spotted on paper and chromatographed (*7*).

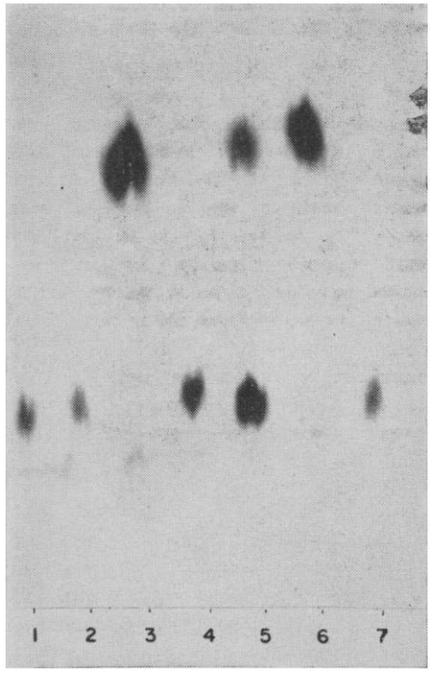

Fig. 1. (1) Lactose standard, 10 μg; (2) bovine milk, equivalent to 0.25 μl of original milk; (3) *Zalophus* milk, equivalent to 250 μl of original milk; (4) lactose standard, 20 μg; (5) *Zalophus* milk and lactose, equivalent to 50 μl of milk with 0.1 percent lactose; (6) glucose, 20 μg; (7) lactose standard, 10 μg.

Recovery of the added lactose in the *Zalophus* milk and of the natural lactose in the bovine milk was good, but no natural lactose could be detected in the *Zalophus* milk. It is estimated that the limit of detection was about 0.025 percent lactose in the original solution.

Aliquots (1 ml) of each of the solutions were evaporated to dryness *in vacuo* over H_2SO_4 and then twice extracted with 1-ml washes of hot pyridine to extract the sugars. The extract from the bovine milk was colorless, but extracts from the *Zalophus* milk were yellow. A yellow flocculent material settled out on cooling and was removed by centrifugation. The pyridine solutions were evaporated to dryness, the bovine material was taken up in 1 ml of water, and each of the *Zalophus* extracts was dissolved in 0.10 ml of water.

Figure 1 shows a chromatogram prepared from 1 μl of this bovine-material solution, 20 μl of the *Zalophus*-material solution with added lactose, and the entire quantity of *Zalophus* material without added lactose, equivalent to ¼ ml of the original milk. Figure 1 shows that if lactose is present in the milk it must certainly be in a concentration of less than 0.001 percent.

A sugar with about the same R_f as glucose appears to be present in a concentration of about 0.025 percent. This is considerably more than could be ascribed to the relatively slight (about 1 percent) contamination with blood (*8*).

M. E. Q. Pilson
Scripps Institution of Oceanography, University of California, La Jolla
A. L. Kelly
Biology Department, Boston University, Boston, Massachusetts

References and Notes

1. *E. Sivertsen, Hvalrådets Skrifter Norske Videnskaps-Akad. Oslo* **26** (1941), 1 (1941).
2. C. R. Schroeder and H. M. Wegeforth, *J. Am. Vet. Med. Assoc.* **87**, 333 (1935).
3. *Official Methods of Analysis of the A.O.A.C.*, W. Horwitz, Ed. (Association of Official Agricultural Chemists, Washington, D.C., 1955).
4. I. G. Macy, H. J. Kelly, R. E. Sloan, *Natl. Research Council Natl. Acad. Sci. (U.S.) Publ. No. 254* (1953).
5. J. B. Martin and D. M. Doty, *Anal. Chem.* **21**, 965 (1949).
6. W. G. Gordon, W. F. Semmett, R. S. Cable, M. Morris, *J. Am. Chem. Soc.* **71**, 3293 (1949).
7. E. F. McFarren, K. Brand, H. R. Rutkowski, *Anal. Chem.* **23**, 1146 (1951).
8. This work was carried out in the laboratory of Dr. Denis L. Fox; we wish to thank both Dr. Fox and Dr. A. Baird Hastings for helpful discussions.

5 September 1961

Decarboxylase Inhibitors Affect Convulsion Thresholds to Hexafluorodiethyl Ether

Abstract. Alpha-methyl dihydroxyphenylalanine (α-MeDOPA) and alpha-methyl meta-tyrosine (α-MMT) do not lower significantly the convulsion thresholds to hexafluorodiethyl ether in the mouse, whereas reserpine and tetrabenazine produce marked lowering of these thresholds. Other workers have shown that α-MeDOPA and α-MMT decrease brain norepinephrine concentrations much more than brain 5-hydroxytryptamine concentrations, while reserpine and tetrabenazine decrease the brain level of these two amines in a parallel fashion. Thus, the fact that α-MeDOPA and α-MMT, even in very large doses, do not lower convulsion thresholds and in some cases raise them suggests that the decline in 5-hydroxytryptamine may be implicated in the lowering of convulsion thresholds produced by reserpine and other, similar agents.

The main difficulty in relating changes in brain concentrations of certain neurogenic amines to the pharmacologic actions of reserpine has been the very parallel depleting action of this drug upon levels of 5-hydroxytryptamine (5-HT) and norepinephrine. Recently it was reported that two decarboxylase inhibitors, alpha-methyl dihydroxyphenylalanine (α-MeDOPA) and alpha-methyl meta-tyrosine (α-MMT) (*1*), produced a persistent decrease, lasting several days, in levels of norepinephrine in mouse brain, while it was also reported that these inhibitors had a smaller and more transient effect on 5-hydroxytryptamine levels (*2*, *3*). This differential action prompted a comparison of the action of these drugs with the action of reserpine, which produces marked lowering of the convulsion threshold to hexafluoro-