

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).

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 H₂SO₄ 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 μ l of this bovine-material solution, 20 μ l of the *Zalophus*-material solution with added lactose, and the entire quantity of *Zalophus* material without added lactose, equivalent to 1/4 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, *Hvalrødets 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-

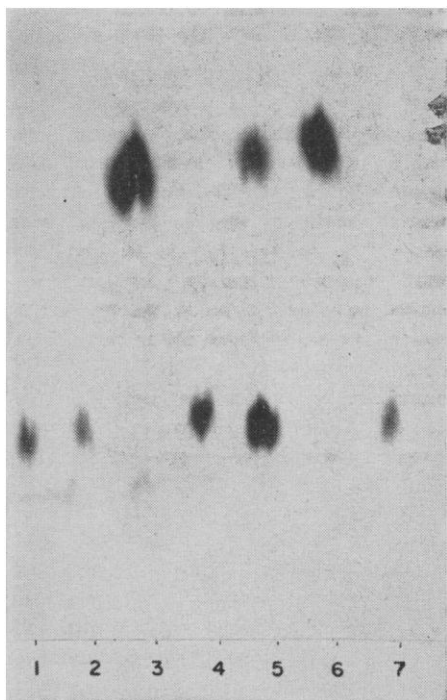


Fig. 1. (1) Lactose standard, 10 μ g; (2) bovine milk, equivalent to 0.25 μ l of original milk; (3) *Zalophus* milk, equivalent to 250 μ l of original milk; (4) lactose standard, 20 μ g; (5) *Zalophus* milk and lactose, equivalent to 50 μ l of milk with 0.1 percent lactose; (6) glucose, 20 μ g; (7) lactose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

diethyl ether (4), and with the action of tetrabenazine (Nitoman, RO 1-9569) (5), a reserpine-like compound which produces similar effects of very short duration on brain 5-hydroxytryptamine and norepinephrine (6).

We have previously described the quantitative measurement of convulsion thresholds to hexafluorodiethyl ether (HFE) (7). The time required for this volatile convulsant to elicit preconvulsive myoclonic jerks or sustained clonic convulsions was measured after the application, every 30 seconds, of 0.05-ml increments of a 10-percent solution (in alcohol) of the convulsant to a gauze wick suspended in a 3.4-liter jar. Reserpine, tetrabenazine, α -MeDOPA, and α -MMT were given intraperitoneally to groups of ten or more mice at various intervals prior to the HFE test. Because one of the solubilizing vehicles (the benzyl alcohol-citric acid-polyethylene glycol 300 aqueous solution for reserpine) had a slight action on the convulsion thresholds, the action of each drug group was compared to that of its corresponding ve-

hicle control group, and alterations in threshold were expressed as percentages of change from these controls.

In Fig. 1 the changes in the thresholds for myoclonic jerks (top) and for clonic convulsions (bottom) are shown. Very large doses of α -MeDOPA and α -MMT do not produce a significant lowering of these convulsion thresholds at any time up to 17 hours. The larger dose of α -MMT produced significant threshold elevation at several points. In sharp contrast, reserpine and tetrabenazine markedly lowered jerking and convulsion thresholds to hexafluorodiethyl ether. Tetrabenazine, as anticipated from other studies (6), produced a short depression of convulsion thresholds and prolonged the effect of reserpine.

These results may be coupled with the observations of Porter (2) and Hess (3) that α -MeDOPA and α -MMT produce a greater and more prolonged depression of brain norepinephrine than of 5-hydroxytryptamine. The fact that those doses which have been shown by them to produce complete and prolonged depletion of norepinephrine do not lower the convulsion thresholds in the way that reserpine does strongly implicates the decrease in brain 5-hydroxytryptamine in the lowering of convulsion thresholds by reserpine. Smith (8) found that α -MeDOPA depletes norepinephrine in mouse brain less than it decreases 5-hydroxytryptamine in guinea pig brain, but his data are not fully comparable because he used subcutaneous injection of the drug and a bioassay determination of the amines. Brodie *et al.* (9) used dimethylaminobenzoyl methylreserpate (SU-5171) as a selective factor on 5-hydroxytryptamine and norepinephrine in rabbit brain and associated the decline in levels of 5-hydroxytryptamine in rabbit brain with the sedation produced by reserpine. Kuntzman *et al.* (10) employed α -MMT to further confirm this association. Although a causal relationship remains to be established, it appears that the lowered convulsion threshold and the central sedation of reserpine and other 5-HT releasing compounds may be temporally correlated with a decrease in brain 5-hydroxytryptamine.

EDWARD B. TRUITT, JR.
ETHEL M. EBERSBERGER

Department of Pharmacology,
University of Maryland School
of Medicine, Baltimore

References and Notes

1. T. L. Sourkes, *Arch. Biochem. Biophys.* **50**, 444 (1954). We thank Dr. Clement Stone at the Merck Institute for Therapeutic Research, West Point, Pa., for supplying these compounds.
2. C. C. Porter, J. A. Totaro, C. M. Leiby, *J. Pharmacol. Exptl. Therap.* **134**, 139 (1961).
3. S. M. Hess, M. Ozaki, S. Udenfriend, R. H. Connamacher, *ibid.* **134**, 129 (1961).
4. E. B. Truitt, Jr., and E. M. Ebersberger, *Arch. intern. pharmacodynamie* **129**, 223 (1960).
5. The tetrabenazine used in this study was kindly supplied by Hoffman-La Roche, Inc., Nutley, N.J.
6. G. P. Quinn, P. A. Shore, B. B. Brodie, *J. Pharmacol. Exptl. Therap.* **127**, 103 (1959).
7. E. B. Truitt, Jr., E. M. Ebersberger, A. S. C. Ling, *ibid.* **129**, 445 (1960).
8. S. E. Smith, *Brit. J. Pharmacol.* **15**, 319 (1960).
9. B. B. Brodie, K. F. Finger, F. B. Orlanese, G. P. Quinn, F. Fulser, *J. Pharmacol. Exptl. Therap.* **129**, 31 (1960).
10. A preliminary report of this work was presented by R. Kuntzman, E. Costa, G. L. Gessa, C. Hirsch, and B. B. Brodie [*Federation Proc.* **20**, 208 (1961)].

2 August 1961

Evidence for Oceanic Frontogenesis off Oregon

Abstract. Bathythermograms recorded off Newport, Oregon, show marked positive gradients during the period of cessation of summer upwelling. It is suggested that these are related to the formation of a shear zone or "front" between oceanic water and shelf water that has been exposed to the surface and modified.

Bathythermograms recorded in the northeastern Pacific Ocean often show positive gradients—that is, small but sharp increases of temperature with depth—at about 100 m. In fact, for waters north of 46°N, this has been shown by Bennett (1) to be the "normal" summer situation. Similar inversions are often found in waters farther south, including those along the Oregon coast. Presumably, most of these inversions are related to the depth and extent of cooling of the water during the previous winter, as suggested by Tully (2).

However, a recent set of observations off Newport, Oregon, showed an inversion that appeared to develop *in situ* during the summer, seemed to have a definite slope from one position to another, and occurred at temperatures between 8° and 11°C. In all of these respects it differed somewhat from the inversions that were described by Bennett.

An analysis of the data, analogous to the frontal concept used by meteorologists, fits the observations very well (see Fig. 1). It is suggested that per-

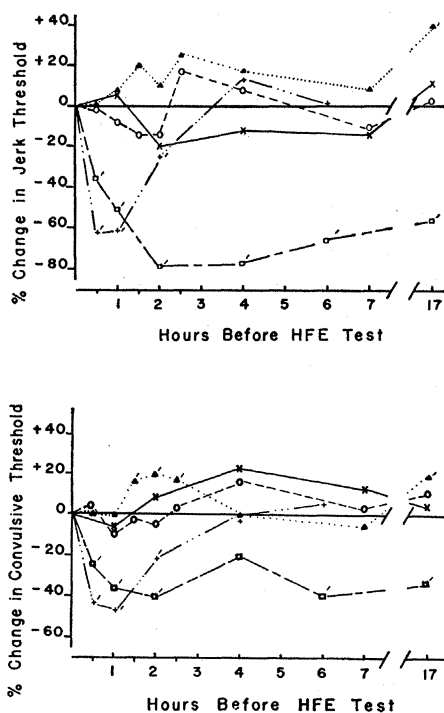


Fig. 1. Change in threshold from that of vehicle-control-group mice for preconvulsive myoclonic jerks (top) and clonic convulsions (bottom), produced by administration of hexafluorodiethyl ether at various intervals after intraperitoneal injection of drugs. / /, $p < .05$; O — — —, α -MMT (100 mg/kg); ▲ — — —, α -MMT (500 mg/kg); × — — —, α -MeDOPA (1000 mg/kg); □ ·····, reserpine (10 mg/kg); + — — —, tetrabenazine (75 mg/kg).