3. Hurley, K. E., Williams, R. J., *Arch. Biochem. Biophys.*, 1955, v54, 384.
4. Blahd, W. H., Bloom, A., Drell, W., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 704.
5. Dinning, J. S., Cosgrove, K. W., Jr., Fitch, C. D., Day, P. L., *ibid.*, 1956, v91, 632.
6. Dinning, J. S., Day, P. L., *J. Exp. Med.*, 1957, v105, 395.
7. Fink, K., Williams, A. D., Fink, R. M., *J. Biol. Chem.*, 1959, v234, 1182.
8. Cavallini, D., Frontali, N., Toschi, G., *Nature*, 1959, v163, 568.
9. Magasanik, B., Umbarger, H. E., *J. Am. Chem. Soc.*, 1950, v72, 2308.
10. Meister, A., Abendschein, P. A., *Anal. Chem.*, 1956, v28, 171.
11. Fink, K., Cline, R. E., Henderson, R. B., Fink, R. M., *J. Biol. Chem.*, 1956, v221, 425.
12. Bonting, S. L., Bonting, S., *Arch. Biochem. Biophys.*, 1955, v58, 100.
13. Fink, R. M., Cline, R. E., McGaughey, C., Fink, K., *Anal. Chem.*, 1956, v28, 4.
14. Fink, R. M., Fink, K., Henderson, R. B., *J. Biol. Chem.*, 1952, v197, 441.
15. Baker, N., Tubis, M., Blahd, W. H., *Am. J. Physiol.*, 1958, v193, 525.
16. Rabinowitz, J. L., *Fed. Proc.*, 1959, v18, 306.
17. Rosenkrantz, H., *ibid.*, 1959, v18, 312.
18. Weinstock, I. M., Epstein, S., Milhorat, A. T., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 272.
19. Simon, E. J. Tessell, I., Gross, C. S., Milhorat, A. T., *Fed. Proc.*, 1958, v17, 311.
20. Zierler, K. L., *Bull. Johns Hopkins Hosp.*, 1958, v102, 17.
21. Milman, A. E., Milhorat, A. T., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 654.

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Toxicity of Sphingosine and Other Long-Chain Amines. (25653)

K. K. CARROLL* (Introduced by R. L. Noble)
Collip Medical Research Laboratory, University of Western Ontario, London, Ontario, Canada

Rosenheim and Webster(1) found that feeding cerebrosides promoted conversion of cholesterol to coprosterol in the gastrointestinal tract. They fed various hydrolytic products of cerebrosides and noted that the basic component, sphingosine, could not be tested because the rats refused to eat diets containing either the free base or its sulfate salt. Recently we also observed that basic sphingosine-containing fractions from hydrolyzed cerebrosides were poorly accepted by rats even at very low levels in the diet(2). Further experiments suggested that toxicity was related to presence of free amine groups in these fractions and feeding tests were conducted with a series of simple aliphatic amines. These studies form the basis of the present paper.

Materials and methods. Methods used to prepare psychosine, ceramide, sphingosine and triacetylsphingosine from cerebrosides of beef brain or beef spinal cord have been described (2). Other amines used in present experiments were obtained from Matheson, Coleman and Bell, East Rutherford, N. J. Sulfate salts were prepared by mixing amines with a solution of sulfuric acid in ethanol as in preparation of sphingosine sulfate(3). Volume of ethanol was adjusted according to solubility of individual compounds. Male Sprague-Dawley rats weighing 80-100 g were used for most feeding experiments. Compounds to be tested were added to a synthetic diet consisting of: casein 21, glucose 58, corn oil 10, salt mixture 5 and celluflour 5 parts by weight, with an adequate supplement of water-soluble vitamins but no fat-soluble vitamins. In some experiments corn oil was omitted and glucose increased accordingly. In liquid diets fed by stomach tube, corn oil was retained but casein was replaced by a mixture of egg albumin and lactalbumin (1:3). The dry diet (150 g) was homogenized with water (100 ml) in Waring blendor.

Results. When young male rats were fed a synthetic diet containing 1 or 2% of free aminolipid prepared from beef spinal cord cerebrosides, they lost weight rapidly and

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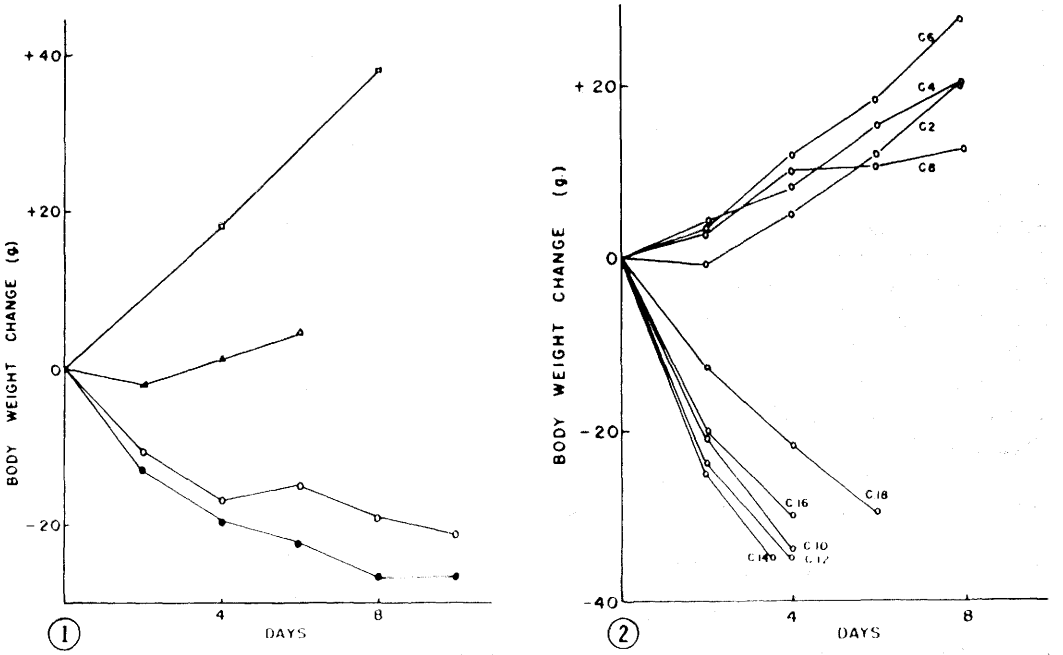

FIG. 1. Growth curves of rats fed diets containing aminolipid fractions prepared from beef spinal cord cerebrosides. —○— Free base (sphingosine + dihydrosphingosine) as 1% of diet. —●— Free base as 2% of diet. —△— Sulfate salt of base as 1.2% of diet. —□— Triacetyl derivative as 5% of diet. Each curve represents avg for 3 rats.

FIG. 2. Growth curves of rats fed diets containing aliphatic amines as sulfate salts. Amount added in each case was calculated to give a level of 1% of free amine in diet. C 2, ethylamine sulfate, 2.1%; C 4, butylamine sulfate, 1.7%; C 6, hexylamine sulfate, 1.5%; C 8, octylamine sulfate, 1.4%; C 10, decylamine sulfate, 1.3%; C 12, dodecylamine sulfate, 1.25%; C 14, tetradecylamine sulfate, 1.25%; C 16, hexadecylamine sulfate, 1.2%; C 18, octadecylamine sulfate, 1.0%. Each curve represents avg for 2 rats.

usually died after about 2 weeks (Fig. 1). Rats also lost weight rapidly when fed a diet containing 1% of free aminolipid from brain cerebrosides. The latter material is mainly sphingosine whereas the aminolipid from spinal cord cerebrosides is a mixture of sphingosine and dihydrosphingosine(3). The sulfate salt of mixed bases from spinal cord cerebrosides did not depress body growth of rats as much as the free bases and the triacetyl derivative appeared to have no growth-depressing effect at concentrations up to 5% of the diet (Fig. 1).

In these experiments gain or loss in body weight was related to food intake and it seemed that the adverse effects of sphingosine-type bases might be caused by unpleasant taste or odor which made the food unpalatable. To test this, 2 groups of rats were given a diet of fox chow *ad lib.* and in addition animals of one group were given 1 ml/day by stomach tube of a suspension containing 10% by weight of sphingosine† in corn oil, while those in the other group were given 1 ml/day of corn oil and served as controls. The group receiving sphingosine ate very little and lost weight while controls showed a continuous weight gain.

In a further experiment sphingosine† was incorporated into a liquid diet fed twice daily by stomach tube, thus ensuring that the rats received sufficient food to maintain body weight. Six male rats weighing approximately 130 g were given 5 ml twice daily of the liquid diet without sphingosine and this was increased gradually over 4 days to 10 ml twice daily. After 7 days rats were divided into 2 equal groups and an amount of sphingosine equal to 1% of total solids was added to the diet of one group. Three days later this was increased to 2%. After a further 3 days, ani-

† This material was prepared from beef spinal cord cerebrosides and is really a mixture of sphingosine and dihydrosphingosine.

mals in the treated group began to appear moribund and it became more difficult to introduce the full 10 ml at each feeding. Autopsy disclosed that the stomachs were very distended with gas and undigested food. The control group continued healthy although did not gain weight rapidly. The results of these experiments suggest that sphingosine exerts a real toxic effect and that the failure of rats to eat diets containing sphingosine is not primarily due to unpleasant taste or odor.

It seemed likely that the amine grouping in the sphingosine molecule was chiefly responsible for the toxic effect. This view was strengthened by feeding experiments with products obtained by partial hydrolysis of cerebrosides. Psychosine, obtained by splitting the amide linkage between fatty acid and sphingosine residues was toxic at the 1% level in the diet while ceramide, obtained by splitting the bond between galactose and sphingosine moieties did not entirely prevent growth when fed at 5% level. It was further found that n-octadecylamine was toxic at the 1% level while n-octadecylalcohol was accepted in the diet at the 5% level. Deichmann *et al.* (4) also describe toxic effects in rats fed diets containing 0.3% of n-octadecylamine.

The degree of toxicity encountered with sphingosine and other long-chain amines was greater than might be expected from comparison with other simple amines. For example, rats gained weight on diets containing as much as 4% of ethanolamine. Because of difference in molecular weights this represents a concentration of amine 20 times greater than 1% of sphingosine. This difference in toxicity between long- and short-chain amines was explored further by feeding a series of simple even-membered amines to rats at the 1% level (Fig. 2). At this level, short-chain amines up to C_8 were accepted and rats grew reasonably well while longer-chain amines from C_{10} to C_{18} were not accepted and rats lost weight rapidly and died. For initial tests the amines were used without purification, but repeat experiments using redistilled octylamine and decylamine gave similar results. However, octylamine appeared to be toxic when fed at the 2% level so the change in toxicity from octylamine to decylamine may not be as abrupt as indicated by Fig. 2.

Discussion. Differences in toxicity between compounds bearing a common functional group may be determined by a number of factors. For example, the compounds may differ in extent to which they interfere with normal metabolic function. They may also be absorbed, transported and excreted in different ways and may be degraded at different rates. Relative toxicities of the degradation products may also play a role.

In aliphatic amines, it is generally assumed that toxicity is due to the amine group and that in animal tissues these compounds are 'detoxified' by oxidative deamination(5). If this generalization holds true, it follows that the substrate specificity of amine oxidases may determine to some extent relative toxicities of different amines. *In vitro* studies have shown that short-chain amines, with the exception of methylamine and possibly ethylamine, are more readily attacked by amine oxidases than are long-chain amines(6,7). However, interpretation of these results is complicated by the low solubility of long-chain amines in aqueous media. Route of absorption of amines may also be important. Long-chain fatty acids are transported from the intestine chiefly *via* the lymphatic system(8) and, if long-chain amines are transported in the same way, they would by-pass the liver, which contains a relatively high concentration of amine oxidase(6,9).

In earlier experiments(2) diets containing 25% or more by weight of cerebrosides supported good growth with no evidence of toxic effects. Since sphingosine and psychosine preparations caused marked growth inhibition at levels of 1% of the diet, it appears that very little free base is liberated during digestion of cerebrosides.

Summary. The free aminolipids obtained by hydrolysis of cerebroside preparations from beef brain or beef spinal cord were poorly accepted by rats at levels as low as 1% of diet. The decreased food consumption and loss of body weight observed when such diets were fed appeared to be related to presence of free amine groups. Long-chain aliphatic amines were more toxic to rats than short-chain amines.

1. Rosenheim, O., Webster, T. A., *Biochem. J.,* 1941, v35, 920.

2. Carroll, K. K., *J. Lipid Research,* 1960, v1, 171.

3. Carter, H. E., Norris, W. P., Glick, F. J., Phillips, G. E., Harris, R., *J. Biol. Chem.,* 1947, v170, 269.

4. Deichmann, W. B., Radomski, J. L., McDonald, W. E., Kascht, R. L., Erdmann, R. L., *A.M.A. Arch. Ind. Health,* 1958, v18, 483.

5. Baldwin, E., *Dynamic Aspects of Biochemistry,* 3rd Ed. Cambridge Univ. Press, Cambridge, 1957, p271.

6. Blaschko, H., *Pharmacol. Rev.,* 1952, v4, 415.

7. Fouts, J. R., Blanksma, L. A., Carbon, J. A., Zeller, E. A., *J. Biol. Chem.,* 1957, v225, 1025.

8. Bloom, B., Chaikoff, I. L., Reinhardt, W. O., *Am. J. Physiol.,* 1951, v166, 451.

9. Davison, A. N., *Physiol. Rev.,* 1958, v38, 729.

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Some Properties of Rattlesnake Venom Following 26 Years Storage.* (25654)

FINDLAY E. RUSSELL, JERRY A. EMERY AND TRUMAN E. LONG

Lab. of Neurological Research, College of Medical Evangelists, Los Angeles County Hospital

Desiccated extracts of some snake venoms retain their lethality for long periods of time when stored in closed containers in the dark (1,2,3,4). However, certain evidence suggests that some desiccated venoms may deteriorate with time even under optimum conditions of light, vacuum and temperature(3,5). While stability of a venom may be a function somewhat dependent upon the family, genus, or species, lethality and specific modes of action of the venom can be altered by the method of storage.

Studies dealing with toxicity of venoms following periods of long storage are usually confined to determinations of m.l.d. or LD_{50} and give no indication of the changes in zootoxicologic properties. The possibility must be considered that fractions of these complex mixtures may be altered without markedly influencing lethality. The present investigation was initiated to determine the lethal doses of certain rattlesnake venoms stored for 26 to 27 years, and to ascertain the status of several of the known zootoxicologic properties of these venoms(6).

Materials and methods. The venoms were extracted and prepared on the following dates after the methods described by Klauber(6): *Crotalus atrox,* lot 451, July 16, 1932; *C. cerastes,* lot 453, July 16, 1932; *C. mitchelli mitchelli,* lot 510, July 4, 1933; *C. ruber ruber,* lot 496, July 17, 1933; and *C. viridis viridis,* lot 462, Sept. 10, 1932. The intravenous and intraperitoneal m.l.d. and LD_{50} of the dried venoms were determined in 20 g mice as described elsewhere(7). Studies of the effects of the venoms on neuromuscular transmission were made using 15 Bülbring phrenic nerve-diaphragm preparations(8) modified for studies on venoms(9). Studies of cardiovascular, respiratory, and central nervous system effects were made on cats as follows.

In 15 adult cats under pentobarbital hypnosis a common carotid artery and femoral vein were cannulated for measurement of arterial and venous pressures. Bipolar electrodes were placed in the skull over the frontal, parietal, and occipital lobes, bilaterally. Conventional limb, unipolar extremity, and precordial leads were affixed. All electrodes were connected to a Medcraft model D, 8-channel electroencephalograph. Respirations were recorded by means of a cantilever pneumograph(10) fed into the electroencephalograph. In 2 animals intermittent artificial respiration was carried out by positive pressure breathing through a tracheal cannula. Cisternal pressure was measured through a 20-gauge needle fixed in the cisterna cerebellomedullaris, and connected to a modified Brodie tambour in parallel with a water manometer. The tambour activated a wire strain gauge transducer which modulated the ampli-

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