*p*H 7.2, at 50°C for 1 hour it became extremely inhibitory with a strength comparable to that of LSD.

Gaddum (*9*) and Woolley and Shaw (*6*) originally proposed that the hallucinogenic action of LSD was caused by its antagonism to serotonin, as could be demonstrated on a smooth muscle preparation. However, there is an anomally in that the nonhallucinogenic BOL 148 (*10*) is also an antagonist of serotonin in such preparations (*11*). By contrast, the fact that LSD is antagonistic toward the binding of serotonin to nerve-binding particles, while mildly solubilized BOL 148 is not, is compatible with the original proposal. Nevertheless, if BOL 148 is converted to a serotonin antagonist in vivo, as it is under relatively mild laboratory conditions, then the anomaly remains. Also, it remains possible that LSD interferes with other neurohumoral agents besides serotonin.

Reserpine inhibits some components of the high-affinity binding, but the effect is much weaker than that of LSD. Calcium ions at high concentration were found to have little effect and chlorpromazine (*12*) could not be shown to be an inhibitor. Attempts to solubilize the LSD-sensitive substance with surface active agents such as Tween and lysolecithin were unsuccessful because the binding constant was changed and the inhibitory effect of LSD was lost. Such changes also occurred slowly on allowing it to stand in buffer at 2°C. Solubilization and precipitation from *n*-butanol with retention of activity and tenfold purification has been achieved. The LSD-sensitive binding is destroyed by cholera vibrio neuraminidase, suggesting that a ganglioside is involved.

Woolley has reported the existence in a variety of tissues of a serotonin lipid receptor, which he has proposed is a participant in calcium transport in vivo (*13*). The property of transporting calcium ions from an aqueous phase to a lipid phase in the presence of serotonin appears to be a fairly general characteristic of many types of lipids (*14*). Although we have not examined the calcium transporting activity of the components with the high-affinity binding, it does not seem likely that it is identical with the serotonin receptor described by Woolley, because the liver is a fairly rich source of the receptor lipid (*13*) whereas we were unable to detect the component with the high-affinity binding in liver preparations.

The existence of serotonin in nerve-ending particles has been shown (*2*), and it is hoped that investigation of its binding to macromolecules in the nerve-ending particles will throw further light on its role in neurotransmission.

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16. This research was supported by ONR contract NONR 401 (40) and by NSF grant GP-971.

19 March 1964

Reduction of Dimethylsulfoxide to Dimethylsulfide in the Cat

Abstract. *A peculiar sweetish odor was noted in the exhaled breath of cats treated with dimethylsulfoxide. By means of gas chromatographic and mass spectrographic techniques, the responsible compound was identified as dimethylsulfide.*

Dimethylsulfoxide has been advocated as a medium for the frozen storage of biological material such as bone marrow (*1*) and whole organs (*2*) intended for transplantation. Recently (*3*) dimethylsulfoxide has been advocated for a wide variety of possible medical uses ranging from the experimental prevention of peritoneal adhesions to tranquilization (*3*).

While investigating the pharmacology of this compound in cats, we noticed a peculiar sweetish odor in the expired air within a minute of the intravenous injection of the compound. A comparison of this odor with those of related chemical compounds led to the suspicion that the gaseous metabolite was dimethylsulfide.

The transformation of the sulfoxide to the sulfide represents an unusual metabolic pathway. The reduction of sulfoxides has hitherto been observed only in microorganisms (*4*). Bennett (*5*), however, showed that rats grew normally when DL-methionine sulfoxide was substituted for dietary DL-methionine. This observation suggests the possibility that DL-methionine sulfoxide may be reduced to DL-methionine.

To verify the occurrence of dimethylsulfide after intravenously administering dimethylsulfoxide to cats, we trapped expired air in an empty absorption bottle immersed in ice. The condensate was analyzed by gas chromatography. The retention time of the unknown sample was identical with

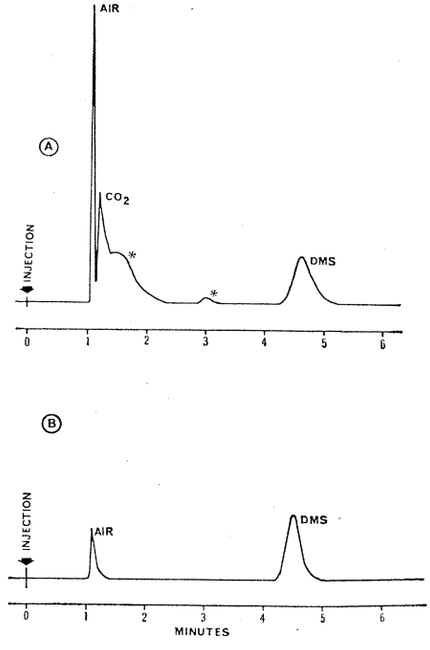

Fig. 1. Gas chromatograms of cat's exhaled breath condensate (*A*) and dimethylsulfide (*B*) (*7*). *A*, 20 μl of condensate, attenuation × 1. Ordinate, recorder excursion; abscissa, time in minutes. *B*, 3 μl of 1 percent of dimethylsulfide in water, attenuation × 16. (*) Unidentified peaks.

that of pure dimethylsulfide (Fig. 1). Mass spectrography provided further evidence.

Analysis was performed on 200 ml of cat breath collected over a 5-minute period shortly after the intravenous injection of 400 mg of the sulfoxide per kilogram of body weight, which is approximately the maximum tolerated single dose. Figure 2 shows that pure dimethylsulfide appears at mass 62. The peak at 35 is quite rare and characteristic of the sulfide. The fragment patterns for the breath sample (Fig. 2*A*) and pure dimethylsulfide (Fig. 2*B*) are identical. The sulfide was absent in a sample of the injected dimethylsulfoxide analyzed by both gas chromatographic and mass spectrographic techniques.

These studies are highly suggestive of the existence of a metabolic pathway in the cat which reduces the sulfoxide to the sulfide. Mazel *et al.* (*6*) have reported that in contrast to some other *S*-methyl compounds, dimethylsulfide is not *S*-demethylated by rat liver microsomes. Although their studies were carried out in vitro, their results lend support to the contention that dimethylsulfide is a terminal metabolite of dimethylsulfoxide. This does not exclude the existence of other metabolic pathways.

Also noted in the course of these studies was the appearance of a deep red coloration of the urine. Preliminary spectrographic analysis of bladder urine from cats treated with dimethylsulfoxide indicates the presence of both hemoglobin and methemoglobin.

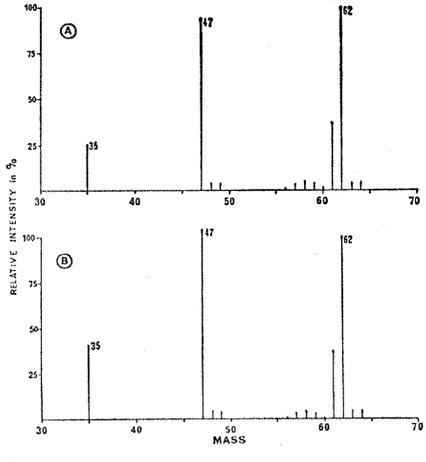

Fig. 2. Mass spectrograms of cat's exhaled breath (*A*) and dimethylsulfide (*B*) (*8*). The sample of exhaled air was exhausted through a liquid nitrogen–chilled trap. The contents of the trap were admitted to the mass spectrometer by warming. *B*, dimethylsulfide introduced by air exhaustion.

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7. Anesthesia: Dial-urethane solution (Ciba), 0.63 ml/kg. Microtek 2500R gas chromatograph; direct injection of liquid samples. Columns: 1 m × 6 mm, 20 percent glycerol on Ultraport; then 3.5 m × 6 mm of diisodecyl phthalate on Ultraport at 85°C. Carrier: helium at 2.4 atm inlet pressure and 100 ml/min flow. Thermal conductivity detector at 150°C and detector current of 640 ma.
8. Anesthesia: Dial-urethane solution (Ciba), 0.63 ml/kg. Magnetically scanned, motor-driven mass spectrometer of the 60° sector type. Peaks normalized to mass 62 = 100 percent in both records.

9. We thank G. P. Happ and David W. Stewart of the Research Laboratories, Eastman Kodak Co., for the mass spectrographic analysis. Supported in part by the U.S. Atomic Energy Commission under contract AT(30-1)-2192.

26 February 1964

Linear Polymerization of a Gastropod Hemocyanin

Abstract. *The decreased solubility of a gastropod hemocyanin in the region of the isoelectric point has been correlated with a sharp increase in light scattering produced by the protein in solution. Electron microscopic observations on negatively stained spray preparations in the isoelectric region demonstrated that the formation of linear polymers of the molecule underlies both the increase in light scattering as well as the decreased solubility of the protein. While the linear polymerization produces chains of varying length with no evidence of interaction between chains, the polymerization proceeds with a high degree of ordering of individual molecules. On either side of the isoelectric region of this hemocyanin two forms of molecules are present, rectangular and circular. The rectangular molecules polymerize in an end-to-end fashion in the isoelectric region. The rectangular molecules which participate in the polymerization appear to be divided perpendicularly to their length into three major segments.*

Hemocyanin, the copper-containing respiratory protein of various invertebrate phyla, was among the first proteins to be characterized by ultracentrifugation (*1*). These and subsequent studies led to the first accurate determination of molecular weights for such macromolecules (*2*). Among these fundamental observations was the demonstration of dissociation of hemocyanin on either side of the isoelectric point (*2*) into particles whose weights are characteristically halves or eighths of that of the original associated molecule (*3*). These changes may be observed visually; for example, the hemocyanin of *Kelletia kelletia* (the sea snail) in associated form is an opalescent gray-blue exhibiting a marked Tyndall effect, but at the *p*H where the dissociation occurs and above that *p*H, it is a clear dark blue.

It has not yet been possible to reconcile the data on size and shape of those proteins in solution from sedimentation and diffusion studies with the appearance of dried hemocyanin in the electron microscope (*4*). Brohult (*5*) concluded, on the basis of the high frictional coefficients, that the hemocyanin of *Helix pomatia* existed in solution as highly asymmetrical particles. Assuming their shape to be elongated prolate ellipsoids, he calculated their length to be 1130 Å and their width to be 136 Å. Dissociation involved splitting in a plane parallel to the long axis and resulted in two half molecules with lengths of 890 Å and widths of 60 Å. Electron micros-

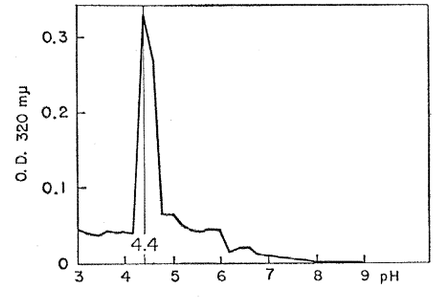

Fig. 1. Light scattering by *Kelletia kelletia* hemocyanin, 100 μg/ml, *p*H 2.1 to 6.0, 0.02*M* acetate buffer; *p*H 6.0 to 9.1, 0.02*M* phosphate buffer; *p*H increments 0.2 units; optical density at 320 mμ.