concluded that DMSO causes glucose and galactose to become sufficiently fat-soluble to bypass carrier mediation.

1. Farquhar, M. G., Palade, G. E., J. Cell. Biol., 1963, v17, 375.
2. Lindemann, B., Solomon, A. K., J. Gen. Physiol., 1962, v45, 801.
3. Alvarado, F., Crane, R. K., Biochim. Biophys. Acta, 1962, v56, 170.
4. Sears, P. G., Siegfried, W. D., Sands, D. E., J. Chem. Eng. Data, 1964, v9, 261.
5. Lindberg, J. J., Pietilä, I., Suomen Kemistilehti, 1962, vB35, 30.
6. Nelson, N., J. Biol. Chem., 1944, v153, 375.
7. Teller, J. D., 130th Meeting, A.C.S., 1956, 69c.
8. Csáky, T. Z., Ho, P. M., Proc. Soc. Exp. Biol. & Med., 1965, v120, 403.
9. Järnefelt, J., Biochim. Biophys. Acta, 1961, v48, 104.
10. Brown, V. K., Robinson, J., Stevenson, D. E., J. Pharmacy & Pharmacol., 1963, v15, 688.

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Dimethyl Sulfone: Isolation from Cows' Milk.* (31272)

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Recently, we observed that normal human urine contained 5-10 mg/24 hr of dimethyl sulfone ($DMSO_2$)(1). It occurred to us that cows' milk might be a source of some of this sulfone. Patton *et al*(2) reported that dimethyl sulfide (DMS) is one of the flavor constituents of cows' milk. $DMSO_2$ has been isolated from dried cows' blood(3) and from beef adrenals(4). We have observed that DMS is oxidized to $DMSO_2$ *via* dimethyl sulfoxide (DMSO) in the rabbit. Thus we thought it would be interesting to see if cows' milk contains DMSO and/or $DMSO_2$.

One liter samples of pasteurized cows' milk, obtained from local markets, were treated with 2 volumes of acetone to precipitate the proteins. The filtrates were freed of acetone with a rotating film evaporator and were then extracted with 3 × 250 ml of hexane to remove most of the fats.

The aqueous residues were extracted continuously with methylene chloride for 24 hours and the extracts were analyzed by gas chromatography as previously described(1). The results are tabulated in Table I. The extraction efficiency was tested by addition of 3500 cpm of ^{14}C $DMSO_2$ to one sample of milk prior to the acetone treatment. All of the radioactivity was present in the final extract.

TABLE I. Dimethyl Sulfone in Samples of Pasteurized Cows' Milk.

Sample	mg $DMSO_2$/liter*
1	8.2
2	7.6
3	6.1
4†	7.8
5	7.1

* By gas chromatographic analysis.
† Taken from the same container as sample #2 but allowed to sour before workup.

Combined extracts containing 38 mg of $DMSO_2$ (by gas chromotographic analysis) were applied to a 13 × 1.8 cm activity grade III neutral alumina column and the column was eluted with 100 ml of methylene chloride. Concentration of the eluent gave 1-2 ml of oil from which needles crystallized. These needles were collected and washed with a little 1:1 benzene:hexane to give 7 mg of colorless crystalline material with mp 108-109°. The infrared spectrum of the crystals in KBr was identical to that of an authentic sample of $DMSO_2$. An additional 11 mg of $DMSO_2$ was recovered from the mother liquors. If any DMSO was in the extract it was below our limits of detectibility (0.5 mg/liter of milk).

After removing one liter of milk for analysis from sample #2 the remainder was allowed

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to sour (room temperature, 4 days) and was then worked up as sample #4. As no increase in $DMSO_2$ was noted, it is probable that the $DMSO_2$ isolated from cows' milk is not a product of micro-organisms in the milk but rather arises from metabolic processes in the intact animal.

Summary. Dimethyl sulfone has been detected and isolated from pasteurized cows' milk. The source of the sulfone is not yet known. Dimethyl sulfoxide was not detected.

1. Williams, K. I. H., Burstein, S. H., Layne, D. S., Arch. Biochem. & Biophys., 1966, v113, 251.
2. Patton, S., Forss, D. A., Day, E. A., J. Dairy Sci., 1956, v39, 1469.
3. Ruzicka, L., Goldberg, M. W., Meister, H., Helv. Chim. Acta, 1940, v23, 559.
4. Pfiffner, J. J., North, H. B., J. Biol. Chem., 1940, v134, 781.

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The Effect of Ethylenediaminetetraacetate and of Lysozyme on Isolated Lipopolysaccharide from *Pseudomonas aeruginosa.** (31273)

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During studies on the endotoxic properties of lipopolysaccharide of *Pseudomonas aeruginosa*, it was noted that its lethal toxicity for mice was reduced or destroyed by incubation with ethylenediaminetetraacetate (EDTA) and with lysozyme. Previous reports have indicated that cells of *P. aeruginosa* died rapidly in the presence of EDTA (6,9). It was suggested by both groups of workers that EDTA acted through its chelation of metal cations and that these metal cations were essential to the structural integrity of *P. aeruginosa*. More recently Eagon, Simmons and Carson(7) showed that Ca^{2+}, Mg^{2+} and Zn^{2+} were components of the cell wall of *P. aeruginosa*.

Lysozyme, on the other hand, did not induce lysis of cells of *P. aeruginosa*. This reagent, however, was not completely without effect since electron microscopy of cells incubated with lysoyzme revealed alterations of the cellular surfaces(6). Furthermore, electron micrographs of thin sections of cells incubated with lysozyme also indicated extensive alterations of the cell walls(4). These observations were interpreted to indicate: (1) that the mucopeptide component of the unaltered cell walls of *P. aeruginosa* is sensitive to lysozyme; and, (2) that the mucopeptide component is not solely responsible for the rigidity of the cell wall of this microorganism.

The investigations described here, therefore, were undertaken to provide further information on the structure of the lipopolysaccharide and of the cell wall of *P. aeruginosa*.

Materials and methods. P. aeruginosa was cultivated for 12-14 hours at 37°C on a rotary shaker in an enriched medium. After collection and washing, cells were killed by suspending in 2% phenol for 1 hour at room temperature with shaking. These cells were then washed twice with sterile 0.85% saline and lyophilized. This material was designated as killed whole cells.

Cell walls were prepared according to the technique of Eagon and Carson(6). Cell membranes were prepared as described by Carson and Eagon(3). Protoplasm was prepared by disruption of a saline suspension of cells at 10,000 psi in the French Pressure Cell followed by centrifugation at 27,000 × *g* for 1 hour to remove walls, membranes, large particles and unruptured cells.

Lipopolysaccharide was prepared by the aqueous ether-ethanol method as described by Foster and Ribi(8). Whole cell lysate was prepared by lysing cells with EDTA plus lysozyme as described by Eagon and Carson

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