Also, theoretical estimates indicate they differ in surface free energy.[6] One may argue with justification that precise values for the surface thermodynamic quantities for a polycrystalline sample cannot be conceptually defined without specifying a shape distribution function and proving that it is invariant with crystal size, for each substance studied. In addition, there are numerous experiments which suggest that the thermodynamic potential of material in small regions surrounding corners, edges and steps on crystal faces differs from that in geometrically plane surfaces. Since the number of such corners, edges, etc., is proportional to the number of crystals, whereas the total area depends on that number times the square of the linear extension of the crystals [so that for a mole of substance divided into n particles of uniform size, $S \alpha (M/\rho)^{2/3}n^{1/3}$], it is clear that any macroscopic quantity which depends on the thermodynamic potential will not be strictly extensive with respect to surface area. As a matter of fact, in a very interesting paper preceding the one under discussion, one of the authors (G. J.) and K. S. Pitzer[7] showed that at low temperatures a significant contribution to the specific heat of *very* small particles comes from the gross motion of the particles; *i.e.*, their heat capacity depends on the *number* of *particles*, in addition to the differences between the vibrational frequencies of atoms in the surface layers and those in the bulk crystal.

In a discussion of the thermodynamic properties of surfaces, one should recognize, however, that even under conditions approaching ideality in composition and configuration, the structures of crystals at temperatures other than 0°K. is determined by minimizing their total free energy.[8] This implies that there must be an entropy contribution arising from lattice defects. Such imperfections will affect the enthalpy as well (and hence the heat of solution), and the magnitude of the increment will depend on crystallite size; it is unlikely that the net effect of the various types of possible defects will in the aggregate depend precisely on the square of the linear extension of the crystals. Indeed, in most laboratory procedures for the preparation of crystals strains, distortions, minute amounts of impurity, etc., will be introduced.[9] Finally, the practical problem of the complete elimination of adsorbed gases will be difficult to solve. We may conclude that the application of the thermodynamic method of Jura and Garland to well crystallized materials will lead to values for thermodynamic functions which only in part are due to the presence of surface. Such magnitudes will be *extensive* with respect to surface area only when the method of preparation, crystal size and shape distributions, etc., are kept strictly unaltered. Such a restriction nullifies the power of the thermodynamic method.

The proposal of Jura and Garland does apply to liquids and glasses, for which the inherent randomness of internal structural minimizes the relative contributions of the very factors which vitiate the method for well crystallized materials.

It is a pleasure to acknowledge an interesting discussion of this paper with Prof. J. A. Krumhansl, Department of Physics.

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[6] R. Shuttleworth, *Proc. Phys. Soc.*, **A62**, 167 (1949); W. D. Harkins, *J. Chem. Phys.*, **10**, 268 (1942). Also, see the excellent summary "Use of Classical Macroscopic Concepts in Surface Energy Problems" by Conyers Herring, presented at a conference on "Structure and Properties of Solid Surfaces". September, 1952, Intn. Union Pure and Applied Physics.

[7] G. Jura and K. S. Pitzer, THIS JOURNAL, **74**, 6030 (1952).

[8] N. F. Mott and R. W. Gurney, "Electronic Processes in Ionic Crystals," Clarendon Press, Oxford, 1948, Chap. II.

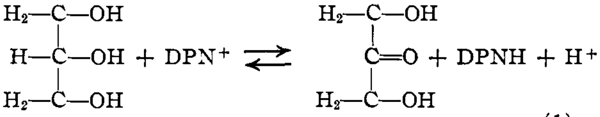
[9] See Chapter IV, ref. 8; W. Shockley, "Electrons and Holes in Semiconductors," D. Van Nostrand Co., New York, N. Y., 1950.

A DPN SPECIFIC GLYCEROL DEHYDROGENASE FROM *AEROBACTER AEROGENES*[1]

Sirs:

A number of microörganisms have been shown to utilize glycerol as the sole carbon source.[2] The major product in the oxidation of glycerol in *Acetobacter suboxydans* has been shown to be dihydroxyacetone (DHA).[3] In *A. aerogenes* glycerol is oxidized past the DHA stage; the products are, in part, organic acids.[2b,4]

We have found that oxidation of glycerol in cell-free extracts of *A. aerogenes* requires diphosphopyridine nucleotide (DPN). This reaction can be followed spectrophotometrically at 340 mμ as shown in Fig. 1. Glycerol was incubated with DPN in the presence of dialysed cell-free extracts of *A. aerogenes*. After the reaction had approached equilibrium, DHA was added and the reaction that followed was due to the oxidation of the reduced DPN (DPNH) by the reduction of DHA to glycerol. Equation 1 expresses this relation as



$$\begin{array}{l} H_2\text{—}C\text{—}OH \\ \quad\ | \\ H\text{—}C\text{—}OH \\ \quad\ | \\ H_2\text{—}C\text{—}OH \end{array} + DPN^+ \rightleftarrows \begin{array}{l} H_2\text{—}C\text{—}OH \\ \quad\ | \\ \quad\ C{=}O \\ \quad\ | \\ H_2\text{—}C\text{—}OH \end{array} + DPNH + H^+ \qquad (1)$$

Reaction 1 proceeds with glycerol in the presence of hydroxylamine which inhibits alcohol dehydrogenase activity.[5] This, therefore, indicates that the glycerol dehydrogenase is a separate enzyme distinct from alcohol dehydrogenase.

At comparable concentrations glyceraldehyde is reduced at a slower rate than DHA. This would suggest that DHA is the primary product of glycerol oxidation by DPN in *A. aerogenes*. Neither α- or β-glycerol monophosphates are oxidized by this enzyme preparation. Triphosphopyridine nucleotide (TPN) is inactive as a substitute for DPN in reaction 1.

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[1] Contribution No. 37 of the McCollum-Pratt Institute. This work was supported in part by grants from the Rockefeller Foundation and the American Cancer Society as recommended by the Committee on Growth of the National Research Council.

[2] (a) H. R. Braak, Thesis, Delft, 1928; (b) R. E. Buchanan and E. I. Fulmer, "Physiology and Biochemistry of Bacteria," The Williams and Wilkins Company, Baltimore, Md., 1930, Vol. III, p. 248.

[3] A. J. Kluyver and F. J. G. DeLeeuw, *Tijdschr. Vergelijk. Geneesk.*, **10**, 170 (1924); L. A. Underkofler and E. I. Fulmer, THIS JOURNAL, **59**, 301 (1937).

[4] H. Kumagawa, *Biochem. Z.*, **131**, 156 (1922); A. C. Baskett and C. N. Hinshelwood, *Proc. Roy. Soc.* (*London*), **B138**, 75 (1951).

[5] N. O. Kaplan and M. M. Ciotti, *J. Biol. Chem.*, in press.

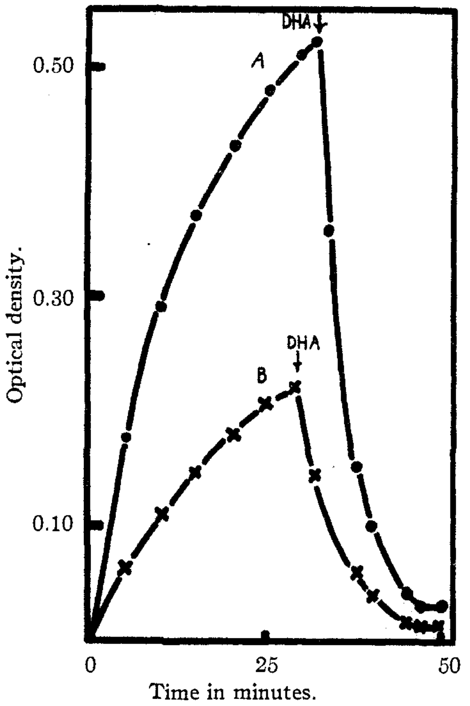

Fig. 1.—The reversible reaction catalyzed by glycerol dehydrogenase: curve A, the reaction mixture contained 100 μM. of potassium phosphate buffer, 0.5 μM. of DPN, 0.6 mg. of dialyzed *A. aerogenes* glycerol dehydrogenase preparation, and 500 μM. of glycerol in a total volume of 3.0 ml. at *p*H 9. The change in optical density was measured at 340 mμ with a Beckman model DU spectrophotometer at 23°. At the arrow 100 μM. of DHA was added. Curve B, the details for curve A apply here except 100 μM. of glycerol was used with 10 μM. of DHA added at the arrow.

During the course of this investigation an interesting non-enzymatic reaction between DHA and DPN was observed.[6] The product of this reaction has an ultraviolet spectrum identical to that of DPNH and possesses other properties of DPNH. That this product is not DPNH is shown by its failure to reduce acetaldehyde in the presences of yeast alcohol dehydrogenase. Further study has shown that the basic requirements for the reaction are an N-substituted nicotinamide structure (as in DPN), and α,β-hydroxy-keto-structure (as in DHA), and an alkaline medium. The details of this investigation and a discussion of the reaction mechanism will be reported elsewhere.

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RECEIVED DECEMBER 22, 1952

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[6] A somewhat similar reaction using glyceraldehyde had been noticed previously by D. M. Needham, L. Siminovitch, and S. M. Rapkine, *Biochem. J.*, **49**, 113 (1951), who presumed that DPNH and glyceric acid were formed.

[7] Public Health Service Research Fellow of the National Heart Institute.

ADDITIONAL COMMENTS ON THERMODYNAMIC FUNCTIONS FOR SURFACES OF CRYSTALS

Sir:

In the preceding letter, S. H. Bauer has criticized a recent article by us. It appears that we were too brief in our discussion of the work which was presented. Our belief was that the data which we had obtained could not be considered final. We were careful to point out several factors which might change the final results. It was the hope of the senior author to reserve the discussion of some of the questions raised in Dr. Bauer's letter until better data were available. Since we knew that several years would be essential for a complete solution to all of the experimental problems involved in the determination, we thought that it would be advisable to publish that which we had done in the hope that these preliminary results would be of value and that others would think and work actively on this problem.

First, concerning the pragmatic test of the invariance of the results with respect to particle size and shape. For the past two years we have engaged in the design and construction of a calorimeter for the sole purpose of making these measurements with respect to the enthalpy. We have hopes that in a year we will be able to make a report of the results of this work. After this project is finished, we hope to make similar measurements of the heat capacity. These of course, will take even more time since the necessary experimental procedures are even more difficult than those involved in the enthalpy determination.

Bauer states that the results should be independent of size and shape. This, of course, will be true only if two conditions are fulfilled: (1) only one crystallographic face is present, and (2) if the particle is sufficiently large that the surface properties are not dependent on particle size. The first of these considerations was an overwhelming factor in the choice of MgO, since the structure is such that we would expect only the 100 face to appear at the temperatures at which the experiments are performed. The few poor electron photomicrographs that we have obtained with our sample of MgO indicated that this was so. Too few individual particles were observed, however, to state that this was certainly true for all of the particles. Since we considered these results as preliminary, we made no really serious efforts to obtain really good pic tures. Actually, it can be shown that under certain circumstances, even if more than one crystallographic face appears, it is possible to obtain the surface tension of each face from the same measurements and a knowledge of the crystal habit; provided that the future work shows that the proposed scheme of measurements is valid. The size at which it may be expected that the thermodynamic functions become size dependent, can be readily computed by the method of Lennard-Jones and Dent.[1]

The question naturally arises as to the possible specific effects mentioned by Bauer. First, the contribution to these functions of the gross motion of the particles studied by Jura and Pitzer[2]: at very low temperatures, say to about 10°K., this effect would be appreciable. However, at room temperature, this quantity is several orders of magnitude less than the observed experimental effects. There is complete justification in its neglect.

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[1] J. E. Lennard-Jones and B. E. Dent, *Phil. Mag.* [7] **8**, 530 (1929).

[2] G. Jura and K. S. Pitzer, THIS JOURNAL, **74**, 6030 (1952).