The Nonelectrolyte Permeability of Planar Lipid Bilayer Membranes

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ABSTRACT The permeability of lecithin bilayer membranes to nonelectrolytes is in reasonable agreement with Overton's rule. That is, $P_d \alpha DK_{hc}$, where P_d is the permeability coefficient of a solute through the bilayer, K_{hc} is its hydrocarbon:water partition coefficient, and D is its diffusion coefficient in bulk hydrocarbon. The partition coefficients are by far the major determinants of the relative magnitudes of the permeability coefficients; the diffusion coefficients make only a minor contribution. We note that the recent emphasis on theoretically calculated intramembranous diffusion coefficients (D_m's) has diverted attention from the experimentally measureable and physiologically relevant permeability coefficients (P_d's) and has obscured the simplicity and usefulness of Overton's rule.

INTRODUCTION

Much attention has been given in the last several years to the nonspecific permeability of nonelectrolytes through plasma membranes. The nonelectrolytes that have been considered are not those that cross the membrane via special transport systems (e.g., sugars and amino acids), but rather those that permeate by a solubility-diffusion mechanism through the bilayer proper. Of particular concern have been two issues: the nature of the diffusion process within the bilayer and the appropriate solvent that models the partitioning of solutes into the bilayer. Enlightenment on these points has been sought by subjecting the logarithms of the permeability data from several cell systems to multivariate linear regression analysis. Such an approach, with its emphasis on theoretically calculated intramembranous diffusion coefficients (D_m's) and their dependence on molecular volume, has tended, in our opinion, to obscure the simple relation between permeability coefficients (P_d's) and lipid solubility enunciated by Overton almost 90 yr ago. Indeed, it has led in one instance to the extreme position that the apparent "sieving" of small hydrophilic solutes by plasma membranes, which has traditionally been interpreted to imply the existence of aqueous pores, arises instead as a natural consequence of the bilayer structure (Lieb and Stein, 1971).

The validity of such a position can be directly tested in planar artificial lipid bilayer membranes. Unfortunately, the same issues of the appropriate

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model solvent for the bilayer and the dependence of intramembranous diffusion coefficients on molecular volume have also generated controversy here (compare Finkelstein [1976] with Wolosin et al. [1978]) and have again succeeded in obscuring the basic simplicity of the results.

In this article we review published permeabilty measurements on planar lipid bilayer membranes and present new data to show that the permeability of bilayers to small nonelectrolytes is reasonably described by Overton's rule; i.e.,

$$P_d \alpha DK_{hc}, \quad (1)$$

where P_d is the permeability coefficient of a solute through a lipid bilayer, K_{hc} is its hydrocarbon:water partition coefficient, and D is its diffusion coefficient in bulk hydrocarbon.[1] It follows from our analysis that large deviations from Relation 1 in the behavior of solutes cannot be attributed to the inherent permeability characteristics of the bilayer structure of plasma membranes, but must be ascribed to other specialized pathways for these solutes.

MATERIALS AND METHODS

Methods

PERMEABILITY MEASUREMENTS Membranes were formed by the brush technique of Mueller et al. (1963) at 25°C ±2° across a 0.8 mm^2 hole in a 125-μm thick Teflon partition separating two Lucite chambers. Both chambers were stirred continuously with magnetic fleas during the course of an experiment. Each chamber contained 3.0 ml of solution, typically unbuffered 0.1 M NaCl (pH ≈ 5.6). In experiments with *n*-butyric acid (pK_a = 4.82 [Dawson et al., 1969]) the solution was 0.1 M NaCl + 5 mM Tris (pH = 7.51); in experiments with codeine (pK_b = 7.95 [Merck Index, 8th edition]) the solution was 0.1 M NaCl + 0.1 M Na acetate (pH = 4.51 or 5.54). The procedure for measuring the permeability coefficient (P_d) of a molecule was that described by Holz and Finkelstein (1970).

An unstirred layer 100 μm thick was calculated from the apparent P_d of *n*-butanol (Holz and Finkelstein, 1970), and this value used to correct all measured P_d values; the correction was generally <20% and never exceeded 60%. Measured P_d values for *n*-butyric acid and codeine were converted to values for the un-ionized forms of these compounds on the assumption that the non-ionic species are the only ones with significant permeability. For codeine, this assumption was confirmed by the observation that the measured value of P_d changed 10-fold for a one unit ΔpH, in the range where over 99% of the compound is ionized. By working at pH's at which most of the compound is ionized (i.e., far from the pK), unstirred layer corrections are minimized (see Gutknecht and Tosteson, 1973).

For most experiments 3.6% egg lecithin in *n*-decane was used as the membrane-forming solution, but in a few experiments *n*-hexadecane was the solvent.

PARTITION COEFFICIENT MEASUREMENTS The partition coefficient (K_{hc}) of a given

[1] Since diffusion coefficients in hydrocarbon for most nonelectrolytes are not available, we use instead the diffusion coefficients in water. This is a reasonable procedure, since diffusion coefficients in water should be more or less directly proportional to diffusion coefficients in hydrocarbon, and we are not concerned with the absolute values of the permeability coefficients but rather with their relative values in a membrane of given composition.

solute between *n*-hexadecane and water was determined at room temperature (25°C ± 2°) by static equilibration as described previously (Finkelstein, 1976). Ethyl ether: water partition coefficients for ethanediol, propanediol, butanediol, and glycerol were determined by the same procedure; the values obtained were in agreement (to within 30%) with those reported by Collander (1954). The observed K_{hc} for butyric acid was determined at pH values of 2.95, 4.85, and 7.24, whereas that for codeine was determined at 5.8, 8.0, and 10.25. The observed values were converted to that of the corresponding un-ionized form on the assumption that this is the only species which partitions significantly into hydrocarbon. This assumption, which is reasonable a priori, is confirmed by the constancy of the calculated K_{hc}'s at different pH values.

Materials

Chicken egg lecithin was obtained from Sylvana Chemical Company (Orange, N.J.) and Avanti Biochemicals, Inc. (Birmingham, Ala.), *n*-decane (99.9%) from Chemical Samples Co., Inc. (Columbus, Ohio), and *n*-hexadecane (spectroquality) from Aldrich Chemical Company (Milwaukee, Wis.).

The same stock solutions of radioactive materials were used for both permeability and partition experiments. [^{14}C]ethyleneglycol (1,2 ethanediol), butyric acid, glycerol, and [^{3}H]glycerol were obtained from New England Nuclear Corp. (Boston, Mass.). [1,2-^{14}C]propanediol (propyleneglycol) was purchased from ICN Corp. (Irvine, Calif.), [^{14}C] codeine from the Radiochemical Centre (Amersham, England), and [1,4-^{14}C]-butanediol from American Radiochemical Corp. (Sanford, Fla.). Significant amounts of a relatively lipophilic radioactive impurity in the [^{14}C]propanediol were removed by repeated extractions into ethyl ether; [^{14}C]butanediol was freed of lipophilic contaminant as described previously (Finkelstein, 1976).

RESULTS

Table I summarizes the results of our present experiments along with previously published data from this laboratory (Finkelstein, 1976). Column 1 lists compounds in the order of decreasing values of hydrocarbon:water partition coefficients (K_{hc}), as recorded in column 2. Column 3 lists the diffusion constants (D) of these compounds in water at 25°C, column 4 presents the values of their permeability coefficients (P_d) for egg lecithin membranes, and column 5 gives the values of P_d $(DK_{hc}/\Delta x)^{-1}$, where Δx, the thickness of the hydrophobic interior of the bilayer, is taken as 50 Å (Fettiplace et al., 1971). Fig. 1 is the traditional double logarithmic plot of the same data; in this case P_d is plotted vs. DK_{hc}. The line in Fig. 1 is drawn with a slope of 1. In Fig. 2 we plot log P_d/K_{hc} vs. log $\bar{V}$, where $\bar{V}$ is molecular volume, leaving the intrepid reader to draw an appropriate straight line.

In order to assess the effect on P_d of the decane retained in the bilayer (Fettiplace et al., 1971), we determined the permeability coefficients for two solutes, butanediol and codeine, in membranes formed from lecithin dissolved in *n*-hexadecane; such membranes retain very little hydrocarbon solvent (Fettiplace et al., 1971). We found a modest reduction in the values of the permeability coefficients; P_d (1,4 butanediol) was reduced by a factor of 1.2 and P_d (codeine) by a factor of 2.4 from the values obtained in membranes formed from lecithin dissolved in *n*-decane.

DISCUSSION

Permeability of Lecithin Membranes

The data displayed in Table 1 and Fig. 1 are well described by the relation:

$$P_d \alpha DK_{hc}. \quad (1)$$

The molecules range in size from water (mol wt = 18) to codeine (mol wt = 299), and the values of K_{hc} span a range greater than four orders of magnitude. Only water and urea deviate significantly from the mean; the value of P_d $(DK_{hc}/\Delta x)^{-1}$ for water is too large by a factor of 3 and that for urea is too small by a factor of 7. (These "anomalies" have been commented upon

TABLE I

PERMEABILITY COEFFICIENTS IN LECITHIN BILAYER MEMBRANES

Molecule	$10^6 K_{hc}$	$10^5 D$	$10^4 P_d$	$P_d(DK_{hc}/\Delta x)^{-1}$
		cm^2/s	cm/s	
Codeine	42,500	0.63*	1,400	0.26
Butyric acid	7,840	1.0‡	640	0.41
1,2-Propanediol	64	1.09§	2.8	0.20
1,4-Butanediol	43	1.0‡	2.7	0.32
H_2O	42	2.44 ‖	22	1.1
Acetamide	21	1.32¶	1.7	0.30
1,2-Ethanediol	17.2	1.25**	0.88	0.21
Formamide	7.9	1.7¶	1.03	0.38
Urea	3.5	1.38‡‡	0.04§§	0.042
Glycerol	2.0	1.09§	0.054	0.12

* Estimated from $D_{butanol}$ according to the relation:

$$\frac{D_{codeine}}{D_{butanol}} \cong \sqrt[3]{\frac{(\text{mol wt})_{butanol}}{(\text{mol wt})_{codeine}}}.$$

‡ Estimated from $D_{butanol}$ (Lyons and Sandquist, 1953).
§ Estimated from $D_{propionamide}$ (Gary-Bobo and Weber, 1969).
‖ Wang et al. (1953).
¶ Gary-Bobo and Weber (1969).
** Estimated from $D_{acetamide}$ (Gary-Bobo and Weber, 1969).
‡‡ Gosting and Akeley (1952).
§§ Vreeman (1966) and Gallucci et al. (1971).

previously [Finkelstein, 1976]. In lecithin:cholesterol and sphingomyelin:cholesterol membranes, P_d $(DK_{hc}/\Delta x)^{-1}$ is unusually large for formamide (Finkelstein [1976]. Poznansky et al. [1976] report this for lecithin membranes as well, but this was not seen by either Gallucci et al. [1971] or Finkelstein [1976].)

It should be noted that it is the partition coefficients that govern by far the relative values of permeability coefficients. Thus, if the abscissa in Fig. 1 is chosen to be K_{hc} instead of DK_{hc}, the points still fall on a straight line with a slope of 1. This is because there can be a much larger spread in values of partition coefficients than of diffusion coefficients. For example, the K_{hc}'s for

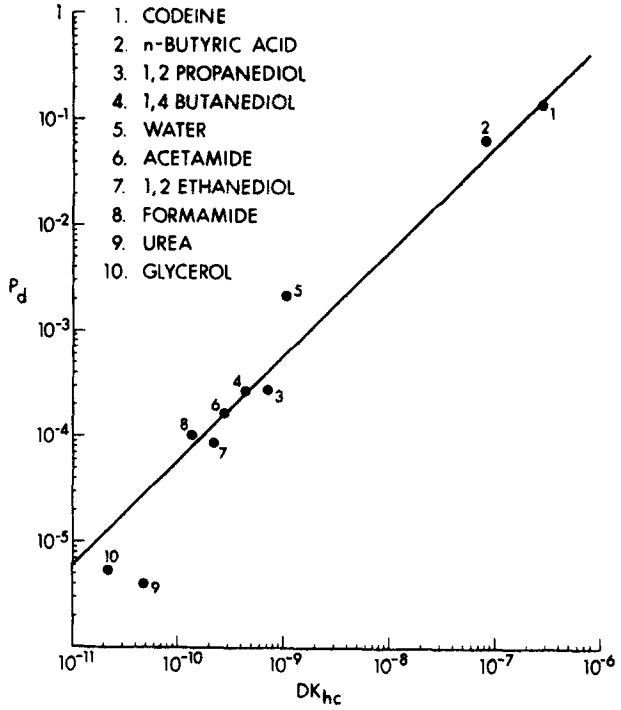

FIGURE 1. P_d vs. DK_{hc}. P_d is in cm/s, D is in cm^2/s, and K_{hc} is dimensionless. The line is drawn with a slope of 1.

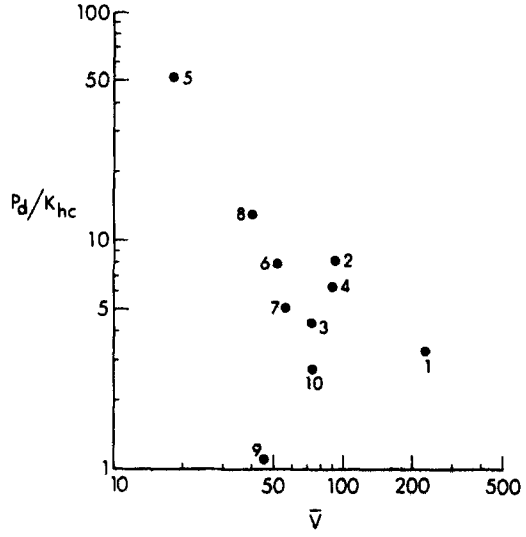

FIGURE 2. P_d/K_{hc} vs. $\bar{V}$. P_d is in cm/s, K_{hc} is dimensionless, and $\bar{V}$ is in cm^3/mol.

the molecules listed in Table I vary by a factor of 10^4, whereas the D's vary by only a factor of 4.

The overwhelming importance of the partition coefficients in determining the relative magnitudes of the permeability coefficients can be lost sight of in pursuit of the dependence of intramembranous diffusion constants on molecular size. It is possible, for instance, to employ multivariate linear regression analysis on permeability data such as ours to determine both an appropriate model solvent for the membrane and also the dependence of diffusion constants within the membrane on molecular size (see Lieb and Stein, 1971). One plots log P_d/K vs. log $\bar{V}$ for several solvents,[2] chooses the solvent that fits the best straight line to the points, and takes the slope of the line as s in the relation:

$$D_m \alpha (\bar{V})^s, \tag{2}$$

where D_m is the diffusion constant of a solute within the membrane.[3] (Relation 2 expresses the dependence of intramembranous diffusion constants on molecular volume.) We feel, however, that handling the data in this way obscures the usefulness and simplicity of Relation 1. This is apparent from a comparison of Figs. 1 and 2.

The issue of the dependence of diffusion constants within bilayers on molecular size initially arose from an attempt to attribute molecular sieving by plasma membranes to the intrinsic properties of the lipid bilayer (Lieb and Stein, 1971). It has since become clear, however, that lipid bilayer membranes do not have sieving properties (Gallucci et al., 1971; Finkelstein, 1976) and that separate, polar pathways (pores) must be invoked instead. No physiologist, for example, would mistakenly conclude from the data in Table I or its plot in Fig. 1 that a lecithin bilayer displayed molecular sieving. Attempts to establish the functional dependence of diffusion constants within bilayers on molecular size are presently motivated by the desire to understand the nature of the diffusion process within the bilayer interior. Although this is a legitimate physicochemical inquiry, we feel that excessive emphasis on theoretically calculated diffusion constants (D_m's) has diverted attention from the experi-

[2] K is the partition coefficient of a solute, in some appropriate model solvent, and $\bar{V}$ is its molecular volume. $\bar{V}$ = molecular weight/density and to a first approximation is directly proportional to molecular weight.

[3] Such an analysis has been carried out for planar lecithin bilayer membranes (Wolosin et al., 1978), but its validity is questionable. The analysis relies heavily on the previous results reported by Wolosin and Ginsburg (1975) for the permeability of organic acids through these membranes. We suspect that the method employed there, involving the titration of the acid that crosses the membrane, does not permit proper corrections for unstirred layers, and hence the data reported from that study are not relevant. This suspicion stems from their use of unbuffered solutions, which allows the build up of significant concentration gradients in the unstirred layers. Whatever the source of the problem, their results deviate markedly from those obtained by us and one other laboratory, as follows: our own determination of P_d (butyric acid) is 6.4×10^{-2} cm/s, a value 60-fold larger than theirs, and J. Gutknecht's (personal communication) determination of P_d (acetic acid) is 5×10^{-3} cm/s, a value 21-fold larger than theirs (see also Gutknecht et al. [1977]).

mentally measurable and physiologically relevant permeability coefficients (P_d's).

Absolute Values of Permeability Coefficients

$(DK_{hc}/\Delta x)$ is the predicted value of P_d for a solute in a hydrocarbon membrane of Δx thickness. Since $P_d\ (DK_{hc}/\Delta x)^{-1}$ is approximately 0.3 in egg lecithin membranes formed from lecithin dissolved in decane, the hydrophobic interior of the bilayer is apparently similar to bulk liquid hydrocarbon. We wish to emphasize, however, that we do not stress the absolute values for P_d. The significant point is that $P_d\ (DK_{hc}/\Delta x)^{-1}$ in a membrane of given composition is relatively constant for small nonelectrolytes (Finkelstein, 1976). It is in this sense that the interior of the bilayer is like liquid hydrocarbon. It is possible that the close agreement of the predicted and experimental values partially results from significant amounts of decane retained in the bilayer (Fettiplace et al., 1971), for the values of P_d are somewhat reduced in membranes formed from lecithin dissolved in hexadecane (see Results), which presumably retain much less hydrocarbon solvent (Fettiplace et al., 1971). If the smaller Δx for these membranes is considered (30 Å instead of 50 Å [Fettiplace et al., 1971]), then $P_d\ (DK_{hc}/\Delta x)^{-1}$ becomes approximately 0.15 and 0.075 for butanediol and codeine, respectively; that is, about one half to one fourth the value for membranes formed with decane as the solvent.

We do not even stress hydrocarbon over other nonpolar solvents in predicting the relative permeabilities of nonelectrolytes. An almost equally good fit of the data is obtained if either $K_{\text{olive oil}}$ or K_{ether} is substituted for K_{hc} in Fig. 1. Partition coefficients in olive oil are about 40-fold larger than those in hydrocarbon, and partition coefficients in ethyl ether are about 200-fold larger than those in hydrocarbon (Table II).

Our emphasis on relative values for P_d was made earlier in considering $P_d\ (DK_{hc}/\Delta x)^{-1}$ for lecithin membranes, lecithin:cholesterol membranes, and sphingomyelin:cholesterol membranes (Finkelstein, 1976). (The latter two, incidentally, should be almost free of hydrocarbon solvent [Redwood and Haydon, 1969].) The cholesterol-containing membranes are "tighter" than membranes formed from lecithin alone; that is, the value of $P_d\ (DK_{hc}/\Delta x)^{-1}$ is smaller—about 0.03 in lecithin:cholesterol membranes and 0.004 in sphingomyelin:cholesterol membranes, as compared to 0.3 in lecithin membranes. Some discrimination among molecules based on size and shape does exist in these "tighter" membranes. For example, P_d (*n*-butyramide)/P_d (isobutyramide) is 1.5 in lecithin: cholesterol membranes and 2.5 in sphingomyelin: cholesterol membranes (Finkelstein, 1976). This effect is more pronounced for a bulky molecule such as codeine. Thus, we find that in lecithin:cholesterol membranes, $P_d\ (DK_{hc}/\Delta x)^{-1}$ is a factor of 3 smaller for codeine than for 1,4 butanediol, and in sphingomyelin:cholesterol membranes it is a factor of 7 smaller. In these more tightly packed cholesterol-containing membranes, the microenvironment seen by the small, straight chain 1,4 butanediol molecule is somewhat different from that seen by the large, bulky codeine molecule. It is not clear, however, whether this difference in microenvironment predomi-

nately effects the diffusion constants within the bilayer or the partition coefficients into it.

Some Remarks on Partition Coefficients of Polyhydroxyl Molecules into Hydrocarbon

An objection that has been raised against the choice of hydrocarbon as a model solvent for the bilayer is that the ratios of permeability coefficients for polyhydroxyl compounds are not consistent with the values expected from the predicted partition coefficients for these molecules into hydrocarbon (Wolosin et al., 1978). Our determinations of both the partition coefficients and permeability coefficients for several polyhydroxyl compounds, however, show no such inconsistency; rather, they demonstrate that the actual values of the partition coefficients of polyhydroxyl compounds into hydrocarbon can be quite different from those *predicted.*

K_{hc} for 1,6 hexanediol (Finkelstein, 1976) is less than K_{hc} for 1-pentanol (Aveyard and Mitchell, 1969) by about a factor of 700. It is not possible to

TABLE II
COMPARISON OF PARTITION COEFFICIENTS IN HEXADECANE, OLIVE OIL, AND ETHYL ETHER

Molecule	$10^6 K_{hc}$	$10^6 K_{olive\ oil}$	$10^6 K_{ether}$	K_{hc}:$K_{olive\ oil}$:K_{ether}
1,2-Propanediol	64	1,700	18,000	1:26:280
1,4-Butanediol	43	2,100	19,000	1:48:440
H_2O	42	700	18,000	1:17:430
Acetamide	21	830	2,500	1:40:120
1,2-Ethanediol	17.2	490	5,300	1:28:310
Formamide	7.9	760	1,400	1:96:180
Urea	3.5	150	470	1:43:130
Glycerol	2.0	70	660	1:35:330

$K_{olive\ oil}$ and K_{ether} are from Collander (1954).

generalize from this, however, that the addition of a $-CH_2OH$ terminal group always has such a large effect. Thus, K_{hc} (glycerol) is smaller than K_{hc} (ethanediol) by only a factor of 8.6 (Table I). The smaller effect on K_{hc} of the $-CH_2OH$ group in this instance may be attributed to two causes. First, the analogy between the two sets of molecules is not accurate. Thus, the transition from pentanol to hexanediol includes both the addition of a $-CH_2OH$ group and the substitution of a $-CH_2$ for a $-CH_3$ group, whereas the transition from ethanediol to glycerol involves the insertion of a $-CHOH$-group between two $-CH_2OH$ groups. Second, there are possibilities for internal hydrogen bonding in glycerol that will tend to make it more hydrophobic than expected for three independent hydroxyl groups. A proper quantitative assessment of these factors is best left to the physical chemist. But whatever the explanation for the ratio of the partition coefficients, P_d (ethanediol)/P_d(glycerol), which is 16.3,[4] is in reasonable agreement with K_{hc} (ethanediol)/K_{hc} (glycerol), which is 8.6, and is therefore consistent with hydrocarbon being a model solvent for the bilayer.

[4] From Gallucci et al. (1971), P_d (ethanediol)/P_d(glycerol) = 3.2; using their revised value of P_d (ethanediol) (Lippe, C. Personal communication), this ratio becomes 6.6, as compared to our ratio of 16.3.

Similarly, although K_{hc} for 1,6 hexanediol (Finkelstein, 1976) is less than K_{hc} for 1-hexanol (Aveyard and Mitchell, 1969) by a factor of 2,000, one cannot generalize from this that an −OH group always has such a profound effect. In particular, K_{hc} (glycerol) is smaller than K_{hc} (1,2 propanediol) by only a factor of 32 (Table I). (Again, we leave the explanation of this to the physical chemist.) Thus, P_d (propanediol)/P_d (glycerol), which is 52,[5] is approximately equal to K_{hc} (propanediol)/K_{hc} (glycerol), which is 32. It would be instructive to compare the permeability of *n*-butanol with that of 1,4 butanediol, where the ratio of the hydrocarbon:water partition coefficients is about 2,000. Unfortunately, the value of P_d for butanol is unstirred layer limited, so that only a lower limit of P_d (butanol)/P_d (1,4 butanediol) can be obtained. We note, however, that in sphingomyelin:cholesterol membranes, this ratio is *at least* 200 (Finkelstein, 1976).

In summary, the effects of $-CH_2OH$ and −OH groups on bilayer permeability coefficients are consistent with their effects on hydrocarbon:water partition coefficients, although the latter are difficult to predict a priori.

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[5] Our value for P_d (propanediol) is a factor of 70 larger than that obtained by Gallucci et al. (1971); we have no explanation for this discrepancy, as our values for P_d's of glycerol, ethanediol, acetamide, and formamide are in reasonable agreement with theirs.

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