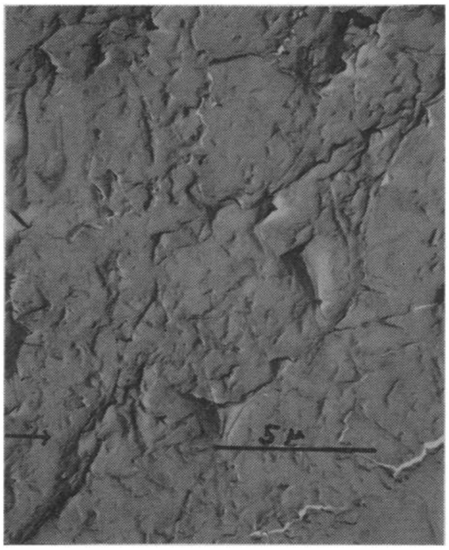

Fig. 2. Quartz-sand grain from core V15-107 taken approximately 110 miles due east of the eastern mouth of the Strait of Magellan in the South Atlantic Ocean; 65 cm from the top of the core. V-shaped mechanical breakage features characteristic of surf action are superimposed on glacial breakage patterns that have been somewhat worn; the latter were probably subjected to mechanical wear before burial. Arrow indicates the shadowing direction.

dence period of the order of 10^6 years might be expected to raise the pH of the solution in which both sand grains and foraminifers were immersed and to promote the etching and removal of silica. That chemical action does occur is evident when sand grains sampled from ancient sediments are examined; in almost every instance there is evidence of features that are not mechanical in origin. This is usually not true of Holocene marine sands.

Fig. 3. Grain of quartz sand from core V16-66 at the 810-cm level. Large-scale, somewhat irregular breakage blocks along with imbricate breakage-patterns characteristic of glacial action are seen; some rounding has taken place, probably resulting from postdepositional action. Arrow indicates the shadowing direction.

Two periods of mechanical breakage on the surface of a single grain of sand have been observed on samples from various parts of the world (*10*). Grains taken from cores from off the eastern coast of South America, near the Falkland Islands, show two breakage sequences, one superimposed on the other (Fig. 2). The initial pattern is glacial and is followed by surf action, suggesting that the grains were first subjected to glacial grinding, were then rolled about in the surf, and were finally transported into deeper water. Pleistocene sands from many other areas show the same sequence. Sand grains from the 270-cm level in core V16-66 show mostly glacial features, but several portions of three grains examined show weakly indented mechanical beach patterns suggesting beach action after glacial grinding. Nichols has shown that beaches developed directly on Antarctic ice (*11*) and that beach processes have been active. However, the beach patterns there are not as common as those found on beaches at lower latitudes; this is to be expected, because beaches in Antarctica are for various reasons not well developed (*11*). Sand grains from the 810-cm level of core V16-66 contain glacial but not beach textures (Fig. 3). Generally, the grains of quartz sand from core V16-66 do not include a beach phase, suggesting that they were carried out to sea by icebergs launched from glaciers of continental proportions.

We believe that examination of sand grains in deep-sea cores from most of the oceans could lead to identification of the point at which glaciers of continental proportions began to launch debris-carrying icebergs into the world's oceans. It seems to us that a good demarcation for the beginning of the Pleistocene would be such a point, because equating any currently known faunal or floral boundary (or both) with the onset of continental glaciation may not be possible. Unfortunately, very few cores representing the approximate Plio-Pleistocene boundary exist at present, but when longer deep-sea cores are taken in the North Atlantic, North Pacific, Arctic, and southern oceans a rock-stratigraphic and geologic-climate boundary (*12*) will probably be delineated for the beginning of the Pleistocene.

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19 May 1965

Optical Activity in the Orgueil Meteorite

Abstract. *Nagy's report of an optical rotation of −0.023° to −0.084° in the Orgueil meteorite has not been confirmed. The highest rotation found in several fatty-acid and hydrocarbon fractions isolated from the meteorite was −0.002°, less than that of optically inactive controls. The "rotations" observed by Nagy may have been caused by scattered depolarized light from colloidal particles, reduced instrument sensitivity due to low transmittance of the solutions, or a combination of both.*

Nagy *et al.* (*1*) have reported the discovery of optical activity in the Orgueil carbonaceous chondrite. They observed a rotation of −0.023° ± 0.005° in an extract consisting mainly of organic acids and hydrocarbons, and concluded: "It seems reasonable to

Table 1. Infrared and ultraviolet absorption maxima of Orgueil meteorite extracts.

Ext.	Spectra				
	Infrared (μ)				UV (mμ)
1,2*	3.3	3.7†	5.85	10.7	220 262 277
A3†	3.34	3.69	5.85	10.69	218
B†	3.34	3.68	5.84	10.68‡	218–220
MA3†	5.75	8.01	8.34	8.56	
MB†	5.77	8.01§	8.39	8.57‖	
A2†	6.11–6.34 (weak, broad)				None between 220–700

* Nagy *et al.* (*1*). Infrared spectra measured in CCl_4. † This work. Infrared spectra measured in CS_2, except A2, which was measured in $CHCl_3$. All ultraviolet spectra measured in CH_3OH. ‡ Weak shoulder at 5.89 μ. § Broad peak extending to 8.1 μ. ‖ Weak shoulder at 5.81 μ.

connect optical rotation in Orgueil with biological activity, either of indigenous origin or perhaps still related to terrestrial contaminations."

In view of the far-reaching implications of this result, I decided to verify the findings of Nagy *et al.*

Through the courtesy of A. Cavaille, I obtained a freshly broken interior sample of a 682-g stone of the Orgueil meteorite from the Natural History Museum, Montauban, France.

To lessen the danger of mutual cancellation of optical activity, and to avoid interference by sulfur, which had been a problem in the work of Nagy *et al.*, I modified their procedure slightly, as follows (*2*): (i) Three separate 6-hour extractions with benzene, chloroform, and methanol, respectively, were substituted for a single 6-hour extraction with a benzene-methanol mixture. (ii) Sulfur and benzene were removed before saponification. (iii) The final sample was split into an acidic and a neutral fraction, which were investigated separately.

Sample A was prepared as follows. A portion (20 g) of finely powdered meteorite was extracted continuously for 6 hours (Soxhlet apparatus), first with benzene, then with chloroform, and then with methanol, 100-ml quantities of solvent being used each time. The three extracts were combined, and, after evaporation of the solvents under reduced pressure, the yellow residue was completely dissolved in boiling benzene, ether, and methanol (2 : 2 : 1), which was then kept at −9°C for 24 hours. Much of the sulfur precipitated on standing. It was removed by filtration and washed with cold ether and *n*-hexane. [To rule out the remote possibility that a small amount of an optically active compound was lost with the sulfur, I dissolved the residue in benzene and passed the solution through a column containing colloidal copper. Evaporation of the now sulfur-free solution gave a minute residue of 0.26 mg (sample A1).] After evaporation of the solvent, the pale-yellow residue was saponified by refluxing it for 3 hours with methanolic alkali (1 g KOH in 12 ml methanol and 3 ml water). The solution was poured into 60 ml of water, with constant stirring, and extracted repeatedly with ether to remove the unsaponifiable material, such as hydrocarbons and traces of sulfur.

The unsaponifiable fraction was purified according to the method of Blumer (*3*) to remove the sulfur. It yielded 24.45 mg of an oily solid (sample A2). The aqueous solution was acidified with 10 percent HCl and extracted with 60 ml of ether in a liquid-liquid extractor for 12 hours. The ether solution was washed with water and dried, and the solvent was removed. The residue, sample A3, weighed 10.76 mg.

Sample B was prepared as follows. A portion (16 g) of finely powdered Orgueil meteorite which had already been extracted with water and a variety of organic solvents (*4*) was hydrolyzed with 10 percent HCl for 10 hours at 100°C. The hydrolyzate was filtered to remove the insoluble residue and extracted with ether five times. The insoluble residue was treated with ether for 3 hours at refluxing temperature, and the ether solution was filtered and combined with the ethereal solution obtained above. The combined solution was washed with water, dried, and evaporated under reduced pressure. The residue consisted of 9.63 mg of a pale-yellow oil (sample B).

To obtain another set of chemically well-defined samples, I treated samples A3 and B with diazomethane, thereby converting the fatty acids to their methyl esters (samples MA3 and MB). The ester samples, weighing 7.07 and 7.37 mg, were colorless. They were purified by chromatography on an alumina column.

The principal absorption maxima of samples A2, A3, B, and their methyl esters are listed in Table 1. The data of Nagy *et al.* are given for comparison. Clearly, there is excellent agreement between the infrared spectra of my samples A3 and B and Nagy's sample. All three, in turn, match the infrared spectra of long-chain, saturated carboxylic acids. This identification is confirmed by the spectra of the methyl esters, MA3 and MB. The strong triplet absorption at 8.01, 8.34, and 8.56 μ is a characteristic feature of the spectra of methyl esters of long-chain acids (*5*). The methyl ester MB seemed to consist largely of saturated acid esters, with a small admixture of nonconjugated, unsaturated acid esters (*6*). The presence of unsaturated esters in sample B (and their absence in sample A3) was confirmed by thin-layer chromatography (*7*).

Three compositional differences between my samples and those of Nagy *et al.* should be noted. First, Nagy *et al.* attached some significance to the absorption bands at 262 and 277 mμ which were present in their samples but not in recent biological material. These bands, which were absent from my samples, seem to have been due to elemental sulfur (*8*). Figure 1 shows a spectrum of pure sulfur along with spectra of Nagy's Orgueil extract and my samples A2 and A3. Most of the features of Nagy's sample, including the peaks at ≈262 and ≈277 mμ, also appear in the sulfur spectrum. The Orgueil meteorite contains between 1 and 2 percent free sulfur, and it seems that this element, rather than some hypothetical organic

Table 2. Optical rotation of Orgueil meteorite extracts and controls. Measurements were made on a Rudolph automatic spectropolarimeter with a sensitivity of 0.001°. Cell length, 1 dm; cell volume, 0.7 ml, except for control 1 (8 ml) and controls 2 and 3 (1.2 ml). +, observed "rotation" large, but irreproducible, owing to strong light absorption by sample.

Sample	Rotation (degrees)						
	Conc. (mg/ml)	560 mμ	500 mμ	440 mμ	400 mμ	360 mμ	340 mμ
A1	0.26	−0.001		±0.000	−0.001		
A2	12.65	−0.001	−0.001	−0.001	−0.004	−0.007	−0.020
A3	7.21	−0.001	−0.003	−0.002	−0.003	−0.006	−0.039
B	8.40	−0.002	−0.001	−0.002	−0.002	−0.008	
MA3	6.35	−0.001	+0.001	−0.001	−0.001	−0.003	−0.004
MB	6.62	−0.002		−0.002	−0.002		−0.004
Control 1	Saturated	−0.001	−0.001	−0.002	−0.005	+	
Control 2	10.02	−0.001	−0.004	−0.005	−0.009	+	+
Control 3	10.17	−0.002	−0.002	−0.009	−0.016	+	+

compound, was responsible for the extra absorptions seen by Nagy *et al.*

Second, yet another type of extraneous material seems to have been present in the samples of Nagy *et al.*, namely, organic sulfur compounds produced during saponification by reaction of benzene, methanol, and meteorite organics with free sulfur and KOH. A control experiment duplicating Nagy's saponification conditions showed that when 260 mg of sulfur was refluxed for 5 hours with 100 ml of a mixture of benzene and methanol (6 : 4) in the presence of 2.9 g of KOH and 1 ml of water, there was evolution of H_2S, with a residue of 93 mg of organic sulfur compounds and sulfur. A typical 22-g sample of Orgueil contains about 400 mg of S, and since Nagy's extract contained a number of meteorite organics in addition to benzene and methanol, an even greater variety of such "secondary" reaction products might be expected. This is an important point, to which I shall return later.

A third compositional difference concerns the disposition of hydrocarbons and other nonsaponifiable compounds, which comprised a substantial part of Nagy's sample. Owing to the lack of distinctive spectral features, they did not contribute significantly to his ultraviolet and infrared spectra, but were detected by thin-layer chromatography. In my procedure, these compounds appeared largely in sample A2, which was investigated separately. Together, my samples A1, A2, and A3 were equivalent to the samples of Nagy *et al.*

The reported optical rotation, though appreciably greater than the nominal sensitivity of the polarimeter, lies in a range where instrumental artifacts can become serious. To guard against such artifacts, three optically inactive controls were prepared. Control 1 was a saturated solution of sulfur in $CHCl_3$. Control 2 was essentially a blank for the procedure of Nagy *et al.*, namely, a chloroform solution of the mixture of sulfur and organic compounds prepared by refluxing 14.2 mg of optically inactive stearic acid and 23.6 mg of *n*-octadecane with S, KOH, benzene, and methanol as already described. The stearic acid and octadecane were added to simulate conditions in the meteorite experiments. Control 3 was a dark-yellow solution of impure acylated melamine.

To duplicate the conditions of Nagy *et al.* as closely as possible, my measurements were likewise performed on three different Rudolph polarimeters at three different institutions: a photoelectric polarimeter with oscillating polarizer at the University of Chicago; a recording spectropolarimeter at the University of Alberta; and an automatic spectropolarimeter at the Argonne National Laboratory. Only the results with the last-named instrument are cited (Table 2), but the other two sets of data were quite similar.

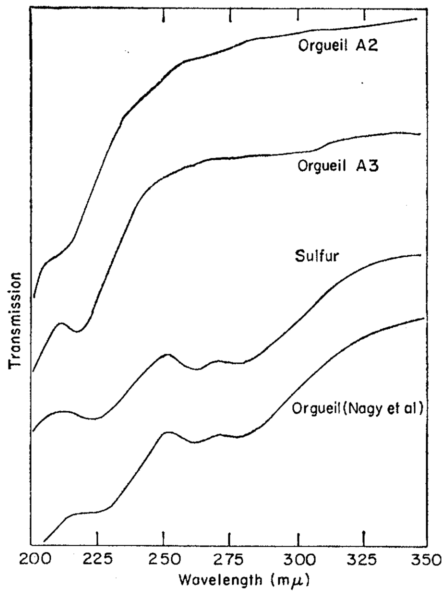

Fig. 1. Ultraviolet absorption spectra of Orgueil meteorite extracts. Unidentified features at 262 and 277 mμ in sample of Nagy *et al.* seem to be due to elemental sulfur, rather than to organic compounds.

None of the six samples investigated showed a significant optical rotation at 440 mμ. The highest result, −0.002°, was an order of magnitude lower than the rotation of −0.023° ± 0.005 and −0.020° ± 0.005 which Nagy *et al.* found in two samples of Orgueil, at 435 and 440 mμ, respectively. Moreover, two of my controls gave higher values than any of my meteorite samples: −0.005° and −0.009°.

Nagy *et al.* had noted that the optical rotation of their samples increased at lower wavelengths, but, owing to strong light absorption by sulfur and its secondary reaction products, they were unable to go below 435 mμ. Neither of these interfering substances was present in my samples, and I was therefore able to extend the measurements down to 340 mμ. As the transparency limit was approached, the samples began to show an increasing "rotation," but since the controls showed the same behavior, this "rotation" seems to have been an instrumental artifact arising under conditions of low light transmission. Rouy and Carroll (*9*) have drawn attention to this effect.

At 340 mμ the transparent sample MA3 gave only one-tenth the rotation of the rather strongly absorbing sample A3, although both were of similar composition and concentration (Table 2). The controls, all of which had appreciable absorption in the near ultraviolet, gave even larger readings at this wavelength, which, however, fluctuated badly. It would seem that strongly absorbing solutions are apt to give a false levorotation.

Evidently, my Orgueil samples do not show the conspicuous levorotation at 440 mμ reported by Nagy *et al.* Before accepting this discrepancy as significant, we must examine some of the more obvious sources of error. At least four possibilities come to mind.

1) *Chemical composition.* Conceivably, the optically active compounds were lost in my procedure. This is hardly possible. Both procedures were based on the same principles: solubility in benzene and methanol, saponifiability, solubility in ether. All material initially extracted from the meteorite was measured on the polarimeter. The infrared and ultraviolet spectra and the thin-layer chromatograms show that all classes of organic compounds identified by Nagy *et al.* were also present in my samples.

2) *Sample size.* Nagy *et al.* did not give the weight of their samples, owing to the presence of an indeterminate amount of sulfur. But there is good reason to believe that my samples were of comparable size. Nagy *et al.* used the extraction procedure of Nagy and Bitz (*10*) which had previously yielded 580 to 675 μg of carboxylic acid methyl esters per gram of Orgueil. This corresponds to (calculated) ester contents of 14.9, 7.8, and 7.5 mg for the three samples of Nagy *et al.* My ester samples initially were of similar size: 7.1 and 7.4 mg, although repeated transfers eventually reduced these amounts to 6.4 and 6.6 mg.

3) *Inhomogeneity of meteorite.* As far as its content of fatty acids and hydrocarbons is concerned, my 682-g stone seemed to be quite comparable to the three stones of Nagy *et al.* One might speculate that the optical activity resides in a trace constituent of very high specific rotation, which is very unevenly distributed throughout the meteorite. It will be shown below, however, that there are other, more probable causes for the discrepancy.

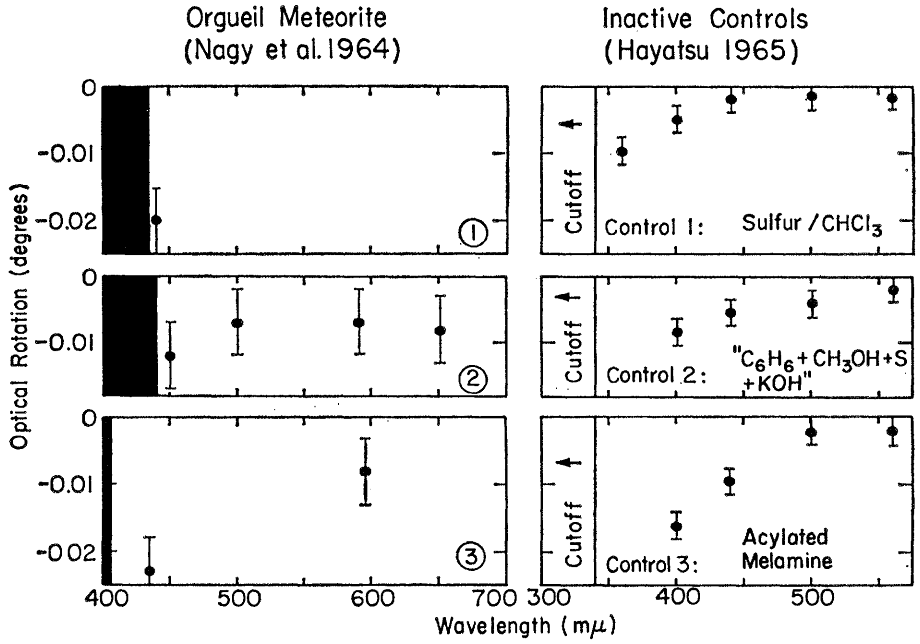

Fig. 2. Spurious optical "rotation" in strongly absorbing solutions. Optically inactive controls show an increasing false "rotation" as their absorption cutoff (lying well below 340 mμ) is approached. The Orgueil samples of Nagy *et al.* also show such an increase. Black area indicates region of "no transmittance," as reported by Nagy *et al.*

4) *Instrumental sensitivity.* The polarimeters used in this study had sensitivities of 0.001°. This corresponds to 4 percent of the rotation, −0.023° ± 0.005°, reported by Nagy *et al.* Apparently these instruments were sensitive enough to detect an optical rotation of the order of 0.02°. Of course, the overall sensitivity also depends on the cell length, cell volume, and concentration. Even though it is not possible, for lack of information, to determine the exact sensitivity of Nagy's measurements, there seems to be no indication that it was significantly higher than that of my study. Considerations of solubility and optical transmittancy alone preclude appreciably smaller volumes and higher concentrations.

Thus a serious discrepancy exists between the results of my study and those of Nagy *et al.* Several causes of this discrepancy might be considered. Nagy *et al.* concede that the levorotation detected by them might "perhaps still [be] related to terrestrial contamination." It seems more likely, however, that the apparent levorotation was an instrumental artifact. Rouy, Carroll, and Quigley (*11*) have pointed out that turbid or colloidal solutions do not obey Malus's law. Light scattered by solid particles becomes depolarized and thus succeeds in passing through the analyzer, where it produces a spurious "rotation." In a typical example cited by Rouy *et al.*, a solution scattering only 10 percent of the incident light gave a "rotation" ten times greater than the signal. This effect was confirmed experimentally. A slightly turbid solution of my sample B gave an apparent levorotation of as much as −0.08° which disappeared completely after the solution was filtered.

Indeed, turbidity seems to have been a problem in the work of Nagy *et al.* They report adding colloidal sulfur to some of their blanks, apparently to compensate for the colloidal sulfur content of their samples. They filtered two of their three samples through a 0.45-μ ultra-filter, but a filter of this pore size cannot remove colloidal particles.

A second source of error may arise from the light absorption of the samples. Rouy and Carroll (*9*) have shown that the sensitivity of the polarimeter is inversely proportional to the light transmittance of the sample, a transmittance of 10 percent corresponding to a tenfold reduction in sensitivity (Eq. 29 of ref. *9*).

It is perhaps very significant that the optical rotation found by Nagy *et al.* always appeared very close to the absorption cutoff (Fig. 2). For some reason or other, the instrument tends to give a "levorotation" under conditions of low light intensity and hence reduced sensitivity. Even my optically inactive controls gave an apparent "rotation" in regions of appreciable absorption. Control 2 (Fig. 2) is especially pertinent to this question. It consisted largely of organic sulfur compounds derived from the reagents in Nagy's saponification procedure. A 10-mg sample sufficed to give an apparent optical rotation of −0.0053° at 440 mμ, that is, one-fifth that reported by Nagy in Orgueil. But the total amount produced under conditions duplicating Nagy's procedure was 93 mg; more than enough to account for the observed rotation.

In an attempt to resolve this discrepancy, Nagy (private communication) has recently repeated my procedure on 10 g of Orgueil. Through a regrettable error in my instructions, the solution was not cooled to a low enough temperature to precipitate the sulfur, and his A3 fraction thus contained at least 89.5 mg of S, in addition to 5.0 mg of organic matter. The solution was opaque below 546 mμ. At 546 mμ, it gave a levorotation of −0.084° ± 0.005°. The original unsaponified extract was "equally opaque," but "was not optically active."

These findings are not inconsistent with the interpretation presented above. Both solutions were "opaque" below 546 mμ, which presumably implies a transmittance of 1 to 5 percent at 546 mμ. According to Eq. 29 of Rouy and Carroll (*9*) the sensitivity of the polarimeter is lowered by factors of 20 to 100 under these conditions, to 0.1° to 0.5°. It would seem that neither the levorotation of −0.084° nor the null result on the unsaponified extract have any significance. Moreover, since sulfur carried through Nagy's saponification procedure apparently gives a colloidal final sample solution (*1*), a false signal from scattered depolarized light might also have been expected (*11*) in the saponified, but not in the unsaponified, extract.

A few comments should be made on the quantitative significance of the negative results obtained in the present study. The highest apparent "rotation," −0.002° at 440 mμ, was on sample A3, corresponding to a specific rotation, $[\alpha]_{440}$ of $\leqq 0.3°$ ml $g^{-1}dm^{-1}$. Most optically active carboxylic acids have large specific rotations, the order of 10° ml $g^{-1}dm^{-1}$. But in a sample containing a mixture of acids, mutual cancellation of activity and dilution by inactive acids will depress this value greatly. Indeed, neither the fatty acids from brown algae nor the naphthenic acids from petroleum measured by Nagy *et al.* gave any detectable rotation in spite of their evident (or presumed) biological origin. Perhaps the most conclusive result to date has been obtained on the amino acids from Orgueil (*12*). Here, the mutual cancellation of activity was explicitly taken into account. If these amino acids were biogenic, and consisted of one optical isomer only, a rotation of 0.0046° would have been expected. The value actually obtained was $\leq 0.001°$.

It seems safe to conclude that the fatty acids and hydrocarbons in Orgueil do not have a detectable optical rotation. Had the measurements of Nagy *et al.* been carried out under conditions where instrumental artifacts can be excluded, one would be led to the inference that a trace constituent of very high specific rotation ($[\alpha]_{440} > 100$) is present in the meteorite; that this trace constituent does not show up on ultraviolet and infrared spectra and on thin-layer chromatograms; and that it was unaccountably lost in my experiments. However, since the levorotations found by Nagy were obtained under conditions where spurious rotations are theoretically predicted and experimentally observed, the case for optical activity in meteorites seems as yet unproven.

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2. All organic solvents were of spectroscopic grade; all other reagents were of analytical grade. Before use, the solvents were freshly distilled through a fractional distilling column. Although the solvents contained 0.0 to 0.5 mg of residue per 100 g solvent, there were no extraneous absorption bands in their infrared and ultraviolet spectra.
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27 May 1965

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Square Root Variations of Reciprocal Graphing of Enzyme Kinetic Data

Abstract. *A variation of the Lineweaver and Burk graph, in which the reciprocal of the square root of the initial reaction velocity is plotted as a function of the reciprocal of the substrate concentration, has been described in the literature and has subsequently been used as the basis of proposals of reaction mechanisms. The utility of this treatment of enzyme kinetic data is examined and has been found to be limited.*

There have been several reports in which enzyme kinetic data have been fitted to a straight line in a variation of the usual Lineweaver and Burk (*1*) graph. This consists of plotting the reciprocal of the square root of the measured initial velocity (v) as a function of the reciprocal of the substrate concentration (s) (*2*). New reaction mechanisms have been proposed on the basis of reasonable fitting of the data to straight lines in such graphs of reciprocal square roots of initial velocities. Before this practice becomes more prevalent it is desirable to examine the validity of this particular treatment of enzyme kinetic data.

On the basis of arbitrarily assumed values of the maximum velocity (V_m) and of the Michaelis constant (K_m) and using the Michaelis-Menten equation, it is possible to generate ideal kinetic data by direct calculation. This has been done, and the results of one such exercise are illustrated in Table 1. These data may now be treated by plotting on either reciprocal coordinates in the classical fashion (*1*) or by plotting the reciprocal of the square root of v (Fig. 1). Deviations from linearity in the graph of $1/v^{\frac{1}{2}}$ plotted against $1/s$ become apparent only at concentrations of substrate which are smaller than K_m. All points calculated from substrate concentrations equal to or greater than K_m fit a straight line equally well when $1/v$ is plotted against $1/s$ or when $1/v^{\frac{1}{2}}$ is plotted against $1/s$. This is made clear by Fig. 2 in which are plotted only those velocities from Table 1 which were calculated from substrate concentrations higher than K_m.

For practical reasons, actual measurements of initial velocities of enzymic reactions become increasingly difficult and imprecise at substrate concentrations which are significantly below K_m. Thus, the ability of actual kinetic data to fit a straight line when the coordinates are $1/v^{\frac{1}{2}}$ and $1/s$ is largely indicative of the insensitivity of this particular plot. This is the case unless special care has been taken to

Table 1. Idealized kinetic data, based on $V = (V_m S)/(K_m + S)$ and assumptions that $V_m = 100$ and $K_m = 1.00$.

$1/s$	$1/v$	$v^{\frac{1}{2}}$	$1/v^{\frac{1}{2}}$
4.0	0.050	4.47	0.223
2.0	.030	5.75	.174
1.33	.023	6.53	.153
1.00	.020	7.05	.141
0.667	.0167	7.73	.129
.500	.0149	8.15	.122
.400	.0140	8.43	.118
.333	.0133	8.64	.115
.250	.0125	8.93	.111
.167	.0116	9.23	.108
.125	.0112	9.41	.106

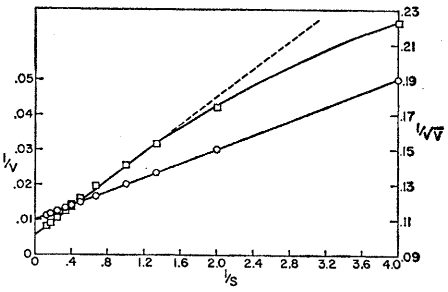

Fig. 1. Data from Table 1 plotted on standard reciprocal coordinates (circles) and on the $1/v^{\frac{1}{2}}$ variation thereof (squares).

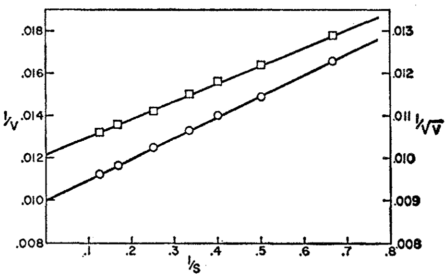

Fig. 2. Data from Table 1 at substrate concentrations greater than K_m plotted on standard reciprocal coordinates (circles) and on the $1/v^{\frac{1}{2}}$ variation thereof (squares).