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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13. I thank Professor Edward Anders for advice and discussion. I also thank Professor W. A. Ayer of the University of Alberta and Drs. L. Katzin and E. Gulyas of the Argonne National Laboratory for helpful discussions, and for permission to use their instrumental facilities for some of the optical rotation measurements. Supported in part by the NASA research grant NsG-366.

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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

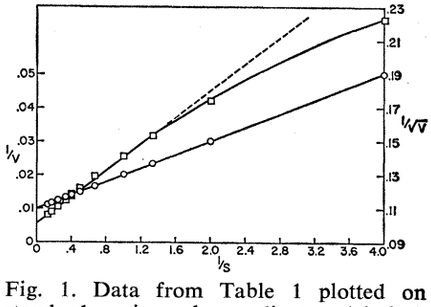

Fig. 1. Data from Table 1 plotted on standard reciprocal coordinates (circles) and on the $1/v^{\frac{1}{2}}$ variation thereof (squares).

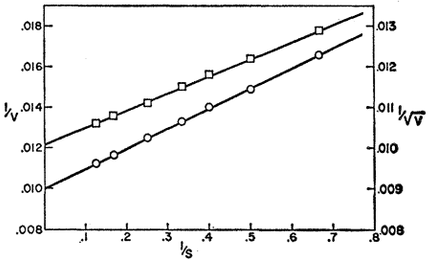

Fig. 2. Data from Table 1 at substrate concentrations greater than K_m plotted on standard reciprocal coordinates (circles) and on the $1/v^{\frac{1}{2}}$ variation thereof (squares).

obtain precise rate measurements at substrate concentrations between $K_m/10$ and $K_m/1$.

This criterion has not been met in reports now appearing in the literature.

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Helium-Glow Photometer for Picomole Analysis of Alkali Metals

Abstract. *The low-power electric-glow discharge in helium can excite effectively the characteristic emission lines of sodium and potassium. The helium-glow photometer used to generate the glow and measure the light is a relatively simple apparatus; it can be used to analyze samples containing 10^{-14} mole or more of sodium and potassium. Overall precision of the apparatus and method is 5 percent or better.*

Improved analytic techniques have permitted more thorough study of the function and performance of subunits of biological systems. For example, micropuncture techniques have permitted renal physiologists to study certain details of the process of urine formation; samples of nanoliter size obtained from renal tubules can be analyzed for their constituents. The total sodium and potassium in such samples is generally less than 10^{-10} mole, and special techniques are required to determine such quantities (*1*).

Operation of the helium-glow photometer (*2*) is based on the fact that electrical excitation of helium generates very energetic, metastable helium atoms that can transfer their energy to impurity atoms and excite them. Sodium or potassium atoms present in the glow will emit their characteristic radiation.

The physical layout of the photometer is sketched in Fig. 1. A pipette of the type described by Prager *et al.* (*3*), with which portions of a sample are placed on a wire electrode, is mounted on the 30× binocular microscope. The pipette mount provides three degrees of translational freedom so that the tip of the pipette can be positioned in the center of the field of the microscope; microscope and pipette move together during the procedure of placing the sample. Samples are held in an oil-filled trough mounted on a rack-and-pinion translating mechanism. The tip of the transfer pipette can be observed through a cover slip window on the side of the trough as the tip is lowered through the oil into a sample drop. The 10-watt radio-frequency (rf) supply is contained within the box that supports the discharge chamber, sample-trough support, and the two photomultiplier housings. A single-envelope twin tetrode is wired as an oscillator tuned to 27.12 Mhz. The output of the oscillator is applied between the upper ring electrode of Teflon-insulated wire and the lower electrode on which sample portions are placed. The lower electrode is an inverted "V" made of iridium wire 0.2 mm in diameter (*4*) and held in clips mounted through a Teflon disc on the oscillator enclosure (Fig. 2). The apex of the "V" is flattened with a fine abrasive stone to facilitate placement of portions of the sample. Line-frequency heating current from an adjustable supply is applied to the lower electrode to heat it and to volatilize the sample into the glow region.

Table 1. Compositions and experimentally determined sodium concentrations of nine artificial renal-tubule fluids. All fluids also contained the following concentrations: $(NH_2)_2CO$, 25 mg/100 ml; $C_6H_{12}O_6$, 50 mg/100 ml; KCl, 5m*M*; $NaHCO_3$, 25 m*M*.

$CaCl_2$ (mg/100ml)	$MgSO_4$ (m*M*)	NaH_2PO_4 (m*M*)	NaCl (m*M*)	Total (meq/lit.)	Na found (meq/lit.)
8	1	1	114	140	144
8	1	1	100	126	127.5
8	1	1	120	146	146.5
8	1	1	109	135	133.5
8	1	1	124	150	151.3
8	1	1	100	126	130.1
8	1	0	125	150	153
0	1	1	125	151	150.2
8	0	1	109	135	135

Table 2. Analyses of five nitric acid extracts of kidney tissue; comparison of results (meq/liter) by regular flame photometry (FP) and helium-glow photometry (HGP).

FP	HGP	Difference (%)
	Sodium	
287	288	+0.35
279	267	−4.30
285	286	+0.35
269	283	+5.21
	270	+0.37
	279	+3.71
264	272	+3.03
	286	+8.34
Mean difference (%) s.d.		+2.12 ± 3.82
	Potassium	
259	276	+6.56
260	244	−6.15
262	262	0.00
220	237	+7.73
	230	4.35
	232	5.55
225	219	−2.66
	218	−3.11
Mean difference (%) s.d.		1.62 ± 5.20

Helium flowing at a few cubic centimeters per second enters through an annular opening at the base of the chamber and flows out through a tube at the top. The effluent gas passes through a rotameter flowmeter and to the atmosphere through an oil-filled bubble trap that reduces the back diffusion of atmospheric gases. The chamber is sealed with an O-ring to the Teflon disc supporting the filament; the opening through which the transfer pipette is passed is sealed during use with another O-ring and a spring-loaded coverplate. The window at the front of the chamber is used to observe the delivery of a sample portion to the iridium wire; the window at the side of the chamber passes light from the discharge zone to the photometric system. These two windows are made of 25-mm diameter cover slips and are sealed to the chamber with a silicone rubber (*5*). A third window, made of Vycor, permits light from a 4-watt ultraviolet lamp (*6*) to fall on the iridium wire; the light insures that the glow starts uniformly.

A lens of short focal length collimates a portion of the light from the region of the discharge near the apex of the iridium "V"; the light passes to a dichroic mirror (*7*) that selectively reflects most of the yellow sodium light and transmits most of the near-infrared potassium light. Multilayer, all-dielectric interference filters (*8*) transmit the desired light to secondary lenses that spread the light over the photocathodes of the photo-