ing 80-percent ethanol within the outer member of a homogenizer, ground with the inner member, and refluxed gently for 10 min. In some cases the roots were frozen and ground in liquid nitrogen prior to extraction, resulting in identical chromatograms. Following concentration in small glass cones, the extracts were transferred to Whatman No. 1 sheets for chromatography. Two-dimensional, ascending chromatograms were made, first using 80-percent phenol and then butyric acid, butanol, and water (2 : 2 : 1) as solvents.

In general, the *complete* coincidence of a spot eluted from the radioactive chromatogram upon two-dimensional chromatography with the pure compound was considered sufficient identification (*3*). However, additional proof was obtained for glyoxylic and glycolic acid by derivation. Carrier glyoxylate was added to the radioactive compound and the phenylhydrazone was prepared. The specific activities remained unchanged through three recrystallizations. Similarly, the specific activity of the *p*-nitrobenzoylate of glycolic acid remained unchanged.

A typical radiogram is shown in Fig. 1. As with barley, by far the most prominent spot was malic acid. Activity was also found in alanine, glutamine, glutamic, aspartic, citric, succinic, isocitric, glyoxylic, and glycolic acids. The presence of the last two compounds is somewhat unusual and generally not included in fixation or exchange reactions accompanying the tricarboxylic acid cycle. In contrast with Poel's results, no spot corresponding to tyrosine was found in any experiments with the onion or soybean, or in a single experiment with barley. There seems, indeed, to be no valid reason, at our present state of knowledge, to expect this compound to possess any appreciable activity. As was reported previously (*1*), the pattern of fixation was not altered by a number of drugs, even when respiration was strongly inhibited. Glyoxylic acid has been found in cell-free bacterial preparations (*4*), and a major role in metabolism has been postulated for this compound. The possibility that glycolic and glyoxylic acids are degradation products of our extraction and/or chromatographic techniques cannot be ignored, but they would have to result from compounds other than those found on our chromatograms.

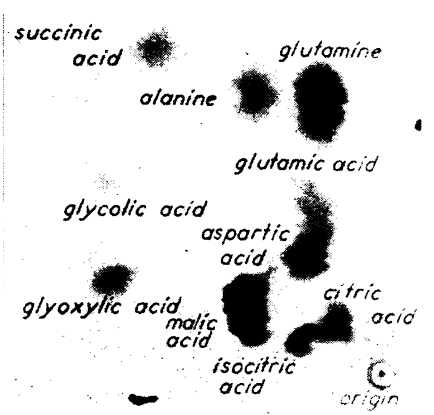

Fig. 1. A typical radiogram.

Poel's finding of the diminution of radioactivity in nutrient solution, as compared with distilled and tap water, is difficult to interpret. We have found that, on immersing soybean roots in increasing concentrations of KCl (0, $2.00 \times 10^{-4}M$, $2.00 \times 10^{-3}M$, and $2.00 \times 10^{-2}M$), there was an increase in radioactivity (1730, 2050, 3010, and 4610, respectively) (*5*). The stimulation of root respiration by increasing salt concentration is a well-known phenomenon. This increased rate of production of (nonradioactive) carbon dioxide is, of course, accompanied by an increased rate of production of organic acids partly capable of exchange and fixation. These effects will oppose each other in terms of the final radioactivity found—that is, whether increased respiration results in increased radioactivity will depend in large measure on the rate of enzymatic exchange and fixation as compared with the dilution of the specific activity of external carbon dioxide by the additional carbon dioxide produced within the roots. If the latter far exceeds the former, then it is conceivable that increased respiration will result in diminished activity. Other factors, however, such as the absolute increase in external carbon dioxide tension (which is reflected, then, in the ratio of root to solution volume), will have to be considered.

References and Notes

1. S. Aronoff and G. E. Graf, *Nature* **172**, 1043 (1953).
2. L. W. Poel, *Nature* **169**, 501 (1952); *J. Exptl. Botany* **4**, 157 (1953).
3. We are indebted to C. A. Hargreaves for the gift of isocitric lactone.
4. J. J. R. Campbell, R. A. Smith, and B. A. Eagles, *Biochim. et Biophys. Acta* **11**, 594 (1953); R. A. Smith and I. C. Gunsalus, *J. Am. Chem. Soc.* **76**, 5002 (1954).
5. We acknowledge with gratitude the assistance of A. Kuksis in this series of experiments.

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Rapid Method for Determining Mean Values and Areas Graphically

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The observed data in many fields of scientific investigation are recorded on continuous graph paper, charted either manually or by various motor-driven devices. It is frequently necessary to determine the mean value of the recorded quantity from such a chart, or to estimate areas bounded by such graphs. A simple method (*1*) was devised for accomplishing either of these ends. This method requires only a pencil and ruler (or straightedge) and can be completed by means of a single broken line without lifting the pencil from the paper. The technique was originally

developed for the analysis of lengthy temperature records, and has since been applied to electroradiograms, ballistocardiograms, electrophoresis patterns, tonograms, and a variety of data recorded at timed intervals. One of the chief advantages of this method is that mean values can be obtained directly on the original graph, without transcribing the numerical values or performing any arithmetical calculations.

Method. As an example, let us take eight values recorded on ordinary rectangular graph paper at points *A, B, C . . . H* (Fig. 1). The technique will be seen to be the same no matter how small or how large the number of points. It is assumed that the horizontal spacing or "timing" between the points is uniform.

Place a ruler so that it passes through points *A* and *B*. Start with the pencil at *A* and draw *Ab,* stopping on the vertical line midway between *A* and *B*. Next, with the pencil held at *b,* turn the ruler so that it passes through *b* and *C*. Draw *bc* along *bC,* stopping on the vertical line through *B*. Next, with the pencil held at *c,* turn the ruler so that it joins *c* and *D,* and draw *cd* as shown. Continue in this manner, each time directing the straightedge toward the next in the series of points and advancing the pencil to the right by half the space between the vertical lines through the original points, until arriving at the final point *h*. The height of *h* above the horizontal axis, measured according to the vertical scale used for the graph, provides the desired mean value.

Although the simplicity of this method renders it relatively free from error, a brief check is readily available and should be performed. Start at *H,* and along *HG* draw *Hg′* (Fig. 1), then *g′f′*, and so on until *a′* is reached. The point *a′* will coincide with *h* if no errors have been made.

Proof. By employing a few properties of centroids (centers of gravity), a nonalgebraic proof can be provided. Assume that a mass of 1 unit is placed at each of the points *A, B, C . . . H*. Then the centroid of all of these points will have a height above the horizontal axis equal to the arithmetic mean value of the height of the individual points. In arriving at the centroid geometrically, points *A* and *B* with mass 1 unit each may be replaced by a mass of 2 units placed at *b*. This may be represented by the symbol b_2. The centroid of b_2 and C_1 is situated at *c*, along the segment *bC*, dividing the length *bC* in proportion to the masses, and nearer to the "heavier" point. Similarly c_3 and D_1 may be replaced by d_4, and so on until h_8 represents the combined mass at the centroid.

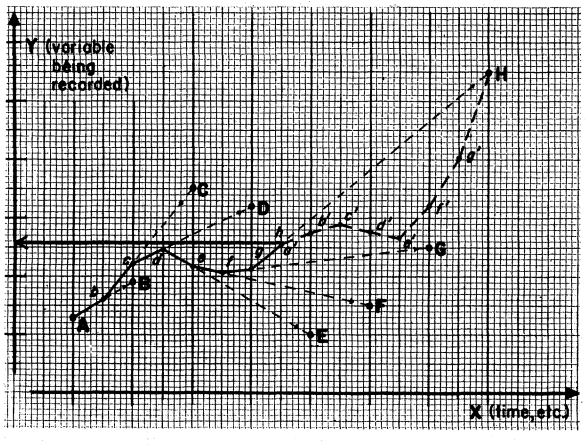

Fig. 1. Graphical determination of mean values.

Comments. The procedure described may be applied to a record consisting of any number of discrete points. In the case of a continuous graph, it is necessary to mark off, along the graph, points equally spaced horizontally, and to apply the method to these selected points. The accuracy of the final result will in general increase as the subdivisions are made finer.

In order to estimate areas under curved graphs (that is, the area between a portion of the curve, two vertical lines and the corresponding portion of the horizontal axis), it is necessary merely to multiply mean value, as derived above, by the length of the horizontal extent.

A review of the relevant literature on graphical methods revealed only a single reference (*2*) to a method at all comparable. However, the present method proved to be considerably easier and more rapid to apply.

References and Notes

1. From the Ophthalmology Research Laboratory (I. H. Leopold, director), Albert Einstein Medical Center, Northern Division. This work was made possible by a grant from the Weinstock Fund.
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Inhibition of Root Growth by Azaserine

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The tumor-inhibitory substance, azaserine, produced by an unidentified *Streptomyces,* has been shown to possess antibiotic activity on a wide range of organisms (*1*). This substance, identified as *O*-diazoacetyl-L-serine (*2*), completely inhibited a wide range of organisms at concentrations of 25 to >100 μg/ml. Some organisms are more sensitive; 50-percent inhibition of several bacilli was obtained at 2 to 4 μg/ml; *Escherichia coli* was inhibited 50 percent by 9.3 μg/ml; two clostridia, *Cl. feseri* and *Cl. hemolyticum* were completely inhibited at 0.5 μg/ml, and *Cl. perfringens* and *Cl. septicum* at 2.5 μg/ml (*3*).

Several experiments have been carried out with azaserine (*4*) on plant systems. They demonstrate the high potency of this substance in inhibiting root growth (*5*). One criterion was the effect on the elongation of the primary roots of germinating cucumber seed, var. Early Fortune, at 25°C for 96 hr (*6*). At $2\times10^{-4}M$ and $5\times10^{-4}M$ root development was com-