ANALOG INTEGRATION TECHNIQUES IN CHROMATOGRAPHIC ANALYSIS

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INTRODUCTION

The need is often encountered in instrumental analysis for recording the integral of the instrument output signal. Especially frequent use has been made of this procedure in recent years in gas chromatography. In another category of analytical techniques, including paper chromatography, it is often desirable to integrate the density of a developed pattern on paper, film or the like. In either case, the signal or pattern to be integrated comprises a series of more or less discrete "events", such as peaks or spots, and it is of interest mainly to know the integral only within the time (or position) intervals defining these separate events. In practice, the integral value has been either (a) continuously recorded, with provision made for correlating the value with the beginning and end of each event, or (b) recorded only at the instant of beginning or end (or both) of each event by use of suitable means for sensing these occurrences.

The method most widely used has been to mark the abscissa axis of the record continuously with a series of pips at a frequency proportional to the signal, the pip count being thereby a measure of the integral between selected abscissa values. The counting of such pips is, however, often laborious, and subject to error. Further, it is difficult to select a pipping rate that will both give sufficient precision on small peaks and avoid the merging of pips on large peaks. In another type of apparatus*, the integrator drives a print-out numerical counter which records the integral value automatically at the start of each new peak (and, by manual triggering, at the end of last peak). A difficulty here is that the trigger circuit must distinguish the occurrence of new peaks from base line noise or zero drift.

The present paper discusses several related analog methods that eliminate both pip counting and the need for any trigger circuitry. Indication of the beginning and end of "events" is automatic and inherent in the techniques. One of these methods is particularly simple and especially suited to gas chromatography. In a modified form, this technique may be applied to the automatic integration of an array of spots of variable shape and density, as in paper chromatography.

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"INTEGRAM" RECORDING

The authors have discussed in an earlier paper[1] a continuous analog integration method in which, in lieu of the conventional chromatogram, a transformed record or "integram" is presented. This record has the property that the base width of each transformed peak is proportional to the integral of the original peak.

Given a typical gas chromatograph output signal as shown in Fig. 1(a), the corresponding integram appears as shown in Fig. 1(b). Here the base width of the pentane peak is proportional to the area under the corresponding peak in Fig. 1(a);

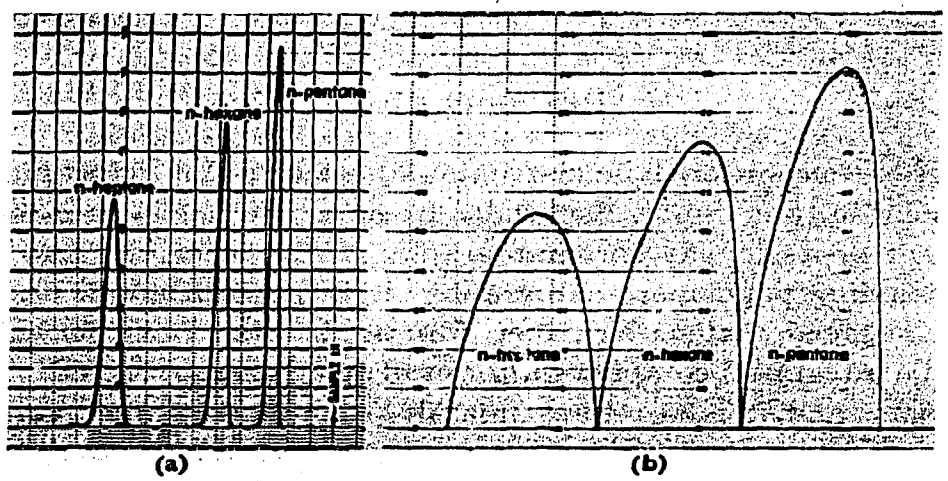

Fig. 1. Chromatogram *vs.* integram. Sample: *n*-pentane, *n*-hexane and *n*-heptane, equal volumes. Chromatogram chart speed: 0.5 in./min. Integram chart speed: 8 in./min/100% signal. Column: 6 ft., silicone 550, 40/60 C-22 firebrick. Temperature: 91°. Detector: thermal conductivity cell. Sample size 0.02 ml.

similarly for the other peaks. The requirement for producing the transformed curve is essentially a simple one: the chart is caused to be driven at a rate *proportional to the signal magnitude.* The pen is servo-driven in the ordinate direction in the usual way. It may be seen that the mechanism needed to drive the chart is an integrator, and that the length of chart paper fed from the recorder between any time limits is proportional to the time integral of the signal between those limits.

"STRIP-INTEGRAM" RECORDING

The authors have now developed a related technique that permits retaining the conventional presentation of signal against a uniform time scale. This offers certain advantages to be mentioned later. At the same time, the system appears to be the simplest yet devised for continuous integration recording in gas chromatography or other techniques such as paper electrophoresis that may have a similar requirement.

From the vantage point of the operator, the manner of presentation is the following: An accessory integrating unit is mounted on or alongside the recorder. From this unit, during the chromatogram run, a narrow paper tape is fed out on which at varying intervals appears a series of lines. On completion of the run, the

strip is torn off and laid next to an ordinary measuring rule. The distance between the first and second lines is proportional to the area of the first peak; distance between the second and third lines measures the second peak area, etc. We have called this taped record a "strip-integram".

The apparatus is shown schematically in Fig. 2. The signal source, *e.g.* the output of the gas chromatograph, drives the pen of a conventional recorder. The pen servo also drives the input of an integrating device which may as shown be the convenient,

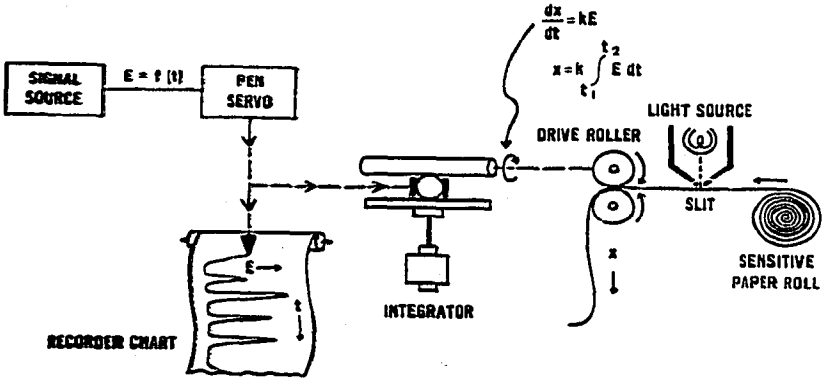

Fig. 2. Apparatus for strip-integram recording.

relatively inexpensive ball-and-disk type integrator. Actually, in the work subsequently shown, the authors used a velocity-servo type of integrator[2] already in their possession and which they described in their earlier paper[1]. The electrical input for integrators of this type is derived either from a re-transmitting potentiometer on the recorder or directly from the signal source. The integrator in any event is of a type that provides a mechanical output, preferably as a rotating shaft, the angular excursion of which is proportional to the integral of the input. The output shaft of the integrator drives a strip of light-sensitive paper past a constantly illuminated slit. The slit, about 0.005 in. wide in the present work, was directly illuminated by a small, commercially available pencil-type mercury vapor source*. The simple power supply furnished with the lamp was connected directly to the a.c. power line.

The paper is a dry, self-developing type that darkens directly on exposure to light and requires no wet development. It is commercially available** and has recently come into wide use in a number of recording oscilloscopes.

It is characteristic of the integrator, as indicated by the equations in Fig. 2, that its output shaft turns at a rate always proportional to the signal magnitude. Thus, at the start of a run or between peaks, where signal level is zero, the sensitive paper is stationary and receives a relatively strong exposure at the slit. At such times a dark, sharp line is impressed on the paper. When a peak appears, the paper is set in motion and exposure thereby much reduced. Lighter background tones are therefore presented between the dark lines. Where a pair of adjoining peaks is not fully resolved, the sensitive strip does not come to a full stop between peaks; hence the

* Ultraviolet Products, Inc., San Gabriel, Calif.
** Kodak Linagraph Direct-Print Paper. Eastman Kodak Company, Rochester, 4 N.Y.

line recorded on the strip is somewhat broadened. Since the length of paper fed past the slit in any time interval is proportional to the integral of the signal during that interval, distances between successive dark lines are a measure, respectively, of successive peak areas.

The precise photometric characteristics of the sensitive paper are of no consequence to the measurement, of course, since only these inter-linear distances are of interest rather than the darkness of any portion of the strip. By the same token, nominal drifts in source intensity and moderate fogging of the strip are without effect on the readings obtained.

Fig. 3 shows the appearance of an actual strip-integram and the corresponding

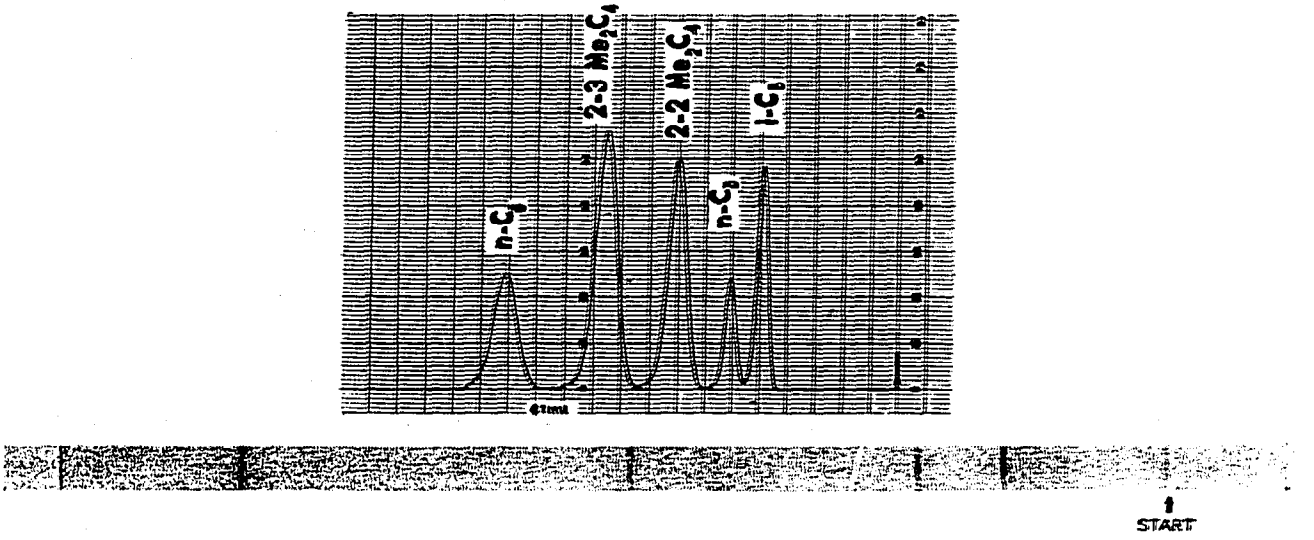

Fig. 3. Chromatogram and strip-integram. Resolved peaks. Sample: isopentane, *n*-pentane, 2,2-dimethylbutane, 2,3-dimethylbutane, and *n*-hexane, respectively 1.0, 0.5, 1.5, and 2.0 and 1.0 parts by volume. Chart speed: ½ in./min. Strip speed: 7.5 in./min/100% signal. Column: same as in Fig. 1. Temp.: 70°. Detector: hydrogen flame. Attenuation: 300,000. Sample size: 0.0025 ml.

conventional chromatogram obtained on a mixture of hydrocarbons. The peaks here are essentially fully resolved. Duration of analysis was about 16 min, and the length of the strip fed out during this time at a rate of 15 in./min per 100% signal was about 11 in. Reading from right to left, the distance between the first two lines is proportional to the area of the isopentane peak; distance between the second and third lines is a measure of the *n*-pentane peak area, etc. The largest of the peak areas indicated here may be read against a steel rule to within about 0.1%, the smallest to within about 0.5%. If desired, a longer strip of paper may be fed out for the same total amount of sample (for example, by driving the integrator disk at higher speed) and reading accuracy thereby increased. This is useful, of course, only so long as the accuracy of the integrating device (or other limiting factor in the system) has not been exceeded.

In Fig. 4 we have deliberately run the same sample mixture at higher column temperature (100° instead of 70°). We obtained in this way varying degrees of resolu-

tion between the peaks. To avoid too short a strip-integram on this run (with sample size reduced about three-fold) we doubled the factor of strip speed *vs.* signal level as compared with Fig. 3. The resulting strip length was about 7 ½ inches. The contrast of the lines is seen to be reduced and the line width increased as resolution is diminished. The reading error introduced by the line broadening appears, however, to be always appreciably less than the uncertainty inherent in the fact of incomplete resolution. Thus, whereas in the chromatogram about 15 % of the true area of the second (*n*-C5) peak overlaps the area of the first peak, and a low-contrast, broad line appears on the strip-integram between these peaks, the area of the second peak is nevertheless readable on the strip to about 1 %. The areas of the somewhat

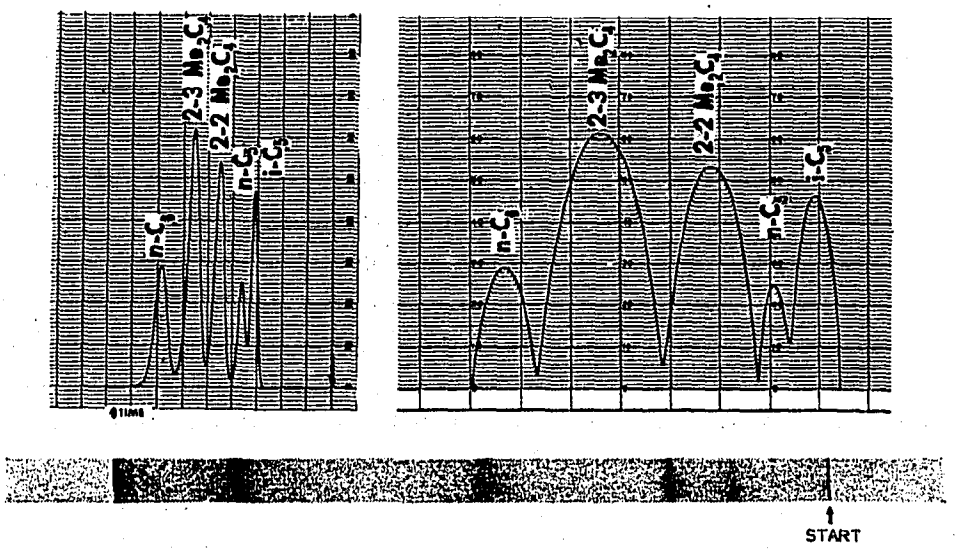

Fig. 4. Chromatogram, integram and strip-integram. Incompletely resolved peaks. Sample: same as in Fig. 3. Chart speed: 1 in./min. Strip speed: 15 in./min/100 % signal. Column: same as Figs. 1 and 3. Temperature: 100°. Detector and attenuation as in Fig. 3. Sample size: 0.0008 ml.

better-resolved peaks, such as the third and fourth (2,2- and 2,3-dimethyl-butane), are readable to within 0.2 to 0.3 %.

In cases such as the preceding, where somewhat poorly resolved peaks are encountered, it may be desirable to increase the exposure level on the strip to bring out the boundaries between such peaks. Fortunately, the sensitive paper has a sufficient latitude of exposure that this will not unduly overexpose and broaden the lines between well-resolved peaks in the same record. Should it prove necessary, however, the latitude of exposure may in effect be easily increased by a large factor. By illuminating the slit non-uniformly, *i.e.* more intensely at one end than the other, there will always be a portion of the slit where exposure is an optimum, whether the peaks be poorly or well resolved.

For purposes of comparison, Fig. 4 includes at the right the corresponding integram run concurrently with the chromatogram and strip-integram. The integram offers the advantage of presenting, in a single record, information on peak height and resolution; at the same time it provides, in the base widths, a rapid and convenient

measure of the time integral of each peak. It does not, however, indicate elapsed time (useful for peak identification), though we have shown[1] that a second pen, driven at constant speed in the ordinate direction, will easily provide this information.

In the strip-integram technique, on the other hand, we retain the familiar, conventional record; and merely by supplementing the recorder with an integrator to drive a sensitive strip, provide an extremely simple and convenient means for continuous integration recording.

A certain advantage that the strip-integram shares with the integram is worth noting. If we consider unresolved peaks, then in contrast to the conventional chromatogram, the extrapolated contours of adjoining peaks in the integram do not overlap. Instead, they may be extrapolated to a common, single point on the axis. This follows from the fact that total integram base width for adjoining peaks is proportional to the sum of their separate integrals, independent of the resolution, on the assumption that detector response is linearly additive for superposed components. This assumption has appeared reasonable at least for the thermal conductivity and hydrogen flame type detectors. Where departure from such behavior is observed, extrapolation to a single point still causes only slight error, because the ratio of overlapping to non-overlapping areas will generally be quite small.

The strip-integram shares the convenience of the integram in the sense that adjoining integration limits for any pair of unresolved peaks may be fairly represented by a single position on the strip, this position being in most cases very close to the center of the broadened line presented on the strip.

The need to change signal attenuation during a strip-integram run presents no particular difficulty. For accurate integration, such change would be made between peaks, when the sensitive strip is stationary or nearly so. Appropriate multiplying factors are of course then applied in reading the successive strip lengths, in a manner analogous to that of other methods of integration.

The sensitive paper may be handled for appreciable lengths of time in ambient light, following exposure in the instrument, without loss of the recorded image. Darkening is, however, more rapid under fluorescent lighting than incandescent lighting. Nevertheless, the line patterns persist for several days of continuous exposure to ordinary ambient fluorescent light levels. Although developing solutions that will permanently fix the images are available from the paper manufacturer, we found in the present use that this results in serious mottling and loss of tonal gradation. There appears to be no compelling need, however, to make the records fully permanent.

The integration accuracy of strip-integram will generally be limited only by the accuracy of the integrating device used. However, in the case of a high-quality ball-and-disk integrator driven by a recorder pen-servo, performance may be limited by the linearity of the recorder slidewire. There appears to be no difficulty, in any event, in obtaining peak area values to $\pm$ 0.2 % or better on peaks for which the length of strip fed from the instrument is about 3 in. or more.

INTEGRATION OF PAPER CHROMATOGRAM SPOTS

The principle of the strip-integram may be extended to the integration of an array of paper chromatogram spots of variable shape and density. An advantage compared with previously reported scanning techniques of spot integration[3] is that the spots need not be presented individually and manually to the instrument. Instead the whole array of spots, regardless of their disposition, is scanned as an ensemble.

Assume an array such as *A*, *B*, *C*, *D* in Fig. 5(a), that may be scanned in a raster pattern for optical density, fluorescence, or radioactivity, etc. The scanning spot may

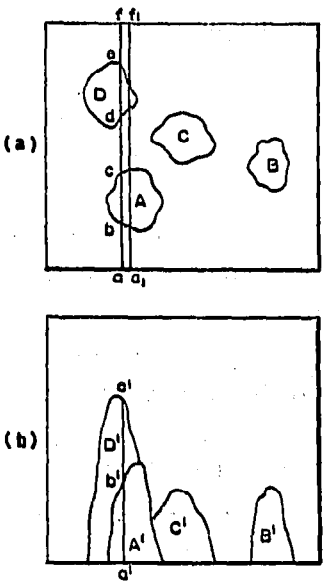

Fig. 5. Chromatogram and transformed chromatogram.

for instance start at the lower left and cover the chromatogram in a series of upward directed scanning strokes af, a_1f_1, etc. Simultaneously, a small spot of light is caused to scan or "write" upon a sheet of light-sensitive paper, represented in Fig. 5(b). Lateral displacement of this writing spot is made synchronous with the lateral displacement of the scanning site on the chromatogram. In the vertical direction, however, the writing spot moves at a variable rate: starting always at the lower margin of the sensitive sheet at the start of each vertical scan on the chromatogram, the writing spot is made to move upward at a rate proportional to the concentration of sample on the chromatogram (as derived from optical density, fluorescence, etc.). After a vertical scan on the chromatogram is completed, the writing spot is brought back to the lower margin for the next scan.

Assuming we scan the chromatogram of Fig. 5(a) for optical density by transmitted light, an electrical signal may be derived proportional to the density. An adjustment or compensation may be made in this signal for the background density of the paper. The corrected density may be integrated, as by a ball-and-disk integrator, and the integrator output caused to drive the writing spot with respect to the paper. Consider scanning of the line af on the chromatogram. The corrected density signal

is zero between points *a* and *b*, hence the writing spot is initially stationary at the lower margin of the sensitive paper and registers a dark mark there. From *b* to *c* the density signal is of finite value. In this interval the writing spot moves from *a'* to *b'*. Since from *c* to *d* on the chromatogram the corrected density is again zero, a dark spot is again recorded at *b'*. The action is repeated as the chromatogram is scanned from *d* to *f*, following which the writing spot is returned rapidly from *e'* to the starting line. Scanning the total array of spots in this manner results in an array of transformed spots *A'*, *B'*, *C'*, *D'*. Since the length of any ordinate such as *a'b'* on the transformed spot is the integral of the density along the corresponding ordinate segment such as *bc* on the original spot, it follows that the total area of any transformed spot is proportional to the total integrated density of sample on the original spot, to the extent that Beer's law applies. It is necessary now only to measure the areas of the transformed spots to obtain values proportional to the amount of sample in each spot. This may be done rapidly with a planimeter, or by cutting out the spots and weighing.

An apparatus that may be used to transform the spots in the case of optical density scanning is shown in Fig. 6. The chromatogram is mounted on a transparent drum I rotated at constant speed by motor *d*. An opaque band *e* bridges the beginning and end of the paper. A source *a* projects a spot of light on the paper, and *b* is a photodetector unit that may include a wavelength-selective filter. Units *a* and *b*

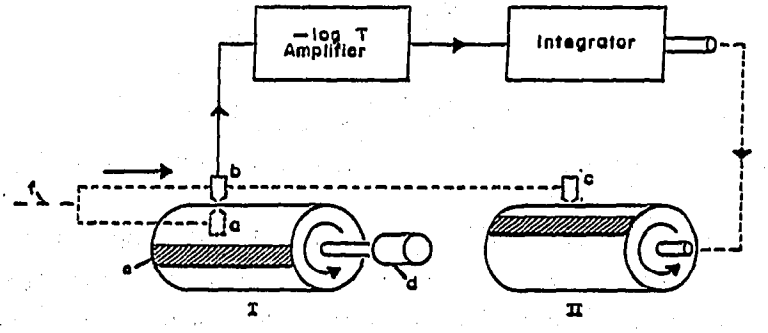

Fig. 6. Apparatus for transforming the chromatogram.

are driven together across the paper at constant speed from a common driving source *f*. The speed of *f* is such that during one revolution of drum I the scanning elements *a*,*b* are displaced laterally an amount equal to the separation of successive scanning lines, such as *af* and a_1f_1 in Fig. 5. A logarithmic amplifier, with output adjustable to compensate for paper background, derives a density signal that is applied to an integrator with a mechanical output. The integrator drives a drum II which carries the sensitive paper. Unit *c*, mechanically coupled to the driving source *f*, projects a small writing spot on the sensitive paper.

At the beginning of each scan, the scanning elements *a*, *b* are aligned with the starting edge of the chromatogram, and unit *c* is aligned with the starting edge of the sensitive paper. When *a*, *b* have completed a scan, as from *a* to *f*, Fig. 5(a), the writing spot at *c* has in general only partially traversed the sensitive sheet, as from

a' to e', Fig. 5(b). As drum I continues to rotate, bringing band e under the scanning site, the starting line on the sensitive sheet is again brought into alignment with the writing spot, in readiness for the next scan, as follows: Opaque band e, moving under the scanner, drives the amplifier to saturation and causes the integrator to drive drum II at relatively high speed. This speed is such as to cause negligible exposure of the paper. The speed is high enough moreover that, if need be (at abscissa positions where there is no chromatogram spot), drum II may turn a full revolution before opaque band e completely traverses the scanning site. A mechanical stop arrests drum II when it reaches the starting line position. At the moment, however, that band e just clears the scanning site, a switch actuated by drum I releases this stop (freeing drum II for another revolution), and so synchronizes the starting time for ensuing scans on both drums.

SUMMARY

Some new analog methods of integration recording are discussed that eliminate the need for pip counting, or for trigger circuitry to sense the beginning and end of "events" in the signal, such as signal peaks. One version especially suited to gas chromatography appears to be the simplest yet devised, requiring only a ball-and-disk integrator driving a light-sensitive paper past a constantly illuminated slit. The paper is a dry, self-developing, commercially available type. By modification of the technique, an array of spots of variable shape and optical density, as in paper chromatography, may be transformed entirely automatically into a configuration from which the integrated density of the original spots may be more directly and rapidly determined.

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