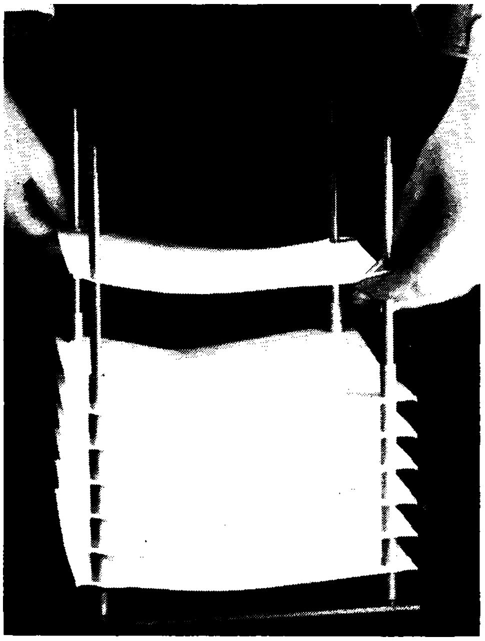

FIG. 2. Method of loading papers on frame.

formed during the phenol run and carries them along at right angles to their previous direction of movement.

When the collidine-lutidine solvent front reaches the top of the paper, there will be a brown line 3–4 cm behind it. No amino acids move in front of this brown line, and in order to increase their resolution in this solvent it may be made to overrun the chromatograms by laying sheets of filter paper horizontally in contact with their uppermost edges. These extra sheets of paper are held in place with thin sheets of glass, thus ensuring good contact. The run should be continued until the brown line almost reaches the top of the chromatograms.

When the second solvent has completed its travel, the frame is removed from the tank and the papers dried in a stream of air. They are then taken out of the frame, sprayed with ninhydrin, and developed by heating in the usual manner.

Using Whatman No. 1 paper, the run takes about 8 hr at 22° C in each direction. The frames may be put in the tanks at night and removed in the morning. Allowing the papers to stand in the tank for a few hours after the run is completed does not lead to any diminution in resolution. They may be dried during the day, and the second run carried out the next night. With 2 frames and 2 tanks it is possible to have 12 chromatograms completed each day, though the result of a particular test cannot be obtained for 48 hr. With Whatman No. 4 paper at 22° C, the run takes about 4 hr in each direction; thus a chromatogram may be completed in one day.

We have used this technique successfully for routine survey of large numbers of urines such as may be required in clinical work, for genetical investigations involving the analysis of urines of many members of the same family, and for physiological experiments involving the sampling of biological fluids over long periods of time. It would also be readily adaptable to semiquantitative work by running serial dilutions of the material to be studied, together with several dilutions of standard solutions on the same frame. Furthermore, there is no reason why the frame should not be lengthened considerably so that many more chromatograms may be run simultaneously.

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Self-Absorption and Backscattering of β Radiation[1]

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The complex nature of the absorption and scattering of β particles causes many difficulties in β counting, especially with low-energy β emitters such as C^{14}, S^{35}, and Ca^{45}, which are much used in biological work. It is often necessary to count sources of appreciable thickness for which self-absorption of the β radiation is significant. A further effect to be considered in the counting of thin sources is the backscattering of β radiations by the container or backing material used to support the source. This effect is strongly dependent on the atomic number (Z) of the backscatterer and is large for materials of high Z. Sources are often made "infinitely thick" for self-absorption, i.e., of thickness at least equal to the range of the β radiation, in order to avoid the large self-absorption corrections required for sources of intermediate thickness. However, for this case (as for any thickness greater than about 5% of the range of the β radiation) the effect of self-scattering (internal scattering) by the source must also be considered. From the experiments reported here it was found that self-scattering by the source increases with the average atomic number of the source material, as in the case of backscattering by the source holder.

An investigation of the above-mentioned effects was carried out with S^{35}, Ca^{45}, and P^{32} obtained from Oak Ridge. These nuclides are simple β emitters with maximum energies of 0.17 mev, 0.26 mev, and 1.7 mev, respectively. Counting was done with a Tracerlab end-window counter with window thickness of 1.5 mg/cm^2 and diameter of 2.8 cm. The counter was attached to a slotted aluminum shelf holder, which permitted counting of sources at various distances from the counter. The whole counter assembly was housed in an aluminum-lined lead shield. The coincidence loss correction for the counter was 0.6%/1,000 cpm.

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Circular flanged dishes stamped out of aluminum, steel, copper, cadmium, and lead were used as source holders. The dishes were 2.8 cm^2 in area and 0.5 cm in depth. Their thickness (0.045 cm) was sufficient to provide saturation backscattering for all sources, as shown by the failure of additional backing (placed against the underside) to increase the counting rate of the source. The dishes were supported by an aluminum shelf with a hole cut out of the center.

The vertical distance between the source and the counter window was 0.8 cm in the shelf 1 position and 2.0 cm in the shelf 2 position, corresponding to calculated geometry factors of 25% and 9%, respectively. The geometry factor determined for shelf 1 by counting a weighed source (5 mg) of U_3O_8 spread over an area of 2.8 cm^2 on a thin collodion film ($\sim 20\ \mu g/cm^2$) was 19%, a value somewhat lower than the calculated value, as would be expected for an extended source.

Self-absorption. The self-absorption of β radiation may be shown (*1, 2*) to follow the equation

$$\frac{A_d}{A_o} = \frac{1 - e^{-\mu d}}{\mu d}, \tag{1}$$

where A_o is the activity (counting rate) of a given amount of the radioactive nuclide with no self-absorption, A_d is the activity of an equal amount of the radioactive nuclide contained in source material of thickness d in mg/cm^2, and μ is the absorption coefficient in cm^2/mg. This equation is based on the assumption that β radiation is absorbed exponentially, which is only approximately true (*3*). Nevertheless, the agreement between equation (1) and self-absorption curves obtained experimentally is remarkably good.

In the present investigation self-absorption curves were obtained by two different methods. The method first used consisted of precipitating constant amounts of the radioactive nuclide with increasing weights of source material, transferring the precipitates to dishes, and counting. After a few preliminary experiments this method was abandoned in favor of another method that gave essentially the same results but was decidedly more convenient. In the latter procedure a supply of source material was prepared that contained a known quantity of the radioactive nuclide. Various weights (thicknesses) of this material were then counted, and the counting rate of each source was divided by the weight of the source to give the specific activity (cpm/mg). A plot of specific activity versus source thickness gives the desired self-absorption curve. In order to obtain uniform and complete distribution of the thinner sources ($< 10\ mg/cm^2$) over the surfaces of the dishes, it was found necessary to slurry the source material in a little acetone, stir thoroughly, and evaporate to dryness. For thicker sources, good results were achieved by simply spreading the source material in the dish and tamping lightly with the flat end of a metal cylinder until the source was smooth and uniform.

The self-absorption of the β radiation of Ca^{45} in $CaCO_3$ is shown in Fig. 1, where the observed specific activity is plotted against source thickness. The data are seen to be well fitted by the curve of equation (1) with a value of 0.09 cm^2/mg for the absorption coefficient, μ. The data in Fig. 1 were obtained with copper dishes in the shelf 1 position. Several other curves obtained with aluminum and steel dishes in both shelf positions gave values for μ in the range $0.09 \pm 0.01\ cm^2/mg$. The point plotted at zero thickness in Fig. 1 is the counting rate of an aliquot of the Ca^{45} stock solution evaporated in a copper dish. This source was essentially weightless (< 0.1 mg) but contained the same amount of Ca^{45} as represented by the other points on the curve. It will be noticed that the activity of the weightless source is somewhat greater than that given by extrapolation of the curve. With aluminum dishes, however, the activity of the weightless source was found to be a little less than that obtained by extrapolation of the curve. This effect is due to backscattering by the dish and was investigated more carefully in the experiments with S^{35} discussed below.

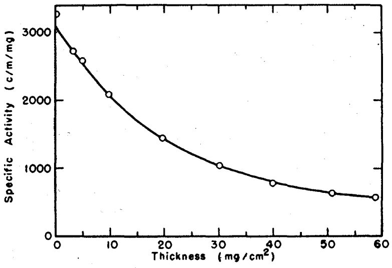

FIG. 1. Self-absorption of Ca^{45} β radiation in $CaCO_3$.

The self-absorption of the β radiation of S^{35} in benzidine sulfate is shown in Fig. 2. The points plotted as squares represent the self-absorption observed with aluminum source holders (dishes). The data obtained with beryllium source holders are plotted as circles. The beryllium source holders were prepared by removing the bottoms from aluminum dishes and replacing with beryllium plates. The important feature of Fig. 2 is the difference in the initial slopes of the self-absorption curves caused by backscattering of the β radiation from the source holders. The relative backscattering by beryllium ($Z = 4$) and aluminum ($Z = 13$) is indicated by the points at zero source thickness, which represent the counting rates of identical weightless sources of S^{35} evaporated on the two metals. It is seen that the specific activities of the sources of thickness less than about

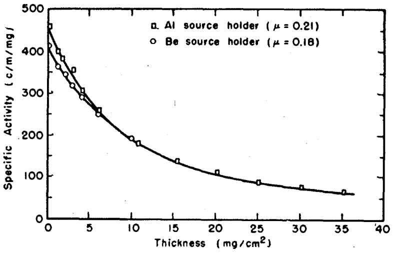

FIG. 2. Self-absorption of S^{35} β radiation in benzidine sulfate using source holders of different atomic number.

6 mg/cm^2 mounted on aluminum are higher than the activities of similar sources on beryllium because of the greater backscattering by aluminum. For thicker sources, in experiments with both S^{35} and C^{45}, the source holder has no effect on the self-absorption curve.

It should also be mentioned that self-absorption curves obtained with $CaSO_4$, $BaSO_4$, and $PbSO_4$ for thicknesses greater than 10 mg/cm^2 were identical in shape with the curve of Fig. 2, but gave greater specific activities because of increased self-scattering by the source materials of higher atomic number. This effect was observed in the self-absorption of C^{14} in wax and $BaCO_3$ by Yankwich and Weigl (*4*), who also found that differences in self-scattering affect the initial slope of the self-absorption curve. From the data in Fig. 2, an absorption coefficient of 0.18 cm^2/mg is obtained for sources of all thicknesses mounted on beryllium. With aluminum source holders, however, the absorption coefficient is initially 0.21 because of the backscattering effect.

Henriques *et al.* (*1*) obtained an absorption coefficient of 0.265 for benzidine sulfate mounted on filter paper. For $BaSO_4$ mounted on lusteroid an absorption coefficient of 0.216 was obtained by Larson *et al.* (*4*). In view of the discrepancies among the various values found for the absorption coefficient and the above-mentioned effects of scattering by source holders and source materials, it would seem advisable for each investigator to obtain a self-absorption curve under the particular experimental conditions being used if accuracy is desired.

Backscattering and self-scattering. In earlier studies of β-counting problems by Engelkemeir *et al.* (*6*), it was shown that sources of identical intensity may exhibit markedly different counting rates, depending on the thickness and atomic number of the source support and on the thickness of source material, as a result of scattering of the β radiation. In the case of backscattering by the support, the counting rate of a weightless or very thin source is observed to increase with increasing thickness of the support (due to reflection of β particles into the counter) to a point beyond which there is no further increase. The source is then considered to have saturation backscattering. This point is generally reached at a thickness equal to about 20% of the range of the β radiation. The increase in counting rate at saturation backscattering is also found to be a function of the atomic number of the source support, the backscattering effect increasing with increasing atomic number. In the case of self-scattering, the observed counting rate of a given activity is found to increase when the activity is incorporated in increasing thicknesses of source material. Beyond a certain thickness, of course, the counting rate decreases because of self-absorption. The self-scattering effect generally reaches a maximum (saturation) at a source thickness equal to 5–10% of the range of the β radiation. Further studies of backscattering have been made by Zumwalt (*7*) and Burtt (*8*) in connection with problems of absolute β counting.

In the present investigation of saturation backscattering and self-scattering effects, the β radiations of S^{35} and P^{32} were employed. In the backscattering experiments identical aliquots of a stock solution of the nuclide were evaporated on various source holders to give essentially weightless sources. Source holders with no backscattering were prepared by removing the bottoms from aluminum dishes and replacing them with thin collodion films of ~ 20 μg/cm^2 thickness, which provide negligible backscattering. Sources with saturation backscattering were prepared both by evaporation of the active solution directly on the various metal dishes and by placing metal backings beneath (and just in contact with) a collodion film on which the active solution had been evaporated.

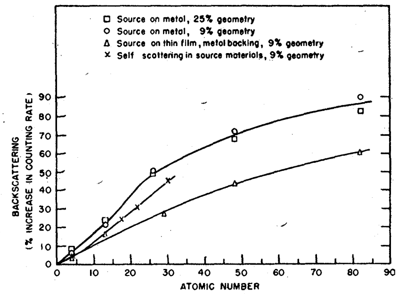

FIG. 3. Saturation backscattering and self-scattering of S^{35} β radiation as a function of atomic number of the scatterer.

The variation of saturation backscattering with atomic number of the source holder for S^{35} is shown in Fig. 3. The backscattering effect is plotted as the percent increase in counting rate over the counting rate of the source mounted on a collodion film, which is considered to have zero backscattering. For sources evaporated directly on metal, the backscattering effect was determined in both shelf positions with essentially the same results. The backscattering for sources on metals is considerably greater than that for sources on films with metal backings. This remarkable effect was first observed by Yaffe (*9*). The most reasonable explanation for the effect is that the relatively irregular surface of a metal provides more opportunity for small angle scattering of β particles in the direction of the counter than does the smooth surface of a plastic film.

Also shown in Fig. 3 are the data on the self-scattering of the radiation of S^{35} as a function of the average atomic number of the source material. In this experiment, thick sources (30 mg/cm^2) of benzidine sulfate (average $Z = 4.5$), $BaSO_4$ (average $Z = 17.3$), $PbSO_4$ (average $Z = 21.7$), and Hg_2SO_4 (average $Z = 29.7$) were prepared to contain the same quantity of S^{35} as used in the sources prepared for the backscattering experiment. The observed counting rates of the thick sources were corrected to zero self-absorption, using an absorption coefficient of 0.18 cm^2/mg (Fig. 2), and compared with the counting rate of the weightless source mounted on collodion film. It is seen that the counting rate of a source is not independent of the composition of the source material, as is often assumed, but increases with increasing average atomic number of the source material in consequence of increased self-scattering.

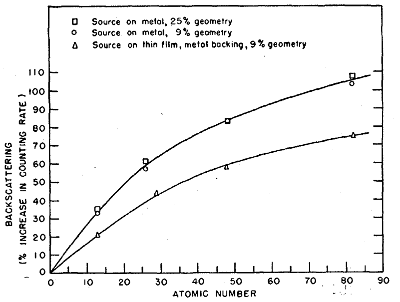

FIG. 4. Saturation backscattering of P^{32} β radiation as a function of atomic number of the backscatterer.

The backscattering data for P^{32} are shown in Fig. 4; the effect is again found to be the same for the two shelf positions, and greater for sources on metals than for sources on films with metal backings. The data for the latter type of source mounting are in reasonable agreement with the data for P^{32} given by Engelkemeir (*6*), Zumwalt (*7*), and Burtt (*8*), who used only this mounting procedure.

It is also seen from a comparison of Figs. 3 and 4 that the backscattering of the high-energy β radiation of P^{32} (1.7 mev) is apparently greater than that of the low-energy β radiation of S^{35} (0.17 mev) for a given atomic number, and that there is a noticeable dip in the curve for S^{35} on metal at low atomic number. These differences were found, however, to be due entirely to absorption of the backscattered β radiation of S^{35} in the air gap and counter window.

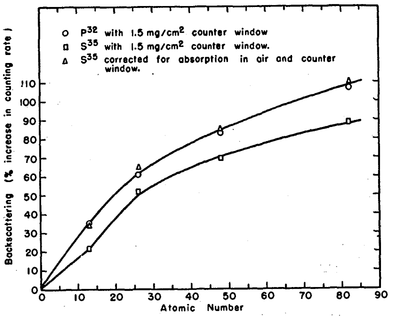

FIG. 5. Saturation backscattering for P^{32} and S^{35} with absorption corrections applied to S^{35}.

Absorption curves were obtained with thin aluminum foils for the S^{35} sources (in the shelf 1 position) evaporated on film, aluminum, steel, cadmium, and lead. From the first absorption curve the initial half-thickness of the primary radiation from the film was observed to be 2.5 mg/cm². By subtraction of this curve from the rest of the absorption curves, the initial half-thickness of the radiations backscattered by aluminum, steel, cadmium, and lead were found to be 1.3, 1.7, 1.7, and 1.7 mg/cm², respectively. The lower value for aluminum is probably responsible for the dip observed in the backscattering curve and indicates that β radiation is more degraded in energy by scattering from materials of low *Z* than by scattering from materials of high *Z*.

Using the above absorption data, the backscattering results for S^{35} were corrected for absorption of the β radiation in the air gap and counter window. The results are shown in Fig. 5, where the squares and triangles represent the uncorrected and corrected data, respectively. The circles represent the data for P^{32}, which require no correction for absorption because of the high energy of P^{32} β radiation. It is seen that the corrected backscattering of S^{35} is in good agreement with that of P^{32}, indicating that the backscattering of β radiation for a given atomic number is independent of the β energy, at least over the range of energy from 0.17 to 1.7 mev. This is in agreement with Zumwalt (*7*) and Burtt (*8*), who have reported the same result for the range of energy from 0.3 to 1.7 mev.

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The Pungency Taste Characteristics of Some Piperazine Derivatives

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Staudinger and Schneider (*1*) found that the piperidides of β-cinnamenyl acrylic acid, 5-phenyl-2-pentenoic acid, 5-phenyl-3-pentenoic acid, and 5-phenyl-*n*-valeric acid have the same pungent taste characteristics of the piperine of black pepper, *Piper nigrum*. Riccomanni (*2*) reported that the piperidine nucleus could be substituted by piperazine and stated that the di-(5-phenyl-3-pentenoyl)-piperazine has a biting peppery taste.

On repeating the work of Riccomanni, it was found by the authors that, although the crude product does have a pungent taste, this is not due to the diacyl piperazine, but to the presence of the di-5-phenyl-3-pentenoyl piperazonium salt, which can be removed from the crude product by water extraction.

The diacyl piperazine and the diacyl piperazonium salts in this investigation were prepared substantially in the manner recommended by Pollard and Adelson (*3*) for these types of compounds. The former were prepared by reacting the corresponding acyl chloride with piperazine hexahydrate, and the latter by heating equimolecular amounts of acid and base. Both series of com-