80° and 100° C. If the temperature is below 70° C when the paper is placed in the oven, the sensitivity of the ninhydrin reaction may be decreased due to yellowing of the paper. The positions of the colored spots developed by the reaction of ninhydrin with the amino compounds on the paper (chromatogram) were revealed by light transmitted through the paper placed over an X-ray illuminator. By using transmitted rather than reflected light, the colored areas on the paper can be located much more readily, and a greater sensitivity can be obtained. In fact, spots which are not visible by reflected light can be readily located by transmitted light.

TABLE 1

Compound	Minimum quantity (μg)	Color of spot	R_f values	
			Phenol 28 hrs	Collidine-lutidine 60 hrs
Alanine	0.2	Purple	0.63	0.41
β-Alanine	0.2	Blue	0.71	0.33
Alanylglycine	3	Pink purple	0.56	0.36
α-Amino-*n*-butyric acid	0.2	Purple	0.77	0.46
ε-Amino-*n*-caproic acid	0.5	"	0.91	0.34
Arginine monohydrochloride	4	Blue purple	0.66	0.14
Asparagine	1	Brown yellow	0.42	0.29
Aspartic acid	0.4	Blue	0.19	0.24
Citrulline	0.5	Purple	0.67	0.31
Cysteic acid	8	Blue	0.10	0.43
Glucosamine monohydrochloride	4	Purple brown	0.52	0.65
Glutamic acid	0.1	Purple	0.32	0.26
Glutamine	2	"	0.62	0.32
Glutathione	10	Blue purple	0.10	0.16
Glycine	0.1	Pink purple	0.42	0.33
Histamine dihydrochloride	12	Yellow brown	0.92	0.46
Histidine monohydrochloride	25	Brown	0.77	0.34
Homocystine	4	Purple	0.38	0.28
Hydroxyproline	1	Brown yellow	0.72	0.42
Isoleucine	0.5	Purple	0.88	0.62
Leucine	0.5	"	0.88	0.65
Lysine monohydrochloride	3	"	0.56	0.14
Methionine	1	"	0.85	0.61
Methionine sulfone	5	Brown purple	0.66	0.51
Methionine sulfoxide	1	Purple	0.84	0.34
Nor-leucine	0.4	"	0.89	0.69
Nor-valine	0.5	"	0.84	0.56
Ornithine monohydrochloride	3	"	0.42	0.13
Phenylalanine	5	Grey brown	0.90	0.67
Proline	1	Yellow	0.90	0.41
Serine	0.3	Brown red	0,37	0.37
Taurine	1	Purple	0.39	0.50
Threonine	2	Pink purple	0.53	0.43
Tryptophane	2	Yellow brown	0.79	0.66
Tyrosine	3	Brown	0.63	0.74
Valine	0.2	Purple	0.82	0.53

Table 1 lists the minimum quantities of several amino compounds which give a visible color with ninhydrin on a two-dimensional chromatogram when viewed by transmitted light.

The R_f value (rate of flow) is the ratio of the distance a compound moves along the paper to the total distance the solvent moves. These values therefore indicate the position a compound will occupy on the paper. They have not been found to be very consistent between different chromatograms, but are useful for comparing the relative positions of compounds on a single chromatogram.

All of these compounds were tested for sensitivity to ninhydrin on filter paper without being run in the solvents. Several were found to be more sensitive than was apparent from the chromatograms, thus indicating a certain amount of decomposition by the solvents. Cystine is completely decomposed when run in the second solvent mixture and must be oxidized to stable cysteic acid in order to give the ninhydrin test (*2*). A decrease in sensitivity to ninhydrin is most apparent with histidine, arginine, phenylalanine, and histamine.

References

1. Consden, R., Gordon, A. H., and Martin, A. J. P. *Biochem. J.*, 1944, **38**, 224–232.
2. Dent, C. E. *Biochem. J.*, 1947, **41**, 240–253.

A Simple Method of Measuring the Surface Area of Small Objects of Irregular Shape[1]

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In the course of experiments with transplantable tumors it was found necessary to measure their surface area. Since the tumors were not symmetrical, no simple formula could be applied, and thus it was necessary to devise a method of measuring small, irregularly shaped objects. The method chosen depended upon determining quantitatively the amount of sodium chloride deposited on the surface of the object after immersion in a salt solution. The amount of sodium chloride thus deposited was then measured by dipping the object in a solution of silver nitrate and finding the amount of the silver salt which combined with the sodium chloride to form a silver chloride precipitate. To establish the reliability of the method, it had to be shown that objects of different sizes and shapes would yield sodium chloride measurements directly proportional to their surface area. If this were found to be true, the method would presumably be suitable for measuring the surface of irregularly shaped objects as well.

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Spheres and cylinders of various sizes were selected as test objects because their surface areas may readily be calculated. The test spheres were ball bearings, 0.63–1.90 cm in diameter; the cylinders were made of wood dowel and were 1.30–1.87 cm in diameter and 0.83–1.56 cm in height. The surface areas were calculated from micrometer measurements of these objects, by appropriate formulas. Each object was then suspended on a thin wire to facilitate manipulation. In order to equalize the wetting properties of different materials, the spheres and cylinders were first coated by dipping them 5 times in a 2% solution of agar at 70° C and permitting them to dry at room temperature in the intervals between dips. The sphere or cylinder was then immersed in 0.9% sodium chloride solution, slowly removed, and dipped in a 25-cc sample of 0.15% silver nitrate solution. Immersion in the two different solutions was done 5 times, using the same sample of silver nitrate. The entire procedure was repeated twice, using two fresh aliquots of silver nitrate, so that for each test three determinations were made.

The amount of silver nitrate remaining in the solution was then determined by the method of Whitehorn (*1*).

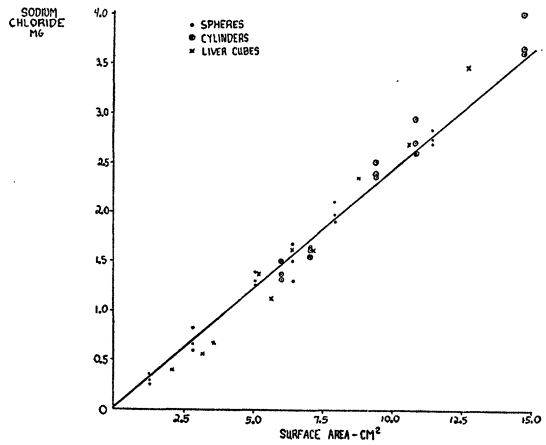

FIG. 1. Graph showing the rectilinear relationship of surface area to the quantity of sodium chloride coating the surface, using objects of different size and shape.

Five cc of concentrated nitric acid and 0.6 gm of ferric ammonium sulfate were added to the silver solution, and the mixture was titrated with 0.15% ammonium thiocyanate. The relative amount of silver nitrate reacting with the sodium chloride was equal to the difference between the amount of thiocyanate used to titrate a control sample of silver nitrate and that amount required to titrate the sample used in an experiment. This value was then multiplied by the calculated factor of 1.16 to determine the milligrams of sodium chloride which had reacted with the silver. The final values obtained for the three samples used for each test object were then averaged.

Measurements of this nature were performed on all spheres and cylinders on three separate occasions, and the results were graphed (Fig. 1). The ratio, k, of square centimeters of surface area to milligrams of sodium chloride was calculated for the spheres, using the expression $\frac{\Sigma (XY)}{\Sigma Y^2}$, where $X = cm^2$ of surface area, and $Y = mg$ of sodium chloride; the k value obtained was 4.06, with a standard deviation of ± 0.517. The k for the cylinders was 3.93, with a standard deviation of ± 0.804, a value which did not differ significantly from that obtained for the spheres, as the difference in ratios was only 1.18 times the standard error of the difference. Therefore, the values for both spheres and cylinders were pooled, the new k becoming 3.99, with a standard deviation of ± 0.34 cm². This new k could now be used to calculate the equation and slope of the line (Fig. 1). The equation was $X = 3.99\ Y$, where X is the surface area (cm²) and Y is the milligrams of sodium chloride as determined by this method.

TABLE 1

COMPARISON OF CALCULATED AND EXPERIMENTALLY MEASURED SURFACE AREAS (cm²)*

Spheres		Cylinders	
Calculated	Measured	Calculated	Measured
1.26	1.04, 1.41, 1.13	6.00	5.61, 5.43, 6.13
2.85	2.74, 3.40, 2.45	7.03	6.32, 6.61, 6.55
5.10	5.66, 5.28, 5.14	9.39	9.77, 9.61, 10.24
6.38	6.14, 6.80, 5.28	10.77	11.04, 10.61, 12.03
7.93	8.60, 8.02, 7.78	14.64	14.95, 14.71, 16.36
11.41	10.98, 11.16, 11.55		

* The standard deviation of the differences between measured and calculated surface areas was ± 0.44 for the spheres, ± 0.63 for the cylinders, and ± 0.53 for the pooled data.

By the use of this equation it was possible to compare the known surface area of the objects used with the surface area found experimentally (Table 1). The average deviation for 33 observations was 0.39 cm².

In addition to the wooden and steel test objects, cubes were cut from fresh liver, as the method was ultimately to be applied to tissue. The surface areas were calculated, the cubes then being coated with agar and tested by the previously described procedure. The values obtained for 10 cubes tended to approximate the calculated line (Fig. 1); the necessarily less accurate measurements used for the calculation of surface area doubtlessly contributed to deviations. The k was 3.89, with a standard deviation of ± 0.84; this value was not significantly different from the pooled k calculated above, as the difference in ks was only 0.67 times the standard error of the difference.

Since it has been shown by these experiments that the reacting sodium chloride per unit surface area does not differ significantly when objects of different regular shapes are used, it seems reasonable to conclude that the method is applicable to objects of irregular shape and that the calculated curve based on sphere and cylinder values may be used in determining the surface area of irregularly-shaped objects.

Reference

1. WHITEHORN, J. C. *J. biol. Chem.*, 1921, **45**, 449.