

the pH at 7.3–7.4. The results have been summarized in Table 1.

In all cases the blood urea nitrogen was appreciably decreased, and significant amounts of urea were removed. In those animals in which the acid-base balance was disturbed, there was a tendency for return to normal. For example, dog No. 20 had repeatedly vomited, and before dialysis the plasma chloride was 86 meq/liter and the CO₂ content 92 volumes %, indicating a moderately severe metabolic alkalosis. Following dialysis the values were restored to 97 meq/liter and 68 volumes %, respectively. The animals showed no apparent harmful effects from the procedure, and in several cases clinical improvement was noted. However, we have not yet been successful in keeping the animals alive for a significantly longer period than the controls.

The apparatus is also useful for many problems in laboratory dialysis. For example, during the preparation of renin (8) from hog kidneys, a solution of the active principle (volume, 8 liters from 3.5-kg kidneys) is dialyzed for 3 days in 3" Visking Cellophane tubes in order to remove trichloroacetic acid and other salts.

TABLE 1
REMOVAL OF UREA FROM NEPHRECTOMIZED DOGS BY
EXTERNAL DIALYSIS

Dog No.	Hrs after nephrectomy	Wt. of dog (kg)	No. of kidney units	Blood urea nitrogen (mg/100 ml)		Hrs of dialysis	Urea removed (gm)
				Before dialysis	After dialysis		
14	72	10.5	6	152	54	7.0	18.8
16	60	8.6	4	216	57	5.6	23.1
17	72	8.0	4	...	88	4.5	21.8
19	84	25.0	8	184	81	5.6	38.0
20	60	16.4	8	179	46	7.6	31.7
20	96	15.0	8	130	80	2.5	...

Using 6 units of the present dialyzer, the same efficiency of dialysis was accomplished in 20 hrs. If more units had been used, the time would have been proportionately shorter. The dialyzing water was cooled by passing through copper coils immersed in an ice bath, and the dialysis was carried out at 2° C without the necessity of working in a large, cold room.

In summary, a continuous dialyzer has been constructed which should find applications both as a laboratory tool and as an "Artificial Kidney." Advantages include a large dialyzing surface relative to the volume of contained solutions, efficient dialysis because the fluids on both sides of the Cellophane are in a thin film and rapidly moving countercurrent to one another, and easy sterilization of the completely assembled unit. The apparatus shows promise for the treatment of acute renal insufficiency.

References

1. ABBOTT, W. E., and SHEA, P. *Amer. J. med. Sci.*, 1946, **211**, 312.

2. ABEL, J. J., ROWNTREE, L. G., and TURNER, B. B. *J. Pharm. exp. Therap.*, 1914, **5**, 275.
3. ALWALL, N. *Acta Med. Scand.*, 1947, **123**, 317.
4. BLISS, S., KASTLER, A. O., and NADLER, S. B. *Proc. Soc. exp. Biol. Med.*, 1932, **29**, 1078.
5. FINE, J., FRANK, H. A., and SELIGMAN, A. M. *Ann. Surg.*, 1946, **124**, 857.
6. FRANK, H. A., SELIGMAN, A. M., and FINE, J. *J. A. M. A.*, 1946, **130**, 703.
7. GOODYEAR, W. E., and BEARD, D. E. *J. A. M. A.*, 1947, **133**, 1208.
8. KATZ, Y. J., and GOLDBLATT, H. *J. exp. Med.*, 1943, **78**, 67.
9. KOLFF, W. J. *New ways of treating uremia*. London: J. and A. Churchill, 1947.
10. KOLFF, W. J., and BERK, H. T. *Acta Med. Scand.*, 1944, **117**, 121.
11. LAM, C. R., and PONKA, J. L. *J. lab. clin. Med.*, 1947, **32**, 1434.
12. MALUF, N. S. R. *Fed. Proc.*, 1948, **7**, 77.
13. MURRAY, G., DELORME, E., and THOMAS, N. *Arch. Surg.*, 1947, **55**, 505.
14. ODEL, H. M., and FERRIS, D. O. *Proc. staff Meet. Mayo Clin.*, 1948, **23**, 201.
15. SELIGMAN, A. M., FRANK, H. A., and FINE, J. *J. clin. Invest.*, 1946, **25**, 211.
16. VERMOOTEN, V., and HARE, D. M. *J. Urol.*, 1948, **59**, 907.

Sensitivity of the Ninhydrin Reaction in Paper Partition Chromatography¹

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The method of Consden, Gordon, and Martin (1) for the qualitative separation of amino acids by partition chromatography on filter paper is being used by the authors in studies of the amino acids in the blood and tissues of insects. This technique is particularly adaptable to physiological investigations with insects because it requires only micro quantities of the substances to be analyzed. Most of the free amino acids present in 25 microliters of insect blood can be qualitatively identified by this method.

In order to estimate quantitatively the free amino acids present in insect blood and tissues, and in order to determine the quantities of blood or tissue extracts necessary to identify qualitatively all of the amino acids present, the sensitivity of several amino compounds to the ninhydrin reaction was determined. This was done after pure amino compounds had been run in the separation chambers under the same conditions as the biological fluids being analyzed. Measured quantities of freshly made, pure solutions were placed on the filter paper, run 28 hrs in water-saturated phenol in one dimension, dried, run for 60 hrs in the second dimension in a water-saturated 1:1 mixture of γ -collidine and 2,4-lutidine, and again dried. The temperature was held between 23° and 26° C during the runs. The paper was then sprayed with a 0.1% solution of ninhydrin in normal butanol and heated in an oven for 5 min between

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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

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
Cystelic 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

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 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 Shape¹

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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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