Comments and Communications

Use of Modified Ninhydrin Reagent in Quantitative Determination of Amino Acids by Paper Chromatography

Procedures for the quantitative estimation of amino acids separated on paper chromatograms have been reported.[1] In this laboratory we have been able to measure quantitatively some free amino acids in tissues of the rat by a method described elsewhere (AWAPARA, J. *J. biol. Chem.*, 1949, in press). Briefly, the procedure consisted in 1) locating the amino acid spots with a 0.05% ninhydrin solution in water-saturated butanol; 2) cutting the spots from the chromatogram and developing full color with 1 ml of 2% aqueous ninhydrin solution in presence of pyridine by heating on a boiling water bath for 20 min; and 3) transferring the colored solution to a volumetric flask and measuring the color intensity in the Beckman Spectrophotometer at 570 mμ.

TABLE 1

Density Values at Various Concentrations of Amino Nitrogen

Amino nitrogen	Optical density			
γ*	Glutamic acid	Aspartic acid	Glycine	Alanine
4	0.124	0.155	0.128	0.105
8	0.343	0.313	0.277	0.218
12	0.551	0.487	0.434	0.410
16	0.741	0.665	0.621	0.555

* Values given correspond to solutions made up to a volume of 25 ml, and corrected for paper blanks.

Recently S. Moore and W. H. Stein (*J. biol. Chem.*, 1948, **176**, 367) have reported a modified ninhydrin reagent. They used a 2% ninhydrin solution in methyl cellosolve water at pH 5 (citrate buffer), which contained stannous chloride as a reducing agent. We have used this reagent instead of ninhydrin pyridine solution and obtained more consistent results and higher intensity of color. The intensity was found to increase considerably when larger amounts of reagent were used, up to 3 ml. Amounts greater than 3 ml were found to give constant values.

Table 1 shows density readings obtained at various concentrations for solutions of glutamic acid, aspartic acid, glycine, and alanine. In all cases the color intensity is a straight line function of the concentration. The greatest source of error in this procedure is the filter paper, which gives blank readings ranging from 0.070 to 0.100. This range of 0.030 units indicates an error of about 7% when 10 γ of amino nitrogen are being determined. The reproducibility of analyses carried out on the same sheet of paper is, however, very satisfactory with variations of about 1 to 2%. Best results were obtained when an aliquot of the colored solution was removed from the tube and made to a convenient volume. By this procedure, the difficult removal of all the color on filter paper remaining in the tube was avoided. The amount of color adsorbed on filter paper was constant.

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[1] AWAPARA, J. *J. biol. Chem.*, 1949, in press; FISHER, R. B., PARSONS, D. G., and MORRISON, G. A. *Nature*, 1948, **161**, 764; NAFTALIN, A. *Nature*, 1948, **161**, 763; POLSON, A., MOSLEY, V. M., and WYCKOFF, R. W. G. *Science*, 1947, **105**, 603; and WOIWOD, A. J. *Nature*, 1948, **161**, 169.

More on High School Biology Requirements

Dr. J. van Overbeek's apprehensions as to the effects of the inadequate teaching of the biological sciences in the secondary schools of this country (*Science*, 1949, **109**, 210) are undoubtedly shared by a good many other biologists. I certainly agree that more adequate instruction in biology is needed in most of our secondary schools. However, Dr. van Overbeek's suggestion that we emulate the admirable Dutch program and require a course in biology in each year of high school is highly impractical. While he might have no difficulty convincing me and other biologists that this would be desirable, he would have little success in convincing most of those educators who are responsible for the secondary school curricula throughout the country. He would first have to convince them that *one* year of biology should be required of all high school students. At present many students are graduating from high school with nothing more in the way of science than a single (and usually inadequate) course in general science. It seems that the trend has been to reduce, rather than to increase, the science requirements for graduation from secondary schools.

Even if Dr. van Overbeek were successful in his efforts to have three or four years of biology required for graduation from high school, the program would fail through lack of qualified instructors. There are not even enough qualified biology teachers to handle the high school biology courses now offered. While there are many excellent teachers of high school biology scattered throughout the country, I believe it is safe to state that the majority, even in large cities, have so little training and interest in the subject that they almost waste their students' time. The prospects of an improvement in this situation are not encouraging. As all college and zoology professors know, their better and more capable majors go on to graduate school and eventually into research or college teaching.

Superior students are encouraged to do this, and perhaps rightly, but the result is that there are not very many really good high school biology teachers. This situation has apparently concerned most professional biologists very little, but I believe that there are now many who will agree with Dr. van Overbeek as to the importance of a biologically literate public and the role of the secondary schools in producing it.

Professional biologists, through their various societies, might well support a program designed to improve the quality and quantity of secondary school biology. While there may be little hope of attaining the goals set by Dr. van Overbeek, I think that substantial progress could be made toward securing the more general requirement of a year of biology for graduation from high school, and toward the improvement of these high school biology courses and the recruiting of more capable and interested teachers for them.

I believe that professional scientists might also take a greater interest in supporting and encouraging the extension of the excellent elementary school science programs which have been established in some cities. It is quite possible that good elementary school science instruction can have a more thorough and lasting influence than secondary school science instruction in developing a public with an interest in and appreciation of science and its methods, attitudes, and problems of general concern. Here again, one of the principal difficulties is adequately trained teachers.

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Errata

My attention has been drawn by correspondents to two incorrect statements in my article "Research and Politics" (*Science*, March 4, 1949). First, it was stated that Vavilov died in a concentration camp. This should have read "died in exile in a remote region of the USSR," since the details are unknown. Second, I said that Kammerer committed suicide after going to Russia. Actually, he did so while still in Vienna, at the moment when he was finally to move to Moscow.

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I would like to call your attention to an error that crept into the paper on neomycin by Waksman and Lechevalier (*Science*, March 25). On page 307, right column, lines 1, 8, and 9, the letter μ should read *u*, or *unit*.

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In the paper "The Effect of Choline-Deficiency on Uterine Activity of Rats," by Kraatz and Gruber (*Science*, March 25), the tenth line from the end, p. 312, ". . . choline every second" should be amended to read ". . . choline every second day."

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(*Continued from page 375.*)

a plausible straight line is obtained which has an intercept on the $y + R$ axis of value R since $y = 0$ when $R = 0$.

The value so derived for R is 14 ± 2 mμ. Careful measurements over a wider range of ionization densities are in progress. The figure agrees well with the figure 18 mμ deduced by Luria and Exner (*3*) from observations of X-ray inactivation.

The results of deuteron bombardment of T-1 phage are thus consistent with the following hypotheses:

1. A target exists, of diameter 28 ± 4 mμ which is very much smaller than the phage itself and indeed smaller than the head of the phage.
2. The measured target size is larger than the true target, due to the fact that radiation from the path of a deuteron which misses the target can inactivate the phage.

References

1. DEMEREC, M., and FANO, U. *Genetics*, 1945, **30**, 119.
2. LEA, D. E. *Brit. J. Radiol.*, suppl. 1, 1947, p. 35.
3. LURIA, S. E., and EXNER, F. M. *Proc. Nat. Acad. Sci., Wash.*, 1941, **27**. 370.