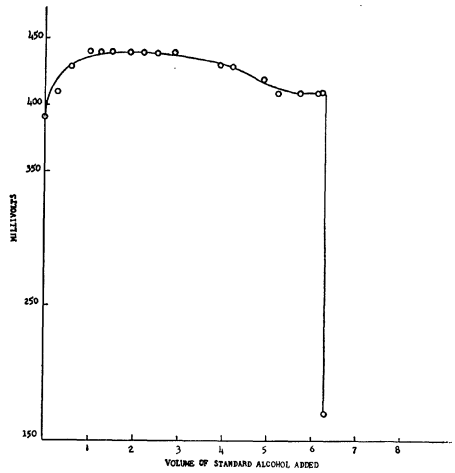

FIG. 2. Electrometric titration of isobutyl alcohol.

including $-OH$, $-NH_2$, R_2CO, RCHO, RCO_2R, and other easily reducible groups based on $LiAlH_4$ titration.[3]

The apparatus used, shown in Fig. 1, consists of a silver electrode *A*, salt bridge *B*, an isolated silver electrode *C*, and an automatic burette *D*. Dried and purified tetrahydrofuran is used to fill the salt bridge and the isolated electrode chamber, LiBr being employed as the electrolyte in both cases. In addition, a small amount of iodine is added to the electrode chamber to act as a depolarizer. The potential of the electrical cell is most conveniently followed by an indicating potentiometer such as Beckman Type H pH meter.

TABLE 1

ELECTROMETRIC TITRATION OF ISOBUTYL ALCOHOL

Wt of sample in g	Ml of alcohol sol.*			Moles of alcohol		
	Run	Blank	Diff.	Present	Found	% error
2.653	6.30	16.70	10.40	0.0359	0.0358	0.3

* Ethyl alcohol 20.00% by volume.

The procedure employed is as follows. A weighed sample of approximately 1 g of the unknown substance to be analyzed is introduced into the reaction vessel. Fifty ml of pure tetrahydrofuran and 20 ml of a solution of $LiAlH_4$ in tetrahydrofuran are added in turn and allowed to stand for a few minutes. The excess hydride is determined potentiometrically by adding a standard solution of ethyl or propyl alcohol in dry benzene. The end point is indicated by a sharp change in the voltage of the electrical cell. The difference between the amount of alcohol consumed during this back titration and that used during a blank run made without the sample represents stoichiometrically the amount of substance in the sample capable of reacting with $LiAlH_4$.

Typical results are shown in Fig. 2 and Table 1. In calculating the molar concentration of various functional types other than an alcoholic hydroxyl, it must be kept in mind that $-OH = -NH_2 = R_2CO = RCHO = -CO_2R = \frac{1}{3} - CO_2H$.

More complete details on the development and application of the titrimetric method described will be presented shortly in another publication.

[3] The use of $LiAlH_4$ for quantitative determination of various functional groups is not new. Hochstein (*1*), Zaugg and Horrom (*3*), Krynitsky *et al.* (*2*), and others have described gasometric methods analogous to the Zerewitinoff method based on measurement of hydrogen gas liberated by the sample on reaction with the hydride. These are, however, not true volumetric methods where a standard solution is added to a stoichiometric end point indicated by some visual or electrical change.

References

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Quantitative Evaluation of Gomori Histochemical Preparations[1]

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Several of the histochemical methods for the demonstration of enzyme activity, which have been developed by Gomori, result in the localized deposition of an insoluble sulfide. The present method permits the quantitative estimation of the enzymatic activity represented in such microscopic sections. There are theoretical and practical objections to methods based on visual or photometric measurement of the blackening (sulfide) as deposited in the section. In the present procedure, the sulfide in a section is extracted and converted to methylene blue by a modification of the method of Fogo and Popowsky (*1*), and the methylene blue in solution is then measured photometrically. The method does not permit comparison between localized areas in any one section unless these are excised, but it will give a measure of differences between separate sections. As given, the procedure deals with volumes 1000 times smaller than those described by Fogo and Popowsky, but the reaction is so sensitive that intermediate scales of volume will serve for most histological preparations.

Procedure. Paraffin sections are placed at the bottom of small (6 × 50 mm) test tubes and deparaffinized with benzene, rinsed with acetone, and dried in air. The section should lie flat against the glass tube without folds. A section previously used for microscopic study is suitable if transfer from the slide to the test tube can be accomplished without loss.

The Gomori substrate as used for microscopic preparations is added and the section incubated for the same period of time as an adjacent serial section, prepared on a slide for microscopic examination. (Sections for mi-

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

croscopy are mounted dry on albumin-coated slides to avoid destruction of some of the enzyme which occurs when water is used to flatten paraffin sections). The enzymatically liberated phosphate is converted to the lead salt, and the usual treatment with ammonium sulfide and careful rinsing with water follows. It is necessary to substitute lead for the cobalt of the alkaline phosphatase procedure, since cobalt sulfide is insufficiently soluble in the HCl required subsequently.

TABLE 1

Duration of incubation (hr)	Optical density of methylene blue*	μg Sulfide (approx.)	μg Sulfide†/hr since previous determination
0.5	.60	.195	
1.0	.75	.244	.098
2.0	.94	.305	.061
4.0	1.21	.393	.044
6.0	1.48	.480	.044
8.0	1.64	.530	.025

* Final volume 277 μl.

† Includes sulfide derived from preformed (nonenzymatic) phosphate.

To the tube containing a dry section stained with lead sulfide, there is added 135 μl of water and 25 μl of a solution of 0.1 g *p*-amino dimethyl aniline sulfate (Eastman Kodak Company) in 100 ml of 5.0 *N* HCl. After standing 20 min with occasional stirring to achieve solution of the sulfide, there is added 5 μl of 0.023 M ferric chloride in 1.2 *N* HCl. This is stirred and allowed to stand for 20 min and the methylene blue is determined in a spectrophotometer at 670 mμ using microcuvettes. For larger or heavily stained sections, final volumes of 3.0 ml may be more suitable. Appropriate lead sulfide standards are also prepared.

Experiments. Serial sections of rabbit appendix 10 μ thick and 14 sq mm in area were incubated in Gomori glycerophosphate substrate for alkaline phosphatase pH 9.5 for from ½ to 8 hr at 26° C. Microscopically, this gave a range from faintly stained sections at ½ hr to generalized overstaining after 8 hr. The amount of sulfide found after the various periods of incubation is shown in Table 1. From the table it is evident that in this material the rate of deposition of sulfide decreased appreciably after 6 hr of incubation under these conditions. Both the amount of sulfide formed and the time scale of decrease in enzymatic activity are in fair agreement with expectation based on independent quantitative microestimations under standard quantitative conditions.

The method should be applicable to any of the histochemical procedures involving deposition of a sulfide soluble in hydrochloric acid.

Reference

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Comparison of Electroencephalographs of Young Rats from Dams on Synthetic and on Normal Diets[1]

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Richardson and Hogan (*1*) observed hydrocephalus in young rats born of dams on a synthetic diet, and concluded that the abnormality was due to a deficiency in the maternal diet. Young rats with the same nutritional history, though free from any sign of hydrocephalus, learn their way through a maze more slowly than do those from the stock colony (unpublished data). In a search for other differences between these two groups of rats, their electroencephalographs were compared. There were no significant differences; however, since to our knowledge observations of this kind on the rat have not been published, it seemed desirable to have

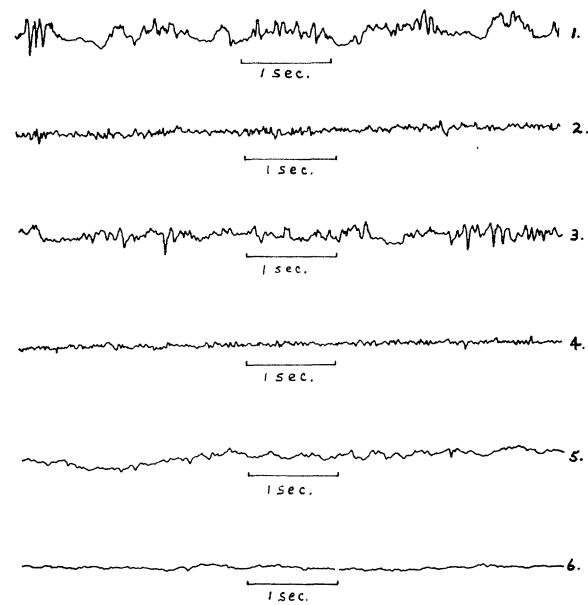

FIG. 1. Electroencephalographs of a normal rat from dam on the stock diet, a rat from dam on the synthetic diet and a hydrocephalic rat from dam on the synthetic diet. 1—Lead I, and 2—Lead II; normal rat from dam on the stock diet. 3—Lead I, and 4—Lead II; rat from dam on the synthetic diet. 5—Lead I, and 6—Lead II; hydrocephalic rat from dam on the synthetic diet.

a typical electroencephalograph of this animal described in the literature.

Fifty-seven young rats, 4–8 weeks of age, from dams on the synthetic diet, were paired according to age, weight, and sex with an equal number from dams on the stock diet. They were given an intraperitoneal injection

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