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New Series of Reagents Useful in Fractionation of Biologically Active Material.

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Aside from the classical procedure of Kossel and Kutscher for the fractionation of nitrogenous bases, we possess no methods which permit a systematic biochemical analysis of nitrogenous substances occurring in nature. The Kossel procedure, although extremely useful for the fractionation of stable substances, interferes very often with the activity of relatively unstable substances, such as vitamines, hormones and enzymes. Various aromatic nitro compounds have been extensively used in biochemistry, such as picric, picrolonic and flavianic acids. These reagents often yield characteristic, slightly soluble compounds with relatively pure substances, but often give little or nothing when we deal with complicated mixtures. The idea occurred to me that by using the salts of these acids a series of more specific reagents could be obtained. This expectation was realized. Where the free acid fails to affect a notable separation of active material, this was accomplished by using a salt of a particular acid, or a series of salts, one after the other. The salts used so far were barium, lead, silver and mercury salts, metals which can be removed from the solution without difficulty. In some cases sodium salts were used with success. The method was used either systematically, starting with picric acid and then using the various salts, or one particular salt was used exclusively. It is hoped that by introducing other salts, or salts of other acids, a systematic biochemical analysis of unknown substances can be effected.

Using this new method, vitamine B was separated quantitatively from yeast as well as from rice polishings, but curiously enough, not by the use of one single salt. A combination of Ag- and Hg-picrates was necessary to attain this end, which corroborates my view perhaps as to the complex nature of this substance. Contrary to the results of Jansen and Donath,[1] no trace of anti-beriberi vitamine could be precipitated from the decomposed picrates by means of platinum chloride in absolute alcohol. This substantiates my earlier findings in 1911. It also seems that vitamine C can be precipitated by a similar procedure, but quantitative data are not yet available.

The method has been applied also to the fractionation of hor-

mones. With insulin no marked success has yet been attained. With pituitrin, on the contrary, the oxytoxic activity could be ascribed to a simultaneous action of several substances[2] which, taken separately, showed little action on the uterus. Finally, by the use of sodium picrate in a very concentrated solution, pepsin could be divided into inactive ferment (propepsin) and the kinase. Detailed reports will be published.

[1] Jansen and Donath, *Gen. Tijdschr. v. Ned-Indie,* 1926, lxvi, 6.

[2] Reported at the Intern. Congress of Physiol., Stockholm, 1926. Comp. Report of Oliver Kamm at the meeting of Amer. Chem. Soc. in Detroit, 1927.

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Determination of Blood pH Without Transferring the Sample.

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The simple procedure described here has been devised to obviate the necessity of transferring the blood and of using mineral oil, both of which tend to complicate the technique for determining the pH colorimetrically. The only special apparatus* required for this consists of a sampling tube identical in shape and size (10x1.5 cm.) with the tubes used to contain the standard solutions. It is provided with a double-holed, hollow, glass stopper, which is ground-in and which has sealed to it a small, two-way stop-cock with two holes. (Fig. 1.) The apparatus is made of clear Pyrex or Nonsol glass and connections are made with special rubber tubing, which will not cause excessive film formation on the mercury. Before use all apparatus, including the mercury, is cleaned and tested for neutrality in a manner similar to that outlined by Austin, Cullen, *et al.*[1]

The general method followed is essentially that of Cullen[2] as modified by Hastings and Sendroy.[3] Whole blood is used instead of plasma in accordance with the method of Hawkins.[4] The necessary correction factor for converting the colorimetric readings to the true pH value is found by the method proposed by Austin, Stadie and Robinson.[5]

When a determination is to be made, the apparatus is assembled as shown in the accompanying figure. Mercury is run from the

* May be obtained from Technical Glass Apparatus Co., 42 Galusha Street, Rochester, New York.