when the precipitated sodium salts were treated with benzene and filtered, the 132° body crystallized from the filtrate. This is to be expected since, just as in Expt. IV, this solution contains the products which are present in the liquid phase.

Further confirmation of the reversibility of the reaction was obtained in Expt. V, in which a succino-malonic ester was formed by replacing succinic ester by succinosuccinic ester. This reaction is probably represented by the equation

$$C_{12}H_{15}O_6Na + C_7H_{12}O_4 \rightleftarrows C_{11}H_{13}O_6Na + C_8H_{14}O_4.$$

In the earlier experiments the succinosuccinic ester was stable to this body in the presence of malonic ester owing to the higher concentration of succinic ester. It would appear that to obtain this body it would be best to use succinosuccinic ester and phloroglucinol tricarboxylic ester. However, it is likely that in these experiments the true equilibria were not reached, because the amorphous bodies obtained are more stable than any other. Too efficient conditions, therefore, are apt to form little else. So that it is not surprising that malonyl-succinic ester was not found among the sodium salts in Expt. VI.

The nature of the crystalline bodies obtained only give the major reaction which has taken place. Without doubt all the other possible substances are present in smaller amounts, at least in the liquid phase.

Owing to the lack of control in these experiments nothing definite can be said with regard to the effect of temperature on the equilibria.

Summary.

1. Equimolecular mixtures of malonic and succinic esters heated with odium give chiefly succinosuccinic ester.

2. When a large excess of malonic ester is used phloroglucinol tricarboxylic ester is found.

3. By using succinosuccinic and malonic esters malonyl-succinic ester is obtained.

4. These results are explained by Dieckmann's theory that the acetoacetic ester condensation is reversible.

[Contribution from the Richardson Chemical Laboratory of Tulane University.]

SALIVARY AMYLASE. I. A PRELIMINARY EXPERIMENTAL STUDY OF ITS STABILITY IN SALIVA.

By Rollin C. Myers and Leonard C. Scott.

Received June 18, 1918.

There appears to be a general view held among chemists that the enzymes as a class of substances are very prone to change, the change being marked by a slow or rapid decline of their power of degrading a given

substrate. This view is upheld by a great number of observations, based on experiments which are especially contrived to study the influence of prescribed conditions, by noting any change in the quantity of substrate transformed in unit time. In most cases, though within narrow limits, variations of the concentrations of electrolytes and nonelectrolytes, of temperature and of the intensity of light, are followed by variations in the activity of the enzymes. While in some cases there appears to be a positive increment activity, *e. g.*, after the addition of amino acids to amylase, or in the presence of a temperature of 40°, the general effect is opposite in character to this. It is only necessary to recall the rapidly destructive action of hydrochloric acid and sodium carbonate in increasing concentrations beyond 0.3 and 0.4% on amylase, peptase, lipase and nuclease in solution. In addition to this, temperatures above 80° for any length of time cause a complete destruction of enzymic activity. From a practical standpoint the notorious difficulties met with in the preparation and purification of the enzymes, because of the sensitiveness of these bodies to precipitants, only strengthen the prevailing view. In fact, without any other evidence, the decided colloidal nature of the enzymes and their complex chemical structure, alone, would be sufficient to favor the view that these bodies are very changeable in nature.

So far as the writers can determine, but little work has been done in respect to the stability of salivary amylase in saliva over long periods of time. Hatta[1] finds that salivary amylase does not change when preserved under a layer of toluene for 6 months, though in the case of formaldehyde and phenol the activity was lost in one month. Statements have been made that scale pepsin suffers a decided loss in activity in the course of several years' standing. The writers examined a sample of ox ptyalin which had been purchased several years ago. It was found to possess little, if any, activity.

In respect to salivary amylase, the writers found that several samples of saliva, over a year old, still possessed amyloclastic activity. No preservative was added to these salivas, though the containers were kept securely corked. This does not exclude mycotic contamination. It is well known that yeast cells, mould spores, and bacteria are always present in the air, so that the possibility of contamination with these organisms cannot be excluded. That these lower forms of vegetable life are known to produce enzymes which have amyloclastic properties to a greater or less degree is beyond question. In order to exclude any possible contamination with enzyme-producing organisms, in other words, to render the saliva sterile, two fresh samples obtained by paraffin stimulation, were first filtered through absorbent cotton to remove the larger particles of food detritus and paraffin, then passed through a sterile Berkefeld

[1] Hatta, *Mitt. Med. Fak. Univ. Tokio*, 14, No. 3 (1915).

filter of the finest grade and received into a sterile flask. The saliva collected in this manner was tested for sterility after standing 48 hours at 37° by inoculating broth tubes with one cc., incubating for 24 hours at 37° and then plating out one cc. on a 0.5% glucose agar. The object of the broth culture was to enable these organisms of low vitality to reproduce in an optimum medium as well as to increase the numbers of those bacteria which might be present. In the event of one cc. of this broth being found sterile, as shown by the agar plate, the certainty of the original saliva being sterile was therefore assured.

Both samples of saliva were found to be sterile after 48 hours' incubation of the agar plates, and were sealed on May 5 and on May 25, 1917. On June 1, 1918, they were again tested in the manner described and found sterile.

Parallel experiments, in which 3 other salivas, preserved, respectively, with toluene, chloroform and thymol, were made.

As an index to the amyloclastic activity of these salivas, the method of Sherman, Kendall and Clark[1] was used.

The following table is a summation of the results obtained:

TABLE I.—AMYLOCLASTIC ACTIVITIES.

Saliva. Sample.	Age.	Vol. used. Cc.	Mg. Cu_2O. 1917.	Mg. Cu_2O. 1918.	% decrease in activity.	Remarks.
Sterile, I	1 yr.	0.1	154.3	48.9	68.3	The prevailing conditions under
Sterile, II	1 yr.	0.1	215.7	213.2	1.2	which these samples were pre-
Thymole	1 yr.	0.1	329.4	68.8	79.1	served were diffused light and
Chloroform	1 yr.	0.1	192.4	35.1	81.8	laboratory temperatures from
Toluene	1 yr.	0.1	369.4	175.0	52.7	18° to 28°.

In addition to the tabular data given, an unprotected saliva which had been allowed to age for 2.5 years in a stoppered flask was examined. This saliva was able to convert starch solution to the acroo-dextrin stage, *i. e.*, to the achromic point, though but little farther, since Fehling's solution was only slightly reduced, a property in sharp contrast to several other protected and relatively fresh salivas, which reduced Fehling's solution. 2.5 cc. of this saliva was not sufficient to reduce 5 cc. of Fehling's solution, in fact the blue color differed but slightly from that of the control tube, according to the Lintner method. The saccharifying property seemed to be absent. The Wohlgemuth value was approximately $D_{60}^{40} = 5.5$. In agreement with the supposition that salivary amylases really consists of two enzymes instead of one, it might be inferred that the saccharifying enzyme was less stable over long periods of time than that which transformed the starch to acroo-dextrin. In view of the lack of convincing evidence on this point, the writers assume that salivary

[1] Sherman, Kendall and Clark, 2 articles, THIS JOURNAL, 32, 1073 (1910).

amylase is a single enzyme which has for its specific function the degradation of starch to maltose.

Conclusions.

1. *Salivary amylase in sterilized saliva without preservative is relatively stable for one year.* This relative stability may vary from practically no change to that of over 50% of its former amyloclastic activity, the variation depending probably on slight differences in the composition of the saliva.

2. The causes which lower the stability of salivary amylase in saliva are not solely the degrading action of bacteria, mould spores, yeast plants and special preservatives. The inherent chemical weakness of the enzyme molecule, rather, must be taken into account, which weakness may be increased by the presence of temperatures from 18 to 30°, diffused light and compounds in the saliva.

3. *Salivary amylase in saliva is relatively stable for a year when preserved with toluene, thymol and chloroform.* Toluene has the least destructive action on the enzyme and thymol and chloroform follow in order.

4. Saliva may be kept for 2.5 years under the ordinary laboratory conditions without preservative and may still show a form of amyloclastic activity.

NEW ORLEANS, LOUISIANA.

[CONTRIBUTION FROM THE OTHO S. A. SPRAGUE MEMORIAL INSTITUTE AND THE DEPARTMENT OF PATHOLOGY OF THE UNIVERSITY OF CHICAGO.]

STUDIES ON PROTEINOGENOUS AMINES.

I. THE SYNTHESIS OF β-IMIDAZOLYLETHYLAMINE (HISTAMINE).

BY KARL K. KOESSLER AND MILTON TH. HANKE.

Received August 12, 1918.

The synthesis of β-imidazolylethylamine (β-aminoethylglyoxaline) reported herewith is based on the synthesis of this substance reported by F. L. Pyman.[1] Diaminoacetone dihydrochloride, obtained from citric acid, is heated with sodium sulfocyanide. The thioglyoxaline thus formed, according to Gabriel's[2] general method, is oxidized with nitric acid and through action of nitrous acid formed the hydroxymethylglyoxaline is obtained; over the chloro-compound the nitril is prepared which on reduction yields the amine. While we have followed in the main Pyman's procedure, several additions and improvements have been made which warrant a detailed report of some of the steps involved in the synthesis of this substance which, on account of its remarkable physiological properties, is of great interest to the biochemist.

[1] F. L. Pyman, *J. Chem. Soc.*, **99**, 668 (1911).

[2] Gabriel, *Ber.*, **26**, 2204 (1893); *Ibid.*, **27**, 1037 (1894).