developed; a clear picture of the whole bone was visible, as is shown by the accompanying photographs. It is convincingly shown in these that the epiphysis contains a larger quantity of the active phosphorus than the diaphysis does. Hence the results of measurements of the radioactivity of the several parts of the bone are fully in accordance with the photographs obtained.

Thus it appears from the radioactivity measurements and also from the radiographs that phosphorus metabolism is more intense in the bone of the rachitic chicken than in the bone of the normal chicken, and also phosphorus metabolism is more intense in the epiphysial part than in the diaphysial part of the same bone.

We are now trying to investigate whether it is possible to estimate photographically the quantity of the active phosphorus present in the several parts of a bone.

Finally, we wish to express our thanks to the Philips Lamp Works for furnishing the radioactive phosphorus used in these experiments, and to Mr. J. C. de Back for his assistance in these investigations.

M. J. L. Dols.
B. C. P. Jansen.
Laboratory of Physiological Chemistry,
University, Amsterdam.
G. J. Sizoo.
G. J. van der Maas.
Natuurkundig Laboratorium,
Vrije Universiteit,
Amsterdam.
Oct. 27.

[1] Brouwers, A., Heyn, F. A., and Kuntke, A., *Physica*, **4**, 153 (1937).
[2] Nature, **139**, 1068 (1937); *Proc. Roy. Acad., Amsterdam*, **40**, 547 (1937).

Enzymic Deamination of Aspartic Acid

From the cell-free solution of *B. fluorescens liquefaciens*, which has a powerful aspartase effect[1], we have isolated aspartase by adding acetic acid up to *p*H 4·6 at 0° C. The precipitate thereby formed (precipitation begins at *p*H 4·8) gives, when rapidly filtered and dissolved in *M*/15 phosphate buffer (*p*H 7), a solution which possesses considerable aspartase and also asparaginase effect. This solution has no fumarase effect, even in experiments of long duration.

Despite the absence of fumarase, the enzyme solution obtained forms malic acid from fumaric acid in the presence of ammonia. The reaction occurs also in the presence of toluene. When a longer time is allowed, the reaction goes so far that only aspartic acid, malic acid and ammonia can be found in the solution; fumaric acid is not detectable. If we start with *l*-aspartic acid, there are formed fumaric acid, malic acid and ammonia. Fumaric acid is not converted into malic acid by our enzyme solution, if ammonia is absent.

The results led to the conclusion that the purified fumarase-free enzyme solution contains two different enzymes. One (I) is the actual aspartase and catalyses the reaction:

$$HO_2C.CH_2.CH(NH_2).CO_2H \rightleftharpoons HO_2C.CH=CH.CO_2H + NH_3;$$

the other (II) catalyses the following reaction:

$$HO_2C.CH_2.CH(NH_2).CO_2H + H_2O \rightarrow HO_2C.CH_2.CH(OH).CO_2H + NH_3.$$

Thus the hydrolytic deamination of an amino acid would be established for the first time. The latter reaction is not reversible. The conversion of fumaric acid into malic acid in the presence of ammonia proceeds through aspartic acid. Consequently, enzymes I and II are required for the reaction. Enzyme II acts more slowly than Enzyme I; therefore at first chiefly fumaric acid and ammonia are formed from aspartic acid.

We report the above results because Gale[2] has recently published investigations on the separation from the raw aspartase in *B. coli*-juice two different enzymes deaminating *l*-aspartic acid. One of his enzymes, however, does not act in the presence of toluene, and both of them contain fumarase, thus preventing elucidation of the mechanism.

In addition, we have also noted that the aspartase effect of the enzyme solution is approximately proportional to the enzyme concentration. Systematic exceptions to this rule are noted in that small quantities of enzyme act more favourably during a long reaction time than do large quantities during a short reaction time. The equilibrium constant for deamination obtained with a fumarase-free preparation was $K_{37} = 100$.

With the enzyme solution or with the suspension of *B. fluorescens liquefaciens* we were unable to accomplish the amino acid synthesis from oxalacetic acid and ammonia, or from succinic acid and ammonia.

A detailed account of this work will be published elsewhere.

Artturi I. Virtanen.
Jorma Erkama.
Biochemical Institute,
Helsinki.
Oct. 24.

[1] Virtanen and Tarnanen, *Biochem. Z.*, **250**, 193 (1932).
[2] Gale, *Biochem J.*, **32**, 1583 (1938).

Oxygen Exchange during Esterification

In a recent paper[1] on the exchange of oxygen between water and acetic acid, we pointed out that the mechanism of the exchange of the carboxyl oxygen atoms of the acid with the oxygen of the water, H–OH, was probably identical with the mechanism of esterification of an acid by an alcohol, *R*–OH, and ventured to predict that "when esterification of an acid occurs in the presence of about equal quantities of alcohol and water an exchange will be observed between the oxygen of the water and that of the acid running parallel to the simultaneous rate of esterification". We mentioned that we were carrying out experiments to test this view.

Meanwhile, Urey and Roberts[2] have esterified benzoic acid with methanol containing excess of the heavy oxygen isotope in order to determine which linkages are broken during esterification. They found it necessary to apply to their results a correction for the exchange between benzoic acid and the water formed during the esterification. They therefore measured also the rate of exchange between benzoic acid and heavy oxygen water under the same conditions as those pertaining during esterification.

Urey and Roberts find that 0·1505 moles of the acid were esterified, while simultaneously 0·0726 moles of the acid exchanged *both* its oxygen with those of the water. Taking into account the relative concentration (or better, the activities) of the water and methanol in the reaction mixture, this result indicates