[CONTRIBUTION FROM THE MAYO CLINIC AND THE MAYO FOUNDATION]

INSULIN AND AMINO ACID CATALYSIS

BY JOHN M. ORT

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Introduction

In a former paper[1] it was shown that in the presence of certain amino acids, the action of very dilute hydrogen peroxide on dextrose is hastened. Under the conditions of the experiments, other amino acids are without effect within six hours. This paper is a report of further studies on this action by the use of the oxidation-potential method. This time both dextrose and levulose were investigated. The effect of insulin on each was also determined, both in the presence and in the absence of the amino acids.

With the same technique as before, a study was made first on the influence of the rate of bubbles of the nitrogen gas which passed through the solutions, stirring them and freeing them from dissolved oxygen. In the former experiments the nitrogen passed through at a rate of approximately 25 bubbles a minute from the fire-polished end of a 6 mm. Pyrex capillary tube of 1 mm. bore, about 4 cm. below the surface of the liquid. In the experiments reported at this time, the rate of bubbles was approximately 2 a second. Doubling this rate did not have a significant effect on the shapes of the time-potential curves, in which form the data are presented.

The slower rate in the former experiments was considered safe since at that rate it was felt the nitrogen would completely carry out any oxygen that might diffuse into the chambers at the top, either through or around the rubber stoppers. On the other hand, substances reacting in the blood streams of animals are subjected to much more violent shaking and mixing than would be given by the twenty-five a minute rate. Therefore, it seems desirable to have present in the experiments the effect of the more rapid stirring, since it is hoped that the results may have some significance in connection with the mechanism of sugar oxidation *in vivo*.

In all experiments the solutions were first freed from most of the dissolved air by the very rapid passage of nitrogen (about 20 bubbles a second) for approximately one hour late in the afternoon. After this the rate was reduced to about 25 bubbles a minute in the former experiments and to about 2 a second in this series, and the nitrogen allowed to bubble through the solutions at the slower rate overnight. The next morning the potentials were found to be changing but very slowly, and the dissolved air was assumed to be completely removed. Hydrogen peroxide was then added and the drifts of the oxidation potentials with time were followed. Longer periods of standing, before the addition of the hydrogen peroxide,

[1] Ort and Bollman, THIS JOURNAL, **49**, 805–810 (1927).

did not change the results significantly. Neither did shorter periods, provided the removal of dissolved oxygen had proceeded far enough to develop within the solution a moderate reducing intensity of approximately −0.15 volt. When hydrogen peroxide was added much before this, the resultant oxidation potentials often rose to 0.3 volt and over. The latent reducing power of the sugars under these conditions seemed to be temporarily paralyzed, for recovery to the previous negative potentials was delayed. The same was true if too much hydrogen peroxide was added at one time.

It is again pointed out that the results of these experiments can have only a qualitative significance. In the absence of a single, definite, reversible, oxidation-reduction equilibrium no exactly reproducible potentials can be

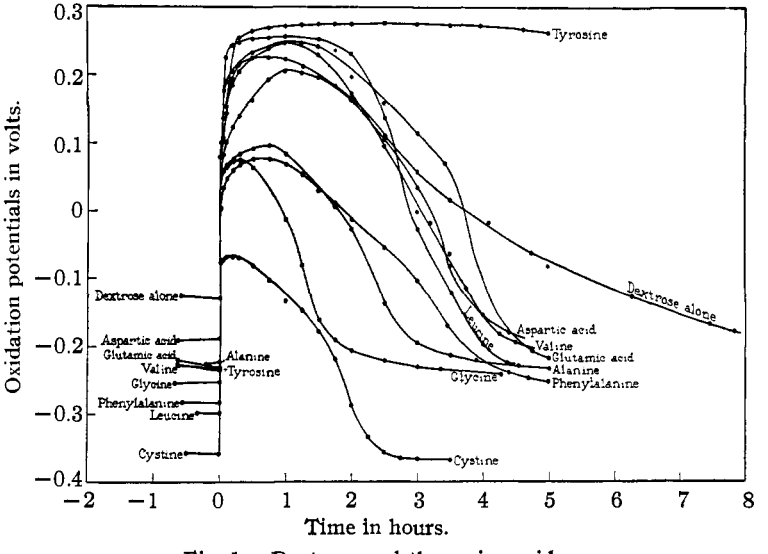

Fig. 1.—Dextrose and the amino acids.

expected. It is only assumed that the differences in the general shapes of the curves correspond to actual differences in the courses of oxidation of dextrose and levulose by hydrogen peroxide under the various conditions studied.

Results of Experiments

Figure 1 shows the effect of several of the amino acids on the action of 0.5 cc. of 0.03% hydrogen peroxide added at time zero to a solution of 200 mg. of dextrose in a buffer at P_H 10 and a temperature of 30°. The amount of amino acid present was 0.001 mole. With the exception of tyrosine, all had at least some accelerating action. This is somewhat different from the results published before. Glutamic acid and aspartic acid had little effect at the slower rate of bubbling, which is the only difference between the two sets of experiments. In Fig. 1, glycine, alanine and phenylalanine

stand together as a group apart from valine, leucine, glutamic acid and aspartic acid. This grouping seems to parallel even closer the grouping, as shown by the latest work, according to specific dynamic action. Tyrosine alone, which has been found to have marked specific dynamic action, seems out of place. But with tyrosine in the experiments as performed *in vitro*, the solutions became more oxidizing than the others, under which conditions, as already mentioned, the reducing power of dextrose becomes temporarily paralyzed. *In vivo* the blood has a posing action, which would prevent such an oxidizing intensity. At least tyrosine seems to have an effect on the oxidation of dextrose somewhat different from that of the other amino acids studied.

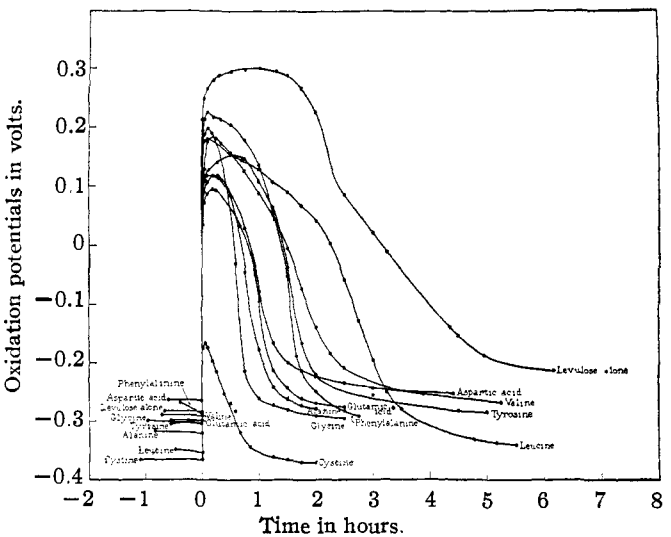

Fig. 2.—Levulose and the amino acids.

Figure 2 shows the results of similar experiments on levulose. As would be expected, recovery occurs sooner and more rapidly than with dextrose. With the exception of cystine, which exists in solution in a reversible system, glycine, alanine, glutamic acid and aspartic acid are the most effective catalyzers. This division seems to have no relation whatever to specific dynamic action. In comparing these curves to those in Fig. 1, it is interesting to note that for levulose as well as dextrose the rate of recovery is greatest in almost all cases in a region of oxidation potentials bordered very roughly by 0.05 and −0.15 volt. It can also be seen that in both cases the rate of fall in potentials drops off sharply in the neighborhood of −0.2 volt.

In Fig. 3 is shown the action of hydrogen peroxide on solutions of insulin and the amino acids, without any sugar. One cc. of Lilly's U 20 Illetin

was added in each case at the start of the run, the other details being as before. With the exception of cystine no action was indicated. These

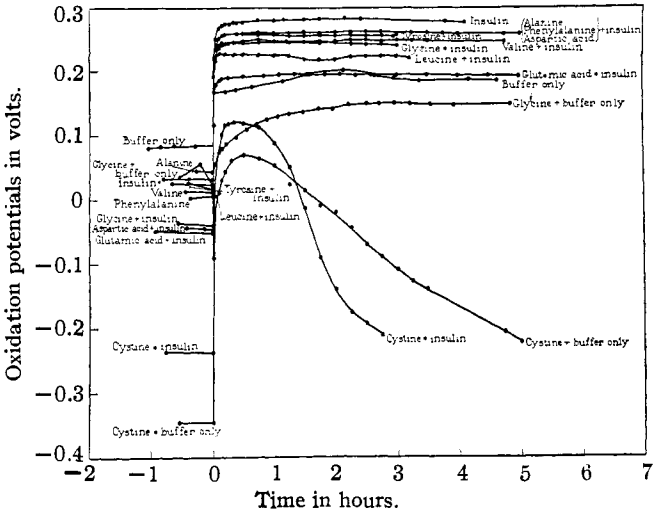

Fig. 3.—Insulin and the amino acids.

experiments were performed as controls for the studies with insulin on dextrose and levulose.

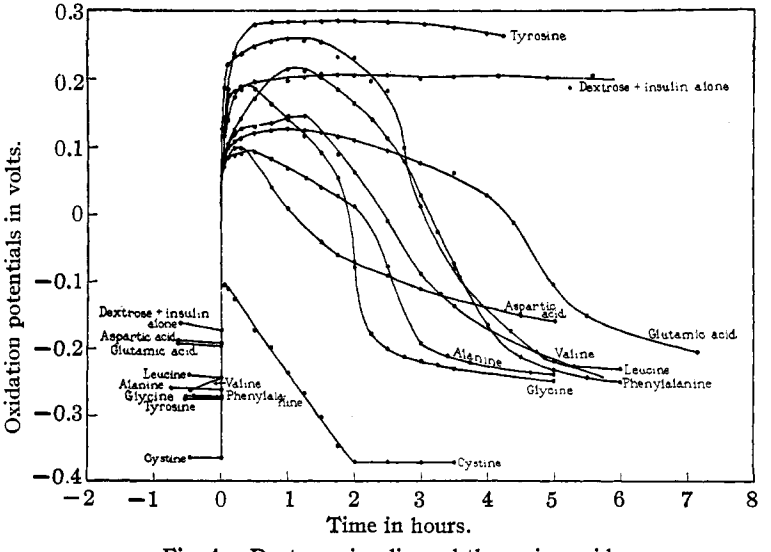

Fig. 4.—Dextrose, insulin and the amino acids.

The effect of insulin on the amino acid catalysis with dextrose is shown in Fig. 4. The action of hydrogen peroxide on dextrose alone seems to be

retarded by insulin. This is true to a less extent also in the presence of glutamic acid, glycine and phenylalanine. The catalysis in the presence of cystine and of aspartic acid was somewhat increased. Little change was found for tyrosine, alanine, valine and leucine. The grouping of the amino acids closely according to specific dynamic action seems somewhat lost in the presence of insulin, although complete recovery to reducing conditions, after addition of hydrogen peroxide, is reached again first in the presence of glycine, alanine and phenylalanine, cystine as usual being excepted.

Finally, Fig. 5 shows the results of similar experiments on levulose with insulin and the amino acids. Again the action of hydrogen peroxide on the sugar alone is retarded by the insulin. There is little suggestion of a

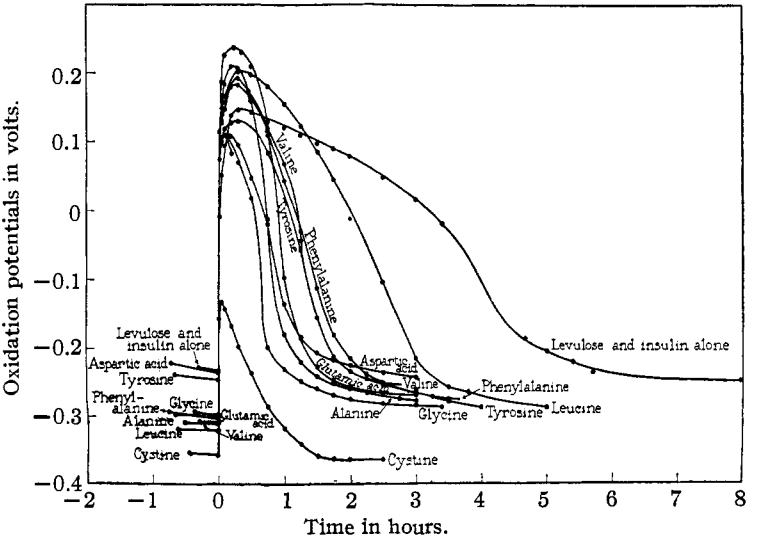

Fig. 5.—Levulose, insulin and the amino acids.

grouping of the amino acids according to specific dynamic action. The presence of insulin seems to be without much effect on the amino acid catalysis, although most of the curves in Fig. 5 are a little closer together than those in Fig. 2. Again, even when insulin is present, as shown in Figs. 4 and 5, attention is called to the fact that the maximal rate of recovery in all cases is in a region of oxidation intensity approximately between 0.05 and −0.15 volt and that the drop in potentials is abruptly slowed down below −0.2 volt.

Summary

A further study was made on amino acid catalysis of the action of hydrogen peroxide on dextrose and this was continued to include the action on levulose. The sole difference between the experiments on dextrose alone and those already published is in the faster bubbling of nitrogen gas in these

reported at this time. The parallelism between the grouping of the amino acids as catalyzers with dextrose and their grouping according to specific dynamic action is even closer than in the former experiments. Tyrosine alone, which yields slightly higher oxidation potentials than the others, is an exception. At these higher potentials whenever they were obtained in any manner with dextrose alone or with other amino acids it was noticed that the reducing power of the sugar was temporarily paralyzed and the return to more negative potentials much delayed. The connection between amino acid catalysis with levulose and specific dynamic action is much less close than with dextrose.

When insulin was added it was found that the action of hydrogen peroxide on both dextrose alone and levulose alone was retarded. For both sugars insulin had no marked effect on the amino acid catalysis. Some effect, however, was noticed in a smoothing out of the differences between the various amino acids.

Attention is called to the fact that in all cases the maximal voltage change towards a return to reducing conditions takes place in a region approximately between 0.5 and -0.15 volt and that below -0.20 volt the drop is rather quickly stopped. It is hoped in a future paper to discuss the significance of these facts in relation to the mechanism of sugar oxidation in alkaline solutions.

ROCHESTER, MINNESOTA

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

A STUDY OF THE OPTIMAL CONDITIONS FOR THE PREPARATION OF TERTIARY BUTYLMAGNESIUM CHLORIDE

BY HENRY GILMAN AND E. A. ZOELLNER

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Introduction

It was previously shown by Gilman and McCracken[1] that the yields of the four butylmagnesium bromides decrease in striking fashion with the increasing complexity of branching of alkyl groups, and to such an extent that *tert.*-butylmagnesium bromide gave a yield less than one-fourth of that obtained with *n*-butylmagnesium bromide. *Tert.*-butyl alcohol is now available at a low cost and the chloride is readily prepared from it in very good yield. Because alkyl chlorides give better yields of RMgCl compounds than the corresponding alkyl bromides[1] a study has been made of several factors affecting the optimal conditions for the preparation of *tert.*-butylmagnesium chloride. As a result of such studies, this tertiary Grignard reagent can now be prepared in quite satisfactory yields.

[1] Gilman and McCracken, THIS JOURNAL, **45**, 2462 (1923). Also, Gilman and Kirby, *ibid.*, **48**, 1733 (1926) for better yields obtained from chlorides over bromides.