

Technical Papers

Anomalous Thermal Behavior of Salivary Apoerythrin Activity

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Eakin and co-workers have adapted a modified vitamin B₁₂ assay to the measurement of apoerythrin (pernicious anemia "intrinsic factor") activity (1) and demonstrated the presence of apoerythrin in saliva (2). The assay depends upon the ability of apoerythrin to bind vitamin B₁₂, making it unavailable for the growth of *Escherichia coli*, which depends upon the vitamin for growth in a specially defined medium. Apoerythrin activity is thus indicated in the assay by inhibition of bacterial growth and is quantitated by comparison of the inhibition with that produced by a standardized solution of gastric mucosa. One unit of apoerythrin is able to bind 1 µg of vitamin B₁₂, and the results are reproducible to within 5%. In studies on the thermal stability of salivary apoerythrin we have now discovered that all activity disappears from saliva heated to 70° C for periods of 1 min or longer, but that samples heated for 5 min at either lower temperatures, or higher temperatures up to 100°, show no such loss in activity. Typical results are shown in Table 1. The data represent the

TABLE 1
EFFECT OF HEATING UPON THE ACTIVITY OF 1
MILLIUNIT SALIVARY APOERYTHRIN

Treatment temperature °C	Apparent activity of 1 milliunit apoerythrin
30	1.00
60	.85
65	.45
70	0
75	.20
80	.80
100	1.00
90-70	.95
70-90	0

effects upon potency obtained by heating 1 milliunit of salivary apoerythrin. All samples were heated for 5 min at the indicated temperatures except the last two, which were heated for 5 min at each of the indicated temperatures and in the order shown.

If samples are first heated to temperatures higher than 70° C and subsequently heated to 70°, there is no loss of activity. Samples heated at 70° C for longer than 1 min lose their activity and cannot be reactivated by subsequent heating to a higher temperature. It appears that the high rate of inactivation at 70° does not influence appreciably samples elevated through the critical temperature range at a sufficiently rapid rate. In our experiments less than 10 sec

elapsed while the samples were being raised through the 65°-75° range. The peak inactivation temperature occurs at slightly over 70°, there being some inactivation at 75° and less at 65°, but no measurable amount at either 60° or 80°. It cannot be construed from these data that the over-70° salivary apoerythrin activity has been unaffected by the thermal treatment, since it differs from normal salivary apoerythrin activity in that it is immune to destruction at 70° for even prolonged periods.

References

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2. BEERSTECHER, E., JR., and ALTGELT, S. *J. Biol. Chem.*, **189**, 31 (1951).

Formation of α-Keto Acids from α-Amino Acids by the Action of Free Radicals in Aqueous Solution

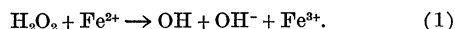
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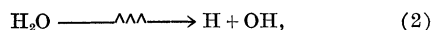
The mechanisms of metabolic processes are, in many instances, still a matter of some conjecture, and there has been some discussion (1, 2) in this respect, as to the possibility of the participation of free radicals. Thus the actual simulation of certain enzymatic processes by reactions involving free radicals *in vitro* may be important in any study of this nature.

It is now well established that the breakdown of α-amino acids by animal tissues consists of an oxidative deamination with the intermediate production of the α-keto acids (3). We have investigated, therefore, the action of OH and HO₂ radicals on a number of α-amino acids. These radicals have been produced in solution as follows:

(a) By the decomposition of hydrogen peroxide in the presence of a ferrous salt (Fenton's reagent), a reaction which proceeds as follows (4):



(b) By irradiation with x-rays of dilute aqueous solutions, where radicals are formed according to the net process (5):



which in the presence of oxygen is followed by:



Previous studies on the oxidation of amino acids by Fenton's reagent (6-8) showed that these substances are deaminated and converted to the aldehydes and the corresponding carboxylic acids. However, with the exception of glycine, which affords glyoxylic acid (6),