Technical Papers

Anomalous Thermal Behavior of Salivary Apoerythein Activity

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Eakin and co-workers have adapted a modified vitamin B_{12} assay to the measurement of apoerythein (pernicious anemia "intrinsic factor") activity (*1*) and demonstrated the presence of apoerythein in saliva (*2*). The assay depends upon the ability of apoerythein to bind vitamin B_{12}, making it unavailable for the growth of *Escherichia coli,* which depends upon the vitamin for growth in a specially defined medium. Apoerythein 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 apoerythein is able to bind 1 μg of vitamin B_{12}, and the results are reproducible to within 5%. In studies on the thermal stability of salivary apoerythein 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 APOERYTHEIN

Treatment temperature °C	Apparent activity of 1 milliunit apoerythein
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 apoerythein. 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 apoerythein activity has been unaffected by the thermal treatment, since it differs from normal salivary apoerythein activity in that it is immune to destruction at 70° for even prolonged periods.

References

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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 HO_2 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*):

$$H_2O_2 + Fe^{2+} \longrightarrow OH + OH^- + Fe^{3+}. \qquad (1)$$

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

$$H_2O \longrightarrow\!\!\!\!\!\!\wedge\wedge\wedge\longrightarrow H + OH, \qquad (2)$$

which in the presence of oxygen is followed by:

$$H + O_2 \longrightarrow HO_2. \qquad (3)$$

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*),