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RADIATION STABILITY OF AQUEOUS SOLUTIONS OF VITAMIN B_3

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Ionizing radiation is rarely used for sterilizing injection preparations, which are dilute aqueous solutions of biologically active substances. The reason for this is that these preparations decompose even with radiation doses that are inadequate for sterilization because of the indirect effect of the radiation on the drug. Recently the physical methods based on elimination or attenuation of the indirect effect when frozen solutions are irradiated have been shown to be highly effective for protecting preparations from decomposition [1-3]. Analysis of the possible mechanisms of this cryostabilization effect revealed that the most complete protection results from the formation of a microheterogeneous structure of the frozen solution [4, 5].

We have now examined the effect of freezing on the radiation stability of aqueous solutions of calcium pantothenate, vitamin B_3.

Kishore et al. [2] have attempted a comparative study of the radiolysis of solutions of pantothenic acid at room temperature and at −80°C. However, they got no quantitative results, since their methods, UV spectroscopy and polarography, were unsuitable for analysis of the irradiated samples. The absorption of the products of radiolysis at room temperature is superimposed on the single absorption maximum in the spectrum of pantothenic acid at 208 nm and so the maximum did not diminish but increased as the dose rose. The acidic nature of these products also precluded measurement of the decomposition of pantothenic acid by polarography in terms of the reduction of hydrogen. After irradiation of pantothenic acid at −80°C the absorption spectrum showed only slight changes, which Kishore et al. believed to be evidence for the enhanced stability of this compound on freezing. We used analytical methods with which the radiation changes in solutions of calcium pantothenate at room temperature and at −196°C can be quantitatively measured.

We examined the radiolysis of calcium pantothenate in aqueous solutions containing 0.9% sodium chloride. The concentration of the vitamin was 5 g/liter (1.1×10^{-3} M). Samples were irradiated in glass ampuls sealed in air at 18 and −196°C in a setup with a ^{60}Co γ-gun (radiation rate 4.3 Mrad/h). We measured the degree of decomposition of calcium pantothenate by thin-layer chromatography (TLC) [6] and biological assay [7].

For TLC we used Silufol UV-254 plates with elution by water. Calcium pantothenate (R_f 0.83) was identified from its quantitative decomposition at 160°C to β-alanine and pantoic acid and the subsequent color reaction of β-alanine with ninhydrin solution (0.5% solution in ethanol). We estimated the quantity of the preparation from the intensity of the violet coloration. The accuracy of the determination of calcium pantothenate at low degrees of decomposition was ±15%. We were also able to measure the amount of β-alanine formed by TLC (R_f 0.64).

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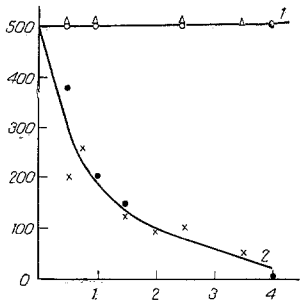

Fig. 1. Plot of vitamin B_3 concentration (mg/liter) in 1.1×10^{-3} M aqueous solution containing 0.9% sodium chloride against radiation dose (Mrad); 1) irradiation at −196°C; 2) at 18°C. Vitamin B_3 concentrations were measured by TLC (filled and open circles) and biological assay (crosses and triangles).

We assayed the biological activity with the organism Saccharomyces ludwigii KM, which is incapable of multiplying when the medium is devoid of vitamin B_3.

We prepared the test suspension by first depleting a culture of S. ludwigii of vitamin B_3 by growing it for two days in nutrient medium lacking this vitamin. Inoculation was carried out with a thick aqueous suspension of the yeast washed from a tube containing wort agar.

A slightly turbid suspension in tap water was prepared from a 2-day depleted culture. One or two drops of the suspension were added to flasks containing Reader's medium. The decomposition time was 42-72 h. We measured the number of cells of S. ludwigii nephelometrically and calculated the vitamin B_3 concentration relative to a standard.

Our results for samples irradiated at 18 and −196°C are shown in Fig. 1, where we compare the results derived by the two analytical methods.

Clearly decomposition of the vitamin occurs during radiolysis of solutions of calcium pantothenate at 18°C; with a dose of 2.5 Mrad its concentration is reduced by 80%. β-Alanine can be detected among the radiolysis products. At −196°C vitamin B_3 is stabilized against radiolysis as effectively as vitamin B_{12} [1]. The biological activity of the radiolyzed solutions was completely preserved after irradiation with a dose of 2.5 Mrad, which is normally taken as sterilizing. Thus our results even for a single example provide evidence that cryoradiosterilization of drug solutions is possible without loss of biological activity.

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