EFFECT OF HIGH ENERGY CATHODE RAYS ON THE CATALASE ACTIVITY OF MICROCOCCUS PYOGENES VAR. AUREUS

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Recent publications have shown that high voltage cathode rays (electrons) can cause lethal mutations in bacteria and hence may be considered as a possible means for sterilization of foods (Brasch and Huber, 1947; Dunn *et al.*, 1948; Proctor, Goldblith, and Fram, 1950). However, if these radiations are to be used for preserving foods, it is necessary to ascertain whether bacteria whose reproductive mechanism has been irreversibly destroyed by ionizing radiations are capable of enzymatic activity that might cause or initiate spoilage in foods. Research by various laboratories has shown that enzymes *in situ* in foods are relatively radioresistant but that crystalline enzymes are relatively radiosensitive (Dale, 1940; Dunn *et al.*, 1948; Forssberg, 1945, 1946; Tytell and Kersten, 1941).

The present study was designed to determine the effect of a lethal dose of cathode rays on the catalase activity of a pure culture of *Micrococcus pyogenes* var. *aureus* (*Staphylococcus aureus*), samples of which were stored at various temperatures.

METHODS

A pressure insulated Van de Graaff accelerator producing monoenergetic 3 Mev electrons was used as the source of the cathode rays (Trump and Van de Graaff, 1948).

A culture of the organism to be irradiated, *M. pyogenes* var. *aureus*, was grown in nutrient broth for 24 hours at 37 C.

The catalase activity of the organism was measured by the permanganate method of von Euler and Josephson as modified by Sumner and Somers (1947). Samples of the bacterial culture were first ground three times in an S-Micro-Waring Blendor, each time for one minute, in order to break down the walls of the bacterial cells without overheating the material during grinding.

Bacterial plate counts were made according to the standard method recommended by the American Public Health Association (1948), and tryptone-glucose agar was used as the culture medium. After irradiation of the culture samples, tests for sterility were made by transferring five 1-ml aliquots of the irradiated samples into fluid thioglycolate sterility medium, incubating the aliquot samples at 37 C for 72 hours, and then examining them for bacterial growth.

RESULTS

From the 24-hour suspension of *M. pyogenes* var. *aureus* (count of 2.0×10^8 bacteria per ml), 25 ml were exposed to 3 Mev cathode rays for doses ranging

from 15,000 to 600,000 rep (roentgen-equivalents-physical). In tests for sterility after irradiation, it was found that a dose of 300,000 rep had resulted in two positive tubes out of five and that a dose of 350,000 rep caused complete sterility of the culture in all tubes.

Other 25 ml aliquots of a 24-hour culture of *M. pyogenes* var. *aureus* were pipetted into polyethylene bags (2.5 inches wide and about 6 inches long), which were then heat sealed at the open end. Half of these bags (18) were irradiated at 700,000 rep, which is twice the sterility dose. The remaining 18 bags served as controls. Six bags of controls and six irradiated samples were stored at each of the following temperatures: 37, 25, and 2 C. Tests for catalase activity were

TABLE 1

Effect of 3 Mev cathode rays on catalase activity of suspensions of Micrococcus pyogenes var. *aureus stored at various temperatures*

DAYS' STORAGE	CATALASE ACTIVITY*					
	Control (not irradiated)			Irradiated (700,000 rep)		
	37 C	25 C	2 C	37 C	25 C	2 C
0	0.00482	0.00482	0.00482	0.00325	0.00325	0.00325
3	0.00497	—	—	0.000355	—	—
5	0.00396	—	—	0.00016	0.00103	—
6	—	0.00548	—	—	0.00141	—
7	0.00325	—	—	0.000213	—	—
8	—	—	0.00184	—	—	0.00137
9	—	—	—	0.000141	0.00055	—
12	—	0.00602	—	—	—	—
13	0.00358	—	0.00170	0.000049	—	0.000601
14	0.00016	0.0063	—	—	—	—
15	—	0.0063	0.00161	—	—	—
16	—	—	—	—	0.000364	0.000579
20	—	—	0.00136	—	—	0.000821
23	—	—	0.00201	—	—	0.00083

* Expressed as Kat. f. values

made at the beginning of the storage period (0 day) and at different intervals thereafter, depending on the temperature of storage. The results are tabulated in table 1, expressed as Kat. f. values calculated according to the equation:

$$\text{Kat. f.} = \frac{K_s \text{ (0 time)}}{\text{ml of bacterial suspension tested}}$$

in which $K_s = \frac{1}{T} \log_{10} \frac{a}{a - x}$, where K_s is the reaction rate, T is the time, a is the initial $KMnO_4$ concentration, and $a - x$ is the $KMnO_4$ remaining. The K_s value for $T = 0$ is obtained by extrapolation.

The data in table 1 show that there was a loss of only about 33 per cent of the catalase activity immediately after irradiation although the radiation dose was twice the lethal amount. The data show, further, that the catalase activity of the

irradiated samples decreased on storage and that the rate of this decrease was a function of the temperature of storage, the rate of decrease being greater the higher the temperature. Preliminary observations on the effect of storage at −18 C showed no loss in catalase activity after 51 days.

With one exception, the catalase activity of the control samples appeared to decrease also during storage, but to a much lesser degree than that of the irradiated samples. In the case of the controls at 25 C, the catalase activity appeared to increase somewhat during storage. Whether it was because this temperature approached that optimum for growth of the organism was not determined.

SUMMARY AND CONCLUSIONS

The effect of high energy cathode rays on the catalase activity of *Micrococcus pyogenes* var. *aureus* has been studied. Doses resulting in lethal mutations of *M. pyogenes* var. *aureus* did not completely inactivate the catalase activity.

The effect of different storage temperatures was studied. It is of interest that the catalase activity of irradiated samples of *M. pyogenes* var. *aureus* stored at high temperatures appears to decrease during storage, whereas that of samples stored in a refrigerator and especially that of samples stored in the frozen state may remain at a high level.

This finding has important practical implications for tissues sterilized by cathode rays and stored in the frozen state, for it indicates that in such cases, if there is need for the inactivation of enzymes, it must be accomplished by some means other than by radiations.

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