animals' hair retained its normal luster; there were no signs of loss of reflexes, anorexia, chemosis, increase or decrease in salivation, gastrointestinal disturbances, or change in the consistency of the feces. The results of the hematologic and histologic examinations performed following the termination of the 45-day extended-toxicity study also indicated that there were no pathologic developments that could be used to differentiate the test animals from the control animals.

References and Notes

1. P. C. Standley, *Publ. Field Museum of Natural History, Botan. Ser.* **18** (1), 540 (1937).
2. ———, *ibid.* **3** (3), 295 (1930).
3. ——— and J. A. Steyermark, *ibid.* **24** (5), 265 (1946).
4. L. H. Bailey. *Manual of Cultivated Plants* (Macmillan, New York, 1949), p. 560.
5. The importation of the Costa Rican plants was through the efforts of Allen I. White, State College of Washington, and was financed by funds provided for medical and biological research by the State of Washington, Initiative 171.

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A Device for Determining Time and Temperature of Sterilization in the Autoclave or Hot-Air Oven

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Since one often is concerned with whether or not sterilization has been properly carried out, a number of indicators have been marketed. Except for continuous-reading electric thermocouples, many of these indicators show only that the temperature has been reached.

Chemical melting points have always been acceptable as reliable indicators of temperature if they remain pure. By enclosing the proper compound in a sealed glass tube so constructed that it permits the flow of the molten material, one may determine the temperature as well as the length of time that the temperature remained constant. This apparatus is small and inexpensive and may be used countless times. It has a definite advantage in that it can be used in any place in the autoclave or oven and included in packages and containers of materials for sterilization.

An hourglass type of apparatus was constructed in such a manner that the solid chemical would melt and flow through a narrow constriction into the bottom tube (Fig. 1). Our idea was that, when molten, the material would flow through a standard-size opening at a uniform speed. Unfortunately, when the liquid formed in the upper part of the sealed hourglass-shaped tube, an air pocket formed and prevented the flow. When a side-arm by-pass tube was connected to the upper and lower sections, the air could pass freely and the molten material could flow. Thus by controlling the constriction in the tube and having the pressure equalized, we had a unit that was physically suited for our purpose.

There are many compounds that will melt at 121°C. Unfortunately, the problem is not quite as simple as merely selecting one of these compounds for our purpose, because some of them will not repeatedly melt and flow at the desired temperature.

In view of these considerations, we have used several compounds, two of which are succinic anhydride, $OCOCH_2CH_2CO$, mol. wt. 100.07, melting at 120°C; and *dl*-mandelic acid —$C_6H_5CHOHCOOH$, mol. wt. 152.14, melting at 120°C.

Mandelic acid is very applicable but unfortunately will melt and flow only once at 120°C. On repeated meltings, the melting temperature is considerably lower. This might be advantageous for a single use in certain operations. On the other hand, succinic anhydride remains constant in its ability to melt and flow repeatedly and is, therefore, our choice for use in this temperature range. Its physical characteristics are listed adequately (*1*).

To evaluate and observe these tubes we constructed an insulated oil bath with thermostatic controls and circulated with motor-driven propellers. Using this equipment, we were able to maintain a temperature of 120.5° to 121.5°C with ease and could determine the flow of various compounds at the desired temperature.

Tubes were constructed by professional glass manufacturers (*2*) with uniform dimensions, and the chemical compound of choice could be accurately weighed into the tube. They were heated in an oven to melt and the tube was sealed. Repeated tests showed that 1 g of

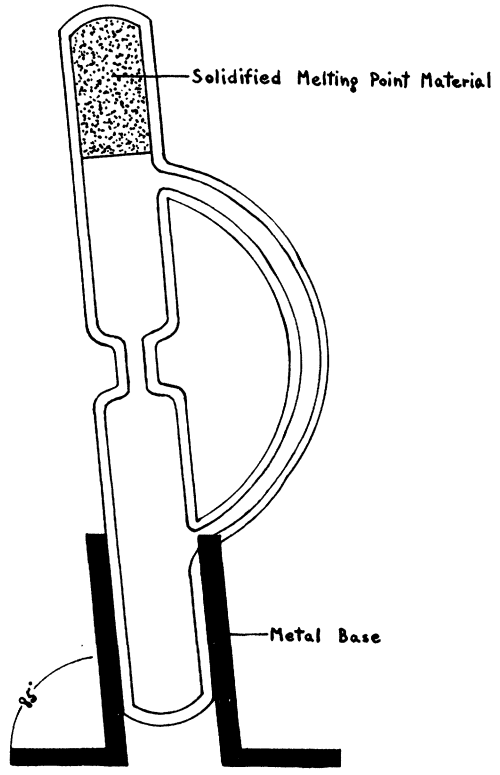

Fig. 1. Line drawing showing cross section of Time-at-Temperature device with metal base.

succinic anhydride would melt and flow at 120° to 121°C in 15 to 17 min. Therefore we feel that we have an apparatus that will truly indicate that the predetermined temperature was reached and held for a minimum sterilizing time. It should be mentioned that at 118° to 119°C, 2.5 hr were required for the succinic anhydride to melt and flow. At 125°C, 1 g would flow in 8 min. It seems from our experiments that these time differences are more or less comparable to sterilizing time.

Although the newer autoclaves are well equipped with automatic time and temperature controls, the arrangement of the load will determine the actual time and temperature required for sterilization and cannot be adequately preadjusted to take care of the widely varying conditions of use in the laboratory. Many of these autoclaves are well equipped with thermocouples and recording instruments to place in varying spots in the autoclave, but for the most part only a few leads are available and one cannot do a heavy spotting of these recording thermocouples because of the expense involved. However, many of these smaller melting-point tubes can be included in every square foot of the sterilizer without adding substantially to the load or occupying valuable space. The Time-at-Temperature (T@T) Tubes can be mounted in special holders and placed on shelves or suspended in the chamber. They could also be affixed to rods of glass or stainless steel and mounted in various positions in large containers of solution. Since the T@T Tube is hermetically sealed, it cannot contaminate the solution and it need not be removed before filling the product into the final container.

To simulate conditions in line with practical usage, 6 lit of solution was placed in an 8-lit Pyrex solution bottle and three T@T Tubes were fastened to a rod in the center of the bottle in such a manner that one tube was above the solution, one just beneath the surface, and one on the bottom. After 15 min at sterilizing temperature, the tube above the solution showed only partial sterilization, and the two tubes within the liquid showed no signs of melting. After 20 min the T@T Tube above the liquid melted completely, and the uppermost tube within the liquid showed partial melting; the lower tube did not melt. It was necessary to keep the autoclave at 120° to 121°C for 30 min to melt the upper submerged tube and for 55 min at 120° to 121°C to melt completely the bottom tube. This showed quite dramatically that the solution heated, not uniformally, but more in a layering manner.

Obviously, by constructing a larger T@T Tube device and placing a higher melting point solid that melts at 166° to 167°C in these tubes, a device for use in the hot-air oven may easily be constructed. We have found that *p*-phenylphenol, which melts at 166° to 167°C, is satisfactory for this purpose, and the tubes with a volume of approximately 3 times that used in the autoclave will check the hot-air sterilization for 1 hr at this temperature.

Because these T@T Tubes are so inexpensive they can be employed in large numbers and also may be used by the small laboratory that cannot afford expensive recording thermocouples.

References and Notes

1. *Handbook of Chemistry and Physics* (Chemical Rubber Publ. Co., Cleveland).
2. Becton, Dickinson and Co., Rutherford, N. J.

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Apparent Simultaneous Adaptive Enzyme Formation in C_{57} Mice

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Adaptive enzyme formation in microorganisms—that is, increases in enzyme concentration induced by the presence of the substrate—has been known for many years (*1, 2*). The existence of a similar mechanism in higher animal forms has been hypothesized (*3*), but only recently has supporting evidence been adduced. Recent studies have indicated that mammalian tissue levels of tryptophan peroxidase and xanthine oxidase (*4, 5*) increase subsequent to administration of the appropriate substrate. Gordon and Roder also reported a similar adaptive increase in the adenosine deaminase activity of chick embryos (*6*).

In vivo experiments indicated that considerable fractions of injected xanthine are metabolized along synthetic pathways (*7*). This suggested that an enzyme concerned with xanthine anabolism, as well as the enzyme xanthine oxidase, might be amenable to adaptive increase. In this study, therefore (*8*), both liver xanthine oxidase and adenosine deaminase levels were determined following prolonged administration of xanthine to C_{57} mice. The findings indicate that both liver xanthine oxidase and adenosine deaminase levels increase following xanthine administration, suggesting that a type of simultaneous adaptive enzyme formation may occur in mammals, as has been demonstrated by Stanier to occur in microorganisms (*9*).

Thirty C_{57}BL/6 male mice were divided into three experimental groups of 10 animals each. Group I, the control animals, received daily intraperitoneal injections of physiological saline; groups II and III were injected with suspensions of xanthine in saline, the daily dosage being 12 and 24 mg, respectively, of xanthine per 100 g body weight. The animals were fed Purina dog chow *ad libitum*. This regime was maintained for 14 to 20 days, at which time the mice were sacrificed. Homogenates prepared from two pooled livers were analyzed for xanthine oxidase by the procedure of Axelrod and Elvehjem (*10*) and adenosine deaminase using a modification of the procedure of Gordon and Roder (*6*).

The results of the *in vitro* analysis of liver enzyme activities are depicted in Table 1 as the mean values and range of values for each experimental group. It is to be noted that there are increases in the liver