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.

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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

Table 1. The effect of xanthine administration on the concentrations of liver xanthine oxidase and adenosine deaminase.

Group No.	I	II	III
Xanthine administered (mg/day, per 100 g body weight)	0	12	24
Xanthine oxidase			
μlO_2/hr, per g wet weight	342(321–361)	422(382–438)	493(415–614)
μlO_2/hr, per g dry weight	1360(1180–1570)	1850(1480–2010)	2110(1780–2940)
μlO_2/hr, per μM nitrogen	0.163(0.148–0.181)	0.208(0.177–0.230)	0.236(0.187–0.313)
Adenosine deaminase			
μM adenosine deaminated/hr, per g wet weight	46.9(28.1–69.8)	61.6(51.2–69.7)	86.6(64.0–141.1)
μM adenosine deaminated/hr, per g dry weight	185(117–245)	272(201–323)	381(263–672)
μM adenosine deaminated/hr, per μM nitrogen	0.0226(0.0136–0.0345)	0.0308(0.0241–0.0403)	0.0416(0.0288–0.0716)

xanthine oxidase and adenosine deaminase activities as the result of prior xanthine administration to the animals. Furthermore, the extent of the adaptive increases are a function of the administered dose of xanthine. Livers of group III animals possess 44 percent higher xanthine oxidase and 85 percent higher adenosine deaminase activities than those of control animals. The increases in xanthine oxidase over the control values exhibited by both groups of xanthine-treated mice are significant at the $p = 0.01$ level, using Wilcoxon's nonparametric test (*11*). Owing to the greater variation in enzyme concentration among animals within the experimental groups, the increases in adenosine deaminase activities of groups II and III over the control animals are significant at only the $p = 0.10$ and $p = 0.05$ levels, respectively. Expression of the enzyme activities on the basis of liver dry weight or nitrogen does not essentially alter these relationships.

It was observed that irrespective of experimental groups, animals with high liver xanthine oxidase activities also manifested high liver adenosine deaminase activities. The coefficient of correlation between the concentrations of these two liver enzyme systems was calculated to be 0.89 which is significant at the $p < 0.001$ level. When the xanthine oxidase activity is plotted versus the adenosine deaminase activity for each animal, the relationship shown in Fig. 1 is obtained.

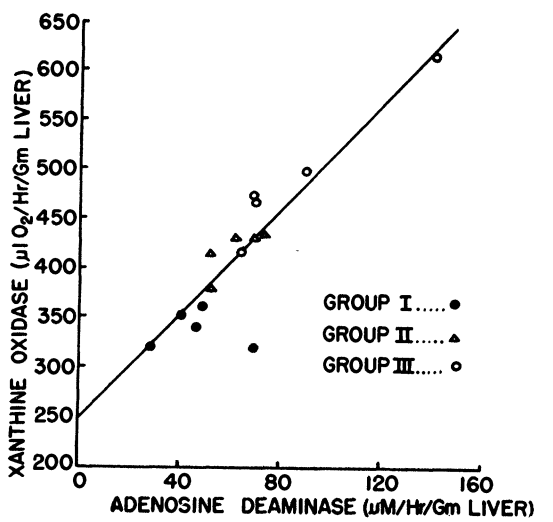

Fig. 1. Relationship between liver xanthine oxidase and adenosine deaminase activities.

These observations strongly imply that a similar mechanism controls the concentrations of both liver enzymes. Possible mechanisms by which this may occur are (i) a sequential adaptation; that is, the increases in anabolic products subsequent to xanthine administration provide increased substrate levels for adenosine deaminase resulting in an adaptive increase in that enzyme; (ii) xanthine itself evokes adaptive increases in adenosine deaminase by possibly combining with the enzyme protein; (iii) xanthine administration indirectly induces increases in both adenosine deaminase and xanthine oxidase, for example, via adrenal stimulation. Experiments are currently under way to explore further these factors.

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