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ACTION OF MERCURIC CHLORIDE UPON TYROSINASE PRODUCED BY VARIOUS ACTIVATORS*

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Protyrosinase, from the diapause egg of the grasshopper, *Melanoplus differentialis,* is activated and converted into tyrosinase by a variety of reagents (Bodine and Carlson, 1940). The active enzyme thus produced has recently been shown to possess similar catalytic properties but to differ greatly in its response to high temperatures (Bodine, Tahmisian, Hill, 1944). The effect of high temperatures is probably related to the type of activator employed as well as to the manner in which the protein enzyme complex is broken down or transformed. Inasmuch as $HgCl_2$ acts as an activator of protyrosinase it becomes of some interest to determine the relative susceptibility to this compound, of the tyrosinase produced by different reagents and to compare this chemical susceptibility with that to high temperatures (Bodine, Tahmisian, Hill, 1944). The present communication deals primarily with the susceptibility of $HgCl_2$ of tyrosinase produced by anionic detergents [sodium dodecyl sulfate, di-ocyl sodium sulfosuccinate (aerosol OT)], urea and heat (70°C).

METHODS

The methods of preparation and standardization of protyrosinase are similar to those already reported (Allen, Bodine, 1941). Warburg manometers were used in a manner previously noted (Allen, Otis, Bodine, 1942). Other experimental procedures were identical with those described for the effects of heat on protyrosinase (Bodine, Tahmisian, Hill, 1944).

RESULTS

As a check on the activation of the protyrosinase sample employed, a complete $HgCl_2$ activation curve was established (Fig. 1, A). From an inspection of this curve, it will be noted that maximum activation (approx. 80%) is attained at 3×10^{-5}M.$HgCl_2$. The ascending or activating limb of the curve rises gradually while the descending or toxic limb drops rather abruptly reaching zero activity at 3×10^{-4}M.$HgCl_2$. This type of activation curve resembles and checks that previously published (Bodine and Tahmisian, 1943). A heat activation curve has also been made and is indicated in Figure 1,B. From these activation curves one may infer that the behavior of the protyrosinase sample resembles that already reported (Bodine, et al., 1944; Bodine and Tahmisian, 1943).

Heat activated tyrosinase (70°-10′) when treated with $HgCl_2$ in concentrations below the minimum for maximum activation (3×10^{-5}M.) shows little effect of the salt (Fig. 1C). With concen-

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trations greater than this a marked toxic action is produced. The relative toxicity of $HgCl_2$ for heat activated enzyme appears less than

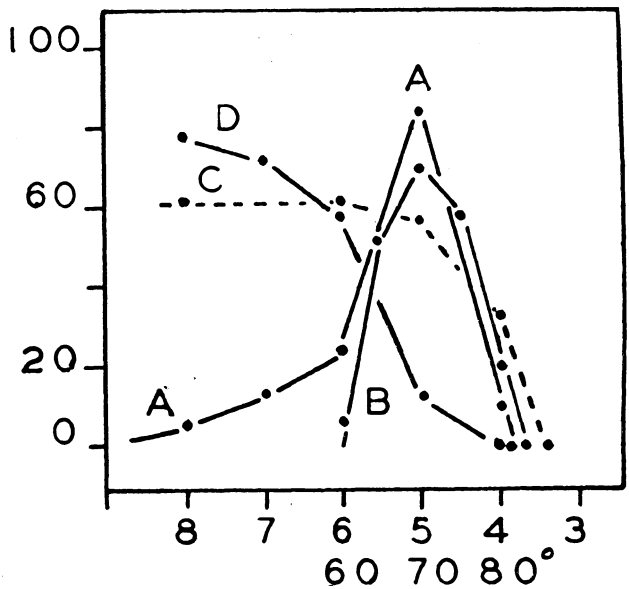

FIG. 1—Shows the effect of $HgCl_2$ and heat upon the activation and injury of protyrosinase. Abscissa: top, concentration of $HgCl_2$ in terms of negative exponents, e.g., 3 x 10^{-8}, 3 x 10^{-7}, etc.; bottom, temperature, degrees centigrade. Ordinate: per cent activity in terms of aerosol OT as 100%. A = $HgCl_2$ activation curve. B = temperature activation curve, 10 minute exposures to temperature. C = heat activated enzyme (70°-10′) plus $HgCl_2$. D = $HgCl_2$ activated enzyme + heat (70°-10′).

for the $HgCl_2$ activated sample. If the reverse experiment is performed, namely, the treating of $HgCl_2$ activated enzyme with temperature (Fig. 1,D) a marked decrease in the activity follows even at temperatures (70°-10′) normally producing maximum activation of the enzyme. The greater toxicity of the $HgCl_2$ at the higher temperatures undoubtedly is conditioned by the increased temperature.

The effect of $HgCl_2$ on tyrosinase produced by different activators is graphically shown in Figure 2. Up to a concentration of 3 x 10^{-5},M. appears conditioned by the activator employed. The order of susceptibility of $HgCl_2$ on this basis is— aerosol OT > NaDS > urea > heat. This order is little different from that for the effects of temperature and one can perhaps reasonably conclude that no significant differences in susceptibility to $HgCl_2$ or heat for the variously activated tyrosinase exists (Bodine, et al., 1944).

$HgCl_2$ activated tyrosinase when subjected to 70°C. for different periods is markedly affected, being completely destroyed in about

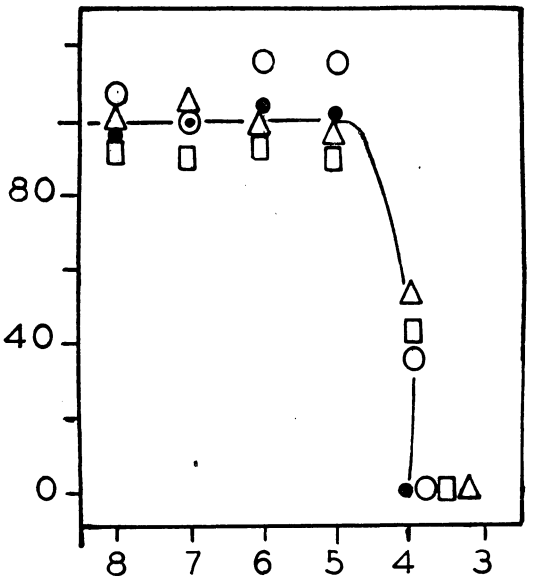

FIG. 2—Shows the effect of $HgCl_2$ on the activity of variously activated enzyme. Abscissa: concentration of $HgCl_2$ in terms of negative exponents, e.g., 3 x 10^{-8}, 3 x 10^{-7}, etc. Ordinate: per cent activity of enzyme. ● = + aerosol OT activated enzyme + $HgCl_2$. ○ = NaDS activated enzyme + $HgCl_2$. □ = urea activated enzyme + $HgCl_2$. △ = heat activated enzyme + $HgCl_2$.

50′ (Fig. 3,B). This is slightly more resistant than urea activated enzyme. In the temperature susceptibility series, $HgCl_2$ activated tyrosinase would be between urea and NaDS (Bodine et al, 1944).

SUMMARY

From the results of the above experiments one may reasonably conclude that marked differences in susceptibility of tyrosinase, variously activated, to a chemical like $HgCl_2$ exist. Activating agents in certain concentrations may destroy or inhibit the enzyme which they activated at lower concentration levels. The transformations upon activation of the enzyme protein molecule, although not necessarily similar for different activators, apparently have some effect upon the susceptibility of the enzyme to temperature or $HgCl_2$. The order of susceptibility to $HgCl_2$ of the enzyme activated by different activators is—

aerosol OT > NaDS > urea > heat.

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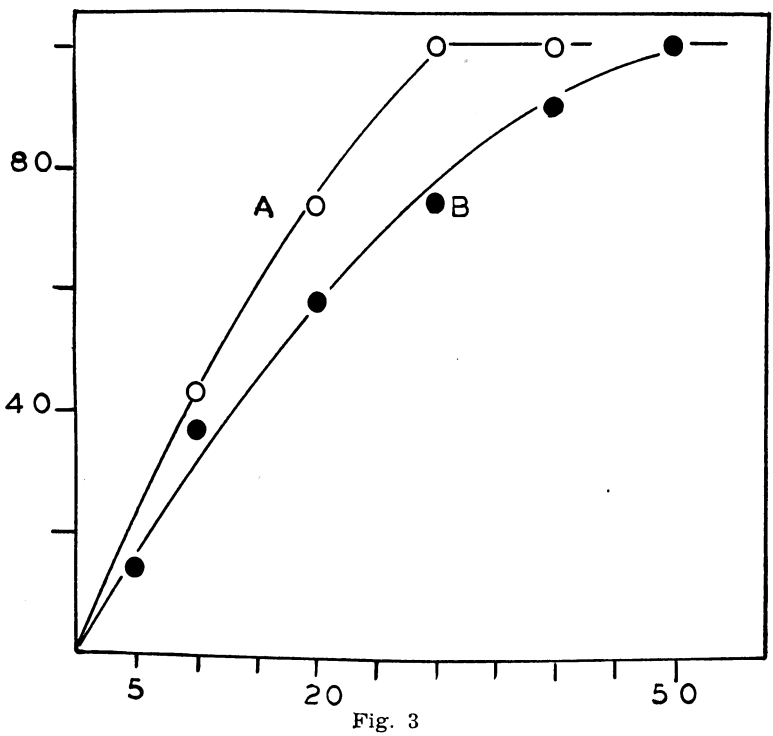

Fig. 3

FIG. 3—Shows effect of 70°C upon injury of $HgCl_2$ and urea activated enzyme. Abscissa: time of exposure to 70°C in minutes. Ordinate: per cent injury. A = urea activated enzyme. B = $HgCl_2$ activated enzyme.