reactions should be carried out in a closed system. A method for the aseptic preparation of blood plasma solutions and the utility of a closed pH stat titration assembly are described.   Data are presented to show the utility of the experimental procedure for following the hydrolysis of substrates such as acetylcholine, benzoylcholine, and phenyl butyrate in solutions of blood plasma.

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_____*Notes*____

Radiation and the Detoxication of Chemicals

By THOMAS J. HALEY* and L. KOSTE

The detoxication of benzoate and resorcinol has been investigated in irradiated animals. In both instances detoxication was inhibited from the third to the seventh post-irradiation days. The common denominator for this action of X-irradiation appears to be co-enzyme A and/or its substrates.

EXPOSURE to X-irradiation is known to increase protein catabolism (1) and increase the free and dynamic glycine pools in the animal (2–4) but Schreier *et al.* (5) found no change in hippurate excretion during the first 3 days post-irradiation. However, Gerber and Remy-Defraigne (6) found that the irradiated liver synthesized more hippurate when exogenous glycine was excluded from the perfusion fluid than when it was present. Chiriboga (7) reported that whole body irradiation caused a decreased glucuronide excretion and suggested that further investigation was necessary to elucidate its role in the radiation syndrome. Inasmuch as both hippurate and glucuronide synthesis involve co-enzyme A and through it the detoxication of benzoate and phenolic compounds, we have investigated the detoxication of benzoate and resorcinol in irradiated rats.

METHODS AND MATERIALS

Hippurate excretion was determined in 18 Charles River CD strain female rats weighing 250 to

300 Gm. and glucuronide excretion was obtained with 6 similar rats. Total 24-hr. urine volumes based upon 137 collections was 22.1 ± 10.5 ml. with a range of 6.5 to 55.8 ml. Urinary output depended upon water consumption which also had the same degree of variation. Normal excretion values for both hippurate and glucuronide were obtained in nine separate experiments and control values after administration of either 100 mg./Kg. of sodium benzoate or 50 mg./Kg. of resorcinol intraperitoneally in three separate experiments after which the animals received 600 rads acute whole body X-irradiation. In all cases urine was collected over a 7-day period by the method of Haley *et al.* (8). Urinary hippurate was determined with an Aminco-Bowman spectrophotofluorometer using the method of Ellman *et al.* (9) and glucuronide was determined by the method of Mead *et al.* (10). Quadruplicated samples of each urine specimen were analyzed. The results were analyzed statistically by the Student *t* test for significance. The radiation characteristics of the industrial unit were: 250 KVP; 15 ma.; FOD 55 cm.; filters 0.21 mm. Cu inherent; 0.5 mm. Cu parabolic and 1 mm. Al; HVL 1.95 mm. Cu; dose rate measured in air, 24.97 rads/min. The machine was calibrated prior to and after each experiment with a Victoreen thimble *r*-meter and during exposure with a Radacon unit.
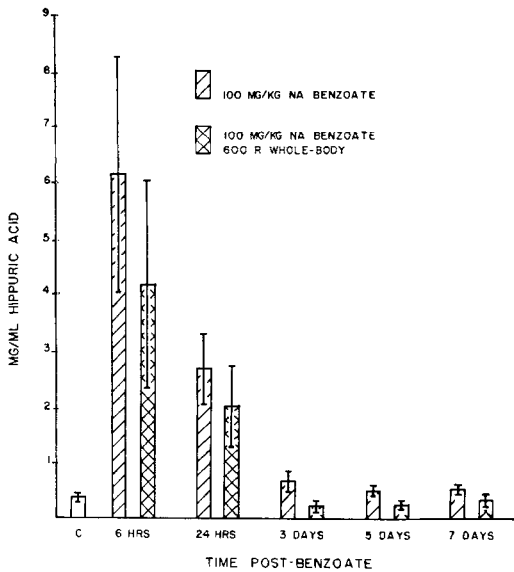
Fig. 1—Hippurate excretion in nonirradiated and irradiated rats. Note nonsignificant decrease in hippurate excretion during the first 24 hr. and significant decrease (P = 0.001) *at the third through the seventh days post-irradiation.*
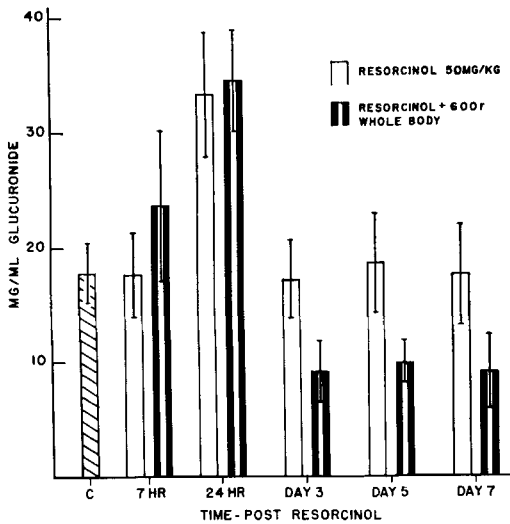

Fig. 2—Effect of 600 rads whole body X-irradiation on glucuronide excretion following 50 mg./Kg. of resorcinol. Compared to the normal excretion all irradiated values from the third to seventh days are significant at P = 0.001. *The increased excretion at 24 hr. is significant at* P = 0.01.

RESULTS AND DISCUSSION

The results obtained are illustrated in Figs. 1 and 2. In confirmation of Schreier *et al.* (5), it was found that hippuric acid was a normally occurring metabolite in rat urine, but contrary to their report it was observed that X-irradiation did affect hip-

purate excretion on the third day post-irradiation. Furthermore, there was no significant difference in hippurate excretion between nonirradiated rats until that time. From the third to the seventh post-irradiation days inclusive, the irradiated group's hippurate excretion was significantly depressed (P = 0.001), although both groups were again approaching the normal hippurate excretion level by the seventh day.

In confirmation of Chiriboga (7), it was observed that urinary glucuronide excretion was significantly depressed (P = 0.001) from the third through the seventh post-irradiation days and had not as yet begun to approach the normal level. Of interest is the observation that both hippurate and glucuronide excretion were unaffected until the third post-irradiation day, indicating that the detoxication pathways for both benzoate and resorcinol seem to be functional in the early post-irradiation period. Perhaps the failure occurring on the third day is related to the intestinal malabsorption of glucose and B-complex vitamins previously reported (11–14) or to exhaustion of the enzyme system(s) involved in the detoxication processes, or both. A clue to one aspect of the problem was the report of Hanel and Wilian-Ulrich (15) that acetylation of sulfanilamide was also depressed on the third day. In every instance the common mechanism of detoxication involved co-enzyme A. Moreover, decreased fatty acid synthesis on the third day also involves this co-enzyme (15). Perhaps other metabolic pathways are activated by irradiation, thus gradually decreasing the co-enzyme's activity or the availability of the substrate required for active detoxication. On the other hand, it is possible that ionizing radiation or the products it produces in cell cytoplasm through free radical formation may have a direct effect on co-enzyme A and/or its substrate. Both are distinct possibilities because ionizing radiation affects sulfhydryl groups and one of the function entities in co-enzyme A is a sulfhydryl group. All of these points remain to be elucidated, if we are to understand why radiation depresses detoxication mechanisms.

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