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PRELIMINARY COMMUNICATIONS

DECREASED ARYL HYDROCARBON HYDROXYLASE AFTER A 15% BURN INJURY

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In the U.S. hospitalization due to serious burns occurs to more than 100,000 persons per year (1). Many of those burned receive drugs and inhale pollutants (2-4) which are metabolized by the mixed function oxygenase system (5,6). It is important to obtain direct measurements of this system since a decrease in activity would prolong the effects of such agents leading in some cases to toxicity. Recently, a report was published suggesting that the burn patient has a diminished hepatic capacity for metabolizing xenobiotics, indicated by a decreased urinary glucaric acid excretion (7). This manuscript reports a preliminary investigation in the rat on hepatic aryl hydrocarbon hydroxylase after a 15% burn injury.

MATERIALS AND METHODS

Male Sprague Dawley rats, weighing 185-200 g, were subjected to a 15% burn while under pentobarbital anesthesia (8). The right side of the rat was shaved and then, with the use of a mold, the protruding skin was dipped into 90° water for 20 sec. This produced a third degree burn that scabbed over and was, by all determinations, not painful to the rat at any time during the experiment. None of the animals died of the injury produced; in fact, the wounds began to granulate and heal by day 25.

Aryl hydrocarbon hydroxylase (AHH) was chosen as the *in vitro* test system because this is the enzyme responsible for the metabolism of many organic pollutants, especially those from incompletely burned materials such as might be found in the environment of burn victims. The method used was a modification of the method of Wattenberg *et al.* (5) and of Kuntzman *et al.* (9), as described in previous papers from this laboratory (10,11). All rats were guillotined and exsanguinated between 11:00 a.m. and 2:00 p.m., with the iced tissues being used immediately. A 15% homogenate was made with a cold 0.1 M K_2HPO_4-KH_2PO_4 buffer (pH 7.4); it was centrifuged at 10,000 x g for 20 min, and then diluted 1 to 15 in the cold phosphate buffer. The incubation mixture for the AHH activity determination contained 125 μmoles KCl, 10 μmoles $MgCl_2$, 3.5 μmoles NADPH, 3.9 μmoles NADH, 0.5 ml of 1% supernatant fraction and sufficient buffer to give a total volume of 3.0 ml. The reaction was then initiated by the addition of 100 μg benzo(a)pyrene in 0.1 ml acetone. After incubation for 15 min at 37°, separation and estimation of the products were performed on an Amino-Bowman spectrophotofluorometer as described previously (10,11). Protein was measured using the biuret method of Lowry *et al.* (12).

RESULTS AND DISCUSSION

The results in Table 1 are illustrative of a number of experiments in this laboratory whereby mixed function oxygenase metabolism is lowered drastically after a burn injury. In the experimental data shown, the hepatic AHH levels per mg of liver protein are lowered 38 percent at 26 hr and 57 percent at 10 days with a probability of less than 0.005 that these occurred by chance, thus supporting the suggestive data of the urinary glucaric acid excretion evidence in man (7).

Table 1. Hepatic aryl hydrocarbon hydroxylase in rats after a 15% burn injury

Time after burn	Controls	15% Burns	Significance*
26 hr	26.57 ± 2.36+ (7)‡	16.60 ± 1.55+ (7)	$P < 0.005$
10 days	26.95 ± 3.16 (8)	11.59 ± 2.72 (7)	$P < 0.005$
Significance*	NS	$P < 0.01$	

*Significance was measured by Student's one-tailed t-test. NS = not significant.

+Values are ng 3-OH-benzo(a)pyrene formed/mg of protein/min. Results are given as means ± S.E.M.

‡Number of rats per group.

Other data obtained, but not shown, indicate that there was no significant difference in either liver wet weight or liver protein levels between control and 15% burned animals. Calculations of AHH per g wet weight of liver were significantly lower in burned animals compared to the controls.

This supportive evidence of decreased mixed function oxygenase in burned patients, indicated originally by a lowered excretion of urinary glucaric acid (7), is of importance not only because of the possible overdose toxicity of pharmacological agents administered to such patients, but also because of the possible resultant toxicity of pollutants metabolized more slowly by a decreased activity of mixed function oxygenases such as AHH. The decreased activity of enzymes, such as AHH, caused by a burn injury appears to fit a pattern. This laboratory and others have reported lower levels of mixed function oxygenases due to other inflammatory agents (10,13,14). As AHH appears to be a fairly ubiquitous detoxifying agent which decreases in response to inflammatory conditions and increases in response to xenobiotics, like benzo(a)pyrene, in many tissues (11), its variation is of extreme importance.

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