4. McQuilkin, W. T., Evans, V. J., Earle, W. R., *J. Nat. Cancer Inst.*, 1957, v19, 885.

5. Sanford, K. K., Likely, G. D., Earle, W. R., *ibid.*, 1954, v15, 215.

6. Pace, D., Aftonomus, B., *ibid.*, 1957, v19, 1065.

7. Mattern, C. F. T., Brackett, F. S., Olson, B. J., *J. Applied Physics*, 1957, v10, 56.

8. Pearson, S., Stern, S., McGavack, T. H., *Anal. Chem.*, 1953, v25, 813.

9. Merck, & Co., Inc., Rahway, N. J.

10. Trout, D. I., Estes, E. J., Jr., Friedberg, S. J., *J. Lipid Res.*, 1960, v1, 199.

11. Zarafonetis, C. J., Seifter, J., Baeder, D. H., Kalas, J. P., *A. M. A. Arch. Int. Med.*, 1959, v104, 974.

12. Wolf, S., *Mod. Concepts Cardiovasc. Dis.*, 1960, v29, 599.

13. Shafrir, E., Steinberg, D., *J. Clin. Invest.*, 1960, v39, 310.

14. Havel, R. J., Goldfien, A., *J. Lipid Res.*, 1959, v1, 102.

15. Simms, H. S., Parshley, M. S., Pitt, R. B., *J. Geront.*, 1947, v2, 205.

16. Kline, I., Leighton, J., Belkin, M., Orr, H. C., *Cancer Res.*, 1957, v17, 780.

17. Weiss, A. K., *Fed. Proc.*, 1960, v19, 137.

18. Henneman, D. H., Henneman, P. H., *J. Clin. Invest.*, v39, 1239.

19. Seifter, J., Baeder, D. H., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 469.

20. Holman, R. L., McGill, H. C., Jr., Strong, J. P., Geer, J. C., *J. Am. Med. Assn.*, 1959, v170, 416.

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Modification of Lethal Effect of Bacterial Endotoxin by Substances Altering the Metabolism of 5-Hydroxytryptamine.* (26677)

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5-Hydroxytryptamine (5HT), which in free form is a potent stimulator of smooth muscle, normally circulates bound to platelets in an inactive state(1). The occurrence of thrombocytopenia and elevated plasma concentrations of 5HT after intravenous injection of endotoxins from Gram-negative bacteria(2-3) suggests that some of the biologic effects of endotoxins might be related to release of 5 HT from damaged or sequestered platelets. The present studies were undertaken to determine the effect of substances that alter the metabolism of 5HT on the lethal action of bacterial endotoxin in mice.

Materials and methods. Three substances that alter 5HT metabolism were investigated: (A) 1-benzyl-2, 5-dimethyl serotonin (BAS),‡ an antimetabolite of 5HT by virtue of similar chemical configuration(4), (B) beta-phenyl isopropyl hydrazine (PIH)§(5), an inhibitor of monamine oxidase which catalyzes the degradation of 5HT to 5-hydroxy indole acetic acid(6), and (C) the metabolic precursor of 5HT, 5 hydroxy tryptophan (5-HTP)‖, administration of which appreciably increases tissue stores and platelet concentrations of 5HT(7). Dosages of BAS, PIH, and 5HTP were based on the reports of Shaw and Wooley(4), Horita(5), and Udenfriend *et al.*(7), and were somewhat larger than necessary for physiologic activity. These substances produced no apparent untoward effects when administered alone to control groups of 10 or more mice.

Eighteen to 25 g male albino mice of the CFW strain (Carworth Farms, New City, N. Y.) were used in all experiments. Groups of 10-20 mice were injected by the intraperitoneal (IP) or subcutaneous (SC) route with the substance to be tested on each of 2 suc-

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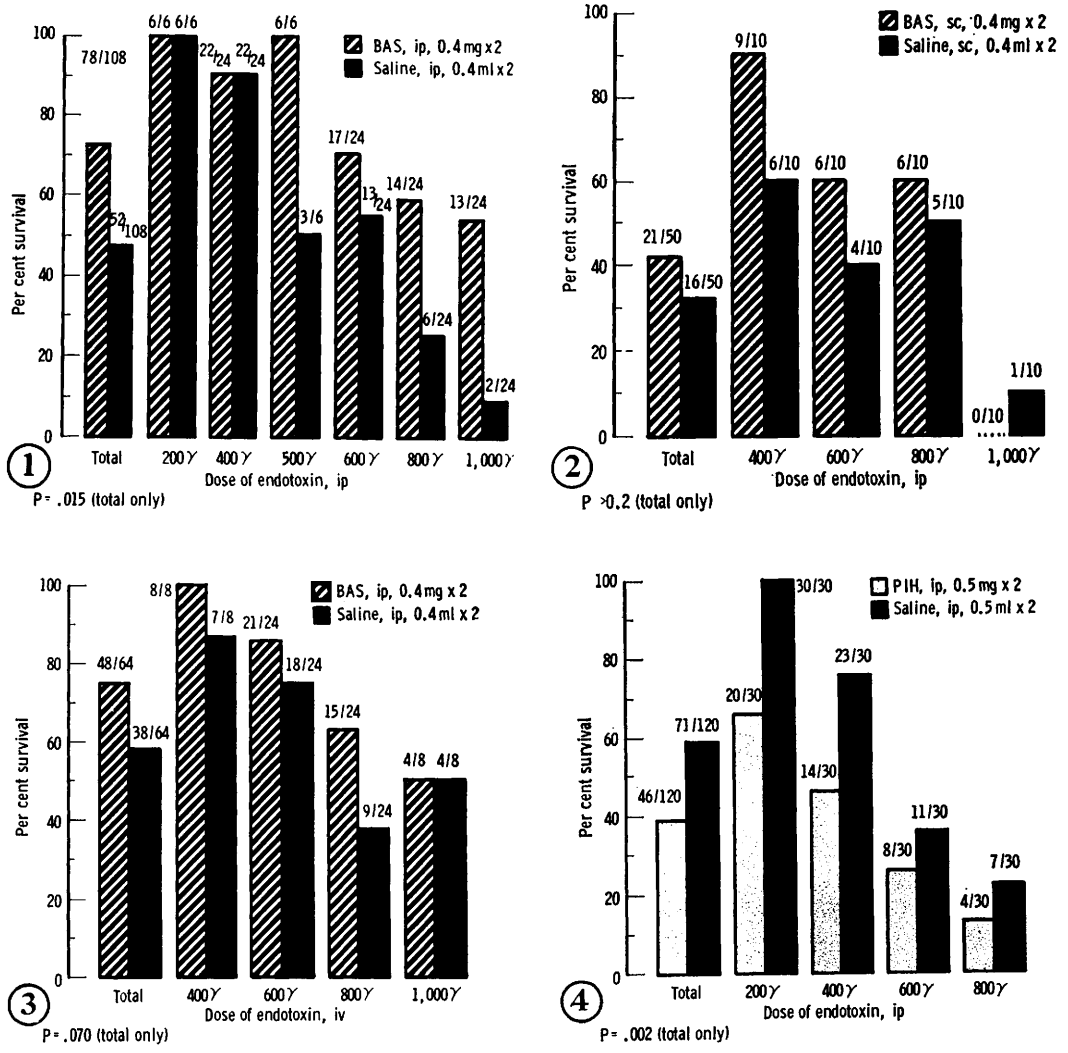

FIG. 1. Effect of BAS inj. IP on response of mice to IP challenge with endotoxin.
FIG. 2. Effect of BAS inj. SC on response of mice to IP challenge with endotoxin.
FIG. 3. Effect of BAS inj. IP on response of mice to IV challenge with endotoxin.
FIG. 4. Effect of PIH inj. IP on response of mice to IP challenge with endotoxin.

cessive days. Controls were injected with normal saline solution. Both pretreated and control groups were then challenged with graded doses of *Escherichia coli* endotoxin (Difco, Lot No. 0127:B8) by the IP or intravenous (IV) route 30-90 minutes after the last pretreatment injection. Mortality was tabulated after 24 and 48 hours. For statistical purposes the groups receiving different doses of endotoxin were combined and the difference in per cent survival between pretreated and control groups submitted to the t test.

Results. As shown in Fig. 1 mice pretreated with BAS by the IP route were significantly more resistant than saline pretreated controls to the lethal action of endotoxin injected by the same route. When either BAS or endotoxin were administered by other than the IP route (Fig. 2 and 3) a protective effect was observed but was less marked and not significant according to this method of statistical analysis. Fig. 4 illustrates that pretreatment with the monoamine oxidase inhibitor PIH rendered mice significantly more susceptible to the lethal action

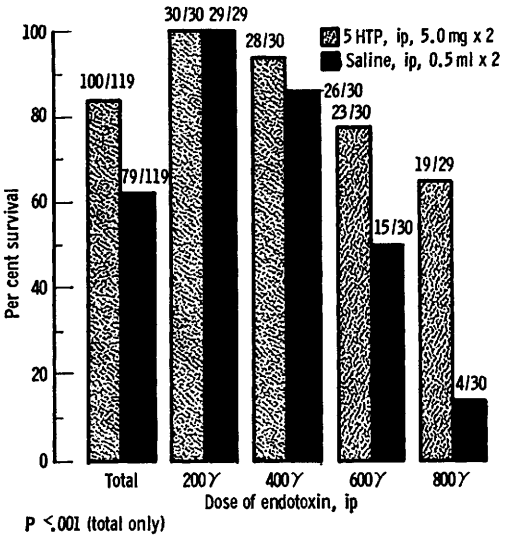

FIG. 5. Effect of 5HTP inj. IP on response of mice to IP challenge with endotoxin.

of endotoxin than saline pretreated controls. Contrary to expectations, pretreatment with the 5HT precursor, 5HTP, significantly enhanced the ability of mice to survive subsequently administered endotoxin injections (Fig. 5).

Discussion. Inhibition of the lethal effect of endotoxin in mice by pretreatment with the anti-5HT compound BAS, and its enhancement by pretreatment with the monoamine oxidase inhibitor PIH suggest that release of 5HT may be a factor in endotoxin lethality. It should be emphasized that these changes were only partial and were influenced by route of administration of both pretreatment and challenge injections.

Shimamoto and associates(8) have reported similar results in rabbits treated with the anti-5HT compounds Brom-lysergic acid diethylamid (BOL) and the monoamine oxidase inhibitor iproniazid. Gilbert(9) reported that the anti-5HT compound lysergic acid diethylamid (LSD) mitigated the acute hypotensive response observed in cats after intravenous injections of endotoxin but in a single instance found oral BAS without effect. Several groups of investigators(10,11, 12,13) have demonstrated that chlorpromazine inhibits the lethal effect of endotoxin, a property possibly attributable in part to its anti-5HT activity. Contrary to our findings, two groups of investigators(11,14) have reported that monoamine oxidase inhibitors are without effect on endotoxin lethality.

The protective effect of 5HTP, the metabolic precursor of 5HT, was not anticipated. However, Gordon and Lipton(15), and Lasker *et al.*(11) observed that mice pretreated with 5HT itself demonstrated enhanced resistance to endotoxin. Although the effects of pretreatment with 5HTP and 5HT are superficially discordant with those described for BAS and PIH, these interrelationships are probably too complex to warrant the assumption that they are contradictory. A similarly confusing situation exists in the case of histamine, another amine with marked physiologic activity which is normally bound to platelets and known to be released after injection of endotoxin(16). Pretreatment with this substance also diminishes the lethality of subsequently administered endotoxin(10,11), as does pretreatment with histamine depletors(17) and antihistaminic compounds(10,11).

Summary. The lethal effect of bacterial endotoxin in mice is modified by administration of the 5HT antagonist, BAS, the monoamine oxidase inhibitor PIH, or the 5HT precursor, 5 HTP. Animals pretreated with BAS and 5 HTP demonstrated increased resistance to endotoxin whereas pretreatment with PIH increased susceptibility to endotoxin.

1. Page, I. H., *Physiol. Rev.*, 1958, v38, 277.

2. Stetson, C. A., Jr., *J. Exp. Med.*, 1951, v93, 489.

3. Shimamoto, T., Yamazaki, H., Ohno, K., Uchida, H., Konishi, T., Iwahara, S., *Proc. Japan Acad.*, 1958, v34, 444.

4. Shaw, E. N., Wooley, D. W., *J. Pharm. and Exp. Ther.*, 1956, v116, 164.

5. Horita, A., *ibid.*, 1958, v122, 176.

6. Udenfriend, S., Titus, E., Weissbach, H., Peterson, R. E., *J. Biol. Chem.*, 1956, v219, 335.

7. Udenfriend, S., Weissbach, H., Bogdanski, D. F., *ibid.*, 1957, v224, 803.

8. Shimamoto, T., Inoue, M., Koizumi, M., Iwahara, S., Konishi, T., *Proc. Japan Acad.*, 1958, v34, 456.

9. Gilbert, R. P., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 346.

10. Abernathy, R. S., Halberg, F., Spink, W. W., *J. Lab. Cin. Med.*, 1957, v49, 708.

11. Lasker, S. E., Fox, C. L., Jr., *Fed. Proc.*, 1960, v19, 103.

12. Noyes, H. E., Sanford, J. P., Nelson, R. M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 617.

13. Thomas, L., *J. Exp. Med.*, 1956, v104, 865.

14. McLean, R. A., Berry, L. J., *Fed. Proc.*, 1960, v19, 245.

15. Gordon, P., Lipton, M. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 162.

16. Hinshaw, L. B., Vick, J. B., Carlson, C. H., Fan, Y., *ibid.*, 1960, v104, 379.

17. Thomas, L., *A.M.A. Arch. Int. Med.*, 1958, v101, 452.

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Alkaline Phosphatase Activity of Human Cell Cultures.* (26678)

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Studies of enzyme activities, nutritional requirements and metabolic activities associated with growth of mammalian cells in culture have in general revealed basically similar patterns regardless of the source from which those cells had been derived(1.2). During studies to characterize the tissue of origin of established human cell cultures, differences in alkaline phosphomonoesterase activity were observed(3). These observations have been extended to other cell lines, including recent isolates, and this report which describes differences in phosphatase activity between clonal strains of several parental lines, reemphasizes the heterogeneity which may exist in cell populations propagated *in vitro*.

Materials and methods. Established cell lines were purchased from Microbiological Associates, Inc., and clonal strains HeLa S_3 and 3213A were provided by Drs. T. T. Puck and M. Bender respectively. The parental Zimmer liver line was derived from a biopsy in a 5 month old infant with congenital atresia of the bile ducts and has been maintained in serial culture for approximately 70 passages. Recent cell isolates were derived according to standard technics from bone marrow aspiration(4) and skin biopsy(5). Clonal strains were derived from one or more consecutive single cell platings according to the procedure of Puck *et al.*(6).

Eagle's medium and M 199 containing 10 or 15% undialyzed human, horse or calf serum or the nutrient medium of Puck containing 20% undialyzed human serum were used for the growth of established cell lines. Recent isolates were cultured in an enriched Eagle's medium with added bovine embryo extract ultrafiltrate(4). After growth for 6-8 days on glass surfaces in an atmosphere of 5% CO_2 in air at 37°C, cells were dispersed with .05% trypsin in bicarbonate-free salt solution(7), and transferred to fresh medium, or harvested by centrifugation at 500 × g at room temperature after neutralization of tryptic action with growth medium. The cells were washed twice in 20 volumes of cold 0.14 M NaCl or 0.25 M sucrose and resuspended in 0.25 M sucrose. Cell-free extracts were prepared by sonic vibration of 2 to 5 ml of cell suspensions in collodion-treated lusteroid tubes for 1 to 5 minutes in an ice water-cooled Raytheon 10 kc oscillator.

Alkaline phosphatase was measured by hydrolysis of disodium p-nitrophenyl phosphate using 2-amino-2-methyl-1-propanol-HCl (AMP) buffer at pH 10.6 and 38°C(8). Specific activity was expressed as μM of p-nitrophenol liberated in 30 minutes per mg of cell protein(9).

Results. Table I summarizes the specific activities for alkaline phosphatase of a variety of established and recently isolated human cell lines. Each value represents the

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