Hormonal Modification of Endotoxin Mortality in Mice.* (26045)

PAUL GORDON AND MORRIS A. LIPTON†
Veterans Administration Research Hospital and Depts. of Pharmacology and Medicine, Northwestern University Medical School, Chicago, Ill.

Activation of the sympathetic nervous system, known to follow endotoxin administration, is held to be in part responsible for endotoxin death(1-4). Demonstrating activation Boquet(2) and Zweifach(3) have observed marked adrenergic vasoconstriction following intoxication, while Heiffer(4) has recorded adrenal depletion of the adrenalines in the rabbit. Antiadrenergic compounds, on the other hand, have been observed to protect several species against endotoxin death(5).

Several recent papers have examined perturbations of non-adrenergic amines following endotoxin administration. They describe an endotoxin-induced serotonin release from blood platelets, a phenomenon that eventuates in a transient increase in plasma serotonin followed by its disappearance from the blood during the shock state(6-8). In this work we are concerned with evaluating the possible influence of this phenomenon on endotoxin mortality. We approximate the condition by studying the effect of injected serotonin on endotoxin lethality, in mice. We attempt to modify this effect, in turn, by use of Compound F. Pertinent to this, glucocorticoids have been found to potentiate the eosinopenic response to serotonin(9).

An examination of thyroxine's modification of endotoxin mortality is appended to these studies. Our interest in such action stems from an observed increased sensitivity of thyroxine-treated animals to adrenaline(10-11). Similarity of subject matter to the main body of the experiment make this inclusion warranted.

These experiments were published some time ago in abstract form(12).

Methods. Swiss Webster and Carworth Farms mice were used, weighing between 22 and 26 g. Food and water were given *ad libitum*. Endotoxin was purified *E. coli* endotoxin (Difco): serotonin, the creatinine sulfate (Sigma): the Compound F, a suspension, Cortef, (Upjohn): and thyroxine, sodium l-thyroxine, (Nutritional Biochemicals).

Exp. 1. Four hundred and fifty Carworth Farms male mice were divided into 3 groups of 150 mice each. One group was injected subcutaneously with 0.8 mg/kg serotonin base, another with 1.6 mg/kg serotonin, and the third group with saline diluent. Thirty minutes later all animals were injected with 32 mg/kg endotoxin intraperitoneally. Mortality data were collected at 48 hours.

Exp. 2. Two hundred Carworth Farms female mice and 100 Swiss Webster female mice were divided into 2 groups each. One group of each strain was injected subcutaneously with 0.8 mg/kg serotonin in 0.1 cc saline and the other group was injected with the diluent alone. Thirty minutes later all animals were given endotoxin intraperitoneally. The Swiss Webster animals were given 24 mg/kg toxin and the Carworth Farms animals were given 32 mg/kg toxin, doses previously found to kill approximately ⅔ of the subjects in the respective strains. At 48 hours mortality data were recorded.

Exp. 3. Four hundred and thirty-five Carworth Farms male mice were divided into 4 groups of from 80 to 130 each. One group was injected subcutaneously with 1.2 mg/kg serotonin, and with 1.0 mg/kg Compound F subcutaneously at another site; another group was injected with serotonin alone and appropriate diluent; another was injected with Compound F alone and appropriate diluent; the fourth group was given 2 injections of diluent. Thirty minutes later all were injected with 32 mg/kg endotoxin intraperitoneally. Mortality data were collected at 48 hours.

Exp. 4. Swiss mice were divided into 4 groups of 70 each and were injected for 0, 1, 3, and 4 days with 50 γ l-thyroxine/day/

* This study supported by grants from U.S.P.H.S. and Ciba Pharmaceutical Co.

† Present address: Univ. of North Carolina, Dept. of Psychiatry, Chapel Hill.

TABLE I. Effect of Serotonin and Compound F on Endotoxin Toxicity in Mouse.

Exp.	Sex	Serotonin, mg/kg	Compound F suspension, mg/kg	Mortality	% mortality	% protection	P
1	♂	0	0	99/150	65		
	♂	.8	"	84/150	52	13	
	♂	1.6	"	64/148	42	23	.01
2	♀	0	"	102/150	67		
	♀	.8	"	48/150	31	36	.01
3	♂	0	"	72/111	64		
	♂	1.2	"	53/114	47	17	.01
	♂	"	1.0	34/130	30	34	.01
	♂	0	"	54/80	67	0	

adult mouse. At end of treatment period each group was given 8 mg/kg endotoxin intraperitoneally. Mortality data were collected at 48 hours.

Results. Table I illustrates serotonin protection against endotoxin mortality, and moderate enhancement of this effect by an otherwise silent dose of Compound F. The degree of protection in these experiments fell between 12 and 36 percent depending upon the dose of serotonin employed, the presence or absence of Compound F, and the sex of the test mice.

The effect of serotonin was moderate but significant at less than the 1 percent level. The increase of protection with F was likewise significant. As well, female mice were significantly more responsive to serotonin than males at the dose tested. Table II illustrates a thyroxine enhancement of endotoxin mortality that increases progressively with the treatment period. Through this influence an LD 11 is increased to an LD 91 after 4 days.

Discussion. Vulnerability to the lethal effects of endotoxin may be modified by the action of diverse circulating hormones. Spink has observed protection in the mouse exerted by glucocorticoids(13). Our studies reveal small amounts of serotonin to be protective in the mouse, and demonstrate potentiation of this effect by a quantity of Compound F suspension not protective in itself. Conversely, a progressive loss of resistance to endotoxin death is observed to develop during thyroxine treatment.

TABLE II. Effect of Thyroxine on Endotoxin Toxicity in Mouse.

Days Rx	Mortality	Mortality
0	8/70	11
1	24/70	34
3	37/71	52
4	64/70	91

As other antiadrenergic compounds have proved to exert some protection the effect of serotonin may be related to an expression of its antiadrenergic action, elaborated on and reported by us elsewhere(14). It is not inordinate to propose that the "anti-endotoxin" action of injected serotonin may be exerted by the serotonin normal to the animal.

Compound F suspension had no effect on endotoxin mortality by itself, a function of the low dose and poorly soluble form in which it was used by us. The same amount of F given as a soluble compound (Solu Cortef, Upjohn) protects against endotoxin death in our hands, conforming to the observations of Spink.

The thyroid principle triiodothyronine has recently been reported to enhance endotoxin mortality in mice by Melby and Spink(15). These workers attributed their results to the augmented oxygen requirement induced by thyroid compounds. With this we are in agreement and suggest as well the parallel contribution of a thyroid potentiation of adrenaline vasoconstriction, forcing a reduction of oxygen available to the peripheral tissues regardless of tissue requirements(10).

Summary. Serotonin reduces endotoxin mortality in mice. This effect is greater in females, and is potentiated by Compound F. Maintained Thyroxine treatment progressively increases toxicity of endotoxin in this species.

1. Thomas, L., *Ann. Rev. Physiol.*, 1954, v16, 467.
2. Boquet, P., Izard, Y., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 254.
3. Zweifach, B., Nagler, A., Thomas, L., *J. Exp. Med.*, 1956, v104, 881.
4. Heiffer, M. H., Munday, R. L., Mehlam, B., *Fed. Proc.*, 1958, v18, 66.
5. Thomas, L., *J. Exp. Med.*, 1956, v104, 865.
6. Davis, R. D., McCyarrie, D. G., Meeker, W. R., *Fed. Proc.*, 1959, v18, 211.
7. Waalkes, J. P., Weissbach, H., Bozicevich, J., Underfriend, S., *J. Clin. Invest.*, 1957, v36, 1115.
8. Rosenberg, J. D., Lillehei, R. C., Moran, W. H., Zimmerman, B., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 225.
9. Halberg, F., *Am. J. Physiol.*, 1954, v179, 309.
10. Smith, D. J., *XIX Internat. Physiol. Congress*, 1953, 773.
11. Swanson, H. E., *Endocrinology*, 1956, v59, 217.
12. Gordon, P., Lipton, M. A., *Fed. Proc.*, 1957, v17, 301.
13. Spink, W. W., Anderson, D. J., *J. Clin. Invest.*, 1954, v33, 540.
14. Gordon, P., Haddy, F. J., Lipton, M. A., *Science*, 1958, v128, 531.
15. Melby, J. C., Spink, W. W., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 546.

Received July 25, 1960. P.S.E.B.M., 1960, v105.

Effect of Urease Injection on Body Weights of Growing Rats and Chicks.* (26046)

H. C. DANG AND W. J. VISEK (Introduced by K. P. DuBois)
Dept. of Pharmacology, University of Chicago, Chicago, Ill.

Antibacterial agents, fed in trace amounts, enhance growth of birds and animals under a variety of environmental conditions(1-4). Some investigators have postulated that these agents suppress toxins produced by gastrointestinal bacteria. Ammonia has been suggested as one of the toxins whose production is inhibited(5-8). We have recently reported that addition of 100 ppm of 3 antimicrobial agents to a casein diet significantly decreased *in vivo* hydrolysis of C-14 urea by rats and reduced production of urease by gastrointestinal bacteria(9). Our preliminary investigations have shown growth stimulation in rats and chicks immunized with jackbean urease (10, 11). Urease stimulates production of antiurease in rabbits which on passive transfer to normal rabbits produces a drop in blood ammonia(12). In the experiments herein reported the effects of jackbean urease injections on growth of rats and chickens fed soybean diets of suboptimal nutrient content are described.

Materials and methods. Urease was crystallized from jackbean meal and recrystallized twice from ethyl alcohol by the method of Kirk and Sumner(12). Enzymic activity was determined and purity of the preparations was checked using paper chromatography. The enzyme dissolved in 0.85% NaCl was injected intraperitoneally into rats and subcutaneously into chickens. Injections were made during the first 4 weeks of each experiment which lasted 8 weeks. In the rat experiments, injections were made every other day whereas in the chick experiment, injections were made on the same 3 successive days each week for the first 4 weeks. Control subjects received 0.85% NaCl in equal volume to that received by those which were immunized. In rats, the starting doses were 10 units† per kg of body weight, progressing to 25 units per kg body weight. Each rat received a total of 30 units of urease. The chicks received initially doses of 0.1 unit per injection and progressed to a dose of 10 units

* Supported by grants from Atomic Energy Comm. Research and Development Division, Office of Surgeon General, U. S. Army, and the Schweppe Foundation, Chicago, Ill. Authors acknowledge Professor Paul Meier for statistical assistance in experiments with rats and the American Meat Institute Foundation for use of facilities.

† One unit of urease activity is that amount of urease required to liberate one mg of ammonia N in 5 min. in a phosphate buffer at pH 7.0 at 20°C.