differs from streptomycin in that it is effective when given prior to and not after irradiation when infection begins. Perhaps the drug also exerts a non-specific beneficial effect such as Treadwell *et al.*(8) demonstrated for estradiol benzoate.

Intestinal lactobacilli formed a formidable component of mouse bacteremia and appeared to have pathological significance in the radiation syndrome in this species. This mouse infection differs from that seen in the rat by Vincent *et al.*(9) where bacteremia lactobacilli counts were relatively low and did not correlate with severe irradiation effects. It is also true that lactobacilli were insignificant or missing from surveys of mouse irradiation bacteremias made by Miller *et al.*(7) and Gonshery *et al.*(10). Differences in lactobacilli concentrations between the present work and that of others(7,9,10) may have been due, in part, to differences in sampling and cultural procedures. Our methods, including homogenization of the liver and culture of the sample in poured, deep-agar plates, favored growth of microaerophilic lactobacilli. Differences in laboratory diet could also have been involved.

Summary. It has been shown that premedication with Quinoxaline-1,4-di-N-oxide is beneficial to irradiated mice, significantly increasing both the ST_{50} day and total number of survivors. The effect has been related to a significant reduction in number of enteric organisms causing the usual irradiation bacteremia. It has been demonstrated that lactobacilli were the predominating invaders under conditions of our experiments. The drug does not appear to have an effect on bone marrow.

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Cardiovascular Responses of Unanesthetized Rats During Traumatic and Endotoxin Shock.* (23547)

CHARLES A. ROSS (Introduced by Karl H. Beyer)
Merck Institute for Therapeutic Research, West Point, Pa.

The available information on the direct measurement of arterial pressures in the unanesthetized rat is scant(1,2), and no data have been found where such studies were made during shock from trauma. Chambers *et al.*(3) determined the apparent systolic pressures of unanesthetized rats before and after physical trauma by an indirect method, but they made no systematic presentation of the data.

Thomas(4) recently pointed out the marked similarities of symptoms and pathological changes reported to occur after endotoxin intoxication and traumatic shock, and Schweinburg *et al.*(5) have suggested endotoxins as a factor in shock. It is the purpose of the present investigation to obtain com-

* Dr. David Tennent prepared the sample of endotoxin used.

parative data by direct measurement of the cardiovascular responses of the unanesthetized rat to traumatic shock and to endotoxin intoxication.

Method. White male Wistar rats weighing from 300 to 500 g were used throughout the investigation. Under ether anesthesia a polyethylene catheter, 0.58 mm ID × 7 cm long, was passed subcutaneously to a ventral incision in the neck from a stab wound caudal to the ear. The left carotid artery, carefully separated from the main vagal trunk, was tied centrally and opened; the catheter tip was passed to lie near its root at the aorta and secured by double ligatures. All incised tissues were infiltrated with procaine. This indwelling catheter was connected to a 15-cm length of similar polyethylene tubing by a 1-cm ferrule fashioned from 21 gauge hypodermic tubing. The entire catheter was connected to a Statham strain gauge (P23Da) or a capacitance pressure sensing head (Lilly) by a short 21 gauge needle stub and a 3-way stopcock. The catheter and pressure sensing head were filled with saline-heparin solution. Following closure of the wound, ether administration was discontinued, the animal was placed in a 20 cm × 25 cm × 12.5 cm metal box, and the pressure sensing head was clamped directly overhead. The negative pressure effect of the liquid column above the heart level was less than 5 mm of mercury and was disregarded. Full recovery from anesthesia was assumed after one hour, at which time the animal moved about the box without ataxia. The indwelling catheter was well tolerated and no discomfort was apparent. The arterial pressures were monitored continuously, and permanent recordings were made at appropriate intervals by a photographic oscilloscope or an ink-writing recorder. The respiratory rates were obtained from the arterial pressure records when these waves were reflected clearly in the tracings or by visual counts with a stopwatch. Following control recordings, the catheter was disconnected at the ferrule, open end was plugged, and protruding tip was taped to skin of the neck. The animal was placed in the drum of the Noble-Collip apparatus(6) and was subjected to a standard tumbling, which prior studies(7) had shown to result in a reproducible mortality rate. After trauma the animal was returned to the box, the catheter was connected to the pressure sensing system, and the arterial pressures, heart rates and respiratory rates were recorded in the post-trauma period. Animals alive at the end of observation period were returned to their quarters. All animals surviving 24 hours after trauma were considered survivors. The endotoxin sample was prepared from a strain of *Salmonella newport* and was determined to have an intravenous LD_{50} of 2.8 mg/kg in the rat by the moving average method(8). A dose of 5 mg/kg in saline was injected into the femoral vein of each rat after the arterial catheter had been placed. The ether was removed and the animal was placed in the metal observation box. The arterial pressures, heart rates and respiratory rates were recorded as described. All animals were heparinized after exposure to trauma or endotoxin injection, to prevent occlusion of the catheter. Gross autopsies were performed on all animals immediately following death.

Results. Responses to trauma. Immediately following trauma no significant changes in the arterial pressures or heart rates were observed; however, the respiratory rates rose transiently (Table I). In the 1½-hour period following trauma, the arterial pressures of each animal in the group which eventually died showed a slight but consistent elevation. Those animals subjected to identical trauma but surviving 24 hours evidenced a progressive decline in arterial pressures. Following this rise in arterial pressures in the group dying from trauma, there was a progressive decline in pressures in 4 of the 5 animals until the pre-terminal period. The heart rates and respiratory rates rose significantly as the terminal period was approached. An abrupt fall in respiratory rate usually signified the beginning of terminal collapse (Fig. 1). The respirations became gasping in type. Before the beginning of respiratory collapse the arterial pressures showed no marked hypotension. As the respiratory rate continued to decrease, the arterial pressures declined abruptly to hypotensive levels. Respiratory arrest, preceded by mild convulsive episodes, was fol-

TABLE I. Cardiovascular Responses of Unanesthetized Rats to Drum Trauma and to Endotoxin Intoxication.
5 rats/group.

Time:	Trauma survivors				Trauma deaths			Endotoxin deaths		
	Control	Post-trauma	Mid-period	Final recording	Control	Post-trauma	Mid-period	Pre-terminal	Control (etherized)	Post-inj.	Mid-period	Pre-terminal
Blood pressure, mm Hg												
Systolic	119 ± 18*	129 ± 21	110 ± 12	103 ± 9	123 ± 18	119 ± 30	115 ± 23	119 ± 14	94 ± 15	89 ± 18	95 ± 16	104 ± 16
Diastolic	83 ± 12	86 ± 14	78 ± 10	64 ± 12	80 ± 10	80 ± 16	86 ± 28	84 ± 27	60 ± 29	52 ± 14	54 ± 15	69 ± 17
Avg arterial pressure, mm Hg	102 ± 14	106 ± 16	93 ± 10	82 ± 13	99 ± 22	98 ± 17	100 ± 26	100 ± 25	77 ± 13	70 ± 14	74 ± 13	86 ± 17
Heart rate, beats/min.	324 ± 39	318 ± 31	392 ± 66	399 ± 48	331 ± 52	326 ± 60	411 ± 78	490 ± 82	344 ± 53	370 ± 53	382 ± 43	463 ± 31
Resp. rate, cycles/min.	70 ± 7	103 ± 17	90 ± 16	96 ± 17	78 ± 15	116 ± 34	100 ± 14	134 ± 24	89 ± 6	98 ± 10	90 ± 7	129 ± 13
Survival time	> 24 hr				271 ± 71 min.				270 ± 69 min.			

* ± stand. dev.

lowed by total circulatory collapse with the heart continuing to beat for a few minutes. The respiratory and heart rates of the surviving group were elevated only slightly by comparison, while the arterial pressures remained slightly below the pre-trauma levels.

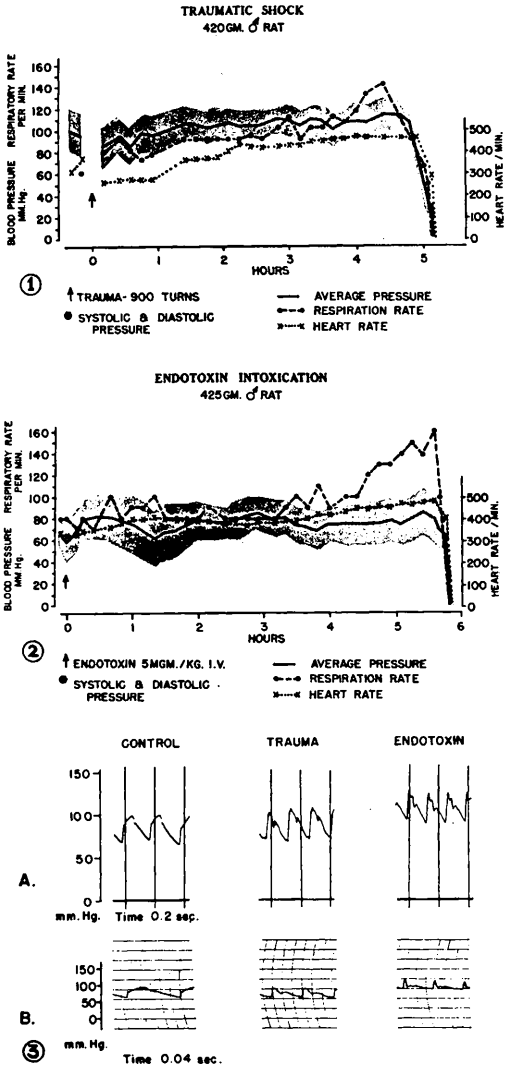

FIG. 1. Cardiovascular and respiratory responses of unanesthetized rat to fatal drum shock.

FIG. 2. Cardiovascular and respiratory responses of rat to a lethal intrav. inj. of endotoxin. Light ether anesthesia was discontinued at zero time.

FIG. 3. Central arterial pulse contours of unanesthetized rats recorded in control period and during early pre-terminal period after standard drum trauma and lethal doses of endotoxin. (A) Photographic recorder (retouched). (B) Ink-writing galvanometer.

* Shaded area.

Fig. 3 presents the individual pressure pulse contours of the unanesthetized rat in the control period and in the pre-terminal period after fatal trauma. Relative to the control, the post-trauma contours show shortened cycle durations, curtailed systolic ejection times, collapse of the pressure curve from mid-systole to aortic valve closure, and a slight positive pressure wave in the diastolic limb. Such changes are indicative of decreased venous return, compensatory vasoconstriction and cardio-acceleration. Cardiac contractility, as indicated by the slope of early systolic upstroke, did not appear markedly altered. Arrhythmias, as indicated by the regularity of the pulses, did not appear until respiratory failure occurred. The pulse contours of rats surviving trauma showed similar but less marked changes, and at the end of the observation period the contours resembled those recorded prior to trauma.

Responses to endotoxin. It may be seen in Table I that the control arterial pressures of this group of rats were on the average lower than the group subjected to trauma, possibly the result of ether anesthesia. The immediate response of the arterial pressures of the etherized rats to the injection of endotoxin was insignificant, although one animal sustained a transient fall of 24 mm Hg. For ½ hour following injection, there was a mixed response of recovery from anesthesia and of endotoxin effect. A moderate depressor response was observed to reach a maximum approximately 1½ hours after injection, the diastolic pressures declining more than the systolic pressures (Fig. 2). This delayed vasodepression also was observed in the anesthetized rat by Zweifach *et al.*(9). Following this response there was a sustained and gradual rise in pressures, with a narrowing of pulse pressures until terminal collapse. The average arterial pressures at the pre-terminal period were lower than the corresponding pressures of the group dying from trauma. Yet an average pressure of 86 ± 17 mm Hg does not indicate hypotension as a major factor contributing to the collapse. The sequence of events at terminal collapse was similar to those described for animals dying from trauma. The pulse contours, as recorded in the pre-terminal period, while differing somewhat, show basically the same changes that were recorded in rats subjected to trauma (Fig. 3).

Gross observations. The gross reactions of rats following fatal trauma and endotoxin injection were similar. Both groups of animals exhibited decreased physical activity, somnolence, pilo-erection, bloody stools and pale, cool extremities. Rectal temperatures were usually subnormal; yet, a hyperthermia was evident when recordings were obtained from the upper descending colon. Urination was uncommon, and the animals often appeared hyper-reactive to auditory or pinch stimuli. The respiratory failure and convulsive episodes were suggestive of hypoxic involvement. The gross changes at post mortem of rats dying from trauma were essentially the same as those described by Noble and Collip(6). Similar pathologic changes in the visceral organs were observed in rats dying from lethal doses of endotoxin, except that the endotoxin-treated rats exhibited petechial hemorrhages in the small bowel, the cecum rarely being involved, which contrasts with the large dark confluent hemorrhagic areas in the small bowel and the cecum observed after traumatic deaths. Pathologic changes in the colon and rectum were not observed after either type of death.

Discussion. The systolic and diastolic pressures of unanesthetized rats were lower on the average than those reported previously(1). However, the heart rates also were considerably lower, indicating that the lack of restraint may have contributed to these differences. The damping effect of the catheter on the frequency response of the recording system is acknowledged; yet, the small volume displacement per pressure increment of the pressure sensing manometers would tend to minimize this error. The average arterial pressures, which differed by less than 10% from the electronically recorded mean pressures, should be relatively accurate values.

The absence of a marked hypotension in rats subjected to fatal trauma was surprising, since such a finding is usually associated with the state of shock. Undoubtedly, the powerful vasoconstrictor response of the rat(2) maintains the central arterial pressures in the

face of a rapidly developing circulatory deficit. While a transient marked hypotensive response was reported previously to occur following trauma in the rat(3), inherent factors in the indirect method(10) coupled with reported difficulties of determining pressure in peripheral arteries in a state of constriction (11) may have been operative. The progressive rise in the central arterial pressures of rats during the 1½-hour period after trauma as observed here is probably a reflection of the intense sympatho-adrenal discharge demonstrated by Young and Gray(12) in the rat after trauma.

The cardiovascular responses of the rat to lethal doses of endotoxin, while differing in several aspects, were not markedly different from the responses evoked by fatal trauma. In both groups of rats, evidence of sympathetic activity, tachycardia, tachypnea, circulatory deficit and similar patterns of collapse at terminus were observed. The endotoxin group differed by showing transient vasodepressor responses, lower arterial pressures throughout and the absence of gross pathological changes in the cecal tissues. The large dose of endotoxin and the absence of physical trauma may account for some of these observed differences.

Profound hypotensive episodes have been reported to immediately follow the intravenous injection of endotoxins in the rabbit(13), cat(14) and dog(15). These responses resembled a histamine-release phenomenon in that tachyphylaxis developed(13). The relative absence of this immediate response in the rat may be a species difference.

The immediate cause of the terminal failure as indicated by the initial collapse of respiratory function implied a primary failure of brain stem function. Such views also have been held responsible for the terminal collapse in hemorrhagic shock in this species(2). However, this functional failure following trauma or endotoxin does not appear to be related directly to the level of central arterial pressure, but more likely to curtailment of effective peripheral blood flow and oxygen exchange at the vital centers.

In general, the studies presented here provide evidence that the various cardiovascular responses, symptoms and pathologic changes in rats subjected to endotoxin intoxication are similar in several aspects to those responses observed in this species subjected to lethal trauma. While certain differences have been observed in these responses, they are considered of insufficient magnitude to exclude endotoxins as possible factors concerned with the irreversible processes of traumatic shock. However, as attractive as the proposals are that endotoxins are operative in traumatic shock, Thomas(16) has reemphasized caution in the interpretation of like responses to dissimilar stresses as involving common factors. At the present, direct evidence is lacking.

Summary. The arterial pressures, heart rates and respiratory rates of unanesthetized rats have been recorded by direct measurement. The effects of traumatic shock and lethal dosages of endotoxin on these responses have been observed. The cardiovascular responses, symptoms and pathologic changes observed in rats subjected to trauma and to endotoxin intoxication were similar and, while certain differences have been observed, they are considered to be of insufficient magnitude to exclude endotoxins as possible factors concerned with their proposed role in irreversible shock.

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Intrinsic Factor Studies VI. Competition for Vit. B_{12} Binding Sites Offered by Analogues of the Vitamin.* (23548)

MARY BARTLETT BUNGE AND ROBERT F. SCHILLING

Department of Medicine, University of Wisconsin Medical School, Madison

The mechanism by which intrinsic factor, a component of normal human gastric juice, enhances absorption of vit B_{12} from the gut has not yet been elucidated. Recently reported evidence(1,2) indicates that vit B_{12} which is bound to gastric juice is absorbed preferentially over unbound vitamin. This observation suggests that binding of vit B_{12} is an essential property of intrinsic factor. However, the ability to bind vit B_{12} is an attribute of several biologic substances which do not possess intrinsic factor activity(3-7). As far as we are aware, there are no published reports of preparations possessing intrinsic factor activity but lacking the property of binding B_{12}. It is possible that binding of vit B_{12} by intrinsic factor is a unique process as compared to binding of the vitamin by substances lacking intrinsic factor activity. To determine if the vit B_{12} binding mechanism of intrinsic factor is more fastidious than that of other vit B_{12} binding proteins, we have measured competition offered by certain vit B_{12} analogues for vit B_{12} binding sites of several biologic fluids. This is a report of such observations.

The effect of an excess of one vit B_{12} analogue, pseudovit B_{12}, on radioactive vit B_{12} binding by human gastric juice has been reported(7). Binding of vit B_{12} was only slightly reduced in the presence of a 50-fold excess of the pseudovitamin. It was concluded, then, that this analogue presents little competition for vit B_{12} binding sites of gastric juice. Gregory and Holdsworth(8) have made somewhat analogous observations on preferential binding of vit B_{12} by an intrinsic factor preparation derived from hog mucosa.

Materials and methods. Vit B_{12} analogues. Ten crystalline analogues of vit B_{12}† were studied. Three of the analogues,‡ sulfatocobalamin (SU-B_{12}), nitrocobalamin (NI-B_{12}), and chlorocobalamin (CL-B_{12}), hold sulfate, nitrate, or chloride ions, respectively, in lieu of the cyanide ion(9). In 4 other B_{12} analogues, desdimethylbenziminazole B_{12} (DDMBI-B_{12}),§ 5(6)-trifluoromethylbenziminazole B_{12} (TFMBI-B_{12}),§ 5(6)-hydroxybenziminazole B_{12} (HBI-B_{12})‖ or Factor III, and 5(6)-aminobenziminazole B_{12} (ABI-B_{12}),§ the side chains on the benziminazole moiety differ from those 2 methyl groups filling comparable loci in B_{12}(10,11). If adenine occupies the position filled by 5,6-dimethylbenziminazole in the B_{12} molecule, the analogue is known as pseudovit B_{12}(pseudo-B_{12})¶ (12,13), the sample of which used for this project was entirely free of B_{12}. Lactam (lactam-B_{12})§ and lactone (lactone-B_{12})§ forms result from cyclization of the acetamide

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† Vit. B_{12} (cyanocobalamin) = B_{12}.

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¶ Kindly donated by J. J. Pfiffner, Parke, Davis, and Co.