Serotonin Release by Bacterial Endotoxin.* (27063)

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Indirect observations have implicated serotonin in shock due to bacterial endotoxin(1,2). Armin and Grant observed that *E. Coli* endotoxin injection produced constriction in the vessels of the rabbit ear which resembled that produced by serotonin(3). During the constriction phase they were able to find minute quantities (0.01 to 0.02 μg/ml) of an ultrafilterable substance in plasma which resembled serotonin in action on the cat uterus, including antagonism by L.S.D. Their findings were later confirmed by others(4) who found 0.004 to 0.01 μg/ml of plasma serotonin 1-2 hours after endotoxin. In addition, many studies had clearly shown that serotonin was released by antigen from platelets of sensitized rabbits (5, 6) and was implicated in anaphylaxis in certain species(7). Previous studies in our laboratories using dogs showed an immediate fall in serum serotonin concentrations, but only small, and not statistically significant elevations in platelet free plasma serotonin(8). The present studies were done to see if immediate release could be demonstrated in rabbits, and if a dose-response relationship existed *in vivo*.

Methods. The carotid artery and external jugular vein of anesthetized (nembutal 30 mg/kg) 3-4 kg New Zealand white rabbits were isolated and cannulated with #240 Clay Adams polyethylene cannulae. Purified *E. coli* endotoxin(9)† was given by intravenous injection. Arterial samples were collected in siliconed tubes containing 50 U.S.P. units of heparin sodium per 6 ml blood for platelet counts and plasma serotonin concentrations. Immediately after collection, blood for assay of plasma serotonin was centrifuged at 4°C for 30 min at 3000 r.p.m. Serum serotonin was collected by the method of Davis(10) and all serotonin samples were assayed fluorometrically(11) using the Aminco Bowman Spectrophotofluorometer. Platelet counts were done in duplicate using Rees-Ecker diluting fluid and a direct counting technique.

Results. Rapid intravenous injection of *E. coli* endotoxin caused thrombocytopenia at all doses employed (Table I). The fall in platelet count was more precipitous with larger doses 15 seconds after endotoxin, but after 5 minutes little difference in counts was present, except at the lowest and highest dose employed. The decrease in total serotonin, measured in serum, also was greater with larger amounts one minute after endotoxin; after 5 minutes the decrease was essentially equal at all doses used (Table II). Plasma serotonin (platelet free) rose to a maximum level 15 seconds after endotoxin, then fell during the first 5 minutes (Table III). No elevation of plasma serotonin occurred with the lowest dose employed (0.01 mg/kg). Separate studies showed that intravenous injection

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TABLE I. Mean Carotid Artery Platelet Counts Following Intravenous Injection of *E. coli* Endotoxin.

Time after Endotoxin	Dose of Endotoxin (mg./Kg.)					
	Saline Control (5)	.01 (5)	.05 (5)	.25 (5)	1.0 (10)	4.0 (10)
Control	326,000	390,000	377,000	291,000	513,000	497,800
15 sec	375,000	485,000	326,000**	207,000**	340,000*	Clumps
60 "	356,000	305,000**	117,000*	30,000*	41,000*	45,600*
5 min	352,000	158,000*	78,000*	38,000*	69,000*	191,000*

No. of animals indicated in parentheses.
* Magnitude of decrease significantly greater than saline control at each indicated time ($p<.05$).
** Magnitude of decrease not significantly greater than saline control at each indicated time ($p>.05$).

TABLE II. Mean Serum Serotonin Concentrations (μg/ml) in Carotid Artery Blood Following Intravenous Injection of *E. coli* Endotoxin.

Time after Endotoxin	Dose of Endotoxin (mg./Kg.)					
	Saline Control (5)	.01 (5)	.05 (5)	.25 (5)	1.0 (10)	4.0 (10)
Control	6.84	6.78	9.22	3.03	9.99	7.92
60 sec	6.22	3.23*	4.57*	.60*	.69*	1.53*
5 min	6.10	1.33*	1.52*	.69*	.90*	.97*

No. of animals indicated in parentheses.
* Magnitude of decrease significantly greater than saline control concentration at each indicated time ($p<.05$).

TABLE III. Mean Plasma Serotonin Concentrations (μg./ml.) in Carotid Artery Blood Following Intravenous Injection of *E. coli* Endotoxin.

Time after Endotoxin	Dose of Endotoxin (mg./Kg.)					
	Saline Control (5)	.01 (5)	.05 (5)	.25 (5)	1.0 (10)	4.0 (10)
Control	.04	.13	.12	.03	.10	.12
15 sec	.05	.07**	.76*	1.25*	2.28*	2.25*
30 "	.07	.15**	.70*	.68*	.93*	1.16*
60 "	.03	.05**	.47*	.22*	.26*	.49*
3 min	.05	.03	.04	.09**	.23**	.38*
5 "	.08	.03	.01	.06	.12**	.28**

No. of animals indicated in parentheses.
* Significantly greater than saline control at each indicated time ($p<.05$).
** Not significantly greater than saline control at each indicated time ($p>.05$).

of 0.01 mg/kg caused no mortality in 15 rabbits, whereas each higher dose produced some mortality, and 4.0 mg/kg was lethal for all rabbits studied.

Discussion. The immediate release of serotonin into plasma following endotoxin injection is strikingly similar to the response of the sensitized rabbit to antigen(6) and correlates well with previously reported *in vitro* release of serotonin after incubation of rabbit blood with endotoxin(12). The lack of a demonstrable rise of plasma serotonin when a non-lethal dose of endotoxin was employed does not imply that serotonin release is responsible for mortality, but shows clearly that massive serotonin release is absent with non-lethal amounts of endotoxin. Serotonin release at a slow rate probably does occur, even with a non-lethal dose, since the fall in serum serotonin was significantly greater ($p<.01$) than the fall in platelet count both 60 seconds and 5 minutes after endotoxin.

Although the release after 15 seconds increased in magnitude between the 0.05 and 1.0 mg/kg doses there was no increase beyond 1.0 mg/kg. The larger dose (4.0 mg/kg) however, produced a more sustained elevation of plasma serotonin since at 60 seconds after endotoxin the plasma concentration in that group was greater than that observed in the group which received 1.0 mg/kg and was the only group in which a significant increase in plasma concentration was observed 3 minutes after endotoxin ($p<.003$). Statistical analysis by rank correlation showed that there was no correlation between the maximal rise of free serotonin and the initial serum serotonin concentration at 3 of the 4 doses used. An inhibitor of serotonin liberation thus may be present *in vivo* as recently demonstrated for histamine an *in vitro* anaphylaxis system(13).

Serotonin release immediately after endotoxin injection in the rabbit may with other vasoactive amines contribute to the initial shock reaction. The lack of further increase in free serotonin concentration between 1.0 mg/kg and 4.0 mg suggests that maximal release occurred with the former dose. Since 4.0 mg/kg is a uniformly lethal dose of endotoxin, but 1.0 mg/kg is not, other factors undoubtedly play a role in the lethal response.

Summary. Within 15 seconds after rapid administration of *E. coli* endotoxin in the rabbit a significant release of serotonin into platelet-free plasma occurs *in vivo*. The magnitude of immediate serotonin release was cor-

related with amount of endotoxin administered, and there was no significant rise of plasma serotonin with a non-lethal dose.

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Hypothermia in Mice Due to Influenza Virus Infection.* (27064)

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Many of the naturally occurring influenzal infections of man are symptomless, and most of the overt infections are characterized by the occurrence of fever without pneumonia. In the mouse, which has been used extensively in studies of the pathogenesis of influenza, the main criteria of infection have been death and pulmonary consolidation. To determine whether the mouse could serve as an experimental model for the less severe infections that usually occur in man, we studied the temperature response of mice to infection with influenza virus.

Hypothermia, rather than fever, proved to be a striking feature of these infections. It was associated with extensive lung lesions. We were unable to produce in mice a satisfactory model for studying the difference between symptomless infection and illness without pulmonary consolidation.

Materials and methods. Mice. Female mice of an inbred strain, BALB/c, 4 weeks old, were used. Three weeks after birth, they were weaned and separated from the males. Twenty serial passages of lungs failed to show evidence of latent pulmonary viruses. Throughout this study, the room temperature in which mice were kept was maintained at 75° to 80°F.

Viruses. Two sublines of the PR8 strain of type A influenza virus were used. They were derived from stock virus which had undergone 8 passages in ferrets, 593 in mice, and 168 in eggs.

An "egg-line" virus was obtained by inoculating 0.1 ml of a 10^{-3} dilution of the stock virus into the allantoic cavity of 10-day embryonated hen's eggs. After 45 hours incubation at 37°C, infected allantoic fluids were pooled and frozen at −48°C. One ml of the pool contained $10^{7.5}$ infectious doses (ID_{50}) by the allantois-on-shell technique(1) and 512 HA units of virus.

A "mouse-passaged line" was obtained by 7 successive mouse-to-mouse passages of the stock virus at 3-day intervals. Infected lungs from mice of the 7th passage were removed aseptically and kept frozen intact at −48°C until used. Immediately before use, a 10% homogenate was made with Standard Medium(1) by grinding the infected lungs in a mortar with alundum. The homogenate was centrifuged at 2500 rpm for 10 min and the

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