ter 2 tissues might be due to the small number of cells sampled for karyotyping.

These results indicate that the close proximity of XY cells has no effect on the ability of XX cells to form chromatin bodies. Similarly the close proximity of XX cells does not appear to cause XY cells to form chromatin bodies. This is evident in the 3 tissues containing an excess of XX cells and in one of the tissues containing an excess of XY cells. In the other 2 tissues containing an excess of XY cells, perecentage of chromatin bodies is higher than percentage of XX cells. If this discrepancy were not due to methodological errors, one might think that the presence of XX cells in these tissues stimulated XY cells to form chromatin bodies. The results found in the other 4 tissues make this seem unlikely. Thus further evidence for the cell autonomy of the formation of chromatin bodies is presented.

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Received June 4, 1962. P.S.E.B.M., 1962, v110.

Evaluation of the Epinephrine Skin Test as a Biological Assay for Endotoxin.* (27631)

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Biological tests for identification and assay of bacterial endotoxins are few. For various reasons any given test may not be suited to the experimental objective. Among those currently in use is the epinephrine skin test introduced by Thomas, who showed that rabbits given an intravenous injection of 1 to 50 μg of endotoxin develop extensive hemorrhagic necrosis in skin sites injected with 100 μg epinephrine at the same time or up to four hours afterward(1). The test is said to be specific for endotoxin, but Nagler and Zweifach have reported that serotonin, given intravenously, produces the same effect(2). If substantiated, this finding would invalidate the test.

The present study was undertaken to determine: a, the sensitivity and reproducibility of the epinephrine skin test (EST); b, the comparative sensitivity of this test and that of the 10-day-old chick embryo for endotoxin; c, the length of time circulating endotoxin can be detected by each test following a lethal dose of endotoxin intravenously; and d, the specificity of the EST for endotoxin, with special reference to the reportedly similar response to serotonin.

Materials and methods. Animals. Adult albino rabbits weighing 1.5 to 2.5 kg were used.

Endotoxin and serotonin assays by the EST. Endotoxin** or serotonin† was injected into the ear vein of rabbits, followed one hour later by separate injections of 50, 100 and 200 μg of epinephrine‡ into the skin of the abdomen, previously shaved and cleansed with 70% isopropyl alcohol. The skin reac-

* Aided by a grant from Nat. Inst. Health, Bethesda, Md., and by a contract with Office of the Surgeon General, U.S. Army.

** Endotoxin: Difco #8, *E. coli,* 1 mg/ml, in sterile saline dilutions of 1, 10, and 100 μg/ml.

† Serotonin: Serotonin creatinine sulfate, Nutritional Biochemicals Corp., in sterile saline dilutions of 1, 10, and 100 μg/ml.

‡ Epinephrine: Epinephrine HCl, 1:1000, Lederle.

tion was read 18 to 24 hours later, and interpreted according to the criteria of Thomas. If there were no skin changes, or only a discrete area of bluish-brown discoloration less than 1 cm in diameter with a central pale area, the test was negative. The test was positive if there was an area of intradermal hemorrhage greater than 2 cm in diameter.

Endotoxin assay by chick embryo. 0.1 ml of test material was injected onto the chorioallantoic membrane of each of six 10-day-old chick embryos. Death within 24 hours in at least 4 of 6 embryos was considered positive for endotoxin(3).

Comparative assay for circulating endotoxin by EST and chick embryo test. A dose of endotoxin expected to be lethal was injected into the ear vein of 5 test animals. At various time intervals thereafter, 14 ml samples of blood were drawn from the femoral artery, and promptly replaced with an equal amount of fresh rabbit donor blood§ *via* an indwelling catheter introduced under novocain anesthesia. Four milliliters of each sample was centrifuged, and the harvested plasma frozen for subsequent endotoxin determination by the chick embryo test. The remaining 10 ml was immediately assayed for endotoxin by injecting the entire amount into the ear vein of a normal rabbit, which received 4 injections of epinephrine intradermally one hour later, 100 μg at 2 sites, and 200 μg at 2 other sites. These rabbits were returned to their cages and the skin sites read 18 to 24 hours later.

Results. A. Endotoxin assay by EST. (Table I). A positive reaction was produced in 13 of 14 rabbits (93%) by 7 μg or more of the endotoxin. Doses varying from 0.5 to 5 μg produced positive reactions in only 3 of 13 rabbits (23%). There was usually no difficulty in reading a skin reaction as positive or negative. Furthermore, all of the 3 or 4 skin test sites behaved similarly in a given animal. The area of hemorrhage was seldom greater with the larger doses of epinephrine. There were no positive reactions to saline given in place of endotoxin.

TABLE I. Endotoxin Assay by Epinephrine Skin Test.

Intravenous endotoxin (μg)	Proportion of animals with positive skin test
0.5	1/3
1.0	0/3
2.0	1/4
5.0	1/3
7.0	1/1
10.0	3/3
15.0	2/2
20.0	3/4
40.0	1/1
50.0	2/2
60.0	1/1

B. Endotoxin assay by the 10-day-old chick embryo. Tests by this method were consistently positive when the amount of endotoxin in the 0.1 ml test sample was 0.5 μg or higher. Occasional positive tests were obtained with as little as 0.1 μg.

C. Assay for circulating endotoxin by EST and chick embryo test. (Table II). In 4 rab-

TABLE II. Duration of Endotoxemia Following a Single Intravenous Injection of Endotoxin.

Exp. No.	1		2		3		4		5	
	EST	Chick assay	EST	Chick assay	EST	Chick assay	EST	Chick assay	EST	Chick assay
20 min.						?*			+	?
40 "					+	—			+	—
1 hr	+	—	+	+			+	+		
80 min.					+	?			+	
2 hr		+		—	+	+	+	+	—	+
4 "	+	?		—						
5½ "	+	—	+	—						

* ? = Borderline result, *i.e.*, 3 of 6 embryos were killed, minimum positive result consists of 4 of 6 embryos killed.

+ positive; — negative.

§ These donor bloods, from 5 normal rabbits, were found to be endotoxin free by the EST.

bits given a lethal dose of endotoxin intravenously (4.0 mg/kg) blood samples at various intervals up to 5½ hours later were positive by the EST. In a fifth rabbit (Exp. 3) given a sublethal dose of endotoxin (3.5 mg/kg), the EST was positive on blood drawn at 20, 40, and 80 minutes, but negative at 2 hours. Results with the chick embryo test were less consistent; often they were negative at time intervals when the EST was still positive.

D. Serotonin assay by EST. Only one of 15 rabbits showed a positive reaction when intravenous serotonin was used in place of endotoxin. The dose of serotonin ranged from 2 to 100 μg.

Discussion. Our results support previous findings(1,2,4) that the EST constitutes a sensitive and highly reproducible bioassay for endotoxin. The sensitivity of the test, as reported by Nagler and Zweifach(4) and by Thomas(1), was apparently greater in their hands than in ours, since they reported a lower limit of 1 μg as compared to our lower limit of 7 μg. Such differences are not inconsistent, because potency of endotoxin differs according to its source and mode of preparation.

Since we were able to obtain only one positive skin reaction in 15 tests with serotonin, we conclude that the EST does not give false positive reactions with serotonin.

Our data indicate that animals dying from endotoxin have a continuing endotoxemia, for the blood was positive as late as 5½ hours after a lethal dose (survival time less than 24 hours).

A comparison of the sensitivity of the chick embryo test with the epinephrine skin test for bioassay of endotoxin must take account of the fact that the chick embryo method does not allow testing of more than 0.1 ml of fluid, whereas a volume of 10 ml and more of test fluid can be employed in the epinephrine skin test. The latter can detect endotoxin in lower concentration than can be detected by the chick embryo test, but the total amount of endotoxin administered must be much greater than is necessary for a positive chick embryo test. Stated the other way, the chick embryo test can detect a much smaller total quantity of endotoxin, but the minimal concentration per ml of the test material must be higher than in the EST. Therefore in any given situation, the limiting factor for the EST may be the amount of test material available, while the limiting factor for the chick embryo test is the concentration of endotoxin. For detection of circulating endotoxin, the EST in our hands gave the more consistent results. This suggests that we were dealing with amounts of endotoxin below or at the minimum sensitivity of the chick embryo test.

Summary. On the basis of these experiments, we conclude: 1, that the EST is a highly reproducible bioassay for endotoxin; 2, that the EST is about 10 times less sensitive than the chick embryo in terms of volume for volume of test material available. But when the concentration of endotoxin per unit volume is very low, the EST can be much more reliable than the chick embryo test for detecting the presence of endotoxin; 3, that serotonin does not give false positive ESTs for endotoxin; and 4, that circulating endotoxin may be detected in rabbits up to 5½ hours after a lethal dose of endotoxin intravenously.

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Received June 4, 1962. P.S.E.B.M., 1962, v110.