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Received April 29, 1964. P.S.E.B.M., 1964, v116.

Effect of Endotoxin on Macrophages of the Adult Chicken. (29437)

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Endotoxin has been shown to have a marked toxic action *in vitro* on splenic macrophages of the guinea pig, rabbit, and man (1). Tissue culture methods have also been used to demonstrate a severe toxic effect on macrophages of the spleen and lung of the rabbit during an 8-hour period following an intravenous injection of endotoxin(2). The significance of these observations has been discussed elsewhere(3).

Avian species appear to be more resistant to endotoxin than mammalian species. Ball *et al*(4) found turkey poults resistant to intravenous injection of 1 to 15 mg of endotoxin. They also observed that hens tolerated the administration of 500 μg of endotoxin without the occurrence of hemorrhage into tumors associated with fowl leukosis. Recently Jordan and Hinshaw reported that adult chickens survived intravenous inoculation of 10 mg/kg of endotoxin(5). Several *in vitro* studies with peripheral blood leucocytes of the chicken indicated that endotoxin had a positive chemotactic effect(6-8) and stimulated leucocyte migration(6-11). Growth in cultures of chick embryo tissues has been found to be unaffected by high concentrations of endotoxin(12-14).

The purpose of the present study was to observe the relative sensitivity of macrophages from adult chickens to different concentrations of endotoxin *in vitro*. The use of culture techniques employed in similar studies on mammalian tissues permitted a comparison of the response of mammalian and avian macrophages.

Material and methods. White Leghorn cockerels from a pullorum-clean flock* maintained on an antibiotic-free diet provided the source of tissue and of blood used in preparation of media. The lot of endotoxin employed was the same as that used in a previous study on mammalian tissues(1). This was a sample of endotoxin from *Escherichia coli* 0127:B8 (Boivin type, Difco). A 0.1% suspension in Tyrode's solution was heated in a water bath at 70°C for one hour. Further dilutions were made in Tyrode's solution for each experiment. Tissue culture methods employed were similar to those previously described(1). Blood was obtained from etherized cockerels by cardiac puncture and 0.5 unit/ml of heparin was added to a portion of the blood for the preparation of plasma. Tissues were removed under sterile conditions and explants prepared and matched for size. Twelve fragments were used for each experimental condition. Streptomycin (100 units/ml) and test substances were added to the medium as in previous studies(1). Cultures, made in D5 Carrel flasks, consisted of 0.5 ml of plasma, 1.0 ml of serum extract of chick embryos and 4 explants placed in the medium before coagulation occurred. The extent of migration of small and large wandering cells was measured with an ocular micrometer at intervals during incubation in a manner previously described(1). Coverslip cultures were prepared for histologic examination and were fixed in Zenker-formol solution at 24 hours of incubation and stained by a modification of the Dominici technique(15).

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TABLE I. Effect of Endotoxin from *E. coli* on Leucocytes in Cultures of Spleen from Adult Chickens. Values are average radius of migration zone in mm ± S.E.

Exp No.	Amt of endotoxin, μg/ml	12-hr migration	Cytotoxic index	24-hr migration	Cytotoxic index
1	0	2.32 ± .06		2.82 ± .07	
	.01	2.39 ± .03	1.03*	2.82 ± .06	1.00
	.1	2.40 ± .01	1.03	2.89 ± .04	1.02
2	0	1.99 ± .07		2.28 ± .07	
	1	2.08 ± .05	1.05	2.47 ± .05	1.08
	5	1.44 ± .13	*.72*†	1.78 ± .15	*.78*
3	0			2.47 ± .09	
	10			2.18 ± .04	*.88*
4	0			2.72 ± .10	
	20			2.34 ± .05	*.86*
	50			1.70 ± .04	*.62*
5	0			2.89 ± .07	
	20			2.53 ± .04	*.88*
	50			2.30 ± .06	*.80*
6	0			3.06 ± .08	
	20			2.30 ± .08	*.75*
	50			1.52 ± .08	*.50*

* Cytotoxic index $= \frac{\text{Avg migration cultures with endotoxin}}{\text{Avg migration controls}}$.

† Values in italics show significant variation from controls at 98% confidence levels.

Results and discusssion. Cultures of spleen showed a sparse but rather wide migration of leucocytes during the first 24 hours of incubation. Few white cells were present compared with cultures of mammalian spleen(1). The extent of leucocytic migration was unaffected by 1 μg/ml or less of endotoxin but was moderately restricted by 5 to 20 μg/ml (Table I). This was considered of limited significance because of the small number of leucocytes usually present in cultures of spleen in these experiments. Studies were not carried out to compare the effect of other test substances in similar cultures, or to study the action of endotoxin in cultures of buffy coat of the blood, in which stimulation of leucocytic migration has been observed by other investigators(6-11). Medium and large sized phagocytic cells migrated earlier than in cultures of mammalian spleen. They usually could be seen at 12 hours, and at 24 hours formed a roughly circular zone of ameboid cells with an average radius approximately ⅓ to ⅔ as wide as that of the zone of leucocytic migration. Monocytes were more numerous initially than in cultures of mammalian spleen. They increased in size on further incubation so that a higher proportion of typical macrophages was present after the first day. Unless otherwise indicated the term "macrophage" has been used to include both the medium sized (monocytic) and large phagocytic cells. Large cell migration during the first 24 hours was unaffected by 1 μg/ml or less of endotoxin, but higher concentrations usually retarded macrophage migration for the first 12 to 24 hours (Table II).

In stained cultures fixed at 24 hours cells in the outer portion of the migration zone consisted principally of intact polymorphonuclear leucocytes. Numerous lymphocytes and degenerated polymorphonuclear leucocytes were seen near the explant. Monocytes and macrophages were also numerous in the inner half of the migration zone and many contained phagocytized material. Monocytes and macrophages in cultures containing endotoxin appeared intact and actively phagocytic in contrast to the appearance of similar cells in cultures of mammalian spleen exposed to endotoxin(16).

On the 2nd day of incubation the migration of macrophages in test cultures was equal to or greater than that in control cultures (Table II). Measurements made on the

4th day showed a definite increase in extent of migration in most of the cultures containing endotoxin. Degree of enhancement varied with different animals and was apparently unrelated to the concentration of endotoxin employed. A significant effect was elicited with concentrations between 0.1 and 50 μg/ml. Morphologic changes due to endotoxin were not observed. These findings contrasted sharply with the effect of endotoxin on mammalian macrophages. A concentration of 1.0 μg/ml which resulted in stimulation of avian cells uniformly decreased the migration and phagocytic ability of mammalian macrophages and caused severe morphologic damage(16). After an initial period of inhibition avian macrophages were stimulated by high concentrations of endotoxin which almost

TABLE II. Effect of Endotoxin from *E. coli* on Migration of Macrophages in Cultures of Spleen of Adult Chicken. Values are average radius of migration zone in mm ± S.E.

Exp No.	Endotoxin, μg/ml	Extent of macrophage migration at different periods of incubation											
		12 hr	Cytotoxic index	24 hr	Cytotoxic index	48 hr	Cytotoxic index	72 hr	Cytotoxic index	96 hr	Cytotoxic index
1	0	.67 ± .04		1.21 ± .06		2.04 ± .05		2.82 ± .07		3.79 ± .12	
	.01	.66 ± .03	.95	1.16 ± .06	.96*	2.04 ± .06	1.00	2.96 ± .08	1.05	3.93 ± .10	1.04
	.1	.66 ± .03	.98	1.21 ± .05	1.00	2.37 ± .04	*1.16*	3.55 ± .13	*1.26*†	4.72 ± .15	*1.25*
2	0	.87 ± .09		1.23 ± .03		1.92 ± .07		2.54 ± .08		3.29 ± .11	
	1	.85 ± .03	.98	1.35 ± .03	1.10	2.60 ± .06	*1.35*	3.88 ± .11	*1.53*	4.64 ± .14	*1.41*
	5	.52 ± .03	*.60*	1.19 ± .02	.97	2.56 ± .06	*1.33*	3.84 ± .09	*1.51*	4.60 ± .08	*1.40*
3	0			.95 ± .03		1.59 ± .06		2.16 ± .05		3.08 ± .09	
	10			.69 ± .03	*.73*	1.52 ± .04	.96	2.30 ± .08	1.06	3.74 ± .11	*1.20*
4	0			1.47 ± .05						4.00 ± .14	
	20			1.40 ± .05	.95					5.85 ± .21	*1.46*
	50			1.02 ± .04	*.69*					5.38 ± .11	*1.35*
5	0	1.18 ± .03								4.07 ± .07	
	20	.92 ± .04	*.78*							3.88 ± .15	1.00
	50	.81 ± .06	*.69*							4.05 ± .17	.96
6	0	1.47 ± .05								4.05 ± .09	
	20	1.19 ± .05	*.81*							4.76 ± .11	*1.18*
	50	.78 ± .04	*.53*							4.62 ± .19	*1.14*

* Cytotoxic index = $\frac{\text{Avg migration cultures with endotoxin}}{\text{Avg migration controls}}$.

† Values in italics show significant variation from controls at 98% confidence levels.

completely prevented the migration of mammalian macrophages(1).

The action of endotoxin on large wandering cells in cultures of lung was similar to that in cultures of spleen in the present study but attempts to culture chicken lung were usually unsuccessful due to bacterial contamination. Few migrating cells appeared in cultures of liver, and none were observed in cultures of testis.

Summary. Different concentrations of endotoxin were added to cultures of adult chicken's spleen growing in a coagulated plasma medium. Endotoxin in concentrations of 0.1 and 1.0 μg/ml did not alter the early migration of leucocytes and macrophages, but stimulated the extent of macrophage migration after the second day of incubation. Concentrations of endotoxin between 5 and 50 μg/ml caused an initial decrease in macrophage migration followed by a definite increase in a majority of experiments. Morphologic changes due to endotoxin were not observed. The low toxicity of endotoxin for avian macrophages was different from the severe toxic action on mammalian macrophages previously observed under similar cultural conditions.

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Received April 29, 1964. P.S.E.B.M., 1964, v116.

Dissociation of Hypophyseal Content and Urinary Excretion of Gonadotropin in Cirrhosis.* (29438)

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By a collaborative investigation of pituitary bioassays and urinary gonadotropins in cirrhotic men, an attempt has been made to resolve the common paradox of estrogenization, testicular atrophy and pituitary basophil hyperplasia that is observed at autopsy (1-4). Increased percentages of anterior hypophyseal basophil cells might have been anticipated to indicate ample gonadotropic hormonal stimulation of the testes and to be associated with elevated levels of urinary gonadotropin. However, the cirrhotics actually have shown low urinary gonadotropins(2), and evidence of pituitary storage rather than secretion of gonadotropins.

Materials and methods. Pituitary glands collected at autopsy from 13 men with advanced hepatic cirrhosis were fixed in ice cold acetone, dried and stored at —20°C. Aliquots of powdered pituitary equivalent to 10 mg of fresh tissue were suspended in physiological saline solution, and these were in-

* This study was supported by grant from Am. Cancer Soc.