Phagocytosis Influenced by Bacterial Culture Medium. (20132)

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During a study of opsonins for *Escherichia coli* in the blood of normal humans and dogs, it was noted that the medium in which the organism was grown influenced its phagocytosis. The possibility that a sensitizing material was present in certain types of culture media, or was elaborated during growth in these media, was investigated.

Methods. A strain of *E. coli* used in this laboratory was carried routinely on Bacto Tryptose Agar enriched as noted below. A comparison was made between phagocytosis of organisms grown in Bacto Tryptose Broth to which were added 10 g dextrose, 10 mg $FeSO_4$, and 0.1 mg thiamine hydrochloride per l, and those grown in Bacto Beef Infusion Broth containing 20 g peptone and 5 g NaCl per l. Cultures were grown 18-20 hours at 37°C, harvested by centrifugation, washed in 0.9% NaCl, and resuspended in saline. All live suspensions were standardized to 1.0 x 10^9/ml turbidimetrically, using a Coleman Model 11 spectrophotometer, and were checked by the dilution plate count method. When killed suspensions were employed, 18-20 hour tryptose broth cultures were centrifuged, suspended in saline, and treated with 1% formaldehyde for 24 hours at room temperature. The formaldehyde was removed by 3 washes of saline, and suspensions were checked for sterility by plating. Ultrafiltration of media was carried out with cellophane dialysis tubing, at 5°C and 10 cm Hg pressure. Dog blood was drawn from the jugular vein into a syringe containing 1 mg heparin (Lederle) per 10 ml blood. The cells were washed in 6 changes of Krebs-gelatin solution(1), and then suspended in an equal volume of Krebs-gelatin solution for the phagocytosis test. In the quantitative estimation of phagocytosis, 0.1 ml washed dog blood cells in Krebs-gelatin solution prepared as described above was added to 0.1 ml saline containing 1 x 10^8 *E. coli*. Details of the measurement of phagocytosis are described elsewhere(1).

Results. 1. *Phagocytosis of E. coli grown in tryptose and in beef media. E. coli* were grown 18-20 hours at 37°C, either in tryptose or in beef infusion broth, harvested, and suspended to 1.0 x 10^9/ml in saline. Phagocytosis was measured after rotation of the blood cell-bacterial mixture for 30 minutes at 37°C. The results are summarized in Table I. The mean percentages and their probable errors of neutrophiles phagocytosing *E. coli* after growth in beef and tryptose media were 87.5 ± 0.8 and 19.4 ± 1.1, respectively.

2. *Phagocytosis of E. coli grown in ultrafiltration fractions of beef.* In order to obtain information concerning the nature of the principle in beef responsible for increased phagocytosis, sterile beef infusion broth was filtered under pressure through cellophane dialysis tubing. Filtrates varying from 10% to 90% of total original volume were collected, and resterilized by autoclaving. Phagocytosis was equally elevated after *E. coli* was grown in beef infusion medium, its ultrafiltrate, or its residue, Table I, indicating that a responsible principle was freely dialysable and stable to autoclaving.

3. *Sensitization to phagocytosis of E. coli during growth in beef infusion medium.* Since greater phagocytosis of *E. coli* occurred after

TABLE I. Percentage of Neutrophiles Phagocytosing *E. coli* Grown in Beef Infusion Broth, Ultrafiltrate, Residue, and Tryptose Broth.

Medium	No. obs.	Mean ± P.E.	S.D.	Diff. / P.E. diff.
A. Beef infusion	32	87.5 ± 0.8	± 6.9	
B. Ultrafiltrate	8	92.8 ± 2.9	±11.4	A–B 1.77
C. Residue	8	89.8 ± 3.4	±13.7	A–C 0.21
D. Tryptose	43	19.4 ± 1.1	±11.3	A–D 48.6

TABLE II. Correlation of Growth in Beef Infusion Broth with Phagocytosis of *E. coli*.

Hr of culture	Mean No. of *E. coli*	% of neutrophiles phagocytosing *E. coli*			Comparison of % of 16 hr	
		Mean ± P.E.	S.D.	Diff. / P.E. diff.	Growth	Phagocytosis
Inoculum	5.58×10^8	25.2 ± 4.4	±19.9			30
0	4.79×10^8	28.8 ± 4.6	±20.7	0–I 0.56	8	35
2	3.92×10^9	45.8 ± 4.8	±21.5	2–I 3.17	63	55
4	5.08×10^9	53.2 ± 4.3	±19.4	4–I 4.52	82	63
6	5.94×10^9	72.4 ± 3.8	±17.2	6–I 8.14	96	87
16	6.23×10^9	84.0 ± 2.2	±10.1	16–I 12.00	100	100

TABLE III. Percentage Neutrophiles Phagocytosing Tryptose Grown *E. coli* Washed in Beef Infusion Broth, in 0.9% NaCl, and in Supernatant of Beef Infusion Cultures.

Washing medium	No. obs.	Mean ± P.E.	S.D.	Diff. / P.E. diff.
A. Beef infusion	41	32.7 ± 2.0	±19.4	
B. Saline	39	27.0 ± 1.6	±15.1	A–B 2.19
C. Beef infusion supernatant	8	38.8 ± 3.6	±14.5	
D. Saline	8	33.8 ± 4.4	±17.9	C–D 0.88

growth in beef infusion than in tryptose broth, experiments were designed to determine when sensitization to phagocytosis took place. To 10 ml beef infusion broth was added 1 ml of a 16-hour tryptose broth culture. The *E. coli* were harvested immediately after mixing, and after 2, 4, 6, and 16 hours growth at 37°C. Total number of organisms was estimated turbidimetrically and by dilution plate counts. The suspensions were standardized to 1 x 10^9/ml for phagocytosis measurements. A summary of 10 experiments is shown in Table II. There was a slight loss of organisms from the measured inoculum at 0 hours. The rate of growth was extremely rapid, an 8- to 9-fold multiplication occurring within 2 hours after inoculation. After 2 hours, growth was slower, and approached maximum in 6 hours. Sensitization to phagocytosis increased somewhat more slowly with time, but correlated closely with growth in beef infusion medium.

4. *Phagocytosis of E. coli grown in tryptose and washed in beef infusion broth.* Since growth in beef media resulted in sensitization of *E. coli*, attempts were made to opsonize tryptose-grown organisms by brief contact with beef infusion. When 1 x 10^9 live or dead *E. coli* were suspended in 1 ml beef infusion broth, the liquid removed, and the organisms resuspended in saline, no increase in phagocytosis over saline-treated controls resulted (Table III).

5. *Phagocytosis of tryptose-grown E. coli washed in supernatant of beef infusion cultures. E. coli* were not opsonized by washing in beef infusion medium but were sensitized to phagocytosis during growth in this medium. It was then determined whether the sensitizing factor(s) thus formed diffused into the medium. *E. coli* were grown in beef infusion and in tryptose broths for 18 hours, and removed by centrifugation. The tryptose-grown organisms were suspended in the supernatant of the beef infusion cultures. Volumes of supernatant medium and numbers of organisms were kept the same. After suspending in beef culture supernatant, the tryptose-grown *E. coli* were centrifuged, washed in saline, and again standardized to 1 x 10^9/ml. Controls were tryptose-grown *E. coli* treated with saline. The same experiments with supernatants of cultures were carried out using formaldehyde-killed *E. coli*. Results are shown in Table III. No evidence of free sensitizing factor was found in the beef medium supernatant.

Discussion. Microscopic examination of live organisms or of the stained smears used in the quantitative estimation of phagocytosis has shown that *E. coli* cells from beef medium

are small and rounded, and cells from tryptose medium are larger, more oblongate, and vacuolated. Electron micrograph studies have confirmed these observations. Fenn(2) found greater phagocytosis of larger than of smaller quartz particles, presumably because the probability of collision of particle and of cell depended on their size. The difference between phagocytosis of *E. coli* cells grown in tryptose and beef infusion media cannot be ascribed to size, since the smaller beef infusion grown organisms are more readily phagocytosed.

The foregoing experiments demonstrate that *E. coli* can be sensitized for phagocytosis by growth in beef infusion medium, and indicate that *in vitro* phagocytosis of microorganisms can be influenced by the medium in which they are cultivated. The sensitizing material cannot be washed free; 10- to 12-fold washing in saline did not decrease the phagocytosis of beef-grown *E. coli*. The absence of a sensitizing system from either the original beef infusion or from culture supernatants, and its appearance during growth, indicate its elaboration by the bacteria.

Summary. 1. The medium in which *E. coli* is grown influences its phagocytosis. Organisms grown in beef infusion are phagocytosed much more than those grown in tryptose medium. 2. The factor(s) in beef infusion is ultrafilterable and stable to autoclaving. 3. The absence of opsonin from beef infusion or from supernatant of beef infusion cultures, and the appearance of sensitization during growth, indicate formation of sensitizing substances by *E. coli* in beef infusion medium.

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Comparison of Effect of Progesterone and 11-Ketoprogesterone upon Glycosuria of Partially Depancreatized Rat. (20133)

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The 11-oxygenated steroid compounds of the adrenal cortex are more potent than their 11-desoxy analogs(1) in affecting carbohydrate metabolism. The data of the present study show that 11-ketoprogesterone has a greater diabetogenic effect in the partially depancreatized rat than does progesterone. The first demonstration that 11-ketoprogesterone is diabetogenic was in unpublished experiments by Dr. R. O. Stafford and Mr. W. P. Baker, Department of Endocrinology, The Upjohn Research Division.

Methods. Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets until they were partially depancreatized at a weight of 275 to 280 g. After diabetes was established all of the rats were placed in metabolism cages and force-fed a medium carbohydrate diet(2) by stomach tube each morning (8:30 to 9:30 a.m.) and afternoon (4:15 to 5:00 p.m.). The technics and diet were modifications of those described by Reinecke, Ball and Samuels(3). The test substances were ground into a fine suspension in peanut oil and were administered by subcutaneous injection twice daily. The animals were kept at a temperature of 74 to 78°F. Twenty-four-hour samples of urine were collected at the same hour (8:00 to 8:30 a.m.) and preserved with toluene. Urine glucose was determined by the method of Shaffer and Williams(4). The 11-ketoprogesterone was obtained from the Department of Chemistry of the Upjohn Company, where it was prepared from 11α-hydroxyprogesterone obtained by the microbiological oxidation procedure recently developed in these laboratories(5).

Experiments and results. Exp. 1 (Fig. 1) involved 12 moderately diabetic rats which were observed during a control period of 14 days. Six rats were each treated with progesterone in doses of 1, 2, 4, 8, 16, 32, and 64