TABLE II. Serum Composition, Body Weights, Blood Pressure, and Mortality from Aortic Rupture Among Turkeys Fed DES and DD (Exp II).

Treatment	Wt (lb)‡	Total lipid‡ (mg/100 ml serum)	Total cholesterol‡ (mg/100 ml serum)	Systolic blood pressure‡ (mm Hg)	% Aortic rupture
0	5.9[a]	673[a]	155[a]	230[a]	0[a]
16 g DES*	5.1[b]	14,536[b]	911[b]	176[b]	39[b]
512 mg DD†	5.4[ab]	12,671[b]	1,150[b]	177[b]	34[b]
1024 mg DD†	4.7[b]	16,548[b]	958[b]	175[b]	47[b]

* Per 100 lb of feed.
† Per pound of feed.
‡ At 10 wk of age.
Means with different superscripts are significantly different according to Duncan's Multiple Range Test.

weight gains. Comparable mortality, hyperlipemia, hypercholesterolemia, and hypotension resulted from the feeding of 16 gm diethylstilbestrol/100 lb and 512 or 1024 mg dienestrol diacetate per pound of feed.

The technical ssaistance of J. W. Carlisle and C. J. Miller is acknowledged.

1. Beall, C. W., Simpson, C. F., Pritchard, W. R., Harms, R. H., Proc. Soc. Exp. Biol. and Med., 1963, v113, 442.
2. Simpson, C. F., Harms, R. H., Neal, F. C., ibid., 1964, v116, 334.
3. Simpson, C. F., Harms, R. H., ibid., 1966, v122, 1162.
4. ———, Poultry Sci., 1964, v43, 681.
5. Sperry, W. M., Brand, F. C., J. Biol. Chem., 1955, v213, 69.
6. Pearson, S., Stern, S., McGavack, T. H., J. Clin. Endocrinol. and Metab., 1952, v12, 1245.
7. Simpson, C. F., Harms, R. H., Proc. Soc. Exp. Biol. and Med., 1965, v119, 509.
8. Carter, R. D., Wyne, J. W., Chamberlin, V. D., McCartney, M. G., Poultry Sci., 1958, v37, 1284.

Received July 25, 1966, P.S.E.B.M., 1966, v123.

Role of the Granulocyte in the Pyrogenic Response to Intra-Cisternal Endotoxin. (31556)

BERNARD DU BUY (Introduced by S. E. Greisman)
Department of Medicine, University of Maryland School of Medicine, Baltimore

Intravenously administered endotoxin may produce fever indirectly by releasing endogenous pyrogen, an intermediate substance presumably derived from granulocytes, into the peripheral circulation(1). That this represents the sole or even major mechanism of the production of fever by endotoxin has been questioned following the observation that endotoxin injected into the basal cistern via chronically implanted catheters regularly produces high sustained fevers with short latent periods(2-4). However, the importance of granulocytic pyrogen cannot be excluded in this model since an inflammatory response about the tip of the chronically implanted catheter could provide a readily available local source of the granulocytic pyrogen. The present study was performed to define the role of the granulocyte in the production of the febrile response to intracerebrally administered endotoxin.

Materials and methods. Glassware was rendered pyrogen-free in a dry-air oven at 180°C overnight. Physiological saline for injection and diluent was sterile and pyrogen-free. *Escherichia coli* endotoxin (Difco, lipopolysaccharide 0127B8) was diluted to 1.0 μg/ml.

* Supported by U. S. Army Medical Research and Development Command, Contract DA-49-193-MD-2867 under the auspices of the Commission on Epidemiological Survey of the Armed Forces Epidemiological Board, and by USPHS grant AI-07052.

Temperature recordings. Male albino rabbits, 1.5 to 3.0 kg, were obtained from a single source. Temperatures were recorded by thermistor rectal probes inserted 6 inches into the rectum and connected to a telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). A polyethylene catheter (PE 90, Clay Adams, N. Y.), previously rinsed with pyrogen-free saline, was taped into a marginal ear vein, and the animals then acclimatized by loose restraint in open stalls for 24 hours. Animals with temperature variations greater than 0.2°F during one hour prior to pyrogen injection or whose initial temperatures were greater than 104°F were rejected. After pyrogen injection temperatures were monitored every 30 minutes for 5 hours. Temperatures immediately prior to injection were taken as baseline and subsequent increments plotted on standard graph paper. Fever indices were determined by measuring the areas under the 5-hour temperature curves.

Basal cistern injections. Acclimatized rabbits were transiently paralyzed with tubocurarine chloride (E. R. Squibb and Sons, N. Y.) administered by slow intravenous injection through the polyethylene tubing; approximately 0.6 mg produced flaccid paralysis without apreciable diaphragmatic involvement. The nape of the neck was shaved and cleansed with 70% alcohol and a 22 gauge spinal needle then inserted midway between the base of the occiput and the first and second vertebral spines and directed toward the lower jaw in the midline (Fig. 1). The needle passed through the cisterna magna and medulla. It was advanced until bony resistance was felt, withdrawn slightly, and 0.5 cc of basilar cistern fluid was aspirated. A comparable amount of endotoxin or saline warmed to room temperature was injected, and the needle was then immediately withdrawn. The cerebrospinal fluid thus obtained generally contained minimal to moderate amounts of gross blood. Animals usually recovered from curare and needling within 5 to 15 minutes without overt neurologic lesions.

Granulocytopenia. Granulocytopenia was produced with nitrogen mustard (Mustargen HCl, Merck Sharp & Dohme, West Point, Pa.) by intravenous injection of 2.5 mg/kg followed by 0.5 mg/kg forty-eight hours later (5). White and differential counts were performed 4 days after the initial injection, at which time pyrogen studies were carried out. The mean granulocyte count in animals so

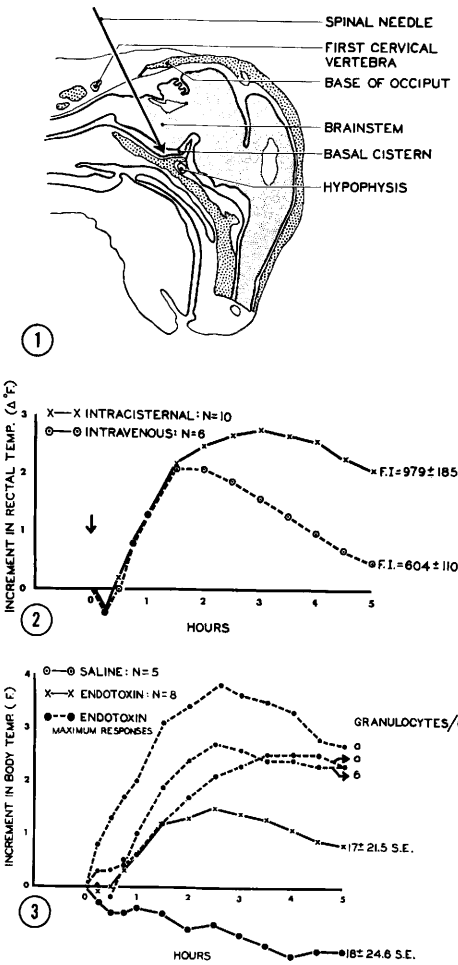

FIG. 1. Transverse section of rabbit head illustrating placement of spinal needle. (Adapted from Craigie, E. H., Bensley's Practical Anatomy of the Rabbit, Blakiston Co., Phila., Pa., 1948.)

FIG. 2. Pyrogenic responses of normal rabbits. Comparison of mean febrile responses to 0.5 μg *E. coli* endotoxin administered either directly into the basal cistern (upper curve) or intravenously into comparably curarized and needled rabbits (lower curve). F. I. = Fever Index.

FIG. 3. Pyrogenic responses of granulocytopenic rabbits. Three individual maximal febrile responses, and the mean febrile response to 0.5 μg endotoxin administered into the basal cistern are depicted. The mean hypothermic response to 0.5 cc saline administered into the basal cistern of control animals is shown for comparison.

treated was 18/cm ± 25 S.E. compared to a normal rabbit granulocyte count of 4,100 with a range of 2,500 to 6,000(6); fifty percent of treated animals had no detectable circulating granulocytes.

Results. Pyrogenic responses of normal rabbits. Injection of 0.5 μg of endotoxin directly into the basal cistern induced febrile responses of greater magnitude and longer duration than when given intravenously to similarly curarized and needled control rabbits (Fig. 2). These results parallel those obtained with chronic basal cistern catheters in rabbits and dogs(2). Curarization and needling *per se* only moderately decreased responsiveness to endotoxin; 6 rabbits so treated responded to 0.5 μg intravenously with a mean fever index of 604 ± 110 S.E. as compared with a mean fever index of 793 ± 64 S.E. of 5 normal untraumatized animals also given 0.5 μg endotoxin intravenously (a dose within the sensitive portion of the dose-response curve).

Pyrogenic responses of granulocytopenic rabbits. Rabbits treated with nitrogen mustard appeared debilitated and approximately one-third died prior to or during the pyrogen studies. Their ability to respond to a pyrogenic stimulus was severely depressed. When 10 cc/kg of plasma containing endogenous pyrogen was injected intravenously into 3 granulocytopenic control rabbits subjected to curarization and basal cistern injection, no febrile responses resulted. Ten cc/kg of this plasma contained sufficient endogenous pyrogen to produce 2.0°F increments in 3 normal rabbits subjected to similar curarization and needling. Moreover, 5 additional nitrogen mustard treated control rabbits given only 0.5 cc of saline into the basal cistern showed a progressive fall in temperature averaging 1.0°F by 5 hours (Fig. 3). Despite such poor physiologic condition appreciable febrile responses ensued following injection of 0.5 μg of *E. coli* endotoxin into the basal cistern; indeed, the 3 animals which developed the greatest febrile responses had minimal or no detectable circulating granulocytes. Since a decrease in temperature occurred in the control animals (Fig. 3), the nitrogen mustard treated animals' febrile responsiveness to endotoxin given intra-cisternally may actually approach that of animals with normal numbers of circulating granulocytes (mean fever indices were −407 ± 158 S.E., +461 ± 179 S.E., and +979 ± 185 S.E., respectively).

Discussion. The hypothesis that endotoxin produces fever by mechanisms other than release of an intermediate substance from granulocytes centers on the finding that endotoxin injected into the basal cistern(2-4) or cerebral ventricles(7-11) produces high and sustained pyrexia. These latter fevers are characterized by short latent periods and by a failure of tolerance to develop upon repeated injection(2). Since such cerebral studies were always performed with catheters chronically implanted in the brain or meninges, objections have been raised that inflammation about the tip of the catheter could place large numbers of granulocytes capable of producing granulocytic pyrogen in close proximity to the injected endotoxin. Indeed, endogenous pyrogen readily produces fever when injected into the cisterna magna(12). Endotoxin injected directly into the cisterna magna produced significant fever in the one reported *acute* experiment(13). The temperature elevation, however, was of lesser magnitude than when comparable amounts were given intravenously (13). While this could be accounted for by the fact that endotoxin injected into the cisterna magna must diffuse comparatively long distances to reach the target area, *i.e.*, the hypothalamus(9), the alternative explanation, absorption of the endotoxin into the peripheral circulation with consequent production of endogenous pyrogen, was not excluded. In the present study, injections of endotoxin were made directly into the basal cistern. The finding that endotoxin injected into a previously untraumatized basal cistern evokes greater fever than when comparable amounts are injected intravenously into appropriate controls excludes the role of systemic absorption of endotoxin and suggests that the cerebral effect of endotoxin is not mediated through release of granulocytic pyrogen. Nevertheless, the role of the granulocyte cannot be entirely excluded by these observations, since granulocytes might enter the area during the trauma of injection or as a result of an inflammatory response induced by endo-

toxin *per se* in the meninges or cerebral ventricles.

To elucidate further the importance of the granulocyte, endotoxin was injected into the basilar cistern of animals previously given nitrogen mustard. Although all such animals were debilitated, their responses to endogenous pyrogen depressed, and hypothermia observed in those given only intra-cisternal saline, appreciable febrile responses occurred following basal cisternal endotoxin instillation. Indeed, several rabbits with few or no circulating granulocytes remained capable of responding with high temperatures. The production of fever by the intra-cisternal injection of endotoxin must therefore represent either a direct central nervous system action of endotoxin as proposed by Bennett and co-workers(2-4), or is mediated by cell types other than the granulocyte.

Conclusion. Endotoxin injected into previously untraumatized basal cisterns of curarized rabbits evoked higher and more sustained febrile responses than comparable amounts given intravenously. Endotoxin injected into the basal cistern also evoked appreciable febrile responses in nitrogen mustard treated rabbits with few or no circulating granulocytes; the highest responses were observed in the absence of circulating granulocytes. Release of granulocytic pyrogen does not appear to constitute a necessary intermediate step for the cerebral pyrogenic activity of endotoxin.

The author wishes to express his gratitude to Dr. Sheldon E. Greisman for providing encouragement and advice, and for critically reviewing the manuscript.

1. Atkins, E., Physiol. Rev., 1960, v40, 580.
2. Bennett, I. L., Petersdorf, R. G., Keene, W. R., Trans. Assn. Am. Phys., 1957, v70, 64.
3. Petersdorf, R. G., Bennett, I. L., Arch. Int. Med., 1959, v103, 991.
4. Keene, W. R., Johns Hopkins Hosp. Bull., 1956, v99, 103.
5. Herion, J. C., Walker, R. I., Palmer, J. G., J. Exp. Med., 1961, v113, 1115.
6. Spector, W. S., ed., Handbook of Biolog. Data, W. B. Saunders Co., Phila., Pa., 1956, 276.
7. Sheth, U. K., Borison, H. L., J Pharmacol., 1960, v130, 411.
8. Feldberg, W., Myers, R. D., J. Physiol., 1964, v173, 226.
9. Villablanca, J., Myers, R. D., Am. J. Physiol., 1965, v208, 703.
10. Cooper, K. E., Cranston, W. I., Honour, A. J., J. Physiol., 1964, v68, (P).
11. Clark, W. G., Borison, H. L., J. Pharm. and Exp. Ther., 1963, v142, 237.
12. Adler, R. D., Joy, R. J. T., Proc. Soc. Exp. Biol. and Med., 1965, v119, 660.
13. Fritze, E., Schneider, H. H., von Voight, I., Z. ges. exp. Med., 1961, v134, 280.

Received August 24, 1966. P.S.E.B.M., 1966, v123.

Turnover Rate and Distribution Pattern of Radiostrontium in the Skeleton of the Laying Hen.*† (31557)

M. N. A. ANSARI,‡ C. R. CREGER, L. B. COLVIN, W. S. ALLEN, AND J. R. COUCH
Departments of Poultry Science and Biochemistry and Biophysics, Texas A&M University, College Station, Texas

Approximately 75% of the Ca required for egg shell formation by the laying hen is dietary in origin, and 25% is obtained from skeletal Ca(1). The exact site of endogenous Ca depletion for egg shell formation is not known. It is feasible, however, to assume that the skeletal structures of the body participate in egg shell formation at different rates. Radiostrontium is a bone seeking isotope and its turnover rate would be expected to be influenced by the mineral requirement of egg shell formation. However, the elimination and turnover rate for Sr^{89} may vary with

* Supported in part by the Division of Radiological Health, USPHS Grant RH 00354NTN
† Contribution of Texas Agri. Exp. Station.
‡ Present address: Univ. of Southern California Med. School, Los Angeles.