multaneous alteration of humoral and cellular factors. Alteration of humoral factors, achievable by killed cells or their components, may suffice to curb the relatively atypical experimental infections produced in such hosts. The situation appears to be more complex in a typical susceptible host like the guinea pig, at least as far as any lasting and significant protection is concerned. Jones *et al.*(13) did observe that a killed S or R adjuvant vaccine can produce slight protection in guinea pigs but the resulting increase in ID_{50} was minor compared with that obtained after use of live vaccines. It will now be of interest to determine whether in typical susceptible hosts, like the guinea pig, full protection can be achieved with the aid of killed brucellae when the animal is simultaneously exposed to other agents, *e.g.*, meprobamate, which recently have been shown capable of modifying cellular responses (14). Under these conditions the drug may lead to the required alteration of cellular properties while the killed brucellae modify humoral factors.

Summary. *In vitro* studies with guinea pig monocytes showed that vaccination of the donor with formalin-killed *Brucella abortus*, failed to result in development of certain cellular properties that are typical for phagocytes obtained from donors that are immune by virtue of innate resistance or prior infection. That is, while brucellae fail to undergo intracellular multiplication in monocytes from immune donors, they do multiply in monocytes from donors treated with killed vaccine, even though the extent of such multiplication was found to be less than in monocytes from normal, susceptible donors. An attempt has been made to relate this finding to the problem of effective immunization against a pathogen like brucella, which presumably requires an effective alteration of both humoral and cellular factors.

1. Live, I., Sperling, F. G., Stubbs, E. L., *J. Infect. Dis.*, 1945, v77, 16.

2. Elberg, S., Herzberg, M., Schneider, P., Silverman, S. J., Meyer, K. F., *J. Immunol.*, 1951, v67, 1.

3. Bang, B., *J. Comp. Path. Therap.*, 1906, v19, 191.

4. Gwatkin, R., *J. Infect. Dis.*, 1931, v48, 331.

5. Traum, J., in Brucellosis, A Symposium. . . ., A.A.A.S., Washington, D. C., 1950, 225.

6. Spink, W. W., *The Nature of Brucellosis*, 1956, Univ. of Minnesota Press, Minneapolis, p268.

7. Herzberg, M., Elberg, S. S., *J. Bacteriol.*, 1955, v69, 432.

8. Pomales-Lebrón, A., Stinebring, W. R., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 78.

9. Braun, W., Pomales-Lebrón, A., Stinebring, W. R., *ibid.*, 1958, v97, 393.

10. Holland, J. J., Pickett, M. J., *J. Exp. Med.*, 1958, v108, 343.

11. Stinebring, W. R., Kessel, R. W. I., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 412.

12. Mitsuhashi, S., Sato, I., Tanaka, T., *J. Bacteriol.*, 1961, v81, 863.

13. Jones, L. M., Thomson, P. D., Alton, G. G., *J. Comp. Path.*, 1958, v68, 416.

14. Kessel, R. W. I., Boughton, J., Braun, W., *Science*, 1961, v134, 1882.

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Effect of Nitrofurazone on Release and Activity of Endogenous Serum Pyrogen.* (27365)

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It is generally thought that bacterial endotoxins produce fever by injury to leukocytes with release of a pyrogen which acts upon the hypothalamic thermo-regulatory centers resulting in an elevation in temperature. This fever-producing material which has been termed *endogenous serum pyrogen* also has been found in animals made febrile with influenza viruses and in the fevers associated with experimental streptococcal, pneumo-

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coccal and staphylococcal infection and tuberculin hypersensitivity(1). Leukocytes obtained from peritoneal exudates or peripheral blood also produced fever when administered intravenously to a variety of animal species. The fever-producing material obtained from these cells has been termed leukocytic pyrogen and by virtue of several common biologic properties has been thought to be identical to endogenous serum pyrogen(1).

Recently Fessler *et al.*(2) reported that leukocytes from rabbits treated with nitrofurazone (5-nitro-2 formaldehyde semicarbazone) did not yield leukocytic pyrogen; incubation of leukocytes with nitrofurazone *in vitro* also rendered the cells non-pyrogenic. On the other hand, animals treated with nitrofurazone responded to challenge with bacterial endotoxins with as much fever as untreated controls. On the basis of these experiments, these investigators cast doubt on the importance of leukocytic pyrogen in the pathogenesis of endotoxin fever.

Since leukocytic pyrogen is presumed to be derived from or closely related to endogenous serum pyrogen, it seemed pertinent to study the effect of nitrofurazone upon the release of endogenous serum pyrogen (E.P.).

Methods. Male rabbits of mixed breed weighing 2 to 4 kg were housed in air-conditioned rooms. Rabbit pellets were obtained from a single source and were constant in composition except that "treated" animals received 10 parts/100,000 nitrofurazone in their feed. To record temperatures, animals were confined to metal stalls with loosely-fitting collars. Temperatures were measured rectally by thermocouples (Telethermometer, Yellow Springs Instrument Co.). Animals were permitted 2 hours for equilibration of temperatures and animals with variations in baseline temperature greater than .2°C were not used. Following injection of the test sera, temperatures were taken every 15 minutes during the first hour and at 30-minute intervals for 2 hours thereafter.

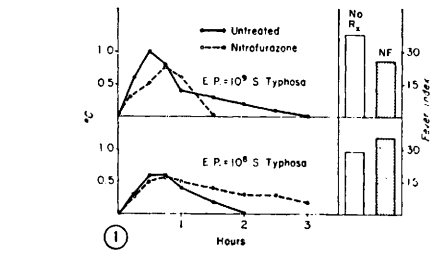
Endogenous serum pyrogen was prepared by intravenous inoculation of typhoid vaccine in dosage of 10^9, 10^8 and 10^7 killed bacteria/kg into nitrofurazone-treated rabbits and untreated controls. Animals were exsanguinated 120 minutes after injection of vaccine. Blood was permitted to clot, serum cleared by centrifugation and cultured in thioglycollate broth. If found to be sterile, sera from several animals were pooled and stored at 4°C until used.

In some experiments, a standard pyrogenic dose consisting of 3 ml E.P. was incubated for 60 minutes at 37°C with an equal volume of nitrofurazone in distilled water to give final concentration of 1.0, 0.1 and 0.01 mg nitrofurazone per ml E.P.

Precautions to avoid contamination by bacterial pyrogens of all solutions, needles and glassware were taken. Fever indices were calculated as described previously(3).

Results. Fifteen rabbits received food containing nitrofurazone for 4 weeks, while 15 were given the same feed without antimicrobial. At the end of this period, 5 rabbits in both treated and untreated groups were given typhoid vaccine containing 10^9, 10^8 and 10^7 killed cells, respectively. All animals were bled 120 minutes later and sera from each group pooled. 5.0 ml aliquots from each group of donors were assayed in each of 4 normal rabbits. The results are summarized in Fig. 1 and indicate that there was no difference in magnitude and duration of fever produced with E.P. from nitrofurazone-treated and normal rabbits given 10^9 and 10^8 killed cells. No E.P. was demonstrated in sera of treated and untreated donors given 10^7 organisms. The minimum pyrogenic dose of E.P. produced with 10^8 organisms was then determined and found to be approximately 2.7 ml. There was no difference in the fever-producing capacity of E.P. from nitrofurazone-treated and normal donors and the minimum pyrogenic dose from each group was the same (Fig. 2).

3.0 ml aliquots of E.P. (= one minimum pyrogenic dose) were incubated at 37°C for 1 hour with an equal volume of distilled water containing nitrofurazone to achieve final concentrations of .1%, .01% and .001% of drug. Four samples of each concentration were assayed for pyrogenicity. There was no significant difference between E.P. mixed with nitrofurazone and E.P. not exposed to this drug (Fig. 3).



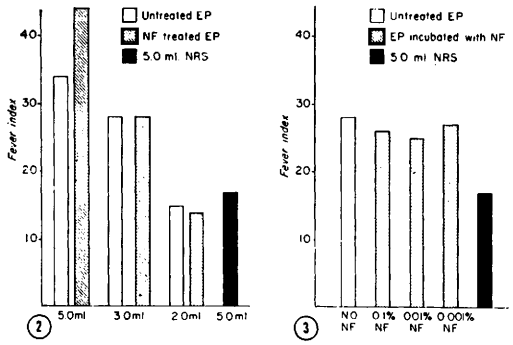


FIG. 1. Magnitude and duration of fever in rabbits given E.P. from nitrofurazone-treated and untreated donors.

FIG. 2. Fever-producing activity of graded doses of E.P. from nitrofurazone-treated and normal donors.

FIG. 3. Amount of fever produced with endogenous pyrogen incubated with varying concentrations of nitrofurazone.

Discussion. These results demonstrate that nitrofurazone did not affect the animals' ability to elaborate potent E.P. following administration of a bacterial endotoxin. This was to be expected in view of the observation that nitrofurazone-treated animals had as much fever following challenge with bacterial endotoxin as untreated controls(2). Furthermore, the fever-producing capacity of E.P. was not impaired following exposure *in vitro* to amounts of nitrofurazone far greater than were present in animals ingesting the antimicrobial. In this respect, endogenous serum pyrogen differs from leukocytic pyrogen, and leukocytes from animals treated with this drug were incapable of releasing pyrogen. Similarly, nitrofurazone interfered with elaboration of pyrogen from leukocytes *in vitro*. These differences, however, do not warrant the conclusion that leukocytic pyrogen does not play an important role in endotoxin fever (2). There is no evidence that tissues other than granulocytes produce a pyrogen(3) and rabbits without circulating polymorphonuclear leukocytes developed no significant fever following challenge with bacterial endotoxins(4). Furthermore, the degree of granulocytopenia in rabbits treated with nitrogen mustard correlates well with the magnitude of the febrile response and the quantity of endogenous pyrogen in the serum(5).

It seems more reasonable to postulate that nitrofurazone exerted a protective effect on leukocytes preventing release of endogenous pyrogen from these cells. Fruhman has shown that neutrophilia in the peritoneal cavity could be evoked only with bacterial lipopolysaccharides and that other irritants were not effective(6). It does not seem unlikely, therefore, that peritoneal exudates which are the usual source of leukocytic pyrogen are produced by means of minute amounts of endotoxin derived from the bacterial flora of the gut which enters the peritoneal cavity in response to instillation of large volumes of fluid. These quantities of endotoxin are in themselves not sufficient to produce fever but injure the leukocyte and release leukocytic pyrogen. This is in keeping with the observation of Collins and Wood that endotoxins stimulate leukocytes to release endogenous pyrogen *in vitro*(7). Nitrofurazone probably affected production of leukocytic pyrogen in two ways. The drug interfered with bacterial multiplication in the gut, leaving less endotoxin to enter the peritoneal cavity and produce injury to leukocytes. Obviously, nitrofurazone could not affect circulating leukocytes in this fashion since it is poorly absorbed from the gastrointestinal tract and in the dosage administered was not present in the bloodstream in measurable concentration. Secondly, in view of nitrofurazone's ability to vitiate the pyrogenic activity of leukocytic pyrogen *in vitro*, the agent may affect leukocytes directly, perhaps by protecting them from the injurious action of endotoxin.

The data do not answer whether leukocytic endogenous pyrogen is identical to endogenous serum pyrogen. More precise biochemical identification of both types is necessary to solve this problem.

Summary. Administration of nitrofurazone to rabbits did not interfere with the animals' ability to release endogenous serum pyrogen following administration of typhoid vaccine.

Incubation of endogenous serum pyrogen with nitrofurazone did not alter its pyrogenicity.

1. Atkins, E., *Phys. Rev.*, 1960, v40, 580.
2. Fessler, J. H., Cooper, K. E., Cranston, W. J., Vollum, R. H., *J. Exp. Med.*, 1961, v113, 1127.
3. Bennett, I. L., Jr., Beeson, P. B., *ibid.*, 1953, v98, 477.
4. Herion, J. C., Walker, R. I., Palmer, T. G., *ibid.*, 1961, v113, 1115.
5. Gillman, S. M., Bornstein, D. L., Wood, W. B., Jr., *ibid.*, 1961, v114, 729.
6. Fruhman, G. J., *Blood*, 1960, v16, 1753.
7. Collins, R. D., Wood, W. B., Jr., *J. Exp. Med.*, 1959, v110, 1105.

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Adenovirus Neutralizing Antibodies in Persons on Taiwan.* (27366)

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"Serological epidemiology" used by Paul to study poliomyelitis in different areas of the world(1) has been followed by application of serological test surveys of different population groups to understand better the epidemiology of other viral diseases. Soon after discovering the adenovirus group, Huebner and coworkers studied the distribution of antibodies against 6 types in children and adults in Washington, D.C.(2). These studies showed that several of the types were very common and appeared to infect very early in life, while other types were less common and infected older children and adults. These observations have been confirmed in the United States(3-5). Recently reports have appeared from Taiwan(6) and Japan(7,8) concerning the presence of adenovirus antibodies. This study is an extension of the earlier one on Taiwan(6) to include 6-7-year-old children and more areas of the island.

Material and methods. Sera. Blood specimens were obtained from residents of 10 different counties of Taiwan, including the nearby Pescadore Islands. Recent primary school entrants (6-7 years old) were bled as part of an islandwide study of eye infections(9). Adult sera (age 16-25) were provided by the laboratories of the Provincial Health Administration after testing for syphilis. The sera were frozen at −20°C prior to testing.

Test procedure. The "improved technic for the neutralization test"(10) was followed closely. The H. Ep. #2 tissue culture cell line (11) was substituted for HeLa cells and calf serum was used in place of horse serum. In this test the serum, virus and tissue culture cells are added to culture tubes at the same time and the test read in 48 hours (also at 72 hours). During this period the virus alone causes typical cytopathogenic effect (CPE), while specific antibody prevents these changes and allows normal growth of the H. Ep. #2 cells. Serum was heat inactivated (56°C for 30 minutes), diluted 2-fold from 1:5 to 1:40 and added in 0.1 ml amounts. Virus suspension was added in 0.2 ml amounts in a concentration in excess of that needed to cause ++++ CPE (one-half $\log_{10}$ greater than the amount needed to damage 50% of the cells(10)). The H. Ep. #2 cell suspension consisted of 100,000 cells in 0.7 ml. Two tubes per serum dilution were employed in each titration.

Virus. Adenovirus prototype strains were used(10). Types 11-18 have recently been obtained from Dr. Wallace Rowe. Four strains isolated in Taiwan and called Taiwan A (#276), B (#1004), C (#6778) and D

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