Falsely Positive Pyrogenic Responses Induced in Rabbits by Latex Particles.* (29352)

GEORGE S. KOBAYASHI† AND LORRAINE FRIEDMAN (Introduced by M. F. Shaffer)
Department of Microbiology, Tulane University School of Medicine, New Orleans, La.

One of the difficulties arising from the use of polystyrene latex particles in biological experiments is that the net changes measured appear similar to some of those manifest by Gram-negative bacterial endotoxin. Such a pitfall was encountered during our own study on the pyrogenic activity of yeasts in rabbits when these particles were employed as presumed inert control material. Since endotoxin-like febrile responses had been elicited by a wide variety of yeasts in our experiments we had begun to consider the possibility that the very particulate nature of the cells contributed to this response. To test this hypothesis, washed latex particles of comparable doses and sizes as the yeast cells were injected. Fevers indistinguishable from those produced by the yeast or by bacterial endotoxin were observed, ostensibly proving that inert particles could induce fever and that the particulate nature of the yeasts participated in the pyrogenicity.‡ In fact, the larger the latex particles, the greater was the response. Dr. Dennis W. Watson suggested the possibility that the particles were contaminated with a pyrogenic substance (*e.g.* endotoxin), so tightly adsorbed that it could not be removed even with repeated washing.

Materials and methods. To explore the possibility that pyrogenic contamination had occurred, the following experiments were performed.

Rabbits were injected intravenously with 1 ml containing:

(A) 10^9 thrice washed latex particles§ of varying sizes;

(B) 10^9 particles of 1.171 μ diameter from (A), acid-treated and washed or alkaline-treated and washed;

(C) 10^9 acid-treated particles from (B), treated with bacterial endotoxin and washed 6 times;

(D) final saline wash from (C).

The acid-treated particles were prepared by treatment with 1 N acetic acid at 100°C for 90 minutes according to the procedure of Haskins *et al*(1) for hydrolysis of endotoxin. Immediately after heating, the particles were recovered by centrifugation, suspended in distilled water, dialyzed against 5 daily changes of distilled water at 4°C, and finally suspended in saline to the original concentration. Alkaline-treated particles were obtained by two 20 minute exposures at 100°C in 10 N NaOH (Watson, personal communication) after which time the particles were recovered and washed as above for acid-treated particles. Finally, a portion of the acid-treated particles was further treated overnight at 4°C with endotoxin of the phenol-water type extracted from *Escherichia coli,* at a final concentration of 10 μg per ml. The endotoxin-treated particles were washed 6 times in non-pyrogenic saline and finally suspended in saline to its original concentration. None of the above treatments destroyed the structural integrity of the particles as determined by microscopic examination.

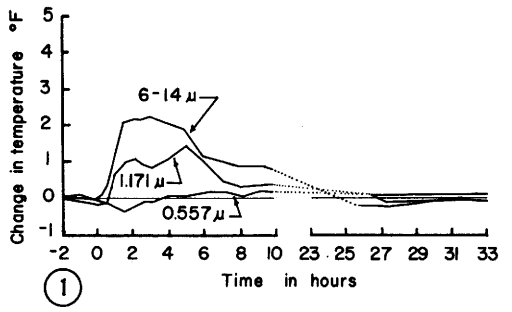
Results. Fevers indistinguishable from those induced with yeasts(2) or bacterial endotoxin were induced with: washed but otherwise untreated latex particles; alkaline-treated particles; and acid-treated particles adsorbed with bacterial endotoxin. In the case of the washed but otherwise untreated

* This investigation was supported by training grant from Nat. Inst. of Allergy and Infect. Dis., U.S.P.H.S.

† Present address: Dept. of Medicine, Division of Dermatology, Washington Univ. School of Med., St. Louis, Mo.

‡ Presented at Annual Meeting, Am. Soc. for Microbiology, Cleveland, Ohio, May 5-9, 1963.

§ Obtained, as in previous studies, from Dr. J. W. Vanderhoff, Dow Chemical Co., Midland, Mich. The following sized particles were employed: 0.557 μ (Run No. LS-063-A); 1.171 μ (Run No. LS-067-A); and 6 to 14 μ (Run No. LS-309-19-3).



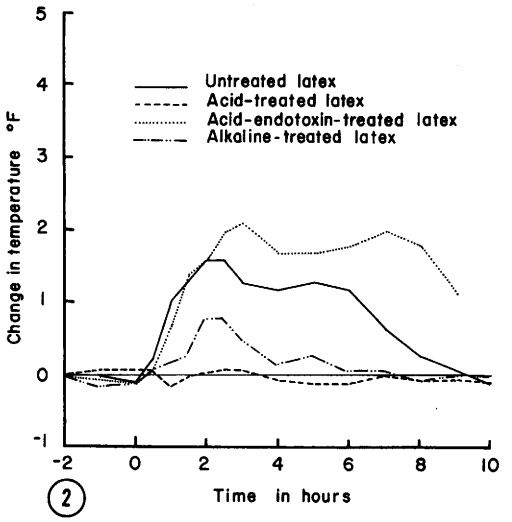


FIG. 1. Febrile responses of rabbits intravenously inoculated with 10^9 polystyrene latex particles of varying sizes. Each curve represents the average response of a minimum of 4 animals. Hours 10-26 were without observation.

FIG. 2. Febrile responses of rabbits intravenously inoculated with 10^9 polystyrene latex particles, 1.171 μ in diameter, treated by various chemical procedures. Each curve represents the average response of a minimum of 4 animals.

particles the response was dose-related, for the fevers of highest intensity were obtained with the particles of greatest surface area, viz. those of 6 to 14 μ. Neither latex particles after acid treatment nor the final saline wash of the endotoxin-treated particles was pyrogenic. These results are shown in Fig. 1 and 2.

Discussion. The reduction in pyrogenic activity of the latex particles by alkaline treatment and the elimination of this activity by acid treatment proves that these particles were contaminated by endotoxin or some other type of pyrogenic substance. This contamination is not a surprising observation when one considers that such particles are known to adsorb many substances tightly and have their widest usage in various laboratory procedures primarily because of this property. Hanna and Watson(3) employed colloidal carbon particles in pyrogenicity and clearance studies in rabbits and obtained data suggestive of contamination of the carbon particles with a substance possessing characteristics of endotoxin.

The use of polystyrene latex particles in other biological experiments may pose problems, therefore, similar to those encountered in our pyrogenic studies. For example, a stimulation of glycolysis during phagocytosis of latex particles by leucocytes has been noted(4,5,6,7,8); this phenomenon also occurs when leucocytes are exposed to endotoxin(9,5). Wallach and Karnovsky(10) reported an increased glucose utilization by ascites tumor cells exposed to latex particles. In the latter experiment the particles were of such a large size that they were not phagocytosed but were adsorbed onto the surface of the ascites cells. The possibility of misinterpretation has not gone unrecognized for Weissman and Thomas(11) suggested that endotoxic contamination may have accounted, at least in part, for the increased glycolysis. While Oren *et al*(6) excercised the extreme caution of washing by a dialytic procedure to remove a preservative which had been added to some batches of their latex particles, it should be emphasized that repeated washing of highly adsorptive particles such as latex, bentonite, and charcoal with nonpyrogenic fluids could actually serve to concentrate ordinarily undetectable amounts of pyrogenic or other endotoxin-like substances from the wash water.

Summary. An endotoxin-like febrile response was elicited in rabbits by intravenous inoculation of washed polystyrene latex particles. Alkaline treatment of the latex particles reduced but did not eliminate the pyrogenic activity, whereas particles treated with acid were incapable of inducing a febrile response. Exposure of the non-pyrogenic, acid-treated particles to a suspension of *Escherichia coli* endotoxin of the phenol-water type restored pyrogenicity to the par-

ticles. Exhaustive washing of these endotoxin-treated particles with non-pyrogenic saline did not remove the fever producing substances.

1. Haskins, W. T., Landy, M., Milner, K. C., Ribi, E., *J. Exp. Med.*, 1961, v114, 665.

2. Braude, A. I., McConnell, J., Douglas, H., *J. Clin. Invest.*, 1960, v39, 1266.

3. Hanna, E. E., Watson, D. W., *Bact. Proc.*, 1963, p56.

4. Sbarra, A. J., Karnovsky, M. L., *J. Biol. Chem.*, 1959, v234, 1355.

5. Strauss, B. S., Stetson, C. A., *J. Exp. Med.*, 1960, v112, 653.

6. Oren, R., Farnham, A. E., Saito, K., Milofsky, E., Karnovsky, M. L., *J. Cell Biol.*, 1963, v17, 487.

7. Sbarra, A. J., Shirley, W., *J. Bact.*, 1963, v86, 259.

8. Dannenberg, A. M., Jr., Walter, P. C., Kapral, F. A., *J. Immunol.*, 1963, v90, 448.

9. Cohn, Z. A., Morse, S. I., *J. Exp. Med.*, 1960, v111, 689.

10. Wallach, D. F. H., Karnovsky, M. L., *Proc. 1st Int. Biophysics Congress, Stockholm*, 1961, p43.

11. Weissman, G., Thomas, L., *Symposium on Bacterial Endotoxins*, 1963, Sept. 4-6, Inst. of Microbiology, Rutgers Univ., New Brunswick, N. J.

Received March 30, 1964. P.S.E.B.M., 1964, v116.

Effect of Cortisone, Amputation, and Food Restriction on Growth of Rat Maxillary Incisors.* (29353)

W. A. WELLBAND† AND L. V. DOMM

Department of Anatomy, Stritch School of Medicine, Loyola University, Chicago, Ill.

We previously observed that cortisone produced an acceleration in the growth (eruption) rate of maxillary incisors in intact, adrenalectomized, and thyro-adrenalectomized rats, concomitant with a reduction in body weight(1,2). The mechanism of this stimulation was not explained and required further investigation. Numerous observations indicate that administration of cortisone in the rat results in a decrease in food consumption(3). Since we had not obtained data on pair fed controls in the previous experiments, it seemed advisable to determine what effect, if any, a decrease in food consumption would have on the eruption rate of the incisor.

It has been reported(4) that factors which increase the attrition rate of rat incisors (lack of sensory innervation, apical amputation, artificial abrasion or hard diet) will increase their growth rate. When the incisor is maintained out of occlusal contact by periodic cutting, its rate of growth is accelerated thereby expressing its true or potential growth rate(5). Although this unrestricted eruption rate has been studied under a variety of experimental conditions(5), we were unable to discover any reports of the effects of cortisone on this rate. The stimulation of this unimpeded eruption rate following cortisone administration would seem to provide indications of a direct effect on the mechanism controlling eruption.

Procedures. Food consistency and restriction. Sixteen female, Sprague-Dawley rats, age 40 days, were housed in metal cages with sawdust bedding and maintained on Purina dog chow pellets and tap water *ad libitum*, for a control period of 3 weeks. Following this period, 6 animals were moved into separate plastic cages, without sawdust, and fed finely ground chow instead of pellets, in an attempt to restrict the number of environmental objects on which the rats could gnaw. Cages were cleaned daily, food and water provided *ad libitum* and amount of daily food consumption recorded. This regime was followed for 4 weeks. At the end of the fourth week the amount of food daily provided was arbitrarily restricted to

* Supported in part by U.S.P.H.S. grant.

Cortisone (Cortone Acetate) generously supplied by Merck, Sharp & Dohme, Division of Merck & Co., Inc., Philadelphia, Pa.

† Present address: Dept. of Gross Anatomy, Med. Coll. of Georgia, Augusta.