

On the mechanism of the adjuvant effect of *Bordetella pertussis* vaccine

C. E. Reed, M.D., M. Benner, M.D., S. D. Lockey, M.D., T. Enta, M.D.,
S. Makino, M.D., and R. H. Carr, M.D. Madison, Wis.

Pertussis vaccine accelerates, rather than retards, clearance of antigen from the circulation and enhances the secondary as well as the primary response. Pertussis vaccine alone has an anamnestic effect and is capable of causing the appearance of homocytotropic antibody which reacts with antigens to which the animal has previously made a primary response. The effects of pertussis and Freund's adjuvant are additive. Increased insulin levels are not necessary for the adjuvant effect, as alloxan-diabetic animals are quite capable of enhanced antibody response after pertussis vaccine. Beta adrenergic blocking drugs increase and adrenergic agonists reduce antibody response slightly. It is concluded that pertussis exerts its effect directly on the precursor of the antibody-forming cells by increasing their rate of cell division. Impairment of beta adrenergic receptors may be the mechanism of the effect.

Although *Bordetella pertussis* vaccine has long been known to act as an adjuvant, the mechanism of the adjuvant action remains undefined. To students of immediate hypersensitivity, pertussis vaccine has special interest as an adjuvant because it not only sensitizes animals to active anaphylaxis by increasing the production of homocytotropic antibody but also sensitizes to passive anaphylaxis and to histamine and other mediators of anaphylaxis. To date it has not been possible to separate the fraction of the organisms responsible for these effects, and it appears probable that a single protein, the histamine-sensitizing factor, is responsible for all of them.¹ The phenomenon of pertussis sensitization has been proposed as an animal model of the physiologic abnormality of asthma and related atopic diseases.² This model could help explain not only the increased bronchial irritability to histamine and other stimuli but also the increased reagin (homocytotropic antibody) production and eosinophilia characteristic of asthma and the other atopic diseases.

Killed pertussis organisms or protein extracts of the organism produce important effects on intermediary metabolism which might account in part for enhanced antibody production. The most thoroughly studied effects are increased blood levels of insulin³ and a diminished hyperglycemic response to epinephrine.⁴ Szentivanyi and colleagues⁴ proposed that the mechanism of the diminished response to epinephrine is an impaired response of the beta adrenergic receptors and were able to mimic the histamine-sensitizing effect of pertussis with beta adrenergic blocking drugs.

From the Department of Medicine, University of Wisconsin Medical School.

Supported by United States Public Health Service Grant Nos. 5-T01-A1-00317-06 and A108106-05.

Received for publication Aug. 3, 1971.

Reprint requests to: Dr. Reed, 1300 University Ave., Madison, Wis. 53705.

Vol. 49, No. 3, pp. 174-182

The present experiments were undertaken to explore the possibility that these metabolic abnormalities might contribute to the adjuvant effect.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats 180 to 200 grams were used for all experiments. Blood for analysis was obtained from the tails.⁵

Antigens

Maximally substituted dinitrophenyl-egg albumin (DNP-EA) and dinitrophenyl-bovine serum albumin (DNP-BSA) containing 7 DNP groups per molecule of BSA were prepared by the method of Eisen and associates.⁶ Bovine serum albumin and DNP-BSA were labeled with ¹²⁵I by the method of Hunter and Greenwood.⁷ The proteins were obtained from Nutritional Biochemicals Corp.

Antibody assay

Total antibody to the DNP hapten was determined by the procedure of Minden and Farr,⁸ with the use of the iodinated DNP-BSA as the test antigen. Homocytotropic antibody was assayed by injecting 0.05 ml. of each serum to be tested into the skin of a normal rat and challenging the recipient 48 hours later with 1 mg. of DNP-EA and one per cent Evans blue dye intravenously. The results are expressed as the area of bluing on the underside of the skin viewed after the recipient animal had been skinned.

Immunoglobulin G levels were measured by a modified Mancini technique.⁹ Rat IgG was prepared by chromatography of rat serum on DEAE-cellulose. Rabbit antiserum was prepared by immunization with rat IgG in Freund's adjuvant.

Drugs

Epinephrine chloride solution, 1/1000, obtained from Parke, Davis & Co. was used without dilution. Propranolol (Ayerst Labs.), butoxamine (Burroughs Wellcome & Co. [U.S.A.] Inc.), an adrenergic blocking drug which somewhat selectively blocks metabolic receptors, and DL-isoproterenol sulfate (Aldrich Chemical) were dissolved in isotonic saline or powdered in a mortar and suspended in cottonseed oil. *Bordetella pertussis* vaccine (Lederle Labs.) was injected intraperitoneally in a dose of 15×10^9 organisms per animal. Control animals were given a similar volume of isotonic saline.

RESULTS

Effect of pertussis vaccine on the rate of elimination of antigen

Freund's adjuvant is commonly believed to act in part by preserving a depot of antigen and increasing the duration of the antigenic stimulus.¹⁰ Therefore, we determined the effect of pertussis vaccine on the rate of disappearance of intravenously injected radioiodine-labeled BSA. Radioiodine-labeled BSA, 5 mg., was injected into the tail vein. Blood samples were obtained 3 minutes, 12 hours, and then daily for 8 days after the injection. The blood was allowed to clot, and the trichloroacetic acid-precipitable radioactivity per milliliter of serum was determined. Potassium iodide, 0.1 per cent, was added to the drinking water throughout the experiment. Six control animals received 0.1 ml. of saline and six experimental animals received 15×10^9 pertussis organisms intraperitoneally at the time of the antigen injection. Two of the pertussis-inoculated group died

TABLE 1. Effect of pertussis vaccine alone given at the time of the secondary injection compared to the effect of pertussis vaccine given with the primary or with the secondary injection of antigen

Group No.	Treatment	$\mu\text{g DNP bound/ml. ABC 33}$ (mean $\pm$ S.D.)	
		Primary Day 15	Secondary Day 9
1	Control (no pertussis)	0.12 $\pm$ 0.09	2.04 $\pm$ 2.2
2	Pertussis with primary immunization	0.88 $\pm$ 0.12	8.40 $\pm$ 5.8
3	Pertussis with secondary immunization	0.13 $\pm$ 0.11	7.82 $\pm$ 4.3
4	Pertussis alone at time of secondary immunization	0.14 $\pm$ 0.12	8.24 $\pm$ 5.6

during the course of the experiment, and the early values of these animals have been eliminated from the data presented in Fig. 1.

Elimination of the antigen was retarded by the pertussis vaccine for the first few hours but subsequently was accelerated (Fig. 1). However, there was no sharp change in the rate of elimination several days after injection as would have been expected from early immune elimination of the antigen. The accelerated elimination occurred at a uniform rate. As pertussis vaccine causes a loss of native albumin from the blood,¹¹ it is likely that the exogenous albumin is lost by similar mechanisms. While the possibility of accelerated and more efficient cellular processing of antigen, particularly aggregated protein, cannot be excluded, this experiment demonstrates that the adjuvant effect of pertussis does not depend upon persistent increased concentration of circulating albumin.

Effect of pertussis vaccine on the secondary response and of pertussis vaccine alone at the time of the secondary response

Twenty animals were divided into four groups of 5 each. On Day 1, all received 1 mg. of DNP-EA intraperitoneally, and Group 2 received 15×10^9 pertussis organisms intraperitoneally. On Day 30, Groups 1, 2, and 3 received booster injections of DNP-EA, and Group 3 received 15×10^9 pertussis organisms. Group 4 received the pertussis vaccine only without the antigen. Blood was collected for antibody assay 15 days after the first antigen injection and 6 days after the second (Table I).

Homocytotropic antibody was found at the time of the secondary response in one of the 5 animals of each of Groups 3 and 4 which received pertussis vaccine but in none of the control animals of Groups 1 and 2. The ultimate level of antibody during the secondary response was about the same whether the pertussis was given with the first or with the second injection of antigen. More interestingly, pertussis vaccine alone without a second injection of antigen had an adjuvant effect of the same order of magnitude as combined antigen and pertussis vaccine. This observation indicates that the pertussis acts on the cells involved in the immune response, probably at some stage subsequent to antigen recognition rather than on antigen-processing steps.

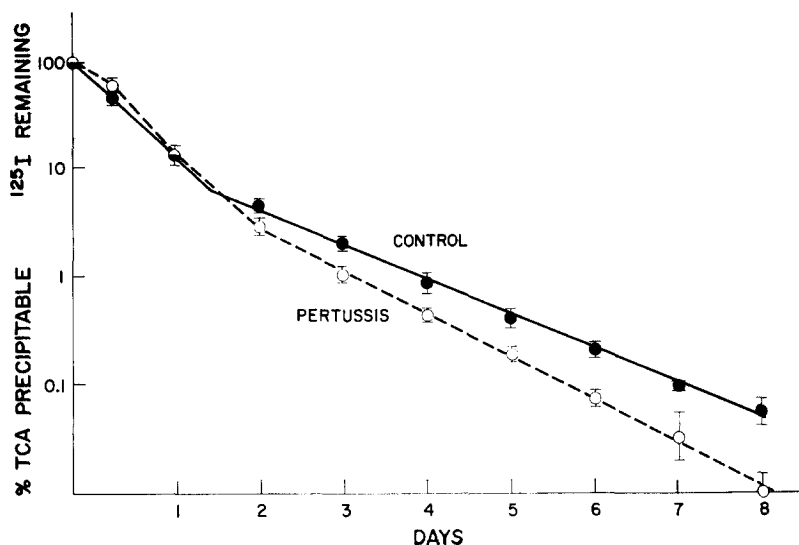


FIG. 1. Elimination of trace-labeled bovine serum albumin from the blood of pertussis inoculated and control rats, mean $\pm$ standard deviation.

Combined adjuvant effect of pertussis vaccine and Freund's adjuvant

Animals were divided into four groups. Groups 1 and 3 received intraperitoneal injections of 1 mg. of DNP-EA dissolved in isotonic saline. Groups 2 and 4 received a subcutaneous injection of the same amount of DNP-EA emulsified in complete Freund's adjuvant (Difco Labs.). Groups 3 and 4 received 15×10^9 pertussis organisms. Each animal received a booster intraperitoneal injection of DNP-EA in saline on Day 30. The combination of pertussis and Freund's adjuvants was more effective than either alone, both in total antibody and homocytotropic antibody response (Table II). Both adjuvants led to a decrease in serum IgG concentration 6 days after the immunization. IgG concentration returned to normal at 15 days and then increased. The increase lasted more than a month. The combined effect of pertussis vaccine and Freund's adjuvant on IgG levels was greater than either alone.

Effect of alloxan on the adjuvant effect of pertussis vaccine

One of the actions of insulin is to increase protein synthesis. As pertussis vaccine causes increased insulin levels, this effect conceivably could account for enhanced antibody production.

Animals were divided into four groups. Groups 1 and 2 consisted of normal animals. Groups 3 and 4 were given 50 mg. of alloxan, a drug which destroys pancreatic beta cells, intravenously 48 hours before the start of the experiment. All developed marked glycosuria and were maintained throughout the experiment by the addition of 5 per cent glucose to their drinking water. Nevertheless, 3 of the animals in Group 3 died before the experiment was completed. All animals were immunized with 1 mg. of DNP-EA intraperitoneally on Day 0. Groups 2 and 4 received 15×10^9 pertussis organisms.

TABLE II. Combined effect of Freund's adjuvant and pertussis vaccine on antibody production

Group No.	Treatment	Total antibody (μg DNP-BSA bound/ml. serum, ABC 33; mean $\pm$ S.D.)		
		Day 6	Day 9	Day 15
1	Control	0.01 ± 0.02	0.05 ± 0.09	0.15 ± 0.08
2	Freund's adjuvant	0.04 ± 0.03	0.18 ± 0.09	0.42 ± 0.10
3	Pertussis	0.02 ± 0.03	0.14 ± 0.10	0.47 ± 0.15
4	Freund's adjuvant + pertussis	0.09 ± 0.04	0.35 ± 0.18	0.77 ± 0.25

TABLE III. The effect of alloxan on the adjuvant action of pertussis vaccine

Group No.	Treatment	No. of animals	Total antibody (μg DNP-BSA bound/ml. serum, ABC 33; mean $\pm$ S.D.)		
			Day 6	Day 9	Day 15
1	Control	8	0.009 ± 0.007	0.10 ± 0.05	0.22 ± 0.14
2	Pertussis	8	0.015 ± 0.012	0.15 ± 0.06	0.33 ± 0.06
3	Alloxan	11	0.007 ± 0.009	0.04 ± 0.03	0.14 ± 0.08
4	Alloxan + pertussis	14	0.013 ± 0.009	0.12 ± 0.06	0.24 ± 0.09

Although the diabetic animals produced somewhat less antibody, the adjuvant effect of pertussis vaccine was fully expressed (Table III). Thus, the adjuvant effect does not depend upon increased insulin secretion.

Effect of adrenergic agonists and antagonists on the antibody response

If, as seems probable, the fraction of the pertussis organism responsible for the adjuvant effect of pertussis vaccine is the same as the histamine-sensitizing factor and the histamine-sensitizing factor acts through suppression of the cellular response to beta adrenergic stimulation, then beta adrenergic blocking drugs might also have an adjuvant effect. Conversely, beta agonists might suppress antibody production.

Animals were divided into five groups of 15 in each. Group 1, the control, received a subcutaneous injection of saline every 6 hours for 10 days after each injection of antigen; Group 2 received epinephrine 600 μg per kilogram; Group 3 received propranolol, 25 mg. per kilogram; and Group 4 received butoxamine, 15 mg. per kilogram every 6 hours instead of saline. Group 5 received 15×10^9 pertussis organisms at the time of the antigen and saline every 6 hours. The antigen, 1 mg. of DNP-EA in saline, was given intraperitoneally on Days 0 and 30. These doses of drugs were determined by preliminary studies to be the maximum doses that the animals could tolerate, without an appreciable mortality rate.

The effects of propranolol and butoxamine were indistinguishable, so, for clarity of presentation, the propranolol data have been omitted from Fig. 2. The animals receiving the beta adrenergic blocking drugs produced more antibody than did the controls, and the animals receiving epinephrine produced less; but the differences were not statistically significant. The difference between the effect of butoxamine (or of propranolol) and epinephrine was significant at the one per cent level (Kruskal-Wallis test).

and IgG levels

Homocytotropic antibody (animals positive/animals tested)			IgG concentration (mg./ml., mean $\pm$ S.D.)			
Day 6	Day 9	Day 15	Day 6	Day 9	Day 15	Day 36
0/10	0/10	0/10	8.5 $\pm$ 1.0	9.5 $\pm$ 3.0	8.8 $\pm$ 1.4	8.8 $\pm$ 1.5
0/8	5/8	5/8	5.0 $\pm$ 2.0	5.4 $\pm$ 0.6	8.2 $\pm$ 1.6	10.5 $\pm$ 1.3
0/10	4/10	4/10	6.1 $\pm$ 1.3	6.8 $\pm$ 2.5	7.8 $\pm$ 2.8	11.8 $\pm$ 1.2
0/8	8/8	8/8	4.3 $\pm$ 0.8	6.5 $\pm$ 0.8	10.0 $\pm$ 1.0	13.2 $\pm$ 2.5

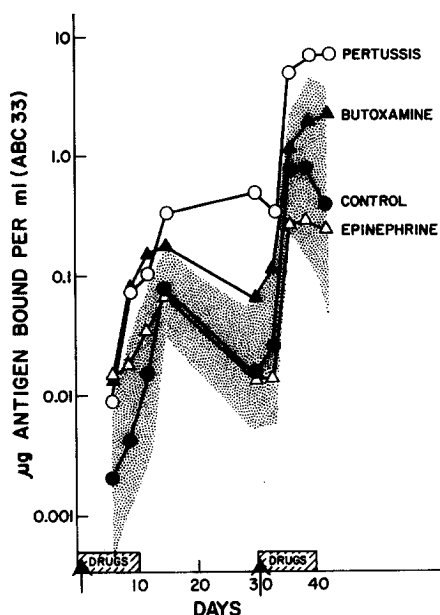


FIG. 2. Antibody response of rats given epinephrine and adrenergic blocking drugs. **Arrows** indicate the injection of 1 mg. of DNP-egg albumin. The drugs were given for 10 days, as indicated in the rectangles. The **points** are the mean values of 15 animals in each group. The **stippled area** represents $\pm$ standard deviation of the control values. Butoxamine increased and epinephrine decreased antibody production slightly, but the differences from the control values are not statistically significant. The difference between the epinephrine- and butoxamine-treated groups is significant at the one per cent level.

Effect of beta agonists and antagonists on homocytotropic antibody response

Twenty-four animals were immunized by a subcutaneous injection of 1 mg. of DNP-EA in complete Freund's adjuvant. Eight animals received isoproterenol sulfate, 10 mg. per kilogram, suspended in cottonseed oil every 8 hours for 10 days. Eight others similarly received butoxamine, 50 mg. per kilogram, suspended in cottonseed oil. The other 8 served as controls and received injections of cottonseed oil alone. Isoproterenol suppressed both total and long-latency homocytotropic antibody formation slightly (Table IV). Butoxamine increased homocytotropic antibody. The difference between isoproterenol and butoxamine on the homocytotropic antibody response was significant at the one per cent level (Kruskal-Wallis test).

Table IV. The effect of isoproterenol and butoxamine on total and homocytotropic antibody production after immunization with Freund's adjuvant

Treatment	Total antibody (μ g DNP-BSA bound/ml. serum, ABC 33; mean $\pm$ S.D.)			Homocytotropic antibody (area of blueing, mm. ² , mean of values of positive animals; numbers in parentheses = number of donors positive/number tested)		
	Day 6	Day 9	Day 15	Day 6	Day 9	Day 15
Control	0.04 $\pm$ 0.03	0.18 $\pm$ 0.09	0.42 $\pm$ 0.10	0 (0/8)	130 (5/8)	125 (5/8)
Isoproterenol	0.02 $\pm$ 0.03	0.07 $\pm$ 0.06	0.38 $\pm$ 0.13	0 (0/8)	101 (5/8)	57 (3/8)
Butoxamine	0.06 $\pm$ 0.04	0.12 $\pm$ 0.11	0.45 $\pm$ 0.11	0 (0/8)	162 (6/8)	146 (6/8)

DISCUSSION

It appears that the adjuvant effect of pertussis vaccine is exerted directly upon the lymphoid cells involved in the antibody response. In part, at least, this effect of pertussis is to increase the rate of cell division. Finger and associates¹² reported that pertussis vaccine accelerated and prolonged the multiplication of antibody-forming spleen cells of mice immunized with sheep red blood cells. We have confirmed this effect (Makino: Unpublished observations). Furthermore, pertussis vaccine alone has an anamnestic effect (Table I). The adjuvant effect is exerted despite accelerated elimination of plasma proteins with reduced concentrations of plasma proteins, including gamma globulin. Eventually, however, gamma globulin levels are increased.

Insulin, which stimulates protein synthesis and could have reasonably been considered as a mediator of the increased antibody formation, is not necessary for the adjuvant effect, although increased insulin secretion could serve as a contributing factor. Fishel and Szentivanyi¹³ and Townley and Szentivanyi¹⁴ have drawn similar conclusions about the possible contribution of insulin in the pertussis-induced increased sensitivity to histamine and the impaired hyperglycemic response to epinephrine. Other hormones, especially growth hormone, conceivably could be altered by pertussis vaccine, but there is no evidence to date that they are.

The other major effect of pertussis on cell metabolism, reduced response to beta adrenergic stimulation, deserves attention. Our data suggest, but do not establish with certainty, that beta adrenergic blocking drugs enhance and epinephrine reduces both the total and homocytotropic antibody responses. If so, a beta adrenergic blocking effect of the pertussis histamine sensitizing factor could be responsible for the adjuvant action. In this case, the result of the postulated beta blockade is presumably an increased rate of mitosis of the precursors of the antibody-forming cells.

More direct evidence of possible impairment of beta adrenergic responses of antibody-forming cells by pertussis vaccine was obtained by Ortez and associates,¹⁵ who found reduced levels of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in the spleens of pertussis-sensitized mice. The pertussis vaccine also impaired the response of splenic-derived adenyl cyclase to epinephrine and fluoride.

While the effects of the adrenergic agonists and antagonists are statistically

significant, they are marginal. Even very large almost lethal doses of beta adrenergic blocking drugs did not increase antibody levels as much as did pertussis vaccine, and unlike pertussis vaccine they did not evoke a homocytotropic antibody response. The adjuvant effects of pertussis vaccine and beta adrenergic blocking drugs thus differ in some important and as yet unexplained way.

Preliminary observations on the effect of adrenergic drugs on antibody formation in vitro indicate that the primary response is suppressed by large doses of epinephrine, theophylline, and cyclic AMP.^{16, 17} Norepinephrine, presumably acting primarily on alpha receptors, enhances antibody formation.¹⁷ On the other hand, Ishizuka and associates¹⁸ reported that cyclic AMP and theophylline enhanced antibody formation. These apparently conflicting results cannot be reconciled at this time, but it is possible that adrenergic drugs have opposing effects on thymic-derived and bone marrow-derived lymphocytes. McManus and co-workers^{19, 20} found that cyclic AMP stimulated mitosis in thymic lymphocytes. In contrast, beta adrenergic stimulation or cyclic AMP inhibits DNA synthesis of peripheral blood leukocytes.²¹⁻²⁵ The situation is further complicated by the apparent difference in the effect of beta adrenergic stimulation on DNA synthesis at different stages of the cell cycle.²⁶

The attempt to account for the adjuvant effect of pertussis vaccine in terms of beta adrenergic blockade or reduced lymphocyte adenyl cyclase must await resolution of these questions of the effect of stimulation of adrenergic receptors of different populations of lymphocytes and at different stages of the cell cycle.

REFERENCES

- 1 Munoz, J., and Bergman, R. K.: Histamine-sensitizing factors from microbial agents, with special reference to *Bordetella pertussis*, *Bacteriol. Rev.* **32**: 103, 1968.
- 2 Szentivanyi, A.: The beta adrenergic theory of the atopic abnormality in bronchial asthma, *J. ALLERGY* **42**: 203, 1968.
- 3 Gulbenkian, A., Schubert, L., Nixon, C., and Tabachnick, I. I. A.: Metabolic effects of pertussis sensitization in mice and rats, *Endocrinology* **83**: 885, 1968.
- 4 Szentivanyi, A., Fishel, C. W., and Talmage, D.: Adrenaline mediation of histamine and serotonin hyperglycemia in normal mice and the absence of adrenaline-induced hyperglycemia in pertussis-sensitized mice, *J. Infect. Dis.* **113**: 86, 1963.
- 5 Enta, T., Lockey, S. D., and Reed, C. E.: A rapid safe technique for repeated blood collection from small laboratory animals, *Proc. Soc. Exp. Biol. Med.* **127**: 136, 1968.
- 6 Eisen, H. N., Carsten, M. E., and Belman, S.: Studies of hypersensitivity to low molecular weight substances. III. The 2,4-dinitrophenyl groups as a determinant in the precipitin reaction, *J. Immunol.* **73**: 296, 1954.
- 7 Hunter, W. M., and Greenwood, F. C.: Preparation of iodine¹³¹ labeled growth hormone of high specific activity, *Nature* **194**: 495, 1962.
- 8 Minden, P., and Farr, R. S.: The ammonium sulfate method to measure antigen binding capacity, in Weir, D. M., editor: *Handbook of experimental immunology*, Philadelphia, 1967, F. A. Davis Company, p. 463.
- 9 Stiehm, E. R., and Fudenberg, H. H.: Serum level of immune globulin in health and disease, *Pediatrics* **37**: 715, 1966.
- 10 Herbert, W. J.: The mode of action of mineral-oil emulsion adjuvants on antibody production in mice, *Immunology* **14**: 301, 1968.
- 11 Bergman, R. K., and Munoz, J.: Hypoproteinemia in mice after treatment with histamine-sensitizing factor from *Bordetella pertussis*, *Proc. Soc. Exp. Biol. Med.* **131**: 964, 1969.
- 12 Finger, H., Emmerling, P., and Schmidt, H.: Accelerated and prolonged multiplication of antibody-forming spleen cells by *Bordetella pertussis* in mice immunized with sheep red blood cells, *Experientia* **23**: 591, 1967.

- 13 Fishel, C. W., and Szentivanyi, A.: The absence of adrenaline-induced hyperglycemia in pertussis-sensitized mice and its relation to histamine and serotonin hypersensitivity, *J. ALLERGY* 34: 439, 1963.
- 14 Townley, R., and Szentivanyi, A.: Secretion of immunoreactive insulin (IRI) by the isolated perfused pancreas of normal and pertussis-sensitized rats, *J. ALLERGY* 41: 122, 1968.
- 15 Ortez, R. A., Klein, T. W., and Szentivanyi, A.: Impairment of the adenyl cyclase system in spleens of mice sensitized bacterially or by beta pharmacological blockade, *J. ALLERGY* 45: 111, 1970. (Abst.)
- 16 Makino, S., and Reed, C. E.: Epinephrine inhibition of hemolytic plaque forming cells following in vitro immunization, *Fed. Proc.* 29: 431, 1970.
- 17 Pearlman, D. S.: The effect of adrenergic agents on the antibody response in vitro, *J. ALLERGY* 47: 109, 1971. (Abst.)
- 18 Ishizuka, M., Gafni, M., and Braun, W.: Cyclic AMP effects on antibody formation and their similarities to hormone-mediated events, *Proc. Soc. Exp. Biol. Med.* 134: 963, 1970.
- 19 McManus, J. P., and Whitfield, J. F.: Stimulation of deoxyribonucleic acid synthesis and mitotic activity of thymic lymphocytes by cyclic adenosine 3'5'-monophosphate, *Exp. Cell Res.* 58: 188, 1969.
- 20 Whitfield, J. F., McManus, J. P., and Gillan, D. J.: Cyclic AMP mediation of bradykinin induced stimulation of mitotic activity and DNA synthesis in thymocytes, *Proc. Soc. Exp. Biol. Med.* 133: 1270, 1970.
- 21 Nowell, P. C.: Mitotic inhibition by epinephrine in leukocyte cultures, *Fed. Proc.* 21: 72, 1962.
- 22 Middleton, E. R., and Hadden, J. W.: Beta adrenergic influences on lymphoblast transformation, *Clin. Res.* 17: 547, 1969.
- 23 Hadden, J. W., Hadden, E. M., and Middleton, E. R., Jr.: Lymphocyte blast transformation. I. Demonstration of adrenergic receptors in human peripheral lymphocytes, *Cell. Immunol.* 1: 583, 1970.
- 24 Smith, J. W., Steiner, A. L., Newberg, W. M., and Parker, C. W.: Cyclic nucleotide inhibition of lymphocyte transformation, *Clin. Res.* 17: 549, 1969.
- 25 Smith, J. W., Steiner, A. L., Newberg, W. M., and Parker, C. W.: The effect of dibutyryl cyclic adenosine 3'5'-monophosphate on human lymphocyte stimulation by phytohemagglutinin, *Fed. Proc.* 28: 566, 1969.
- 26 Hadden, J. W., Hadden, E. M., Middleton, E. R., Jr., and Good, R. A.: Lymphocyte blast transformation. II. The mechanism of action of alpha adrenergic receptor effects, *Int. Arch. Allergy Appl. Immunol.* 40: 526, 1971.