STUDIES ON THE LYMPHOCYTOSIS INDUCED IN MICE BY BORDETELLA PERTUSSIS*

By STEPHEN I. MORSE, M.D.

(From The Rockefeller Institute)

Lymphocytosis rarely accompanies acute bacterial disease. A striking exception is found in many cases of whooping cough, as first noted by Fröhlich in 1897 (1). In patients with pertussis the peripheral leucocyte count may be greater than 175,000/mm³. and the majority of cells are usually morphologically normal small lymphocytes. Despite clinical familiarity with the phenomenon, comparatively little is known regarding the nature of the component of *Bordetella pertussis* that induces this unique response. It is known that active disease is not prerequisite since lymphocytosis occurs following the injection of killed pertussis vaccine into man (2) and experimental animals (3); sonic extracts of the bacterium have also been reported to induce lymphocytosis, but the active factor has not been defined (4). The dynamics of the response in the host have also not been delineated and it is not certain whether the increase in circulating lymphocytes is the result of increased production of cells or a consequence of mobilization of cells from lymphoid and other tissues. It is apparent that an understanding of the mechanism by which *B. pertussis* induces lymphocytosis might extend our comprehension of the regulation and control of the level of circulating lymphocytes.

The results of studies on the leucocytosis, particularly the lymphocytosis, produced in mice by injecting pertussis vaccine are presented in this report. Sequential changes in the quantity and kinds of leucocytes in the circulation after inoculation of animals with vaccine were examined. The properties of the vaccine which made it effective in producing leucocytosis were also analyzed. The histopathology of tissues of mice injected with pertussis vaccine suggested that lymphocytosis may result primarily from release of cells into the circulation from tissue depots, particularly the lymphoid organs.

Materials and Methods

Mice.—Four- to 6-week-old NCS mice (5) of both sexes were utilized. During the latter part of the study many of these specific pathogen-free mice were found to carry *Escherichia coli* and such contaminated animals responded somewhat differently to the injection of pertussis vaccine (see Results).

Pertussis Vaccine.—Commercially available pertussis vaccine (Eli Lilly and Company, Indianapolis) containing 50 to 100 × 10⁹ phase I organisms/ml, suspended in physiological saline with 1:10,000 thimerosal, was initially used. Concentrated suspensions of the killed organisms were also kindly supplied by Dr. J. M. McGuire of Eli Lilly and Company. Before injection these were diluted to achieve the same concentration of organisms as in the conventional preparations.

Leucocyte Counts (WBC).—Blood for peripheral leucocyte counts was obtained by severing the tail a few millimeters from the distal end. Differential counts were performed on Wright-stained films. It was frequently difficult to distinguish monocytes from large lymphocytes and only those cells which were typical monocytes were classified as such. Cells were also classified as polymorphonuclear leucocytes (PMN's), small lymphocytes and large mononuclear cells (including both monocytes and large lymphocytes) by direct examination in the counting chamber using ×430 and phase contrast objectives. The wet differential count was reproducible, correlated well with stained films, and had the advantage of achieving representative cell distribution. Unless otherwise indicated 4 to 6 mice were utilized for each determination.

Agglutination Tests.—0.2 ml of serum was serially diluted twofold in phosphate-buffered saline, pH 7.2, in plastic cups. 0.2 ml of vaccine diluted 1:2 in the same buffer with 10 per cent normal rabbit serum was added and the degree of agglutination was recorded after 90 minutes at 25°C.

Operative Procedures.—Thymectomy was performed under light ether anesthesia by the method of Kaplan (6). Intraperitoneal pentobarbital was the anesthetic agent for splenectomy.

The excellent technical assistance of Marie B. Picard is gratefully acknowledged.

RESULTS

Peripheral Leucocyte Counts of Mice Injected Intravenously with Pertussis Vaccine.—Mice were injected intravenously *via* a lateral tail vein with either 0.3, 0.1, or 0.05 ml of pertussis vaccine. The lower doses were brought to 0.3 ml volume with diluent (physiologic saline containing 1:10,000 thimerosal); another group of mice received 0.3 ml of diluent alone. WBC's were then performed at specified intervals.

As depicted in Fig. 1, striking leucocytosis occurred in all animals receiving the vaccine. At the higher doses, an elevation in white cell count was apparent within 24 hours and the peak response for all groups was 3 to 5 days after injection. At the height of the leucocytosis following the injection of 0.3 ml of pertussis vaccine, there were between 135,000 and 200,000 circulating white cells/mm³. The counts remained elevated for more than 2 weeks. More concentrated vaccine did not result in significantly greater leucocytosis indicating that 0.3 ml was a maximum dose. Since animals receiving diluent alone did not develop leucocytosis it was concluded that neither the diluent nor repeated cutting of the tail had any appreciable effect.

Morphology of the Circulating Leucocytes in Pertussis-Injected Mice.—The nature of the cellular elements which contributed to the observed leucocytosis was determined by examination of Wright-stained blood films obtained at various time periods after the injection of 0.3 ml of pertussis vaccine.

Two hours after the intravenous injection of vaccine, a pronounced leuco-

FIG. 1. The leucocyte response of mice injected intravenously with different amounts of pertussis vaccine.

penia was evident (Fig. 2). Unlike the initial granulocytopenic response to endotoxin, the main acute effect of pertussis vaccine was lymphopenia. There was an average reduction of 70 per cent in the number of circulating small lymphocytes, and the large mononuclears had decreased by approximately 40 per cent. In contrast the number of circulating PMN's had decreased by less than 10 per cent.

Within 4 to 6 hours the leucocyte count rose and the PMN's were predomi-
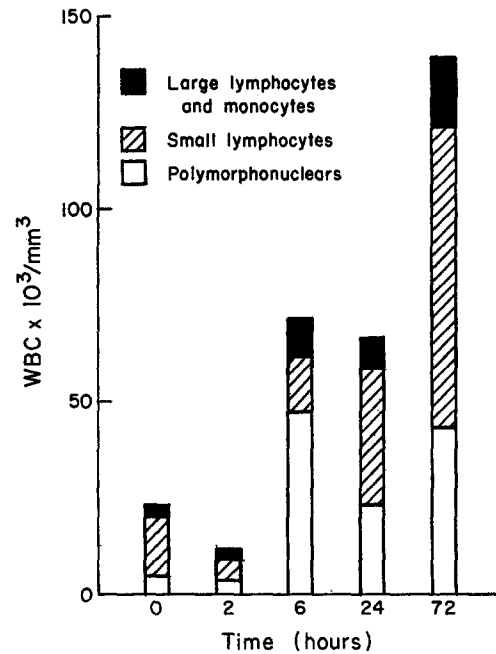

FIG. 2. Changes in the leucocyte counts of mice during the first 72 hours after administration of 0.3 ml of pertussis vaccine.

nant. The total leucocyte count then remained stationary or declined slightly during the next 18 hours. However, during that interval the granulocytic elements decreased whereas the number and percentage of small lymphocytes increased.

At the time of the peak response, 4 days after injection, the small lymphocytes comprised 50 to 70 per cent of the leucocyte population (Fig. 3). These cells appeared morphologically normal. The granulocytes accounted for 25 to 40 per cent of the white blood cells at this time, and these cells were mature with typical multilobed nuclei and normal granulation of the cytoplasm. The remainder of the cells were large lymphocytes, a few with vacuolated cytoplasm, and monocytes. Eosinophiles and basophiles were rarely seen.
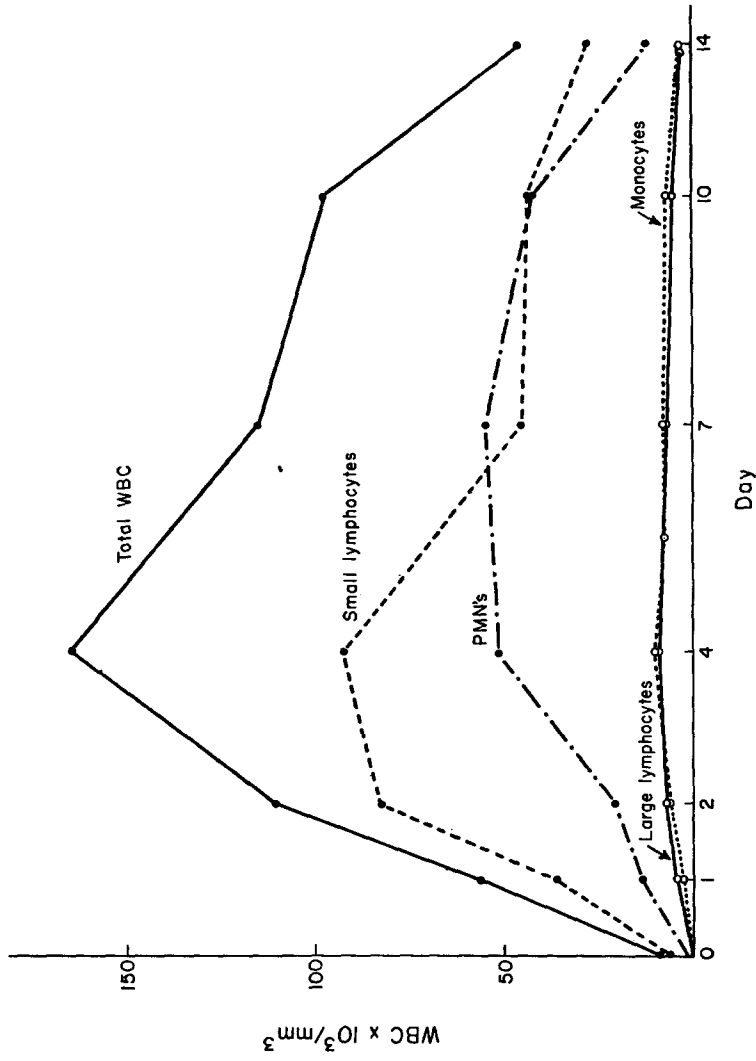
FIG. 3. Changes in the differential leucocyte counts of mice after the intravenous injection of 0.3 ml of pertussis vaccine.

As evidenced by the lack of nuclear staining with trypan blue or eosin Y, as well as by the brisk locomotion of the leucocytes on glass surfaces, greater than 99 per cent of the white cells were considered to be viable.

After the period of maximal leucocytosis, the number of small lymphocytes declined, whereas the number of PMN's remained relatively stationary so that 7 days after injection of pertussis vaccine, the total number of circulating leucocytes had decreased and the PMN's predominated. During the next 7 days, there was a parallel reduction of all cell types. At no time were abnormal or immature cells conspicuous.

E. coli-contaminated NCS mice responded somewhat differently to the injection of 0.3 ml of pertussis vaccine. Although the magnitude of the lymphocyte response was identical with that observed in NCS mice, they tended to have a higher total WBC and a more vigorous granulocytic response.

Hematocrits and reticulocyte counts of mice injected with pertussis vaccine were also determined. These values were essentially identical with those of mice injected with diluent alone. Moreover, there was no qualitative difference in the platelets.

Comparison of the WBC of Ventricular and Tail Vein Blood of Mice Injected with Pertussis Vaccine.—It is well known that the tail vein leucocyte count of mice is greater than that of ventricular blood (7). It was therefore necessary to compare leucocyte counts from these two sites after administration of pertussis vaccine to determine if comparable responses occurred, and to eliminate anatomical factors as significant components of the observed hyperleucocytosis.

Mice were injected intravenously with 0.3 ml of pertussis vaccine. At specific time intervals, tail vein blood was obtained from each of 4 mice. These mice were then killed with chloroform and blood was rapidly sampled from the left ventricle.

As shown in Table I, both leucocytosis and lymphocytosis were demonstrated in ventricular as well as in tail vein blood. The ventricular WBC was lower both before as well as after injection of pertussis vaccine. However, the relative increase in both the total number of leucocytes and small lymphocytes was greater in the case of the ventricular samples. These observations indicated that tail vein WBC's were satisfactory indices for determining the leucocyte response to pertussis vaccine. The differences between the central and peripheral leucocyte concentrations in the mouse made it impossible to estimate the absolute number of circulating leucocytes.

Response to Pertussis Vaccine Injected by Different Routes.—Injection of pertussis vaccine into mice renders them sensitive to the lethal effects of histamine whereas uninoculated animals are quite resistant (8). It is known that histamine sensitization is more readily demonstrated following intravenous administration of vaccine than after other routes of injection (9, 10). Therefore, the leucocyte counts of mice inoculated with vaccine intravenously, intraperitoneally, and subcutaneously were compared.

Mice were injected with 0.3 ml of pertussis vaccine either by the intravenous, intraperitoneal, or subcutaneous routes; leucocyte counts were performed 1, 4, and 7 days later.

As depicted in Fig. 4, subcutaneous injection of vaccine resulted in only a slight elevation of the leucocyte count. Four days after injection, animals receiving an intraperitoneal dose of vaccine had total leucocyte counts which were 75 per cent of that observed when the intravenous route was used. However, the number of small lymphocytes was only 50 per cent of that seen after intravenous injection and the majority of cells were granulocytes. The greater polymorphonuclear response to intraperitoneal injection may have been a consequence of local inflammation.

TABLE I

Leucocyte Concentrations in Ventricular and Tail Vein Blood of Mice
after the Injection of Pertussis Vaccine

Day	Total WBC/mm[a]		No. of small lymphocytes/mm[a]	
	Ventricle	Tail	Ventricle	Tail
0	8,400	18,400	6,200	14,200
1	20,700	55,500	15,300	32,100
4	121,000	191,000	80,300	116,700
7	93,500	156,000	36,800	65,200

* Averages of 4 animals.

Thus, although leucocytosis was seen in both cases, the lymphocyte response to pertussis vaccine was twofold greater following intravenous injection than following intraperitoneal inoculation. The subcutaneously administered dose was essentially ineffective.

It was of particular interest that cells did not accumulate in the peritoneal cavity after injection of pertussis vaccine into that site.

Properties of Pertussis Vaccine Which Relate to the Induction of Leucocytosis and Lymphocytosis.—Although a complete survey of the leucocyte response to pertussis vaccine was not undertaken, significant leucocytosis and lymphocytosis was found to follow the intravenous injection of vaccine into C_{57} black mice, Sprague-Dawley rats, guinea pigs, and New Zealand rabbits.

The injection of comparable amounts of pertussis vaccine obtained from other commercial sources (Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, and Parke, Davis and Company, Detroit) evoked in mice a leucocytosis in every way comparable to that seen with the Lilly product. However, leucocytosis was not produced by the intravenous injection of a soluble antigen prepared from phase I pertussis cells (UBA, Eli Lilly and Company). Injection of equivalent numbers of *E. coli*, *Staphylococcus aureus*, or cells of a rough strain of *B. pertussis* did not induce hyperleucocytosis.
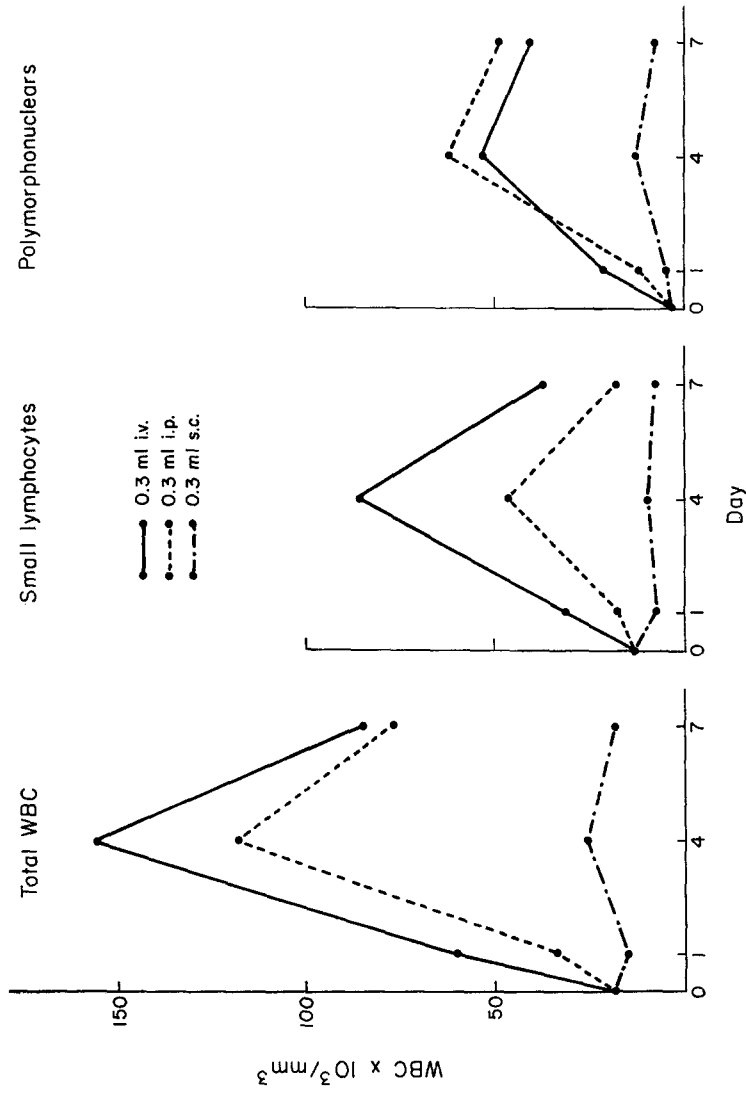
FIG. 4. Leucocyte counts of mice receiving 0.3 ml of pertussis vaccine by the intravenous, intraperitoneal, or subcutaneous route.

It was found that the leucocytosis-promoting property of pertussis vaccine was associated with the bacterial cells and not with the suspending medium. The bacilli were sedimented at 10,000 RPM for 20 minutes and resuspended to volume. 0.3 ml of resuspended bacterial cells or clear vaccine supernatant fluid were injected intravenously into mice. Leucocytosis followed administration of the bacteria but not the injection of the supernatant fluid.

Vaccine heated at 100°C for 30 minutes no longer induced leucocytosis whereas complete potency was retained after heating at 56°C for 30 minutes.

*Production of Leucocytosis-Promoting Activity during Growth of B. Pertussis.—*Occasional lots of pertussis vaccine were less effective than others with respect to their ability to produce leucocytosis. It was possible that variations in the level of active material might be a reflection of variations in cultural conditions. One aspect of this problem was investigated by studying the leucocytosis-inducing activity present in cultures of *B. pertussis* harvested after different growth periods.

B. pertussis strain 3779B, utilized in production of the Lilly vaccine, was grown on a modified Cohn-Wheeler charcoal agar medium at 35°C (11). The organism fulfilled the morphological criteria for phase I and did not grow on conventional nutrient media. Slants were inoculated, and at varying time intervals the growth was washed off with 5.0 ml of physiological saline containing thimerosal (1:5000). The optical density at 650 mμ of the suspension was determined on an appropriately diluted aliquot.

After storage at 4°C for 5 days the organisms were no longer viable. The suspensions were then diluted to contain 100 $\times$ 10^9 cells/ml, and 0.3 ml was injected intravenously into 4 mice. Leucocyte counts were determined 4 days later. In addition, the ability of the bacterial cells used for challenge to agglutinate with pertussis antiserum was examined. Pertussis antibodies were prepared in rabbits by the subcutaneous injection of 0.5 ml of pertussis vaccine three times a week for a total of nine injections; the rabbits were bled 7 days after the last inoculation. Aliquots of the pertussis suspensions were mixed with an equal volume of antiserum in capillary precipitin tubes and the speed and extent of agglutination were qualitatively estimated.

As indicated in Table II maximum growth was obtained after 72 hours, but the maximum capacity of the organism to induce leucocytosis was present after 48 hours of growth and then diminished during further incubation. After 6 days of growth no activity was present. It was not clear whether the mechanism of inactivation was based upon thermal or other physical or chemical instability, or the production by the organisms of an inactivating substance. Organisms harvested at 24 hours could not be adequately tested because they were too toxic. Agglutinating capacity of the bacillus seemed to parallel the ability to induce leucocytosis in mice.

Induction of Active Immunity against the Leucocytosis Stimulating Properties

of B. pertussis.—In order to determine whether the factor responsible for the development of leucocytosis was related to an antigenic component of *B. pertussis*, groups of mice were injected with vaccine subcutaneously, and, when serum antipertussis antibody was demonstrable these animals were challenged intravenously.

Mice were injected subcutaneously with two doses of 0.2 ml of pertussis vaccine 1 week apart. Seven days later 4 animals were killed and the agglutinin content of their sera individually measured. All had titers between 1:320 and

TABLE II

The Effect of Incubation Time on the Ability of B. Pertussis Cultures to Evoke Leucocytosis

Incubation	OD$_{680}$	Agglutination	WBC × 10³/mm³	PMN's	Small lymphocytes
days				*per cent*	*per cent*
2	1.8	++	145.6	30	60
			150.4	24	68
			120.0	35	52
			—*	—	—
3	5.2	+	53.4	32	64
			79.2	22	72
			72.4	25	68
			80.8	22	71
6	2.6	±	22.6	24	68
			21.8	15	80
			24.6	17	80
			24.8	24	71

* One animal died 48 hours after injection.

1:1280. Of the remaining mice, 4 received 0.1 ml of pertussis vaccine intravenously, and 4 received 0.3 ml. Control animals received subcutaneous injections of saline rather than vaccine before intravenous challenge.

As shown in Fig. 5, actively immunized mice did not respond to either dose of the challenge vaccine whereas the control animals had a typical response.

In other experiments, it was found that animals repeatedly injected intravenously with pertussis vaccine developed leucocytosis after each injection. This is in contrast to the protective effect produced by prior subcutaneous inoculations. Preliminary evidence indicated that the levels of antibody produced after intravenous injection were much lower than those attained after subcutaneous administration of the vaccine.

Passive Protection against Pertussis-Induced Leucocytosis.—Attempts to block the effects of pertussis vaccine by the prior administration of hyperimmune

rabbit antisera gave variable results unless the serum was admixed with the vaccine immediately before injection. Under those circumstances there was a 37 per cent reduction in the peak WBC response; no effect occurred when normal rabbit serum was employed. However, passive protection was readily induced by antipertussis mouse serum.
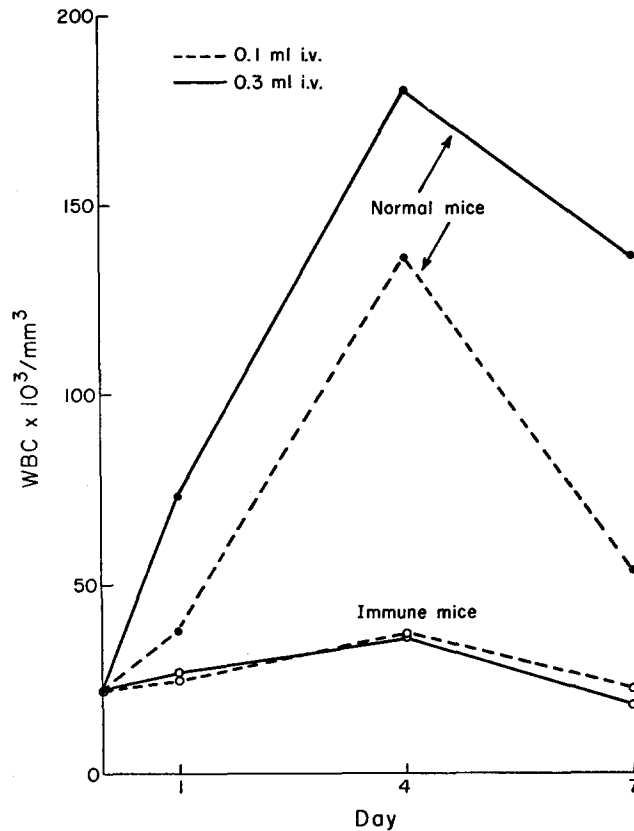

FIG. 5. Leucocyte counts of mice challenged after immunization with subcutaneous injections of pertussis vaccine.

A serum pool was obtained from mice 7 days after the second of 2 weekly subcutaneous inoculations of 0.2 ml of pertussis vaccine. The agglutinating titer of the pooled serum was 1:320. 0.2 ml of either antipertussis mouse serum or normal mouse serum was injected intravenously into 4 mice 24 hours prior to the administration of 0.1 ml of pertussis vaccine by the intravenous route.

As shown in Fig. 6, the prior administration of antipertussis serum reduced the total WBC rise by 30 per cent and the rise in lymphocytes by 44 per cent

at the time of the peak response. The results, although showing less than complete protection were significant.

The Effect of Thorotrast on the Leucocyte Response to Pertussis Vaccine.—It was of interest to investigate the response to pertussis vaccine following the injection of inert particles in order to determine if an adequately functioning reticuloendothelial system was required. Thorium dioxide (thorotrast, Testagar
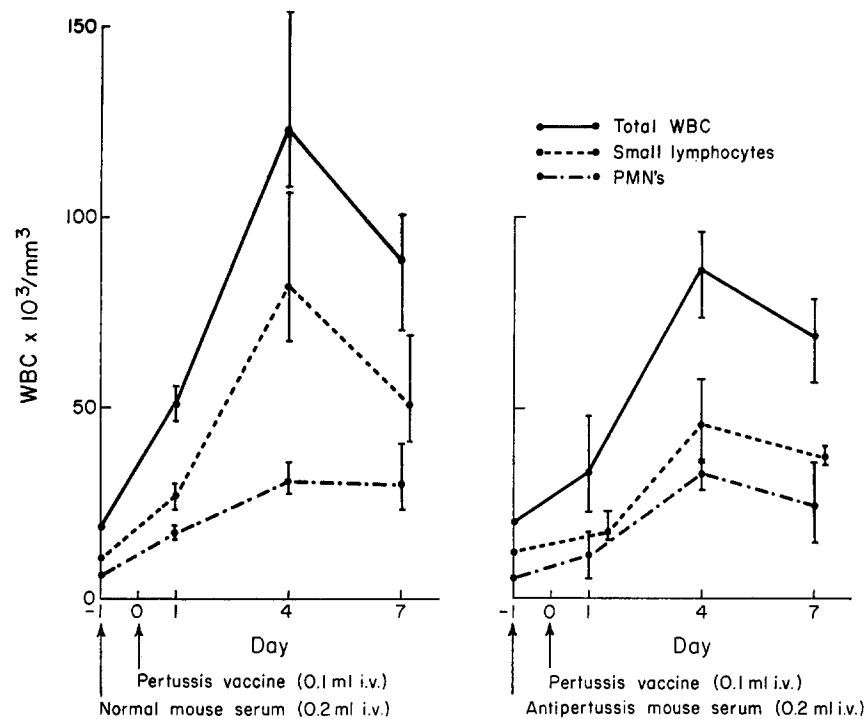

Fig. 6. The effect of 0.1 ml pertussis vaccine given intravenously to mice which had received antipertussis mouse serum 24 hours previously.

and Co., Detroit), frequently used for the production of "reticuloendothelial blockade," was chosen as the inert material.

0.02 ml of thorotrast was injected intravenously 45 minutes before the intravenous injection of 0.1 ml of pertussis vaccine. Leucocyte counts were performed 1, 4, and 7 days later.

As indicated in Fig. 7, thorotrast administration reduced but did not abolish the leucocytosis.

Attempts to Transfer Leucocytosis with Serum and Organ Extracts of Pertussis Injected Mice.—The results with thorotrast suggested that before leucocytosis can develop, the organism must be phagocyted by the cells of the reticuloendo-

thelial system. Perhaps either a soluble bacterial product is then released which induces leucocytosis directly or there is indirect stimulation of the production of an endogenous leucocytosis factor. Numerous studies were devised in an attempt to demonstrate a soluble leucocytosis factor in the reticuloendothelial tissue or circulation of mice which had received pertussis vaccine. Samples were obtained at time periods ranging from a few hours to 3 days after vaccine administration. In no instance was serum, or extracts of liver and spleen from inoculated animals capable of transferring leucocytosis.
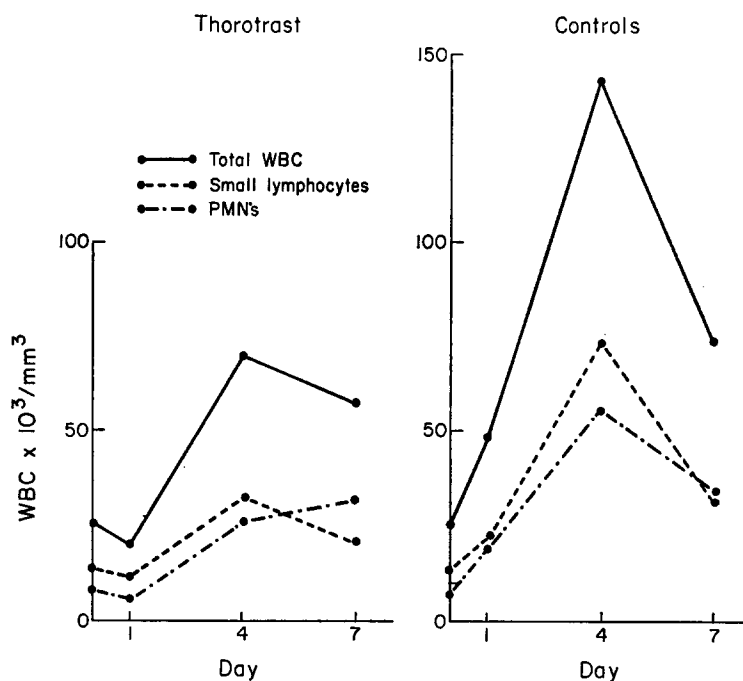

FIG. 7. The effect of thorotrast on the leucocyte response of mice injected intravenously with pertussis vaccine.

The Effects of Thymectomy and Splenectomy on the Response to Pertussis Vaccine.—If the effect of pertussis vaccine was to stimulate the production of an endogenous leucocytopoietic or leucocyte releasing factor, removal of the target organ might modify the leucocyte response. Therefore the effects of extirpation of two organs known to affect leucocyte regulation, the spleen, and the thymus, were studied.

Two groups of mice were thymectomized at 4 weeks of age. One group was given pertussis vaccine 9 days after operation and the other 13 days after

thymus ablation. The challenge dose was 0.1 ml of pertussis vaccine in both instances.

Although a slight lymphopenia was present at the time of vaccine injection, both groups of mice responded with a leucocytosis and lymphocytosis comparable to that in normal animals.

Six splenectomized and 6 sham-operated mice were also challenged with 0.1 ml of pertussis vaccine 10 days after operation. The resulting leucocyte response is shown in Fig. 8.

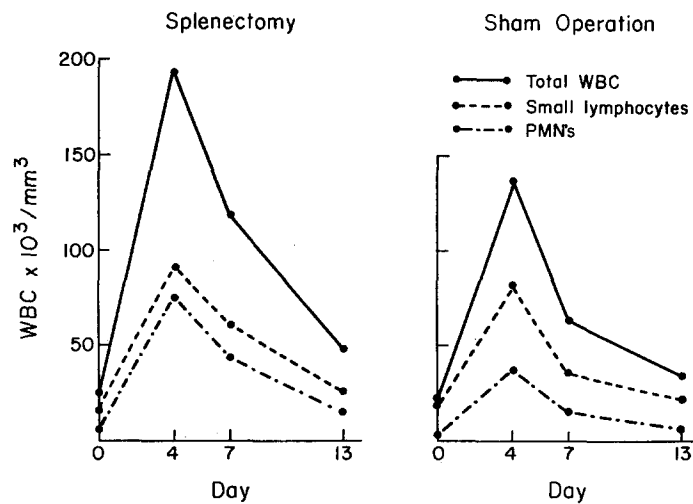The total WBC was higher in the splenectomized animals, and the rate of

FIG. 8. The effect of splenectomy on the leucocyte response of mice injected with pertussis vaccine.

decline was slower than in the sham-operated controls. The difference in total leucocytes was virtually all due to a greater increase in circulating PMN's in the splenectomized animals. Histological studies indicated that PMN's were present in large numbers in the splenic red pulp of the intact animals. Therefore the lack of splenic sequestration of cells might have caused both the greater elevation and slower fall of the leucocyte counts of splenectomized animals.

Changes in the Lymphoid and Hematopoietic Organs of Mice Injected with Pertussis Vaccine.—In order to provide more intimate knowledge of the mechanism of leucocytosis and lymphocytosis produced by *B. pertussis*, gross and microscopic studies of various tissues of injected mice were performed.

Twenty mice were injected intravenously with 0.3 ml of pertussis vaccine. One, 4, 7, and 14 days after injection, 5 mice were killed by chloroform anesthesia. The weights of the thymus, spleen, and mesenteric node (pancreas of
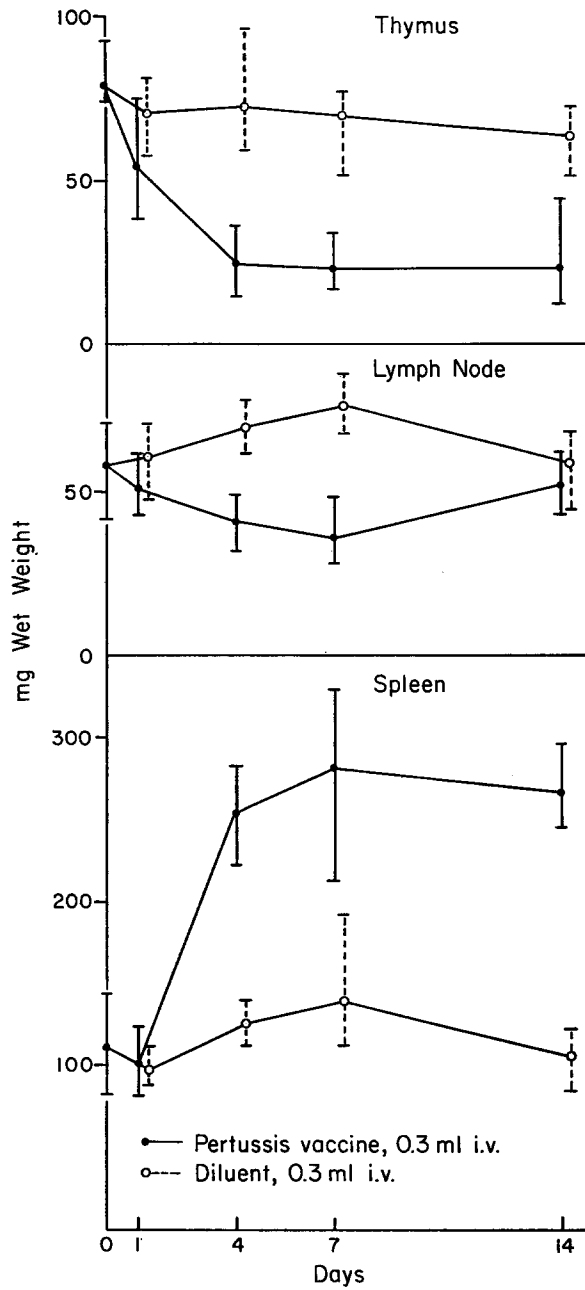
'Fig. 9. Changes in the weights of the thymus, mesenteric nodes, and spleen of mice receiving pertussis vaccine.

Aselli) were determined. The lymphoid organs, as well as samples of liver, lung, kidney, and adrenal gland were fixed in 10 per cent formalin for histologic examination. Twenty litter mates received 0.3 ml of diluent injected intravenously and were killed at the same time intervals. These mice served as experimental controls. Another group of 5 litter-mates served as zero time controls.

Fig. 9 illustrates the changes in the weights of the lymphoid organs after the intravenous injection of vaccine. There was a dramatic fall in thymus weight and by the 4th day after vaccine injection, the average thymus weight was approximately 35 per cent of the control. The loss of thymic weight persisted for the duration of the experimental period.

The mesenteric nodes which were removed *en bloc* and trimmed of excess fat also decreased in weight. However, the extent and duration of the weight loss was less than that of the thymus. The nodes weighed 70 and 57 per cent of normal at the 4th and 7th day after injection respectively. However, by the 14th day they were of normal weight.

In contrast to the response of the thymus and nodes, the spleens of pertussis-inoculated animals markedly increased in size and weight (Fig. 9).

On microscopic examination, the most obvious changes in the lymph node were disruption of follicular architecture; loss of distinction between cortex and medulla; and decrease in the quantity of lymphocytic elements. The changes were not seen at 1 day after injection but were prominent 4 days after the injection of pertussis vaccine. By the 14th day a few normal appearing follicles were present.

In the case of the thymus, there was a striking loss of normal thymocytes by the 4th day and the reticular cells were prominent. There was also little demarcation between the cortex and medulla; again by the 14th day groups of small mononuclear cells, poorly organized, were found.

The spleens of the experimental animals showed the same changes with respect to disorganization of follicles and diminution in quantity of lymphoid cells as seen in the lymph node and thymus. In addition, however, there was a marked increase in red pulp with numerous polymorphonuclear leucocytes present in the sinusoidal spaces. The increase in red pulp almost certainly accounted for the observed increase in spleen weight.

Representative sections were also stained by the methyl green–pyronine method. Mitotic figures were no more numerous in the thymus, lymph node, or spleen of experimental animals than in the controls during the course of the experiment.

It was of great interest that typical germinal centers were not seen, an observation that correlated with the finding that normal levels of antibody were not formed after intravenous injection of pertussis vaccine. In contrast sections of lymph nodes and spleens of mice receiving subcutaneous injections of pertussis vaccine did show changes characteristic of stimulation of antibody production.

Examination of bone marrow from pertussis-injected mice showed moderate increase in the cells of the granulocytic series and virtual absence of small lymphocytes. There were no significant gross or microscopic abnormalities noted in the other organs examined and no abnormalities were found in the tissues of mice receiving diluent alone.

DISCUSSION

Phase I cells of *Bordetella pertussis* are capable of inducing a variety of biological changes in experimental animals. These changes include enhanced sensitivity to histamine, serotonin, and other pharmacological agents as well as increased susceptibility to anaphylaxis (8, 12–14). In addition, pertussis vaccine has an adjuvant effect (15).

Another property of the organism is the ability to induce leucocytosis with a predominating lymphocytosis. This type of reaction is rarely seen in bacterial diseases although it does occur occasionally in patients with tuberculosis or either secondary or congenital syphilis. Lymphocytosis is commonly observed in certain viral infections particularly mumps, measles, and disease caused by type 12 adenovirus (16).

Many hypotheses have been presented to account for the pertussis-associated lymphocytosis. An early theory suggested that the paroxysmal coughing literally squeezed the lymphocytes from the lymphatics into the circulation. This postulate is untenable since lymphocytosis can be induced in man and experimental animals by the injection of killed vaccine.

Adrenal cortical insufficiency has also been invoked as an explanation for the lymphocytosis. However, lymphocytosis of this degree is not noted in patients with Addison's disease. Moreover, adrenalectomized rats maintained only on mineral corticoid give a response comparable to normal rats after an injection of pertussis vaccine (17). In addition, in the present study no histological changes were noted in the adrenals.

It has previously been shown that injection of sonic extracts of *B. pertussis* into rabbits results in a leucocytosis and lymphocytosis (4). Heating of the sonic extract for 30 minutes diminished the lymphocytosis and a predominately granulocytic response occurred. Bradford and his coworkers studied the leucocyte changes in mice consequent to the intraperitoneal injection of sonic extracts of *B. pertussis* cells 3 weeks after initiation of intrapulmonary infection. They observed a striking leucocytosis 3 days after intraperitoneal injection of unheated sonic extract (18). Many of the observed responses were primarily granulocytic.

In the studies presented in this report, several observations have been made regarding the nature of the leucocyte response in mice which follows the injection of pertussis vaccine. Although the relative increase in granulocytes is large after the injection of pertussis bacilli it is the mature, small lymphocyte that shows the greatest increase in absolute numbers. The response, with

respect to both the PMN's and lymphocytes, is far greater than that seen after the injection of other organisms. There is also an increase in the numbers of circulating large lymphocytes and monocytes. There is no significant change in cells of the erythrocytic series.

The route of injection is of considerable importance. Intraperitoneal injection is less effective than the intravenous route in inducing leucocytosis, but the results are complicated by the development of an inflammatory focus in the peritoneal cavity. Subcutaneous injection is virtually without effect on the peripheral leucocyte count. It is difficult to assess the factors which make the subcutaneous route ineffective. The local destruction of bacterial products by tissue enzymes, or a different distribution of injected material may be responsible.

That the distribution of the inoculum is of importance is suggested by the results obtained with thorotrast. Whether the "reticuloendothelial blockage" induced by thorotrast is due to loading of phagocytic cells, or removal of circulating opsonins, the net effect is to retard uptake of particles by fixed phagocytes. Therefore the diminution of both granulocytosis and lymphocytosis strongly suggests that the factor(s) involved is the product of an interaction between these cells and the injected bacteria.

Such an interaction might result in the release of an endogenous substance which in turn stimulates leucocytosis. Leucocytosis was not induced in mice receiving serum or organ extracts from previously inoculated animals suggesting that the hypothetical factor may be present only in small quantities in the circulation or is rapidly fixed to tissues. Since neither prior thymectomy nor splenectomy reduced the leucocytosis, an endogenous substance could not arise solely from these organs.

Alternatively, the interaction between the organism and the phagocytic cells might result in the liberation of a soluble bacterial product which then evokes the white cell response. Highly active material has been obtained in this laboratory following fractionation of sonic extracts of *B. pertussis* cells and studies are in progress to elucidate the first step in the pertussis-induced leucocytosis.

The inability of actively or passively immunized mice to respond with the usual leucocytosis indicates that the component of *B. pertussis* that induces the reaction may be antigenic. Alternatively, the presence of antibody could affect the distribution of the organisms and their processing by host tissues and cells so that active material is not released.

The leucocytosis-promoting properties of pertussis vaccine bears many resemblances to the histamine-sensitizing properties: heat lability; time of peak response; and heightened effect by the intravenous route of injection (9, 10). However, purified preparations have not been isolated and it can not be determined if the leucocytosis factor is related to any known constituent of *B. pertussis*.

It is not known whether the leucocytosis is due to the release or production of new cells, or a combination of both events. Bone marrow examination showed increased granulocytopoietic activity, but immature PMN's were not apparent in the circulation suggesting that the granulocytic phase may be due to the operation of both factors.

Histologic changes in the bone marrow and the lymphoid organs,—thymus, lymph node, and spleen,—suggested that the lymphocytosis may be due to the release of formed small lymphocytes into the circulation. The tissues were virtually denuded of small lymphocytes and there was no evidence of cellular debris and mononuclear phagocytosis which is seen when lymphoid tissue is destroyed by x-irradiation (6). Moreover mitotic figures were no more prominent in the experimental group than in the controls again suggesting that production of new cells is not a major factor. It has been estimated that there is an accessible reserve of small lymphocytes which is 40 times greater than the number of these cells in the circulation (19). Thus far there is no evidence that lymphatic blockage plays a role in the observed phenomena.

The fate of the lymphocytes which appear in the circulation is not clear. Two weeks after the injection of pertussis vaccine, there was beginning repopulation of the lymphoid organs with lymphocytes, but the number of mitotic figures was not increased; it is therefore possible that the circulating lymphocytes may "home."

Studies now in progress are designed to isolate and characterize the component of *B. pertussis* which produces the leucocyte response and to elucidate further the dynamics of the phenomenon.

SUMMARY

1. Intravenous injection into mice of phase I *Bordetella pertussis* vaccine resulted in a striking hyperleucocytosis with a predominating lymphocytosis. Intraperitoneal inoculation was less effective, and subcutaneous administration was inactive.

2. Active immunization prevented the hyperleucocytosis; passive immunization was less effective.

3. Reticuloendothelial blockage reduced the effect of the vaccine.

4. Extirpation of the spleen or thymus did not alter the leucocyte response.

5. Histologic studies suggested that the increase in circulating lymphocytes resulted from release of cells from lymphoid organs, including the thymus.

BIBLIOGRAPHY

1. Fröhlich, J., Beitrag zur Pathologie des Keuchustens, *J. Kinderkrankh*, 1897, **44**, 53.

2. Sauer, L. W., Whooping cough, a study in immunization, *J. Am. Med. Assn.*, 1933, **100**, 239.

3. Tuta, J. A., A study of the lymphocytosis following intravenous injection into

rabbits of suspensions and extracts of *Hemophilus pertussis*, *Folia haematol.*, 1937, **57**, 122.

4. Ehrich, W. E., Bondi, A., Jr., Mudd, S., and Flosdorf, E. W., The tolerance of rabbits for the agglutinogen and the toxins of *Hemophilus pertussis*, *Am. J. Med. Sc.*, 1942, **204**, 530.

5. Dubos, R. J., and Schaedler, R. W., The effect of the intestinal flora on the growth rate of mice and on their susceptibility to experimental infections, *J. Exp. Med.*, 1960, **111**, 407.

6. Kaplan, H. S., Influence of thymectomy, splenectomy, and gonadectomy on incidence of radiation-induced lymphoid tumors in strain C57 black mice, *J. Nat. Cancer Inst.*, 1951, **11**, 83.

7. Simonds, J. P., The blood of normal mice, *Anat. Rec.*, 1925, **30**, 99.

8. Parfentjev, I. A., and Goodline, M. A., Histamine shock in mice sensitized with *Hemophilus pertussis* vaccine, *J. Pharmacol. and Exp. Therap.*, 1948, **92**, 411.

9. Maitland, H. B., Kohn, R., and MacDonald, A. D., The histamine sensitizing property of *Hemophilus pertussis*, *J. Hyg.*, 1955, **53**, 196.

10. Munoz, J., Effect of antiserum on sensitization by *B. pertussis* to histamine, *Fed. Proc.*, 1964, **23**, 404.

11. Powell, H., Culbertson, C., and Ensminger, P., Charcoal agar medium for preparing *Hemophilus pertussis* vaccine, *Pub. Health Rep.* U.S.P.H.S., 1951, **66**, 346.

12. Munoz, J., Effect of *H. pertussis* on sensitivity of mice to serotonin, *Proc. Soc. Exp. Biol. and Med.* 1957, **95**, 328.

13. Kind, L. S., The altered reactivity of mice after immunization with *Hemophilus pertussis* vaccine, *J. Immunol.* 1953, **70**, 411.

14. Kind, L. S., The altered reactivity of mice after inoculation with *Bordetella pertussis*, *Bact. Rev.*, 1958, **22**, 173.

15. Munoz, J., Comparison of *Bordetella pertussis* cells and Freund's adjuvant with respect to their antibody inducing and anaphylactogenic properties, *J. Immunol.*, 1963, **90**, 132.

16. Olson, L. C., Miller, G., and Hanshaw, J. B., Acute infectious lymphocytosis presenting as a pertussis-like illness: its association with Adenovirus type 12, *Lancet*, 1964, **1**, 200,

17. Fichtelius, K. E., and Hassler, O., Influence of pertussis vaccine on the lymphocyte production in adrenalectomized rats, *Acta Path. et Microbiol. Scand.*, 1958, **42**, 189.

18. Bradford, W. L., Scherp, H. W., and Tinker, M. R., Effect of extracts of *Hemophilus pertussis* on leucocyte counts in normal and sensitized mice, *Pediatrics*, 1956, **18**, 64.

19. Cronkite, E. P., Jansen, C. R., Cottier, H., Rai, K., and Sipe, C. R., Lymphocyte production measured by extracorporeal irradiation, cannulation, and labeling techniques, *Ann. New York Acad. Sc.*, 1964, **113**, 566.