THE EFFECT OF HYDROCORTISONE AND X-IRRADIATION ON THE LYMPHOCYTOSIS INDUCED BY BORDETELLA PERTUSSIS*

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PLATES 45 TO 47
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Extreme leukocytosis characterized by a predominating lymphocytosis is a striking feature of clinical whooping cough. In studies reported previously, it was shown that intravenous injection of thimerosal-killed Bordetella pertussis into mice also results in marked elevation of the numbers of circulating leukocytes; when large numbers of organisms are administered the leukocyte count reaches levels as high as 150,000 to 200,000 cells per cubic millimeter (1). As in the case of the leukocytosis found in patients with active pertussis, the majority of cells are mature small lymphocytes. Further studies have been designed to isolate the factor which induces this unique response and to attempt to delineate the mechanism of the reaction.

The presence in the blood of pertussis-treated mice of large numbers of normal, mature small lymphocytes also affords the possibility of precise analysis of the effects of various pharmacologic agents and environmental conditions on these cells in vivo. In the studies reported herein, this model has been utilized to examine the action of adrenal cortical hormones and X-irradiation with respect to the circulating small lymphocyte. Administration of either to normal animals causes profound lymphopenia but the results obtained in the present studies indicate they do so by quite different means (2).

Materials and Methods

Production of B. Pertussis-Induced Leukocytosis.—Methods utilized for the induction and analysis of the leukocytosis produced by B. pertussis have previously been described in detail (1). In brief, mice of the NCS colony were injected intravenously via a lateral tail vein with 0.3 ml of commercially available pertussis vaccine or a specially prepared suspension (both obtained from Eli Lilly and Company, Indianapolis). The numbers of killed organisms administered was between 1 to 5 × 10⁶. Total leukocyte counts (WBC) were performed on tail vein blood; differential counts were determined either by direct examination of cells diluted in 1% acetic acid, 0.01% gentian violet in the counting chamber at 430 magnification, or of Wright-stained blood films. At least six mice were employed for each experimental determination.

Adrenal Cortical Hormone.—Saline suspensions of hydrocortisone acetate (Hydrocortone

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acetate, Merck, Sharp and Dome, Philadelphia) were diluted appropriately in sterile saline and injected subcutaneously.

X-Irradiation.—Mice were irradiated through the courtesy of Dr. W. Burnet, Radiotherapy Division, New York Hospital. 650 R was administered with a 250 kv unit at a working distance of 50 cm through 0.5 and 1.0 mm aluminum filters. The half value layer was 1.5 mm copper. The mice were kept in individual compartments of egg cartons during the 4 to 5 min of irradiation.

Histology.—Organs were fixed in 10% formalin and stained with hematoxylin-eosin or methyl-green pyronine.

RESULTS

The effect of Hydrocortisone Acetate on the Leukocyte Count of NCS mice.—Following a single subcutaneous injection of 15 mg of hydrocortisone acetate into NCS mice, a marked reduction in the number of circulating leukocytes rapidly occurred (Table I). Within 24 hr the average leukocyte count was only 12% of normal. This change was primarily accounted for by a reduction of greater than 90% in the number of circulating small lymphocytes. The WBC depression lasted for more than 7 days without associated mortality. It was of note that polymorphonuclear leukocytosis did not occur and indeed moderate neutropenia was regularly observed.

The Effect of Prior Administration of Hydrocortisone Acetate on the Subsequent Development of Pertussis-Induced Leukocytosis.—Since hydrocortisone produced long lasting depression of the lymphocyte count it was of interest to determine in what fashion prior administration of the steroid might modify the lymphocytosis usually induced by pertussis vaccine. As indicated in Text-fig. I, the intravenous injection of pertussis vaccine 24 hr after the subcutaneous administration of 15 mg of hydrocortisone was followed by marked leukocytosis. However, in contrast to the response in animals pretreated with physiological saline only, the predominating cell type was the polymorphonuclear leukocyte (PMN). Thus in the normal animals, 4 days after the injection of pertussis vaccine the total leukocyte counts averaged 175,000 cells/mm³; 60.6% of the
cells were small lymphocytes. In contrast, in animals pretreated with hydrocortisone the leukocyte count 4 days after administration of pertussis vaccine averaged 151,000 cells/mm$^3$ but 70% of the cells were polymorphonuclears. The granulocytes were normal in appearance and neither immature nor hypersegmented cells were noted.

Text-Fig. 1. The leukocyte response of mice to pertussis vaccine injected 24 hr after hydrocortisone acetate administration.

Although the absolute number of circulating lymphocytes was far less than that found in normal animals injected with pertussis vaccine, the relative rise in the face of pretreatment with hydrocortisone was more than 30-fold, a value three times greater than that found in the controls.

A striking feature of these studies was that over 75% of the animals pretreated with hydrocortisone and then given pertussis vaccine died between the 4th and 7th day after the administration of the vaccine. The deaths were associated with extreme polymorphonuclear leukocytosis. At autopsy no overt lesions were seen nor could conventional organisms be cultured from a variety
of tissues sampled. As previously indicated, deaths did not occur following the injection of either hydrocortisone or pertussis vaccine alone.

The Effect of Hydrocortisone on the Early Phases of Pertussis-Induced Leukocytosis.—In the previous experiments it was clearly demonstrated that hydrocortisone markedly depressed the circulating lymphocyte count of normal mice. Moreover, the characteristic lymphocytotoxic effects of the hormone were seen in the lymphoid tissues (3). Further experiments were designed to evaluate whether destruction of circulating cells also occurred. It was felt that examina-
tion of the effect of hydrocortisone on the kinetics of pertussis-induced lymphocytosis might provide substantial information on this question which has been the subject of controversy.

![Graph showing the effect of hydrocortisone acetate administered 48 hr after pertussis vaccine](image)

**Text-Fig. 3.** The effect of hydrocortisone acetate administered 48 hr after pertussis vaccine.

Groups of mice were injected with pertussis vaccine and at various times 15 mg of hydrocortisone was administered subcutaneously. White blood counts were performed at frequent intervals and the viability of circulating cells was assayed.

Results of such experiments performed during the early phases of the pertussis
reaction are illustrated in Text-figs. 2 and 3. When the hormone was administered 1 day after the injection of pertussis vaccine, the continuing increase of the lymphocyte count which occurred in the control group receiving pertussis vaccine alone was completely prevented. The same was true when the steroid was given 2 days after the vaccine. Of note was that the lymphocyte count was not depressed and the numbers of these cells in the circulation remained constant at the elevated level attained before hydrocortisone was given. Numerous and careful examinations of blood samples obtained at intervals after hydrocortisone administration revealed the circulating cells to be of normal morphology. Moreover, viability seemed unimpaired as measured by the nuclear exclusion of trypan blue and eosin Y, and normal motility.

Although the lymphocyte response to pertussis vaccine was in effect suddenly "switched off" by hydrocortisone, the polymorphonuclears in the circulation, and hence the numbers of total white blood cells increased. In these groups of mice occasional deaths occurred but only after 5 to 7 days had elapsed following hydrocortisone injection.

The Effect of Hydrocortisone Administered at the Time of Peak Lymphocytosis Induced by Pertussis Vaccine.—The previous experiments, in which it was shown that hydrocortisone prevented the progressive lymphocytosis induced by pertussis vaccine but did not produce a fall in the numbers of circulating lymphocytes, suggested that the hormone did not destroy circulating small lymphocytes. However, it was possible that the stability of the lymphocyte count reflected a complex balance between destruction of cells in the blood, destruction of cells in lymphoid tissue, and release of viable cells into the circulation. It was possible to circumvent some extent these complex interactions by administering hydrocortisone at the time of the peak response to pertussis vaccine. In normal animals given pertussis vaccine lymphocytosis reaches a maximum 4 days after injection of the organisms. Then the numbers of lymphocytes gradually declines. As is seen in Text-fig. 4, the administration of hydrocortisone 4 days after pertussis vaccine did not alter the kinetics of the disappearance of lymphocytes from the circulation.

In order to be certain that the apparent lack of effect of hydrocortisone on the numbers of circulating small lymphocytes was not a dose-dependent phenomenon, 25 mg of hydrocortisone was administered at the time of peak response to pertussis vaccine and the results were identical to those obtained when the lower dose was employed.

It was possible that the small lymphocytes in the circulation were killed by the adrenal steroid but not lysed. Again, the ability of the cells to exhibit normal motility, exclude trypan blue and eosin Y from the nuclei, and their capacity to take up neutral red indicated that the cells were viable.

Histologic Changes in the Lymphoid Tissue of Mice Receiving Pertussis Vaccine and Hydrocortisone.—As shown above there was no evidence that
hydrocortisone destroyed circulating lymphocytes in pertussis-treated animals. However, it was clear that the steroid prevented maximal lymphocytosis without causing demonstrable effect on lymphocyte dynamics once the peak

Text-Fig. 4. The effect of hydrocortisone acetate administered at the time of the peak leukocyte response to pertussis vaccine.

response was obtained. It was therefore of considerable interest to determine the effect of hydrocortisone on the lymphoid tissue of pertussis-treated animals. Mice were injected intravenously with pertussis vaccine. 2 or 4 days later 15 mg of hydrocortisone was given subcutaneously and animals were killed
4 and 24 hr after administration of the steroid. Other mice received either hydrocortisone or pertussis vaccine alone.

Histologic changes were confined mainly to the lymphoid organs; spleen, lymph node, and thymus. As reported previously the intravenous administra-

![Graph showing leukocyte response to pertussis vaccine](image)

**TEXT-Fig. 5.** The leukocyte response of previously irradiated mice to the injection of pertussis vaccine.

tion of pertussis vaccine is followed by a marked diminution in the population of small round cells in these organs and disruption of follicular architecture of the spleen and lymph node (1). The changes are minimal 2 days following vaccine administration but are marked at 4 days, the time of maximal lymphocytosis. Necrosis is not observed. Concomitantly, thymus and lymph node mass decrease whereas the spleen size increases because of engorgement of the red pulp.
The observed effect of hydrocortisone alone on the lymphoid tissue of mice were similar to those described by Dougherty and White in their classic studies (3). Pyknotic nuclei, karyorrhexis, and necrotic debris of small lymphocytic elements were prominent 4 hr after hydrocortisone administration. Large phagocytic mononuclear cells were also seen. 24 hr after hydrocortisone administration the lymphoid organs were markedly decreased in size and there was a dramatic diminution in the number of small lymphocytes. Necrosis, however, was no longer evident.

<table>
<thead>
<tr>
<th>Pretreatment*</th>
<th>Time after 650 R</th>
<th>WBC/mm³</th>
<th>Small lymphocytes</th>
<th>PMNs</th>
<th>Large mononuclears</th>
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<tbody>
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<td></td>
<td><strong>day</strong></td>
<td></td>
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<tr>
<td>Pertussis vaccine</td>
<td>0</td>
<td>146,800</td>
<td>92,700</td>
<td>39,000</td>
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<td>7</td>
<td>1,380</td>
<td>310</td>
<td>950</td>
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* 0.3 ml i.v. 3 days before irradiation.

The histologic changes in the organs of mice pretreated with pertussis vaccine and then given hydrocortisone were an amalgamation of the abnormalities produced by either agent alone. Thus in those animals given vaccine alone the lymphoid organs were essentially normal 2 days later but if they were then injected with hydrocortisone changes were apparent in 4 hr which were similar to those observed when hydrocortisone was given alone, i.e. necrosis of tissue lymphocytes was marked (Fig. 1). When hydrocortisone was administered 4 days after pertussis vaccine, the number of tissue lymphocytes had already diminished, but nevertheless 4 hr after steroid injection, there was marked necrosis of the remaining cells (Fig. 2). In both instances, 24 hr after hydrocortisone administration the lymphoid organs were shrunken and virtually complete depopulation of small lymphocytes had occurred.

These results indicated that the prior injection of pertussis vaccine did not inhibit the necrotizing effect of hydrocortisone on small lymphocytes within the lymphoid tissues. Despite this, neither necrotic changes nor evidence of impaired viability of circulating small lymphocytes was seen.

The finding that hydrocortisone acetate, which causes lymphopenia in
normal animals, did not alter the viability of circulating small lymphocytes made it of interest to study another lymphocytopenic agent, X-irradiation in the same system.

The Effect of Prior X-Irradiation on the Leukocyte Response to Pertussis-Vaccine.—A dose of 650 R given to NCS mice produces a profound leukopenia which lasts for more than 2 wk (4). In this strain of mice 650 R is not, however, lethal. The effect of pertussis vaccine on irradiation-induced leukopenia was studied in experiments in which mice, which had received 650 R 5 days previously, were then injected with 0.3 ml of pertussis vaccine intravenously.

As indicated in Text-fig. 5, within 24 hr after the administration of pertussis vaccine, the average leukocyte counts of previously irradiated animals rose from 1200 cells/mm³ to 8250 cells/mm³. This represented approximately a 7-fold increase. 4 days after the injection of the vaccine the counts had risen more than 13-fold and the total leukocyte counts were within normal limits. Although there was a marked increase in the numbers of circulating lymphocytes the predominating cell type was the polymorphonuclear leukocyte, whereas in normal mice the small lymphocyte predominates. As noted in Text-fig. 5 there was no essential change in the total or differential leukocyte counts in X-irradiated animals given saline intravenously rather than pertussis vaccine.

Although neither this dose of irradiation nor pertussis vaccine alone produced lethal effects significant mortality was observed when both agents were employed. Deaths began to occur 5 to 7 days after vaccine administration to the X-irradiated animals and no overt cause was determined.

The Effect of X-Irradiation on the Leukocytosis Induced by Pertussis Vaccine.—In the experiments described above, it was found that administration of adrenal cortical hormones did not cause an accelerated loss of lymphocytes from the blood of pertussis-treated animals. In contrast to those findings, X-irradiation (650 R) when administered near the time of peak response to the intravenous injection of pertussis vaccine was followed by extensive disappearance of circulating small lymphocytes (Table II). Within 24 hr the average leukocyte count had decreased from 146,000 to 43,900 cells/mm³. The alteration in total and differential cell counts 1½ hr, 5½ hr, and 20 hr after X-irradiation are indicated in Text-fig. 6.

Over the next 72 hr the count fell to an average value of 6000 (Table II). It was difficult to calculate accurately the absolute number of cells which had disappeared from the circulation since the leukocyte counts of mice differ in the peripheral and central blood vessels (5). However, considering the tail vein counts to represent between 140 to 170% of the mean value, a conservative estimate of the number of leukocytes removed from the circulation during this 4-day period was between 82 and 100 million cells/ml. Of these at least 75% were small lymphocytes.
Morphologic Changes of Circulating Cells in Pertussis-Treated Mice Given 650 R.—Morphologic abnormalities of leukocytes in X-irradiated subjects have often been noted (6) and were occasionally seen in the white blood cells of irradiated normal mice in these studies. However, because of the rapidly occurring leukopenia, these cells were often difficult to find. In animals with marked leukocytosis due to the effect of pertussis vaccine, radiation induced changes of the leukocytes were readily identified. Examination of blood films revealed the presence of obviously degenerated leukocytes as early as 1 to 2 hr after irradiation (Fig. 3). In many instances the cell morphology was so bizarre that identification of the original cell type was precluded. Some of these clearly nonviable cells were rarely present in the circulation 3 days after irradiation.

Histologic studies revealed that at least a portion of the radiation-damaged cells were removed by the fixed mononuclear phagocytes lining the hepatic sinusoids. 5 hr after irradiation the sinusoids contained numerous cells with pyknotic nuclei as well as cell fragments (Fig. 4). The Kupffer's cells were swollen, and the leukocyte debris could be clearly seen within the cytoplasm of many of them (Fig. 5). 24 hr after irradiation, necrotic cells both inside
Kupffer's cells and free in the sinusoids could still be seen, but within 48 hr these abnormalities were noted infrequently.

Examination of the lungs revealed that cell debris was only occasionally present in the alveolar capillaries, but in some instances engulfment by alveolar macrophages appeared to have occurred. These changes were seen 5 hr after irradiation but not after 24 hr. It was difficult to evaluate by histologic methods the role of the spleen in the clearing process because of the drastic primary effects of irradiation on the in situ population of small lymphocytes.

DISCUSSION

There are few reagents which can induce significant lymphocytosis; none produce the degree of change that follows the intravenous injection of pertussis vaccine. The phenomenon provides a unique experimental model for the study of the circulating lymphocyte and the effects of various agents on this cell. Not only can accurate observations of direct effects on the circulating lymphocyte be made but studies on certain aspects of the physiology of the lymphoid system are facilitated.

It is clear from studies on granulocyte kinetics that the numbers of circulating polymorphonuclears can be markedly increased by such agents as bacterial endotoxins, even when severe marrow damage has been produced by X-irradiation (7, 8). Similarly, as reported here, granulocytopenia in mice given sublethal X-irradiation was reversed by the administration of pertussis vaccine. More importantly, the numbers of circulating small lymphocytes were restored to approximately 50% of normal values after falling to a level of 6% of normal. In like manner hydrocortisone-induced lymphopenia was reversed by pertussis vaccine.

The cells appearing in the blood of mice given pertussis vaccine after pretreatment with either X-irradiation or hydrocortisone were morphologically normal. Blast or other immature cells were not seen. This observation, coupled with unpublished observations derived from experiments utilizing tritiated thymidine, support the previously stated thesis that pertussis-induced lymphocytosis is not a manifestation of an increased rate of mitosis of precursor cells. From the studies performed it appears highly probable that administration of pertussis vaccine is one method of determining the residual readily mobilizable pool of lymphocytes in animals rendered lymphocytopenic.

It is pertinent to reemphasize that polymorphonuclear leukocytosis, as well as lymphocytosis, was a feature found in hydrocortisone or X-ray pretreated mice as well as in normal animals given pertussis vaccine. Indeed, in the case of hydrocortisone pretreatment the granulocytosis was extreme.

Mortality of 80 to 90% of experimental animals pretreated with either the adrenal hormone or X-ray occurred several days after subsequent administration of pertussis vaccine. Mice given steroid, X-irradiation, or pertussis vaccine
alone were not similarly affected. The cause of the delayed death is unknown. Although it is apparent that pertussis vaccine cannot be utilized as a therapeutic measure for radiation-induced leukopenia, as suggested above it might be a useful tool to evaluate the residual extracirculatory pool of cells which can be mobilized after various doses of X-ray.

The existence of large numbers of circulating lymphocytes in normal animals after the injection of pertussis vaccine makes it possible to assess accurately the effect of various agents on these cells. Thus it is well known that at sufficiently high dosages, X-irradiation is cytotoxic for small lymphocytes within the circulation (6, 9). The studies reported herein clearly showed the rapid and dramatic effect of X-irradiation on the morphology, viability, and numbers of circulating small lymphocytes. Within 90 min after 650 R was given to animals which had received pertussis vaccine 3 days previously, a significant reduction in the numbers of circulating small lymphocytes had occurred. Within 24 hr, the average lymphocyte count had decreased from 92,700/mm³ to 12,700/mm³, and in the next 72 hr the count had fallen to 1800/mm³. Many damaged cells were found in the circulation within the first few hours after irradiation. No typical morphologic changes were identified and a wide variety of bizarre abnormalities were found.

Histologic studies revealed that the destroyed cells were phagocytosed by the Kupffer's cells of the liver and to a lesser extent by the lining cells of the alveolar capillaries. It was difficult to assess microscopically the role of the spleen in clearing the circulation of necrotic cells because of the extent of primary radiation damage.

Administration of glucocorticoids to normal animals results in involution of lymphoid tissue and profound lymphopenia. Characteristic histologic changes are found in the lymphoid tissue within a few hours of injection of the adrenal hormone and death of tissue lymphocytes is clearly evident (3). An effect of hormones of the adrenal cortex on the viability of circulating small lymphocytes is less certain. Thus, in vitro studies indicate that cortisol succinate is cytotoxic for blood lymphocytes of the rat (10) but there is conflicting evidence regarding an effect of glucocorticoids on human blood lymphocytes (10, 11). It seemed clear that an experimental animal with induced hyperlymphocytosis might be an excellent model for studying the in vivo effect of corticosteroids on circulating small lymphocytes.

From the data presented, it can be seen that when hydrocortisone acetate was administered in the early phases of the response to pertussis vaccine, the lymphocyte count did not rise further. Instead the number of circulating lymphocytes remained stationary for several days at the level obtained when the hormone was administered. Blood samples were taken at various time periods after hydrocortisone acetate injection and at no time were morphologic changes, such as were seen following X-irradiation, found. Moreover, sensitive
tests for viability such as nuclear exclusion of trypan blue or eosin Y, motility, and uptake of neutral red revealed the circulating cells to be normal. In contrast to the lack of findings in the blood lymphocytes, the lymphocytes within the spleen, lymph node, and thymus were extensively destroyed.

More conclusive evidence of the lack of effect of hydrocortisone acetate on the circulating small lymphocyte was obtained when mice were given the drug at the time of peak lymphocytosis following pertussis vaccine. The lymphocyte counts of these animals did not acutely fall and for the period of observation were superimposable on those of animals given vaccine alone. Again, tests for cell viability showed no alteration in the circulating lymphocytes. At the same time, there was histologic evidence of destruction of many of the residual small lymphocytes within lymphoid tissues. Destruction of cells within lymphoid tissue is not found when pertussis vaccine alone is given.

The experimental observations strongly indicate that glucocorticoids did not destroy or devitalize circulating small lymphocytes. Several lines of evidence made it unlikely that the prior administration of pertussis vaccine per se decreased the in vivo potency of the hormone. Larger doses of hydrocortisone acetate did not affect the results; destruction of small lymphocytes within lymphoid tissue occurred in animals given hydrocortisone whether or not they had previously received pertussis vaccine; and polymorphonuclear leukocytosis occurred following the administration of adrenal steroid.

An extension of these findings may provide an explanation for the lymphopenia occurring in normal animals after the use of adrenal hormones. Normally, small lymphocytes leave the blood to enter lymphoid tissue and from these organs, via the lymphatic systems, cells enter the circulation to maintain the steady state number of cells. In the case of the rat and the mouse it has been estimated that ten to fifteen times the number of small lymphocytes present in the circulation at any one time enter the blood through the thoracic duct each day (12, 13). Following adrenal cortical hormone administration small lymphocytes are destroyed within lymphoid tissues and obviously do not enter the circulation. Those that leave the circulation may then be destroyed within the lymphoid organs, or they may not reenter the circulation in sufficient numbers for other reasons, e.g. they may serve as the basis for a new population of small lymphocytes. Too little is known of the precise events of recirculation of a given small lymphocyte to resolve this problem.

**SUMMARY**

1. In mice rendered lymphocytopenic by X-irradiation or hydrocortisone acetate, pertussis vaccine evoked both lymphocytosis and polymorphonuclear leukocytosis.

2. When mice with lymphocytosis induced by pertussis vaccine were X-irradiated, prompt and extensive destruction of circulating as well as tissue
small lymphocytes occurred. The devitalized circulating cells were cleared from
the blood primarily by the Kupffer’s cells of the hepatic sinusoids.
3. Hydrocortisone acetate administered to mice with lymphocytosis did not
cause acute lymphopenia nor was there any evidence of destruction of circu-
lating small lymphocytes. However, destruction of these cells within lymphoid
tissues was apparent. These observations suggested that adrenal cortical
hormones are not “lymphocytolytic” with respect to circulating lymphocytes.

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EXPLANATION OF PLATES

PLATE 45

Fig. 1. Microscopic section of the spleen of a mouse autopsied 2 days after pertussis vaccine and 4 hr after hydrocortisone acetate administration. A portion of a follicle and perifollicular region with cellular necrosis is shown. Hematoxylin-eosin. × 600.

Fig. 2. Microscopic section of the spleen of a mouse autopsied 4 days after pertussis vaccine and 4 hr after hydrocortisone acetate administration. Note the disorganization and decreased cellularity of the follicular region which is a characteristic finding at this time period following injection of pertussis vaccine. Cellular necrosis due to hydrocortisone is evident. Hematoxylin-eosin. × 600.
PLATE 46

Fig. 3. Abnormal cells which appeared in the circulation 2 hr after X-irradiation of mice which had received pertussis vaccine 3 days previously. Wright stain. × 1500.
PLATE 47

Fig. 4. Section of the liver of a mouse 3 days after the administration of pertussis vaccine and 5 hr after receiving 650 R. Hematoxylin-eosin. × 600.

Fig. 5. Higher magnification of a Kupffer cell from a section similar to that shown in Fig. 4. Cellular fragments are present within the cytoplasm. Hematoxylin-eosin. × 1400.