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Experimental Pathology of Measles in Monkeys* **

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SUMMARY

The experimental pathology of measles was studied in cynomolgus monkeys using epidemiological (or wild) virus obtained from human cases. Specific pathological changes were observed in many organs and tissues such as the lymphatic tissues, respiratory tract, liver, spleen and urinary bladder. Giant cells detected in lymphatic tissues were differentiated into four types on the basis of their cytological features. So-called Warthin-Finkeldey cells were found to be disseminated in the lymph nodes throughout the body in the prodromal stage of the diseases. In six cases, inclusion bodies were detected in the lymph nodes, where hitherto, inclusions have only been detected in reticular cells. Lymphoid giant cells disappear about ten days after virus inoculation which was earlier than the disappearance of reticular giant cells. The relation between the intensity of specific pathological changes and the level of the immunological response was studied. In monkeys showing specific pathological changes, a large increase in antibody titer has not yet been observed. In the parenchyma of the lungs of three cases, lesions rich in giant cells were observed. This may correspond to the pathological changes of giant cell pneumonia.

Monkeys were infected by specimens obtained from patients within 3 days after the onset of a rash, as well as from those who showed Koplik's spots but not a rash. However, specimens lost their infectivity to monkeys if they were obtained from human cases 4 days or later after the onset of a rash.

INTRODUCTION

There have been many reports on findings from autopsies of cases of human

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measles. Some of the pathological changes revealed by many authors have been generalized as specific to measles and of these the appearance of Warthin-Finkeldey cells in lymphatic tissues has been recognized as the most characteristic change.

However, a detailed picture of the pathogenesis of measles is still lacking. This is due firstly to the uncertainty of virological procedures for epidemiological virus and secondarily to the very restricted host range of measles other than the experimentally adapted strain.

There are only a few reports on the pathological changes and clinical features of measles in monkeys (Blake and Trask, 1921a, 1921b; Gordon and Knighton, 1941; Shaffer *et al.*, 1941; Taniguchi *et al.*, 1954; Sergiev *et al.*, 1960; Nii and Kamahora, 1963), and to date Sergiev *et al.*, (1960) have made the most extensive investigations of the virological and pathological features of the disease.

In recent years there has been great progress in research on the virology of measles (Blattner, 1957). Enders and Peebles (1954) first succeeded in isolating measles virus in tissue culture cells and showed its cytopathogenic changes *in vitro*, *i. e.* syncytial formation as well as intranuclear and cytoplasmic inclusion bodies. These cytological changes caused by the agent *in vitro* were valuable in understanding the histological changes observed *in vivo*.

These *in vitro* investigations provided a successful way to prevent measles infection by using vaccines.

Nowadays live attenuated vaccines are also used in practice in Japan (Okuno, et al., 1960a, 1960b). For practical and epidemiolgical reasons it is nceessary to see the biological effects induced by vaccines on human beings and animals. Also it is of interest to find their pathological effects and to compare epidemiological and vaccine viruses.

This report describes the pathological changes in monkeys induced by epidemiological measles (or wild) virus, with parallel observations on the immunological responses of these animals.

MATERIALS AND METHODS

1. *Monkeys*

Cynomolgus monkeys weighing 1-2.5 kg were used and were originally obtained from Java. Since it was possible that the monkeys had suffered from a natural infection of measles before the experiments, virus was inoculated, as soon as the animals were imported.

Just before inoculation, a blood sample was taken to see whether animals were immune to measles using the neutralization test or complement fixation test, or both. The results of these tests are summarized in Table 1. Groups of monkeys were imported at five different times. About 15 per cent of the monkeys were found to be immune and the data for each group are shown in the table.

2. *Virus*

The measles epidemic of 1963 in Japan was more extensively than usual. At this time viral samples, which were nasal washings or swabs of nasopharyngeal secretions, were collected from thirteen patients living in Osaka and neighbouring cities. Among them, eight samples were obtained

Table 1. Antibody Status of Cynomolgus Monkeys at the Time Just Imported

Month	Year	number of monkeys having no definite antibody *	number of immune monkeys▲
May	1963	13	0
June		6	1
July		10	1
September		2	4
October		8	1
Summation		39	7

* The monkeys having a titer of <4 by the neutralization test or by the CF test are shown, although this titer may be a little questionable in relation to their real degree of immunity.

▲ The monkeys having a titer of $\geqq 4$ by the neutralization test or by CF test are shown.

from patients who had shown Koplik's spots as well as a rash, while five samples were derived from cases showing only Koplik's spots. The swabs were immersed in saline or Hanks' solution to resolve the secretions.

The resulting solutions or nasal washings were mixed with streptomycin and penicillin. After low speed centrifugation they were used for inoculation.

3. *Virus inoculation*

Aliquots of 1 to 2 ml of the above samples were injected into the right arm of the monkeys.

4. *Antibody titration*

Blood was taken just before virus inoculation and just before death and sera were tested to see the level of immunological response to measles virus. This was tested by neutralization with the Toyoshima strain of measles, or the complement fixing reaction. An antibody level of $\geqq 4$ was regarded as positive and one of <4 as negative, although the latter was always questionable (Ruckle, 1957).

5. *Autopsy and histological examinations*

Killing of monkeys was made by chloroform inhalation. Organs and tissues were fixed with Bouin's fixative or 10 per cent formalin. Histological preparations were stained with haematoxylin and eosin. In order to see cytological features of affected cells in lymph nodes a silver staining method was also used.

RESULTS

1. *Susceptibility of monkeys to samples derived from patients at various stages of the disease*

It should be considered that the inoculation dose has much correlation with the incubation period, the whole course of the disease, as well as the immunological response. However, in experiments on monkeys using epidemiological measles virus quantitative studies are difficult to make. It has already been reported that

the isolation of the agent was much affected by the stage of illness and failure to isolate it was correlated with the presence of serum antibody that emerged soon after the appearance of a rash (Ruckle, 1957).

Specimens were collected from 13 patients with measles at different stages of the disease. Patients were grouped as those who showed Koplik's spots as well as a rash and those who showed only Koplik's spots. In the former group, the number of days after the appearance of rash was considered. The effects of the 13 samples were tested on 21 monkeys, which had antibody levels of <4 shown by the CF test or neutralization test, and a single sample was inoculated into 1 or 2 monkeys. Monkeys seldom died after infection with measles and positive signs of infection were the existence of specific pathological changes or immunological responses.

Table 2. Relations between the Antibody Response and the Appearance of Specific Pathological Changes in Monkeys* after Inoculation of Virus Specimens (Virus specimens were derived from cases who had shown Koplik's spot but no rash.)

Animal number	Kind of virus specimen	The number of days from virus inoculation to death of monkeys	CF antibody titer		Neutralizing antibody titer		Specific pathological changes
			just before virus inoculation	just before death	just before virus inoculation	just before death	
No. 13	A	12	<4				##
No. 1	〃	14	<4	<4			##
No. 2	B	12	<4				##
No. 3	〃	14	<4	<4			#
No. 4	C	14	<4	64			0
No. 5	〃	14	<4	256			0
No. 32	D	5			<2	<4	##
No. 24	E	7	<4	16	<4	4	#
No. 34	〃	14	<4		<4	>64	0

* cynomolgus monkeys

The results are shown by Tables 2 and 3. All viral specimens derived from the group of patients showing Koplik's spots but no rash, caused specific pathological changes or a rise of antibody level. On the other hand specimens taken from the other group of patients ,who showed a rash, did not always cause effects in the monkeys. Whether the specimens had infectivity in monkeys or not was very closely related with the times when the specimens were taken from the patients after the onset of the rash. The samples which were collected within 3 days after the onset of a rash infected monkeys, while those taken after 4 days did not. This means that the latter samples did not have enough infective virus to cause the disease in monkeys and this is interesting from the epidemiological point of view.

Table 3· Relations between the Antibody Response and the Appearance of Specific Pathological Changes in Monkeys * after Inoculation of Virus Specimens (Virus specimens were derived from cases who had shown rash as well as Koplik's spot.)

Animal number	Virus specimens		The number of days from virus inoculation to death of monkey	CF antibody		Neutralizing antibody titer		Specific pathological changes
	Kind of specimen	Time of collection of specimen in relation to onset of rash		just before virus inoculation	just before death	just before virus inoculation	just before death	
No. 25	F	+1 day	10			<4		+
No. 31	〃	〃	11			<4	>64	0
No. 28	G	+2 days	9			<4	< 4	++
No. 21	〃	〃	11	<4	32	<4	>64	0
No. 23	H	+2 days	8	<4		<4	< 4	++
No. 30	〃	〃	11	<4	64	<4	>64	0
No. 14	I	+3 days	16			<4	< 4	+
No. 15	〃	〃	17			<4	< 4	+
No. 16	J	+3 days	13	<4		<4	< 4	+
No. 17	K	+4 days	14			<4	< 4	0
No. 18	L	+5 days	13			<4	< 4	0
No. 19	M	+7 days	13			<4		0

* cynomolgus monkeys

2. *Relationship between the extent of pathological changes and the level of immunological response*

Studies on the antibody response in monkeys inoculated with measles have already been reported by various workers (Enders, 1956; Mutai, 1964).

Most monkeys with Warthin-Finkeldey giant cells in the lymphatic tissues, still showed no definite rise of antibody titer in the serum, but case No. 24, which revealed specific pathological changes throughout the body, showed an antibody titer of 4 by the neutralization test, though this titer did not imply a high level of immunity. On the other hand, in those animals which showed a marked rise of antibody titer, specific pathological changes were not seen. This relation were also seen in four pairs of monkeys, when each pair was inoculated with equal aliquots of four samples. These animals, showing a rise of titer but no speficic histological changes, were thought to have recovered from the disease, although the initial antiboy titer of <4 just before sample inoculation should be questioned and therefore a subsequent booster effect may be considered.

The above correlations between the existence of pathological changes in monkeys and the immunological responses of the animals provide exact proof that these changes were caused by measles virus itself.

3. *Pathological changes revealed in tissue preparations*

A) *Lymph nodes*

Characteristic changes seen in the lymph nodes are the appearance of socalled Warthin-Finkeldey type giant cells and intranuclear and cytoplasmic inclusion bodies. In addition to these changes, the hypertrophy of reticular cells, degneration of lymphoid cells and reticular cells, the appearance of an area of lymphoid exhaustion and retrogressive changes of the germinal center are considered to be caused by the agent and therefore these histopathological changes are called "Lymphadenitis rubeolae".

1) *Giant cells*

a) *Warthin-Finkeldey cells*

The occurrence of Warthin-Finkeldey type giant cells has been recognized as specific to measles and its histological features are very peculiar. However the histogenesis of these cells is still uncertain, and there has been no histological definition on this type of giant cell.

The typical morphological features of these cells are shown by Figs. 1 and 2, and many different types of giant cells can be seen. Apparently they are formed originally from different kinds of cells, and one type is thought to be formed by agglutination or fusion of small lymphocytes, another to be derived from primitive reticular cells and yet another to be formed by fusion of lymphoblastic cells (Figs. 3, 4, 5, 6 and 7).

Although the giant cells are generally distributed in the cortex as well as the medullary cord, they are often located in the germinal centers. (Fig. 8)

They are variable in size. In the lymph nodes derived from one autopsy case, giant cells having a hundred nuclei in each cell could be seen (Fig. 9), while in lymph nodes obtained from other autopsy cases many small giant cells with three to five nuclei resembling those of small lymphocytes were seen. (Fig.s 10 and 11)

It is interesting that the frequency of appearance of giant cells and their size were found to be very similar in lymph nodes in different sites in the body of individual monkeys.

b) *Reticular giant cells*

As described above, some Warthin-Finkeldey giant cells have been found to have arisen from reticular cells, although many seemed to have originated from lymphoid cells.

In this section typical giant cells derived from reticular cells are described. They were often seen in the cortical area adjacent to the marginal sinus and also in sites which were thought to correspond to retrogressive germinal centers. As reported in the preceding paper (Nii and Kamahora, 1963), they are formed by fusion of reticular cells and contain approximately twenty or fewer nuclei. They could be differentiated by their morphological appearance from so-called Warthin-Finkeldey giant cells originating from lymphoid cells. (Fig.s 12, 13, 14 and 15)

The characteristics of these giant cells is that intranuclear and cytoplasmic inclusion bodies were often detected in them and that their appearance had a close relation to the course of the disease and perhaps to the stage of onset of the immunological response in the body, as described later.

In one case (Monkey No. 13), reticular giant cells with inclusions were disseminated in the lymph nodes of the whole body. (Fig. 16)

c) *Phagocytic giant cells*

Essentially, this type of giant cell may be reticular. It was often found in the intermediate and medullary sinuses and characteristically contained many phagocytic granules. Therefore this type of giant cell seems to be formed by the fusion of many macrophages.

In the lymph node of monkey No.14, phagocytic giant cell of varying appearance were observed. Some contained small phagocytic granules and their nuclei were inlayed throughout the whole cytoplasmic area. This arrangement of nuclei was undoubtedly different from that shown in Langhans' giant cells which appear in the Lymphadenitis in tuberculosis. On the other hand other giant cells had slightly different morphological features; they contained many granules and their nuclei were grouped together in one region of the cytoplasm, and the rounding of their cytoplasm was more or less pronounced (Fig. 17 and 18). Round cytoplasmic masses without any nuclei were also seen. It seems that these phagocytic giant cells with the different appearances described above, are merely due to differences in the stage at which they were observed between their first appearance and their destruction.

d) *Plasmacellular giant cells*

This type of giant cell is thought to be formed by the fusion of plasma cells. It is found in the spleen as well as in the lymph nodes (Fig. 19).

2) *Detection of inclusion bodies in the affected cells in the lymph nodes*

Of the four types of giant cells mentioned above, only the reticular type showed definite inclusion bodies. In this type of giant cell, intranuclear inclusion bodies as well as cytoplasmic inclusion bodies are shown. Inclusions were also found in single cells in the affected area, where exhaustion of lymphoid cells and hypertrophy of reticular cells was seen.

Of 12 monkeys showing pathological changes, 6 monkeys had inclusion bodies in their lymph nodes, although the frequency and the appearance of these varied

in each monkey. Besides giant cells, detection of inclusion bearing cells provides a positive proof of measles virus growth in these tissues.

3) *The mode of appearance of lymphoid giant cells and reticular giant cells*

To avoid confusion in cytological meanings, we used the term "lymphoid giant cell" for the so-called Warthin-Finkeldey cell in this section in distinction to the reticular giant cell. The exact cytological identification of each giant cell is not always easy, but we distinguished four types of giant cell by cytological features. Among these, lymphoid and reticular giant cells were more frequently observed and therefore the appearance and frequency of the latter two kinds of giant cells were studied in correlation with the course of the disease. Dissemination of both types of giant cells in the lymph nodes of the whole body was surveyed in relation to the number of days after the inoculation of specimens until the death of the animals. The results are shown in Table 4.

As the physiological condition of each monkey may differ and the viral dose inoculated into each animal may not be equal, the whole course of the disease varies in each animal. Therefore a slight discrepancy may be found between the results of these and other similar experiments. With these considerations in mind, the following facts are stressed. Lymphoid giant cells disappear after a 10 to 12 day period of infection, which may correspond to the incubation and prodromal stages. On the other hand reticular giant cells persist for much longer. This variation in the cytopathological changes in the lymph nodes during the course of infection may depend on the appearance of the antibody response in the animals. In our experiments, however, the monkeys showing characteristic pathological changes in their bodies were found not to have much antibody in their sera. Therefore it is suggested that a low level of antibody, such that it is still hardly detectable in the serum by the routine neutralization or CF tests, is acting against affection of measles in the lymph nodes.

It is also suggested that degenerative changes of lymphoid giant cells occurs earlier than that of reticular ones.

Quantitative changes in the dissemination of giant cells are also noticed during the course of infection. In the earlier stages of the disease the wide distribution of the two types of giant cell was seen in the lymph nodes throughout the body and also in most microscopic fields of each node, while in the rather later stages (from the 10th to the 12th day after inoculation) only reticular type giant cells are apparent and finally these are found only in restricted areas of certain lymph nodes. This qualitative and quantitative cytological change during the course of the disease suggests that there is an immunological response of the body.

However it must be pointed out that the earliest pathological changes in the lymphatic tissues in the early incubation period were not shown from our autopsy cases.

Table 4. The Mode of the Appearance of Lymphoid and Reticular Giant Cells in the Lymphatic Tissues of Monkeys in Correlation to the Times after Virus Inoculation

number of days after virus inoculation	5 days		7 days		8 days		9 days		12 days		12 days		13 days		14 days		14 days		16 days		17 days	
experimental case number of monkeys	No. 32		No. 24		No. 23		No. 28		No. 2		No. 13		No. 16		No. 1		No. 3		No. 14		No. 15	
type of giant cell	○ lym.	● ret.	lym.	ret.	lym.	ret.	lym.	ret.	lym.	ret.	lym.	ret.	lym.	ret.	lym.	ret.	lym.	ret.	lym.	ret.	lym.	ret.
right profound axillar lymph node	##	+	+	+	##	+	##	—	##	+	—	##	—	—	—	+	—	—	—	##	—	—
left profound axillar lymph node	+	+	+	+	n.t.	n.t.	##	+	##	+	—	##	—	—		+	—	—	—	##	—	—
right profound inguinal lymph node	+	—	+	+	##	+	##	—	##	+	—	+	—	—	—	—	—	+	—	+	—	—
left profound inguinal lymph node	##	—	+	+	##	##	##	—	##	+	—	+	—	—	—	—	—	—	—	+	—	—
mesenterial lymph node at the root of mesenterium	##	+	+	—	##	##	##	+	##	+	—	##	—	+	—	##	—	##	—	##	—	##
mesenterial lymph node in the small intestine	+	+	+	+	##	+	##	—	##	+	—	+	—	—	—	##	n.t.	n.t.	—	—	—	—
peribronchial lymph node	##	+	n.t.	n.t.	##	+	##	—	n.t.	n.t.	—	+	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	—	+
submandibular lymph node	##	+	—	—	##	+	##	+	n.t.	n.t.	—	+	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	—	—
lymphatic tissues of the small intestine	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	##	—	—	—	—	—	—	+	—	—	—	—	n.t.	n.t.
spleen	+	—	—	—	+	—	##	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—

○ .....lymphoid giant cell
● .....reticular giant cell
+～## .....frequency of appearance of giant cells
n.t. .....not tested
— .....giant cells could not be found.

4) *Other pathological changes in lymph nodes*

Hypertrophy of reticular cells as well as exhaustion of lymphatic cells was detected to a greater or lesser degree. Proliferation of macrophages in the marginal sinuses and medullary sinuses was often marked.

In the prodromal stage of the disease, inflammatory cells such as leucocytes, monocytes, erythrocytes and so on were rarely seen in the affected area, although many Warthin-Finkeldey cells were conspicuous. On the other hand, in the later stage of the disease (about 14 days after virus inoculation) infiltration of the above cells was seen in the lesions, and as already mentioned, lymphoid giant cells were not found in these lymph nodes, only reticular giant cells being seen. Therefore the latter lesions seemingly resembled the histological changes of granulomatous inflammation and thus "inflammation" in the usual sense occurs after the onset of destructive changes of infected cells.

Infiltration of plasma cells was sometimes seen, and as will be described later, this seemed to have a close relation with the immunological response of the whole body.

5) *Healing of measles lesions in lymph nodes*

Monkeys as well as humans usually recover from the disease. Therefore pathological changes caused by measles must disappear in the convalescent stage of the disease. The following findings may show one aspect of the healing process in the lymph nodes.

As described earlier, Warthin-Finkeldey cells decrease in number from about the 12th day after virus inoculation due to the onset of the antibody response, which suppresses further invasion of the agent, and due to the destruction of the preesisting giant cells themselves. When these cells degenerate in the surrounding lymph containing antibody, antigenic substances released from them will combine with antibody and these complexes as well as the cell fragments will be removed by the action of macrophages.

Other biological changes which may have a relation with the healing process in affected lymph nodes were observed. Focal lesions, which consist of several reticular giant cells as well as moderate infiltration of inflammatory cells were often found to be surrounded by a layer of fibroblasts, and in some of them the latter fibroblastic layers were seen to be fused with the capsule of the lymph node. Therefore, at first sight, this lesion was mistakenly thought to be situated in the capsule wall. This proliferation of fibrolbasts plays a role in protecting the unaffected lymphatic area from the lesion.

Another of the most peculiar findings in lymph nodes was caseous lesions demarcated by a thick wall of the fibroblasts (case No. 16). In Figs. 20 and 21, a large round lesion is seen and the caseous masses, which apparently consist of degenerated syncytial cell debris, remain in the central empty area. The thick fibroblastic mass separates the lesion distinctly from the rest of the lymph node.

6) *Effects of measles virus infection on germinal centers in lymph nodes*

It is well known that a cyclic appearance and disappearance of lymphatic nodules (secondary nodules) occurs in lymphatic tissues, showing lymphocytopoiesis as well as other biological activities. The features of such nodules in the lymph nodes affected with measles were investigated. The existence of germinal centers as well as mitotic figures was examined microscopically in many lymph nodes of the body. It was found that the appearance of germinal centers was much influenced with the intensity of the pathological changes. In lymph nodes where specific cytological changes were seen, germinal centers were absent or appeared in an inactive phase and in such cases hypertrophy as well as degeneration of reticular cells were seen.

As reported by other authors and as seen in some of our autopsy cases, Warthin-Finkeldey cells were more frequently situated in the germinal centers than in the primary nodules. In these affected germinal centers, few mitotic figures were found. The more frequent appearance of giant cells in these regions indicates a higher susceptibility of the cells to measles virus. It is suggested that on infection with virus the preexisting undifferentiated cells in the germinal centers may easily become affected. The inhibition of the appearance of lymphatic nodules (or germinal centers) in severe "lymphadenitis rubeolae" seemed to be caused by an unbalance in the regulation of the general biological mechanism of the body, and not merely by a direct effect of infection of measles virus on each cell in the nodule. It is interesting to see that they reappear in the convalescent stage, when pathological changes in the lymph nodes and in other parts of the body become less or disappear.

B) *Respiratory organs*

Syncytial formation as well as the appearance of inclusion bodies in the mucosal epithelium in the trachea, bronchi and bronchioli were usually observed, accompanied by systematic changes in the lymph nodes throughout the body. Degenerating syncytial cell masses were also observed in the lumina of these organs. In these detached cells cellular cilia were often seen (Figs. 22 and 23).

Nuclei in the syncytia are usually grouped together in one area of the cytoplasm and looked like a mass of grapes. Halo formation of intranuclear inclusion bodies was not marked and "full inclusion bodies", that is dense masses staining purplish pink, which occupied the whole of the nuclei, were usually observed (Fig. 24).

Affection of tracheal and bronchial glands was also noticed (Fig. 25). In subepithelial lymphatic nodules, Warthin-Finkeldey cells were often observed, but it must be noted that extensive phathological changes in the mucosal epithelium are not always accompanied by the formation of specific giant cells in the adjacent lymphatic nodules. This has also been noted by other authors (Davidsohn *et al.*, 1932).

Parenchymal cells of the lungs often revealed interstitial pneumonitis and proliferation of alveolar cells and septal cells was seen. In case No, 23 , an extensive interstitial pneumonitis was seen, and severe pathological changes in the mucosal epithelium of the respiratory tract were also observed (Fig. 26). In the lesions of such pneumonitis revealed at the earlier stage of the disease, infiltrative changes of inflammatory cells were hardly detected, except in the haemorrhagic lesions of the lung.

In some cases syncytial formation in the parencyhma was also observed. In case No. 1 changes in the mucosa of the trachea, bronchi and bronchioli were very mild, while many giant cells were detected in the parenchyma, and this seemed to correspond to the "giant cell pneumonia" referred to as Hecht's disease (Figs. 27, 28 and 29). Giant cell lesions were also detected in the lungs in case Nos. 3, 16, 23 and 24, and these were accompanied by infiltration of neutrophile or eosinophile leucocytes and other mononuclear cells in greater or lesser degree. In our experiments, however, intranuclear and cytoplasmic inclusions in the giant cells were not definite. These findings show that the pathological change, which is essentially the same as "giant cell pneumonia", occurs frequently in animals infected with measles, although its intensity may vary in each animal.

Other changes which suggest secondary bacterial infection were also seen in the lungs.

C) *Small intestine*

Lymphoid giant cells as well as reticular giant cells were observed in solitary lymph nodes and Peyer's patches of the ileum (Fig. 30). In the lamina propria of the villi, small giant cells containing three or four nuclei were detected and degenerative changes of cells in the stroma were also seen (Fig. 31). These changes were the same as those in other lymphatic tissues of the animal, suggesting that almost the same stages of affection occurred in the lymphatic tissues throughout the body. In case No. 1 reticular giant cells were observed in the submucous lymphatic nodule. These changes in morphological appearance of giant cells from lymphoid giant cells to reticular giant cells through the course of the disease were found to be true in all lymphatic tissues.

D) *Liver*

Pathological changes in the liver caused by measles infection have only been reported by Shultze (1943). In our experiments lesions in the liver were revealed in three cases (case Nos. 1, 2 and 3). It is interesting to see that in this organ also two kinds of giant cells could be seen. In case No. 2, Warthin-Finkeldey type giant cells were seen (Fig. 32). In the lesions in the intermediate or central portion of the lobules, many mononuclear cells, a few giant cells and a few plasma cells were seen. The cellular origin of the Warthin-Finkeldey cells in these areas is quite uncertain, and at least three kinds of cells, *i. e.* undifferentiated lining cell, Kupffer cell and lymphocyte derived from the blood may give origin to these cells. Degene-

ration of Kupffer cells and marked proliferation of undifferentiated cells in the lesions was distinct. Dilatation of sinusoids was marked. Another type of giant cells resembling reticular giant cells in lymphatic tissues was also seen (Figs. 33, 34 and 35). The origin of the latter cells is also uncertain. In lesions where reticular cell type giant cells were seen, infiltration of inflammatory cells such as polymorphonuclear leucocytes, plasma cells and erythrocytes was very pronounced.

E) *Spleen*

In the white pulp of the spleen Warthin-Finkeldey cells were very apparent, their appearance being correlated with their appearance in other lymphatic tissues of the body (Fig. 36), but typical reticular giant cells could not be seen in any autopsy cases. Occasionally small giant cells originating from plasma cells were seen in Billroth cords (Fig. 19).

One of the most interesting histological changes in the spleen may be the development of the plasma cell reaction. It is not easy to see the dynamic aspect of the reaction quantitatively on infection with measles, but generally speaking the appearance of plasma cells was related with the intensity of specific pathological changes revealed in the body. At the stage of the disease when the animal was severely affected, showing a number of Warthin-Finkeldey cells in the lymphatic tissues or many syncytial cells in the respiratory tract, the infiltration of plasma cells in the spleen was only moderate, while this was most pronounced at the stages of the disease when such pathological changes had just disappeared or only few if any remained (Fig. 37). However, this response of plasma cells seemed to subside after the monkeys showed a high level of antibody. The cells disappeared gradually, in spite of the persistence of a high grade of immunity for a longer period. The response of germinal centers, *i. e.* the appearance of active centers in the spleen as well as in the other lymphatic tissues, occurs only a little later than the marked appearance of plasma cells. These facts suggest that plasma cells, as well as lymphoid cells in the lymphatic nodules (*i. e.* secondary nodules) may play an important role in the defense of the measles-infected animals.

F) *Kidney and urinary bladder*

In the kidney, specific pathological changes were not observed. However, among our autopsy cases in the severely affected monkeys exudative glomerulitis was shown and this seemingly resembled the histological changes reported by Bolande (1961) in the glomerulus of a human case of measles. In the kidney of case No. 32, a moderate infiltration of plasma cells was recognized (Fig. 38).

Of the bladders tested, two (those of case No. 28 and No. 24) showed definite specific pathological changes. In some places in the mucosal epithelium, syncytial formation was noticed. Some syncytia contained about ten nuclei grouped together in one region of the cytoplasm (Figs. 39 and 40), while in other syncytia, there was a distorted appearance of all nuclei but the nuclei did not have an abnormal arrangement. In the urinary bladder of case No. 24, several small giant cells were

found in regions between the epithelial layer and the lamina propria, where infiltration of many mononuclear cells was also marked, but the cellular origin of these giant cells was uncertain.

Giant cells were also observed in the bladder of cases No. 2 and No. 13 but their features were not so characteristic as those described above.

Cytoplasmic inclusions just like those reported by Bolande (1961) were often found, but they could not be considered as specific to measles (cases Nos. 4 and 17) (Melamed, 1961).

G) *The mucous membrane of the cheek*

In the subepithelial layer of the buccal membrane, syncytia were found and infiltration of lymphoid cells was also seen. Hypertrophy of epithelial cells in these lesions was marked (Figs. 41 and 42). However these lesions were rarely found (case No. 23).

DISCUSSION

Since the first description of giant cells in measles by Ciaccio (1910) and Alagna (1911) a great number of similar findings have been made. These are summarized in Table 5.

From these pathological findings, the major role played by mesenchymal cells in the growth of the virus during the incubation period has been suggested (Burnet, 1959; Robbins, 1962). However the pathogenesis of measles is not yet completely understood and there have been only a few experimental studies on this problem.

Sergiev *et al.* carried out parallel investigations on the clinical, virological and pathological features of the disease and showed that virus growth occurred in the reticulo-endothelial and lymphoid tissues of monkeys in the early period after virus inoculation and later, morphological changes occurred in the mucous membranes which were of haematogeneous origin or were due to the spread of the pathological process from lymph nodes to the adjacent epithelial linings of mucous membranes.

Our experiments also showed the extensive dissemination of Warthin-Finkeldey cells in the lymph nodes throughout the body of monkeys inoculated with measles. However, the earliest pathological changes in the animals were not studied because animals were not killed during the early incubation period. In our work, the appearance of Warthin-Finkeldey cells in the lymphatic tissues was always accompanied by pathological changes in the respiratory tract; this was shown even in a monkey that was killed on the 5th day after inoculation with the specimen. Therefore, it is unknown whether pathological changes spread from lymph nodes to the adjacent epithelial lining of the mucosal membranes, as suggested by Sergiev *et al.* (1960).

We could find definite pathological changes in the spleen, liver, small intestine, the mucosal epithelium of the oral cavity, the respiratory tract, urinary bladder and

Table 5. Distribution of Measles Giant Cells in the Lymphatic Tissues in Correlation with the Stage of the Disease *

Year	Author	Host and numbers of Cases	Location of giant cells	Stage of disease
1910	Ciaccio	human, 8 cases	tonsil	?
1911	Alagna	the same 8 cases as above	〃	〃
1931	Warthin	human (four cases) case 1	〃	2 days before Koplik's spot and 4 days before rash
		case 2	〃	3 days before Koplik's spot and 5 days before a fully developed attack of measles
		case 3	〃	36 hours before rash
		case 4	〃	day before rash
1932	Finkeldey	human (two cases) case 1	〃	3 days before rash
		case 2	appendix	2 days before rash
1932	Herzberg	human (one case)	appendix	4 days before rash
1932	Davidsohn and Mora	human (four cases) case 1	appendix	3 days before rash
		case 2	〃 very small number of giant cells	2 days after rash
		case 3	〃	2 days before rash
		case 4	not found in appendix	9 days after rash
1933	Fischer	human (one case)	appendix	1½ days before rash
1933	Schultze	human (one case)	appendix	2 days before rash
1935	Hathaway	human (one case)	spleen, peribronchial, retroperitoneal and peripancreatic lymph nodes, but lymphoid tissue of the gastro-intestinal tract failed to show giant cells	no rash, no Koplik's spot (This case was exposed to measles)
1937	Bullowa et al.	human (three cases)	found in the mucosal and submucosal layers of the appendix,	preeruptive stage
		human (two cases)	No giant cell was noted in any of the sections of the appendixes	during the eruptive or posteruptive stage
1937	Gräff	human (one case)	tonsil and cervical lymph node, but none in the appendix or other organs	no rash, no Koplik's spot, died 15 days after exposure
1937	Wegelin	human (three cases) case 1	appendix	4 days before rash
		case 2	〃	3 days before rash
		case 3	〃	rash appeared on the day of operation
1938	Masugi and Minami	human (one case)	not found in spleen and lymph node, (but found in bronchus, pharynx, oral cavity, oesophagus and salivary gland)	4 days after rash
1939	Semsroth	human (one case)	paratine tonsils, peribronchial and hilar lymph nodes, spleen, thymus, (bronchus and bronchial gland)	Koplik's spot was not found and rash was also not observed, died four days after exposure to a patient with measles.
1940	Stryker	human (one case)	tracheobronchial lymph node, interstitial connective tissue about the lymph node and mucous glands, spleen, lymphatic tissues of the ileum (bronchial mucous glands, pulmonary alveoli, bronchi)	The patient has a history of contact with measles
1941	Gordon and Knighton	monkey (at least nine experimental cases)	many lymph nodes in the body	during the third day and the 10th day after virus inoculation
1943	Schultze	human case 1	tonsil, cervical lymph node, thymus, hilar lymph node, spleen, (lung, spleen, liver, meninges)	prodromal stage
		case 2	tonsil, cervical lymph node, thymus, hilar lymph node, spleen	〃
1944	Mulligan	human (one case)	appendix	3 days before rash
1945	Corbett	human (one case)	peribronchial lymphoid tissue, thymus, spleen, lymph nodes of abdomen and thorax, Peyer's patches, colon (bronchi and bronchioles)	Death on day rash appeared
1948	Simon and Ballon	human (four cases) case 1	appendix	3 days before rash
		case 2	〃	4 days before rash
		case 3	〃	5 days before rash
		case 4	〃	5 days before rash
1950	Bunting	human (one case)	thymus	one day after rash
1954	Taniguchi et al.	monkey (many experimental cases)	lymph nodes throughout the body (many other tissues and organs)	from the 8 th day to the 16 th day of infection
1958	Roberts and Bain	human (three cases) case 1	cervical, mesenteric and pulmonary hilar lymph glands, spleen, appendix, small intestine, solitary lymph follicles of the large intestine. (buccal mucosa, alveolar epithelium, bronchial epithelium)	Koplik's spot appeared, but no rash
		case 2	spleen, smallintestinal, mesenteric paraaortic, paratracheal, and hilar lymph glands and appendix	Neither Koplik's spot nor rash appeared
		case 3	spleen and lymph nodes	3 days after rash
1958	Sherman and Ruckle	human (four cases) case 1	lymphnodes, tonsil, thymus, spleen, intestinal lymphoid tissue (respiratory mucosa)	Neither Koplik's spot, nor rash appeared.
		case 2	(urinary bladder) not found in lymphatic tissues	8 days after rash
		case 3	not found in lymphatic tissues	4 days after rash
		case 4	not found in lymphatic tissues	3 days after rash
1960	Sergiev et al.	monkey (many experimental cases)	reticuloendothelial and lymphoid tissue. (tracheal and bronchial mucosa)	detected as early as the 3rd day of experimental infection and seen during the second period (from the 5th-6th to the 9th-11th day)
1963	Nii and Kamahora	monkey (many experimental cases)	lymph nodes throughout the body (several other tissues and organs)	from the 5th day to the 17th day after virus inoculation

In this table the same classification as made by Dr. Corbett (1945) is adopted.
Pathological changes in other tissues and organs are shown in Parenthesis.

in the lymph nodes throughout the body. These findings show that measles causes systematic changes in many organs and tissues in the body of monkeys and from these it is understood that measles is a serious disease among human children.

In the epithelium of the respiratory tract the same histological changes were observed as reported by many other authors. Recently Mottet *et al.* (1961) have found exfoliated measles giant cells in nasal secretions of patients, and Nagahama *et al.*, (1963) have recognized measles antigens in nasopharyngeal smears using the fluorescent antibody technique. However, it must be noted that multinucleated epithelial cells appear in the respiratory tract in many other diseases also (pneumonia, tuberculosis and carcinoma), as shown by cytological examination of bronchial washings (Hoch-Ligeti *et al.*, 1963).

The detection of giant cells in the epithelium of the urinary bladder is in good accordance with the studies of Hinuma *et al.* (1962), in which exfoliated cells in the urine with viral antigen of measles were found by the fluorescent antibody technique. Our results also agree with the data by Gresser (1960), who isolated measles virus from the urine of patients.

It is interesting from the epidemiological point of view to study the infectivity of nasal secretions of patients in relation to the time of onset of a rash. Monkeys could be infected with specimens obtained from patients within 3 days after the onset of a rash, as well as from patients who showed Koplik's spots but no rash. This is in good accordance with the findings by Nagahama *et al.* (1963) who reported that viral antigen was detected in nasopharyngeal secretions from patients within 3 days after the onset of a rash. On the other hand specimens lost their infectivity to monkeys if they were obtained from human cases 4 days or later after the onset of a rash. It is also interesting to compare this finding with the data on isolation of virus made by Ruckle *et al.* (1957), who reported that virus could be isolated only from those subjects whose blood specimens and throat secretions were collected between 48 hours before and 32 hours after the onset of a rash.

The formation of giant cells in the lymph nodes has been found to be induced by other viruses besides measles (Tomlinson, 1935: Sommers *et al.*, 1951) and this pathological finding may be considered as one of the characteristic changes caused by viruses. However, such peculiar morphological features and appearances as Warthin-Finkeldey cells do not seem to be found in lymphadenitis induced by other viruses.

We would like to discuss four problems in relation to giant cell formation in measles. The first is "What kinds of cells in the lymph nodes form giant cells?" In spite of the existence of many reports concerning Warthin-Finkeldey cells, opinions differ on their cellular origin. In Table 6 the histogenesis of giant cells reported by many authors is summarized. The different types of measles giant cells were also reviewed by Lennert (1961) and they were differentiated as follows. One type is seemingly derived from reticular cells (reticuläre Riesenzellen), another is the lymphoid giant cell which is formed by the fusion of lymphocytes or lymphoblastic cells (Riesenzellen mit lymphoiden Kernen) and the third is the plasma

Table 6. Cytological Geneses of Measles Giant Cells (Warthin Finkeldey Cells) Presented by Earlier Authors

Authors	Year	Possible geneses presented by authors
Warthin	1931	◦ by amitotic nuclear divisions in hyperchromatic cells resembling lymphocytes, or from cells of lymphoblast type.
Finkeldey	1932	◦ a fusion of lymphocytes or derived from reticulum or from a kind of undifferentiated mesenchymal cell.
Herzberg	1932	◦ made up apparently of aggregates or coalescing cells of lymphoid origin.
Davidsohn and Mora	1932	◦ strongly suggesting lymphocytic or plasma cell.
Hathaway	1935	◦ from the reticulo-endothelial cells of the sinuses.
Semsroth	1939	◦ amitotic division of plasma cells.
Stryker	1940	◦ Giant cells in the lung were differentiated into four types. 1) fused epithelium 2) from phagocytosis of fat cells 3) megakaryocytes 4) a fourth type ?
Gordon and Knighton	1941	◦ derived from monocytes which had multiplied by amitotic division.
Mulligan	1944	◦ The result of polynuclear abnormal development of stem cells was in parallel with the mononuclear normal development of the lymphocyte from stem cells.
Simon and Ballon	1948	◦ by fusion of lymphocytes.
Bunting	1950	◦ Origin of smaller giant cells···macrophages loaded with nuclei of lymphocytes which were attacked by the virus.
Roberts and Bain	1958	◦ Nuclei of giant cells are very similar in appearance to those of lymphocytes.
Sherman and Ruckle	1958	◦ Origin of some of the smaller giant cells···by phagocytosis of small round cells resembling lymphocytes by macrophages. The larger giant cells could not be proved to be of phagocytic origin.

cell type (Plasmacelluläre Riesenzellen). These preceding reports have shown that Warthin-Finkeldey cells vary in appearance and a variety of cells participate in their formation.

Although four types of giant cells were differentiated by appearance in our experiments, their exact differentiation was not very easy. To clarify this point silver staining of the lymph nodes was sometimes carried out (Figs. 44 and 45), but even by this method the identification of cells participating in the formation of giant cells was not exact. In preparations stained with silver it was found that owing to severe pathological changes of affected cells, the original organization of each cell in giant cells seemed to be distorted and reticular fibers were often seen to be fragmented. Although there was difficulty in such cytological determinations, some giant cells seemed to be formed by the agglutination and fusion of different kinds of cells. For example reticular cells and lymphoid cells fused together to form a giant cell. Thus, it is suggested that a variety of cells in the lymph

nodes have the ability to form giant cells in measles.

The second problem is "By what mechanism are giant cells formed ?" As suggested by many other authors, three mechanisms are possible. One is the fusion of cells, another is amitotic nuclear division of infected cells, and a third is phagocytosis of affected cells or nuclei by macrophages. *In vitro* experiments have supported the first possibility. The data by Toyoshima *et al.* (1960) especially showed that syncytia were formed by a massive dose of measles virus inactivated by ultraviolet light. A high dose of virus may cause giant cell formation in the lymph nodes regardless of intracellular virus growth, and this means that infected cells can fuse with neighbouring cells or recruit other non-virus producing cells by releasing virus or similar substances at the cellular membranes of these cells. On the other hand a few smaller giant cells may appear as the result of the second or third mechanism.

The third problem is "Is there any relation between giant cell formation and virus growth in the lymph nodes?" In *in vitro* experiments on measles Enders *et al.* (1954) showed the existence of intranuclear and cytoplasmic inclusion bodies in infected cells. Therefore the detection of inclusions in cells presents a proof of the growth of the virus in them. Although it has been suggested that lymphatic tissues are the sites for virus growth, no one has shown inclusion-bearing cells in the lymph nodes. However, in the preceding paper Nii and Kamahora (1963) reported the detection of giant cells bearing inclusions in the lymph nodes of monkeys that had been experimentally infected with measles. Further observations have been presented in this report. Moreover recent work using the fluorecsent antibody technique showed the definite existence of measles viral antigen in the lymph nodes of infected monkeys (unpublished). These data, as well as the detection of inclusion bodies, indicate that virus growth undoubtedly occurs in lymphatic tissues.

Cytologically such inclusions, however, have been shown only in the reticular giant cells and not in the so-called Warthin-Finkeldey cells (or lymphoid giant cells). Therefore it is uncertain whether lymphoid giant cells or plasma cell type giant cells can support virus growth. Presumably the ability of cells to support virus growth depends much upon the extent of cellular differentiation or upon functions correlated with their organization *in vivo*. Undifferentiated lymphoid cells at least are supposed to be susceptible to measles virus, because a higher frequency

Table 7. Relation between the Appearance of Giant Cells and the Sites of Germinal Centers in the Lymphatic Tissues.

Authors who found giant cells more frequently at the germinal centers.	Authors who did not find giant cells at the germinal centers, but in the surrounding lymphoid tissues
Ciaccio (1910), Warthin (1931), Finkeldey (1932), Herzberg (1932), Davidsohn and Mora (1932), Wegelin (1937), Semsroth (1939), Mulligan (1944), Simon and Ballon (1948), Roberts and Bain (1958)	Hathaway (1935)

of appearance of Warthin-Finkeldey cells was seen in the portions related to the germinal centers, as had been shown previously by many workers (Table 7). Among the great number of lymphoid giant cells seen in histological preparations of lymphatic tissues in our autopsy cases, only one was found to contain a definite intranuclear inclusion body (Fig. 46). However even in this exceptional case it was considered that the giant cell was formed by the fusion of one reticular cell having an inclusion and other lymphoid cells. This great difficulty in detecting inclusions in lymphoid giant cells may be explained by one of the following two possibilities or by both. One possibility is that some of the lymphoid cells such as small lymphocytes do not participate essentially in virus growth, but they can join in the formation of giant cells caused by a massive dose of measles virus released by other virus producing cells. The second possibility is that some other lymphoid cells such as lymphoblasts begin to produce mature virus after infection and form giant cells by themselves but they begin to degenerate before typical inclusion bodies are formed in them. Perhaps the two phenomena occur in the affected lymph nodes.

Recent fluorescent antibody studies showed that many single cells in lymph nodes contain viral antigens (unpublished data). Therefore, these single cells also participate in the formation of virus regardless of the concomitant appearance of giant cells.

The last problem is "Why do lymphoid type of giant cells disappear earlier than reticular giant cells?" Generally it is said that Warthin-Finkeldey cells can be detected in the prodromal stages, but not during or after the eruptive stage. The correlation of the appearance of the giant cells with the stage of the disease is shown in Table 4. After the appearance of antibody in the blood and lymph, further infection of cells is inhibited and the preaffected cells degenerate and disappear. One reason for this phenomenon may be the early destruction of lymphoid giant cells after their first interaction with the virus. If some lymphoid cells do not participate essentially in producing virus but only join in the formation of giant cells, neutralization of virus by antibody would block the further formation of new lymphoid giant cells and then reticular giant cells would remain by recruiting noninfected neighbouring cells even in the presence of the antibody.

It is well known that measles sometimes develops into pneumonia (Steinhaus, 1901;Kohn *et al.*, 1933). Many reports have also been presented on the correlation of giant cell pneumonia with the disease. They are summarized in Table 8. Extensive virological studies on this type of pneumonia have been made by Enders and his colleagues, who isolated the so-called giant cell pneumonia virus from such cases and identified it as measles virus by comparative immunological studies between the pneumonia virus and the measles virus that had already been isolated and identified. (Enders *et al.*, 1956, 1959; Cheatham *et al.*, 1958; Mitus *et al.*, 1959).

However, pathologically, giant cell pneumonia may have other etiologies and is not specific for measles. This is shown in Table 9. It is said that in giant

Table 8. Reports of Giant Cell Pneumonia Caused by Measles

Authors	Year of publication	detection of inclusion bodies	virus isolation
Kromayer	1889	n. d. *	n. a. ⁺
Hecht	1910	n. d.	n. a.
Denton	1925	n. d.	n. a.
Goodpasture et al.	1939	nuclear inclusions	n. a.
Milles	1945	n. d.	n. a.
Pinkerton et al.	1945	cytoplasmic and nuclear inclusions	n. a.
Adams et al.	1956	cytoplasmic and nuclear inclusions	n. a.
Enders et al.	1956	n. d.	+
Roberts et al.	1958	cytoplasmic inclusions	n. a.
Adams et al.	1958	cytoplasmic and nuclear inclusions	n. a.
Sherman et al.	1958	cytoplasmic and nuclear inclusions	n. a.
MacCarthy et al.	1958	n. d.	+
Cheatham et al.	1958	n. d.	+
Enders et al.	1959	inclusions	+
Mitus et al.	1959	nuclear inclusions	+

n. d. *.....not described n. a. ⁺.....not attempted
+ .....succeeded in virus isolation

Table 9. Reports of Giant Cell Pneumonias Caused by Other Agents

Authors	Year of publication	Comments on etiology
Friedländer	1873	found in the pneumonic process caused by cutting the recurrent laryngeal nerves
Karsner et al.	1913	due to a variety of causes and not characteristic of the pneumonias following measles and pertussis
Moore et al.	1930	no specific cause
Chown	1939	vitamin A deficiency
Adams et al	1941	Viral nature was considered (cytoplasmic inclusions were found)
Weller	1952	Virus origin was considered (cytoplasmic inclusions were found)
Wolman et al.	1952	due to virus ? (cytoplasmic inclusions were found)

cell pneumonia the measles rash is poorly developed suggesting some inadequacy in the immunological process.

In our autopsy cases interstitial pneumonitis was observed to a greater or less degree in severely affected monkeys and syncytial formation by parenchymal cells was also detected. Therefore, it is considered that essentially the same pathological changes which are seen in the typical giant cell pneumonia may be found more or less in most cases of measles, especially in the later period of the disease. As suggested by other authors, a defect in the normal immunological

response of the animals may have a close relation to the appearance of giant cell pneumonia in them. Our three autopsy cases at least have shown lesions in the lungs where many giant cells were detected and these pathological changes may correspond to Hecht's giant cell pneumonia, but the reason for its induction is still unknown.

Proliferation of plasma cells in the spleen as well as the appearance of active centers in the lymphatic tissues seemed to be closely related to the course of infection. Infiltration of plasma cells into the spleen was pronounced in the stage corresponding to the convalescent period of the disease. The monkeys in this stage showed no specific pathological changes or only mild ones. The reaction of plasma cells after infection with antigen has recently been reported by Langevoort (1963). Their dynamic response on infection with measles virus seems to occur essentially in the same manner as described by the author.

Two aspects of the pathology of the virus infection in animals are understood. The first is the direct interaction between virus and host cells. In measles infection, the appearance of specific giant cells as well as inclusion bodies is seen. The second aspect is the general inflammatory changes and in measles this type of histological change is observed after the onset of destruction of infected cells and also after the appearance of antibody. Proliferation of fibroblasts demarcating measles lesions in the lymph nodes is recognized as a typical feature of the inflammatory changes and some giant cells may arise as the result of such inflammatory changes.

To understand more of the pathology of measles it seems necessary to find the exact sites of virus growth in the body other than the lymphatic tissues and respiratory tract and the course of infection in them during the disease, and from these consideration studies by the fluorescent antibody technique should show more of the pathology of measles.

ADDENDUM

The above studies were performed by inoculating epidemiological virus (or wild virus) isolated from human cases into monkeys. As described in Materials and Methods some of the monkeys used has already suffered from the disease before these experiments. It is generally known that sometimes monkeys with no measles antibody upon arrival in the laboratory are found to have antibody later (Ruckle, 1956; Peebles *et al.*, 1957). Therefore the possibility of their natural infection just before arrival in the laboratory or just before the beginning of experiments cannot be excluded.

The following case was found to have been naturally affected, although the route of infection is unknown.

One cynomolgus monkey (case No. 43) was designed to be used in an experiment to see the biological effect of live attenuated measles vaccine on monkeys. However, its serum just before inoculation with the vaccine virus was found to have a neutralizing antibody titer of >5. The animal was killed two days after inocula-

tion with the vaccine. The exact antibody titer of the serum just before death could not be determined, owing to accidental contamination during the test in tissue cultures, but parallel tests *in vitro* suggested that the serum contained a definite antibody titer.

Histologically, dissemination of Warthin-Finkeldey cells was shown in the lymphatic tissues, and reticular giant cells with typical intranuclear inclusion bodies were clearly seen in the mesenterial lymph nodes (Fig. 43). In the lungs typical pneumonitis accompanied by syncytial formation in the bronchial epithelium was also observed.

These findings indicate that the monkey had been naturally infected approximately ten days before its arrival in this institute, but it is unknown whether the agent which caused the disease in case No. 43, was the same epidemiological type of measles as that prevailing in human beings, or whether it originated from MINIA (Monkey-Intra-Nuclear-Inclusion-Agent), that may be ecologically maintained among monkeys and cannot be differentiated from human measles immunologically (Ruckle, 1958a, 1958b). Such problems modify and complicate experimental results on monkeys, but it is necessary to consider these facts to clarify the pathology of measles in animals.

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EXPLANATION OF FIGURES

Fig. 1. Extensive pathological changes characteristic of measles in the mesenterial lymph node. Syncytia seemingly formed by reticular cells and primitive lymphoid cells are shown, and a grape-like assemblage of lymphocytic cells are also seen.
Case No. 23, ×520, H-E stain

Fig. 2. Warthin-Finkeldey cells in the paratracheal lymph node.
Case No. 23, ×2000, H-E stain

Fig. 3. A giant cell seemingly formed by lymphocytes.
The mesenterial lymph node of case No. 28. ×1440, H-E stain

Fig. 4. A lymphoid type of giant cell in the paratracheal lymph node.
Degenerative changes of its nuclei are shown. Case No. 23, ×1440, H-E stain

Fig. 5. A Warthin-Finkeldey cell, which consists of a grape-like assemblage of lymphocytic cells is shown.
The mesenterial lymph node of Case No. 28, ×1440, H-E stain

Fig. 6. A part of Fig. 1 under higher magnification.
A grape like assemblage of lymphocytes and syncytial formation of reticular cells are shown.
The mesenterial lymph node of Case No. 23, ×1440, H-E stain

Fig. 7. A Warthin-Finkeldey cell seemingly formed by the fusion of primitive reticular cells.
The paratracheal lymph node of Case No. 23, ×2500, H-E stain

Fig. 8. Several Warthin-Finkeldey cells at the germinal center in the mesenterial lymph nodes.
Case No. 2, ×720, H-E stain

Fig. 9. A large Warthin-Finkeldey cell, seemingly derived from undifferentiated lymphoid cells, just inside the edge of the germinal center.
It contains about a hundred nuclei. Case No. 2, ×1440, H-E stain

Fig. 10. Two small Warthin-Finkeldey cells in the mesenterial lymph node. One is at the marginal sinus and the other is in the cortex. Each has only three or four nuclei.
Case No. 28, ×1440, H-E stain

Fig. 11. One of the Warthin-Finkeldey cells shown in Fig. 10 under higher magnification. This is in the cortex of the lymph node.
The mesenterial lymph node of ease No. 28, ×3600, H-E stain

Fig. 12. Reticular giant cells in the mesenterial lymph node.
Case No. 14, ×1440, H-E stain

Fig. 13. A focal lesion with many reticular giant cells and a slight extent of infiltration of inflammatory cells. The lymph node is at the root of the mesenterium.
Case No. 16, ×360, H-E stain

Fig. 14. A part of Fig. 13 under higher magnification.
Reticular giant cells and moderate infiltration of leucocytes are shown.
Case No. 16, ×1440, H-E stain

Fig. 15. A focal lesion, consisting of many reticular giant cells and infiltration of inflammatory cells.
The mesenterial lymph node of Case No. 14, ×360, H-E stain

Fig. 16. Two reticular giant cells with intranuclear inclusion bodies as well as cytoplasmic inclusion bodies in the mesenterial lymph node at the root of the mesenterium.
Case No. 13, ×2000, H-E stain

Fig. 17. Many phagocytic giant cells in the right axillar lymph node.
Case No. 14, ×360, H-E stain

Fig. 18. Two phagocytic giant cells with many granules. In each, only two or three nuclei are shown.
The right axillar lymph node of Case No. 14, ×1440, H-E stain

Fig. 19. A plasmacellular giant cell in the cord of Billroth of the spleen.
Case No. 2, ×2500

Fig. 20. A round caseous lesion surrounded by a thick layer of fibroblasts. Two large amorphous degenerated cell masses are in the caseous portion.
The mesenterial lymph node, Case No. 15, ×140, H-E stain

Fig. 21. A part of the caseous lesion in the mesenterial lymph node. An amorphous degenerating cell mass and small cell debris are surrounded by a thick wall of fibroblasts.
Case No. 15, ×360, H-E stain

Fig. 22. Detachment of many syncytia in the lumina of the bronchus.
Case No. 32, ×360, H-E stain

Fig. 23. Syncytial formation and agglutination of nuclei at the mucous membrane of the trachea.
Case No. 23, ×1440, H-E stain

Fig. 24. Exfoliated syncytia in the lumina of the bronchus.
Nuclear assemblage in one part of the syncytium is shown. All nuclei have inclusions.
Case No. 32, ×1440, H-E stain

Fig. 25. Characteristic syncytial formation in the bronchial gland.
Case No. 23, ×1440, H-E stain

Fig. 26. Many epithelial giant cells as well as interstitial pneumonitis are shown. The proliferation of the parenchymal cells of the lung is remarkable.
Case No. 23, ×360, H-E stain

Figs. 27, 28. Syncytial formation and cellular proliferation in the parenchyma of the lung.
Case No. 1, ×360, H-E stain

Fig. 29. A part of Fig. 27 under higher magnification.
The lung of Case No. 1, ×1440, H-E stain

Fig. 30. A warthin-Finkeldey cell in Peyer's patch of the small intestine.
Case No. 2, ×360, H-E stain

Fig. 31. A small Warthin-Finkeldey cell and degenerating cells in the lamina propria of the small intestine.
Case No. 2, ×1440, H-E stain

Fig. 32. A Warthin-Finkeldey cell in the sinusoid of the liver.
Dilatation of the sinusoid is remarkable.
Case No. 2, ×2500, H-E stain

Fig. 33. A focal lesion in the liver; Giant cells and inflammatory cells are shown.
Case No. 1, ×360, H-E stain

Fig. 34. A focal lesion at the intermediate portion of the liver. Giant cells and many inflammatory cells are shown.
Case No. 1, ×360, H-E stain

Fig. 35. Giant cells at a focal lesion in the liver
A moderate extent of infiltration of polymorphonuclear leucocytes is also shown.
Case No. 1, ×1440, H-E stain

Fig. 36. A Warthin-Finkeldey cell in the white pulp of the spleen.
Case No. 32, ×360, H-E stain

Fig. 37. Proliferation of plasma cells in the cord of Billroth.
Case No. 14, ×2000, H-E stain

Fig. 38. Infiltration of plasma cells in the medulla of the kidney.
Case No. 32, ×2500, H-E stain

Fig. 39. Syncytial formation in the epithelium of the urinary bladder and exfoliated degenerated cell masses.
Case No. 24, ×1440, H-E stain

Fig. 40. Syncytial formation in the epithelium of the urinary bladder. A grape-like assemblage of nuclei in the syncytium is shown.

Case No. 24, Urinary bladder, ×1440, H-E stain

Fig. 41. A fused giant cell and cell agglutination in the subepithelial layer of the buccal membrane.
Case No. 23, ×2500, H-E stain

Fig. 42. Agglutination of lymphoid cells in the subepithelial layer of the buccal membrane.
Case No. 23, ×1440, H-E stain

Fig. 43. Reticular giant cells with intranuclear inclusion bodies in the mesenterial lymph node.
Case No. 43, ×2500, H-E stain

Fig. 44. A grape-like assemblage of lymphoid cells and syncytial formation of reticular cells in the mesenterial lymph node.
Case No. 23, ×1440, silver stain

Fig. 45. A lymphoid giant cell in the paratracheal lymph node.
Case No. 23, ×1440, silver stain

Fig. 46. A Warthin-Finkeldey cell in a mesenterial lymph node of Case No. 32. Only one nucleus has an intranuclear inclusion, while the others do not.
×5500, H-E stain

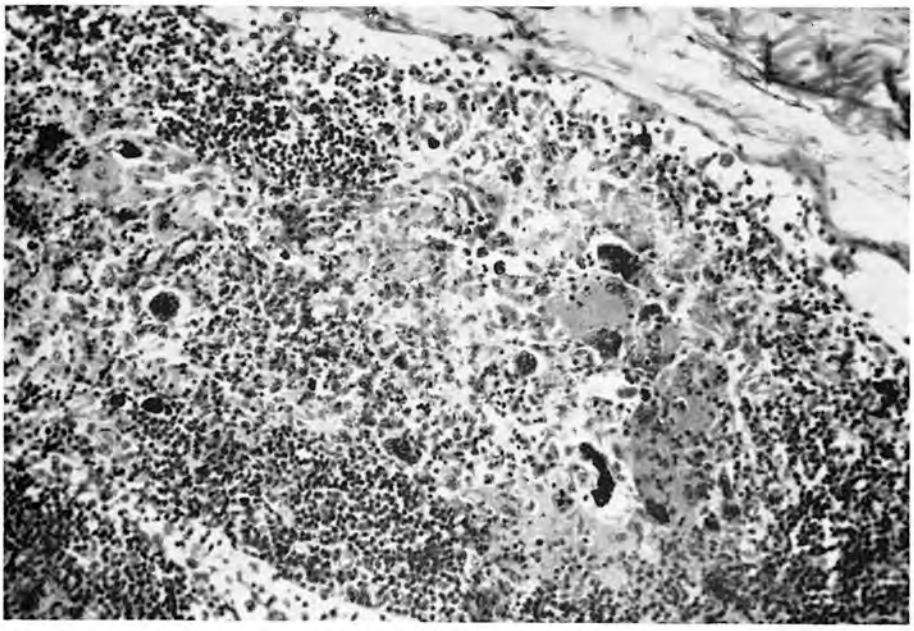
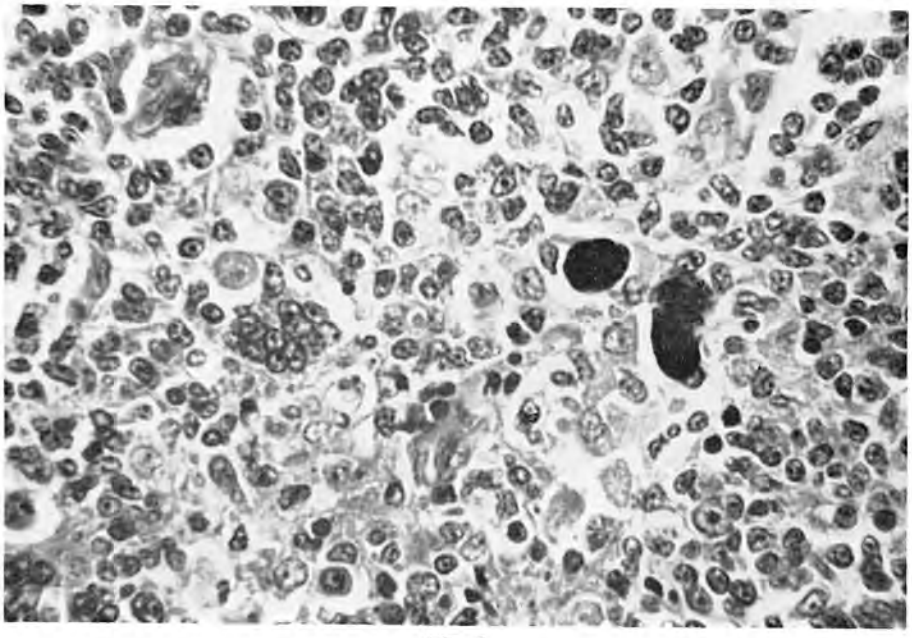


Fig. 1



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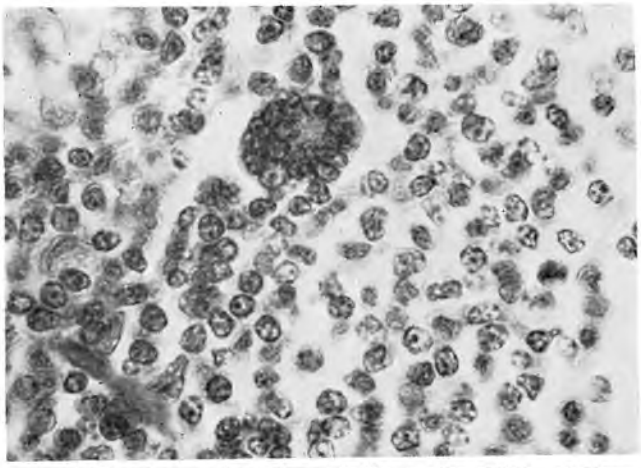

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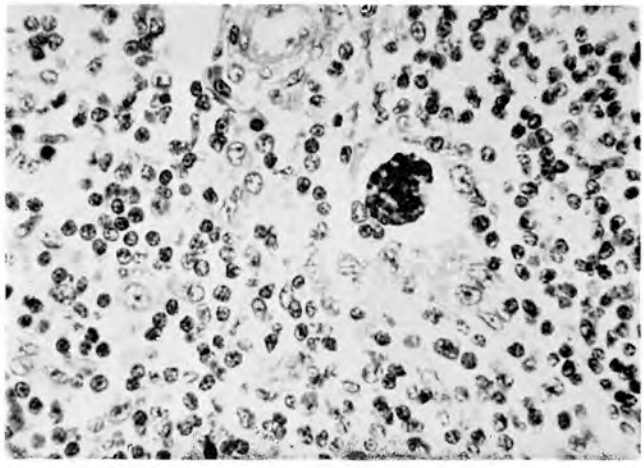

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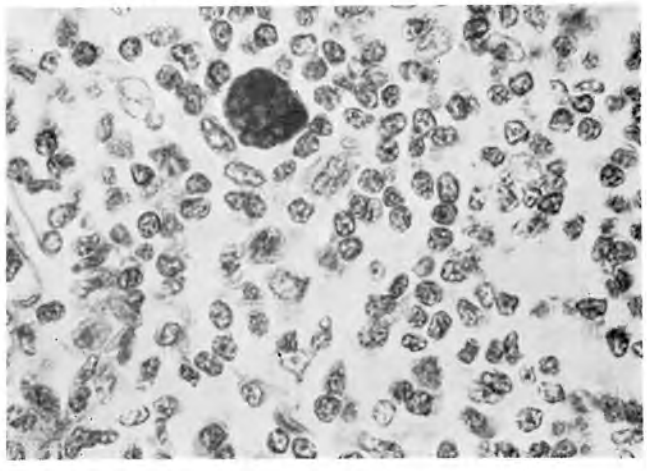
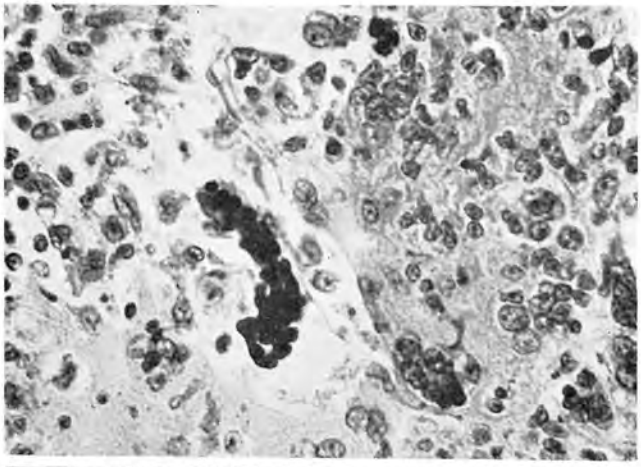


Fig. 5



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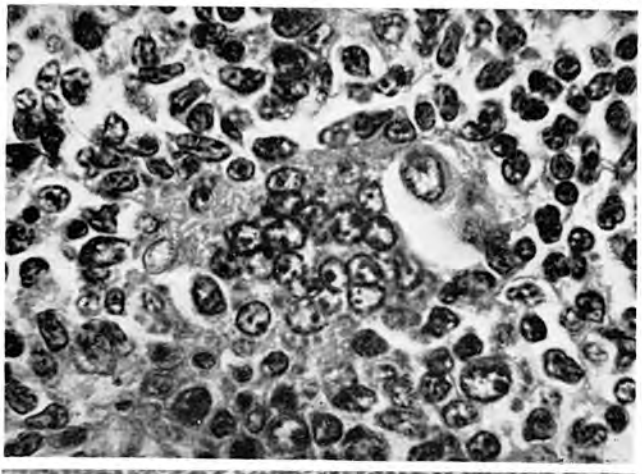

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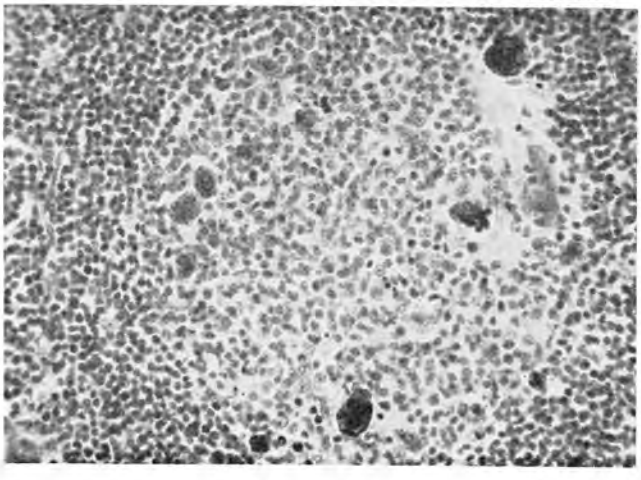

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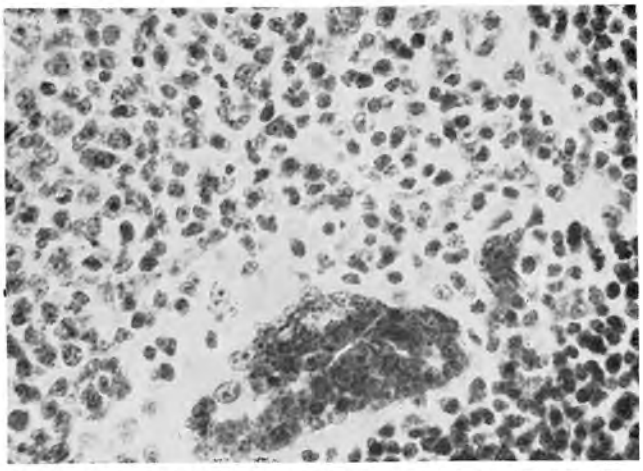

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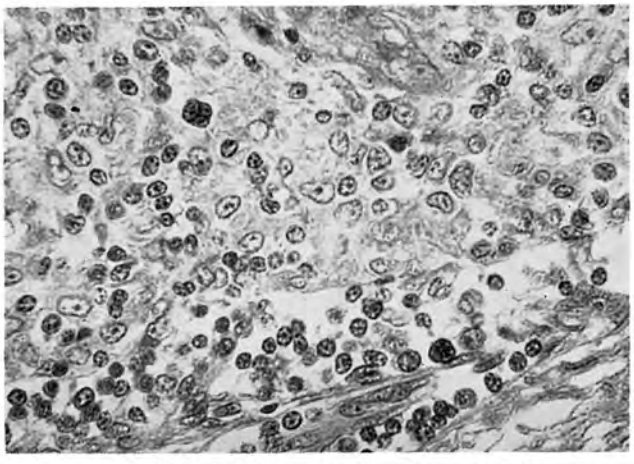

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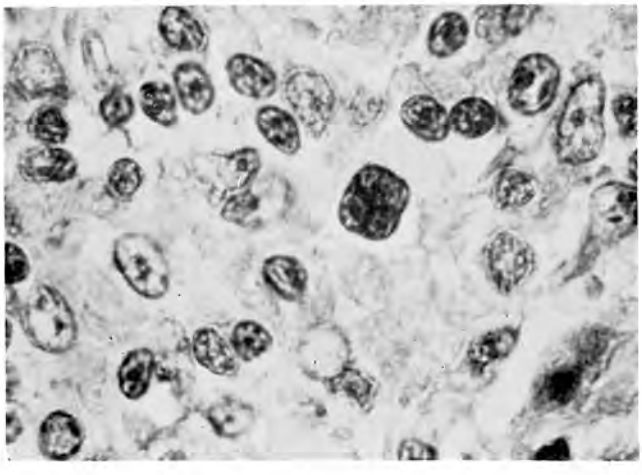

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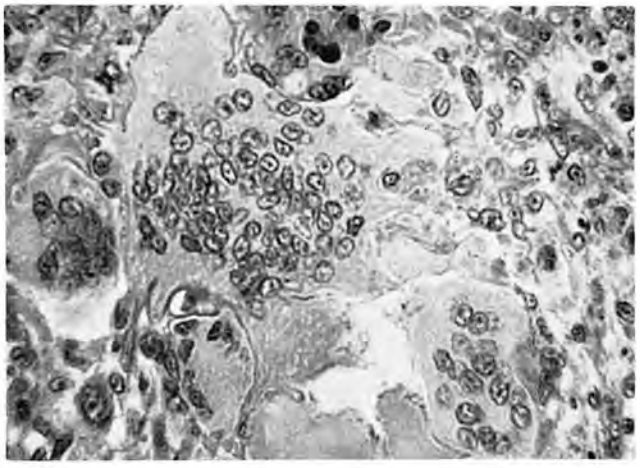

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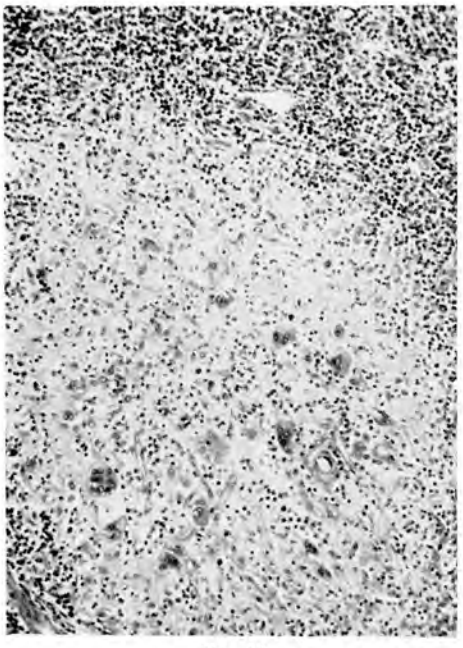

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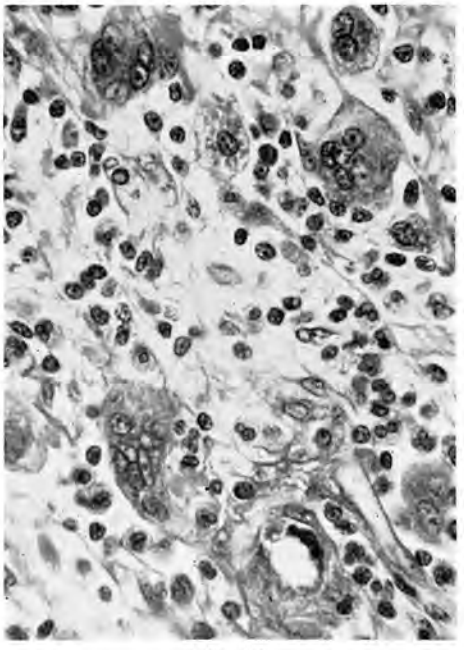

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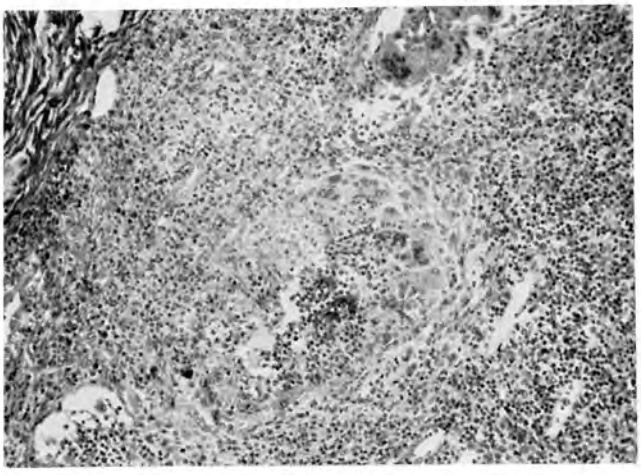

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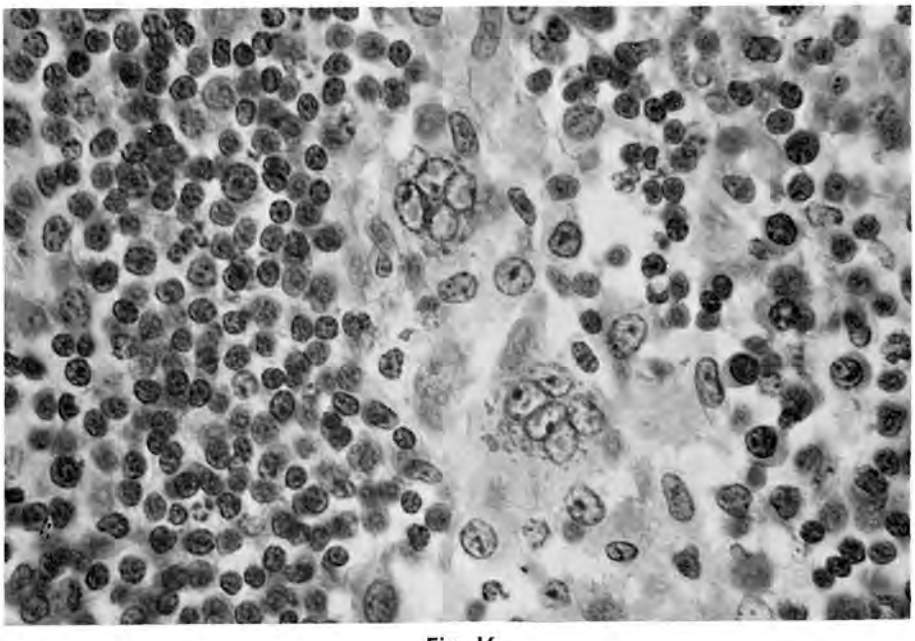

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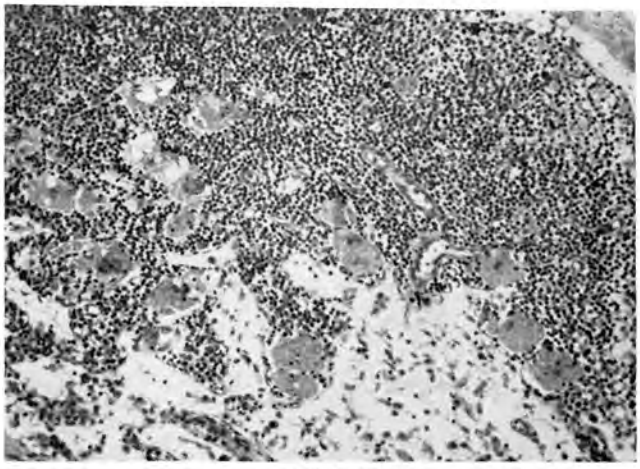

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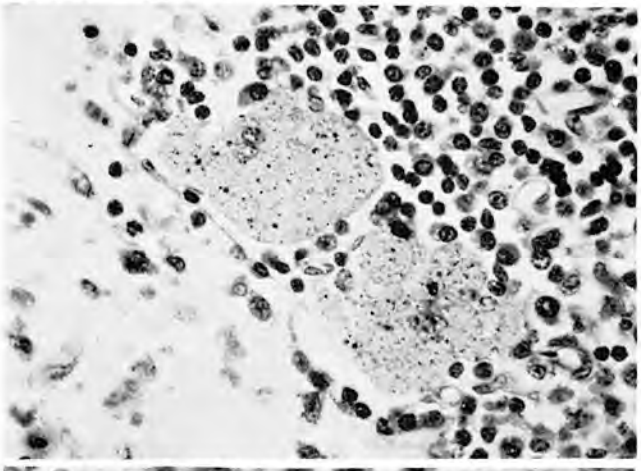

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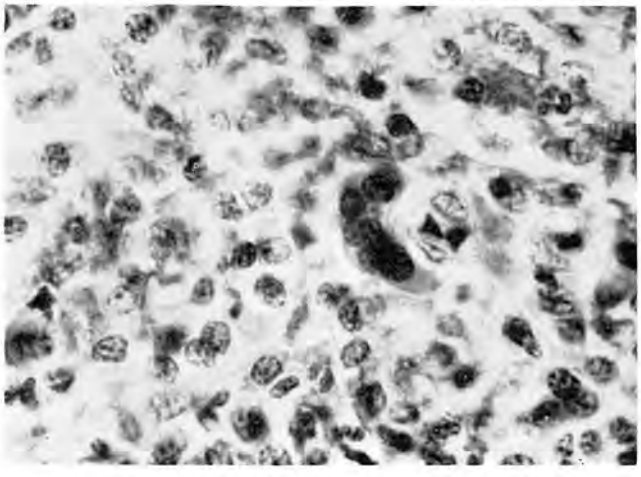

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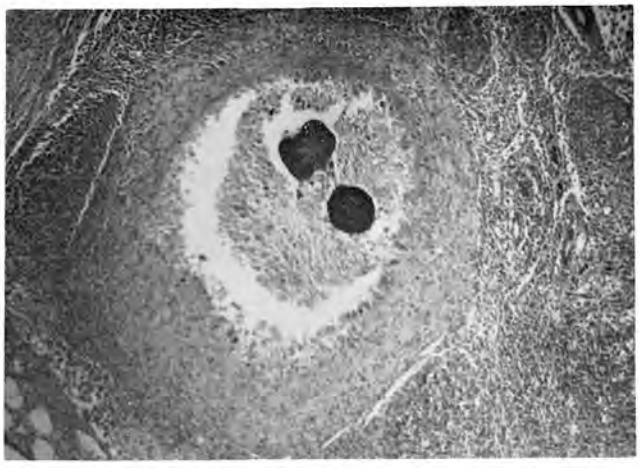

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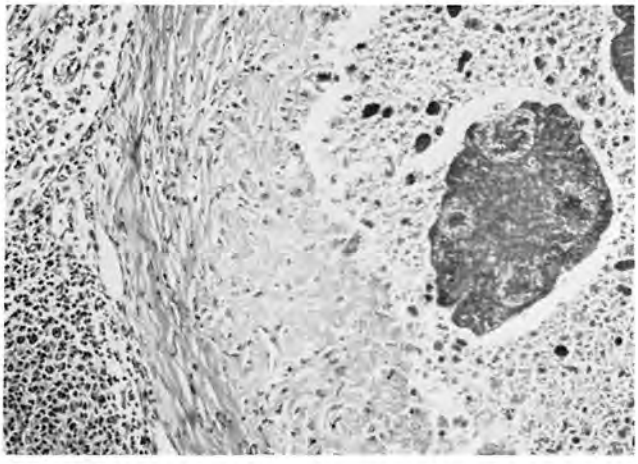

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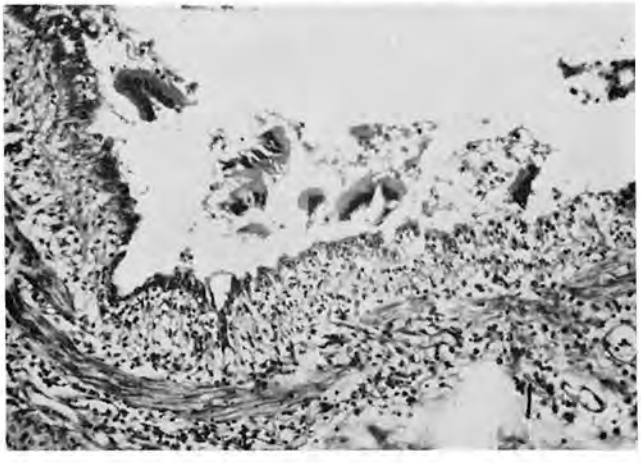

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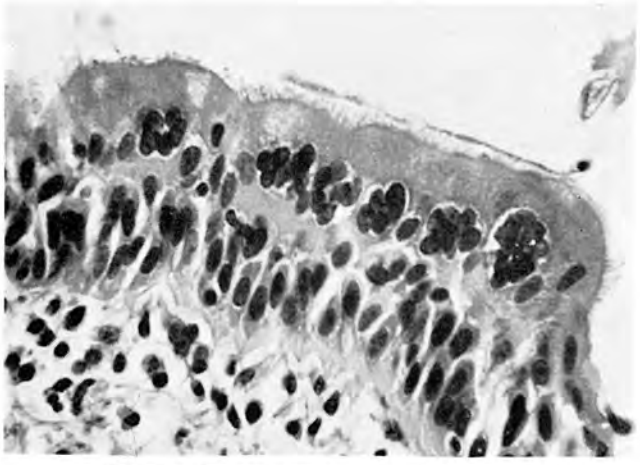


Fig. 23

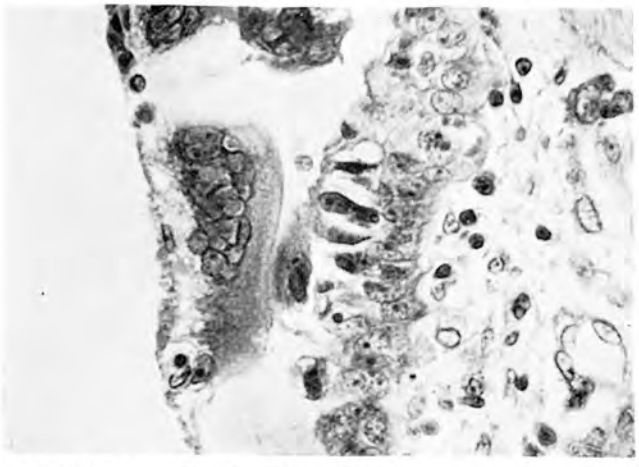
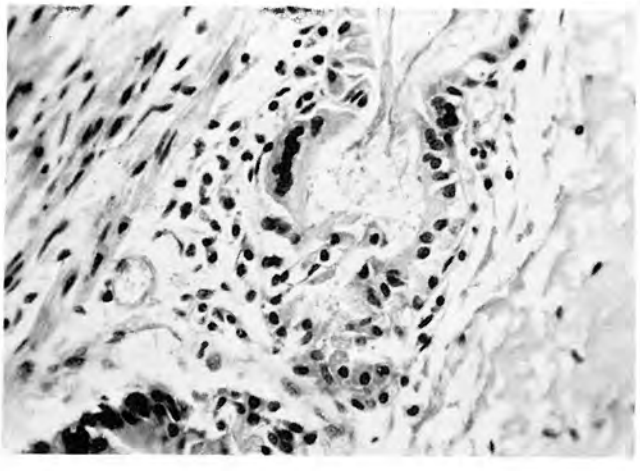


Fig. 24



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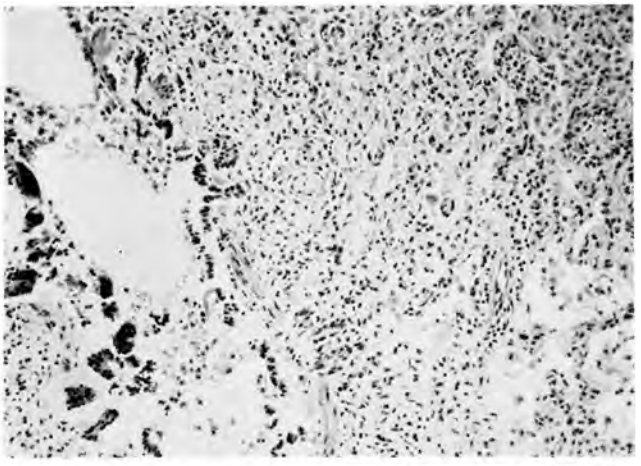

Fig. 26

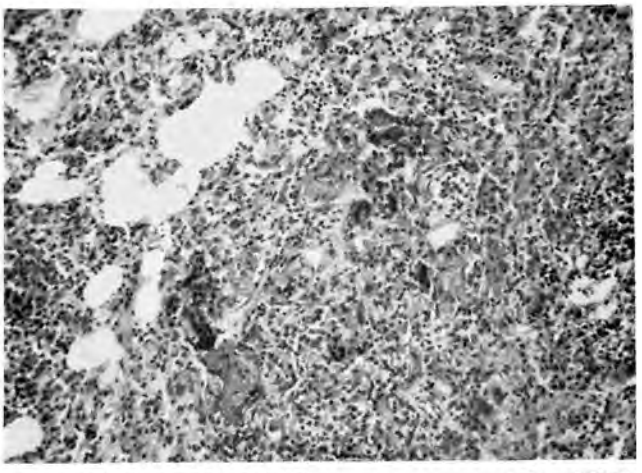

Fig. 27

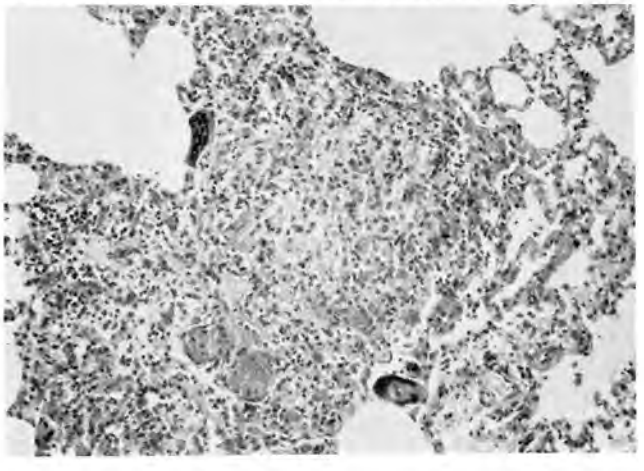

Fig. 28

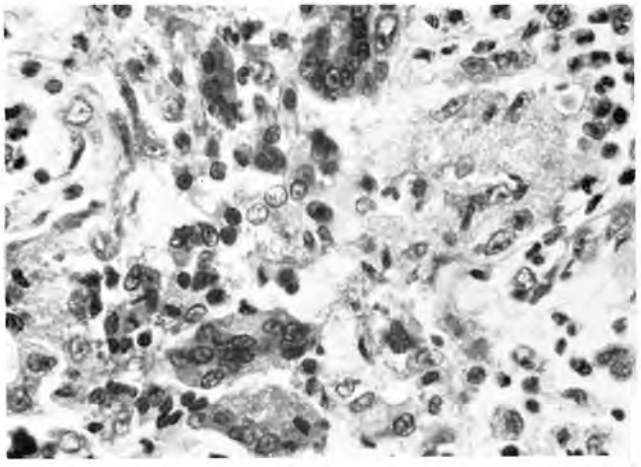

Fig. 29

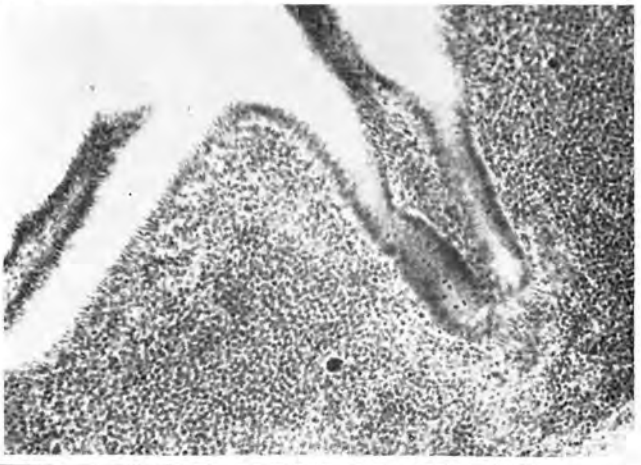

Fig. 30

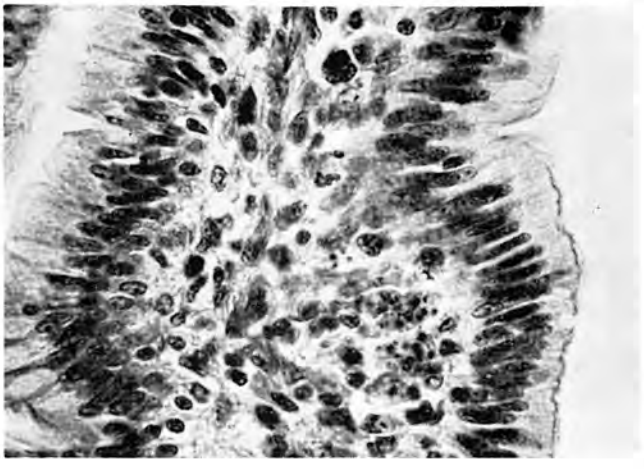

Fig. 31

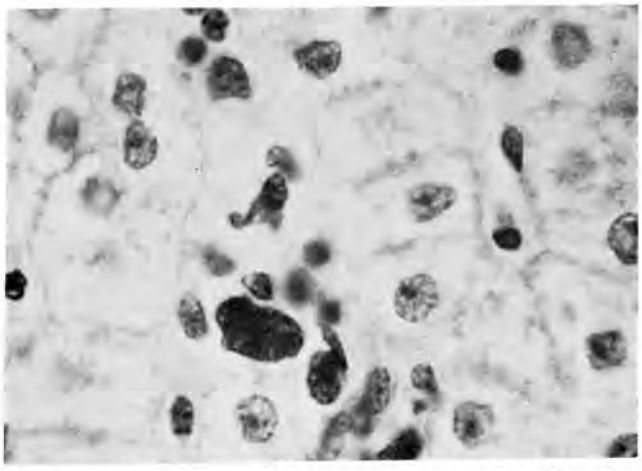

Fig. 32

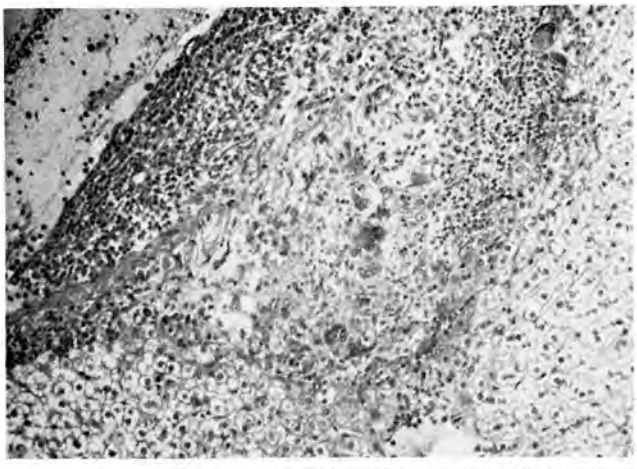

Fig. 33

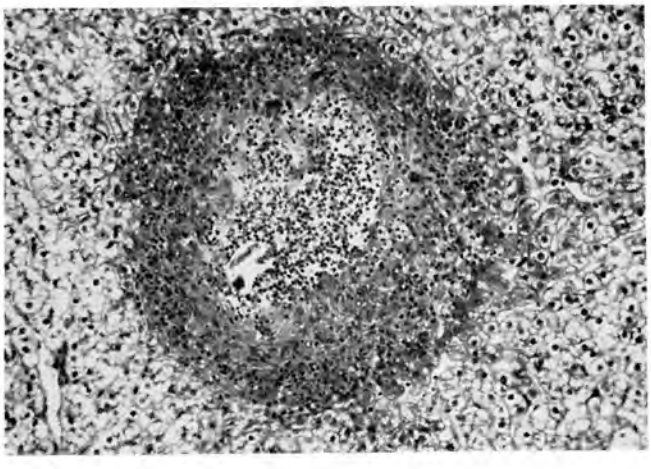

Fig. 34

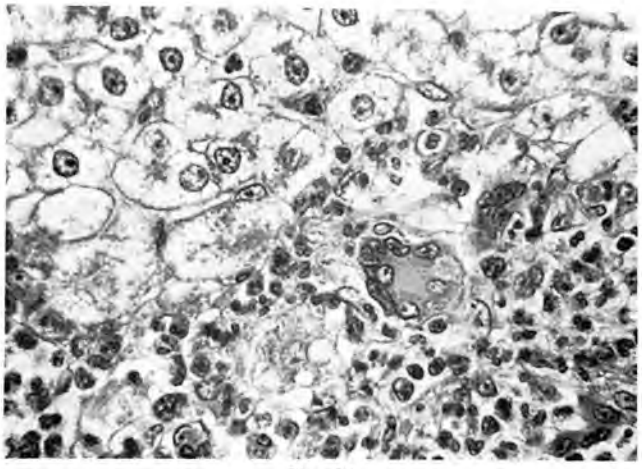

Fig. 35

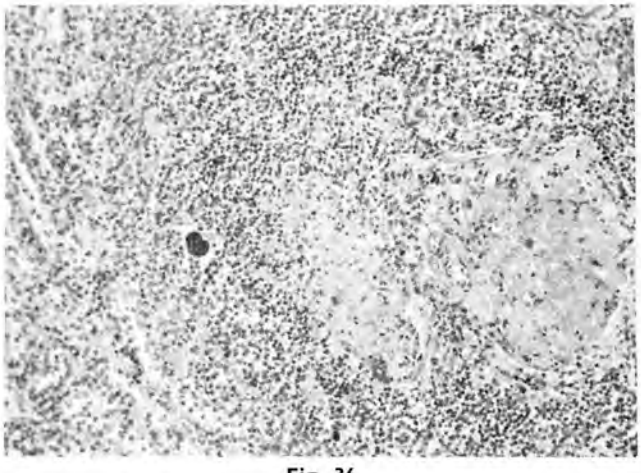

Fig. 36

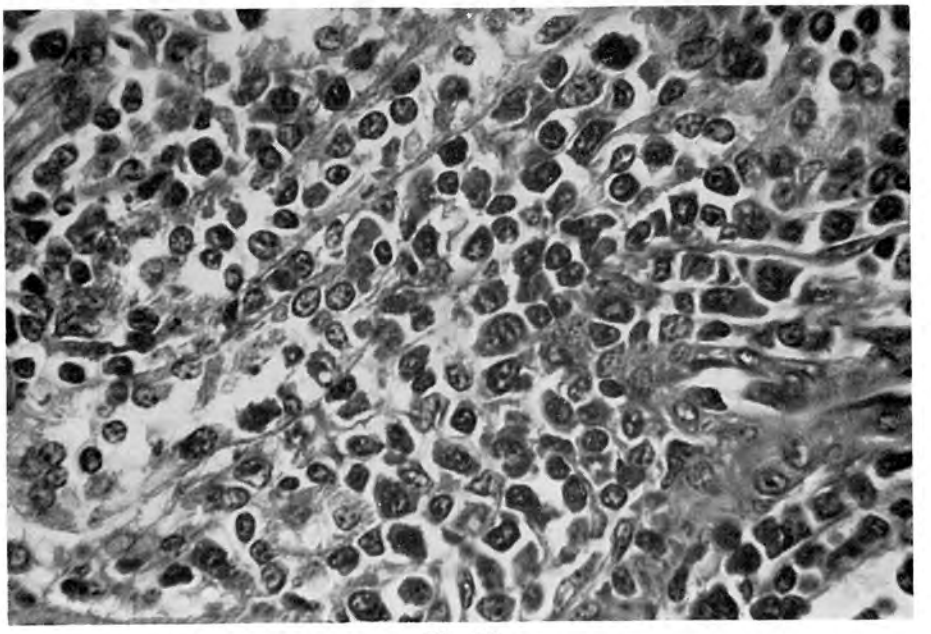

Fig. 37

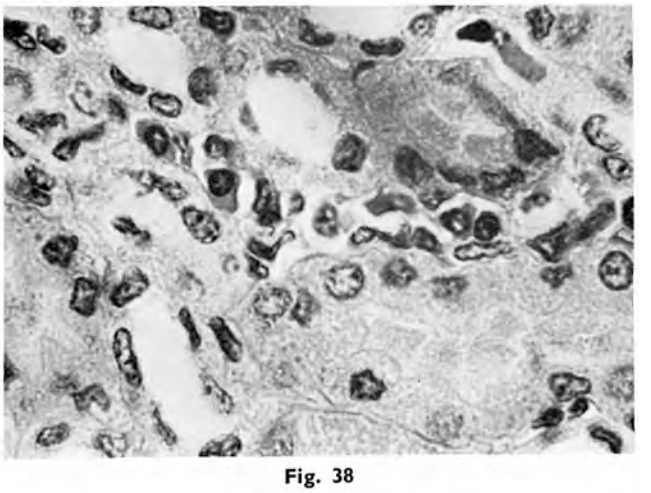

Fig. 38

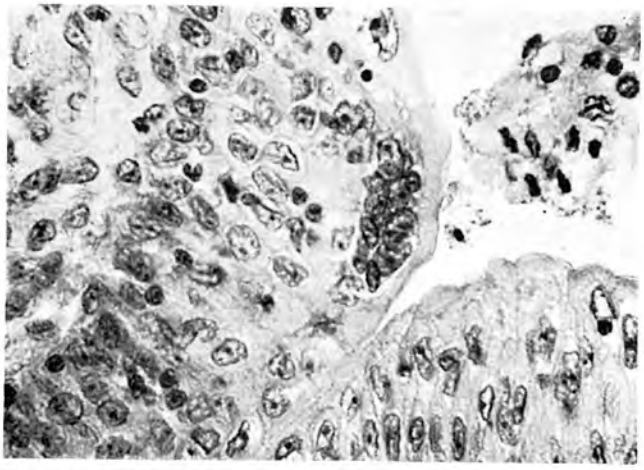

Fig. 39

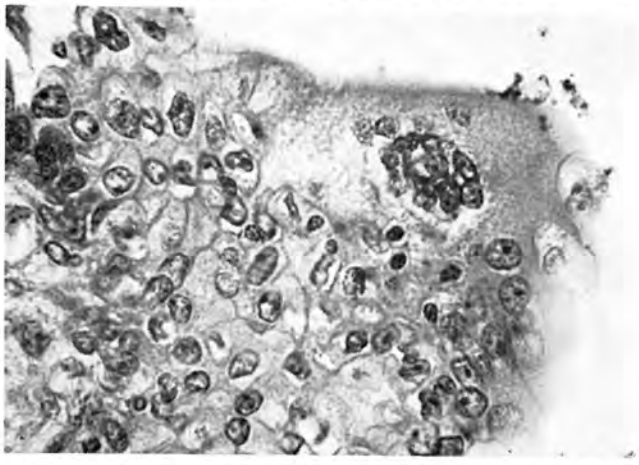

Fig. 40

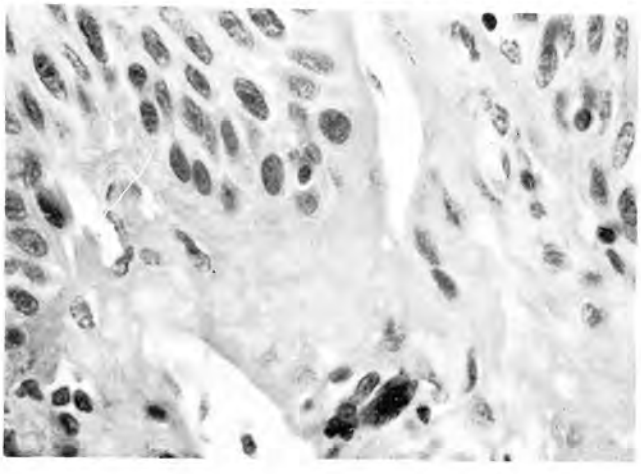

Fig. 41

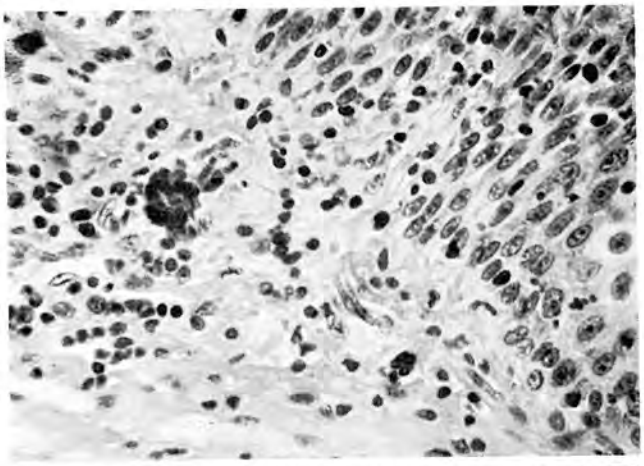

Fig. 42

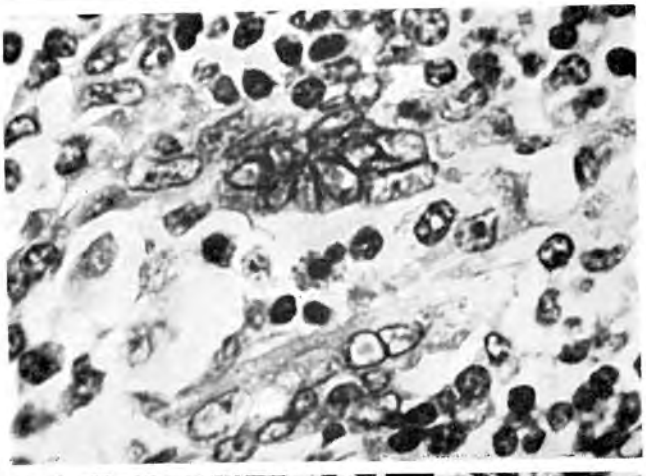

Fig. 43

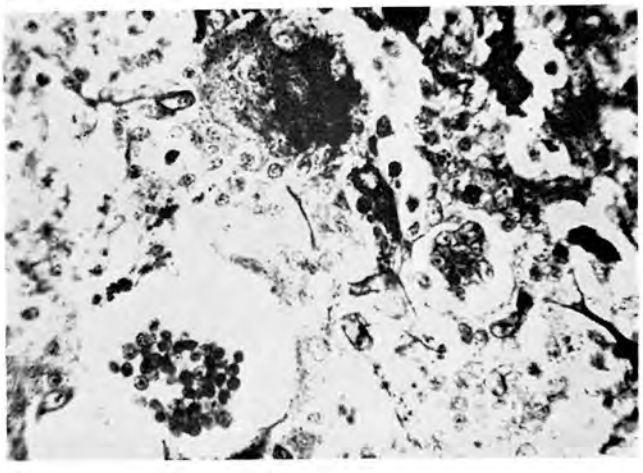

Fig. 44

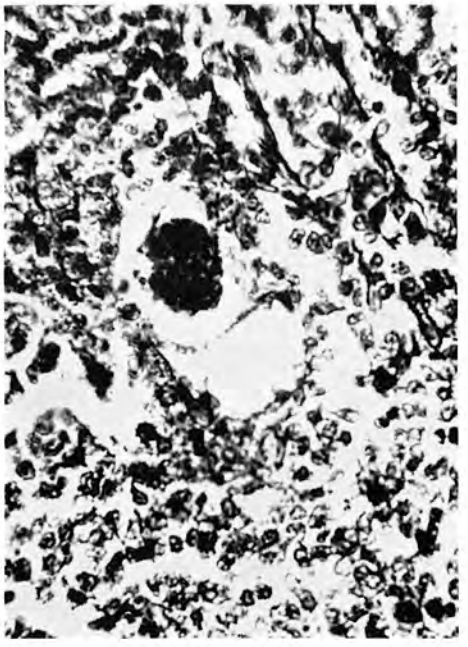

Fig. 45

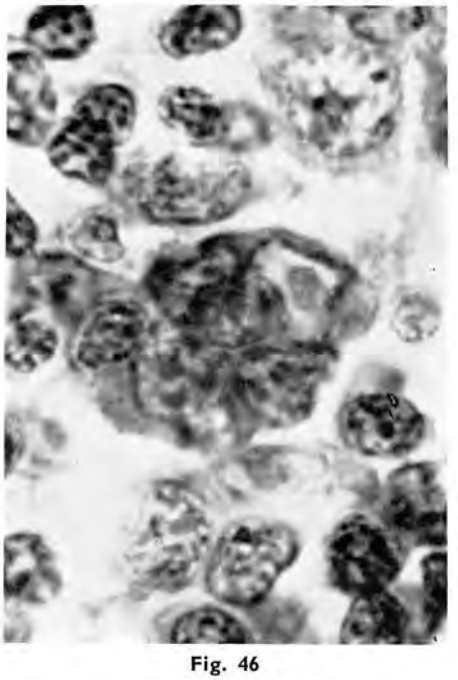

Fig. 46