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INTRANUCLEAR INCLUSIONS IN VISCERAL DISEASE *

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The presence of intranuclear bodies with specific tinctorial properties has been widely accepted as indicative of infection with a virus having special affinity for tissues of neural or ectodermal origin. It is the purpose of this paper to present morphological evidence that similar bodies may be found in lesions of internal organs, and to bring forward for consideration the possibility that viruses of this type may localize in tissues other than skin and central nervous system.

REVIEW OF LITERATURE

The history of such nuclear inclusions in mammalian tissue cells dates back to a publication by Jesionek and Kiolemenoglou [1] in 1904, on the finding of protozoön-like structures in the organs of an hereditary syphilitic. Their illustrations and descriptions show that they were observing bodies very similar to, or identical with, those with which this paper is concerned. They were found in the kidneys, lungs and liver of an eight-month syphilitic stillborn fetus. In the kidneys the cells containing them were irregularly scattered through the interstitial tissue of the cortex, often in groups of ten to forty. In the liver and lungs they occurred singly; in the lung they were found free in the bronchi and alveoli.

The intranuclear bodies are described as oval in form, with a fairly definite cuticular zone suggesting a capsule. The nucleus consisted of a central body, separated as if by a shell from the cytoplasm; on

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the inner surface of this shell (*i.e.* the nuclear membrane) were darkly stained granules of various sizes. The cell body was spongy and the pole near the nucleus filled with granules. As regards staining, the authors noted that the central body, that is to say the inclusion, stained rather palely and with a reddish tinge in hematoxylin-eosin preparations, after sublimate fixation, whereas the peripheral granules were dark blue.

The authors believed that they could exclude the possibility that these bodies arose as modifications from preformed structures. Their appearance suggested some animal or vegetable parasite, and after consultation with Professor R. Hertwig, it was concluded that they were probably Gregarines. The possibility that they were the cause of syphilis — this was before the days of the *Treponema pallidum* — was discussed, but not seriously entertained.

Shortly after the appearance of this paper Ribbert,[2] recalling that he had seen similar bodies some twenty years previously, published a short paper on protozoön-like cells in the kidney of a syphilitic newborn and in the parotid of children. In the kidneys Ribbert found large cells in the tubules but not in the glomeruli or stroma. The preparations were twenty years old and somewhat faded, but it was still evident that there were no transitions between these cells and the renal epithelium. They are described as having a round or oval nucleus containing a large homogeneous body, separated from the nuclear membrane by a clear zone. The cell body was slightly vacuolated or finely granular.

In Ribbert's second case similar intranuclear bodies were found in the parotid of a non-syphilitic year-old child. They occurred in the ducts singly or in groups, often pushing aside the epithelial cells. In his third case identical cells were found in even greater numbers in the parotid ducts.

As regards their significance Ribbert agreed with Jesionek and Kiolemenoglou that they were elements which in no way resembled any of the normal or pathologically altered body cells. This suggested at once their parasitic nature and the preparations were submitted to the zoölogists Rhumbler and Ehlers, who rather inclined to the opinion that they were amoebae or sporozoa.

In 1907, Loewenstein,[3] at Ribbert's suggestion, studied the parotids of thirty children from two months to two years old and found inclusions in four. In the last case they were observed in fresh un-

stained preparations. The slides were submitted to Professor Ludwig, the Director of the Zoölogical Institute, who was of the opinion that they were protozoa, either coccidia or other sporozoa. Loewenstein found no transitions to normal body cells.

Pisano,[4] in 1910, reported the finding of similar intranuclear bodies in the tissues of a stillborn fetus. The viscera in this case showed pronounced lesions — the liver, a gummatous hepatitis, while the kidneys, spleen, thyroid and lungs were all the seat of interstitial fibrosis. The large cellular elements containing inclusions were present in great number in the kidneys, fairly numerous in the liver and rare in the lungs. They were found in the crevices of the connective tissue and free in the lumina of the tubuli contorti of the kidneys. As to their significance various possibilities were considered and rejected. The most specious hypothesis, and the one adopted by Pisano, and also by Perrando [5] in a subsequent article, was that the cells were of epithelial origin but "arrested in their development by the dystropic and paraplastic effect of the syphilitic infection." The paper makes no reference to previous German observations. The following year Mouchet[6] found similar bodies in the bile ducts of an eight-day syphilitic infant, with icterus. They were regarded by him as sporozoa, the sporocysts of which were enclosed within hypertrophic epithelial cells.

Smith and Weidman [7] in 1910 published in the University of Pennsylvania Medical Bulletin a paper entitled "Infection of a Still-born Infant by an Amebiform Protozoan (Entameba mortinatalium), N. S." The bodies which they depicted and described were obviously identical in nature with those reported by Jesionek and Kiolemenoglou, and by Ribbert. One other fact of interest is brought out in their paper, that in the liver and kidney the supposed parasites were surrounded by a definite inflammatory reaction of lymphoid and polymorphonuclear leucocytes. In the light of subsequent events it is perhaps unnecessary to review the detailed steps by which Smith and Weidman excluded all the hitherto known amoebae as a result of which they were forced to create a new species for their parasite.

In 1914, the same authors,[8] having in the meantime become acquainted with the previous German reports, published a new observation. The case was that of a two-months' old child, dying of pneumonia. There were ulcerous eruptions about the mouth and nose and scaly lesions of the buttocks, no definite luetic parental

history, a negative Wassermann reaction, but fibrosis of thymus and of pancreas; so that there was a strong suspicion that the child, like the previous cases, was syphilitic. The "parasites," having the same appearance as those in the previous case, were found this time only in the lungs. There was an organizing pneumonia, and a few areas of caseous necrosis in which no tubercle bacilli could be found. The writers conclude that the "parasites" are "doubtless harmless for the mother, but for the fetus, especially when impaired by luetic taint, they may prove pathogenic and capable of destroying life."

Bodies of this type next reappear in the literature in 1921, being described this time as "An Intracellular Protozoan Parasite of the Duct of the Salivary Glands of the Guinea-pig." Leila Jackson [9] found it in 26 of 48 pigs examined, and described it as follows: "an encysted organism of irregular round or oval contour. In its most conspicuous and fully developed form, it practically replaces the host cell which still retains its relations to the duct wall. The center is occupied by a round or oval body, which stains deeply and unevenly, around which is a wide, lightly stained zone and outside of this a peripheral capsule." The illustrations which accompany the paper show a very close resemblance to the bodies described by the previous writers. Jackson, however, interpreted them as coccidial in nature, and thought they were situated within the cytoplasm of the epithelial cells, rather than within the nucleus. There may therefore perhaps be some question as to whether she was dealing with the same type of structure.

In the same year, 1921, Goodpasture and Talbot [10] published an excellent paper, "Concerning the Nature of the Protozoan-like Cells in Certain Lesions of Infancy." In a six-weeks' old male child, which had had green stools from birth, glucose in the urine, edema of the feet, and cough, they found large cells with acidophilic intranuclear bodies, in the alveoli of the lungs, in the chronically inflamed bronchi, and impacted in the glomeruli of the kidneys. Their illustrations leave no doubt that the bodies were identical with those in our own case, and with the bodies reported previously.

Goodpasture and Talbot remark that these inclusion-containing cells resemble no normal element of the body. They believe, however, that their origin can be traced to large mononuclear cells situated just outside the endothelium of small veins and capillaries, and that they may gain entrance into the blood stream, penetrating

the capillary walls. No cells of this type were observed in organs other than the kidneys, lungs and liver. The possibility that other types of cells, particularly epithelium, might be transformed into cells of this type, could not be excluded. Indeed, early stages of the transformation could be seen in the alveolar epithelial cells.

The authors found it difficult to form an opinion as to the nature of this remarkable change, which suggested the intranuclear bodies described by Tyzzer [11] (1906) in varicella. They felt very certain that whatever they might be, they were not protozoa.

De Lange [12] in 1922 recorded a similar finding in a three-day old icteric infant with cirrhosis of the liver. Syphilitic infection could not be proved, the viscera showing no spirochaetae by the Levaditi method, and the parents giving a negative Wassermann reaction. The inclusion-containing cells were found in the convoluted tubules of the kidneys; in their vicinity, there was round cell infiltration of the stroma. They were interpreted as some undetermined form of cell degeneration.

Three further cases were reported in 1922 by J. Müller,[13] one in a stillbirth, one in a child eight weeks old with hydrocephalus and slight interstitial nephritis, and a third in a two-months' old child with congenital syphilis. The inclusions were found only in the kidneys; their appearance conforms to that described by previous observers.

Müller excludes the possibility that the cells are protozoan because of their occurrence in stillbirths, and because it does not seem possible that they could have passed the placental barrier. He is therefore forced to believe that they originate from tissue cells which have undergone a peculiar degeneration, characterized by the dissociation of the oxy- and basi-chromatin within the nucleus, and by hypertrophy of the affected cells as a whole. The foregoing cases are summarized in Table 1.

We should hardly be justified in reviewing these isolated findings at length were it not for the fact that the matter of these intranuclear bodies takes on new interest and importance with the discovery of B. Lipschütz [14] in 1921 that similar structures are constantly and characteristically associated with the lesions produced by the herpes virus, both in man and rabbits. Although it had been previously shown by Grüter [15] and Loewenstein [16], that the virus of herpes febrilis is transmissible in series to rabbits, Lipschütz was the

first to make a careful study of the intracellular inclusions in this disease, and in herpes zoster and herpes genitalis, and to interpret these bodies as the expression or result of an intranuclear virus. Since Lipschütz's first publication, numerous articles have appeared, dealing not only with the question of cell inclusions, but with the most interesting and still obscure problem of the relation of the herpetic virus, and the encephalitis produced by certain strains of it in rabbits, to the virus of epidemic encephalitis in man. We shall attempt to discuss only the herpetic inclusions, and their possible bearing on the interpretation of the case which is here reported.

Lipschütz showed first of all that these bodies are not artifacts, inasmuch as they may be easily recognized in fresh preparations. They are round, oval, or even slightly irregular structures ranging from 2 micra to such as completely fill the nucleus, reaching to the nuclear membrane but leaving usually a small clear zone. Smaller inclusions lie against a clear background. Usually there is only one within a nuclear membrane, but sometimes several. Practically every nucleus at the site of the herpetic lesions may contain an inclusion. Frequently the inclusions appear homogeneous, but in properly differentiated iron-hematoxylin preparations, they appear to be composed of numerous minute granules embedded in a homogeneous matrix.

Although they typically occur within the nuclei, they may in early lesions be occasionally found in the cytoplasm in the vicinity of the nuclear membrane. They are present both in the nuclei of epithelial cells, and in the swollen hydropic nuclei of connective tissue cells in the corium, and in the cells about blood vessels. Once an inclusion was seen within a mast cell, and once within the proliferating epithelium of a hair follicle.

The staining reactions are summarized as follows:

Stain	Inclusions	Nucleoli
Giemsa	Red	Dark blue
Hematoxylin-eosin	Dark red	Blue-black
Heidenhain iron-hematoxylin	Yellow-gray	Black
Pappenheim	Green or blue	Red

The inclusions may thus be readily differentiated tinctorially from the nucleoli.

Lipschütz [14] discusses at length in this and a subsequent paper [17] the possible interpretation to be placed upon these and similar

TABLE 1

Résumé of Reported Cases

No.	Year	Author	Age	Pathological diagnosis	Location of inclusions	Interpretation
1	1904	Jesionek and Kiolemenoglou	Stillbirth	Congenital syphilis	Kidneys, lungs, and liver	Gregarines (R. Hertwig)
2	1904	Ribbert	Newborn	Congenital syphilis	Kidneys	Amoebae or Sporozoa? (Ehlers — Rhumbler)
3	1904	Ribbert	One year	——	Parotids	
4 5 6 7	1907	Loewenstein	Two months to two years	——	Parotids	Coccidia or other sporozoa (Ludwig)
8	1910	Pisano	Stillbirth	Congenital syphilis, Gummatous hepatitis, etc.	Kidneys, liver and lungs	Embryonic epithelial cells
9	1910	Mouchet	Eight days	Congenital syphilis	Bile ducts	Sporozoa
10	1910	Smith and Weidman	Stillbirth	Focal nephritis	Kidneys, liver, and lung	Entamoeba mortinatalium
11	1914	Smith and Weidman	Two months	Pneumonia	Lungs	Entamoeba mortinatalium
12	1921	Goodpasture and Talbot	Six weeks	Green stools, edema of feet, bronchitis	Lungs and kidneys	Degenerative change in the nuclei of endothelial leucocytes
13	1922	De Lange	Eight days	Congenital syphilis? icterus, cirrhosis	Kidneys	Undetermined form of cellular degeneration
14	1922	J. Müller	Eight weeks	Hydrocephalus, focal interstitial nephritis	Kidneys	Degenerative change, with dissociation of oxy- and basi-chromatin
15	1922	J. Müller	Stillbirth	?	Kidneys	
16	1922	J. Müller	Two months		Kidneys	

intranuclear inclusions, and reaches the conclusion that they represent the reaction of the nuclear plasm to a living virus classed with the Chlamydozoa-Strongyloplasma. To the group of these intranuclear viruses, which includes causative agents of the various forms of herpes, Borna's disease of horses (epidemic encephalomyelitis), varicella, fowl-pox, etc., is given the name "karyooikon group," distinguishing them from the cytooikon group, in which the inclusion is present in the cytoplasm. The trachoma bodies, the Guarneri corpuscle in smallpox, and the Negri bodies in the ganglion cells in rabies are representative of this latter group.

Lipschütz's arguments in support of the view that the inclusions are to be interpreted as a specific reaction to a living parasitic virus, are briefly the following:

1. Their general resemblance to the well-known inclusions of variola and vaccinia.
2. Their constant occurrence in the pathologic tissue, and their limitation to that tissue.
3. The impossibility of identifying them with hypertropic nucleoli or any known degenerative products of the cell.
4. The possibility of reproducing similar bodies by experimental inoculation of the virus.

Subsequent investigation has almost unanimously confirmed Lipschütz's work so far as the regular occurrence of the intranuclear bodies in spontaneous and experimental herpes lesions is concerned. There have, however, been differences of interpretation. Thus Lauda,[18] who studied the inclusions both in the herpetic lesion and within the ganglion cells and glia cells of the encephalitic rabbits, believes that the inclusions are the result of a degenerative, destructive process of the nucleus, whereby the oxychromatin comes to lie in the center of the cell, and the basi-chromatin moves to the periphery. Against the parasitic nature of the inclusions, he brings the following arguments: (1) the absence of evidence that the virus is really situated within the nucleus; (2) the absence of elementary bodies within the inclusions (he was unable to confirm Lipschütz's observation as to the presence of minute granules within the inclusion); (3) the lack of specificity, since bodies of apparently identical morphology are found in such diverse conditions as herpes labialis, zoster and varicella. Zdansky,[19] also, in a recent paper on

the pathologic anatomy of the encephalitis produced in rabbits by the herpes-encephalitis virus, ascribes no etiological importance to the inclusions found in the ganglion cells and glia cells, but regards them rather as degeneration products of the karyoplasma. Levaditi[20] and Da Fano[21] believe that the virus is more probably to be found in certain minute granular bodies scattered through the affected nerve tissue, both within the cells or between them. The larger intranuclear bodies (the "neurocorpuscules" of Levaditi, Harvier and Nicolau[22]) are regarded as products of nucleolar degeneration. Cowdry and Nicholson,[23] in studying cytologically experimental herpetic encephalitis material given them by Flexner, Noguchi and Amoss, attribute little importance to intranuclear inclusions of the Lipschütz type, and most of their attention appears to have been directed to the smaller types of granules emphasized by Da Fano. In conclusion, they state their belief that the inclusions which are so abundant in herpes do not represent a concrete class of granulations *sui generis*, but that they are of variable composition and derived from several sources.

Quite the opposite point of view is taken by Goodpasture and Teague. In one of the most recent of the brilliant series of studies in this field, Goodpasture[24] makes a very strong argument in favor of the view that the nuclear inclusions represent a change brought about specifically by the virus of herpes or closely related viruses. As the virus passes from the peripheral nerve endings to the central nervous system along the nerve trunks, a progressive involvement of the neurolemma cells and then of the ganglion cells in the corresponding region of the brain, may be demonstrated. When the lesion is unilateral, only the cells on the affected side show inclusions. While it is not maintained by Goodpasture that the intranuclear inclusion is itself the active agent of the disease, he does believe with Lipschütz that it indicates an intranuclear localization of the infective substance.

Case Report

F. S. (history 60991 — autopsy 9582), male, white, age 36 years. Entered Presbyterian Hospital September 27, 1924.

Chief Complaints. Fever for 3½ weeks. Pain in chest for 10 days.

Past History. Gonorrhoea 9 years ago. In 1908, an acute illness (appendicitis ?) since which time he has been subject to attacks of indigestion, characterized by acute pain across the upper abdomen and lower chest, coming on ½ hour after meals, and relieved by rhubarb and soda. At times the pain has been

localized in the right lower costal region and is relieved by lying on the affected side. No history of herpes was obtained on careful inquiry.

Present Illness. The present illness began gradually about one month ago with afternoon fever. Seven days after the onset a motile gram-negative bacillus was obtained from blood culture. This was at first believed to be *B. paratyphosus* but was later identified as *B. coli*. Ten days ago he began to have pains in the lower anterior part of the chest. The pain was burning in character and worst when fever was highest. There were no abdominal pains and no blood in the stools.

Physical Examination. Thin, well-developed man, acutely ill. Heart and lungs negative. Abdomen flat, with slight fullness in the right upper quadrant where there was definitely increased resistance and tenderness on pressure. Liver palpable 3 cm. below the costal margin. No abnormal neurological signs. Pulse regular, not dicrotic, 100. Blood pressure 105/55. Blood: R. B. C. 4,000,000, Hg. 92 per cent, W. B. C. 16,900, P. M. N. 79 per cent, Lym. 20 per cent, Eosin. 1 per cent. Wassermann reaction negative. Stool negative for blood (Guaiac test). Blood culture: negative on one occasion; on another, *B. coli* was again recovered.

Course. The temperature curve suggested a pyogenic infection rather than paratyphoid fever. The tenderness in the region of the liver persisted, and the diagnosis lay between subphrenic and hepatic abscess. On October 9th, exploratory coeliotomy disclosed an abscess in the right lobe of the liver, which was drained. Culture of the pus yielded *Staphylococcus pyogenes albus*.

Operation was followed by only temporary improvement. Transfusion (750 c.c. of blood) failed to influence the temperature or leucocytosis. Examination of the chest was negative.

On November 4th, because of the failure to improve, he was again operated upon, and a subphrenic abscess found and drained. The septic temperature persisted. An X-ray on November 8th showed a shadow in the right lower chest and obliteration of the phrenic angle. Dullness was found over this area. The leucocyte count was 24,850, P. M. N. 86 per cent.

Upon aspiration of the chest, a small amount of blood and fluid, sterile on culture, was obtained. On a second aspiration of the chest 10 days later, a small amount of blood-streaked pus was withdrawn, from which was grown a non-hemolytic streptococcus in pure culture. The margins of the draining operative wound at this time had become necrotic over a wide area, and masses of necrotic liver tissue were discharged from the abscess.

On November 19th, a piece of tissue was removed from the wall of the liver abscess. Sections of this showed bile ducts, some compressed, some distended with polymorphonuclears. There was much dense granulation tissue in the midst of which were a few degenerating liver cells; also many phagocytes, some containing hemosiderin. No amoebae could be found. No organisms could be found in a Levaditi preparation. Smears from the abscess stained with methylene blue, and by Fontana method, showed many cocci, often in chains, and many bacilli but no spirochaetae or spirillae, or filamentous organisms.

On December 1st, the patient developed bloody diarrhea and on December 5th *amoeba histolytica* was reported as present in the stools. The administration of emetin hydrochloride (0.63 gm. in twelve days) was followed by a decrease in the number of the stools. No amoebae were found after the emetin therapy was begun. The margins of the liver wound showed signs of more active

healing, with the appearance of granulation tissue, and the abscess cavity became smaller. The fever, however, persisted, and also the pulmonary signs.

On December 18th he had two bloody stools, the second one consisting of almost 500 c.c. of blood. He became weaker, and died two days later, having been ill approximately 3½ months.

Autopsy 9582. Anatomic diagnoses: Abscess of the liver; ulcerative colitis with hemorrhage; suppurative pleurisy, right; lobular pneumonia, organizing; small bronchiectases; fibrous pleural and peritoneal adhesions; sclerosis of pulmonary venules.

The body is that of a fairly well-developed, moderately emaciated white man, 165 cm. in length. There are no cutaneous lesions. There is rigor of the jaw, neck and extremities, and some lividity of the dependent portions. There are no abnormalities over the calvarium; no discharge from the eyes, ears, nose or mouth. The pupils are round, equal and in mid-dilatation. The conjunctivae and buccal mucous membranes are quite pale. The teeth are in good condition. There is no enlargement of the thyroid; the superficial lymph glands are not palpable. The chest is long and narrow. On the right side of the chest, in the posterior axillary line, over the 8th rib, is a recent surgical incision, through which a finger can be introduced directly into the pleural cavity. From this wound there flows a small amount of thick, milky-white, purulent material. There is a long incision over the lower anterior chest on the right, following the course of the ribs. The cartilages of the lower ribs project into the wound and are necrotic. There is considerable erosion of the epithelium around the margin of this wound, especially at the inner angle, where there is a granulating surface 2 cm. in breadth, extending back from the edge. The wound is widely gaping, and through it one can see directly into a cavity which extends into the liver. In the upper right quadrant of the abdomen are two gray, glistening scars which touch each other at their upper ends and form an angle of about 60°. The external genitalia are normal. There is no edema of the feet or ankles. The subcutaneous fat is practically absent.

Abdominal Cavity. There is no excess of fluid in the peritoneal cavity. There are fairly recent adhesions between the almost fat-free omentum and the anterior abdominal wall, and also in the region of the cecum. These adhesions become denser in the region of the liver and the anterior surface of the right lobe is bound to the anterior abdominal wall by tough adhesions which completely wall off the abscess from the peritoneal cavity. The liver edge extends 9 cm. below the xiphoid.

The *liver* weighs 1480 grams and measures 20 x 20 x 10 cm. After dissecting off the adhesions, there is seen in the right lobe on the anterior surface a cavity with an opening about 2 cm. in its greatest diameter. The surface of the right lobe of the liver is for the most part devoid of adhesions except in the region of the cavity; the left lobe is covered with a smooth glistening capsule. There is no accumulation of pus between the liver and diaphragm. On section it is seen that the cavity extends into the liver for a distance of 3 cm. The wall is composed of gray, glistening, dense fibrous tissue, superficially bile-stained and covered with a little exudate. Above the cavity, which is in the dome of the liver, there is an infarct-like area of atrophy and congestion, slightly sunken below the level of the surrounding parenchyma. This wedge-shaped area has its base toward the capsule and its apex is against a large branch of the hepatic vein. Contrasting with this, the lobules in other portions of the liver are considerably

larger; there is a very narrow red zone about the efferent vein, but about the portal vessels is a broad grayish-yellow zone. Surrounding the abscess cavity and extending for a variable distance toward the inferior border on the anterior surface is again seen an irregular zone, identical in appearance with the large one described. A communication between the larger bile ducts and the abscess cavity cannot be demonstrated, but there are seen in the wall of the cavity several oval openings from 1 to 2 mm. in diameter, which appear to be the openings of bile ducts. The portal vessels and bile ducts elsewhere appear normal. There are no other abscesses or infarcted areas found. There is no general increase in connective tissue.

The *gallbladder* is small; its wall is thin. It contains a small amount of pale viscid bile.

The *spleen* weighs 225 grams and measures 16 x 7 x 3 cm. The capsule is smooth and glistening. The organ is moderately soft. On section the pulp is reddish-gray in color; the Malpighian bodies are small but easily seen.

The *pancreas*, *adrenals*, *kidneys*, and *pelvic organs* are normal.

Gastro-intestinal tract. The stomach, duodenum, jejunum and ileum are normal.

In the cecum are found discreet ulcers of varying size with clean bases; only a few of these show slight undermining. These ulcers extend down to the inner layer of muscle and tend to encircle the gut; they measure 0.5 cm. to 4 cm. in greatest dimension. The wall about them is not indurated, and clinging to the margin of one of these ulcers are blood clots. In the ascending colon also are a few ulcers, to one of which a blood clot is adherent. They are similar in appearance to those in the cecum, but somewhat smaller. The bases of the ulcers are covered neither by exudate nor by definitely recognizable granulation tissue. There are fifteen of these ulcers. No other ulcers are found in the remainder of the large intestine.

The *lymph glands* in the mesentery are not enlarged; on section they appear normal.

Thoracic cavity. There are numerous adhesions between the lower lobe of the right lung and the parietal pleura. These adhesions tend to wall off the small cavity communicating with the incision in the lateral chest wall. A few adhesions are present between the apex of the upper lobe on the left and the parietal pleura. There is no excess of fluid in either pleural cavity.

The upper lobe of the *right lung* is everywhere air-containing and fluffy, as is also the middle lobe. The lower lobe is much collapsed, dark red and flabby; its pleura roughened by the fibrous adhesions and by a fibrino-purulent exudate. On section the cut surface of the upper and middle lobes is pale. There is no consolidation. The lower lobe on section is dark red, firm, but not uniformly consolidated. The pleura is quite obviously thickened; it seems also somewhat wrinkled, yet the exudate fills in the spaces between these wrinkles, producing a level surface. Scattered throughout the lower lobe are small grayish areas which appear to be more or less confined to the immediate region of the smaller bronchi. The bronchi are not thick walled.

The upper lobe of the *left lung* is everywhere air-containing. The lower lobe is heavy. The posterior portion is dark bluish-red in color, and this area is somewhat sunken below the level of the air-containing lung adjacent to it. The lower lobe feels flabby; it does not have the consistence of a firmly consolidated lung but is more or less elastic. On section there are found in the

upper lobe a few areas, lobular in size, which are consolidated, and the cut surface of these is yellowish in color. In the lower lobe are small areas of consolidation which are for the most part close to the smaller bronchi; they rarely measure more than 0.5 cm. in diameter, and are not confluent. The bronchi in the centers of these in some instances contain a little purulent fluid, but for the most part they are empty and their walls are not especially thickened. Some of the medium-sized bronchi are dilated; they are, however, lined with smooth mucosa. The lymph glands at the hilum of the lung are not enlarged; they contain a considerable amount of pigment.

The *heart* and *aorta* show no significant changes.

Diaphragm. There is no demonstrable communication between the abscess in the liver and the right pleural cavity.

Bacteriologic report. The culture from the content of the large intestine was negative for dysentery bacilli.

Microscopic examination: Liver. The wall of the abscess is composed of dense connective tissue in its deeper part where there are many widely distended capillaries. At the outer margin of the abscess wall are small accumulations of liver cells undergoing atrophy, and also many mononuclear wandering cells of considerable size, many containing yellow pigment, others having vacuolated cytoplasm. Bile ducts are also found; some of these have a wide lumen, in others the lumen is very small. In the more superficial part of the abscess the wall is less dense; it is infiltrated with small round cells and a few polymorphonuclear leucocytes. Upon the surface are large colonies of cocci. Throughout the entire abscess wall are many large cells, often with basophilic cytoplasm, and having oval or round, vesicular nuclei. In each of these nuclei is a very prominent deeply staining mass. These cells are to be seen both within the capillaries and in the connective tissue. Frequently they appear to be passing through the wall of the capillary or are lying against the endothelial lining of the vessel; in places also they seem to have just penetrated the capillary wall and are lying immediately adjacent to it on the outer side. Some of the endothelial cells lining the capillaries are swollen. The liver lobules immediately about the abscess are flattened, and the liver cells atrophic. There is some increase in connective tissue in the portal areas near the abscess, and groups of phagocytes containing hemosiderin are to be seen. At a distance from the abscess the liver parenchyma appears normal. An occasional small accumulation of polymorphonuclear leucocytes grouped around a necrotic cell is found in the sinusoids; these degenerating cells are apparently not in continuity with the liver cells or endothelium of the sinusoids but lie free within the lumen.

Intestine. The ulcers extend down to the submucosa. The base is composed of loose granulation tissue infiltrated with small mononuclear wandering cells. In the granulation tissue and within small blood vessels are large cells with vesicular nuclei, in each of which is to be seen a very striking intranuclear mass. The infiltration with wandering cells does not involve the underlying muscle. In one or two instances an arteriole lying in the superficial part of the ulcer has undergone necrosis and the hemorrhage probably came from such vessels. There is no regeneration of the epithelium at the margin of the ulcer.

Lung. The smaller bronchi are filled with an acute inflammatory exudate and the epithelium has disappeared in many instances from at least a portion of the wall. In other places the epithelium has become flattened and more squamous in type. The walls of these bronchi are infiltrated with wandering

cells of all varieties, and the infiltration extends into the adjacent alveolar septa which are wide. Many of these alveoli are filled with polymorphonuclear leucocytes. In some of the bronchi the exudate has undergone organization and the lumen is partly filled with a fibrous plug. The exudate in some of the alveoli also is undergoing organization. The capillaries are engorged.

In other areas the alveoli are filled with a recent acute inflammatory exudate. The septa of these alveoli are not infiltrated with wandering cells. In many alveoli the epithelium is distinctly cuboidal, and apparently attached to or continuous with the lining epithelial cells are very large cells with vesicular nuclei, each containing a prominent, deeply staining mass. Some of these cells lie free in the alveoli or in the exudate within them. Large cells, at times multinucleated, with intranuclear masses, are found within the smaller branches of the pulmonary artery, or lying just beneath the endothelium of these vessels. These cells are identical in appearance with the striking cells found in the liver and in the intestinal ulcers.

In the section from the right lower lobe the pleura is greatly increased in width, and here is found granulation tissue which is very dense. The capillaries in the granulation tissue are quite large; adjacent to the capillaries, and at times within them, are to be found many of the large cells with the prominent intranuclear masses. The alveoli in the immediate vicinity of this granulation tissue have thickened walls, they are lined with cuboidal epithelium, and their lumina are much smaller than normal.

In the section from the left lower lobe, the pleura is only slightly thickened, and there is a delicate fibrinous exudate upon it. In many parts of the section the alveoli are filled with polymorphonuclear leucocytes, and the epithelial cells lining these alveoli are larger than normal, but less cuboidal than in many parts of the section from the right lower lobe. These epithelial cells are deeply basophilic in their staining reactions. The alveolar septa are frequently thickened. In many places the alveolar space is filled with edema fluid, and in such alveoli the lining epithelium is quite prominent. These alveolar septa are not especially thickened. In yet other alveoli is compact fibrin with a few polymorphonuclear leucocytes. The smaller bronchi are frequently dilated, and their lumina are filled with polymorphonuclear leucocytes; the epithelium has undergone metaplasia. Throughout the section are many of the prominent cells with the large intranuclear bodies. These are found in the smaller branches of the pulmonary artery, in the capillaries or just beneath the endothelium of these vessels, and also free within the alveoli or in direct continuity with the epithelial cells lining the alveoli.

With the Gram stain, many gram-positive diplococci, often in pairs, are seen in the areas of acute inflammatory exudate.

Spleen. In the pulp are many polymorphonuclear leucocytes and hemorrhages. None of the large cells are found.

Adrenals. The cortical cells are depleted of lipoid. In one of the capillaries is a single large cell apparently undergoing degeneration.

Testes. There is no spermatogenesis. The membrana propia of some of the tubules is thickened and the interstitium is edematous. Small accumulations of lymphocytes, many of which are undergoing karyorrhexis, are found in the interstitial tissue.

Heart, kidneys, pancreas, and *prostate* are essentially normal.

Nuclear Inclusions

In the foregoing protocol, brief reference has been made to the intranuclear bodies present in intestine, liver and lungs. They were found, as has been noted, in large cells for the most part, isolated from the surrounding tissue, although in the lungs they appeared in some cases to be continuous with contiguous alveolar epithelial cells. Even with low magnification the inclusion-containing cells were conspicuous by virtue of their large size, measuring 25 micra. Usually they contained a single nucleus, but cells with two, three or four nuclei were seen. In these multinucleated cells inclusions were sometimes present in only one or more of the nuclei (Fig. 3).

The inclusions themselves varied in size, shape and, to a certain degree, in their staining reaction. The largest forms measured 11 micra in greatest dimension and completely filled the nuclear area with the exception of a narrow, clear zone sharply separating them from the deeply stained nuclear membrane. Projecting into this clear zone from the nuclear membrane were the deeply stained remnants of the chromatin material, often aggregated into a single lenticular clump. In addition smaller and less definitely stainable chromatin granules could be seen lining the nuclear membrane on its inner surface. The smaller forms, of which there were often several within the nucleus, were at times difficult to distinguish from the nucleoli and, as will be pointed out later, stained less distinctively.

The shape was most commonly spherical or ovoid, but elongated sausage-shaped forms were seen (Figs. 1 and 2). The structures appeared to have a certain plasticity, conforming to the shape of the nucleus. They generally were sharply outlined, but occasionally their margin was somewhat fuzzy and indistinct. Forms which were interpreted as degenerative showed fusion with the nuclear membrane, the latter also becoming indistinct.

The bodies showed little evidence of internal structure. At times the central portion stained more intensely; at times also one could see irregular areas of rarefaction. By none of the staining methods used, including Heidenhain's iron-hematoxylin, was it possible to identify minute granules within the inclusions.

The staining reactions conform closely to those given by Lipschütz [14] in his original article on herpetic inclusions. In addition to the reactions given by Lipschütz, it was found that the bodies stained

brilliantly red with Mallory's acid fuchsin — aniline blue, orange G, and that they did not retain the gentian violet in the Gram stain. With the Levaditi stain they became a uniform chestnut brown in contrast to the yellow of the remaining tissue. With Bensley's modification of Altman's mitochondrial stain the inclusions were vividly red. The cytoplasm of the inclusion-containing cells stained purplish with the hematoxylin-eosin, being in general more basophilic than the surrounding tissue cells. The cytoplasm appeared finely granular; but indistinct and well-defined granules were demonstrated only with the Mallory stain, and when present they were intensely fuchsinophilic. These cytoplasmic granules were not found in all of the inclusion-bearing cells. Stained with Scharlach R, some of the larger cells in the lung sections were found to contain finely divided fat globules within the cytoplasm; these did not extend completely to the surface but left a clear superficial zone. The outline of the cell was usually sharp, but it was sometimes possible to discern a paler staining fringe suggesting an ectosarc. One or two cells were found with plasmatic pseudopods, devoid of granules.

Location and Origin of the Cells

In the intestinal lesions the inclusion-containing cells are found in the stroma of the granulation tissue forming the base of the ulcers. Frequently they are near the blood vessels and indeed are often seen lying immediately beneath the endothelium, penetrating it, or within the lumen.

In the liver, they are most numerous in the granulation tissue lining the wall of the abscess, both intra- and extra-vascularly. A few degenerating forms were found in the hepatic sinuses at a distance from the lesions.

In the lung they are found projecting from the alveolar wall and interposed between unaffected alveolar epithelial cells; free in the alveolar cavities; and in the walls of the arteries and veins and within and without the lumina of the alveolar capillaries (Fig. 4). They are also found in the granulation tissue of the pleura. The large forms are not present in the bronchial epithelium or within the lumina of the bronchi.

The bodies as they have been described above, present little difficulty in their recognition. A closer study of the sections, especially those of the lung, discloses the presence of smaller intranuclear

inclusions within a large proportion of the alveolar and bronchial epithelial cells. These are distinguishable with difficulty from nucleoli. In sections stained with aniline fuchsin-methyl green, however, they retain the fuchsin whereas the nucleoli of the bronchial and alveolar epithelial cells, studied in twenty other cases, failed to give this reaction. One is therefore led to believe that these smaller forms may represent early stages in the formation of the larger inclusions.

As regards the origin of the cells it seems certain that there is more than one type of cell affected. In the lung the large inclusion-containing elements are frequently seen in direct continuity with neighboring alveolar epithelium. On the other hand the presence of cells with intranuclear bodies in the granulation tissue and within the blood vessels points to another source, obviously not epithelial. So alien in their appearance, however, are these cells that it is impossible to assign to them a definite origin from any one particular type of mesenchymal element. There is a striking tendency of those cells which are not of epithelial origin to localize in the vicinity of the blood vessels, or immediately beneath the endothelium, or even within the lumen. This suggests, but does not establish, their origin from the adventitial wandering cells.

Discussion

Since there is no possibility of carrying on an experimental study with the material from this case, an interpretation of the nature and significance of the inclusions must for the present rest upon the basis of similar observations recorded by others. There can be no doubt that the inclusions are identical in their morphology and staining reactions with the bodies seen by previous observers in the viscera of infants, and by Lipschütz and others in the tissues of spontaneous and experimental herpes, and in the various neural and visceral lesions produced by the herpetic and related viruses. Various possible interpretations thus present themselves:

1. The bodies (including those occurring in herpes and related conditions) represent merely a peculiar form of nuclear degeneration, not produced by a specific virus.
2. The bodies indicate the localization of the herpes virus or a similar virus within the nucleus of certain visceral cells.

It seems hardly necessary to consider seriously the possibility that the inclusions themselves or the inclusion-containing cells represent protozoan parasites. The mere fact that bodies of this appearance can be produced at will in a variety of tissues by the injection of herpetic virus, effectually disposes of this theory.

While it may be admitted that no positive proof has yet been brought that inclusions of this type are due to an intranuclear virus, there are several strong arguments against the first possibility, namely, that they are merely non-specific nuclear degenerations. They are present in cells which are evidently actively mobile. It seems unlikely that cells showing such marked nuclear degeneration should preserve their capacity for ameboid motion. Furthermore, individual cells are selectively involved whereas in the ordinary degeneration large numbers of the cellular elements are simultaneously affected. Again there are no obvious degenerative alterations in the cytoplasm of the inclusion-containing cells apart from the occasional presence of fat. On the basis of the above considerations, and above all because of the infrequency of their occurrence in routine pathological material, it seems safe to infer that the inclusions are not produced by a banal, non-specific nuclear degeneration.

There is but one argument, a very convincing one, however, in favor of the second possibility, that the inclusions in this case are caused by a virus identical with or closely related to the herpetic group. That argument is the morphological and tinctorial identity of the structures with those occurring in spontaneous and experimental herpetic lesions. We have recently had opportunity to compare the inclusions with those produced in the rabbit cornea and brain by the inoculation of the contents of a vesicle from a case of herpes labialis, occurring in one of the laboratory workers. The inclusions correspond in every particular.

Recent work has made it probable that viruses of this nature are more widely distributed, and perhaps more persistent, than had hitherto been suspected. Although it could not be established that the virus isolated by Rivers and Tillett [25] from cases of varicella was actually the cause of that disease, the virus obtained did produce in the skin, testicles and corneae of rabbits intranuclear inclusion-bodies apparently identical with those described in herpes, and situated for the most part within endothelial leucocytes as well as within the epithelial cells. Most interesting also is the transmissible virus

obtained by Miller, Andrewes and Swift [26] from the testes of rabbits inoculated with the blood and joint fluids of patients suffering from acute rheumatic fever, which had the power of producing inflammatory lesions with typical nuclear inclusions in the testes, skin, pericardium and heart muscle of inoculated animals. Still more interesting and puzzling is the isolation by Andrewes and Miller [27] of an apparently identical virus from the testes of supposedly normal rabbits. Flexner and Amoss [28] have also reported obtaining a very virulent neurotropic virus from the spinal fluid of a case of vascular and neural syphilis.

That man may harbor the herpes virus in a latent state is suggested by the experiments of Bastai and Busacca.[29] They examined the blood and spinal fluid of a large series of patients who had had no herpetic eruption for a long period. Positive results were obtained by corneal inoculation in rabbits in a large proportion of cases.

We are obviously at the beginning of our knowledge of this interesting group of diseases and it would be ill-advised and premature to say that the case presented illustrates a hitherto unrecognized disease caused by a virus akin to the herpes viruses. And yet, after studying the rather confused literature in this field, it seems less improbable than it did at first glance. There is first of all the closest possible morphological identity between the nuclear inclusions found in our case and those which are coming to be generally recognized as characteristic of the herpes-encephalitis type of virus. Secondly, there is growing evidence that the lesions produced by viruses of this type are not necessarily restricted to neuro-ectodermal structures, as maintained by Levaditi,[30] but may under suitable conditions be produced in such diverse tissues as trachea, bronchi, ovary, testis, adrenal (Goodpasture and Teague [31]). The virus may be present in the blood, spinal fluid or buccal secretions, and may be introduced into the body by direct inoculation of liver, pancreas, ovaries, thyroid, salivary glands, kidneys and spleen (Teissier, Gastinel and Reilly [32]). Even if we assume, as is probable, that the peculiar large cells with inclusions are to be interpreted as an infection with a virus of this group, it is still impossible to prove that this virus is the primary cause of the intestinal ulcers, liver abscess and pneumonia. The presence of bacteria in the liver abscess and pulmonary lesions makes it still more difficult to arrive at a conclusion. In favor of the view that the supposed virus is concerned in the lesions are:

First, the occurrence of the inclusion cells in numbers only at the site of the lesions; second, the fact that viruses of this type are capable of inciting an inflammatory reaction, in some cases of considerable severity; and third, that we are unable to attribute the changes to any other discoverable agent.

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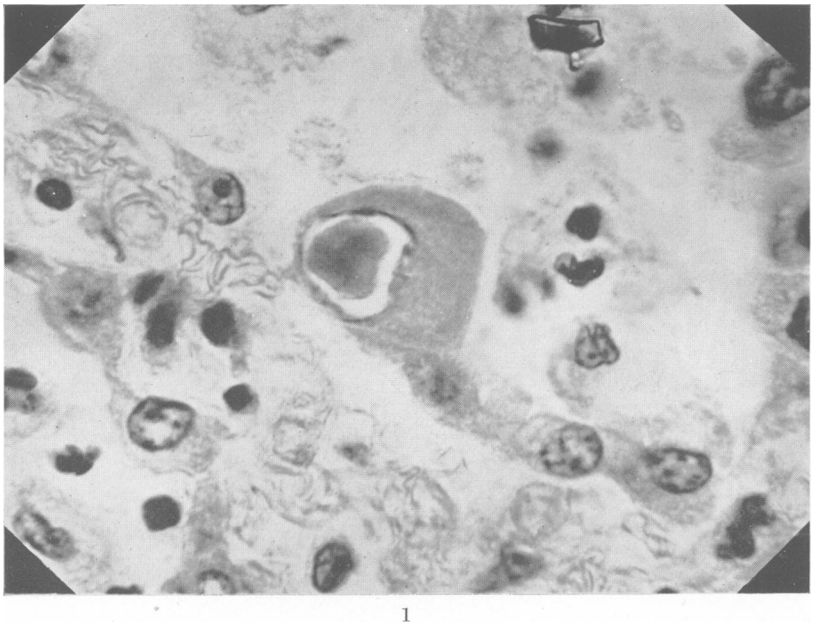
DESCRIPTION OF PLATES LXXII–LXXIII

Fig. 1. Cell with intranuclear inclusion, continuous with lining epithelium of lung alveolus. (Hematoxylin-eosin stain.) X 1050.

Fig. 2. Inclusion-containing cell lying within capillary in wall of liver abscess. (Hematoxylin-eosin stain.) X 1050.

Fig. 3. Lung: Multinucleated cell in alveolus. Two of nuclei contain typical inclusions. (Eosin-methylene blue stain.) Oc. 10x. Imm. 1/12.

Fig. 4. Lung: Two inclusion-containing cells lying just outside endothelium of a capillary. (Eosin-methylene blue stain.) Oc. 10x. Imm. 1/12.



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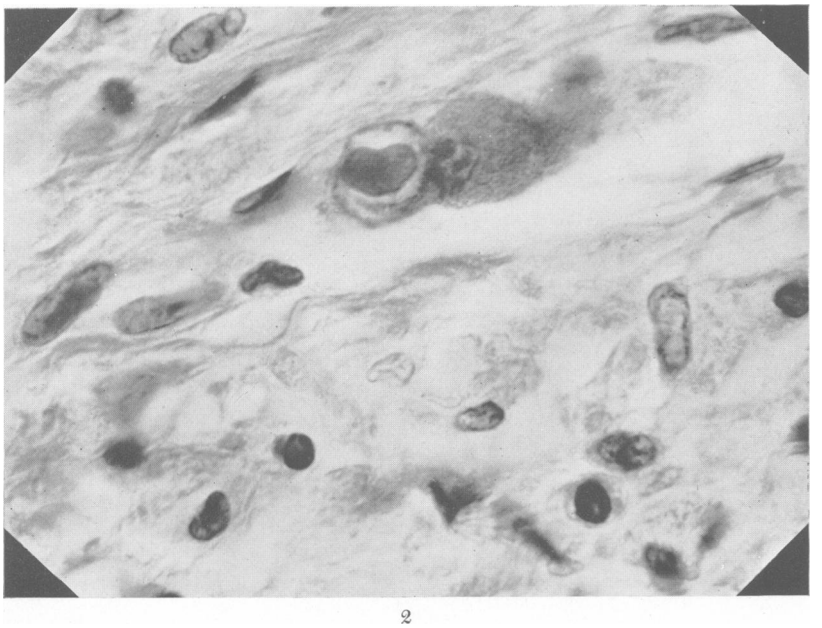

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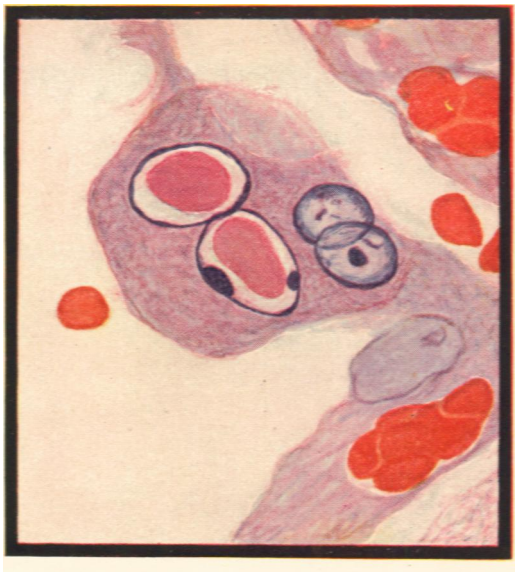

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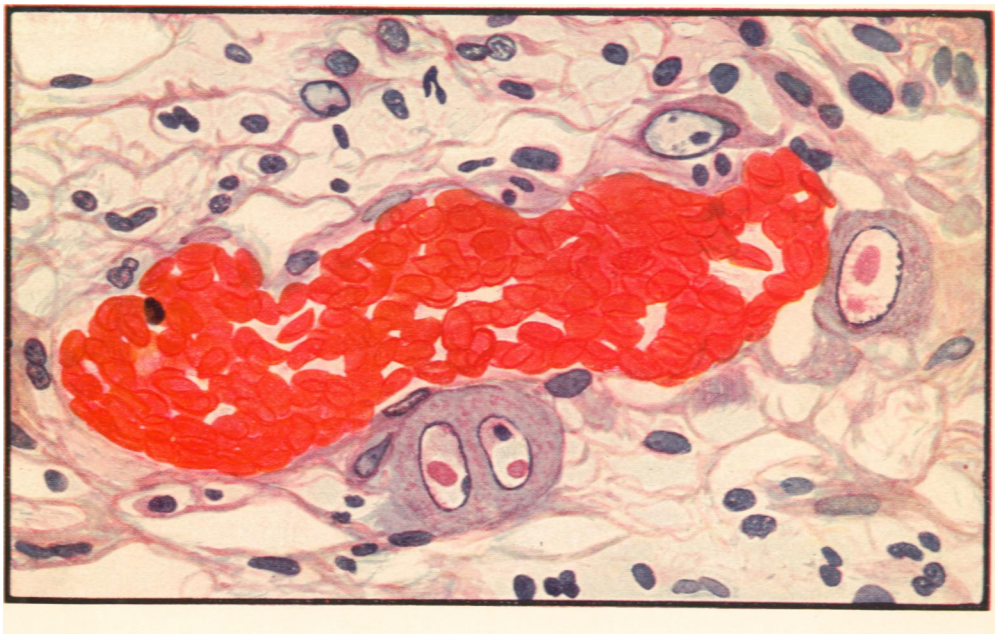

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