A FILTERABLE VIRUS PRESENT IN THE SUBMAXILLARY GLANDS OF GUINEA PIGS.

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During recent years much attention has been paid to peculiar alterations in the morphology of certain cells in lesions associated with the presence of filterable viruses. These changes are especially constant and well marked in the lesions of spontaneous and experimental herpes simplex. Indeed, they occur so constantly in this condition that Goodpasture and Teague (1) have used the presence of these cells as a guide in tracing the passage of the virus through the tissues of the infected animal. The uniformity of the cellular changes in herpes simplex and the constancy with which they occur renders this the most satisfactory of the virus diseases to be used as an example in discussing analogous and related conditions. Cells derived from the endothelium, epithelium, or mesenchyme may show these changes and although there may be variations in the appearance of the alterations in the different cells, on the whole they are very similar, whether the cells involved be connective tissue cells, epithelial cells of the cornea, or large ganglion cells of the central nervous system.

The characteristic features of these changes, as seen in tissues stained with eosin and methylene blue, are the following. The nucleus takes on a vesicular character and the limiting membrane is deeply stained with the basic dyes. Usually the inner surface of the membrane is irregular, as though the basic staining material were collected in granules or small clumps. A few of these granules are much larger than the others and these are considered to represent the nucleoli of the unaltered nucleus. Within the nucleus, usually at the center, is a round or oval body which stains either faintly or, more often,

deeply with acid dyes. The depth of staining depends to some degree, however, upon the length of exposure. The red-staining material may occupy not more than half, or even less, of the nuclear space or it may occupy almost the entire nucleus. In any case it is sharply limited from the surrounding nuclear material by a clear unstained halo. The material itself is usually granular, never hyaline or dense; it appears as though composed of a multitude of very fine granules compressed into a ball or mass.

Nuclear changes which cannot at present be differentiated from those occurring in the lesions of spontaneous and experimental herpes simplex also occur in the skin lesions of herpes zoster, in the skin lesions of varicella, and in the lesions experimentally produced in rabbits by the Virus III of Rivers and Tillett (2). Nuclear changes resembling to some extent those occurring in herpes simplex are also found in a variety of conditions, especially diseases of animals, such as, epidemic encephalomyelitis of horses, or Borna's disease, fowl-pox, certain diseases of fishes, etc.

Except for a few reported instances, nuclear changes like those occurring in herpes simplex have so far been found only in conditions in which the presence of a filterable virus has been demonstrated or in which the association of a virus of this group is very probable. The experimental production of these lesions except by the injection of filterable viruses has, in our hands, been unsuccessful. It is true that Luger and Lauda (4) have mentioned the occurrence of similar structures in a case of salvarsan dermatitis. But even though these lesions should be present in isolated instances of this kind, it would be necessary to demonstrate the absence of a filterable virus in the given instance before the present conception of the direct relationship between these nuclear changes and filterable viruses would become untenable.

Lipschütz (3) has considered that these nuclear changes represent "nuclear inclusion bodies" in the sense of Prowazek. He has collected all the conditions in which nuclear inclusions occur, and also the conditions in which unusual bodies or structures are found in the cytoplasm of the cells, into a great group of virus diseases, the causative agent of which he classes with the *Chlamydozoa-strongyloplasma*. Lipschütz has maintained that the bodies or structures seen within the nucleus represent a specific reaction of the cells to a living virus.

The bodies are not considered to be masses of parasites but are held to represent reaction products, associated with which is the virus.

This conception of Lipschütz has not been universally accepted, however. Luger and Lauda (4) who have devoted much study to the nature of these nuclear changes, maintain that they do not represent "inclusion bodies" in the sense of Prowazek, but that they are the result of a non-specific type of nuclear degeneration, which these authors call "oxychromatic degeneration." They refer to the observations of Heidenhain, who described two varieties of nuclear, chromatic substance, basic chromatin and oxychromatin. During nuclear degeneration occurring under certain conditions, the oxychromatin tends to collect in the center of the nucleus and the basic chromatin at the periphery. According to these writers the typical nuclear changes seen in the lesions of herpes simplex represent the final stages in this separation of oxy- and basic chromatin.

At the present time the evidence in favor of either of these views is not convincing. Our own observations suggest that the inclusion bodies are produced by the accumulation of a finely granular material which at first is scattered throughout the nucleus. This material which, in its scattered form, is very faintly acidophilic, takes on a deeper and deeper red stain as it accumulates into a mass, until the typical nuclear changes become manifest. The earlier stages of this process, however, are difficult to detect and usually only the fully developed nuclear "inclusion body" is seen. Until further knowledge concerning the chemical nature of these structures is obtained, it is not important, at least in the present connection, to decide whether we shall speak of "nuclear inclusions" or of "oxychromatic degeneration" of the nucleus. It is very important, however, to know whether these changes represent characteristic lesions due to the action of filterable viruses or whether they represent a form of degeneration which occurs under a great variety of conditions producing a non-specific injury to the cell. The burden of evidence at present points to the former concept, for it would be very surprising, in view of the careful study which has been made of cellular changes under various pathological conditions, that this striking nuclear alteration should have been frequently overlooked.

In a few isolated instances, pathologists have reported finding peculiar nuclear changes in human pathological material. VonGlahn and Pappenheimer (5) have recently collected the reports of sixteen human cases, and have added a case of their own, in which, at autopsy, in various viscera, cells with striking nuclear abnormalities were present. While the descriptions of these nuclear changes in the individual cases differ to some extent, in most instances they are sufficiently alike to justify the conclusion that the various writers were dealing with identical or closely related conditions. All of the cases reported, except that of VonGlahn and Pappenheimer, were in infants under 1 year, five of these were still-born. Five of them probably suffered from congenital syphilis, in one nephritis was present, in one pneumonia, one had "green stools," edema of feet, and bronchitis, another one hydrocephalus and focal interstitial nephritis. The case of VonGlahn and Pappenheimer was that of a male aged 36 years, who suffered from an abscess of the liver. Certain cells in the intestines, liver, and lungs were found to be of very large size, measuring at times 25 micra in diameter. Some of these were multinucleated. Within the nuclei of these cells were large acidophilic masses. In the lung these large cells were usually in continuity with the epithelium, but cells of granulation tissue and of the blood vessels also contained the nuclear inclusion masses.

In addition to these cases collected by VonGlahn and Pappenheimer, L. Jackson (6) has reported finding an ameba-like organism in the kidneys of a child. This child, 15 months old, suffered from diphtheria and died suddenly. At autopsy lesions of bronchopneumonia were found. In the kidney tubules were large cells which the writer interpreted as amebæ. It seems from the description that these may have been structures similar to those present in the cases collected by VonGlahn and Pappenheimer. Indeed, Jackson stated that the structures were similar to those described by Ribbert (7) and by Smith and Weidman (8), whose cases were included by VonGlahn and Pappenheimer in their series.

In most of the earlier human cases the nuclear inclusions were thought to be due to the presence of parasites, amebæ, or sporozoa, in the later cases they were described as nuclear degenerations. One of the cases in infants was reported by Goodpasture and Talbot (9) in 1921. These writers drew attention to the similarity between the nuclear changes in this case and the nuclear changes in the epithelial cells in varicella which were first described in 1906 by Tyzzer (10). They also called attention to peculiar cellular changes present in the epithelial cells of the salivary glands of guinea pigs which were first described by L. Jackson in 1920. Goodpasture and Talbot studied these cells in guinea pigs and concluded that the condition was an example of cellular transformation similar to that occurring in the lesions of infancy. They thought that there was no evidence that these cellular and nuclear changes were due to some intracellular infection. Von Glahn and Pappenheimer were the first to fully identify the large cells occurring in human cases with the abnormal cells met with in herpes simplex and related conditions. They regarded the intranuclear masses seen in their own case as nuclear inclusions, "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 tissues of spontaneous and experimental herpes, and in various neural and visceral lesions produced by the herpetic and related viruses." These writers were unable to carry on any experimental study, but offer the suggestion that the lesions in this case may have been related to the presence of an unknown virus.

Cellular Changes in the Submaxillary Glands of Guinea Pigs.

Unusual changes in the submaxillary glands of guinea pigs were first described by L. Jackson (11) under the title, "An intracellular protozoan parasite of the ducts of the salivary glands of the guinea pig." These structures were found in 26 of 48 pigs examined. She interpreted them as protozoa, probably coccidia. We have had no difficulty in confirming the observation of Jackson that in the ducts of the submaxillary glands of guinea pigs there are found unusual and striking structures which on first observation suggest a parasitic origin. However, we have been unable to differentiate the different stages of development resembling those of protozoa which Jackson described. The structures we have observed, however, conform in all particulars with those shown in the illustrations in Jackson's paper and there can be no doubt that we are dealing with the same abnormalities. We have examined the submaxillary glands of 75 guinea pigs over 6 months of age. Sections from these glands have been stained in eosin and methylene blue, and in 63 of the glands, or 84 per cent, these unusual structures have been found in larger or smaller numbers. From our own study we have identified them as swollen epithelial cells (Fig. 1). The nucleus of each of these cells contains a mass of granular material which is definitely acidophilic. The altered cells are found chiefly in the ducts of the serous portion of the gland, though, in a few instances, they have been seen in the mucous portion. The cells lie either on the basal membrane contiguous to the unaltered epithelial cells or within the lumen of the duct, evidently having pushed forward during the process of hypertrophy. The altered cells occasionally are not more than twice the usual size (Fig. 2) but in most instances they are much larger than this, up to 40 micra in diameter. The large size and red staining of the nuclear inclusions render them easily visible under the low power of the microscope. In ducts cut obliquely or longitudinally, not infrequently six or eight, or even more,

of these structures can be seen within a single duct; in other ducts not more than one or two of these cells are present. In the sections from certain glands only one or two groups of ducts containing the altered cells are seen, in other glands large numbers of ducts are involved. About the ducts containing these cells there is usually a cellular reaction and this is often of assistance in locating the areas in which the altered cells are present. The tissue reaction about the involved ducts consists of mononuclear cells, lymphocytes, and large cells with vesicular nuclei (Fig. 3). Polymorphonuclear leucocytes are rarely seen and never predominate. The cytoplasm of the involved cells usually takes on a light blue color which, however, is considerably more intense than that of the cytoplasm of the unaltered cells. The nuclear membrane is somewhat irregular, and stained deeply with the basic dye. At or near the center of the nucleus, and separated from the membrane by a clear unstained area or halo, is a mass which is stained red. The color of this mass is usually definitely deeper than that of the typical herpes simplex inclusion bodies. This mass may occupy not more than one-fourth the nuclear space but it not infrequently fills almost the entire space, leaving a very narrow halo between it and the membrane. Within this clear space there are seen two, three, or even more, irregular masses, 0.5 to 2 micra in diameter. These masses stain deeply with the basic dye. They may lie approximated to the inner surface of the membrane, or upon the acidophilic mass, or free in the space. Upon careful focussing in almost all instances radiating irregular lines passing from the mass to the inner surface of the membrane can be observed. These bands also stain with the basic stain. Occasionally a very faint network of strands between the various radiating bands is apparent. The central mass is usually oval or round, the margin is slightly irregular, and the mass appears finely granular. At times some of the granules appear larger and somewhat refractile. The presence of these granules may have been responsible for the interpretation of these structures as protozoa and for the descriptions of an organized structure. It is evident from this discussion that in their main features the cells resemble those occurring in the lesions of herpes simplex. On account of their much greater size and the darker color of the inclusion bodies the resemblance is, at first, not evident.

Sections have been made from the submaxillary glands of a number

of animals of other species to determine whether identical or analogous structures are also present in them. The glands of eight full grown rabbits, three rats, a mouse, a dog, and a cat, have been studied but no structures resembling the large cells seen in guinea pigs have been found. Although these peculiar cells were present in the submaxillary glands of 84 per cent of the guinea pigs over 6 months old, in very young pigs they were present only occasionally. Of 43 young guinea pigs, most of them less than 1 month old, the large cells were found in the submaxillary glands in only three instances and then they were few in number and confined to one or two groups of ducts. It seems, therefore, that if these lesions are due to an infection with a virus, the infection usually occurs only after the first few weeks of life but finally almost all guinea pigs become infected.

EXPERIMENTAL.

Transmission of Infection to Young Guinea Pigs.—An attempt was first made to demonstrate whether or not an infectious process is responsible for these lesions. The most obvious mode of procedure would be the inoculation of material from glands in which the lesions were presumably present into other glands which were not already the seat of the lesion. Since, however, the lesions are found in almost all older guinea pigs it would be necessary to inject the material into the glands of very young guinea pigs free from infection. Injections into the submaxillary glands of very young guinea pigs, however, are not without difficulties, and moreover, there is always the chance that even the young guinea pigs are already infected. Nevertheless, a number of experiments have been made in which material from the glands of old guinea pigs was injected into the glands of young ones. The pigs were killed at varying periods, from 2 to 12 days, following the injection and sections were made from the submaxillary glands. In nine out of eleven injected glands a marked mononuclear reaction involving the interstitial tissue was present. In certain areas cells were found which showed nuclear changes, with acidophilic inclusions, exactly resembling those seen in the lesions of herpes simplex. Occasionally similar nuclear changes were seen in duct cells of normal size. In a very few instances the duct cells showing these changes were

somewhat swollen but they did not equal in size the very large cells in the glands of full grown guinea pigs. Most of the transmission experiments, however, have been made by injecting the material in other locations in young pigs where these cells do not occur, such as the testicle, brain, lung, and tongue. The most striking results were obtained when the injections were made into the brain and in most of the experiments this has been the site of inoculation.

Minor modifications of the technique of the brain injections were made in certain experiments and the results were not always identical but the following description is typical of this experimental method and of the results obtained. A full grown guinea pig is killed and the submaxillary glands removed under sterile precautions. A small piece is removed from each gland and placed in Zenker's solution, to be later examined in order to make certain that typical lesions are present. Aerobic and anaerobic cultures of the submaxillary gland are made at this time to exclude the possibility of bacterial infection. The remainder of the glands is cut into small pieces with scissors and ground in a mortar in about 2 cc. of Locke's solution. This is then centrifuged at low speed for a few minutes, to remove the large particles, and the supernatant fluid is used for injection. 0.1 cc. of this suspension is then injected directly into the brain[1] of a guinea pig less than 1 month old. 24 hours following this injection the temperature of the injected pig is usually not elevated and the animal appears normal. After 48 hours, however, the temperature frequently becomes elevated, 105–106°, but without the animal showing any marked symptoms. On the 3rd day the guinea pig appears sick, the hair is raised, the animal fails to move about in the cage, and the temperature continues elevated. On the 4th day, the symptoms have become more marked. The animal now begins to show signs of heightened nervous irritability as indicated by tremors and slight convulsive movements. On the 5th day it is usually very ill, has irregular jerking movements, is unable to rise when placed on its side, and death ensues. When the brain is removed no gross abnormalities beyond congestion are seen. Cultures are made to rule out the possibility of bacterial infection, and the brain is placed in Zenker's solution.

[1] Guinea pigs were anesthetized with ether before injection.

Later when microscopic sections are examined there is found well marked exudate over the surface of the entire brain, including the cerebellum (Fig. 4). The exudate consists chiefly of mononuclear cells, lymphocytes, and large cells with vesicular nuclei. There is considerable edema and the blood vessels of the membranes are distended and filled with blood. In contrast with the lesions resulting from intracerebral infection of the brain with herpes simplex virus, very slight if any changes can be detected in the brain itself, the blood vessels of which appear normal. The most striking feature is the presence in the meningeal exudate of large numbers of cells, each of which contains an acidophilic mass within the nucleus. These cells resemble in all particulars the cells containing nuclear inclusion bodies which occur in herpes simplex and related conditions (Fig. 5). The number of abnormal cells present in the guinea pig lesion is much greater than the number usually present in the lesions following injection of herpes simplex virus or Virus III of Rivers. In no case have very large cells like those found in the submaxillary gland been found. 54 young guinea pigs have received intracerebral inoculations of an emulsion of the submaxillary gland of full grown guinea pigs. Most of these animals showed symptoms similar to those described, though in some the symptoms were delayed. The animals died or were killed at various times from the 2nd to the 12th day following the injection. In 48 of the guinea pigs, or 89 per cent of those injected, lesions as described above were found.

Emulsions of the submaxillary gland of full grown guinea pigs have also been injected into the testicles of sixteen young guinea pigs. In all cases a histologic examination of the submaxillary gland was made to make certain that the specific lesion was present in the material used for injection. 0.1 cc. of the emulsion was usually injected into each testicle. The inoculated guinea pigs showed some elevation of temperature for several days, but they developed no other symptoms. The animals were killed at various intervals from 4 to 10 days following the injection, and both testicles were removed. Histologic examination was later made of one testicle from each animal and in almost all cases some degree of cellular infiltration was found. In twelve instances, or in 75 per cent of the animals inoculated, cells containing typical nuclear inclusion bodies were present. These

were either in cells of the interstitial tissue, or in cells of the tubules, or in both.

Injections of an emulsion of the submaxillary glands of full grown guinea pigs were also made into the tongues of young guinea pigs. These animals developed no symptoms. They were killed and the tongues were removed on the 3rd, 5th, and 9th days following the injection. Microscopic study of these tongues showed a localized cellular reaction in the stroma, and cells containing typical nuclear inclusion bodies were present. A tongue removed on the 9th day after inoculation showed the most marked cellular infiltration. In no instance was the epithelium of the tongue involved.

Inoculations were also made into the lungs of three guinea pigs. One of these animals showed a rise of temperature on the 7th day, and was killed. The lung showed no gross changes but on microscopic examination a circumscribed mononuclear cellular infiltration was found. In a few of the alveoli in the involved area, large mononuclear cells containing typical nuclear inclusion bodies were present. Two other guinea pigs inoculated in the lungs were killed on the 11th and 22nd days after injection. Although a mononuclear cell infiltration was present in the lungs, no cells with nuclear inclusion bodies were found. The injection of emulsions of submaxillary glands of full grown guinea pigs into young guinea pigs therefore has quite regularly resulted in the production of a subacute inflammatory reaction and the appearance of cells containing nuclear inclusion bodies morphologically identical with those seen in herpes simplex and allied conditions.

Control Experiments.—These experiments suggest that the submaxillary glands of full grown guinea pigs contain a virus which is responsible for the reaction in the young guinea pigs, and it seems probable that the large cells and the reaction seen in the older pigs is a manifestation of natural infection with this virus. This view is supported by the following observations. Four young guinea pigs were inoculated in the brain with an emulsion of the submaxillary glands of other young guinea pigs, presumably tissue containing no large cells with nuclear inclusions. Subsequent microscopic examinations showed that there were actually no lesions in these glands. The results of these inoculations were entirely negative. Nevertheless, it seemed possible that the reaction observed in the tissues of young guinea pigs

following the injections of the glands of old guinea pigs was of a non-specific nature, and that any glands of external secretion might contain irritating substances which on injection into the brains of young guinea pigs might give rise to an inflammatory reaction, and that under these conditions cells containing nuclear inclusions might appear. Consequently, two young guinea pigs were injected with emulsions made from the pancreas of an old guinea pig and seven young guinea pigs were inoculated with emulsions made from the submaxillary glands of full grown rabbits. No symptoms resulted from any of these inoculations and the microscopic examination of the brains failed to reveal any lesions. Moreover, nine young rabbits, inoculated intracerebrally with an emulsion made from the submaxillary glands of full grown rabbits, remained well and no lesions were found in the brain. The results so far reported, therefore, indicate that an infectious agent is probably responsible for the lesions found in the submaxillary glands of full grown guinea pigs and that the lesions in young guinea pigs resulting from the inoculation of the emulsion of submaxillary glands of full grown guinea pigs are due to this infectious agent.

Transmission in Series.—This conclusion would only be justifiable, however, if it were possible to reproduce the lesion after passage through a series of animals. But in spite of the fact that young guinea pigs which have received intracerebral inoculations of emulsion of submaxillary glands of old guinea pigs almost invariably show severe symptoms with marked cerebral lesions, the inoculation of emulsions of the brains of these experimentally infected animals into other young guinea pigs has invariably failed to cause symptoms or to give rise to lesions. But even though the second animal has shown no lesions or symptoms the inoculations have been continued from animal to animal in series through as many as six guinea pigs with the hope that in this way the virus might gradually acquire greater virulence. But the results have all been negative. Attempts were also made to transmit the virus by inoculating from testicle to testicle or from salivary gland to salivary gland. Except in one instance, to be mentioned later under Series C, when a testicle to testicle inoculation gave a mildly successful result, these experiments were also unsuccessful. Recourse was then had to the expedient of varying

the seat of inoculation, inoculating first in the brain and then in the testicle or *vice versa*. A large number of these experiments have been carried out and in seven instances has it been possible to obtain a positive result on the second transfer. In three of these experiments the transfer was made from brain to testicle, in three others from testicle to brain, and in one from testicle to testicle. In one series it has been possible to reproduce the lesions through a series of three transfers (Series T). In this instance the transfer was made through testicle, brain, testicle. The following are abstracts of the protocols of the experiments in which positive results were obtained.

Series M—Brain to Testicle.—March 1, 1926, Guinea Pig 1 received an injection into the brain of 0.1 cc. of an emulsion of the submaxillary glands of two full grown guinea pigs. In this animal the temperature was elevated from the 3rd to 7th days following the injection, reaching 105.8°. The guinea pig showed marked symptoms, became prostrated, and was killed on the 8th day. Sections from the brain of this animal showed a marked meningeal exudate, and typical nuclear inclusions were found in many of the cells. The remainder of the brain was emulsified and 0.1 cc. of the emulsion was injected into each of the testicles of Guinea Pig 2. In this animal the temperature was elevated to 104.4–105° from the 3rd to the 11th days. It was killed on the 11th day. Sections from the left testicle showed a mononuclear infiltration and a few cells contained typical nuclear inclusions.

Series N—Brain to Testicle.—March 26, 1926, Guinea Pig 3 received an intracerebral injection of 0.1 cc. of an emulsion made from the submaxillary glands of two full grown guinea pigs. The animal became very sick on the 3rd day and was killed. Sections from the brain showed a marked meningeal infiltration with numerous cells showing typical nuclear inclusions. The remainder of the brain was emulsified and 0.1 cc. of the emulsion was inoculated into each of the testicles of Guinea Pig 4. The animal showed an elevation of temperature from the 7th to the 9th days, ranging from 104.4–105.2° and was killed on the 9th day. Sections from the testicle showed a slight cellular reaction and in a few cells typical nuclear inclusions were found.

Series P—Brain to Testicle.—March 18, 1926, Guinea Pig 5 received an intracerebral injection of 0.1 cc. of an emulsion of the submaxillary glands of two full grown guinea pigs. The animal showed an elevation of temperature and marked symptoms, and died on the 4th day. An emulsion made from the brain of this animal was injected into both testicles of two young guinea pigs, Nos. 6 and 7. On the 7th day Guinea Pig 7 showed a temperature of 105.4° and was killed. In sections of the testicles, however, no lesions were found. Guinea Pig 6 had a temperature ranging from 104.9–105.6° on the 7th to 9th days. The animal was killed on the 9th day and in sections of the testicle a circumscribed mononuclear

reaction was seen and in the cells of the tubules in this area there was found a small number of cells, in the nuclei of which were typical acidophilic masses.

Series C—Testicle-Brain: Testicle-Testicle.—January 5, 1926, Guinea Pig 8 received an injection into the left testicle of 0.1 cc. of an emulsion made from the submaxillary gland of an adult guinea pig. This animal had an elevated temperature from 104.8–105.7° on the 6th, 7th, and 8th days, and it was killed on the 8th. Sections made from the testicle showed a marked infiltration with mononuclear cells, and in a small number of the tubule cells, typical nuclear inclusions were found. The remaining portion of the testicle was emulsified in Locke's solution and 0.1 cc. was injected into the brain of Guinea Pig 9 and into the left testicle of Guinea Pig 10. Guinea Pig 9 showed an elevation of temperature on the 5th to the 9th days, ranging from 104.7–105.8°. On the 9th day this animal was killed and sections made from the brain showed a circumscribed meningitic exudate in which were numerous cells showing typical changes with nuclear inclusion bodies. The testicle of Guinea Pig 10 was removed on the 9th day. Sections from this showed a very slight interstitial reaction, a few of the cells of which showed typical nuclear inclusions.

Series T—Testicle-Brain-Testicle.—April 15, 1926, Guinea Pig 11 received an inoculation into the left testicle of an emulsion made from the submaxillary glands of two full grown guinea pigs. The temperature was elevated on the 6th and 7th days, rising to 106° on the 8th day. On this day the animal was killed, the testicle removed, and placed in 50 per cent glycerol. On the following day the testicle was washed free of glycerol and emulsified in Locke's solution. 0.1 cc. of this emulsion was injected into the brain of each of two guinea pigs, Nos. 12 and 13. Beginning with the 3rd day following the injections, both animals showed an elevation of temperature, ranging between 104° and 106°. On the 7th day Guinea Pig 12 was killed. A small piece of the brain was retained for microscopic study and the remainder was placed in 50 per cent glycerol. On the 9th day following the injection, Guinea Pig 13 was killed, the brain removed, a small piece placed in Zenker's fluid for microscopic study, and the remainder placed in 50 per cent glycerol. The examination of the sections of the brains of Guinea Pigs 12 and 13 revealed, in each case, a localized meningeal reaction, and a moderate number of the cells showed characteristic changes with the nuclei containing acidophilic masses.

The brains of Guinea Pigs 12 and 13 were preserved in glycerol for 25 and 27 days, respectively. An emulsion was made from this glycerolated material on May 28 and 0.3 cc. of this was inoculated into the brain of Guinea Pig 14 and 0.1 cc. into each testicle of Guinea Pig 15. Although Guinea Pig 14 had some fever there were no marked symptoms and it was killed on the 11th day following the injection. Sections made from the brain of this animal showed no lesions of any kind.

Guinea Pig 15 had moderate fever up to 105°. It was killed on the 11th day, and the testicles were removed. The left testicle was preserved for further inoculation and the right placed in Zenker's solution. Sections made from this testicle

showed a slight cellular infiltration and edema and in the tubules a small number of cells with nuclear inclusions were found.

It will be seen from these protocols that in seven instances it has been possible to produce lesions in two animals in series and in one other instance in three animals in series. Many variations in the technique have been made in the hope of transmitting the virus indefinitely. Transfers were made at various periods following the infection, even as early as the 2nd day. In other experiments, instead of employing the entire brain tissue for the emulsion, only scrapings from the surface of the brain were used, since the lesions containing the cells with nuclear inclusions are found only in the meningeal exudate. In other experiments it was thought possible that some stimulating or accessory substance present in the submaxillary gland might be necessary for infection and that when transfers were made from brain to brain this factor would of course be lacking. Consequently, emulsions of submaxillary glands of very young pigs were added to the brain emulsions of infected guinea pigs which were used for transfer. The results with none of these methods, however, proved successful.

Although it was not possible to transmit the virus indefinitely, the results obtained offer considerable evidence that in this condition we are dealing with an agent which reproduces itself, and, therefore, presumably is a living virus. There has been no indication so far obtained that the virus on passage tends to become more virulent. Indeed, the opposite effect has been observed. In all cases when any effect has resulted from the second transfer the lesions have been less well marked than those following the first transfer. Although the virus may be preserved, at least for short periods, in glycerol, no evidence was obtained that any increase in virulence or infectivity occurs during this time.

Infectiousness for Other Species of Animals.—Attempts have been made to reproduce the lesions in other species by the inoculation of an emulsion of the submaxillary glands of full grown guinea pigs into the brains of nine young rabbits, five young rats, and two young kittens. These animals all remained well and sections of the brains of these animals, which were killed at varying intervals, showed no lesions.

Properties of the Virus.

Thermolability of the Infectious Agent.—On February 3, a full grown guinea pig was killed and the submaxillary glands removed with sterile precautions. Histologic sections prepared from small pieces of these glands were subsequently shown to contain the specific lesions. Aerobic and anaerobic cultures of the glands made at this time remained sterile. The glands were ground thoroughly in a mortar and suspended in 5 cc. of Locke's solution. After centrifuging for a few minutes at low speed the suspension was divided into two parts; one half was heated at 54°C. for 1 hour and the other was stored on ice during this period. 0.1 cc. of the unheated suspension was then injected intracerebrally into Guinea Pigs 16, 17, and 18, and 0.1 cc. of the heated suspension was injected intracerebrally into each of the guinea pigs, Nos. 19, 20, and 21. All the pigs were less than 1 month old. One of the animals inoculated with the heated material, Guinea Pig 20, was found dead on the 2nd day after inoculation. The brain was removed and prepared for histologic examination. One of the animals which had been inoculated with the unheated material, Guinea Pig 18, was killed on the same day and sections were prepared from the brain for comparison with the sections from Guinea Pig 20.

On February 6, a marked contrast was noted between Guinea Pigs 16 and 17, inoculated with the unheated suspension, and Guinea Pigs 19 and 21, inoculated with the heated suspension. The former appeared unsteady on their feet and their hair was ruffled, whereas Guinea Pigs 19 and 21 appeared perfectly normal. All four guinea pigs were killed on the 3rd day following injection, and histologic sections were prepared from the brains. Microscopic examination of the brains of Guinea Pigs 16, 17, and 18 all showed a marked mononuclear exudate into the meninges in which numerous cells with characteristic nuclear inclusion bodies were found. On the other hand, sections from the brains of Guinea Pigs 19, 20, and 21 showed no meningeal exudate and no cells containing nuclear inclusions.

Resistance of the Virus to 50 Per Cent Glycerol.—On May 17 small pieces of the submaxillary gland of three full grown guinea pigs were placed in a small sterile bottle containing equal parts of glycerol and Locke's solution. The bottle was then stored on ice. A small piece of each gland was prepared for histologic examination and was subsequently shown to contain the specific lesion. The remainder of the glands was emulsified in the usual manner and 0.1 cc. was injected intracerebrally into each of two young guinea pigs, Nos. 22 and 23. Guinea Pig 22 was moribund on the 5th day following injection and was killed. Guinea Pig 23 was found dead on the 7th day. Microscopic study of the brains of Guinea Pigs 22 and 23 showed the usual brain lesion with typical nuclear inclusion bodies. On May 28, 11 days after placing the submaxillary glands in 50 per cent glycerol, the pieces of tissue were washed free of glycerol, ground in a mortar, and suspended in Locke's solution. After centrifuging a few minutes at low speed, 0.1 cc. was injected intracerebrally into each of the guinea pigs, Nos. 24 and 25, both less than 1 month old. Guinea Pig 25 was found dead on the 7th day following the

injection and Guinea Pig 24 was found dead on the 10th day. Microscopic study of the brains of both of these animals showed a meningeal exudate containing cells which showed typical nuclear inclusion bodies.

In another experiment in which the submaxillary gland was exposed to 50 per cent glycerol for 7 days, a similar result to that described above was obtained. In another instance the submaxillary gland was exposed to 50 per cent glycerol for 28 days. The injection of this material into the brains of young guinea pigs failed to produce the characteristic cerebral lesions.

Filterability of the Virus.—On April 15 two full grown guinea pigs were killed and the submaxillary glands removed with sterile precautions. Sections prepared from small pieces of these glands were subsequently shown to contain the specific lesion in the ducts of the glands. Aerobic and anaerobic cultures of the glands made at this time remained sterile. The glands were ground thoroughly in a mortar and suspended in a total volume of 15 cc. of Locke's solution. The suspension was centrifuged at moderate speed for 15 minutes. Half of the supernatant fluid was then filtered through a new Berkefeld N filter. The material filtered rapidly. The filter was subsequently tested and found to be impermeable to *B. coli.* 0.1 cc. of the unfiltered suspension was inoculated intracerebrally into each of three guinea pigs, Nos. 26, 27, and 28. 0.15 cc. of the filtered material was inoculated intracerebrally into each of three guinea pigs, Nos. 29, 30, and 31. All the guinea pigs were less than 1 month old. The results following injection of the unfiltered material were as follows: On April 19, the 4th day following injection, Guinea Pig 26 was found dead, No. 27 was moribund, and No. 28 seemed sick and was killed and the brain removed for histologic examination. Microscopic study of the brain of No. 28 showed an intense meningitis containing numerous cells showing typical nuclear inclusion bodies.

The results following the injections of the filtered material were as follows: Guinea Pigs 29, 30, and 31 all showed on the 2nd day, a rise in temperature ranging from 105–105.6°. On the 4th day Guinea Pig 29 had a temperature of 105.2°. On the 5th day the temperature began to drop and on the 6th day it was subnormal and the animal was killed. The brain was removed and prepared for histologic examination. Guinea Pig 31 showed a rise in temperature, ranging from 104–105° from the 4th to the 8th days. The animal was killed on the 8th day and the brain was removed for histologic examination. The temperature of Guinea Pig 30 ran an irregular course. This animal was killed on the 12th day and the brain removed. A microscopic study of the brains of these three guinea pigs showed a moderate meningitis and in every instance cells containing typical nuclear inclusion bodies were found.

In a second filtration experiment a new Berkefeld N filter was used which was tested during the course of the filtration by the addition of 0.5 cc. of an 18 hour broth culture of *B. coli* to the suspension of submaxillary gland. Cultures of the filtrate remained sterile. The same result as the one described above, was obtained.

Relation of the Infection of the Submaxillary Glands to Other Diseases of Guinea Pigs.

The only other disease affecting guinea pigs known to the writers which may possibly be related to the infectious process in the submaxillary glands is a condition described in 1911 by Römer (12). He observed sporadic cases of paralysis of the extremities in guinea pigs. By intracerebral inoculation of healthy guinea pigs with emulsions of the brains of the diseased animals he was able to transmit the infection without difficulty. After an incubation period of from 9 to 23 days the inoculated animals developed paralysis and after 2 to 10 days of severe illness, died. The brains of both the spontaneously and experimentally infected animals showed a marked infiltration of the meninges with an exudate containing many mononuclear cells and also many polymorphonuclear cells. No mention was made of the presence of cells containing nuclear inclusions.

The brain lesions in this condition resemble to some extent the lesions observed after intracerebral injection of the virus from the submaxillary glands. However, the ease with which the infection could be indefinitely transmitted and the long incubation period observed render it unlikely that the agents concerned in the two conditions are identical, though this possibility should be borne in mind.

SUMMARY AND CONCLUSIONS.

In the lesions of herpes simplex and similar conditions due to filterable viruses, cells are present which show characteristic alterations, particularly in the nucleus. The nucleus of these cells contains a mass which stains with acid dyes. Surrounding this mass is a clear space or halo, within which there are large granules staining with basic stains. These cells are little if at all enlarged.

In a few human cases, especially in infants, enlarged cells have been found which contain nuclei showing changes similar to those seen in the abnormal cells of herpes simplex.

In the ducts of the submaxillary glands of guinea pigs, Jackson observed structures which she considered to be protozoan parasites. Our own studies indicate, however, that these structures are greatly swollen epithelial cells with nuclei having the same characters as the

nuclei of the atypical cells in the lesions of herpes simplex. These cells are usually surrounded by a mononuclear cellular reaction. They were found in 84 per cent of the full grown guinea pigs examined but they were present in only three of forty-three young guinea pigs less than 1 month old. The resemblance of these cells, except as regards size, to the atypical cells present in lesions due to filterable viruses suggested that they also may be the result of an infection with a similar agent. That they are usually not present in guinea pigs less than 1 month old indicates that natural infection usually occurs after this period.

Experiments were therefore undertaken to determine whether or not an infective agent is concerned in this condition and if so to learn something of its nature. When an emulsion of the submaxillary glands of full grown guinea pigs is injected into the brains of young guinea pigs the animals have fever and exhibit symptoms of cerebral irritation. They usually die in 5 to 7 days and in sections of the brain a diffuse subacute meningitis is found. In the exudate there are large numbers of cells having all the characteristics of the abnormal cells of herpes simplex. Similar cells are present in the lesions resulting from the injection of the same emulsion into the testicle, lung, tongue, and submaxillary glands of young guinea pigs. In none of these lesions, however, are the cells greatly enlarged as they are in the lesions in old guinea pigs.

These results support the view that the lesion in the submaxillary gland of old guinea pigs is due to an infective agent. Attempts were therefore made to transmit this agent through a series of young guinea pigs. When the injections were all made into the same organ all the experiments but one gave negative results, but when the site of injection was changed at each transfer it was possible in a number of instances to reproduce the lesions through two animals in series and in one experiment through three animals in series. By modifying the technique, efforts were made to transmit the infection indefinitely but these attempts were unsuccessful. No explanation can be offered for this failure.

Studies made to determine some of the properties of the infective agent have shown that it is destroyed by heating at 54° for 1 hour, and that it is not injured by preservation in 50 per cent glycerol for as long as 11 days. After the material had remained in 50 per cent glycerol

for 28 days, however, it was found to be no longer infective. The infective agent was not held back by a Berkefeld N filter which was impermeable to bacteria. It seems probable therefore that the infective agent belongs in the group of filterable viruses, though further work will be necessary to learn more of its exact nature. These observations present additional evidence that the presence of cells with nuclear inclusions in any lesion indicates that the injury is probably due to an infective agent belonging in the group of filterable viruses.

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EXPLANATION OF PLATE 33.

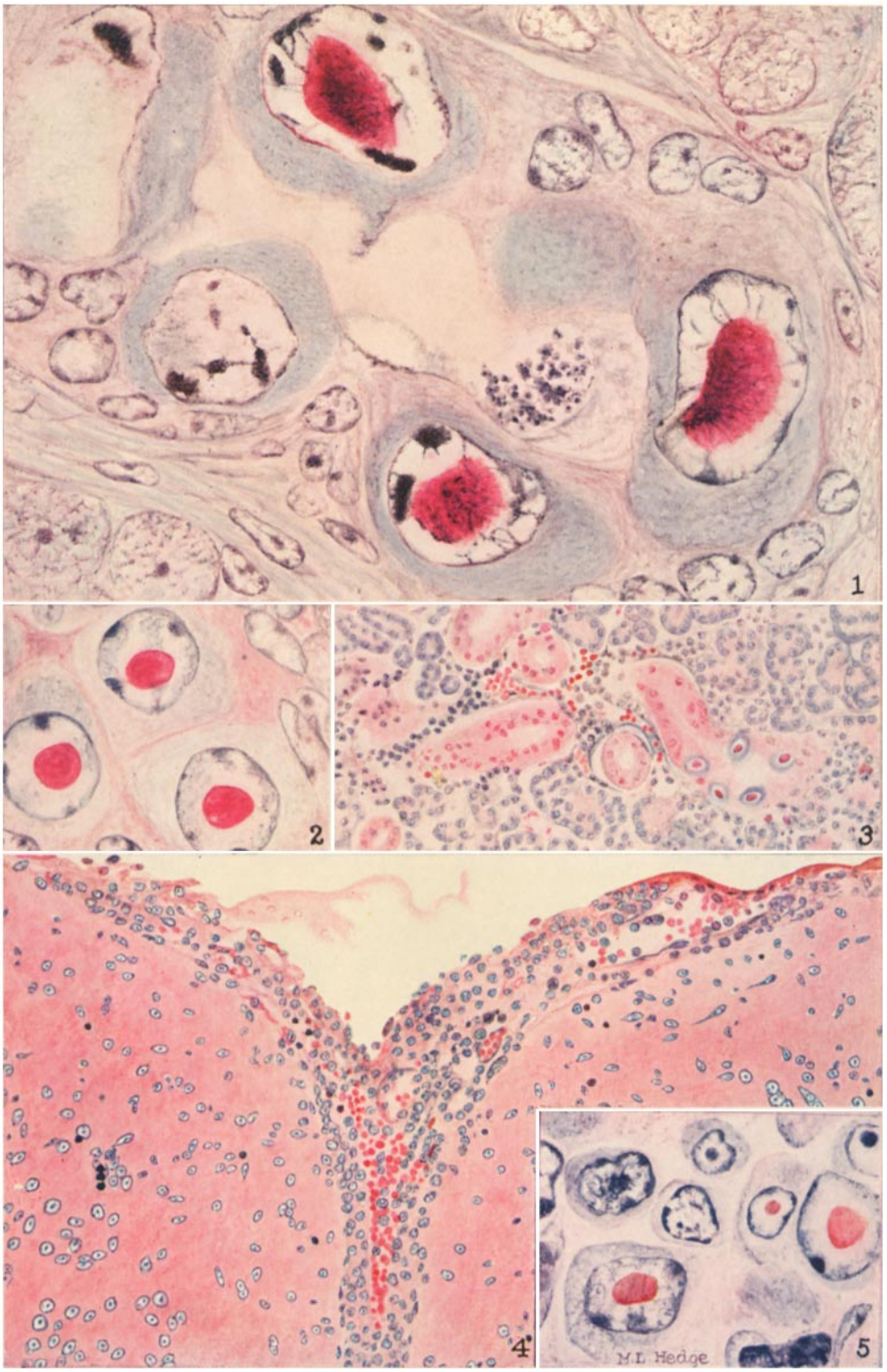
FIG. 1. Swollen epithelial cells containing nuclear acidophilic inclusions within a duct of the submaxillary gland of a full grown guinea pig. Magnification × 1700.

FIG. 2. Duct cells of smaller size containing acidophilic nuclear inclusions. This illustration was made from a section of the submaxillary gland of a young pig, exact age not known. Duct cells of this size containing inclusions have been found only rarely. Possibly these cells are in process of transformation. Magnification × 1700.

FIG. 3. Low power drawing from a section made from the submaxillary gland of a full grown guinea pig. Shows a duct swollen with epithelial cells containing nuclear inclusion bodies. There is a moderate degree of cellular reaction in the vicinity of the infected duct.

FIG. 4. Low power drawing of a section of the brain of a young guinea pig inoculated with an emulsion of the submaxillary gland of a full grown guinea pig. A well marked meningeal exudate is shown.

FIG. 5. High power (× 1700) drawing of the cellular meningeal exudate seen in low magnification in Fig. 4.



(Cole and Kuttner: Filterable virus in guinea pigs.)