THE PROBLEM OF THE SIGNIFICANCE OF THE INCLUSION BODIES FOUND IN THE SALIVARY GLANDS OF INFANTS, AND THE OCCURRENCE OF INCLUSION BODIES IN THE SUBMAXILLARY GLANDS OF HAMSTERS, WHITE MICE, AND WILD RATS (PEIPING)

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In recent years inclusion bodies have been observed with increasing frequency both in man and in animals. Although in many instances these pathological changes have been associated with definite virus diseases, for example poliomyelitis (1, 2), Rift Valley fever (3), yellow fever (4, 5), infectious ectromelia (6), etc., they have also been frequently reported as accidental findings.

The constant association of inclusion bodies with well established viruses such as those of variola, vaccinia, herpes, sheep pox, fowl pox, Virus III, the submaxillary gland virus of guinea pigs, etc., has led many workers to assume as a working hypothesis that the finding of these cytoplasmic or intranuclear changes means the presence of a filtrable virus. Thus recently Feyrter (7), McCordock (8), Rich (9), and McCordock and Smith (10) have found inclusion bodies in the lungs of children dying of postpertussis pneumonia, and have therefore suggested that whooping cough may be due to the action of a virus, or to the combined action of a virus and the Bordet-Gengou bacillus.

Cowdry (11) takes exception to this point of view. It is, of course, entirely possible, as he states, that eventually we shall be able to produce inclusion bodies by agents other than filtrable viruses, but in the meantime it seems logical to continue searching for a virus whenever these characteristic lesions are found. At the present time our knowledge of filtrable viruses of low pathogenicity is practically nil,

TABLE I

Inclusion Bodies Reported as Accidental Findings, Unaccompanied by Specific Symptoms of Disease

Species	No. examined	No. positive	Per cent positive	Age	Tissue	Attempts at transmission	Author
Man		51		5 still-births 45 infants 1 adult	Kidney, lung, liver Salivary glands, etc. Lung, liver, intestine	None " "	15 observers summarized by Farber and Wolbach (12)
Man		14		Infants	Lung, salivary gland, liver, etc.	Negative	McCordock and Smith (10)
Man	84	51	61		Brain: spongioblastoma multiforme	None	Russell (15)
	108	12	11		Brain: miscellaneous gliomas	"	
	36	10	36		Liver: Hodgkin's lymphogranuloma	"	
	56	5	10		Liver: death due to miscellaneous causes	"	
Monkey (*M. rhesus*)	31	19	61		Nasal mucosa	"	Stewart and Rhoads (16)
Monkey	60	20	33		Lung, nasal mucosa, trachea, bile duct, bronchiole	"	Covell (17)
Monkey (*M. rhesus*)					Kidney tubule	"	Hindle and Stevenson (18)
Monkey (*Cercopithecus*)					" "	"	

Dog	68	2	3		Liver	Negative	Cowdry (19)
Dog					Brain: nerve and glia cells	"	Dawson (20)
Rat (wild)			90		Kidney tubule	None	Hindle and Stevenson (18)
Rat (white)	70	10	14	2 mos.	Ducts of submaxillary gland	"	Thompson (21)
Mouse (Clacton strain)	25	25	100		Liver	Positive	Findlay (14)
Guinea pig			70	Full grown	Ducts of submaxillary gland	"	Cole and Kuttner (22)
Rabbit			20	" "	Testes	"	Rivers and Tillett (23)

and it is especially in this type of disease that inclusion bodies may prove of aid. The finding of inclusion bodies in various internal organs of still-births (12) is difficult to attribute to an infectious agent, but it may be that we are dealing with a filtrable virus of low virulence, and that infection has taken place *in utero*. Wagner (13) states that the occurrence of these peculiar structures in still-born and premature infants is against their being of protozoan origin, since the human placenta is impermeable to parasites of this size. A filtrable virus, on the other hand, could pass the placental barrier. Whether this agent, if it is a filtrable virus, is frequently present in the circulation of adults, or has a greater tendency to invade the blood stream during pregnancy, and thus infect the fetus, remains a matter of speculation. The fact that these lesions occur in infants in the complete absence of symptoms, cannot be held to exclude the possibility of a virus, since Virus III and the submaxillary gland virus of guinea pigs were discovered accidentally in the course of experimental work, and did not produce symptoms. Findlay (14) has recently demonstrated a filtrable virus in the parenchymal cells of the liver in a certain strain of mice, which is also symptomless.

In spite of the fact that inclusion bodies have been observed as accidental findings in man and in many species of animals, relatively few attempts have been made to determine their significance. Table I summarizes the occurrence of inclusion bodies reported in the absence of specific symptoms, and the number of instances in which attempts at transmission have been made.

It will be seen that 6 of the 13 workers who have recorded these observations attempted to demonstrate the presence of a virus. 3 of the 6 attempts proved successful.

In analyzing the cases of infants previously reported, it is of interest that in those instances in which the submaxillary and parotid glands are reported as involved, inclusion bodies are not found in other tissues of the body. It is also worthy of note that in still-births and in the very youngest infants, the submaxillary and parotid glands are often negative, and the kidneys, lungs, and liver most frequently show the hypertrophied cells containing intranuclear inclusion bodies. This suggests that if these changes are due to a filtrable virus and if the infection has taken place *in utero*, as must be assumed, the virus first

attacks the viscera and then gradually localizes in the salivary glands. On the other hand, it may be that we are dealing with different viruses.

In view of the fact that the pathological findings in the submaxillary gland disease of guinea pigs are so similar to those found in the submaxillary and parotid glands of infants dying from miscellaneous causes, it seemed of interest to try to determine whether a virus could be demonstrated in autopsy material of this kind. Moreover, before any special importance can be attached to the finding of inclusion bodies in pathological conditions occurring in children, it would be important to try to discover the significance of intranuclear changes found in the salivary glands of young children in a fairly high percentage of routine autopsies (Farber and Wolbach, 12 per cent (12)). At the time the present work was started, no experiments to demonstrate a virus from human material of this kind, had been made. In a recent publication McCordock and Smith (10) state that they have been unable to transmit a virus from the salivary glands of infants showing inclusion bodies, to young guinea pigs. Their experiments are not reported in detail.

The Occurrence of Inclusion Bodies in the Submaxillary Glands of Chinese Still-Births and Infants Less than 2 Years of Age

Method.—The submaxillary glands, and whenever possible the parotid glands, were obtained from autopsies on still-births and infants less than 2 years old. The autopsies were performed anywhere from 12 hours to 4 days after death. No precautions for asepsis were observed in removing the glands, but they were carefully washed with sterile saline. A small piece of tissue was removed from each gland for section, and the remainder was placed in 50 per cent glycerine. After 3 to 5 days exposure to glycerine in the ice box, some of the material was washed, ground with sand, and centrifugalized at low speed. The supernatant fluid was then injected intracerebrally or directly into the submaxillary glands of a variety of animals, young guinea pigs, hamsters, mice, rats, rabbits, and monkeys. All intracerebral injections and removal of glands from animals were carried out under ether anesthesia. The rest of the tissue was stored in glycerine until the histological sections had been examined. In the earlier experiments all the material was inoculated into animals as soon as possible without waiting for the completion of the sections; in later experiments the tissue obtained from still-births was stored in glycerine until the sections had been examined, and if these were negative, no animal injections were made. Aerobic and anaerobic cultures of the supernatant fluid were made before inoculation. In spite of the exposure to glycerine some of the material was not sterile, and a small number of organisms

TABLE II

Summaries of the Clinical and Pathological Data of the 4 Positive Cases

Case	Age	Sex	Gland		Clinical data	Interval between death and autopsy	Autopsy No.	Anatomic observations*
			Submaxillary	Parotid				
	mos.					*days*		
1	2½	♀	+	+	Attacks of turning blue for 5 days, no fever, W.B.C. 10,000, Wassermann negative. X-ray: bronchopneumonia of entire right lung, and left upper. Throat: *Strep. hemolyticus*	3	1433	Interstitial pneumonia; interstitial emphysema and edema of lung
2	7	♀	0	+	Cough, fever, cyanosis, convulsions; died in Outpatient Department	4	1488	Edema and hemorrhage of lungs; slight interstitial pneumonia
3	7	♀	+	+	Cough, fever, anorexia; pulmonary tuberculosis (right upper), bronchopneumonia. Kline test for syphilis negative	1	1532	Acute caseous pulmonary tuberculosis, disseminated tubercles in liver, spleen, and mesenteric lymph nodes; interstitial lobular pneumonia
4	6	♀	+	+	Fever, diarrhea, dehydration, malnutrition, otitis media. X-ray of chest negative. (Wassermann not done)	1	1558	Otitis media, acute ulcerative enterocolitis, acute bronchitis, lobular pneumonia

No inclusion bodies were found in the lungs, liver, or kidneys of these 4 cases.

+, inclusion bodies present.

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grew out, usually Gram-positive cocci and Gram-negative bacilli. However, the contaminating organisms were rapidly killed off in the animal body, and only very rarely produced abscesses or other complications following injection.

In this way the material from 49 autopsies was examined, including 13 full-term infants, 11 premature infants, and 25 still-births. The glands obtained from the premature infants and still-births were uniformly negative. Of the 13 full-term infants, 9 were negative, and 4 showed foci of mononuclear cells and hypertrophied duct cells with acidophilic intranuclear inclusion bodies. In 3 of the cases the characteristic cells were numerous, occurring both in the submaxillary and in the parotid glands, in the fourth case they were found only after considerable search in the parotid gland. This lesion in the salivary glands of infants, dying from miscellaneous causes, has been observed by many authors both in Europe and America (9). As far as we know, this is the first time these findings have been reported in Chinese infants. The pathological changes were similar in every way to those previously described.

Animal Inoculations with Emulsions of Human Salivary Glands Showing Inclusions

Case 1.—The submaxillary and the parotid glands were stored in 50 per cent glycerine for 3 days, Dec. 19 to 22, 1933. A part of each gland was washed, ground with sand, and a small amount of saline added. Cultures of the supernatant fluid showed no growth. The material was injected intracerebrally into one young guinea pig, one hamster, and one mouse. All the animals remained well, and were discarded after 2 weeks.

On Jan. 11, 1934, it was found that the sections of the glands from this case showed numerous inclusion bodies (see Fig. 1), and it was therefore decided to inject more animals. The material had at this date been stored in glycerine for a period of 23 days. The tissue was emulsified in the usual way. The supernatant fluid was injected intracerebrally, and directly into the submaxillary gland of young rabbits, guinea pigs, and mice. At the time that the submaxillary gland of each animal was injected, the other submaxillary gland was removed for histological examination. All the animals remained well. Some of those injected intracerebrally were killed on the 6th day following injection, and sections made of the brain. In some instances although the first injection had failed to produce any symptoms, part of the brain was emulsified and injected into a second animal. Attempts were also made to reduce the resistance of a few animals by injections of benzol. The results were, however, entirely negative.

In the case of the intraglandular injections, the injected gland was removed from some of the animals on the 5th day, and from others on the 12th day. A piece of the gland was prepared for histological examination, and the rest injected intraglandularly into other animals. The material was thus carried through 3 series of rabbits, 4 series of guinea pigs, and 4 series of mice. Hamsters were not used in the second attempt for reasons to be discussed later. Sections were made

of a part of each gland at the time of transfer. Although some of the glands showed a slight amount of cellular reaction, most of them were negative.

No evidence was obtained that the agent which had produced the characteristic changes in the submaxillary and parotid glands of this infant had been transmitted to rabbits, guinea pigs, or mice by intracerebral or intraglandular injection.

Case 2.—The submaxillary and parotid glands were stored in glycerine for 3 days, Feb. 26 to Mar. 1, 1934. A part of each gland was emulsified and the supernatant fluid injected directly into the submaxillary glands of 2 guinea pigs and 2 mice. The glands were removed after 2 weeks, and sections prepared. Both the mice and the guinea pigs failed to show any characteristic reaction.

Case 3.—The submaxillary and the parotid glands were stored in glycerine for 5 days, May 2 to 7, 1934. A part of each gland was emulsified, and 0.1 cc. of the supernatant fluid was injected intracerebrally into 3 young guinea pigs. 2 of the guinea pigs were on a normal diet and 1 was on the vitamin-deficient diet used by Zinsser *et al.* (24) in their work on typhus. Cultures made from the supernatant fluid showed a few colonies of *B. coli.* All 3 of the animals remained well and were discarded.

On June 6, 1934, it was found that the sections of the glands from this case showed many inclusion bodies (see Fig. 2). The material had, on this date, been stored in glycerine for a period of 34 days. The tissue was emulsified in the usual way, and the supernatant fluid was injected intracerebrally into one young (*Macacus rhesus*) monkey, approximately 2½ years of age, and intraglandularly into another, older monkey of the same species. Both monkeys remained well. The monkey that had been injected intracerebrally was killed on the 7th day. The brain was removed for histological examination. The inoculated gland was removed from the second monkey on the 15th day, and sections made.

Microscopic sections of the brain were negative. At the time that the submaxillary gland of the second monkey was injected, the other gland was removed for histological examination. Sections of the uninoculated gland showed very occasional small areas of lymphocytic infiltration. The inoculated gland showed a similar reaction which was somewhat more marked. The capsule of the inoculated gland was infiltrated with lymphocytes at one point. However, the contrast between the inoculated and uninoculated glands was not striking, and no inclusion bodies were found in either one.

No evidence was obtained that the salivary glands from this case contained a virus which could be transmitted to monkeys (*Macacus rhesus*).

Case 4.—The submaxillary and parotid glands were stored in glycerine for 18 days, June 18 to July 6, 1934. Attempts were made to reduce the resistance

of the animals to be inoculated, by exposure to X-ray, since Zinsser and Castaneda (25) have found this the most satisfactory method of reducing the resistance of rodents to typhus. Rabbits, guinea pigs, and rats were given the following dose immediately before inoculation: kv. 160, ma. 8, filter 5.0 cm. oil, 0.25 mm. Cu, 1.5 mm. Al, (effective wave length 0.19 Å. u.) distance 50 cm., 19 minutes, 400 Roentgen units. The tissue was emulsified in the usual way, and the supernatant fluid injected intracerebrally into 1 young rabbit, 2 young guinea pigs, and 3 young rats. All the animals remained well, and were discarded.

No evidence was obtained that rodents could be made susceptible to an infectious agent in the salivary glands by exposure to X-ray.

The Occurrence of Inclusion Bodies in the Submaxillary Glands of Hamsters, White Mice, and Wild Rats

In the course of the attempts to transmit a presumptive filtrable virus from the salivary glands of infants to animals, the submaxillary glands of hamsters, wild and white mice, wild and white rats, squirrels, rabbits, and monkeys (*Macacus rhesus*), were examined histologically.

Hamsters.—It was found that the submaxillary glands of nearly all full grown hamsters showed characteristic pathological changes consisting of scattered areas of cellular infiltration composed mainly of mononuclear cells. In the vicinity of these areas, although usually not in them, one or two cells situated in the center of an acinus were greatly hypertrophied. The nucleus was also enlarged and contained an acidophilic inclusion body surrounded by a halo (see Fig. 3). The staining of the inclusion body tended to be less dense at the periphery, but no definite structure could be made out. The inclusion bodies found in the acinal cells of the hamster are usually more regular in outline than those found in the guinea pig. There are often one or more small irregular basophilic masses lying close to the nuclear membrane. The halo is always well defined. The cytoplasm of the cell stains blue or purplish with eosin-methylene blue. In most instances the staining of the cytoplasm is irregular, some parts staining more deeply than others. Occasionally somewhat more definite basophilic masses could be made out in the cytoplasm. These changes suggest degenerative processes in the cytoplasm, rather than cytoplasmic inclusion bodies. The lesion differed from that found in the submaxillary glands of guinea pigs in that the acini and not the ducts, were primarily affected. In only three instances were these hypertrophied cells with acidophilic inclusion bodies, found in the duct as well as in the acini of the submaxillary glands of hamsters. Although the inclusion bodies found in full grown hamsters are smaller than those found in full grown guinea pigs, they can often be identified under the low power of the microscope. The fusion of two hypertrophied cells, each with its separate inclusion body, occurs fairly often in the

center of an acinus. As in the guinea pig, the serous portion of the gland in the hamster is more often affected than the mucous. Occasionally the characteristic cells were found in glands which showed very little reaction, although in most cases foci of mononuclear cells were present.

Since over 90 per cent of the submaxillary glands of full grown hamsters showed these pathological changes, it seemed futile to try to use these animals in attempting to transmit a possible virus from human submaxillary glands, since the lesions were so similar, and there might be a cross-immunity. It did, however, seem of interest to determine whether the submaxillary gland which showed these lesions contained a filtrable virus.

To carry out experiments of this nature, it was essential to use young hamsters before they had become infected. It has never been possible to breed the Chinese hamster (*Cricetulus griseus*, M. Edw.) in captivity. It was therefore only possible to work on this problem during short periods in the fall and spring, when young hamsters could be caught in the burrows. Although the exact age of the hamsters was not known, it was found that in most instances, the submaxillary glands of very small hamsters failed to show any pathological changes.

Intracerebral Injection of Submaxillary Glands of Full Grown Hamsters into Young Hamsters

Method.—The submaxillary glands of three or more full grown hamsters were removed, with precautions for asepsis. A piece was then removed from each gland for histological examination, and the rest was ground with sand and a small amount of saline added. The emulsion was centrifugalized at low speed for a few moments. Aerobic and anaerobic cultures of the supernatant fluid were made to rule out the presence of bacteria. 0.05 cc. of the supernatant fluid was injected intracerebrally into small hamsters under ether anesthesia. Sections of the glands were examined later for the presence of hypertrophied acinal cells with acidophilic inclusion bodies, which were uniformly present. The material for inoculation was always prepared in the same way and will be referred to subsequently as the "hamster virus."

The animals remained well for 2 to 5 days after the inoculation. Some hamsters died suddenly without any marked preliminary symptoms on the 5th to 7th day, and others died following generalized symptoms of meningeal irritation. Sections of the brain showed a meningeal exudate which was often localized (see Fig. 4). The exudate consisted mainly of mononuclear cells, lymphocytes, and large mono-

nuclear cells with vesicular nuclei. Many of the cells in the exudate showed acidophilic intranuclear inclusion bodies (see Fig. 5). The brain tissue itself showed practically no changes. The reaction obtained was similar to that produced by the intracerebral injection of the submaxillary gland virus of guinea pigs into susceptible guinea pigs.

As in the case of the submaxillary gland virus of guinea pigs, it was found impossible to transfer the hamster virus from brain to brain in hamsters. It was thought that if it were possible to establish the hamster virus in another animal species, it might become more virulent. All attempts to transmit the hamster virus to rabbits or young guinea pigs, either by intracerebral or intraglandular (submaxillary gland) injections, using large doses and making several transfers, failed. Intracerebral inoculations into white mice were also unsuccessful.

Localization of the Hamster Virus in the Submaxillary Gland Following Subcutaneous, Intraperitoneal, and Intraglandular Injection. Thermolability of the Virus; Resistance to Glycerine

The hamster virus was injected into a series of young hamsters from which one submaxillary gland had been removed for histological section,—subcutaneously, intraperitoneally, and intraglandularly (submaxillary). At the same time hamster virus which had been heated at 56°C. for 30 minutes was similarly injected into another group of young hamsters. 2 weeks later, the remaining submaxillary gland was removed for section from both groups of animals. Histological sections of all the animals that had received the unheated hamster virus, showed varying degrees of cellular reaction and hypertrophied acinal cells with acidophilic intranuclear inclusion bodies, whereas the glands removed before injection were negative. Sections of the glands removed from the hamsters which had been injected with the heated material were negative with one exception. These experiments indicated that exposure to 56°C. for 30 minutes destroyed the hamster virus.

A single attempt to filter the hamster virus through a Mandler filter was unsuccessful. No small filters were available at the time this experiment was done, so that the material had to be greatly diluted.

The hamster virus survived for 6 days in 50 per cent glycerine. Longer periods of glycerinization were not tried. Attempts to transfer the virus from gland to gland were not undertaken.

Intracerebral Injection of the Submaxillary Glands of Full Grown Mice into Young Mice Less than 1 Month Old

The submaxillary glands of full grown mice were examined histologically and it was found that a small proportion of them showed pathological changes similar to those observed in hamsters. About 20 to 25 per cent of the submaxillary glands of full grown mice obtained from the animal room stock of white mice (originally imported from the United States) showed scattered foci of mononuclear cells. In the vicinity of these areas, one or two cells, usually in the center of an acinus, were greatly hypertrophied, and the nucleus contained an acidophilic inclusion body surrounded by a halo (see Fig. 6). In mice these hypertrophied cells were never found in the ducts, only in the acinus. The lesion in mice was so similar to that in hamsters that it cannot be differentiated morphologically. Stock white mice in this laboratory were less frequently infected than hamsters, and the hypertrophied cells with the intranuclear inclusion bodies were never very numerous. In addition to the laboratory-bred white mice, the submaxillary glands of 6 wild brown mice were examined histologically. Although in four instances the glands showed foci of cellular infiltration, no hypertrophied cells with intranuclear inclusion bodies were found.

Attempts to demonstrate a virus in the submaxillary glands of mice which showed these lesions, were made. Young mice, less than 1 month old, usually failed to show any pathological changes and were used for inoculation.

The submaxillary glands of full grown white mice were prepared in the same way as the hamster virus, and will subsequently be referred to as the "mouse virus." Although the submaxillary glands of 3 to 6 mice were combined, the sections made from a portion of each gland showed that they were never very heavily infected, and the characteristic cells were only found after considerable search. Intracerebral injections into young mice were difficult, and many of the animals died as the result of inoculation. The successfully injected mice remained well for several days before they became sick. A few of them died on the 6th to 8th day. Sections prepared from the brains showed a slight localized meningeal exudate, consisting of mononuclear cells. A few of these showed acidophilic intranuclear inclusion bodies.

Localization of the Mouse Virus in the Submaxillary Gland Following Subcutaneous, Intraperitoneal, and Intraglandular Injection. Thermolability

The mouse virus was injected into a series of young mice, about 1 month old, from which one submaxillary gland had been removed for histological section—subcutaneously, intraperitoneally, and intraglandularly (submaxillary). At the same time mouse virus which had been heated at 60°C. for 30 minutes was similarly injected into another group of young mice. 2 weeks later the remaining submaxillary gland was removed for section from both groups of animals. Histological sections of all the mice which had received the unheated mouse virus showed varying degrees of cellular reaction and hypertrophied acinal cells with acidophilic intranuclear inclusion bodies, whereas the glands removed before injection were negative. The mice which had been injected with the heated material were also negative. These experiments indicated that exposure to 60°C. for 30 minutes destroyed the mouse virus.

No attempts to filter the mouse virus were made and its resistance to glycerine was not determined. Transfers from brain to brain or gland to gland were not undertaken.

A single attempt was made to transmit the mouse virus to guinea pigs which had been on a vitamin-deficient diet, but no characteristic lesion was produced.

Experiments with the Rat Virus

Thompson (21) has reported the occurrence of intranuclear inclusion bodies in the duct cells of the submaxillary glands of 2 months old white rats. These lesions were present in 10 of the 70 rats examined. They were absent in 12 rats 6 months of age. Hindle and Stevenson (18) found inclusion bodies in the kidney tubules of wild rats caught in London. They do not state whether the submaxillary glands were also examined. Neither Thompson nor Hindle and Stevenson attempted to demonstrate a virus.

It seemed of interest to see if the stock white rats used in this laboratory showed similar lesions to those described by Thompson. Sections of the submaxillary glands of both full grown and young rats

aged between 6 and 8 weeks have been entirely negative. On the other hand, about 50 per cent of the full grown wild rats caught in Peiping have shown lesions in the submaxillary glands, but not in the kidneys. The pathological changes in the submaxillary gland consisted of foci of mononuclear cells, and hypertrophied acinal or duct cells with acidophilic intranuclear inclusion bodies (see Figs. 7 and 8). In some of the wild rats only the acinal cells seemed to be involved, in others mainly the duct cells. The changes in the duct cells are similar to the lesions found in man and guinea pigs (see Fig. 9), and those in the acinal cells to the lesions found in hamsters and mice. A few experiments were undertaken to determine whether these pathological changes in the submaxillary glands of wild rats indicated the presence of a virus. Since young wild rats were not available, the submaxillary glands of wild rats were emulsified in the usual way, and injected intracerebrally and intraglandularly (submaxillary) into young white rats. Two of the white rats injected intracerebrally became sick on the 8th day and were killed. Sections prepared from their brains showed a slight meningeal exudate consisting of mononuclear cells, a few of which contained acidophilic intranuclear inclusion bodies. The submaxillary glands which had been injected directly were removed after 2 weeks and showed a marked reaction consisting of a mononuclear infiltration with fairly numerous hypertrophied acinal and duct cells with acidophilic intranuclear inclusion bodies. The submaxillary gland removed before injection was entirely negative. These experiments indicate that the submaxillary glands of full grown wild Peiping rats sometimes harbor a virus which is transmissible to young white rats.

In the course of this work the submaxillary glands of 3 other animal species were examined: 3 normal full grown monkeys (*Macacus rhesus*), 3 large squirrels, and 5 rabbits. Scattered foci of mononuclear cells were found in all 3 of the monkeys, but no intranuclear inclusion bodies. The submaxillary glands of the 3 large squirrels were negative, but the submaxillary gland of 1 young squirrel showed an occasional focus of mononuclear cells, but no inclusion bodies. The submaxillary glands of the rabbits were negative, with the exception of 1 animal which showed a few mononuclear cells in one area.

DISCUSSION

The occurrence of protozoa-like cells in the parotid glands of infants was first described by Ribbert (26) in 1904. Since that time, these structures have been frequently observed both in Europe and America. In examining the salivary glands obtained from routine autopsies on 24 Chinese infants, dying of various causes (exclusive of still-births), this lesion was found 4 times. This is as far as we know the first time that this lesion has been observed in Chinese children. Although our series is too small to draw any conclusions, it suggests that these pathological changes are fairly common in China.

In their discussion of the significance of intranuclear inclusion bodies frequently associated with whooping cough, McCordock and Smith (10) consider the possibility that the lesion found in the salivary glands of infants may be due to a virus closely related to the submaxillary gland virus of guinea pigs. Inoculation of human material into guinea pigs was, however, unsuccessful in their hands. In our attempts to demonstrate an infectious agent in the salivary glands obtained from 4 Chinese infants with characteristic pathological findings, guinea pigs, rabbits, hamsters, white mice, white rats, and 2 monkeys (*Macacus rhesus*) were inoculated intracerebrally or directly into the submaxillary gland. The results were entirely negative. Repeated transfers in rabbits, guinea pigs, and mice in the hope of adapting the "human virus" to a new host, were without avail. Efforts to reduce the resistance of the rodents by diet and X-ray failed. It is of course possible that the virus deteriorated quickly after death (the autopsies were performed 1 to 4 days after death), or that it is easily destroyed by glycerine. The material inoculated into monkeys had been stored in glycerine for 34 days.

In the course of these experiments, the submaxillary glands of several different species of animals were examined. It was found that the submaxillary glands of hamsters, white mice, and wild rats showed pathological lesions similar to those found in the submaxillary glands of man and the guinea pig. In contrast to the guinea pig, the lesion in hamsters and white mice usually involved the acinal rather than the duct cells. In wild rats (Peiping) both the acinal and duct cells showed the characteristic changes.

Emulsions of the submaxillary glands of full grown hamsters, white mice, and wild rats which showed these lesions, when injected intracerebrally into a young susceptible animal of the same species, often produced symptoms of meningeal irritation and death. Histological examination of the brain showed a localized, meningeal exudate consisting of mononuclear cells, some of which contained acidophilic intranuclear inclusion bodies. The lesion produced was analogous to that obtained with the submaxillary gland virus of guinea pigs. It was not possible to transmit the hamster virus or the mouse virus serially by intracerebral injection. It is an interesting fact that although intracerebral injections of the inclusion-containing submaxillary glands of rodents give rise to a fatal meningitis, the potency of the virus is decreased rather than increased, and it is only in the submaxillary gland itself that the virus can persist.

It was thought that if the hamster virus could be adapted to other rodents, it might become more virulent. The injection of the hamster virus by various routes, into rabbits, guinea pigs, and white mice, was without success. The intracerebral injection of the submaxillary gland virus of guinea pigs into hamsters was also negative.

The localization of the hamster virus and the mouse virus in the submaxillary glands follows subcutaneous, intraperitoneal, and intraglandular (submaxillary) injection in the respective species. The hamster virus is destroyed at 56°C. for 30 minutes, and is preserved in glycerine for at least 6 days. Longer periods of exposure to glycerine were not tried. The mouse virus is destroyed at 60°C. for 30 minutes. The thermolability of the rat virus was not determined.

The occurrence of inclusion bodies in the submaxillary glands of so many different species of animals, man, guinea pigs, hamsters, white mice, wild rats, is of interest. Although no inclusion bodies were found in the submaxillary glands obtained from 3 normal monkeys (*Macacus rhesus*) examined, areas of lymphocytic infiltration were present in all of them. In the various rodents studied, the submaxillary glands of very young animals were usually not infected, whereas in the human species typical lesions have been found in the viscera of still-births and infants that lived less than 48 hours. The age of the youngest child in whom inclusion bodies have been observed in the salivary glands is 2 months. The submaxillary gland viruses of

rodents are extremely specific, and it has been impossible to transfer the hamster virus to rabbits, guinea pigs, or mice, or the submaxillary gland virus of guinea pigs to hamsters. It is possible that the failure to transfer an infectious agent from the submaxillary glands of infants to animals is due to a very limited specificity and it may be necessary to inoculate higher apes before a positive result can be obtained.

The submaxillary gland virus as it occurs in rodents is not very virulent, and it is only by intracerebral inoculation of large doses that it produces any symptoms which we can recognize. Since we have found it impossible to demonstrate a virus in human salivary glands, no statement can be made at the present time as to whether the inclusion bodies found in the lungs of children dying of pertussis are due to the activation of an endogenous virus or to invasion by a specific virus which is the cause of whooping cough.

CONCLUSIONS

1. Acidophilic intranuclear inclusion bodies occur in the salivary glands of Chinese infants dying from miscellaneous causes. The lesion is similar to that previously described in infants in Europe and America.

2. Attempts to prove that this lesion is due to an infectious agent by its production in animals have been unsuccessful.

3. Acidophilic intranuclear inclusion bodies have been found in the submaxillary glands of hamsters, white mice, and wild rats.

4. Evidence is presented to show that the lesion in hamsters, white mice, and wild rats is due to a virus, which is specific for each species, being transmissible to normal individuals of this breed, and which is very similar to the submaxillary gland virus of guinea pigs.

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EXPLANATION OF PLATES

PLATE 47

FIG. 1. The submaxillary gland from Case 1 showing two hypertrophied duct cells with inclusion bodies surrounded by well developed halos. Eosin and methylene blue. × 1290.

FIG. 2. The submaxillary gland from Case 3 showing a greatly hypertrophied duct cell which has been extruded into the lumen. The nucleus is almost completely filled by the inclusion body and the halo is not well defined. The cytoplasm of the cell contains numerous basophilic masses. × 1290.

FIG. 3. Submaxillary gland of a full grown hamster showing hypertrophied acinal cells, two of which have fused. The nuclei are greatly enlarged and contain large inclusion bodies surrounded by halo. The cytoplasm of the cells stains more deeply in certain areas than in others. × 1290.

FIG. 4. Low power section of the brain of a young hamster inoculated with an emulsion of the submaxillary glands of full grown hamsters. A well marked meningeal exudate is shown. × 155.

PLATE 48

FIG. 5. High magnification of Fig. 4 showing meningeal exudate, composed of mononuclear cells, with an inclusion body surrounded by a wide halo. × 1290.

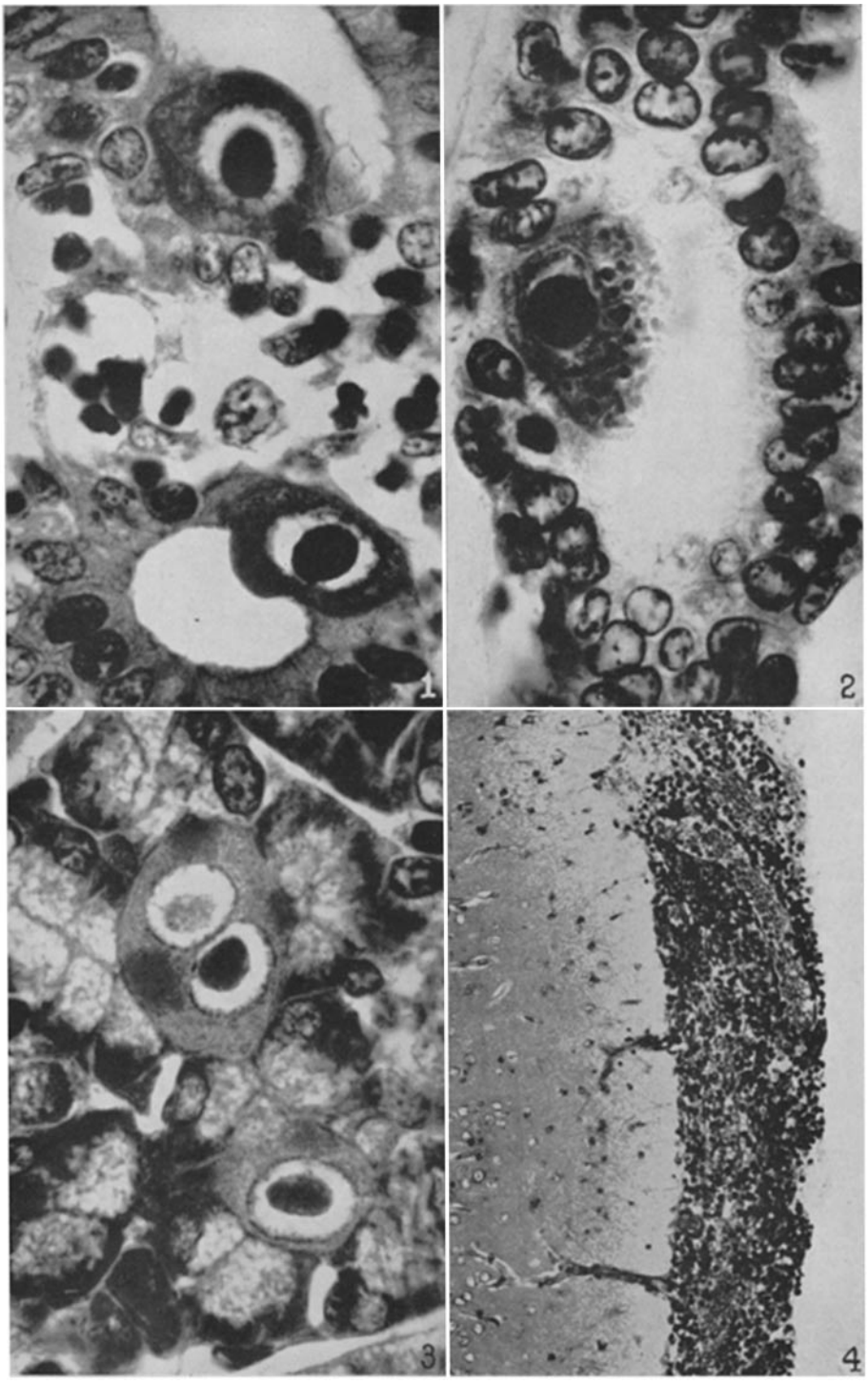
FIG. 6. Submaxillary gland of a full grown white mouse showing a hyper-

trophied acinal cell. The nucleus is greatly enlarged and contains a large inclusion body surrounded by a clear space. The nuclear chromatin is collected in several small masses at the surface of the nuclear membrane. × 1290.

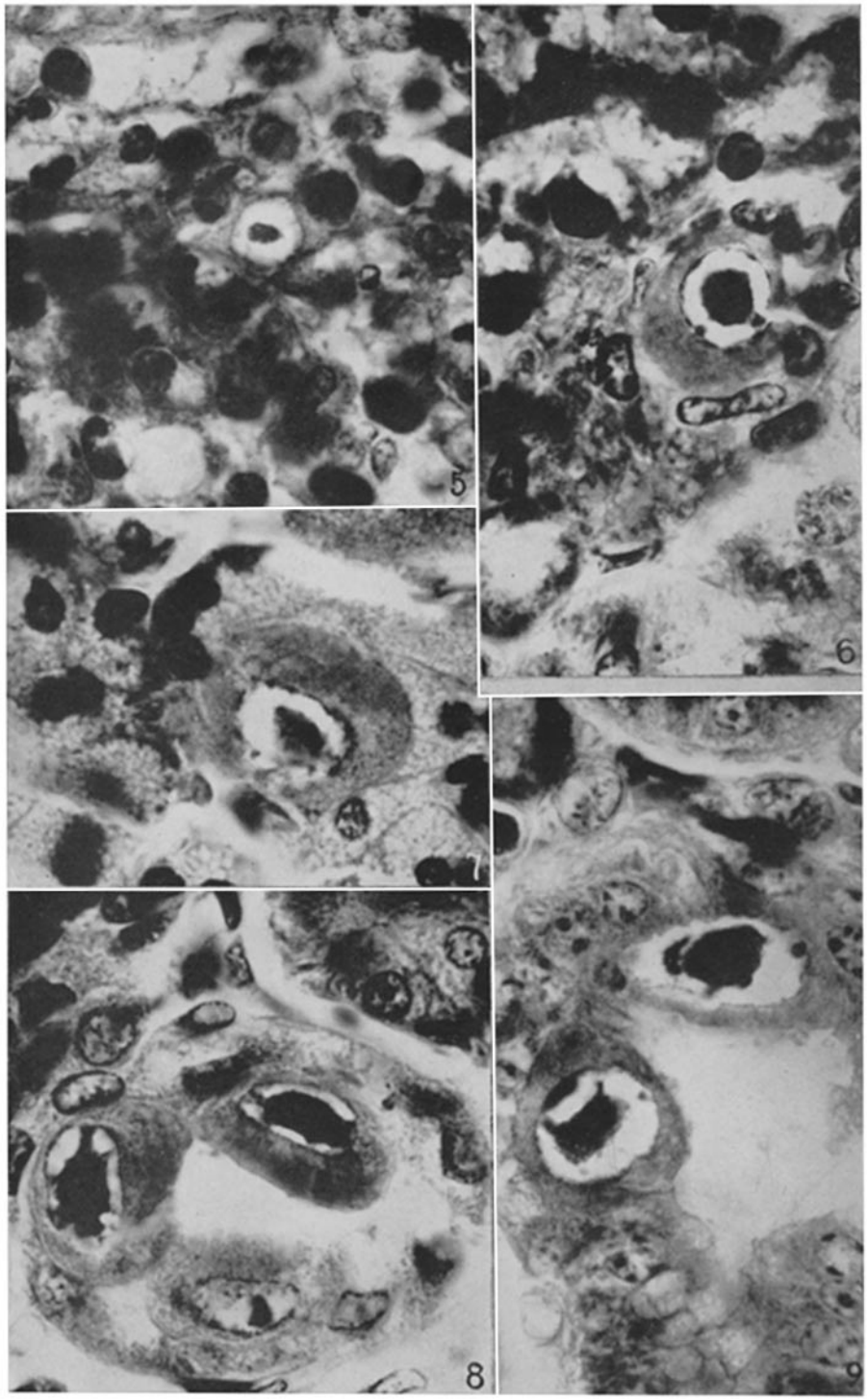
FIG. 7. Submaxillary gland of a wild rat showing a hypertrophied acinal cell. The nucleus is enlarged and shows an inclusion body surrounded by a wide halo.

FIG. 8. Submaxillary gland of a wild rat showing two hypertrophied duct cells. Both cells contain large, irregular inclusion bodies. × 1290.

FIG. 9. Submaxillary gland of a guinea pig showing hypertrophied duct cells with inclusion bodies, for comparison with Fig. 8. × 1290.



(Kuttner and Wang: Inclusion bodies in salivary glands)



(Kuttner and Wang: Inclusion bodies in salivary glands)