Published December 1, 1935

FURTHER STUDIES ON THE SUBMAXILLARY GLAND VIRUSES OF RATS AND GUINEA PIGS

By ANN G. KUTTNER, M.D., and T'SUN T'UNG, M.D.

(From the Department of Medicine, Peiping Union Medical College, Peiping, China)

PLATES 35 AND 36

(Received for publication, September 3, 1935)

In a previous article, the occurrence of inclusion bodies in the submaxillary glands of wild rats, white stock mice, and hamsters, similar to those found in the salivary glands of guinea pigs and infants, was described (1). A virus specific for each of these three species of rodents, analogous to the submaxillary gland virus of guinea pigs (2), was demonstrated. The intracerebral injection of uninfected white rats, mice, or hamsters with emulsions of the homologous submaxillary glands showing characteristic pathological changes, usually killed the animals in 5–8 days. Histological examination of the brains showed a meningeal exudate consisting of mononuclear cells, some of which contained acidophilic intranuclear inclusion bodies. As in the case of the submaxillary gland virus of guinea pigs, in spite of the fact that the intracerebral inoculation almost invariably killed the animal, it was impossible to pass the mouse or hamster viruses from brain to brain. Each of these rodent submaxillary viruses proved to be specific: the hamster virus could not be transmitted to guinea pigs or mice, or the guinea pig virus to hamsters.

It was thought that further information in regard to these submaxillary gland viruses of rodents might be obtained by studying the wild rat virus in white rats. Although Thompson (3) previously reported the occurrence of inclusion bodies in the submaxillary glands of 2 months old rats, we have never observed a spontaneous infection in our stock white rats of any age. The wild rat virus would, therefore, be easier to work with than either the hamster or mouse virus, since in both hamsters and mice spontaneous infections are common.

Furthermore, it was thought possible that the wild rat virus might become more virulent by passage in the white rat.

The most striking characteristic of the guinea pig virus, which has also been shown to be true of the hamster and mouse viruses, is its marked predilection for the submaxillary gland. No matter where the virus is injected, it localizes in the salivary glands and produces typical pathological changes. It is only following intracerebral injection that it is possible to produce obvious symptoms or death, and then it is impossible to transmit the virus in series from brain to brain. Recently Hudson and Markham (4) have been able to carry the guinea pig submaxillary gland virus from brain to brain in one instance through 3 generations, and in another through 2. The virulence of the virus, however, decreased rather than increased, and it was impossible to continue intracerebral passage.

The intracerebral injection of the wild rat submaxillary gland virus into white rats produces symptoms of meningitis in the way characteristic of the other submaxillary gland viruses, but as with the guinea pig, hamster, and mouse viruses it has been impossible to transfer the rat virus from brain to brain in series. It was thought of interest to see whether serial intracerebral passage might be made possible either by reducing the resistance of white rats and guinea pigs by exposure to X-ray radiation, or by increasing the virulence of these two viruses by the addition of organ extracts (Duran-Reynals factor). Zinsser and Castaneda (5) have shown that the resistance of rats to typhus can be reduced by X-ray. Duran-Reynals (6, 7) has proved that certain organ extracts, particularly testicular extract, have the effect of enhancing the virulence of vaccinia. Hoffman (8) has confirmed Duran-Reynals' findings in regard to vaccinia, and has extended them to other viruses such as herpes, vesicular stomatitis of horses, and Borna disease.

Attempts at Brain to Brain Transmission of the Virus

(*a*) *Following Exposure to X-Ray.*—Young white rats approximately 6–8 weeks old and young guinea pigs, weighing about 180–220 gm. were exposed to 400 r units at 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., for 19 minutes 1–10 days before inoculation. This dose is somewhat less than that recommended by Zinsser and Castaneda. The animals, however, were very young, approximately 1–2 months old. The

rats withstood this dose of X-ray well, but many of the guinea pigs died following exposure.

In the case of the rats, the virus consisted of an emulsion of submaxillary glands showing the characteristic lesion, obtained from several full grown wild rats. In the case of the guinea pigs, an emulsion of the submaxillary glands of several full grown guinea pigs was used. Normal animals were inoculated at the same time as those exposed to X-ray. Under ether anesthesia, 0.1 cc. of the supernatant fluid obtained from the virus emulsion was inoculated intracerebrally into guinea pigs, and 0.05 cc. into rats. In the first generation no striking difference between the normal and X-rayed animals, either in severity of symptoms or duration of life was observed in guinea pigs or rats. In the first generation X-rayed animals were killed as soon as they appeared sick. After the removal of a piece of each brain for the preparation of histological sections, the rest of the brain tissue was emulsified and injected intracerebrally into other guinea pigs and rats which had been exposed to X-ray radiation. In a few instances it was possible to pass the guinea pig submaxillary gland virus and the rat submaxillary gland virus through 2 generations, but the inoculation of the brain emulsions of the second generation animals into other X-rayed animals gave negative results. Histological sections prepared from the brains of the X-rayed guinea pigs and rats did not show more extensive or striking lesions than those of the normal controls.

No evidence was obtained to indicate that the exposure to this dose of X-ray reduced the resistance of guinea pigs or rats sufficiently to make it possible to transmit these submaxillary gland viruses from brain to brain in series.

(*b*) *With the Addition of Testicular Extract (Duran-Reynals Factor).*—Testicular emulsions were prepared from the homologous species. Male rats and guinea pigs were killed and one testis was removed with sterile precautions. Each testis was cut up finely with scissors and emulsified by grinding with sand in 5 cc. of saline. Duran-Reynals suggests suspending the testicular material in approximately an equal volume of saline, but the suspension prepared in this way, proved too thick for intracerebral injection. After centrifugalization, the supernatant fluid was combined with an equal quantity of virus emulsion and injected intracerebrally into rats and guinea pigs. Control animals were injected with equal quantities of saline and virus emulsion.

No evidence was obtained to indicate that the Duran-Reynals factor (testicular extract) enhances the virulence of these two submaxillary gland viruses. It was impossible to transmit either the guinea pig or rat submaxillary gland virus from brain to brain with the addition of testicular extract.

(*c*) *Following Exposure to X-Ray and with Addition of Testicular Extract.*—In another series of animals the effect of X-ray and testicular extract were combined; equal quantities of virus emulsion and testicular extract were injected into rats and guinea pigs that had been exposed to 400 r units of X-ray. Attempts were made to carry the viruses through 5 generations in this way from brain to brain, transferring regularly on the 5th day. No evidence was obtained that the

Published December 1, 1935

submaxillary gland viruses could be enhanced in virulence by these means. Two animals were injected in each transfer. Only the animals of the first generation appeared sick. In subsequent generations the animal not used for transfer, was observed for 2–3 weeks and then killed, and the submaxillary glands removed for histological examination. The glands of the 4th and 5th generations failed to show the specific lesion, indicating that by this method of rapid transfer from brain to brain, the virus was lost and failed to localize in the salivary glands in the usual way.

Has Brain Tissue Any Inhibitory or Neutralizing Action on the Submaxillary Gland Viruses?

In his original articles, Duran-Reynals (6, 7) compares the effect of other tissue extracts to testicular emulsions in enhancing the virulence of vaccinia. He found that kidney, liver, skin, and brain also had a slight tendency to increase the activity of vaccinia. On the other hand in the case of the submaxillary gland viruses, it seems possible that brain tissue might exert the opposite effect. In spite of the fact that animals injected intracerebrally with these viruses usually died, transfer from brain to brain in series was impossible. To test out the possible neutralizing action of brain tissue on the submaxillary gland viruses of guinea pigs and rats, the results of injecting equal quantities of submaxillary gland virus and brain emulsion, and equal quantities of submaxillary gland virus and testicular extract subcutaneously were compared. The subcutaneous inoculation of the submaxillary gland viruses, followed 2–3 weeks later by histological examination of the submaxillary glands for the presence of the specific lesion, is a more delicate method of testing for small amounts of these viruses than intracerebral injection.

Method.—In this instance, the rat virus was obtained from the submaxillary glands of 9 young rats which had been inoculated either subcutaneously or intratesticularly 2–4 weeks previously. Only one gland was obtained from each animal as the other one had been removed for histological section at the time of inoculation. Although as previously stated, no spontaneous infection in either young or full grown rats has been observed in this laboratory, nevertheless one of the submaxillary glands was usually removed as a control before any kind of injection was made. Whereas in the case of guinea pigs, hamsters, and mice it was necessary to use very young animals before they had become spontaneously infected, it was found that full grown white rats from our stock were as susceptible as young animals to intracerebral and subcutaneous injection. However, young animals be-

tween 1–2 months of age were used almost entirely, since it was thought that the chances of establishing the wild rat virus in them might be better than in adult animals.

A group of 6 young rats from each of which one submaxillary gland had been removed, was divided into 2 lots, 3 were inoculated subcutaneously with equal quantities of rat submaxillary gland virus and a freshly emulsified normal rat brain. The other 3 rats were injected subcutaneously with equal quantities of rat virus and freshly prepared rat testicular extract. The mixtures of rat virus and organ extracts were not allowed to stand, but were injected immediately. The animals were killed 2–3 weeks later, and the remaining submaxillary gland from all 6 animals was removed for the preparation of histological sections. All the submaxillary glands showed typical lesions and no differences were observed in the cellular reaction, or in the number of acidophilic intranuclear inclusion bodies, between the 2 lots of rats.

No evidence was obtained to indicate that extracts of brain tissue reduced the activity of the rat submaxillary gland virus, or that the addition of testicular extract produced any striking generalization of the virus following subcutaneous injection.

A similar experiment was carried out in guinea pigs with identical results.

The Distribution of the Submaxillary Gland Viruses Following Intracerebral Injection

The simplest explanation of the fact that it has been found impossible to date to transmit the submaxillary gland viruses from brain to brain in series, is that these viruses have no tendency to neurotropism. The invasion is limited to the meninges, and it is only when large doses are injected into the brain, that the local reaction is sufficiently intense to cause symptoms and death. If the animal survives the acute meningitis, the virus leaves the brain and invades the submaxillary glands. Even at the height of the meningeal reaction and in spite of the fact that inclusion bodies are numerous in the meningeal exudate, the virus does not seem to multiply to any great extent in the meninges. The inclusion bodies occur for the most part in large endothelial wandering cells. In the meninges of rats they are common in so called foreign body giant cells, such as may occur in many tissues in response to the injection of irritating, extraneous substances. It seems possible that these wandering cells may partially neutralize the activity of the

Published December 1, 1935

virus, so that when the brain of an animal dying of meningitis is injected intracerebrally into another animal, only a very slight reaction occurs, and the virus is soon lost on further passage from brain to brain. The virus is not, however, completely destroyed in animals dying of meningitis, since there is still sufficient virus in the brain emulsion to produce the specific lesion in the submaxillary gland of another animal when the infected brain tissue is injected subcutaneously.

The distribution of the virus was studied in a guinea pig showing symptoms of meningitis the 5th day following intracerebral inoculation of the guinea pig submaxillary virus.

The animal was killed and histological sections prepared from the brain and submaxillary glands. The brain showed a slight, localized meningeal reaction in which inclusion bodies were fairly numerous. The sections of the submaxillary glands failed to show any cellular reaction or specific lesion.

The brain, submaxillary glands, and one kidney were removed with sterile precautions and emulsified separately. Each of the 3 emulsions was tested for the presence of bacteria, and injected intracerebrally into 2 young guinea pigs. All 6 of the guinea pigs remained well and failed to show any appreciable symptoms. They were killed 16 days after inoculation, and the submaxillary glands removed for the preparation of sections. Only the glands of 2 animals that had been inoculated with the brain tissue showed small, very early lesions with typical intranuclear inclusion bodies. The glands from the 4 animals inoculated respectively with the submaxillary glands and kidney, proved negative.

This experiment indicates that the concentration of the virus in the brain of a guinea pig showing symptoms of meningitis 5 days after intracerebral injection, is too small to produce meningitis when injected into a second animal. The virus is, however, present in sufficient quantities to induce a localization of the virus in the submaxillary gland following subcutaneous injection. On the other hand the submaxillary gland and kidney did not in this instance contain enough virus either to produce meningitis when injected intracerebrally or to infect the submaxillary gland when injected subcutaneously.

In another experiment the distribution of the virus was studied in a rat inoculated intracerebrally with submaxillary gland virus on the 10th day following inoculation.

The animal was moribund and was killed with chloroform. Histological sections prepared from the brain showed a fairly extensive meningeal exudate con-

Published December 1, 1935

taining cells with acidophilic intranuclear inclusion bodies. Sections from the submaxillary glands showed a small early lesion with a few inclusion bodies. As in the previous experiment, the brain, submaxillary glands, and kidney were removed with sterile precautions, and emulsified separately. Each of the 3 emulsions was injected intracerebrally into 2 young rats. All 6 rats remained well and were killed on the 20th day after inoculation. The submaxillary glands of all 6 animals were removed for the preparation of histological sections. Slides from the glands of all the animals showed typical lesions with intranuclear acidophilic inclusion bodies in the duct cells.

This experiment indicates that when an animal lives as long as 10 days after intracerebral injection, the virus may be widely distributed in the body, but in none of the tissues was it in sufficient concentration to produce characteristic symptoms or death following intracerebral inoculation.

Occurrence of the Virus in the Kidneys of Spontaneously Infected Animals in the Absence of Kidney Lesions

The occurrence of the virus in the kidney of animals which had been inoculated intracerebrally, seemed to indicate either that the submaxillary gland virus might be eliminated through the kidney or that it might possibly localize in the epithelium of the kidney as well as in the submaxillary gland. Hindle and Stevenson (9) described the occurrence of intranuclear inclusion bodies in the kidney tubules of wild (London) rats. A spontaneous lesion in the kidneys of rats, guinea pigs, hamsters, or mice has not been observed in this laboratory. Nevertheless, it seemed of interest to try to determine whether there was any virus present in the kidneys of rats and guinea pigs with spontaneously infected submaxillary glands in the absence of demonstrable histological lesions.

The kidneys of several wild rats whose submaxillary glands were subsequently shown to contain typical lesions with acidophilic intranuclear inclusion bodies, were removed with precautions for asepsis. A piece of each kidney was put aside for the preparation of histological sections, and the rest of the kidneys were emulsified in the usual way. The emulsion was tested for the presence of bacteria, and then injected intracerebrally into 2 rats, and subcutaneously into 2 rats from which one submaxillary gland had been removed. The rats that had been inoculated into the brain, remained well. The animals that had been inoculated subcutaneously were killed 14 days after injection, and the remaining submaxillary

Published December 1, 1935

gland removed for the preparation of histological sections. These sections showed typical lesions with acidophilic intranuclear inclusion bodies, whereas the glands removed from each animal before inoculation proved to be negative. No lesions were found in sections prepared from the kidneys used for inoculation.

In a similar experiment with guinea pigs, identical results were obtained. Although no demonstrable lesions were found in sections prepared from the kidneys of full grown guinea pigs with positive submaxillary glands, the subcutaneous injection of the kidney emulsion nevertheless produced characteristic changes in the submaxillary glands of young susceptible guinea pigs.

These experiments indicate that the submaxillary gland viruses of spontaneously infected wild rats, and full grown guinea pigs, are present in the kidney in spite of the lack of demonstrable pathological changes. The concentration of the viruses in the kidney seems to be much less than that in the submaxillary gland.

Distribution of the Viruses in Rats and Guinea Pigs after Subcutaneous Injection

In view of the findings stated above, it seemed of interest to determine how widely these viruses were distributed in the animal body following subcutaneous injection. A series of experiments was therefore done in young white rats and young guinea pigs to show in which tissues these viruses were present. The results in guinea pigs and rats were the same, so only one typical experiment will be cited.

The blood, cervical lymph nodes, submaxillary gland, spleen, liver, lung, and kidney were examined in the following way.

A young rat injected with virus subcutaneously 2 weeks before was anesthetized. 3–4 cc. of blood were obtained by cardiac puncture and placed in a sterile bottle containing sodium citrate. The animal was then killed, soaked in 5 per cent lysol, and the various organs removed with precautions for asepsis. Histological sections were prepared from each tissue. Pieces of the liver, lung, spleen, and kidney of approximately equal size were emulsified in the same quantity of saline. The amount of tissue derived from the submaxillary gland and cervical lymph nodes was smaller, but the same quantity of saline was added. All the tissues were ground with sand in the usual way. Cultures were made from each kind of tissue to rule out the presence of bacteria. 0.5 cc. of each organ emulsion and 0.5 cc. of citrated blood were injected subcutaneously into each of 3 rats, from which one submaxillary gland had been removed. The animals all remained well, and were

killed 2–3 weeks after inoculation. The remaining submaxillary gland was removed and histological sections prepared. No specific lesions were found in any of the tissues used for inoculation, except the submaxillary gland.

The submaxillary glands of the rats which had been injected with blood, liver, and spleen were uniformly negative in contrast to the submaxillary glands of the rats which had been injected with submaxillary gland, cervical lymph nodes, kidney, and lung which were usually positive. The lesions in the glands of the animals injected with submaxillary gland were nearly always the most extensive, and contained the greatest number of acidophilic intranuclear inclusion bodies. The concentration of the virus in the lung seemed very slight as judged by the fact that the inoculated animals were not uniformly positive and the lesions were always small. In order to obtain such a wide distribution, it is obvious that the blood of the animals which had been inoculated subcutaneously, must at some time have contained virus, but apparently these viruses do not persist in the circulation. In view of Thompson's recent observations (10) that intranuclear inclusions occur in the liver of apparently healthy rats, it is of interest that in these experiments no evidence was obtained of the virus being present in this organ.

Direct Injection of the Viruses into the Kidneys of Rats and Guinea Pigs

Since these viruses were found in the kidney of infected animals so frequently, it was thought possible that they might proliferate easily in the epithelium of the kidney following direct injection.

Direct injections of the homologous virus into the kidney of both rats and guinea pigs were made. The animals that survived operation, suffered no obvious ill effects from the injection, and were killed at various intervals. Often the scar made by the inoculation was visible in the gross. Sections were prepared both from the scarred area and from areas that appeared normal. The microscopic lesions produced by direct injection into the kidney were always circumscribed and more or less confined to the needle tract. The rest of the kidney did not become involved. The number of intranuclear inclusion bodies found was small and they were limited to the cells of the tubules in the area of scar formation (see Figs. 1 and 2). The glomeruli never contained inclusion bodies following direct injection into the kidney.

Published December 1, 1935

The marked predilection of these viruses for the submaxillary gland was also apparent in these experiments. Sections of the submaxillary glands of the animals inoculated directly into the kidney, nearly always showed definite lesions if the animals were killed 8 or more days after inoculation. The number of inclusion bodies in the submaxillary gland was consistently greater than that found in the kidney itself.

In this connection it is of interest to note that on 2 occasions in guinea pigs which had been exposed to X-ray and which had reacted severely to radiation, microscopic lesions were found in the kidney as well as in the submaxillary gland as the result of subcutaneous injection of the guinea pig submaxillary gland virus. In one of these guinea pigs the lesion in the kidney was fairly extensive: typical acidophilic intranuclear inclusion bodies were present in the cells of tubules, in the interstitial tissue, and in the glomeruli (Fig. 3). In this animal, the site of inoculation was hemorrhagic and necrotic. Sections prepared from this area showed typical inclusion bodies in the subcutaneous tissue (Fig. 4). A local reaction of this kind was never obtained in normal guinea pigs following subcutaneous injection.

The subcutaneous injection of the rat submaxillary gland virus into rats that had been exposed to a similar dose of X-ray did not produce any gross local reaction. As stated before, young rats seemed consistently more resistant to X-ray than young guinea pigs.

Can "Virus" Pneumonia Be Produced with the Submaxillary Gland Viruses?

In recent years the concept (11) that certain epidemic diseases presumably due to filtrable viruses, such as measles, epidemic influenza, and whooping cough, are often complicated by a characteristic type of pneumonia, has been accepted by many workers. This so called virus pneumonia differs from the usual pyogenic lobar or lobular pneumonia by certain well defined pathological findings. It is essentially an interstitial pneumonia in which the more acute stages are characterized by edema and hemorrhage into the alveoli. The alveolar walls tend to become thickened by the invasion of mononuclear cells of various kinds. Whatever purulent exudate is present, is in most cases confined to the bronchi. There is a marked thickening of

Published December 1, 1935

the bronchial walls due to the presence of a "collar" of mononuclear cells. These collars are sometimes so conspicuous that they can be observed with the naked eye.

Several observers (12–14) have recently emphasized the occurrence of this type of pneumonia in children dying of pertussis. In addition to finding pathological changes consistent with a virus pneumonia, acidophilic intranuclear inclusion bodies have been found in a fairly high proportion of the cases coming to autopsy. In order to evaluate the significance of the inclusion bodies occurring in pertussis, McCordock and Smith (14) have also studied the incidence of intranuclear inclusion bodies in the submaxillary glands of children dying of this disease. These authors are of the opinion that inclusion bodies occur more frequently in the submaxillary glands of children dying of whooping cough, than in the submaxillary glands of children dying from other causes. McCordock and Smith raise the question whether the inclusion bodies found in the lungs of pertussis cases, could be due to the activity of a filtrable virus present in the salivary glands, or to a specific filtrable virus which is the cause of whooping cough.

In a previous paper (1) one of us attempted unsuccessfully to demonstrate a filtrable virus in the submaxillary glands of children dying from miscellaneous causes, which showed typical acidophilic intranuclear inclusion bodies. In spite of the fact that no infectious agent has to date been shown to be present in the salivary glands of children which show these changes, it seemed of interest to study the effect of intratracheal injections of the submaxillary gland viruses of rats and guinea pigs.

It is often considered that virus pneumonia as it occurs in man, is the result of the combined action of a virus and bacteria. The virus is thought to facilitate the entrance of the bacteria into the lung. Shope (15) has conclusively proved the dual etiology of swine influenza.

Methods.—In these experiments, the animals were divided into 4 groups: one received virus alone, one virus and bacteria, one heat-killed virus, and one bacteria alone. The methods used in guinea pigs and rats were essentially the same. The virus was prepared in the usual way from the submaxillary glands of animals that had been injected with virus subcutaneously 2 or more weeks previously. In order to estimate the potency of the particular lot of virus used, intracerebral injections were made at the same time as the intratracheal. The viruses were killed by heating at 60°C. for 30 minutes.

Bacteria.—A strain of small Gram-negative bacilli closely resembling human influenza bacilli was isolated from the throat of a normal rat. These organisms grew more luxuriantly on "chocolate" agar than on blood agar, but failed to grow on plain agar. The same culture was used for guinea pigs and rats and will hereafter be designated as the rat "influenza" strain. Several chocolate agar slants were washed off with saline and a moderately heavy suspension was used.

The animals to be injected were anesthetized, and one submaxillary gland removed for the preparation of histological sections. At the same time the trachea was exposed and a direct injection made, 0.3–0.5 cc. into rats and 0.7–1 cc. into guinea pigs. The animals injected with the mixture of virus and bacteria, received equal quantities of virus emulsion and bacterial suspension, the animals receiving virus alone, an equal quantity of virus and saline. Irrespective of what had been inoculated, many of the animals died shortly after injection. The animals that died soon showed in the gross extensive hemorrhages in the lungs. Microscopically, the main findings were hemorrhage and edema into the alveoli, and purulent exudate in the bronchi.

Most of the animals that survived for several days did not appear to be sick. One guinea pig which had received virus alone was found dead on the 8th day. The lungs of this animal appeared slightly hemorrhagic at autopsy, but no definite consolidation was apparent. Microscopically, a moderately extensive interstitial pneumonia was present, and many of the alveolar and wandering endothelial cells contained acidophilic intranuclear inclusion bodies (Figs. 5 and 6). The trachea also showed areas of cellular reaction in the interstitial tissue and intranuclear acidophilic inclusion bodies were found in large mononuclear endothelial cells. The tracheal epithelium itself was never involved. Sections of the submaxillary gland showed a very small area of reaction in which two typical small intranuclear inclusion bodies were found. No well defined bronchial collars were observed in this animal.

Two rats, one injected with virus alone, and one with virus and bacteria, were killed 7 days after inoculation, and sections prepared from the lungs and the remaining submaxillary glands. In the gross the lungs appeared more or less normal except for one small area of hemorrhage in the rat injected with virus and bacteria. Microscopically, the lesions in the animal receiving virus and bacteria were more extensive than those in the animal receiving virus alone, and there was a marked purulent bronchial exudate present. Both these animals showed a small number of well marked collars around the bronchi consisting mainly of large, wandering mononuclear cells, some of which contained typical intranuclear inclusion bodies (Figs. 7 and 8). The trachea of both these rats showed areas of cellular reaction in the interstitial tissue. Acidophilic intranuclear inclusion bodies were present in large mononuclear endothelial cells (Fig. 9). The tracheal epithelium itself was not involved. In the rat that received the mixture of virus and rat influenza bacilli no masses of organisms, such as are commonly seen in children dying of pertussis, were found amidst the cilia of the tracheal epithelium.

Published December 1, 1935

The submaxillary glands of both rats showed areas of cellular infiltration, and in the gland of one of the animals typical inclusion bodies were found.

Some of the animals were killed at longer intervals, 12–14 days after injection. The lesions in both rats and guinea pigs were less striking than those in animals killed after 7–8 days, although a few inclusion bodies were found in the alveoli.

Inclusion bodies were never found in the lungs of the animals that had been injected either with heat killed virus, or with the strain of rat influenza bacilli.

Intratracheal injection of the virus in almost every instance led to a rapid invasion of the submaxillary gland. Sections from the submaxillary glands of nearly all the animals surviving 7 days or longer that had been injected with living virus were positive. The glands of the animals that received either heat-killed virus or bacteria alone were uniformly negative.

These experiments indicate that it is possible to produce the picture of virus pneumonia by intratracheal inoculation of the submaxillary gland viruses in rats and guinea pigs provided large doses of virus are injected. The results obtained are similar to those of Muckenfuss *et al.* following intratracheal injection of vaccine virus into rabbits (16). No evidence was obtained that these viruses facilitated the invasion of the strain of rat influenza bacilli used. The injection of foreign material of any sort intratracheally produces considerable reaction. These viruses show a much greater tendency to proliferate in the submaxillary gland than in lung tissue.

Does Transfer in Young Rats Increase Virulence?

Stewart and Duran-Reynals (17) have shown that vaccine virus tends to become generalized following intradermal injection when combined with testicular extract.

The wild rat virus was transferred subcutaneously at regular intervals of 10–14 days, with and without the addition of testicular extract through 6 generations of young white rats. All the animals remained well, and the virus localized in the submaxillary glands in the usual way. No evidence was obtained to indicate that the virus increased in virulence.

Injection of the Wild Rat Virus Intratesticularly

The wild rat virus was injected directly into the testis of young white rats. The injected testis showed no definite local reaction. Sections prepared from the testis showed small areas of cellular reac-

tion, but no intranuclear inclusion bodies were found either in the interstitial or parenchymal cells. Sections prepared from the submaxillary glands of the animals 2 or more weeks after intratesticular injection, usually showed characteristic lesions.

Does Generalization Occur if the Salivary Glands Have Been Removed?

The predilection of the submaxillary gland virus for the salivary glands is so characteristic that it seemed of interest to try to determine how the rats would take care of the virus if these glands were removed as completely as possible. Under ether anesthesia, all the recognizable salivary gland tissue and cervical lymph nodes were excised. After 1 or 2 days when the animals had recovered from the operation, the rats were injected subcutaneously with virus emulsion. The animals remained well, and were killed after 2–3 weeks. At autopsy a small fragment of salivary gland could always be found, which showed typical lesions.

As in the case of guinea pigs, it was found that total extirpation of the salivary gland tissue was impossible.

Do the Lesions in the Salivary Glands of Infants Persist to Adult Life?

In rodents the lesions in the salivary glands, usually acquired in the first few months of life, seem to persist indefinitely. No one to date has described lesions in the salivary glands of human adults similar to those found in infants. In one instance VonGlahn and Pappenheimer (18) have described visceral lesions such as occur in infants, in a man 36 years of age.

It seemed of interest to study sections of surgical specimens containing fragments of more or less normal submaxillary gland removed during the course of various operations. About 25 specimens of this kind have been examined, but no inclusion bodies were found.

DISCUSSION

The submaxillary gland viruses as they occur in rodents represent a benign infection. The most striking characteristic of these viruses, as stated before, is their marked predilection for the salivary glands. Once infected, the animals harbor the infectious agent throughout life, and the virus can be isolated from salivary glands that show the characteristic lesions at any time, although the host himself is immune.

One of the important characteristics of certain filtrable viruses is their tendency to invade the nervous system. In many diseases in which a virus etiology is proven or suspected, *e.g.* vaccinia, variola, measles, and pertussis, encephalitis is fairly common. This aspect of filtrable viruses has been intensively studied in recent years. It is worthy of note that in mumps, an infectious disease of the salivary glands, meningitis is not an infrequent complication. Johnson and Goodpasture (19) have been able to transmit mumps to monkeys, but no inclusion bodies have been found in the infected submaxillary glands of these animals. To date no work has been reported as to the effect of intracerebral or intraspinal injection of the mumps virus into monkeys.

Although the submaxillary gland viruses of rodents, when injected intracerebrally, produce a characteristic type of meningitis, it has been impossible to modify them in such a way as to make them attack the nervous tissue itself, either by the addition of the Duran-Reynals factor, or by reducing the resistance of the host by X-ray.

The distribution of the submaxillary gland viruses in the body of spontaneously and artificially infected animals seems to be fairly wide. The viruses have been found in tissues (kidney and lung) in which no pathological evidence of their presence is demonstrable. They are apparently disseminated *via* the circulation, but do not seem to persist for any length of time in the blood. Attempts to adapt the submaxillary gland viruses of rats and guinea pigs to other organs such as the kidney, lung, and testis, have not met with very striking results. Whether or not the acidophilic intranuclear inclusion bodies reported by Findlay (20) in the livers of Clacton mice, and in the livers of both mice and rats by Thompson (10), are due to the submaxillary gland viruses, or some other viruses, remains undetermined. In our experiments following subcutaneous injection, the liver apparently did not tend to harbor the virus.

The submaxillary gland viruses as they occur in guinea pigs, hamsters, mice, and rats are remarkably uniform in their properties, and no significant variations have been observed. Cowdry (21) has recently reported the occurrence of acidophilic intranuclear inclusion bodies in the submaxillary glands of a certain species of monkey (*Cebus fatuellus L.*). It would be of interest to determine the presence of a virus in this higher form, and compare it to the rodent viruses.

In the case of man, the virus etiology of the lesions found in the salivary glands of infants has yet to be proved. However, certain differences in the pathological changes as they occur in man and in rodents are apparent at the present time. In rodents it has been emphasized that new-born animals are uniformly free of the infection, and there is no evidence indicating intra-uterine infection. In man, on the other hand, as previously pointed out (1), although lesions in the submaxillary glands of infants have not been reported before the age 2 months, visceral lesions with hypertrophied cells and typical acidophilic intranuclear inclusion bodies have been found in still-births and infants that lived only 1–2 days. Of course it may be that we are dealing with 2 different viruses, but if it is a virus at all, we have to assume that the still-births were infected *in utero*.

The other interesting difference between man and rodents in respect to these pathological changes in the salivary glands, is the tendency for these lesions to persist in full grown animals, whereas in human beings as far as we know, they have never been reported in adults. On one occasion (18) visceral lesions similar to those of infants have been described in a man of 36 years.

The most important aspect of the submaxillary gland viruses is the one emphasized by the work of McCordock and Smith (14): Provided that the lesions in the salivary glands of infants are due to an infectious agent, can this virus under certain circumstances invade the lung? Although it is always dangerous to draw analogies from one animal species to another, it seemed of interest to determine what kind of lesions resulted from the intratracheal injection of the submaxillary gland viruses of rats and guinea pigs. By the injection of large quantities of virus, pathological changes suggestive of a virus pneumonia could be produced in the lungs of guinea pigs and rats. These viruses, however, showed no marked tendency to invade lung tissue, and even when mixtures of virus and bacteria were injected intratracheally, no severe lesions developed. These viruses did not seem to facilitate the multiplication of bacteria in the lung. The virus in salivary glands of man, however, may be more virulent than that of rodents. Perhaps further light can be thrown on this question by studies in the monkey (*Cebus fatuellus L.*).

CONCLUSIONS

1. It has not been possible to increase the virulence of the submaxillary gland viruses of guinea pigs and rats, either by reducing the resistance of the animals by exposure to X-ray, or by the addition of testicular extract (Duran-Reynals factor).

2. In guinea pigs and wild rats with spontaneously infected submaxillary glands, the kidney has been found to contain the virus in the absence of demonstrable pathological changes.

3. Direct injection of these viruses into the kidney produces only mild, circumscribed lesions.

4. The viruses, following subcutaneous injection into white rats and guinea pigs, are widely distributed 2 weeks after injection. They are present in the submaxillary glands, cervical lymph nodes, kidney, and lung. They were not demonstrable at this time in the blood, liver, or spleen.

5. By the intratracheal injection of large doses of virus in guinea pigs and rats, an interstitial bronchopneumonia with thickening of the alveolar and bronchial walls and the presence of acidophilic inclusion bodies, can be produced.

6. No evidence was obtained to indicate that the multiplication of bacteria in the lung is greatly enhanced by the injection of these viruses.

BIBLIOGRAPHY

1. Kuttner, A. G., and Wang, S. H., *J. Exp. Med.*, 1934, **60**, 773.
2. Cole, R., and Kuttner, A. G., *J. Exp. Med.*, 1926, **44**, 855.
3. Thompson, M. J., *J. Infect. Dis.*, 1932, **50**, 162.
4. Hudson, N. P., and Markham, F. S., *J. Exp. Med.*, 1932, **55**, 405.
5. Zinsser, H., and Castaneda, M. R., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 840.
6. Duran-Reynals, F., *Compt. rend. Soc. biol.*, 1928, **99**, 6.
7. Duran-Reynals, F., *J. Exp. Med.*, 1929, **50**, 327.
8. Hoffman, D. C., *J. Exp. Med.*, 1931, **53**, 43.
9. Hindle, E., and Stevenson, A. C., *J. Roy. Soc. Trop. Med. and Hyg.*, 1930, **23**, 327.
10. Thompson, M. J., *Am. J. Path.*, 1934, **10**, 676.
11. McCordock, H. A., and Muckenfuss, R. S., *Am. J. Path.*, 1933, **9**, 221.
12. McCordock, H. A., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 1288.
13. Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 346.

14. McCordock, H. A., and Smith, M. G., *Am. J. Dis. Child.*, 1934, **47**, 771.
15. Shope, R. E., *J. Exp. Med.*, 1931, **54**, 373.
16. Muckenfuss, R. S., McCordock, H. A., and Harter, J. S., *Am. J. Path.*, 1932, **8**, 63.
17. Stewart, F., and Duran-Reynals, F., *J. Exp. Med.*, 1929, **50**, 341.
18. VonGlahn, W. C., and Pappenheimer, A. M., *Am. J. Path.*, 1925, **1**, 445.
19. Johnson, C. D., and Goodpasture, E. W., *J. Exp. Med.*, 1934, **59**, 1.
20. Findlay, G. M., *Brit. J. Exp. Path.*, 1932, **13**, 223.
21. Cowdry, E. V., and Scott, G. H., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 709.

EXPLANATION OF PLATES

PLATE 35

FIG. 1. The kidney of a guinea pig injected directly with the guinea pig submaxillary gland virus 8 days after injection. Inclusion bodies are visible in the tubule just above the glomerulus. × 155.

FIG. 2. The same tubule under high power showing several small inclusion bodies. × 1290.

FIG. 3. Inclusion bodies in the glomerulus of an X-rayed guinea pig inoculated with virus subcutaneously, 9 days after injection. × 1145.

FIG. 4. Inclusion bodies in the subcutaneous tissues at the site of inoculation of the same guinea pig as Fig. 3. × 1145.

PLATE 36

FIG. 5. The lung of a guinea pig found dead 8 days after intratracheal injection of guinea pig submaxillary gland virus, showing hemorrhage and thickening of the alveolar wall. × 145.

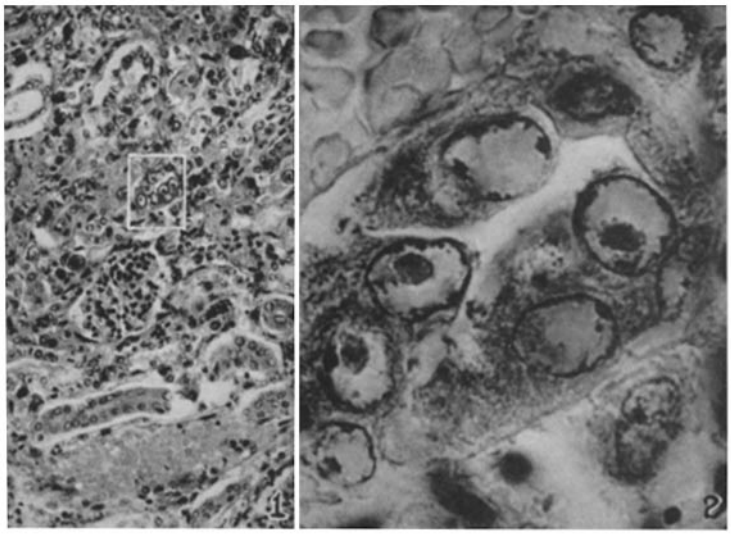
FIG. 6. High power of Fig. 5 showing an intranuclear inclusion body in the alveolar wall. × 1210.

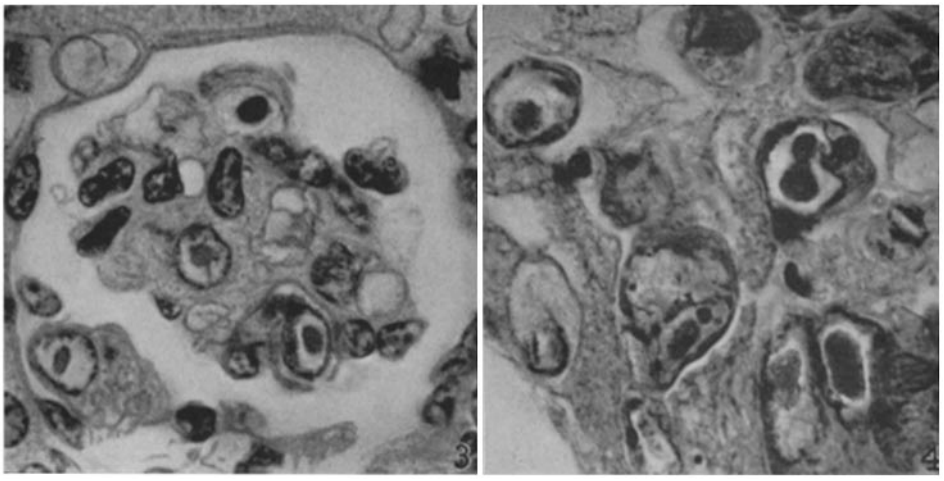
FIG. 7. The lung of a rat 7 days after the intratracheal injection of the rat submaxillary gland virus and bacteria showing the thickening of the bronchial wall. Several inclusion bodies are visible in the collar surrounding the bronchus. × 145.

FIG. 8. High power of Fig. 7 showing inclusion bodies. × 1210.

FIG. 9. The trachea of the same rat as Fig. 8 showing inclusion bodies in 2 endothelial cells lying just below the tracheal epithelium. × 1210.

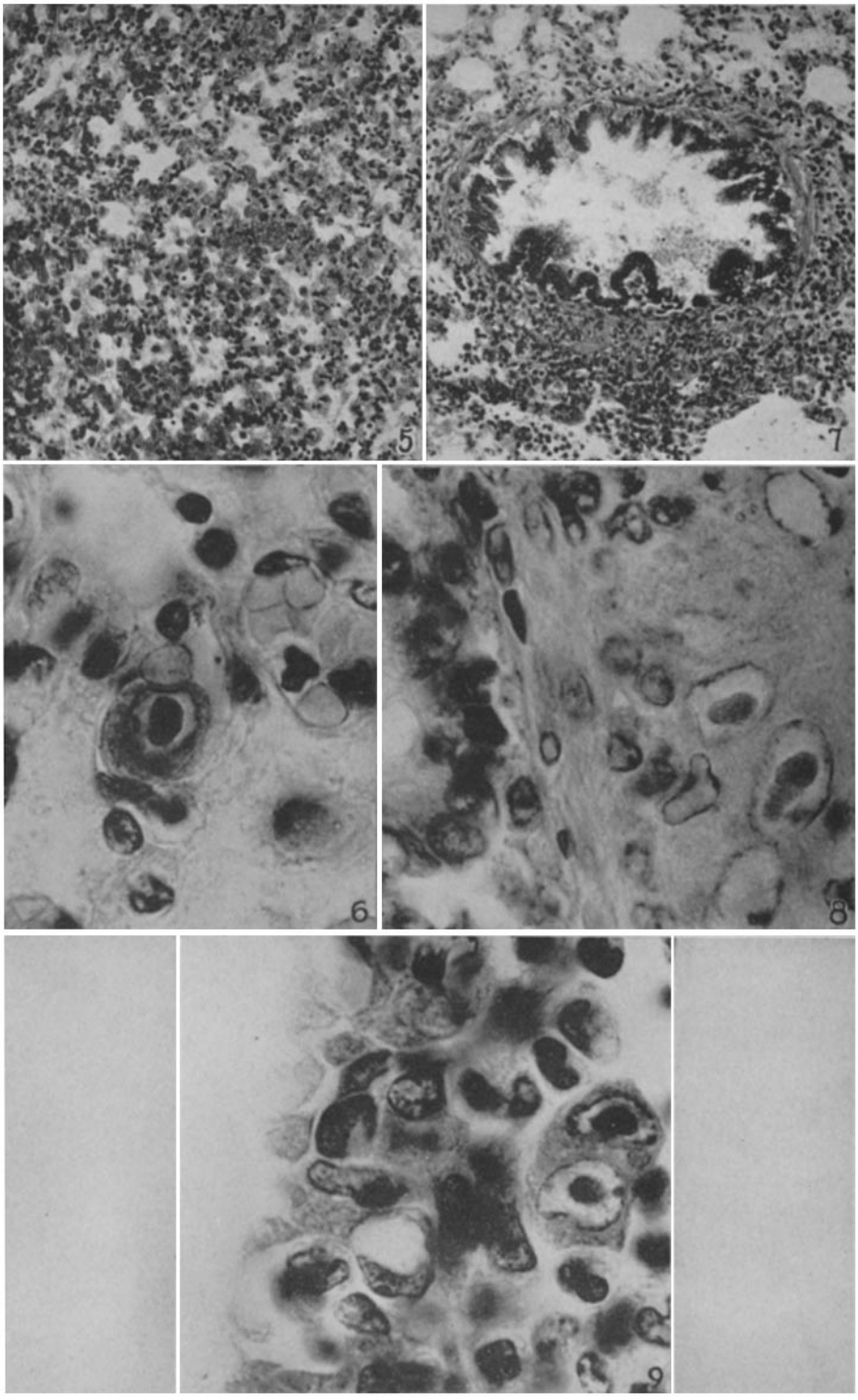
Published December 1, 1935





(Kuttner and T'ung: Submaxillary gland viruses)

Published December 1, 1935



(Kuttner and T'ung: Submaxillary gland viruses)