SOME GENERAL CONSIDERATIONS ON THE PATHOLOGY OF SMALLPOX.

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I shall endeavor to give in this paper the results of an investigation of smallpox which was undertaken by members of the Pathological Department of the Harvard Medical School. The work on the disease in man was carried out in this city during the epidemic of 1901 and 1902. The experimental work was carried out in the Philippine Islands. Funds for this purpose were provided by the liberality of three gentlemen in Boston and by the Rockefeller Institute in New York. The results of the investigation are incomplete. There are many questions still to be solved and the solution can only come from further clinical and experimental study of the disease. There are great difficulties in the way of the investigation of smallpox. The disease only comes at intervals. The patients are not treated in well-organized hospitals, where there are facilities for and the habit of the study of disease. The hospitals in which the disease is treated are, as a rule, used only at intervals and are unprovided with laboratories. The great stimulus to research, clinical teaching, has no place in them. The energies of the physicians in charge are entirely taken up in controlling the exigencies of an unusual situation. The very atmosphere of a smallpox hospital is unfavorable for the careful determination of the questions to be solved and for quiet investigation. The isolation of the disease is unfavorable, in that the valuable aid given by constant comparison with other diseases is lost. There is frequently an attempt to keep the presence of the disease below the surface and to have nothing done which may in any way call attention to it. In the epidemic of 1901 and 1902 the health authorities of the city gave us every facility for investigation which was possible. Autopsies were held in fifty-two cases, embracing all forms of the disease, and provision was made that members of the department could live in the hospital.

Difficulties also attend the experimental study of the disease. Such study cannot be carried out in a laboratory, where there would be the possibility of the infection extending from the laboratory. The laboratory animals, as the rabbits and guineapigs, are immune to the disease. The only animal, so far as known at present, which is susceptible to smallpox is the monkey. These are expensive, difficult to acquire, and

in this climate very susceptible to disease. The majority of monkeys obtained from animal dealers are infected with tuberculosis. It was on this account that the expedition was sent to the Philippine Islands, where animals for experimentation could be easily obtained, and where smallpox virus was available from the occasional cases of the disease among the natives. The study of the disease in Boston was carried out mainly by Drs. Magrath, Brinckerhoff and Tyzzer, assistants in the Department of Pathology; by Professor G. N. Calkins, of Columbia University, and myself. The study of the disease in the Philippines was carried out by Drs. Brinckerhoff and Tyzzer. A number of articles on the disease have already appeared in the *Journal of Medical Research,* in 1903 and 1904, and articles embracing the work of the Philippine expedition will shortly appear.

From the middle of the last century up to the present there have been three periods in which the disease has been studied, and in which important contributions to knowledge have been made. In 1873, by the work of Weigert and other German observers; in 1902, by the work of Guanieri and other Italian observers; and in 1903, in this country, by the work of Howard, in Cleveland; of Ewing and Park, in New York, and by the work in Boston.

If material taken from a smallpox lesion on man be inoculated on an epithelial surface of a calf, after a definite period of incubation, a lesion which anatomically closely resembles the parent lesion, the pock, is produced, and marked constitutional disturbances, the most prominent of which is fever, take place. The material from the place of inoculation transferred to an epithelial surface on another calf develops in the same way, and after a series of transfers from calf to calf may be returned to man, and it develops not the original disease, smallpox, but the incomparably milder disease, vaccinia. This is no longer in doubt. Many of the strains of vaccine virus now used have been originally derived from smallpox. Just how many transfers from smallpox are necessary before the original virus loses its power of producing the parent disease is not known. One of our experiments in this regard is interesting. The contents of a smallpox vesicle from a monkey was used to inoculate the cornea of a rabbit. After five successful transfers from animal to animal, a monkey was inoculated and not vaccinia but smallpox was produced. This disease, vaccinia, confers immunity not only against vaccinia, but against smallpox. The immunity, though not absolute, is stronger than is developed in the course of most of infectious diseases. It persists a variable time, the variability being due to the individual. All the evidence which we have regarding the immunity shows that it is due to a germicidal power which the blood-serum has

acquired by the disease enabling the serum to destroy the virus of both vaccinia and smallpox when brought in contact with it. The disease, vaccinia, differs from smallpox in three striking ways: 1. The period of incubation is shorter, being in man five days and in the calf three days. The incubation period of variola is twelve days. It is possible to give a partial explanation of this difference by the fact that a very much greater amount of virus is introduced in vaccinia and the means for absorption of the virus or its products are facilitated by the trauma of vacciniation. We have no means of knowing the amount of smallpox virus necessary to produce the disease nor the facilities which the primary focus affords for systemic infection. 2. In vaccinia the general exanthem is absent. There may be a few vesicles around the point of inoculation, which may be explained by the supposition that at the time of inoculation a small amount of the virus entered the superficial lymphatics and was distributed around a small area. Such satellite vesicles are exceptional. They are not due to the distribution of virus after its development at the point of vaccination, for they develop simultaneously. 3. For the development of vaccinia it is necessary that the virus be placed in contact with a susceptible epithelial surface. Vaccinia may be transferred from individual to individual by accidental contact or by means of intermediate objects, but I know of no cases in which the infection has been carried by the air.

If material from a smallpox lesion be placed in contact with a susceptible epithelium of man, not previously immunized, or of the monkey there develops at the site of inoculation a lesion larger but having the general characteristics of the pock, with constitutional disturbances, and an exanthem less abundant but having all the other characteristics of the exanthem of variola vera. All that we know of variola inoculata in man has come down to us from the old literature. Inoculation of smallpox to confer immunity from the disease is now obsolete in civilized lands. Plehn mentions that it is still carried out among the natives in Central Africa. All that we know from the old literature is to the effect that the disease was incomparably milder than smallpox and was attended by a remarkably low mortality when skilfully carried out. One of the general rules for successful inoculation was that the virus should be introduced superficially, never directly, into the skin or subcutaneous tissue. Among the Chinese it was the habit to inoculate by placing smallpox crusts in the nostrils. So far as can be ascertained the period of incubation is eight days. There is no doubt that the practice of inoculating smallpox to confer immunity would have been extensively used and probably still used were it not for the fact that the individual with the mild disease, variola inoculata, is capable of transmitting to

others the true disease. There is not the fundamental difference which is found in vaccinia. There is not a qualitative difference in the virus of variola inoculata as compared with variola. It is capable of air transmission, and in this way produces variola. It was formerly the custom for the individual before inoculation to undergo a certain regime of food and medicine, which was supposed to have an influence in resisting the virus, enabling the milder disease to be produced. There is no reason to believe that this was the case. With our present vastly increased knowledge of disease and nutrition, it can hardly be held that any regime would make an individual more resistant to a particular disease. Smallpox epidemics have not shown that the robustness of an individual diminishes the chance of infection or the virulence of the disease. Variola inoculata only differs from variola vera in its milder course and shorter period of incubation. A disease in all respects comparable to variola inoculata in man save that the period of incubation is shorter, is produced in monkeys by inoculating them with smallpox virus.

When smallpox prevails epidemically, individuals not immune acquire the disease. The susceptibility of man to the disease is almost absolute. Before vaccination, when smallpox prevailed endemically, with occasional epidemic extensions every twelve or fifteen years, it had become almost a disease of childhood. Adults had acquired immunity, by having passed through the disease in childhood. There is great difference in the severity of the disease in different cases and in different epidemics. The virulence of the disease bears a definite relation to the intensity of the exanthem, which has made possible the classification into variola without exanthem, discrete variola and confluent variola. A tendency to hemorrhage in the lesions is indicative of severity, the acme of virulence being seen in the type purpura variolosa, which differs so much from ordinary forms that its nature has been in dispute. To what these different degrees of severity are due we do not know. In an epidemic the most severe cases can apparently take their origin from the mildest cases. In the recent epidemic of the disease in this country, the general type was mild, and in certain parts of the country the mildness of the type was phenomenal. I have had the opportunity of studying the eruption in certain of these very mild cases, and have found the same histologic changes with the same types of organisms as in the severe cases, but the organisms were much less numerous. The period of incubation in smallpox is twelve days, this being the main point of separation between variola vera and variola inoculata. Vaccinia, variola inoculata, and variola vera have three points in common: The production of a local lesion, which in all three has the same general type,

swelling of the lymph-nodes adjacent to the lesion and the production of immunity. Variola vera and variola inoculata differ from vaccinia, in that a virus capable of air transmission is produced in the two latter, and the local lesion is followed by an exanthem. Variola inoculata differs from variola vera in the milder course of the disease and in the shorter period of incubation.

The study of smallpox in the last epidemic has thrown some light on these various conditions. The histology of the vaccine process is best studied by inoculations made on the cornea, a method which was introduced by the Italian investigator, Guanieri. The tissue of the cornea is extremely simple; there are no blood-vessels to complicate the picture, the epithelial cells are large, few in number, and all changes taking place in them are readily studied. The lesion differs from vaccination of the skin in the absence of vesicle formation, easily explained by the anatomic structure of the cornea which does not permit of it. Owing to the absence of vessels, the vascular reaction takes place around the cornea only, and fluid exudation passes through the thin conjunctiva and through the periphery of the cornea before reaching the lesion. The vaccination is made by passing a lancet-shaped needle obliquely into the tissue, making a slight trauma, of which there is no macroscopic evidence in the absence of the specific infection. In a successful vaccination, a small elevated, slightly opaque spot appears in twenty-four hours. Microscopic study of the cornea after sixteen hours shows the presence of small bodies, round or irregular in shape, within the epithelial cells. They are extremely small and of fairly constant size, the round ones varying about one micron in diameter.

By the examination of numerous corneas at varying intervals of time, it has been made out that these bodies increase in size, and with this growth a differentiation of structure takes place in the previously homogeneous body. Small points staining differently appear, they become more numerous, and finally the body breaks up in a number of small forms, which are similar to the smallest bodies first observed. The small forms again enter the adjacent cells, and the development is repeated. After fifty hours the small, the developing, and the segmenting forms are found in the same section. Similar bodies having the same course of development have been found in the vaccine lesion of the skin, cornea, and mucous membrane of the calf, in the skin and mucous membrane of the monkey, and in the skin of man. Their appearance constitutes the sole anatomic criterion for determining the specific nature of the process. They are found in no other condition. They can be differentiated from other accidental inclusions in cells. They have a definite relation to the nucleus lying in a space in the cell adjacent to

this. They grow, showing differentiation of structure with growth, and finally segment, this resulting in the production of bodies similar to the small forms first found. Similar bodies having the same development are found when variola virus instead of vaccine is used for inoculating the rabbit's cornea. We have elsewhere given the reasons which have led us to regard these bodies as living organisms, and as forming the specific contagium of vaccine. It has not been possible to find, or at least to recognize, such bodies in the vaccine lymph, but this is probably due to technical difficulties in the examination.

We have seen that the virus of smallpox differs from the virus of vaccinia, (1) in producing in man and in monkeys, not vaccinia but smallpox; (2) in being capable of air transmission; that, when smallpox virus is used for inoculating the calf or rabbit, a process in all respects resembling vaccinia is produced. When used for inoculating a monkey, a process identical with the pock is produced. We are concerned not with the anatomic structure of the pock, but with the specific bodies which are found there. With the development of the pock there first appear, included within the cells adjacent to the nucleus, bodies of the same size, structure, and undergoing the same course of development as in vaccinia.

Their study is attended with more difficulty in the skin than in the cornea, owing to the greater complexity of the tissue. In addition to the bodies already described in vaccinia and following them, other bodies begin to appear, which undergo their development not in the protoplasm, but in the nuclei of the epithelial cells. The development of the nuclear body finally results in the formation of a structure comparable to a sporoblast, in which spore-like bodies from one-third micron to one micron in size are formed. These differ in size and structure from the products of segmentation of the vaccine organism. In the earliest lesions in smallpox only the bodies in the cytoplasm of the cells are found. In the later lesions both are present, the intranuclear forms then occupying the center and oldest part of the pock. We believe as the result of our studies that the organism which constitutes the virus of vaccinia and smallpox is the same; that in vaccinia it undergoes a definite cycle of development, resulting in a structure, the gemmule, arising from simple growth and segmentation; that in smallpox a further and more complicated cycle of development, in which probably sexual forms occur, is added to the vaccine cycle. It is only in man and in the monkey that the conditions are favorable to the development of the cycle which constitutes smallpox. The intranuclear parasites are just as characteristic for smallpox as are the cytoplasmic forms for vaccinia. They are found in both variola inocu-

lata and in variola vera. They are not found in any other process. There is no doubt that in various pathologic conditions certain changes may take place, both in the nucleus and cytoplasm of a cell, which may have a certain resemblance to some forms of the parasites. Every one who has had any experience in the study of unicellular organisms in tissues knows that it is often impossible to say whether a single object belongs to the host cells or is a parasite. Not only must form and size and structure of the single individuals be studied, but their relation to their surroundings and their stages of development must be borne in mind. We believe that the spores which arise from the multiplication of the intranuclear bodies constitute the contagion of smallpox, which is capable of air transmission. This introduced into a susceptible animal develops the typic disease, smallpox, both cycles of the organism taking place in the lesions. In the non-susceptible animal, such as the calf or rabbit, only the single, and probably asexual cycle is developed, constituting vaccine. Even though the organism only undergoes this simple development, the potentiality for a complete development may be retained for a time, as is shown by the experiment of Dr. Tyzzer, in which a smallpox virus from a case of variola inoculata of the monkey, after passing through five generations in the cornea of rabbits, was still capable of producing variola inoculata with an exanthem in the monkey.

The experimental study of smallpox in the monkey was undertaken with the view of the investigation of the relationship between vaccinia, variola inoculata, and variola, the ascertaining of the mode of infection in man, the mode of production of the exanthem and the possible relationship between the exanthem and immunity.

Monkeys are susceptible to variola inoculata. It has not been found possible to give them the human disease, the true variola. This, so far as we know, only appears in man and is produced by a mode of infection which we do not know, but there are strong grounds for the hypothesis that the place of primary infection is in the respiratory mucous membrane. Attempts were made to give monkeys variola by means which would have certainly proved successful in man. They were exposed to smallpox patients in the wards of the hospital, material which had been in contact with the patients was placed in their cages and non-infected monkeys kept in the same cage with infected, but no effect was produced. The common monkey of the Philippines is the Macacus cynomologus which had been proved to be susceptible to both vaccinia and variola inoculata. Thinking that the higher anthropoid apes would be more susceptible to the disease and might develop variola vera, the same experiments were carried out with orang-utans procured from

Java, and these proved no more susceptible than the common monkeys. There is some evidence from the literature that monkeys can acquire variola. Andrew Anderson, in his book on "Fevers," gives an excerpt from a letter which he received from a friend who was traveling in Central America. He says:

In the year 1841 I was in the Province of Veragüe in New Grenada to the north of the Isthmus of Panama and left the town St. Jago on the western coast for David in Chiriqui a town in the interior about 60 or 70 miles to the northeast and leeward of St. Jago. The smallpox was raging with great violence in St. Jago, but there was no appearance of it in David. A few days after my arrival there, taking my accustomed morning ride in the forest which teems with animal life, I was struck by observing one or two sick and apparently dying monkeys on the ground under the trees. The next morning I was struck by the same singular appearance and by thinking that I perceived several on the trees moping and moving about in a sickly manner. I consequently dismounted and carefully examined two which were on the ground — one dead and the other apparently dying; and after careful examination no doubt remained in my mind that they were suffering and had died from smallpox. They presented every evidence of the disease, the pustules were perfectly formed, and in one instance, that of the dying one, the animal was quite blind from the effects. A few days afterward (I think about four or five days) the first case of smallpox appeared among the inhabitants of David and in the course of a fortnight half the population was stricken.

In 1858 Dr. Furlong, taking part in a discussion upon diseases in animals, stated that he had received a letter from the wife of a prominent physician of the Island of Trinidad who mentioned that during epidemics of smallpox in that island the wild monkeys suffered from the disease. Both of the statements, it will be observed, concern the monkeys of America, and it is not impossible that they may be more susceptible to the disease, but at the same time it must be acknowledged that the evidence is very imperfect. In explanation of the nonsusceptibility of the monkey to variola, we would have to assume either that there is a qualitative difference between the virus of variola inoculata and smallpox, or that the monkey has a relatively greater immunity which prevents an infection from the very small amount of virus which the air may convey to a susceptible surface or that there are anatomic or physiologic differences which may prevent the virus reaching such a surface. The only evidence for a qualitative difference in the virus is that the monkey is susceptible to one only, and that the disease produced differs in the monkey and in man from variola vera in its milder type and shorter period of incubation. Variola inoculata is the same in the monkey as in man, save that period of incubation is shorter. The

constitutional reaction takes place in man on the eighth day, and in the monkey on the sixth or eighth. In the monkey a well marked pock develops at the site of inoculation and reaches its acme on the eighth day. The exanthem begins on the eighth or ninth day. The extent of the exanthem varied greatly. In some animals only one typic lesion was present, while in others over a hundred were found. In the monkey, as in man, the distribution of the exanthem showed a partiality for certain regions. The face was most often the site of an eruption. Elsewhere in the order of frequency it was present upon the wrists, the scrotum of the male, the region about the anus and base of the tail, on the palms of the hands and the soles of the feet, or the inner aspect of the arms and thighs. The eruption seemed to avoid the trunk and the outer hairy surface of the limbs.

It seems possible that the shorter period of incubation of variola inoculata may be explained by the greater amount of virus which is used for inoculation, compared with the probably small amount in ordinary infection, the greater primary amount enabling the increase which is necessary to produce the constitutional disturbance and the exanthem to take place more quickly. But it must also be evident that if the period of incubation depended on the amount of virus primarily used, it would vary in duration with the amount of virus used. It is also possible, as Brinckerhoff and Tyzzer suggest, that the period of incubation may be short in variola inoculata in consequence of the virus being introduced into the circulation at the time of infection.

Attempts were also made to produce variola vera in the monkey by varying the place of inoculation. It is generally believed that in man a primary infection takes place on some mucous surface from which systemic infection follows. There is no anatomic evidence for this assumption. No one has ever found such a protopustule. In our 52 autopsies, careful search was made for such a lesion, but in vain. In variola vera there are no symptoms of such a lesion, but it is possible that a simple pock situated in the lungs, for instance, could pass through its development without giving rise to symptoms. We all know how extensive a tuberculous process can exist in the lungs without the production of symptoms. This set of experiments yielded interesting results and which are capable of explanation. Variola inoculata with exanthem and immunity follows primary inoculation of the skin. Immunity is not produced, or very rarely produced, by inoculation of the cornea, the mucous membrane of the nose, mouth, or palate. It is produced by inoculation of the trachea or by blowing the virus into the lungs without the production of a lesion. The study of the character

of the primary lesion in these places gives the explanation. The exanthem and the immunity are due to the absorption of products from the primary focus. In the case of the skin such products are retained by the impermeable horny layer, and absorption is facilitated. In the mucous membrane and in the cornea the absence of the horny layer causes an ulcer and not a vesicle or pustule to be produced, and the products escape on the surface. In the trachea and in the lungs, the thin layer of epithelium renders the absorption of the products easy, both from the lesion and from the mucous surface. But by none of these experiments was variola vera produced. It seems also probable that if a protopustule be developed in variola vera it must be in the lungs or in the larynx or trachea, because only in these places can the necessary absorption take place. The absence of symptoms would probably exclude the larynx and trachea.

Comparative study of the vaccine and variola virus was undertaken in the course of the Manila work. While the series of experiments was not sufficiently extensive to permit final conclusions as to the comparative resistance of the two viruses, they have shown that there is a difference, the vaccine virus being more resistant than the variola.

Variola virus dried on glass rods at room temperature was found inactive after two weeks, while the vaccine virus under such conditions was unchanged. The variola virus seemed to undergo an attenuation both after prolonged exposure to glycerin and after passing through a series of monkeys. The attenuated virus finally lost the power of producing an exanthem, though a typical local lesion was produced. It was not ascertained whether by the attenuated virus only, the vaccine cycle of the parasite was produced. Only one filtration experiment was made, and this showed that the virus of variola did not pass through the Berkefeld filter.

The study of immunity which was produced by the two viruses also gave interesting though at present inexplicable results. From the experiments it has been shown that the immunity produced by vaccine virus against both vaccinia and variola inoculata is stronger than the immunity produced by variola inoculata. Locus of the primary lesion had influence on the subsequent immunity. The most effective immunity was produced by variolations, or vaccination of the skin where the conditions best favor absorption. Vaccinia was also shown to have greater power for infection than variola. It was found possible to produce immunity for variola inoculata and not for vaccinia. There was a certain interesting local immunity produced. Even vaccination of the skin did not always produce perfect immunity toward the corneal infection. This tends to show that the immunity is due to the

action of the serum on the parasites, and that when the conditions are unfavorable for the serum to exert its action on the virus there may be infection in an animal otherwise immune.

The study of the histology of variola inoculata in the monkey, has also given interesting differences as regards variola. In variola in man, one of the most striking anatomic facts is the small part which leucocytes play in the lesions. In the early process in the skin, and before the lesions become infected, there may be no leucycytes present. The blood count shows a marked diminution in the neutrophile leucocytes. Examination of the blood-forming organs shows the same diminution in these leucycytes and an increase in the cells belonging to the lymphoid series. In our earlier work, it seemed probable that the diminution in the neutrophile leucocytes was due to the imperfect differentiation of young forms. In variola inoculata, this striking leucocyte reaction was absent. Numbers of leucocytes were present in the local lesions and an active leucocyte formation in the blood-forming organs.

We feel that in our work on smallpox, which is nearing its temporary conclusion, the fundamental questions relating to the disease have not been answered. These questions are: 1. The parasite and its complete life cycle. We feel confident of the hypothesis of the vaccinia and variola cycle which we have brought forward, but it remains for the present a hypothesis. 2. The relationship between vaccinia, variola inoculata and variola vera. Our work has served to accentuate the differences between these conditions. We cannot advance even a tenable hypothesis with regard to the internal relationship of variola inoculata and variola vera. The difference in the period of incubation in the two diseases, the non-susceptibility of the monkey to variola vera and the absence of the leucocyte reaction in variola inoculata, is striking. 3. The mode of infection in variola vera. Our work suggests a primary infection by air-borne virus and in the lungs. The only evidence is that the lungs are susceptible to a primary infection by means of insufflated virus. But this in the monkeys produced variola inoculata and not variola vera. 4. The mode of production of the exanthem. The specific lesions of smallpox are only developed on epithelial surfaces. The Manila work has shown that the specific lesions in the testicle are associated with the presence of the organism. It is assumed, and there is good ground for believing, that after an increase of contagium at a primary focus the organisms enter the blood and are carried as emboli to the skin, where they produce the eruption. But this is again a hypothesis. We have found that the organisms in variola inoculata pass from the primary lesion into the endothelial cells of the blood vessels and enter the blood. We have not proved

at what stage of the disease the blood is infectious nor how long. 5. The immunity, its mode of production, and the relationship between the natural and acquired immunity.

The answers to these questions involve long and arduous work by skilled investigators on both the human and the experimental diseases in monkeys. For the work a constant supply of variola virus and experimental animals is necessary. We believe that the work can only be carried out in places where both these conditions can be fulfilled. It is probably true that we shall always have smallpox with us. Notwithstanding the fact that we have in vaccination a mode of defense which leaves little to be desired, there will always be opposition, more or less effective, from people who are utterly incapable of appreciating the value of evidence. These people furnish the material for small epidemics which give opportunities for investigation. Investigation in smallpox is costly. It demands the entire time of well-trained investigators, who are to be found associated with universities, and the compensation for such men should equal that of a competent clerk. The investigations carried out by the Harvard Medical School have been brought to a temporary close by the lack of further funds and by the necessity of rounding up present work. Smallpox is a disease which has been of the most influence on the human race, it is the only one of the exanthems which is open to animal experimentation, and with the full solution of the questions which it brings up, it is not too much to hope that light may be shed on the allied diseases.