SUSCEPTIBILITY OF THE GUINEA PIG FETUS TO VACCINIA *

JOSEPH STRITAR, M.S., AND N. PAUL HUDSON, M.D.

(From the Department of Hygiene and Bacteriology, University of Chicago, Chicago, Ill.)

INTRODUCTION

Vaccinia is one of a group of virus diseases that under natural conditions present lesions of similar appearance in a variety of animals. The experimental host-range of vaccinia itself is also wide, including man, monkeys, cattle, fowl and rodents. Animal susceptibility is not necessarily a property common to the members of an allied group, however, for the receptivity on the part of a single member may be of only a relatively mild degree. The response of the guinea pig to vaccinia is an example; while the rabbit is highly susceptible, the former is generally regarded as comparatively non-reactive.

The cultivation of the vaccine virus in tissue cultures has introduced a semi-artificial condition in which cells even of embryonal nature serve as a medium. The demonstration of this principle by Rivers [1] and his associates has been extended more recently by Goodpasture, Woodruff and Buddingh [2] to the successful cultivation of the virus in membranes of the chick embryo. They have found a greater susceptibility of the embryonic tissues than of the hatched chick to vaccinia.

Various agents capable of inducing disease have been reported as more invasive in avian embryonic tissues than in hatched or adult birds. As long ago as 1912, Murphy and Rous [3] reported that their chicken sarcoma grew more favorably in the embryo (chicken, pigeon, duck) than in the adult. The same has been found true by Rivers and Schwentker [4] in the case of a parrot disease virus. Mackenzie,[5] studying Rift Valley fever, Burnet,[6] working with canary pox, and Syverton, Cox and Olitsky [7] in equine encephalomyelitis experiments, likewise noted the growth of their respective viruses in chick embryo tissue cultures while young or adult fowl were not responsive.

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Woolpert,[8] in seeking to demonstrate any peculiar susceptibility of the fetus in a species whose adults are relatively resistant, found in preliminary experiments that the guinea pig fetus reacted to vaccinia virus. We have investigated the problem and this paper reports our studies on the susceptibility of this type of experimental animal to the virus. In short, it was found that lesions appeared, death sometimes ensued, and the virus increased in the fetal animal, while the same virus had little or no effect on the guinea pig after birth.

Experimental

Materials and Methods

The neurovaccine strain of Levaditi was used throughout these experiments. It was carried free of bacteria by testicular passage in rabbits, and its virulence, as measured by the ability to produce cutaneous lesions in the same animal, was constantly maintained. The operative technique was in general as described by Woolpert.[8] Most fetuses were inoculated intracerebrally, only a few intraplacentally.

About a third of the fetuses were injected with rabbit testicular material, the remainder receiving suspensions of guinea pig fetal tissues proved to contain virus. The potency of testicular and fetal virus was regularly determined by rabbit skin titration. The titer varied from 1:100 to 1:100,000, three-fourths the preparations having a titer of 1:10,000 and 1:100,000. The fetal virus usually measured 1:10,000 and the testicular material was somewhat more potent. The doses used were 0.1 cc. for each fetus inoculated, and when a preparation was injected in a number of fetuses it was commonly employed undiluted and diluted 1:10 and 1:100.

Fetuses in various stages of gestation were employed. The range was from 27 to 53 days of intrauterine life, the average was 39 and the median 37 days. The experimental period varied widely, being from 2 to 35 days; the majority of experiments were terminated between 5 and 7 days after inoculation. In addition to delivery of the fetuses by cesarean section, experiments were ended by such factors as threatened or actual abortion, birth, or fetal resorption. In a few instances the young that were born alive furnished other material

for examination. Excluding the fetuses that were not available for study because of early abortion, intrauterine maceration, resorption or calcification, and maternal destruction of aborted fetuses, data were obtainable on 115 fetuses inoculated with potent vaccine virus.

Controls on the virus employed in inoculation consisted of repeated demonstration of cutaneous vaccinial lesions in the rabbit and their prevention by mixture with antivaccinial rabbit serum. Furthermore, the results in the fetus were controlled by the recovery of the virus from fetal organs by rabbit skin inoculations and again its neutralization by specific immune serum. Occasionally a fetus of a litter was uninoculated and served as a control on the infectiousness of the material used in its litter mates. All operative procedures were carried out with strict aseptic technique and infrequent bacteriological examinations of the inoculum and the tissues of infected fetuses were negative.

Throughout the fetal experimentation the virus was repeatedly tested for its action on adult guinea pigs. The rabbit testicular virus was tested by the cutaneous and intracerebral routes, while the infected fetal tissues, controlled by rabbit skin reactions, were examined by the cutaneous, intracerebral, intravenous and intratesticular routes. The only observable lesion elicited in the guinea pig was a mild hyperemia with papule formation at the site of intracutaneous inoculation. This appeared only occasionally and with the highest concentration of inoculum. In no instance was a febrile reaction obtained. These results indicate the relative non-reactivity of the adult guinea pig to the test virus employed in the fetal experimentation and furnish a basis for comparison with the results to be described.

Results

The action of the virus on the fetus could best be judged by the gross and microscopic pathology. Death as a criterion of virus effect was not dependable, since fetal mortality may result from natural causes and we are not aware of its incidence under ordinary conditions. In our experimental series dealing with 80 mothers there were 10 fetuses that were found in various stages of resorption, dehydration and calcification. This is not a complete picture, however, as no doubt many killed by virus action were aborted and lost.

Gross Pathology

The lesions seen in the infected fetuses appeared in the skin and various viscera. Because of the small size of the organs, the presence of lesions was judged mainly by the external changes in each part. For the same reason, pathology in the intestines was not definable. Changes were recorded in various parts of 88 of 115 fetuses inoculated with virus proved potent by rabbit skin test and observed under conditions suitable for detection of lesions. The 27 fetuses not showing any gross pathology included those injected with high dilutions of virus material. Of the 88 animals, lesions were seen in the skin in 33, on the surface of the brain in 11, in the lungs in 54, in the liver in 25, in the kidneys in 55 and in the spleen in 9 instances. The placenta and heart showed gross changes in each of 2 fetuses. It is clear that the distribution of lesions was irregular and that they occurred most commonly in the lungs and kidneys.

The lesions in most parts were circumscribed and usually with a regular margin. They varied in size from being barely discernible to the naked eye to 2.5 mm. in diameter. The largest number of lesions were flat, although a few were elevated in the form of vesicles and some were depressed or pitted. The kidneys of 3 fetuses were marked by small ulcerating foci. Most of the lesions were gray or gray-white and with a sharp outline; these occurred in all the tissues but were most conspicuous in the skin. Minute hemorrhagic areas appeared on the lung surfaces in 11 fetuses, and occasionally irregular areas of hemorrhage 2 mm. in diameter surrounded by diffusely pale grayish tissue were found involving the kidney. Rarely, gray lesions elsewhere were tinged with red or surrounded by narrow zones of hemorrhage. In general, no differences in the gross changes in individual sites were attributable to the source of virus, although lesions of the brain were more common in the fetuses inoculated with rabbit material, and splenic alterations were seen only in the animals that received fetus virus. Representative lesions are pictured in Figures 1 to 7.

Microscopic Pathology

The microscopic features characterizing the acute effect of vaccine virus on the fetal tissues are necrosis, edema and hemorrhage. There is a noteworthy lack of cellular infiltration, although in older lesions

a mesenchymal reaction is apparent. The detailed study of the histopathology will be made the subject of another communication.

Vaccinia in the Fetus

Thirty-three of the inoculated fetuses were examined in detail for the effect of vaccinia. Suspensions of their tissues were reinoculated into rabbit skin for the identification and titration of virus and to determine its distribution in the fetus. Twenty-six had been injected with suspensions of infected fetal organs (15 with renal, 7 with brain, 2 with lung and 2 with placental suspensions) and 7 received rabbit testicular virus. The results cannot be correlated with the type of material injected and the data will be considered together. Further, the titer of the virus in the organs examined was not referable to the strength of the original inoculum. The period varied widely, as previously indicated; the tissues of 22 of these fetuses were examined from 4 to 7 days after inoculation. The duration of the experiment under these conditions did not seem to have a direct bearing on the demonstration of virus and its distribution and titer.

Vaccine virus was recovered from fetuses by rabbit inoculation and the rabbits showing positive skin reactions to this virus were proved immune to subsequent infections of virus of rabbit origin. Virus was demonstrated in all but 3 of the 33 fetuses, and in 2 of these, lesions in the lungs were apparent. Virus was recovered from 18 of 24 brains, 20 of 31 lungs, 13 of 26 livers, 6 of 17 spleens, 23 of 26 kidneys, 2 of 3 skin lesions, and 11 of 17 placental tissues tested. The blood of 10 fetuses was examined and only 2 yielded virus and they in low dilution. In all, suspensions of 146 organs (excluding blood) were tested quantitatively in tenfold dilutions. Ninety-four gave positive results in the following titers: 13 to a titer of 1: 1; 10 to 1: 10; 36 to 1: 100; 15 to 1: 1000; 18 to 1: 10,000; and 2 to 1: 100,000. Not only did the kidney furnish the highest incidence of virus recovery, but it also more often gave the highest titer among the organs, usually being 1: 10,000. It was used most frequently as a source of passage virus.

When the incidence of virus recovery was compared with the presence of lesions in various parts, it was observed that of the 146 tissues examined for virus, lesions and virus both were found in 57 tissues, neither was in 37, the virus and no lesions were in 39, and

lesions but no virus were demonstrable in 13. In this series of 33 animals, gross changes were thus noted in 70 tissues. In 3 fetuses no pathology was seen, but in 2 of them the virus was isolated from some part. The virus was found to persist to the limit of the experimental periods and was recovered as late as 32 days after fetal inoculation. Likewise, lesions were observed after the maximum interval of experimentation.

Fetal Source of Virus

We have already indicated that the virus of fetal origin behaved in fetuses as that of rabbit passage in all the respects noted in the experiments. That the virus increased in the fetus was demonstrated by the effective transfer of virus through series of fetuses. In one group the fetuses of 9 mothers were serially inoculated, beginning with 1: 100,000 titer of testicular neurovaccine and ending with 1: 10,000 titer of virus in the fetal kidney, with lesions present. Besides establishing the multiplication of vaccinia virus with the development of lesions in the fetus, it was found that the highest concentration and the most frequent effect of the virus was in the kidneys. With all this, however, there seemed to be no selective adaptation of the virus to the fetus, since the virus was constantly transmissible to the rabbit with typical manifestations, its characteristics in the guinea pig fetus did not change during the year of experimentation and it was not modified in its relative non-infectivity for the adult guinea pig.

Vaccinia in the Newborn

All gradations of effects were noted among the 35 animals that were delivered at term after inoculation *in utero*. Six died soon after birth and some displayed lesions with virus demonstrated in low titer in rabbit skin. Nine others lived from a few days to 3 months; of these, 2 had vaccinial lesions in the lung and kidney, 3 showed softening of the cord and brain after symptoms of paralysis and weakness, and the others died showing pathology not referable to experimentation. The remaining 20 animals survived. Although the skin in all animals born was examined thoroughly, often from the undersurface as well, no lesions were seen in the group that was born alive.

The susceptibility of the newborn was tested, with the same nonreactivity resulting as was obtained in the inoculation of adult guinea pigs. Eleven animals from 4 to 10 days old were injected intracerebrally with potent virus and none showed fever or symptoms.

Discussion

Much experimental work has been done on the parasitization of mammalian hosts with viruses. The range of animals in which disease is induced has been greatly extended by the introduction of special techniques, as well as by the use of animals not naturally exposed to specific infection. Little has been done, however, on the susceptibility of the mammalian fetus. The whole question of fetal reactivity has been only slightly explored, due perhaps to hesitancy in operative procedures. Although there are definite restrictions on account of the essential circumstances, the field is worth exploring on the bases of parasitism and fetal physiology.

We are here reporting the successful propagation of the virus of vaccinia in the guinea pig fetus, with the development of typical lesions. This prenatal susceptibility, as compared with the relative resistance of the adult, is consistent with the work already cited of the successful growth of viruses in cultures of embryonic tissues. Such cultures apparently furnish a circumstance sufficiently favorable for intracellular growth. That this is possible in tissue cultures of the natural host is logical, but evidently some other factors enter that allow virus growth in the fetus whose adult is relatively insusceptible. This may be due to the fact that the fetus furnishes a different type of tissue marked by the two properties of comparative immaturity and rapidity of cellular growth. The principle of virus increase in rapidly growing cells may be thought of as the reverse of the property of viruses to stimulate host cell growth, as pointed out by Rivers [9] in connection with infectious myxomatosis of rabbits, and as observed to be an essential result of viruses inducing tumor growths.

The microscopic examination of affected tissues, so far as it has been carried, does not disclose a marked cellular reaction. It might be supposed that fetal susceptibility to vaccinia depends on this relative lack of cellular response. That the fetus reacts to some infectious agents with the proliferation and infiltration of inflamma-

tory cells is illustrated by the descriptions of the response of the fetus to the tubercle bacillus [10] and to the submaxillary gland virus.[11] It thus appears that the infectivity of the fetus does not depend on the absence of demonstrable cellular defense. Susceptibility is based on some other type of cellular reactivity, as in postnatal animals, and the relative susceptibility in this case is apparently referable to the properties of fetal cells already mentioned. On the other hand, the lack of universal susceptibility, as pointed out by Woolpert,[8] furnishes a situation equally inexplicable.

Most fetuses were inoculated intracerebrally. The virus content of brain tissue, however, was found to be low at the termination of the experimental periods, much lower in fact than in distant organs. The kidneys, and to a less degree the lungs, were sites of predominant virus localization. The predilection of the skin for pock development is significant in view of the dermal nature of the virus. Judging by the wide distribution of lesions and by the high virus titer of tissues distant from the site of injection, it appears that the fetal guinea pig is parasitized by vaccinia virus in a general way, much like generalized vaccinia in the rabbit and in the chick embryo in the shell, as described by Goodpasture and Buddingh.[12] The virus content of tissues was not dependent, however, on its supply of infected blood, since the blood contained demonstrable virus only twice in ten specimens and then only in low dilutions.

Necrosis was the chief pathological finding. Areas involved were mainly circumscribed with, in the skin, the development of a definite pock. Superficial lesions in the viscera were likewise sharply outlined, except in the kidney where blanched areas indicated a more diffuse process. Hemorrhage occurred frequently in the center of necrotic lesions and sometimes constituted the main gross alteration in the lungs. The lesion in the fetus reached a peak macroscopically in 3 to 5 days and did not seem to progress appreciably during the remainder of the experimental period. A distinct disadvantage in this work was the inability to observe the development of lesions.

The use of vaccine virus propagated in tissue culture and in the chick embryo in the shell has been proposed for human vaccination purposes. Virus of fetal guinea pig origin might be useful to the same end because of its bacterial sterility and high potency, but much must yet be done to standardize and regulate its production.

It should be pointed out that we were unable, by repeated fetal passage, to modify the relation of the virus toward the postnatal guinea pig, on the one hand, and toward the rabbit, on the other. After as many as nine transfers from fetus to fetus, the virus still was ineffective when injected into young or adult guinea pigs by any route and yet retained its ability to induce typical lesions in the rabbit by skin or testicular inoculations. This points to the stability of the virus under these conditions, a property that has been commented on by Goodpasture and Buddingh.[12]

Summary and Conclusions

The vaccine virus was successfully propagated in the fetal guinea pig. The effect of the virus was to induce typical lesions in various organs and tissues. The same virus strain caused only the slightest reaction in the postnatal animal. These results indicate a markedly greater susceptibility of fetal tissues for an infectious agent. The cultivation by other workers of certain viruses in embryonic tissues, when adults of the same species were not reactive, points to the same phenomenon. The mechanism of this comparative effect is not yet clear, although general immaturity and rapidity of cell growth in the fetus are suggested as possible factors.

Lesions were found irregularly distributed in the principal organs, including the skin, and were most common in the lungs and kidneys. Of all the fetal tissues the kidney yielded virus most constantly and in the highest titer, usually measuring 1: 10,000.

The vaccine virus was passed through a series of fetuses and was not appreciably modified in its cutaneous activity in the rabbit or in its failure to induce effects in the postnatal guinea pig.

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DESCRIPTION OF PLATE

PLATE 19

FIG. 1. Fetus delivered dead by cesarean section 6 days after intracerebral inoculation with 0.1 cc. of 1: 10 dilution of tissue culture vaccine virus. Multiple, circumscribed cutaneous lesions, some pitted. Patches of adherent fetal membrane around left eye and over scalp. × 1.

FIG. 2. Fetus delivered alive by cesarean section 10 days after intracerebral inoculation with 0.1 cc. of 1: 10 dilution of rabbit testicular vaccine (titer 1: 1000). Round gray lesions on eyelid, back, side, heel (not in focus), placenta and in adhesion of fetal membranes. Fetus still enclosed in amniotic sac. × 1.

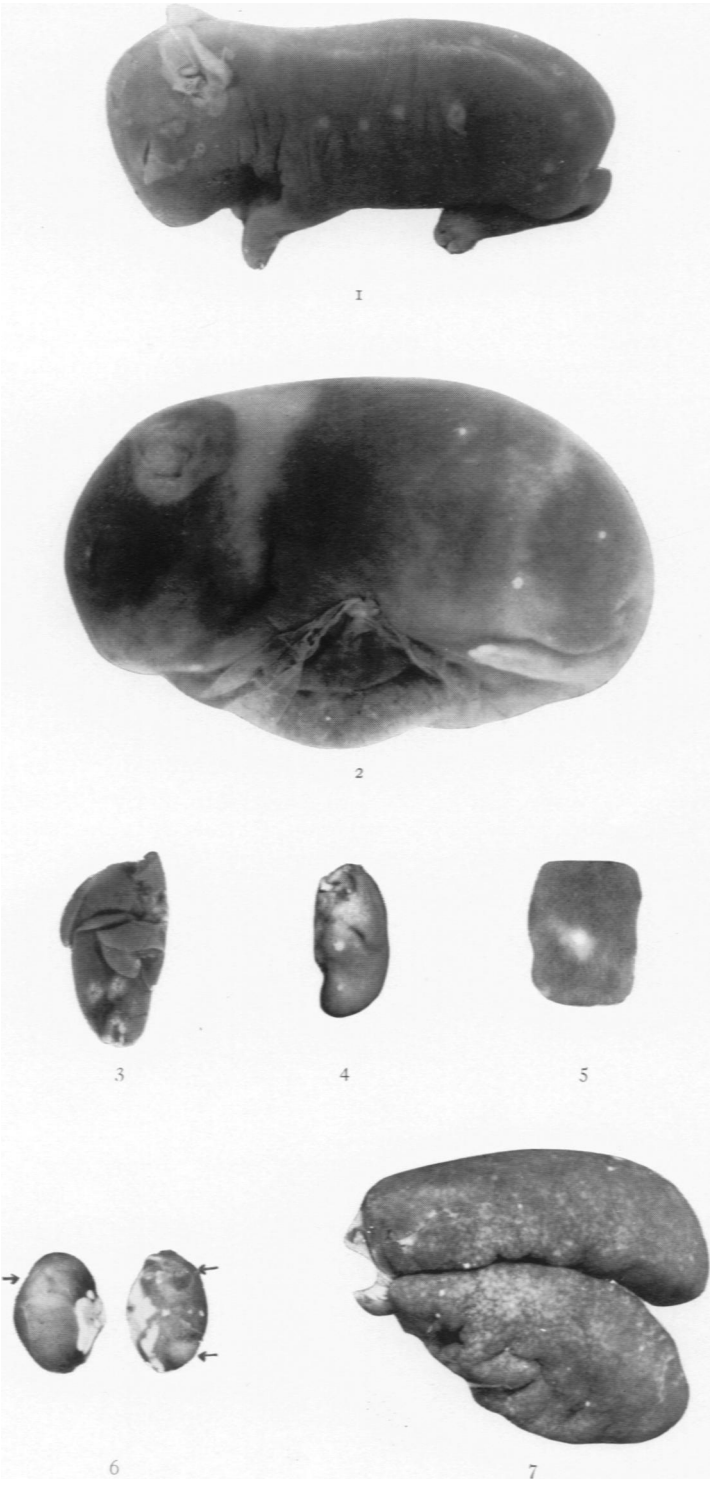
FIG. 3. Lung of fetus delivered alive by cesarean section 7 days after intracerebral inoculation with 0.1 cc. of 1: 100 dilution of rabbit testicular vaccine (titer 1: 10,000). Diffuse gray areas with hemorrhagic centers on diaphragmatic surface. × 2.

FIG. 4. Lung of fetus of Fig. 2, showing two gray lesions on costal surface. × 1.5.

FIG. 5. Skin lesion of fetus of Fig. 2, in patch of white hair. × 1.5.

FIG. 6. Diffuse necrotic areas in upper pole of left kidney and in both poles of right kidney of fetus of Fig. 2, with hemorrhagic centers in lesions of both upper poles. (White areas are recognized as bits of fetal fat.) × 1.5.

FIG. 7. Two lobes of liver (others removed) of fetus of Fig. 2, showing ten minute gray lesions on surface. × 1.5.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