THE EXPERIMENTAL TRANSFORMATION OF VARIOLA TO VACCINIA

By E. S. HORGAN
Stack Medical Research Laboratories, Khartoum

The variation or, as some writers prefer, mutation, of variola to vaccinia virus has for so long been the subject of careful and extensive research, and as successful claims that it has been effected have been by no means infrequent it might have been expected that some measure of agreement, at least on the experimental technique, had been reached.

On the contrary, a detailed survey of the literature reveals not only the most marked differences of opinion but the actual denial, especially by French workers, that such a change ever takes place under experimental conditions.

No attempt has been made in the present paper to present a complete survey of the voluminous literature on the subject; there is an excellent summary of the older literature by Copeman (1898). The literature quoted below deals for the most part with more recent work, but some of the earlier and classical investigations have been also quoted, in order to emphasize the curious and striking discrepancies which have persisted for more than 70 years and which are yet unsolved.

In 1862 following discussions in the Academy of Medicine, la Société des Sciences Médicales de Lyon named a commission presided over by Chauveau to investigate the problem. These workers (1865) never succeeded in effecting the change from variola to vaccinia on cattle, and although they occasionally obtained papular eruptions, the variolous nature of these was proved by the retransfer to man. Several of the human subjects developed typical variola and one of them died.

Similar unsuccessful experiments were carried out on the horse, and the commission finally decided that the two infections of variola and vaccinia were independent and that one could not be converted to the other. Martin (1860) of Boston, quoted by Copeman, had a similar result; he variolated a cow and carried the lesions on to children who developed small-pox.

The work of Pasteur on the attenuation of viruses gave a fresh stimulus, and various workers, Voegt, Stumpf, Fischer, Freyer, Eternod and Haccius, claimed successes. Chauveau reinvestigated the problem, using calves but again failed, and concluded that variola inoculated on to bovines either died out, or remained variola. Confirmation of this was furnished by Juhel Renoy, Pourquier and Ducamp.

Klein (1894), who in 1879 had obtained negative results in thirty-one trials, succeeded in 1892 by successive passages through four calves and from the

https://doi.org/10.1017/S0022172400011554 Published online by Cambridge University Press

fourth calf to a child, and Copeman (1909), who completely failed in each of four separate series of experiments in inoculating calves with human small-pox lymph, succeeded eventually, by using variolous material from monkeys, which after two or three passages on the calf produced vesicles indistinguishable from typical vaccinia.

Since that time there has been continuous opposition between the claims of the French and German schools, the latter claiming frequent successes, and to try and solve such contradictory results the classical researches of Kelsch *et al.* (1910) were undertaken. Using variolous lymph or fresh or old crusts ground in glycerol, they inoculated both rabbits and calves in different ways; all were unsuccessful, the results in calves being either negative or at the most an eruption of doubtful nature, while the rabbits only showed a mild dermatitis. Subsequent vaccination, however, of the rabbits showed that they were immune to vaccinia.

Kelsch and his co-workers reported twenty failures during 1909 and 1910.

In further experiments carried out in a vaccine lymph institute these workers rubbed sterile glycerol into the scarified skin of white calves, but in spite of all precautions a typical vaccinial reaction developed, suggesting that in lymph institutes, vaccinia virus was a widespread contamination, and that this possible fallacy should be borne in mind when considering the successful claims of other workers. Teissier *et al.* (1913) attempted to variolize the pig but only obtained a papular eruption which did not produce immunity to vaccination and which was of a very doubtful nature.

One of the few successful claims from French sources is that of Gauducheau (1911), who used a mixture of variola virus from the monkey and a strain of *Staphylococcus aureus*. He inoculated this first on to a monkey, and then by successive passages through the buffalo he finally obtained pustular lesions of a vaccinial type.

Chaumier & Belin (1913) inoculated areas of the skin of a donkey with variola direct from a human case and the same virus passaged through three monkeys. All insertions succeeded, and the resulting material from each was passed on to a heifer but only the material derived from human variola produced a typical vaccinial eruption: the material from monkey passage failed.

This curious experience, which is a reversal of the usual experience with monkey variola, does not seem to have been repeated.

Tomarkin & Carrière (1913) believed in the possibility of the change after three or four passages on calves or rabbits.

Ruediger, quoted by Ashburn *et al.* (1913), passed the virus from a fatal case of small-pox in 1908 through two monkeys, from the second monkey to a heifer, and after five successive passages through heifers on to man. He used this strain of vaccinia with great success on almost 7 million human vaccinations in the Philippine Islands.

Similar anomalies have been noted in the results of workers handling the virus from cases of alastrim. Thus while Cleland & Ferguson (1915), working in

New South Wales with material from cases of the mild Australian small-pox, obtained typical vaccinia after three passages through calves, Green (1915), working with human variolous material from the same outbreak and first passage monkey variola, failed, although primary inoculation on to calves produced in some cases small yellow vesicles unlike vaccinia. Two attempts were made to produce vaccinia by successive passage using four calves in each series, but both attempts failed to improve the quality of the vesicles.

Blaxall (1923) comparing the material obtained from mild small-pox in the north of England with that obtained from virulent small-pox in Poplar occurring at the same time found that in neither case was direct inoculation on to rabbits or calves successful. Inoculation of monkeys in each instance produced an eruption with typical vesicles, and this vesicular material collected from the monkeys inoculated from either source transferred to calves and rabbits produced, after three passages, definite vesiculation in both kinds of animals.

Successful results were also reported by Sobernheim & Zurukzoglu (1928) using alastrimic material from cases in England. In two series of rabbits typical vaccinia developed after the third and fifth passages respectively.

On the other hand, Ledingham (1935) and his colleagues appear to have had considerable trouble in securing a vaccinia-like variant from variola of the alastrim type in England, either by rabbit passage alone or by monkey passage followed by rabbit. His comments are as follows:

"The monkey material like the human material produces invariably when inoculated into the rabbit dermis an inflammatory node which is specific in that a mixture of the same material with anti-vaccinial serum produces no response. This I showed in 1928. For a short series of passages the virus can be kept going from dermis to dermis, but attempts to secure good surface takes on the scarified skin of the rabbit with dermal material generally failed."

Aragão (1911), using a mixture of material from various cases of alastrim, inoculated five young calves and numerous rabbits but failed to secure a take by scarification on the shaved skin of the animals. Successive corneal passages on the rabbit and intradermal injection of rabbits with material from corneas infected with alastrim were equally unsatisfactory.

Baujean (1923) inoculated the contents of the vesico-pustules of alastrim on to the rabbit, guinea-pig, calf, monkey—all with negative results: the monkey (a coaita) alone showed small non-inflammatory nodules which never suppurated and disappeared in 15 days.

Moody (1922) inoculated twenty-six rabbits and four calves (scarification) with material from lesions at different stages of the disease—all were negative.

Jorge (1924) using rabbits had equally negative results.

Within recent years the method of intratesticular passage has been used by several workers. Thus Nayoshikii *et al.* (1920) had successful results by serial intratesticular passages in the rabbit, then inoculation on to the skin of the rabbit and finally on to the calf.

Teissier *et al.* (1931) used two strains of variolous origin, one of which had undergone fourteen and the other two passages on the monkey.

In a series of experiments they employed emulsions of the virus from the testicles (orchitis) of the monkey, dog, cat, donkey and the spleen of a monkey with generalized variola.

Young bulls were inoculated both cutaneously and intratesticularly with varying mixtures of the emulsions, but in no case was either a cutaneous eruption or an orchitis obtained, although the animals inoculated intratesticularly when subsequently tested proved completely immune to vaccinia. Those inoculated cutaneously had a marked but not a complete immunity.

The same authors attempted to repeat the successful results of Chaumier & Belin by using the donkey as the intermediary animal. No less than thirteen donkeys over a period of 4 years were employed, the cutaneous or testicular routes being used, both of them on six of the animals. Although there was an orchitis in four out of the six, emulsions of the testicles into the testicles of ten rabbits failed to give any reaction nor did they produce any eruption when rubbed on the skin of rabbits or calves. Five of the thirteen donkeys inoculated cutaneously showed a dry papular eruption, very different from vaccinia, and which gave no subsequent takes on the skin of either calves or rabbits but when inoculated cutaneously on to the monkey produced an abundant variolous eruption.

Dr C. R. Amies of the Lister Institute (personal communication 26 November 1936) inoculated vesicle fluid from a case of mild small form of variola prevalent in London in 1930–1 on to the skin (scarification) of a *M. rhesus* monkey. "A typical local reaction occurred and scrapings of this were emulsified, filtered through a Berkefeld V candle and inoculated intratesticularly into rabbits. A mild orchitis was produced and by serial intratesticular passage a considerable increase in virulence resulted. Material from the sixth serial passage produced a confluent eruption when rubbed into the freshly shaved skin of rabbits and the strain has since been maintained as a dermal lapine."

These curious discrepancies between the almost constant failures of the French and the comparatively successful English, German and American workers are hardly sufficiently emphasized in English text-books, and in any consideration of the problem it is obvious that the failures as well as the successes must be considered. In this connexion it may be apposite to quote briefly the opinions of two recent writers. Blaxall (1930) remarks: "It is now recognized that calves can without doubt be inoculated cutaneously with variola either by the repeated passage method, or more certainly through the intermediary of another animal such as the monkey. Rabbits react in the same way as calves to primary variolous inoculation; primary vesiculation is rare, but by passage success is obtained though several passages may be required. Sheep, horses, fowl have similarly been variolated and probably all animals susceptible to vaccinia are inoculable with variola." This statement would probably represent to-day the opinion of a large number, perhaps the majority of workers on the subject.

Gastinel (1938), while frankly admitting the speculative nature of his remarks, concluded that the "qualities" of the variola viruses used explain certain of the discordances. "La connaissance actuelle d'épidémies de variole très bénignes souligne qu'il existe de par le monde des souches de virulence spontanément atténuées, et l'on pourrait formuler l'hypothèse que, dans un tel état le virus variolique serait peut-être susceptible de végéter expérimentalement sur la genisse. Si à l'origine les virus variolique et vaccinal ont été un seul et même virus, *l'adaptation ultérieure* à l'homme et à la génisse les ont séparés et rendus quasi irréductibles dans leur positions actuelles.

"On conçoit, en effet, l'impossibilité d'une plus ou moins brutale mutation quand chacun de ces virus est parvenu au terme extrême de sa différenciation; mais que se recontrent des races varioliques plus proches des qualités originelles, et la transformation apparaît au contraire plus réalisable."

This hypothesis would apparently deny the possibility of variation from virulent small-pox to vaccinia but would admit it in the case of alastrim and is somewhat reminiscent of the old theory which assumed that outbreaks of cow-pox were due to infection from the hands of milkers with lesions of the mild *inoculated* small-pox on their arms. Ledingham (1935) has recently discussed in detail this theory from the epidemiological side, and pointed out the baselessness of such an assumption, while the literature certainly lends no support from the experimental side, for numerous failures, as well as successes, have been reported in the case of both viruses.

On the whole, judged from the available evidence, there appears to be no relation between the natural pathogenicity of the virus of variola or alastrim for man and its infectivity for animals other than the monkey.

Methods

During an outbreak of virulent small-pox (February 1938: 177 cases, 64 deaths) in the Gezira cotton-growing area of the Blue Nile Province about 80 miles from Khartoum, the opportunity was taken to collect variolous material, chiefly pus from some of the cases. The pus was collected into capillary tubes which were packed in ice in vacuum flasks, brought immediately to Khartoum and stored in the freezing chamber of a refrigerator.

Technique. The pus was ground up with a piece of the capillary tube in a mortar and mixed with distilled water *p*H 7·0 in an approximate dilution of 1 : 10—the material from several of the cases being mixed. This diluted pus was immediately used for inoculation of the animals.

Three experiments were carried out:

(I) The pus was rubbed thickly on to the scarified abdomen of a *Cercopithecus sebaeus*—typical pustules formed and were scraped on the sixth day. The pulp (0·2 g.) was emulsified with distilled water to a dilution of 1 : 5 (approximately) and rubbed on the scarified sides and abdomen of a young calf. All calves used in the Stack Laboratories are of the zebu (Asiatic) breed, 6–9 months old, and all males. The calf was examined daily, and on the fifth day the skin carefully washed and inspected.

Results. There was no sign of either redness, papules or vesicles, and the lines of the scarifications were barely visible.

The whole surface was gently scraped with a scalpel but only a very small quantity of what appeared to be epithelial scales was obtained. This material was ground up with distilled water and at once rubbed on one scarified flank of a second calf.

The other flank was mapped out into three areas and inoculated as follows, five inoculations of 0·2 c.c. being made in each area:

(1) The suspension of skin scrapings from the first calf.

(2) The original suspension of monkey variola.

(3) The area was infiltrated with testicular extract prepared from the testicle of a young bull and the suspension of monkey variola inoculated intradermally into this prepared area.

Duran Reynals (1929) and McClean (1930) have shown the value of testicular extract in enhancing the virulence of vaccinia inoculated intradermally, and it was thought that it might provide a similar adjuvant effect with variola.

Results. This calf had a fine delicate pink skin which would have enabled one to detect the slightest reaction, but there was a complete absence of take on either flank.

The scarified flank was again scraped, and as the amount of material was very small it was rubbed on the freshly shaved and scarified skin of a rabbit. The latter was examined daily but there was complete absence of take and the experiment was discontinued at this point.

The calves and rabbit were subsequently tested with ordinary calf lymph and each showed a profuse and typical take.

(II) In connexion with another series of experiments on the reaction of non-immune rabbits to intradermal inoculations of human variola (Ledingham, 1926; McKinnon & Defries, 1928), the monkey variola and the scrapings of the two calves were inoculated intradermally into a rabbit: two control inoculations with human variola were also made.

Results. The animal showed typical reactions in the insertions of both human variola and those of monkey variola, but the insertions of the calf material were completely negative.

(III) A mixture of pus from two more cases of small-pox with confluent eruptions was rubbed as before on the skin of the right flank of a large *C. sebaeus*. The left flank was used to titrate the potency of the material. Examination on the sixth day showed a confluent pustular take on the right side, while the left side gave the following reaction:

Dilutions	... 1	1 : 10	1 : 100	1 : 1000	1 : 10,000
Result	+ +	+ +	±	(6)	(1)

+ + = confluent. ± = semi-confluent.

The figures in brackets indicate the number of pustules.

The pulp was scraped, 0·38 g. being collected and emulsified with distilled water *p*H 7·0 to a dilution of 1 : 10.

Rabbit passage. Both intradermal and intratesticular routes were employed; to avoid confusion they will be discussed separately.

Intratesticular passage. Amies method (as above) was followed with the omission of filtration through a Berkefeld V candle. The experience of the writer with filtration of vaccinia through these candles was so discouraging on previous occasions and possibly due to some defect of the candles in stock that this part of the technique was omitted. In its place the suspension was centrifuged for ½ hr. at 3000 r.p.m. to bring down coarse particles and most bacteria and cultures of the supernatant showed only a few colonies of *Staph. albus*.

First rabbit passage. 0·25 c.c. of the supernatant was inoculated into one testicle. There was no sign of orchitis, and the animal was killed and the testicles removed on the fifth day—neither showed any sign of inflammation.

The inoculated testicle was ground up with distilled water to a dilution of about 1 : 10 and the thick suspension centrifuged at 3000 r.p.m. for 15 min. Cultures of this supernatant were sterile.

Second rabbit passage. It was inoculated as before into one testicle and rubbed on one scarified side (right). The inoculated testicle became slightly swollen and tenser than the other but the reaction was slight, and when the animal was killed on the fifth day and the testicles removed there was no obvious inflammation. The scarified side showed a tiny papule which developed about the third day and, without forming any vesicle, had completely disappeared by the eighth day.

Third rabbit passage. The testicular emulsion from the second rabbit was inoculated into both testicles and rubbed on one scarified side of a third rabbit.

Result. Both testicles became slightly swollen, tense and congested.

The scarified side showed on the fifth day a confluent inflammatory reaction on which soft crusts were beginning to form. There were no definite papules nor vesicles, and although the reaction resembled vaccinia it was unlike a typical vaccinial reaction of the fifth day.

The animal was killed, the testicles removed, emulsified as before and injected on to a fourth rabbit.

Fourth rabbit passage. The skin reaction was scraped, emulsified and rubbed on the right side of the fourth rabbit—the testicular suspension being rubbed on the left.

Result. Definite orchitis particularly in the left testicle: right and left sides typical reaction of confluent vaccinia more marked on the right side, a few isolated and typical vesicles on the periphery of each eruption.

The animal was killed on the fifth day and the testicles, which were moderately swollen and congested, ground up, desiccated, and stored in sealed ampoules. The lesions on the sides were scraped, the pulp mixed (1 g.), desiccated *in vacuo* and stored in ampoules for further use.

The potency of the fresh pulp tested by scarification on the skin of a rabbit was:

Dilutions: ...	1 : 10,000	1 : 20,000	1 : 40,000	1 : 80,000	1 : 160,000
Result	+ +	+ +	±	+(6)	+(5)

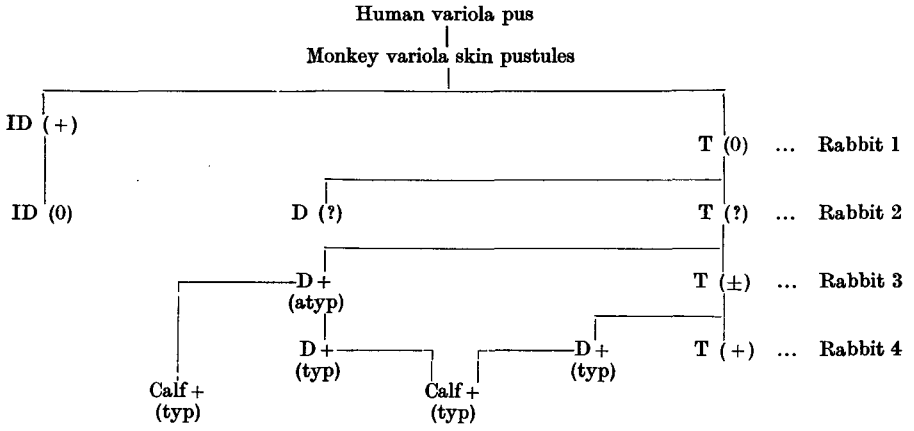
(+) The isolated vesicles were large and well developed on the fifth day.

Reactions on calves. One calf inoculated with the atypical dermal lesions of the third rabbit and one inoculated with the typical dermal lapine of the fourth —both showed typical takes with well-developed lines of pearly vesicles.

Both calves and the rabbit used for potency testing were tested after 1 month with the routine vaccine lymph (strain obtained from Lister Institute). All animals showed complete immunity to the undiluted lymph.

Intradermal passage. Rabbit 1 received two intradermal insertions (0·2 c.c.) of the monkey variola suspension; both gave a very slight reaction with redness and swelling, and when the animal was killed on the fifth day both areas of skin were excised, ground up thoroughly with distilled water, centrifuged in the usual manner, and the supernatant inoculated as before (four insertions) into the left flank of rabbit 2. With the exception of a slight redness about the point of each inoculum fading away after 24 hr., none of the insertions showed a trace of reaction, and it was considered useless to carry on further intradermal passage.

The following table summarizes the results of the third experiment:



D = dermal (scarification).
ID = intradermal.
T = intratesticular.
Symbols in brackets express degree of reaction.
typ = typical vaccinia.
atyp = atypical vaccinia.

DISCUSSION

The principal points of interest which emerge from the above results are: (1) The complete failure to passage variola virus or to bring about its variation to vaccinia in the calf or rabbit using dermal or intradermal methods of inocu-

lation, as compared with the success of intratesticular passages. (2) The increasing degree of orchitis in successive passages. (3) The increasing adaptation of the virus to the skin of the rabbit with successive intratesticular passages. This is evidenced by the appearance of one very small papule which dried up without becoming a vesicle and faded away rapidly (first passage) followed by a confluent but not typical eruption (second passage) and followed by a confluent typical vaccinia (third passage). Both confluent eruptions when passaged on to the skins of the rabbit, calf and sheep gave a typical vaccinial eruption.

Before regarding the above as a successful effort to bring about variation or mutation of virulent variola to vaccinia several important criticisms must be considered: (1) The possibility of a contamination of the material by vaccinia virus. It is true that a vaccine lymph institute is attached to the Stack Laboratories, but the greatest care was taken by keeping the rabbits in a separate house, and all manipulation of the testicular suspensions and inoculations were carried out with instruments sterilized by heat. No special precautions were taken in the case of the calves which owing to lack of accommodation were stabled in the same building with the routine vaccinifers, but in spite of this the results were completely negative. (2) The possibility of spontaneous rabbit-pox. No case of this disease has been known to have occurred among the stock rabbits, and in the personal knowledge of the author no epidemic of any kind has occurred for the past 11 years. More than a hundred have been used in the last 2 years for routine potency tests of vaccine lymph, and while the susceptibility varies, in no case has an immune animal been encountered. (3) The contamination would have presumably occurred between the first and second rabbit passages, that is, if one assumes the vaccinial nature of the small papule. Judging by the ease with which vaccinia proliferates in the testicle one might have reasonably expected a much more definite and typical eruption than this small, atypical lesion, especially as a dense testicular suspension was rubbed on the skin. As a final check the suspensions of the human variola and the first passage monkey variola were rubbed on to the scarified skin of another rabbit, using heavy inocula, but, as in the first experiment, the result was completely negative.

It is the experience of the writer that accidental vaccinial contamination of calves does not easily occur in Khartoum. On one occasion three calves which had been shaved and ready for inoculation developed symptoms which subsequently proved to be cattle plague and the animals were not inoculated. Although they were in contact in the same stable with inoculated calves developing vaccinial lesions, there was no appearance of any eruption on their skins, at least for 5 days, when, cattle plague having broken out also in the other animals, all were slaughtered.

Having carefully considered the above criticisms, the writer is of the opinion that contamination can be definitely excluded and that a true variation or mutation of variola to vaccinia took place.

An attempt will now be made to consider very briefly the present position of the problem of variola-vaccinia or alastrim-vaccinia variation.

(1) There is general agreement with regard to the difficulty of producing lesions in animals other than the monkey with human variola or alastrim.

(2) The assistance afforded by passage through a monkey towards securing subsequent transfer to other animals, is generally admitted.

(3) Evidence is lacking that continued passage in the monkey can ever bring about the vaccinial variation—the infection seems to remain variola indefinitely.

(4) Are there differences in the susceptibilities of various genera and species of monkey or in individuals of a species?

The question of genus has not been sufficiently considered, and it is not uncommon to find the specific name omitted: e.g. one cannot decide definitely from the existing literature whether the genera *Cercopithecus* and *Macacus* differ in susceptibility to variola or alastrim. With regard to the difference in individuals of a species the literature could also be more definite. Thus in a recent series of ten *Cercopithecus sabaeus*, marked individual differences in susceptibility to the same strain of human variola were noted (unpublished observation). The use of one or two animals which give negative results or only poor takes may lead to wrong conclusions with regard to the communicability of the particular strain of virus and it is possible that some of the negative results reported are due to this variation in individual susceptibility.

(5) The potency of the human variolous material (as determined on a susceptible monkey). The potency of the pus and scabs from cases of human small-pox seems to vary greatly. This has been noticed on several occasions during the above-mentioned epidemic, the contents of typical pustules of confluent cases either failing to take on a monkey or only producing a poor take in the undiluted state. Other variolous suspensions tested on the same animal produced typical pustules at a dilution of 1 : 1000 showing that the lack of susceptibility of the monkey was not the cause of failure. This question of potency has been insufficiently taken into account and may explain some of the negative results. It is attractive to speculate that one important advantage in the use of monkey variola for subsequent transfer to other animals may lie in its higher potency as compared with that of the original human inoculum.[1]

(6) *The animal or animals most likely to bring about the variola-vaccinia variation.* The majority of successful results reported have been obtained with calves or rabbits, but there seems no conclusive evidence in favour of either, although German workers claim that the rabbit is the animal of choice. It appears that the calf is more likely to be successfully inoculated and react with a pustular or papular eruption than the rabbit.

[1] This speculation is of course not new. Teissier, Duvoir and Stevenin (1911) failed to infect rabbits or calves with monkey variola and concluded that their previous lack of success was not due to want of virulence in human variola. Their results rather demonstrate that variolous material from any source is unlikely to produce a visible infection in the calf or rabbit in its first passage.

There is insufficient evidence to decide whether the employment of other animals in which occasional successes have been recorded would be of advantage. The successful result of Chaumier & Belin on the donkey has not been confirmed, although it appears that this animal can be readily infected with variola either by the dermal or intratesticular route (Teissier *et al.* 1931).

Breed, age and sex (*bovines*). The evidence is scanty and the breed of calf is so frequently unrecorded that no conclusion is possible. There is some evidence that certain calves which can be infected with variola from human sources or by successive passage carry on the virus as variola, as the monkey is believed to do, e.g. the early results of Chauveau or the equivocal results of Green (1915). It would be interesting to know whether such results are more likely to occur with certain breeds or whether they are due to purely individual characteristics.

It is possible that the particular breed might be of some importance, for although it is probably true that all cattle are equally susceptible to vaccinia it does not necessarily follow that all equally provide the environment in which variola-vaccinia variation occurs.

Age and sex. Copeman (1909) believed that young male calves were more likely to give successful results than young heifers or milch cows, and he suggested that in this way some of Chauveau's anomalous results may be in part explained. Unfortunately, as many workers do not specify the sex and merely mention (English literature) "calves", a study of more recent work fails either to confirm or disprove this belief. For vaccinial susceptibility at least there is nothing to choose between the sexes.

(7) *The route for inoculation. Skin.* The evidence is conclusive that the skin is an unsuitable tissue for either dermal or intradermal inoculation, but it is perhaps scarcely surprising that owing to the long-held belief in the epiblastic affinities of the vaccinia group of viruses, this route has been the favourite one for most workers.

Ledingham has long since pointed out (1924) there is no evidence of this elective affinity for epiblastic tissue, the primary attack being on the reticulo-endothelial system, and it is possible that the viscerotropic variola virus finds great difficulty in adapting itself to the epiblastic tissue (skin) of an entirely different animal as the rabbit or calf.

It is true that the skin of a rabbit may be infected by an intradermal inoculation of variolous material producing a local inflammatory reaction similar to that of vaccinia and that subsequently the animal develops some degree of immunity, but the virus does not appear to multiply readily as judged by the difficulties experienced by various workers to secure successive intradermal passages. A study of the literature also shows that the immunity produced by the inoculation of variolous or alastrimic material on the shaved or scarified skin is of a variable character and is often absent.

The production of anti-vaccinial immunity (in rabbits) by the intradermal inoculation of variola virus generally needs fairly large and repeated doses over a considerable period. These considerations would also suggest that there is

little tendency for variola viruses to multiply in the skin of animals other than the monkey.

Tissues and organs other than the skin. The testicle has long been known to be extremely susceptible to infection with vaccinia, and Levaditi and others have shown that by continued intratesticular passage the variant neuro-testicular vaccinia, usually called neuro-vaccine, can be obtained. This term is somewhat of a misnomer as the strain is really a highly virulent viscerotropic or pantropic virus (particularly for the rabbit) which seems to attack especially tissues of mesodermic origin (Levaditi *et al.* 1938). Passages through the testicle of vaccinia seem then to be particularly favourable for the selection of the viscerotropic elements, and with the viscerotropic variola virus it is possible that the most important action of the testicle is to allow the virus a favourable culture medium in which to multiply sufficiently and so have a chance of acclimatizing itself to a new environment. The variation to vaccinia —whatever be its nature—would be more likely to occur under such conditions than in the skin. The evidence from the present experiments suggests that while the virus is still variola in the early testicular passages the variation to vaccinia may rapidly occur.

Findlay (1936) has discussed more fully the question of variation in relation to skin passage, and one of his suggestions—that the particles of variola become heterogeneous as a result of spontaneous variation and that some vaccinia particles are then selected by the tissues of the animal—is equally applicable to testicular passage.

The published results, although few in number, are on the whole very favourable to intratesticular passage as the present method of choice, and it might well be adopted as a standard technique in future work on the problem. The negative results of Teissier *et al.* (1931) in their experiments on young bulls might be explained by the fact that only one intratesticular passage was carried out in each case. The negative results from their series on donkeys strongly suggest that the virus causing the orchitis was still variola.

Passage through other glandular organs such as liver, kidney or suprarenal does not appear to have been attempted in the case of variola, but judging from the experimental work with vaccinia there is no evidence that they would be a more suitable environment than the testicle.

Cornea. Aragão (1911) appears to have been the only author to use successive cornea passages, and with negative results. This is of interest, as the cornea of the rabbit is susceptible at least in some animals to variola virus (Paul's test), but the same explanation might apply as in the case of the skin.

Eye (anterior chamber). Komiyama (1932) claims this is more susceptible to variola than either the cornea, skin, or testicle, and to have carried on cultures for eight successive passages. This work has yet to be confirmed, and there is no evidence to show whether the final passage virus was variola or vaccinia.

(8) *Number of passages.* All workers who obtained success have recorded that several passages, usually from three to five, were necessary, and Blaxall

and others have emphasized the importance of this factor, but it is doubtful if repeated skin passages would invariably be a guarantee of success (e.g. the failure of Green (1915) to secure a vaccinia variant).

There is no doubt, however, that failure to do so must be largely responsible for some of the negative results, but in an analysis of the literature a distinction must be carefully made between the results of those workers who were attempting to bring about the variola-vaccinia variation and those who were only testing the susceptibility or immunity reactions of various animals to variola or alastrim. It will be noted that a considerable number of the published results come under this latter category.

(9) *Difference in the infectivity of various strains of variola or alastrim.* This point has been briefly discussed above in a comparison of the susceptibility of animals to these diseases, but it has been suggested also that a similar difference may exist between individual strains of variola or alastrim isolated from different epidemics and explains some of the anomalous results.

The evidence on this point is quite insufficient to enable any conclusion to be drawn, although it will be noted that in one of the few comparable series of experiments with full data, Cleland & Ferguson (1915) working in New South Wales, and Green working in London, obtained different results with the same virus.

Future work on the problem of variola-vaccinia variation would greatly benefit if some agreement were reached between different workers in various laboratories throughout the world, using a standardized technique or techniques. The results would be particularly valuable if supplies of variolous human material from different epidemics of small-pox could be interchanged. The variolous material could be frozen and desiccated *in vacuo* to preserve its virulence, and in these days of rapid aerial communication its transport would present no difficulties. Experiments with the same strain of virus under different environments or with strains from different origins under the same environment would solve many of the contradictions in the present literature, and may throw new light on the more general problems of variation and mutation in viruses.

Summary

1. The more recent literature on the experimental methods employed in variola or alastrim-vaccinia variation is reviewed and briefly discussed.

2. An account is given of attempts to produce a vaccinia variant from variola of the virulent type and a successful result, by employing the method of intratesticular passage, has been described.

3. The reasons for accepting this as a genuine variation and not a vaccinial contamination are discussed.

4. An attempt has been made to analyse some possible factors underlying the discrepancies in the literature, and a plea is made for more co-operation in future work on the subject.

REFERENCES

ARAGÃO, H. DE B. (1911). *Estudos sobre Alastrim. Mem. do Ins. Ozwaldo Cruz*, p. 311.

ASHBURN, P. M., VEDDER, E. B. & GENTRY, E. R. (1913). *Philipp. J. Sci.* **8**, 17.

BAUJEAN, R. (1923). *Bull. Soc. Path, exot.* **16**, 676.

BLAXALL, F. R. (1923). *Bull. Acad. Méd., Paris*, **89**, 146.

—— (1930). *System of Bacteriology*, **7**, 84. London: H.M. Sta. Office.

CHAUMIER, E. & BELIN, M. (1913). *C.R. Soc. Biol., Paris*, **74**, 138.

CLELAND, J. B. & FERGUSON, E. W. (1915). *Proc. R. Soc. Med.*, Sect. Epidem., **8**, 19.

COPEMAN, S. M. (1898). *Vaccination—its Natural History and Pathology*. Macmillan.

—— (1909). In Allbutt and Rolleston, *A System of Medicine*, **2**, pt. 1, p. 753.

DURAN REYNALS, F. (1929). *J. exp. Med.* **50**, 327.

FINDLAY, F. M. (1936). *J. roy. Micr. Soc.* **56**, 213.

GASTINEL, P. (1938). Art. Virus Variolique, in LEVADITI & LÉPINE (1938).

GAUDUCHEAU, A. (1911). *Bull. Soc. Med. Chir. Indochine* (1911–12). Quoted in *Bull. Inst. Pasteur*, 1911, **9**, 940.

GREEN, A. B. (1915). *J. Hyg., Camb.*, **15**, 315.

JORGE, R. (1924). *Bull. Off. Int. Hyg.*, Publ. 16, No. 10.

KELSCH, M., TEISSIER, P., CAMUS, L., TANON L. & DUVOIR M. (1910). *Bull. Acad. Méd., Paris*, **62**, 27.

KLEIN (1894). *Rep. med. Offr loc. Govt Bd* for 1892–3, p. 391.

KOMIYAMA, T. (1932). *J. Orient. Med. Jan.* p. 6. Quoted in *Bull. Inst. Pasteur*, 1933, **31**, 204.

LEAKE, J. P. & PORCE, J. N. *Publ. Hlth Rep. Wash.* **36**, 1437.

LEDINGHAM, J. C. G. (1924). *Brit. J. exp. Path.*, **5**, 332.

—— (1926). *J. State Med.* **34**, 125.

—— (1935). *Proc. R. Soc. Med.* **29**, 736.

LEVADITI, C. & SCHOEN, R. (1936). *C.R. Soc. Biol., Paris*, **122**, 736.

LEVADITI, C., FASQUELLE, R. RENNIE & SCHOEN, R. (1938). *Ann. Inst. Pasteur*, **60**, 142.

LEVADITI, C. & LÉPINE (1938). *Les Ultravirus des maladies humaines*. Paris: Maloine.

MCCLEAN, D. (1930). *J. Path. Bact.* **33**, 1046.

MCKINNON, N. & DEFRIES, R. D. (1928). *Amer. J. Hyg.* **8**, 93.

MOODY, L. M. (1922). *Ann. trop. Med. Parasit.* **16**, 21.

NAYOSHIKII, TANESABURO SHIMATANI & HISAO, KASAI (1920). *Rev. int. Vacc.* **1**, 73. Quoted by LEVADITI & LÉPINE (1938).

SOBERNHEIM, G. & ZURUKZOGLU (1928). *Dtsch. med. Wschr.* **54**, 339.

TEISSIER, P., WURTZ, R., CAMUS, L., TANON L. & MARIE, A. (1913). *Bull. Acad. Méd., Paris*, **69**, 65.

TEISSIER, P., DUVOIR, M. & STEVENIN (1911). *C.R. Soc. Biol., Paris*, **70**, 654.

TEISSIER, P., RIVALIER, E., REILLY, J. & STEFANESCO, V. (1931). *C.R. Soc. Biol., Paris*, **108**, 1105.

TOMARKIN & CARRIÈRE (1913). Kolle and Wassermann's *Handbuch der pathog. Mikroorganismen*. Jena.

(*MS. received for publication* 9. VII. 38—Ed.)