CROSS IMMUNITY EXPERIMENTS IN MONKEYS BETWEEN VARIOLA, ALASTRIM AND VACCINIA

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PART I. VARIOLA AND VACCINIA

In spite of the voluminous literature, much of it of a polemical rather than scientific nature, on the immunity relationships between variola and vaccinia, comparatively little work has been carried out on experimental animals, and the results of different workers are not altogether concordant. Even in the important article by Blaxall (1930) in the *System of Bacteriology* only a few lines were devoted to a discussion of the existing experimental evidence, and also in the more recent article by Gastinel (1938) this particular problem has been very briefly treated.

Present experimental work

Opportunity was taken during an outbreak of virulent smallpox in the Gezira cotton-growing area of the Anglo-Egyptian Sudan in 1938 to collect variolous material from different patients, and to inoculate a series of monkeys.

Technique

The contents of the vesicles or pustules were aspirated into capillary tubes, which were immediately packed in ice in a vacuum flask and brought directly to Khartoum, 80 miles from the epidemic zone. The capillary tubes with the pus were ground up in a mortar with distilled water (*p*H 7·0), the suspension lightly centrifuged to throw down the powdered glass, and the turbid supernatant fluid pipetted off and stored in the freezing chamber of a refrigerator. Inoculation was made on to the scarified bellies of monkeys (*Cercopithecus sebaeus*), the amount of inoculum and area varying in different animals, and the subsequent immunity tests were carried out with both vaccinia and variola.

Details of the treatment of each animal are given in the following notes:

Inoculation with human variolous material

First series

No. 1. The belly was shaved and scarified all over with material from case (D) in order to obtain sufficient material for further work. The take, however, was poor, as only a series of isolated papules developed along the lines of scarification, and hence were not scraped as intended.

No. 2. Scarified on the belly in four places; each insertion with material from the same case (D), being approximately an inch in length.

Result. A good take with confluent papules.

No. 3. Scarified all over the belly with same material.

Result. A poor take. Only three papules appeared.

This monkey was again inoculated 8 days later with material from another case, four scarifications being made on the flanks to avoid the area previously inoculated. Three of the insertions were negative, the other showed three abortive lesions.

No. 4. Scarified all over the belly with variola D.

Result. A very good take which was scraped on the 8th day, the animal being anaesthetized; healing was rapid and uneventful.[1]

In all these animals, except the second inoculation in No. 3, the appearance of the take was typical; appearing as papules about the 4th day, becoming vesicles by the 7th, rapidly developing as a raised scab and healing completely by about the 14th day. The surrounding reaction was less than in vaccinia and the animals did not appear to be ill, or refuse food at any stage.

Nos. 5, 6. Inoculated with calf vaccine lymph (No. 43, potency 1 in 50,000) derived from the sheep strain of the Lister Institute, diluted 1 in 10, in four small insertions of about 1 cm. each.

These six animals were tested for immunity with both vaccinia and variola.

No. 7. Inoculated at the same time as Nos. 5 and 6 with a mixture of variolous material (cases A+B+D) to determine the potency. This animal served as a control.

Immunity tests

In all the experiments reported in this paper, the method of testing for the immunity was the same. The shaved belly and part of the flank were divided into a number of areas, each 14 sq. cm., which were lightly scarified with a needle and 0·1 c.c. of the appropriate dilution rubbed in with a glass rod. The animals were lightly anaesthetized with ether.

Table I

The results shown in this table were read on the 4th and 5th days.

No. of animal	Immunized with	Tested with: Vaccinia (No. 43) 1/1	1/10	1/100	1/1000	Variola (cases A+B+D) 1/1	1/10	1/100	Period after first immunizing dose days
1	Variola	± (10)	(1)	0		0	0	0	35
2	,,	(10) Accelerated reaction with poor vesicles	(4)	(4)	(1)	0	0	0	31
3	,,	+ Good take with vesicles	±	(10)	(3) typical	0	0	0	18
4	,,	0	0	0	0	0	0	0	15
5	Vaccinia	0	0	0	0	0	0	0	32
6	,,	0	0	0	0	0	0	0	32

No. of animal	Immunized with	1/10	1/100	1/1000	1/10000
7	Control	+	+	(3) (marked reaction)	0

Not tested

+ = confluent. ± = semi-confluent. Numbers in brackets denote number of separate vesicles.

[1] The experimental work on the production of a vaccinial variant from this monkey variola has been already published (Horgan, 1938 *a*).

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Owing to shortage of monkeys further experiments had to be temporarily postponed.

Second series

No. 8. Scarified all over the belly with material from four smallpox cases (K, L, M, N). The supernatant fluid had been stored in a refrigerator for approximately 3 months.

Result. Completely negative.

This animal was re-scarified with monkey variola (from No. 4), which was titrated as follows:

$\frac{1}{1}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{10000}$
+	+	±	(1)	0

The take in all insertions showed typical vesicles.

Hence it seems that the virus in the first inoculum had become completely inactive in spite of optimal conditions for storage. Although no titration had been carried out when the material was fresh, it was collected from typical confluent cases and presumably had contained active virus.

No. 9. Used to duplicate the titration of the monkey variola in No. 8.

	$\frac{1}{1}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{10000}$
Results.	+	±	(8)	(1)	0

This result is closely comparable to No. 8.

No. 10. Scarified all over the belly with material from smallpox cases (C+D) in order to obtain more variolous material for another set of experiments.

Result. A very good confluent eruption which was scraped on 8th day. Healing was uneventful.

Table II

No. of animal	Immunized with	Tested with: Vaccinia (No. 43) $\frac{1}{1}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$	Variola (monkey) $\frac{1}{1}$	$\frac{1}{10}$	$\frac{1}{100}$	Period after immunizing dose (days)
7	Variola	0	0	0	0	(6)	0	0 (M.V.)	34
						0	0	0 (H.V.)	
						Tiny abortive papules			
8	,,	(10)	(3)	0	0	0	0	0 (M.V.)	29
		Poor take with abortive vesicles							
9	,,	±	±	(4)	0	0	0	0 (M.V.)	29
		Vesicles smaller than normal				0	0	0 (H.V.)	
10	,,	0	0	0	0	0	0	0 (M.V.)	25

		$\frac{1}{1000}$	$\frac{1}{10000}$	$\frac{1}{100000}$	$\frac{1}{10}$	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{10000}$	
11	Control	+	+	5	+	+	5	0 (M.V.)	
					+	±	1	0 (H.V.)	

(M.V.) = Monkey variola. (H.V.) = Human variola.

COMMENTS

Two monkeys in this series were tested with monkey as well as human variola, and it is of interest to note that the undiluted monkey variola produced a few abortive lesions in No. 7, although the undiluted vaccinia gave a completely negative result.

Table III. *Cross immunity experiments of previous workers*

date	Primary inoculation	Animals: No.	Animals: Species	Subsequent test: Vaccinia	Subsequent test: Variola	Subsequent test: Alastrim	Comments
	Vaccinia	6	*M. rhesus*	4 (−)	2 (−)	—	—
	Variola	3	,,	2 (−)	1 (−)	—	—
	Vaccinia	7	*M. cynomolgus*	7 (−)	Subsequently 7 (−)	—	—
02)	Variola	3	*M. rhesus*	3 (±)	2 (−)	—	—
	subcutaneous	3	,,	3 (2 − 1 ±)	—	—	Inoculum of variolous
zer &	Vaccinia	13	,,	13 (−)	—	—	—
5–6)	Variola	6	,,	—	6 (−)	—	—
	,,	7	,,	6 (3 ± 3 −)	1 (−)	—	—
	,,	5	,,	—	5 (+)	—	Tested on cornea
	,, (cornea)	1	,,	—	1 (−)	—	,, ,,
	,, (palate)	5	,,	5 (+)	—	—	—
	,, (palate)	3	,,	—	3 (2 − 1 +)	—	—
	,, (palate)	1	,,	1 (−)	—	—	Tested on two occasio
: Stevenin	Variola cutaneous intratracheally (3) digestive route (3) intravenously (1)	14	*M. nemestrinus*	? (±)	? (−)	—	Nos. tested are not s paper, but all tested solid immunity to va partial to vaccinia
914)	Variola	3	*M. macacus* ?	3 (+)	—	—	—
on (1915)	Alastrim (Australian)	1	*M. rhesus*	1 (±)	—	—	—
	,,	4	Calves	4 (3 + 1 −)	—	—	—
	Vaccinia	1	Calf	—	—	1 (+)	—
	Alastrim (Australian + variola)	8	*M. rhesus*	7 (+)	5 (4 + 1 −)	—	Poor primary take of
		1	,,	1 (+)	—	—	—
	Vaccinia	4	,,	1 (−)	3 (−)	3 (2 + 1 −)	—
	Vaccinia and variola	1	,,	—	—	1 (±)	—
	Variola	5	,,	5 (2 + 3 −)	—	5 (3 + 2 −)	—
	Alastrim	1	Calf	1 (+)	1 (−)	—	—
921, 1927)	Alastrim (W. Indies)	2	*M. rhesus*	2 (−)	—	—	—
	,,	2	,,	—	—	2 (−)	—
				—	1 (−)	1 (−)	1 monkey re-tested 84 d first inoculation
	Variola	1	,,	1 (±)	—	—	—
	,,	1	,,	—	1 (−)	1 (−)	—
	Vaccinia	1	,,	—	1 (−)	1 (−)	—
	Alastrim (England)	2	,,	2 (−)	—	—	—
	,,	2	Calves	2 (+)	—	—	First passage with material
	,,	2	,,	2 (±)	—	—	Second passage in calv
	,,	2	,,	2 (−)	—	—	Third passage in calve
	,,	1?	,,	1? (+)	—	—	First passage with material
	,,	1?	,,	1? (+)	—	—	Second passage with material

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	Variola	2	*M. rhesus*	2 (−)	—	—	—
	,,	2	Calves	2 (±)	—	—	First passage with material
	,,	2	,,	2 (±)	—	—	Second passage with material
	,,	2	,,	2 (−)	—	—	Third passage with material
ɔri (1922)*	Neuro-variola (intracerebrally)	6	Rabbits	6 (≡)	—	—	—
	Neuro-variola ?	6 (?)	,,	? (+)	? (−	—	No. of animals is not cl context
	Neuro-variola (cornea) ?	6 (?)	,,	? (+)	—	—	,, ,,
	Neuro-variola ? (intracerebrally)		,,	? (−)	—	—	Fixed neuro-variola !
	Neuro-variola ? (intratesticularly)		,,	? (+)	—	—	Neuro-variola after f sages only
	Neuro-variola ? (intratesticularly)		,,	? (−)	—	—	Fixed neuro-variola !
	Vaccinia (intracerebrally)		,,	—	? (−)	—	? Neuro-variola !
	Alastrim (England)	2	*M. rhesus*	2 (±)	—	—	—
	Alastrim + variola	1	,,	1 (±)	—	—	—
	Vaccinia	2	,,	—	—	2 (≡)	Vaccinated 11 months
	Vaccinia	1	,,	1 (−)	—	—	—
	Alastrim (B. Congo)	6	*Cercopithecus* spp. various	6 (+)	—	—	One intratesticular, fi fied on skin and muco branes. No visible re
	intravenous	2	,,	2 (+)	—	—	Inoculated intravenou virus and belly scarifi showed good take, th few papules
t (1926)	Alastrim (India)	2	*M. sinicus*	2 (+)	—	—	Passaged first to seco key. No visible react
	,,	5	,,	5 (2 + 3 −)	—	—	Passaged through seri two no visible react three showed vesicul
ɔira (1934)	Alastrim (Brazil)	2	*M. rhesus*	2 (±)	—	—	—
	intravenous	1	,,	—	—	1 (−)	—
	Vaccinia	1	,,	—	—	1 (−)	—
	Variola	1	,,	(−)	(−)	—	Tests for vaccinia w calf lymph and chick

as otherwise mentioned primary inoculation was by the cutaneous route.

s of so-called "neuro-variola" in rabbits should be accepted with considerable reserve. It must be noted that alternate t
was used through many passages and as the testicle of the rabbit is shown to be a tissue in which variola-vaccinia varia
2, personal communication; Horgan, 1938*a*), it is highly probable that in the later passages, the authors were really dealing
ıia variant. It is at least suggestive, that while the early intratesticular inoculation failed to prevent a subsequent vaccin
conferred complete immunity.

e noted that Teissier, Reilly & Rivalier (1929) completely failed to carry on variola in rabbits, guinea-pigs, white rats or
age, and even continued intracerebral passage in monkeys failed to fix variola.

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Summary of results

In a series of seven monkeys inoculated cutaneously with smallpox material (contents of vesicles and pustules) all subsequently showed complete immunity to human variola. Two of these were also tested with fresh monkey variola (first passage). One showed a few abortive papules on the site on which the undiluted material was applied, while in the other immunity was complete.

Two monkeys inoculated with monkey variola showed complete immunity to both human and monkey material.

Of the nine animals immunized with variola, three showed complete immunity to vaccinia and in the others, except in No. 3, the vaccinial take was poor and only with the lower dilutions and undiluted lymph, and tended to dry up by the 5th day.

In No. 3 the immunity, although complete to variola, was of comparatively low degree to vaccinia as judged by the appearance of typical and not abortive vesicles, which reached their maximum development about the 8th day. This result seems to be correlated to the low susceptibility of the animal to variola as judged by the poor primary reaction, which failed to protect completely against the second inoculum of variola 8 days later.

Two of the fully immune animals had each been scarified over a considerable area and had developed a well-marked reaction. The remaining one, No. 7, which had been used for titration, also showed a very good take in the 1/1 and 1/10 dilutions of variola.

Two monkeys immunized with vaccinia showed complete immunity to both vaccinia and variola.

For the sake of simplicity the literature since 1894 is summarized in Table III (pp. 618–19). It was hoped to obtain a full record, giving such particulars as the area of inoculation, the immunizing dose, the strength of resulting reaction, the period elapsing before testing for immunity, etc. but unfortunately such data are often lacking and, in some of the papers, it is not always clear how many animals were used for any one procedure.

Discussion

In this section the discussion is concerned only with variola and vaccinia, but in the table the results with alastrim have also been included, partly to present a complete chronological summary of previous work, and partly because certain workers (Green, 1916; Gordon, 1925) used mixtures of alastrim and variola for the inoculation of some animals.

Taking into consideration the present results with those of previous workers, the answer to the question: Is the immunity between vaccinia and variola in the experimental monkey reciprocal? can be given with reasonable certainty. It is *not completely* reciprocal. To avoid ambiguity the term "reciprocal immunity" is defined in the present paper as follows: "The mutual and complete cross protection in a susceptible animal inoculated cutaneously in linear incisions with amounts of the respective viruses of a corresponding order to that used for human vaccination."

All workers are in agreement that vaccinia can confer complete immunity in monkeys both against itself and variola, but there are some discrepancies in the results from variolization. The majority of workers are in agreement that variola can confer complete protection against itself and very strong protection against vaccinia. In certain circumstances, however, the resultant immunity is complete and the reasons will now be considered.

There is scarcely any doubt from the above experiments that when the cutaneous route is used the production of immunity by variola against vaccinia is directly related to the strength of the primary reaction, and for complete protection a well-marked reaction over a considerable area of skin appears to be essential. This is well exemplified in Nos. 4 and 10, while 7, although variolized over a lesser area, developed strong reaction to the primary inoculum. These results correspond fairly well with those of Green (1916), who mentions that in three of the six animals immune to vaccinia, the take was fairly good to good, while two of the three which showed a partial take with vaccinia had poor primary vesiculation from variola. However, in one monkey, in spite of very marked reaction, subsequent vaccinial immunity was not complete.

Area inoculated and reaction

Only some authors state the actual area or number of insertions variolized. Thus Brinckerhoff *et el.* (1905–6) variolized six animals cutaneously by a number of scarifications (generally 12) over the abdomen. All insertions showed typical primary reactions and in some animals a secondary rash appeared. Three of the six animals subsequently showed a full immunity to vaccinia. Blaxall (1923) adopted a technique of shaving the animal's back in four or five places, then lightly scarifying and rubbing on the material, a procedure which probably gave a considerable total area. Green (1916) inoculated only two linear incisions in shaved areas.

Probably the factors concerned in the production of a strong reaction are (1) area, (2) dosage, and (3) individual susceptibility, and presumably the desideratum is to obtain the maximum proliferation of virus in an epiblastic tissue (skin) which experience has shown to be only indifferently adapted to the mesodermic or viscerotropic virus of epidemic variola. Teissier, Duvoir & Stevenin (1911) had shown that human variola inoculated into monkeys by routes other than the skin, e.g. intratracheally or intravenously, brought about an "inapparent infection" with partial immunity to vaccinia, but the amounts of variolous material inoculated in any case were not stated. Teissier, Reilly & Rivalier (1929) and Teissier, Reilly, Rivalier & Stefanesco (1932), have shown clearly that active proliferation of variola virus takes place in the testicle, and, if an intratesticular inoculation is made, an acute orchitis is produced, accompanied in some cases by a generalized cutaneous exanthem, and the serum becomes strongly viricidal to vaccinia. With small inocula (amounts not stated) in young animals (*M. sinicus* and *C. callithrix*), there was an absence of orchitis or other symptoms, but an "inapparent infection" was produced with the development of viricidal antibodies. A similar state was produced in another

animal by injection into the femoral artery of a smaller dose and its serum became actively viricidal. The same authors carried out numbers of similar experiments on cats and dogs and in a series of rabbits, and in the case of the latter demonstrated that large inocula of variola virus intratesticularly brought about partial immunity to vaccinia. The monkeys were not apparently directly tested with vaccinia virus, but it would have been interesting to note whether, as might be expected, the animals which developed acute orchitis and rash, were subsequently fully immune to vaccinia.

That the amount of virus is the important factor is also borne out in the work of McKinnon & Defries (1928), who rendered rabbits completely immune to vaccinia by repeated intradermal inoculations of variolous material over a considerable period. These results in rabbits have been fully confirmed by one of us (E. S. H., 1938, unpublished experiments). Of four rabbits which received repeated intradermal doses of human variola over a period of 35 days, three were fully immune when subsequently tested with calf-vaccine lymph of high potency, while the other showed an accelerated reaction.

It appears, therefore, that when the cutaneous route is used for variolization, the reaction produced is the most important factor, but the total area inoculated is also important, for the larger the area the greater the possible immunizing dose and the greater the chance of a strong reaction.

Dosage and potency

By dosage is meant the total amount of active virus (elementary bodies) used for immunization. Unlike vaccinia virus whose potency can be accurately titrated beforehand on rabbits, it is rarely possible to secure enough vesicular fluid or pus from smallpox cases for preliminary titration in monkeys, and little work appears to have been done on the potency of such material, but in the present paper an attempt has been made. It will be noted that the potency was quite low, a dilution of about 1 in 1000 being the average end-point. Gordon (1925), working with human alastrim material, found active virus was still present at a dilution of 1 in 10,000. A good vaccine lymph has an end-point on the monkey of from 1 in 100,000 to 1 in 500,000. Unless this considerable difference is borne in mind, the nominal amount of variolous material used may be quite misleading. To secure a variola virus with a titre of a corresponding order to a very potent vaccine lymph would probably be very difficult, but might be effected by concentration of the elementary bodies of variola. It is hoped shortly to undertake experiments on this point.

Potency has also to be considered in the subsequent tests for immunity, and it is strange that this factor often appears to have been neglected, even in vaccinial immunity, although years ago the Japanese workers, Kasai & Kii (1926), pointed out the value of a semi-immune animal, calf or rabbit, for the determination of differences in potency of vaccine lymphs.

This point has been especially stressed in the recent paper of Rivers *et al.*

(1939) in their experimental work on the amount and duration of immunity in man following vaccination by a cultured vaccine virus.

From the evidence it seems clear that, irrespective of route, the larger the amount of active variola virus, either introduced directly, or resulting from a proliferation of an inoculum of the virus in any susceptible tissue, e.g. testicle or skin, the more complete is the resultant immunity to vaccinia.

Vaccinia virus, on the other hand, has a so much greater "infectivity", or capacity for proliferation in the tissues of most animals, that very small inocula are sufficient.

Period elapsing between the immunizing and test doses

The period elapsing between the immunizing and test dose has varied from 8 days to several months with different workers, but most of those who mention the time interval have tested in a period ranging from 15 to 40 days.

Brinckerhoff *et al.* (1905–6), in their most careful and complete series of experiments, proved that immunity to both variola and vaccinia was strong or complete about the 4th day, and the general opinion seems to be that vaccinial immunity in monkeys remains for about a year or longer. It is consequently most unlikely that this time factor is of any importance as an explanation of the discrepancies in the literature.

Susceptibility

Individual susceptibility is undoubtedly of some importance, for, if the take is poor and reaction slight, even with a large inoculum of variola virus the resultant vaccinial immunity is likely to be of low degree as seen in No. 3. As an inference from such data, a complete absence of immunity might be expected in an insusceptible animal, in which there was no visible reaction following cutaneous variolization. Hitherto direct proof has not been obtained by us as no monkey completely insusceptible to *active* variolus material has been encountered, but, as mentioned later in this paper, the inference appears to have been justified at least in the case of some African strains of alastrim virus. However, Teissier, Duvoir & Stevenin (1911), who inoculated monkeys cutaneously with ground-up smallpox scabs, found that, in spite of a negative reaction, a partial immunity to vaccinia virus was subsequently produced ("inapparent infection"). This result does not appear to have been confirmed by other workers.

Judging from present series ordinary African monkeys (*C. sebaeus*) are fairly uniformly susceptible to variola virus, only one, No. 3, being so far encountered in which the susceptibility was very low. Most previous workers have used various species of *Macacus* with similar successful results. A completely insusceptible animal of the genus *Macacus* or *Cercopithecus* seems to be very rare.

Bearing these various factors in mind, explanations of the results of those

workers who obtained a completely reciprocal immunity in all animals might be hazarded. The most probable are:

(1) The inoculation of a sufficiently large area and the employment of potent human variolous material to produce a good reaction. These factors may possibly have accounted for Blaxall's successes.

(2) The use of vaccinia test lymph of low potency.

(3) The employment of a hypersusceptible monkey in which conditions for the rapid proliferation of variola virus were at a maximum.

Conclusions

It remains to be considered what light the cross immunity tests made hitherto have thrown on the variola-vaccinia relationship.

In the present paper stress has been laid on the amount of immunizing variola virus in the body, whether resulting from multiplication in a susceptible animal, such as the monkey, or from a sufficiently large inoculum in a non-susceptible animal, such as the rabbit, the latter really being an example of hyper-immunization.

The results of Teissier *et al.* (1911) also show the importance of dosage of variola in obtaining immunity to vaccinia.

The evidence is in favour of the hypothesis of Gordon (1925) that the basic antigen responsible for cross-protection is identical in both viruses but varies quantitatively, forming a smaller proportion of the complete antigenic structure of variola. It is also in accordance with the well-known fact of rapid development of vaccinial immunity, after 4 or 5 days, in smallpox cases. In man, the naturally susceptible host, the virus must be under optimal conditions for rapid multiplication and circulation and, as Blaxall (1930) remarks, the facts suggest that the natural disease evokes antibody much more rapidly than when the inoculation is artificial and traumatic.

Ledingham's (1925) comparison of vaccinia to a Vi-containing avirulent strain of *B. typhosum* is a most attractive hypothesis, and accords closely with the results of cross-immunity tests. Unfortunately the term "Vi antigen", which is essentially an immunity antigen, still tends to be closely associated with the concept of virulence, and its use may lead to some ambiguity in the present application. Hence a slight modification of Ledingham's hypothesis is here put forward, that variola contains a certain amount of basic group antigen (vaccinia) combined with a highly specific antigen or antigens, the combination being responsible for virulence in man and to a lesser extent in monkeys. The group antigen is responsible for the production of viricidal antibodies and immunity, while the specific antigen, although capable of evoking specific antibodies such as agglutinins, probably plays little, if any, part in immunity. It could be compared to the specific H antigens of Salmonella organisms.[1]

[1] One might press the Salmonella analogy slightly further; a pure (as judged by agglutination test) Vi strain of *B. typhosum* (No. 6S or Vi I) still contains traces of H and O antigens as proved by immunization of animals (Felix) whereas Amies (1934) has shown that the sera of rabbits hyperimmunized with vaccinia virus agglutinate to a low titre the elementary bodies of variola.

Under suitable conditions, such as passage through a non-susceptible animal, variation takes place resulting in the loss of the specific antigen, but the group antigen remains unchanged. According to this view, vaccinia virus is a permanently degraded antigenic variant of variola virus.

Findlay (1936), in discussing Ledingham's hypothesis, points out that whereas variola virus is virulent for man, vaccinia virus is more so for the rabbit. The present authors are unable to agree with Findlay's statement concerning the virulence of vaccinia virus for the rabbit. The difficulty probably lies in the different meanings of the term "virulent". The meanings which we attach to the terms "virulence" and "infectivity" are illustrated by the two diseases, variola and varicella in the human subject. Both are highly infective, but while variola is very virulent, varicella is almost non-virulent. In this sense, variola virus is devoid of infectivity for the rabbit, while vaccinia virus has a high degree of infectivity for this animal, but normally very little virulence. If epizootics of rabbit pox be accepted as due to vaccinia virus—an assumption which is more than doubtful—this would be an instance of a strain of vaccinia virus with both high infectivity and high virulence for the rabbit.

The authors' views can be summarized as follows: variola virus contains a specific antigen, the presence of which is associated with a high degree of virulence for man and perhaps some monkeys, and a low degree of infectivity for most other animal species. When this specific antigen is lost, a group antigen is exposed and in this condition the virus is identical with that of vaccinia. Such a degraded virus has a lower virulence for man, but a wider range of infectivity for different animal species, a range which extends from the very susceptible rabbit at one end of the scale to the strongly resistant cat or white rat at the other.

SUMMARY

A series of cross protection tests in monkeys between variola and vaccinia is described. The results, which are in accordance with those of the majority of previous workers, indicate that, while the cross immunity is strong, it is not completely reciprocal in most cases. The protection induced by vaccinia against both viruses is complete; that of variola is complete against itself, but varies from strong to complete against vaccinia.

Complete immunity is directly related to the degree of primary reaction to variola, and it is suggested that the amount of active virus is the important factor.

The influence of dosage, potency and susceptibility is briefly discussed.

The results of immunity tests are best explained by an hypothesis which assumes that vaccinia is a degraded antigenic variant, which has lost its specific non-immunizing factors, but retains intact the group antigen essential for immunity.

PART II. ALASTRIM AND VACCINIA

Foreword

The term alastrim is used in this paper to denote the virus causing the clinical conditions variously named alastrim, mild smallpox, milkpox, variola minor, amaas, etc.

The first part of this section deals with a strain (St Louis) of American origin. In the second part a brief account is given of efforts to obtain the virus from local cases in Khartoum.

A. THE ST LOUIS STRAIN OF ALASTRIM

The source of the strain

The strain used in these experiments was very kindly sent by Dr Markham, Ohio State University, Columbus, who furnished the following particulars: "The strain was isolated a few months ago during a small outbreak of smallpox in St Louis. All of the patients had the mild form of the disease which prevails in this country (U.S.A.). The strain was isolated by direct inoculation of pustule content onto the chorio-allantoic membrane of the developing chick embryo (Goodpasture's method) and the material sent was egg membrane desiccated *in vacuo* and represented the 4th passage on the chick embryo."

Experimental details

The dried material was ground up with a little buffered distilled water (*p*H 7·0) and inoculated cutaneously on to the shaved bellies of two monkeys (*C. sebaeus*), A and B. There was no visible reaction on either until the evening of the 5th day, when along the lines of scarification small papules could just be seen, but were felt more readily. They developed rapidly, reaching their maximum in less than 48 hr. and, without forming definite vesicles or pustules as is the case with a virulent strain of smallpox (variola), commenced to dry up as yellow crusts or scabs, which finally fell off about the 13th or 14th day. At no time did the animals show any sign of illness or refuse food.

Both the authors had experience of the lesions of variola on monkeys during an outbreak of smallpox in the Gezira area near Khartoum in 1938 and during the latter part of this work another strain of variola was isolated (see p. 633). There was a perceptible difference between the naked-eye appearance of the lesions caused by such strains of variola and those caused by the St Louis strain, the reaction with the former being more marked and with a small zone of congestion around the papule. The papules were larger and later developed into more definite vesicles and pustules, which dried as heaped up yellow crusts.

On the 6th and 7th days respectively the monkeys were anaesthetized with ether, the papular eruption gently scraped with a sharp scalpel and the pulp ground up with buffered (McIlwaine's) distilled water (*p*H 7·0). The material from each animal was necessarily small in amount. That from A was kept as a

suspension for stock virus and that from B, which gave the better take, was used to prepare a suspension of E.B. (elementary bodies) by Salaman's method (1938). This suspension (3 c.c.) was of moderate opacity, similar to that of rather dilute milk, and appeared moderately homogeneous by dark ground examination. Half of it was used for titration and further inoculations, and half was desiccated *in vacuo* over P_2O_5 and stored in a sealed evacuated ampoule.

All virus material was stored in a refrigerator at about 4° C.

Monkeys C and D. One side of each was lightly scarified and inoculated with the suspension of E.B. The other was used for titration of the potency of the suspension. Both animals showed typical reactions from the 5th day and on the side inoculated all over with the E.B. the take was almost confluent throughout, and about the 8th day formed into small yellow crusts from under some of which tiny beads of purulent looking fluid could be expressed.

Monkey C was anaesthetized with ether and gently scraped on the 7th day in order to obtain material for a fresh E.B. suspension and two of small snips of the well developed lesions were excised for histological examination.

Titrations of the E.B. suspension from B, by the same technique as that detailed for variola in Section I, gave the following results, the readings being made on the 7th day.

	Dilutions			
	1/1	1/10	1/100	1/1000
Monkey C	+	±	18	4
D	+	±	9	5

Monkeys E and F were each inoculated with the E.B. suspension in four scratches each about 1 cm. long.

They were lightly inoculated in order to compare their resultant immunity with that of the previous animals more heavily inoculated over a larger area.

Each scratch showed a slight papular reaction on the 6th day which developed in the usual way.

Monkeys G and H were each vaccinated in two scratches with a suspension of E.B. of vaccinia (Amies' strain). Its potency had been tested a short time before the present series and gave a titre of 10^{-8} intradermally on rabbits. It

Table IV

	Alastrim (E.B.)			Vaccine (calf lymph)			Days after immunization
	1/1	1/10	1/100	1/1	1/10	1/100	
Monkey A	0	0	0	0	0	0	30
B	0	0	0	0	0	0	30
C	0	0	0	0	0	0	23
D	0	0	0	(10)*	0	0	23
E	0	0	0	±*	(12)*	(1)*	16
F	0	0	0	+	+	(6)	16
G	0	0	0	0	0	0	16
H	0	0	0	(5)†	0	0	16

* Vesicles small and abortive, drying into crusts about the 5th day.

† Pin-point papules which never formed vesicles and had completely dried up by the 5th day.

had not been titrated to the end-point on monkeys, but 0·1 c.c. of 10^{-6} dilution caused a typical intradermal reaction. All inoculations developed the typical vesicular eruption of vaccinia.

Cross-protection tests were carried out as follows:

One side of the shaved belly was inoculated with dilutions of the desiccated E.B. prepared from monkey B (this desiccate was not retitrated at the time, but when used a fortnight later to inoculate a fresh monkey it gave a confluent take) and the opposite side similarly inoculated with a standard vaccine calf lymph (titre 1 in 100,000 produced isolated papules on rabbits).

Summary of results

Four monkeys inoculated over a fairly large area with alastrim showed full immunity to alastrim. Three showed full immunity to vaccinia while, in the other, protection was almost complete, only an abortive take being obtained with the undiluted potent lymph.

All animals showed good reactions, the takes of the second passage (monkey material) being more marked than those of the first passage, inoculated with the chorio-allantoic material.

Two animals inoculated by four scratch insertions with E.B. of alastrim (first passage) subsequently proved immune to alastrim; one showed strong immunity to vaccinia but, in the other, protection was poor.

Two animals vaccinated with E.B. suspension of vaccinia showed complete immunity to alastrim; one showed complete and the other almost complete immunity to vaccinia, the take with an undiluted vaccine lymph of high titre being negligible.

The results indicate that the immunity relations of the St Louis strain of alastrim and vaccinia are in all probability of the same quantitative order as those previously discussed for variola and vaccinia; namely, the immunity is not completely reciprocal with small inocula of alastrim, and with localized reactions, but becomes so if sufficient virus is inoculated over a sufficiently extensive area to produce a well-marked reaction. The factors to be borne in mind when evaluating the results have already been discussed in the previous section.

B. LOCAL (KHARTOUM) STRAIN OF ALASTRIM

It is well known that while the smallpox in the Northern Sudan is of the classical virulent epidemic type with a high mortality, the prevailing form in the Equatorial Province is an alastrim, which causes at times widespread epidemics of a mild character with a negligible mortality. Small outbreaks or sporadic cases of a similar nature occasionally occur also in the north and during the progress of the present work four such cases occurred in the Khartoum urban area. The first case occurred in March 1939, the second a month and the third 5 weeks afterwards. The patients were young adult males, but it is curious that in spite of careful enquiries by the Medical Officer of Health, no kind of

connexion could be traced between them. The rash was well marked on face and neck in the second and third patients, being semi-confluent and showing a characteristic centrifugal distribution. Constitutional symptoms in all were negligible. Umbilication was noted in very few of the vesicles; the progress to scabbing was fairly rapid, and the scabs fell off leaving areas of depigmentation on the black skins with very slight pitting in a few places. The fourth patient, who was the wife of the second and was admitted to hospital 9 days after her husband, had a very mild attack with only a few scattered vesicles, insufficient for obtaining material, over her body.

EXPERIMENTAL TECHNIQUE

The turbid fluid was collected from a considerable number of vesicles from each case into capillary tubes, which were immediately brought to the laboratories, and ground up in just sufficient distilled water to produce a thick suspension, which was lightly centrifuged for a few minutes to throw down the powdered glass. The supernatant fluid was rubbed on as a heavy inoculum over the entire scarified bellies of monkeys (*C. sebaeus*). Two monkeys were used for the first case, one for the second, and two for the third. In the first monkey used for case 3, an attempt was made to give an intravenous injection also, but failed owing to the small loose veins, and 2 c.c. of the turbid supernatant fluid were given subcutaneously instead. The second monkey for case 3 was inoculated intradermally in five insertions (0·25 c.c. each) with a ground-up suspension of the moist scabs.

Apart from the slight initial reaction due to trauma, none of the animals showed the slightest evidence of a take. Two used for the first patient, G and H, were inoculated with calf lymph and gave typical well-marked reactions as also did the monkey inoculated intradermally from case 3. The other two were heavily inoculated cutaneously with the St Louis pulp from monkey A and one, K, showed a dry scabby take developing three small typical papules, but as it was proved shortly afterwards in connexion with other work, that the St Louis material had almost completely lost its potency for some unknown reason,[1] this result is of no significance, and, if anything, could be regarded rather as a positive take. In spite of rigid precautions observed in all these experiments the other animal (P) must have been accidentally infected with vaccinia at the time of inoculation, for three large typical vesicles developed which rendered it fully immune to a subsequent test with calf vaccine lymph. It may be stated that, as far as is known, this has been the only animal accidentally infected in these Laboratories.

SUMMARY OF RESULTS

Attempts to inoculate five monkeys with large inocula of human alastrim material from three patients failed completely. There was no sign of any reaction and, as judged by subsequent tests with vaccinia, no evidence of any "inapparent infection".

[1] A similar rapid deterioration in potency of a saline suspension of variola was noted in 1938.

These results are in close accord with the experiments of Van Hoof (1925) in the Belgian Congo, although this worker, after a considerable number of failures, finally succeeded in infecting a *Cercopithecus* by intravenous injection, having previously shaved and scarified the belly.

This animal, which developed a well-marked rash, and another subsequently infected with alastrim, which showed only a single papule at the point of inoculation, were later tested with vaccinia and reacted with typical takes. Six other monkeys, inoculated with alastrim material with negative takes, reacted equally well to subsequent vaccination thus showing no evidence of "inapparent infection".

Discussion

The experimental evidence from cross-protection tests between alastrim and vaccinia is more conflicting and inconclusive than is the case with variola and vaccinia, and the problem has been fully discussed by Jorge (1924) and Ledingham (1925), the latter of whom gives a lucid and detailed analysis of the experimental evidence up to the present time, and points out the various factors which have to be borne in mind in appraising such experiments. These factors have already been discussed in the present paper (p. 624).

Since 1925 there appears to have been little fresh experimental evidence, but a brief general summary of the relevant literature has been tabulated (pp. 618–19) in the same form as given in Ledingham's paper.

A review of the evidence including the present results suggests that strains of varying antigenic constitution are included under the general name, alastrim, and there seems to be some evidence that such differences may be related to geographical distribution. Findlay (1936) has pointed out that slight differences may exist between the various alastrims with respect to animal susceptibility. Based on these views the following very tentative grouping has been compiled from the literature:

Table V. *Susceptibility of animals**

Country of origin	Authors	Monkeys	Others
Australia	Cleland & Ferguson (1915) Green (1916)	*M. rhesus* +	Calves ±, G. pigs –
India	Turkhud & Pandit (1926)	*M. sinicus* – (earlier passages)	Calves –, rabbits – (± in one case)
Africa (Congo)	Van Hoof (1925)	*Cercopithecus* sp.? ± (animal infected with great difficulty)	Rabbits –, goats –
Africa (Sudan)	Horgan & Mansour (This paper)	*C. sebaeus* –	—
America (St Louis)	Horgan & Mansour (This paper)	*C. sebaeus* +	Calves –, rabbits –
America (Brazil)	da Cunha & Teixeira (1934)	*M. rhesus* +	—
America (W. Indies)	Leake & Force (1921, 1927)	*M. rhesus* +	Rabbits – (but later two animals partially immune to vaccine)
England (North)	Blaxall (1923)	*M. rhesus* +	Calves –, rabbits – (first passage)
England (Gloucester)	Gordon (1925)	*M. rhesus* +	—

* Included under this head might be growth on the chorio-allantoic membrane of chick embryo. North American strains (including St Louis) have been isolated in this way and Torres & Teixeira (1935) report success with a case of Brazilian alastrim.

Broadly speaking, all American and English strains appear to take fairly readily on the monkey but not on any other animal. The Indian strain failed to produce a reaction without passage. With the African strains the difficulties of a primary take are very great and an animal could only be infected by means of the artifice of intravenous injection with scarification (Van Hoof, 1925). Baujean (1923) is the only author who appears to have had a doubtful reaction in one monkey (a Brazilian Coaita) with an African strain.

The Australian type seemed to have been readily inoculated to monkeys, but differs from all other strains in its capability of infecting at least some calves from the first passage.

Vaccinial variation appears to have been obtained readily with English strains (Blaxall, 1923; Sobernheim & Zurukzoglu, 1928; Amies, personal communication), in the hands of both English and continental workers, as was the case also with the Indian strain (Turkhud & Pandit, 1926).

Experiments, still in progress, to bring about vaccinial variation in the St Louis strain have so far been a failure. These will be published later. No previous work appears to have been carried out on American or African strains, and it seems doubtful if true vaccinia was obtained from the Australian strain (Green, 1916).[1]

Correspondingly there appear to be certain differences in cross-immunity tests, but the recorded experiments are few and still fewer monkeys were employed in each test, so that it would be most unwise to draw any definite conclusions.

The present work suggests that immunity relations between the St Louis strain and vaccinia are similar to those between variola and vaccinia, and that most probably the difference is quantitative. The results of Leake & Force (1921, 1927), who found fully reciprocal immunity with a West Indies strain, are scarcely dissimilar considering the small number of animals (three) used.

With the Brazilian alastrim, Cunha & Teixeira (1934) obtained strong protection against vaccinia (two monkeys) with a full protection of the latter against alastrim in the one animal employed; results which correspond closely with our own.

With the English virus, Blaxall (1923) obtained full immunity (two monkeys) to vaccinia, and here the same considerations may apply as already discussed under variola. Gordon (1925) obtained strong but not complete protection (two monkeys). He also obtained a slight take of alastrim following immunization by vaccinia, but as the animal had been vaccinated 11 months previously, it is possible that immunity was beginning to pass off.

Taken as a whole the results with both the American (North and South) and English alastrims are in close agreement, and suggest the existence of a common group antigen identical with vaccinia, but varying quantitatively.

The Indian workers, Turkhud & Pandit (1926), obtained full immunity to vaccinia, but it was first necessary to adapt the virus to the monkey by passage and to secure a definite reaction with vesiculation. No cross-protection tests appear to have been carried out.

The immunity relations of the Australian type have been fully discussed by Ledingham (1925). Here again this type stands by itself, and as Table V shows, only a low degree of cross-protection was obtained, suggesting considerable antigenic differences.

The few experiments carried out with an African alastrim (Van Hoof, 1925) suggest an inability to protect against vaccinia. No cross-immunity tests were reported.

To sum up: there is no reason to doubt the usually accepted view that immunity relationships between the alastrims and vaccinia are very close and comparable to those of variola and vaccinia.

The evidence from experimental animals is, however, far too scanty to enable any general conclusions concerning the antigenic identity or relation-

[1] The results on this point are conflicting, as Cleland & Ferguson (1915) reported they obtained typical vaccinial vesiculation after calf passage.

ships of various alastrims to be drawn, and for the present it would perhaps be wiser to regard results obtained from a strain of alastrim responsible for a particular outbreak, or epidemic, as being strictly applicable to that strain only, and to be used with caution when dealing with a strain of different provenance.

Some of the difficulties (at least) in discussions on alastrim seem to arise from the assumption, perhaps an unconscious one, that the very uniform epidemiological and clinical features of outbreaks of alastrim throughout the world are necessarily associated with antigenic identity of the infecting strains. The position may not be unlike that of the Salmonella group where enteric fevers, often with an identical clinical picture, are due to Salmonella strains antigenically related but with marked specific differences.

It is obvious that, before the problem is finally solved, a direct antigenic comparison of different strains of alastrim in experimental animals will be required. Owing, however, to the varying susceptibility of monkeys, such a comparison may be attended by certain difficulties, for it does not follow that a strain such as an African or Indian one, which may need several passages before being adapted to monkeys, will retain its specific antigenic features completely unchanged during the process of adaptation.

SUMMARY

The results of cross-immunity tests in monkeys between an American (St Louis) strain of alastrim and vaccinia are described.

The cross-immunity between these viruses is strong but not completely reciprocal, suggesting a common antigen which varies quantitatively.

All attempts to isolate a Sudan strain of alastrim were unsuccessful.

The relevant literature is briefly discussed, and the tentative conclusion is reached that strains of varying infectivity and immunizing properties in monkeys may be associated with geographical distribution of epidemics or outbreaks.

PART III. VARIOLA AND ALASTRIM (ST LOUIS STRAIN)

During the progress of our experiments on alastrim and vaccinia, a small outbreak of variola (six cases), traced to a case from Abyssinia, occurred in a village named Suki on the Blue Nile about 200 miles upstream from Khartoum. Three of the cases were clinically severe; the others were moderate to mild. The rash was well marked on the face and limbs, although the umbilicated vesicles remained discrete. When the scabs dried and fell off, pitting was noted in some places.

The contents of pustules of two of the cases were collected in capillary tubes by the Senior Medical Inspector, Dr N. Corkill, to whom the authors are much indebted. The tubes were packed in ice in a vacuum flask and brought with as little delay as possible to Khartoum, where they were ground up in the usual way, and two monkeys immediately inoculated.

EXPERIMENTS

Monkey I (*C. sebaeus*) was inoculated all over the scarified belly. A good take of fair-sized papules appeared on the 4th day, which developed into small vesicles on the 8th day. There was a considerable area of inflammation around and between the papules, although the animal did not show any general symptoms of illness, or refuse its food. The lesions were scraped on the 8th day, the animal being anaesthetized with ether. Part of the pulp was ground up with buffered distilled water (*p*H 7·0) and part desiccated and tubed *in vacuo* as stock virus.

Monkey II was inoculated on the belly in four linear insertions each approximately 1 cm. in length. The take was good, with a well-marked secondary exanthem on the belly and flanks from the 4th day.

Monkeys III and IV were large animals, and used to titrate the pulp, the usual technique being employed.

The results read on the 7th day were:

	Dilutions			
	1/1	1/10	1/100	1/1000
Monkey III	+	(8)	(1)	—
IV	+	±	(6)	—

Both monkeys developed severe general infections with secondary exanthems on the belly and flanks from the 6th to 7th days, the most marked sign being swelling of the face and especially the lower eyelids, and both were quite ill for three or four days, refusing food and remaining huddled in a corner of their cages. Monkey III developed a small abscess underneath the right eye, but the relation of this abscess to the variola was doubtful. It was opened under an anaesthetic, and the pus contained a mixture of various organisms.

Both animals had completely recovered by about the 14th day, when the lesions were small dried scabs in process of falling off.

Monkey IV had been reinoculated on the 4th day in a small scarified patch around the umbilicus between the previous insertions, as the first inoculation did not appear to have taken when the animal was examined on this day. It is interesting to note that immunity was already strong, only a few abortive vesicles appearing on the area.

Monkey V was inoculated in the same way as II in four linear insertions. The take was good, but there was no secondary exanthem, and this animal did not show any symptoms of general infection.

Monkeys J to M were inoculated with alastrim (St Louis monkey material).

Monkey J was scarified all over the belly and inoculated with monkey alastrim (elementary bodies).

The take was good with small semi-confluent papules, which became tiny vesicles by the 8th day. Part of the area was scraped on the 8th day to obtain fresh virus material.

Monkey K, which had been inoculated with alastrim (see p. 629) was

reinoculated in four linear insertions, none of which appeared to take, and 5 days later the belly was scarified all over and reinoculated with material from J. The area showed a diffuse scurfy appearance, probably due to the trauma on the 2nd day, but there were no signs of a true take, nor did any papules form. The fact is of some interest, as it indicated that the first inoculation of St Louis virus, in spite of a feeble reaction with a few papules, had produced solid immunity to itself.

Monkey L was inoculated in four linear insertions with virus from J. All insertions showed typical takes with small confluent papules.

Monkeys M and N were used to titrate the virus of J.

	Dilutions			
	1/1	1/10	1/100	1/1000
(M)	+	±	(7)	– (7th day)
(N)	+	±	–	– (,,)

+ Confluent; ± semi-confluent; – absence of take.

None of these animals showed the slightest signs of illness, and no secondary papules or exanthem were ever noticed with the St Louis strain.

Tests for immunity

In order to have virus of the highest possible potency, two fresh animals were inoculated on the same day, monkey VI being inoculated with variolous material from monkey I and monkey O with alastrim material from J. The take on the latter was confluent but moist, and when scraped on the 7th day it was noted that some areas were covered with yellowish scabs. Slides from these showed the presence of pus cells and large numbers of cocci, chiefly staphylococci. As the typical alastrim lesions on the 7th day are always dry papules and the pulp shows remarkably few organisms, it is probable that the condition was due to a mild infection with skin cocci, which owing to passage may have increased in virulence.

The variolous reaction on monkey VI was only moderate, consisting, on the 8th day, when it was scraped, of numbers of well-developed, but not semi-confluent, vesicles.

In order to obtain material of maximum potency, each pulp was ground up with the minimum of buffered distilled water (*p*H 7·0), the resulting suspension being semi-solid, like thick cream, and part of this was desiccated for future work.

The usual technique for immunity tests was employed, the belly being marked out in four areas, two of which were inoculated with the thick suspensions of alastrim and variola, and two with small quantities of the respective desiccates, the skin being moistened slightly and the powder rubbed well into the scratches. The use of the dried viruses appeared to be of no particular advantage, the results being exactly the same as with the suspensions, and hence they are omitted from Table VI.

In most of the animals, both insertions of the alastrim showed a moist sero-purulent exudate after about 48 hr., and smears of this showed large numbers of cocci and pus cells. The presence of the exudate was rather confusing for the first 2 days, but it had dried up completely by the 4th day as thin yellowish scabs, under some of which were reddened areas. On the 5th day there was little trouble in reading positive reactions, as these scabs were desquamating and the small papules of alastrim with their pin-point crusts could be seen quite easily, and did not appear to be influenced in any way by the coccal infection.

Table VI. *Immunity tests (5th day)*

Monkey	Immunized with	Tested with: Alastrim	Tested with: Variola	Days after immunization
I	Variola (Suki)	0	0	37
II	,,	≡	0	29
III	,,	0	0	25
IV	,,	≡	0	25–21
V	,,	≡ (3)	0	25
J	Alastrim (St Louis)	0	0	35
K	,,	±	±	52–28–23
L	,,	0	0	25
M	,,	0	0	25
N	,,	0	0	25

≡ Small abortive papules drying about 5th day and only visible as tiny crusts on 7th day.

± Papules smaller than normal.

SUMMARY OF RESULTS

Five monkeys immunized with variola virus showed complete protection to variola. Two of these were also negative to alastrim, while the other three showed strong immunity with an accelerated reaction.

Four out of the five monkeys immunized with alastrim virus showed complete immunity to both alastrim and variola, while the remaining one only exhibited partial protection to both. This result in the latter, K, was rather curious for, as mentioned in the text, this animal when previously inoculated with alastrim virus appeared to have had already a complete immunity resulting from the inoculation 24 days before. Nevertheless, when tested 52 days after the first inoculation, it showed only partial immunity to both alastrim and variola; the papules of each developing to approximately the same degree.

Whether experimental alastrim immunity is short-lived as a general rule, or whether its partial loss in the present case was associated with some idiosyncrasy of the particular monkey, it is impossible to say in default of further evidence.

DISCUSSION

Previously published results on cross-immunity in experimental animals between alastrim and variola are surprisingly few, and, as far as the authors are aware, the results tabulated in Part I represent all the available evidence.

It will be seen that Green (1916), working with the Australian alastrim and

a strain of variola isolated from a case in England, found the degree of cross-protection very slight; thus of five monkeys previously inoculated with alastrim, four reacted subsequently to variola, and of five monkeys variolized three reacted when tested with alastrim. Leake & Force (1921) found complete reciprocal immunity between the West Indies' alastrim and variola, but only one monkey was used for each test.

Analysis of our findings indicates that (*a*) immunity between variola and alastrim is not fully reciprocal and that (*b*) alastrim is the better immunizing agent. These facts are of considerable interest for the variola strain was the most virulent for monkeys yet isolated in the Sudan, while the alastrim was incapable of producing more than a local reaction. These results lend additional support to the previously enunciated hypothesis that the factor which confers virulence on the complex variola antigen is not associated with immunogenic capacity.

The relation between the strength of the immunizing reaction and subsequent immunity does not appear to be so close as in the previous series of experiments, for all animals inoculated with variola developed good reactions. However, in spite of these, only two exhibited complete immunity to alastrim, while monkey IV, which developed an acute general infection as well as a strong local reaction, still showed a slight susceptibility to this latter virus.

Nevertheless there is nothing in these findings which is against the conclusion reached in the previous sections, that solid immunity is dependent on a strong local primary reaction.

Our experiments also suggest that cross-immunity between alastrim and variola is due to a common antigen, which varies quantitatively, being apparently present in lesser amount in variola. Since it has been shown that either virus can protect completely against vaccinia, it is only reasonable to identify this basic (non-specific) immunity factor as vaccinia.

In conclusion, it must once more be emphasized that the results in the present section are those from a particular strain of alastrim in relation to variola and, while in accordance with a certain general hypothesis, it does not follow they will necessarily be paralleled in future work with different strains.

Bearing in mind the equivocal evidence from the Australian strain, there is certainly no justification at present for drawing the general conclusion that alastrim is a better immunizing agent than variola.

The present paper has been rigidly confined to an examination of the evidence from experimental animals, but the conclusions reached may be of some use in any future analysis of the clinical and epidemiological relationship of alastrim and variola.

Summary

Cross-protection tests between alastrim (St Louis) and variola indicate that immunity is not fully reciprocal, but that the degree of protection induced by alastrim is greater than that by variola.

The relationship is, however, of a quantitative character, and the results are in accordance with the previously suggested hypothesis that immunogenic capacity is directly related to the amount of basic antigen (= vaccinia) present, but is quite independent of the virulence of the particular virus.

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