IMMUNISATION WITH INACTIVE VACCINIA VIRUS.

By J. O. W. BLAND, M.B., *Freedom Research Fellow.*

(*From the Freedom Research Laboratory, the London Hospital, Whitechapel, E.* 1.)

(With 2 Charts.)

The recognition in recent years of post-vaccinal encephalitis has made it of urgent import to find some safer way of immunising against small-pox than the present one of scarification with active vaccinia virus. On the assumption that post-vaccinal encephalitis is due to vaccinia itself immunisation with a killed or inactive vaccine would be the ideal. That such a method should be possible, granted the discovery of the right technique, is suggested both by work on vaccinia virus itself and by the success obtained with inactive vaccines of some other filterable viruses.

A study of the literature leaves no doubt that some immunity from vaccinia can be obtained by the use of inactive vaccines. The earlier work on this subject, dealing almost exclusively with vaccines inactivated by various degrees of heat, is well summarised in the handbook of Lentz and Gins (1927) and will not be dealt with in detail here. The degree of immunity was invariably slight. Of the more recent work the most important papers are those of Gordon (1925), Kraus (1930), Iwanoff (1927) and Bussel and Mayzner (1930). Gordon (1925) used mainly heated lymph for his vaccines and he claims to get complete, though transient, protection with them if enough is used. In two experiments a phenolated vaccine was tried and found to be at least as good as the heated, but Gordon appears not to have pursued this subject. In these experiments the possibility is not excluded that the vaccines contained a small amount of living virus. Gordon did indeed show in one experiment that the degree of heat he used (56° C. for 30 minutes) was enough to make a lymph active before treatment at a dilution of 1/50,000, inactive at a dilution of 1/100, but it will be shown later that such treatment does not always kill the virus. Kraus (1930) describes experiments on monkeys, rabbits and guinea-pigs with carbolised and formolised vaccines. Guinea-pigs did not give satisfactory results, but in the other animals Kraus claims good protection. An analysis of his data shows that he worked on a basis that was only roughly quantitative and that, in fact, the degree of immunity he obtained was slight. Iwanoff's (1927) results with formolised vaccine disagree with those of Kraus. In his hands guinea-pigs were much more easily protected than rabbits and could be made solidly immune. Rabbits showed a weak skin immunity but formed viricidal antibody in their blood. Bussel and Mayzner (1930) did their

work directly in man. With a formolised vaccine they produced a slight immunity in a small series of infants. They also proved the presence of vaccinial antigen in their vaccine by comparing its effect on vaccinated and non-vaccinated persons when it was inoculated in the skin of the arm. The non-vaccinated gave little or no reaction, while the vaccinated showed an intense reddening of the skin and the formation of a pustule.

It is possible that the reason for the low degree of immunity hitherto obtained with killed vaccinia virus may be the attention given to heat as the inactivating agent. It is noteworthy that in the case of those viruses for which effective killed vaccines have been made heat has not been used. Examples are the carbolised vaccine of Semple (1911) for rabies; the formolised vaccine for foot and mouth disease (where heat-killed vaccine is ineffective); the carbol-glycerol vaccine used by Todd (1928) in fowl-plague; Laidlaw's and Dunkin's (1928) formolised vaccine in canine distemper; Hindle's (1928, 1929) carbolised and formolised vaccine in yellow fever. The success achieved in these diseases shows that we should not rest content with the present position of vaccinia and provides sufficient excuse for reopening the question.

Plan of experiments and technical methods.

The present series of experiments was designed to study three aspects of the problem: (1) To compare the relative merits of phenol, formalin and heat as inactivating agents. (2) To compare the relative merits of rabbits, guinea-pigs and monkeys as experimental animals. (3) To compare the relative merits of the subcutaneous, intracutaneous and intravenous routes of inoculation. A general plan was adopted throughout the work, but the details of this varied slightly in individual experiments. Some notes on this plan may be given under the following heads.

(i) *Preparation of the vaccines.* Homologous virus was always used. That is to say rabbit virus was used for rabbit experiments, guinea-pigs virus for guinea-pigs and monkey virus for monkeys. The diluent for making the suspensions, and for all other purposes, was $M/50$ phosphate buffer, pH 7·6. The suspensions were clarified before use by centrifugation or sedimentation.

(ii) *Test of vaccines for inactivity.* This point requires special emphasis. From a practical point of view a vaccine that depended for its efficacy on a small amount of living virus would not be very satisfactory, for the time during which it would remain potent would probably be of brief duration and laborious to estimate, whereas an effective killed vaccine should prove more stable. Since so much of the previous work on this subject is open to the suspicion that some active virus was left in the vaccines used particular attention has been paid to this point.

In the present work a vaccine has been considered killed only when it passed a rigorous test which was used unchanged in all the experiments but one. (1) The vaccine is inoculated in full strength and in adequate amount into a susceptible animal (0·2 c.c. intradermally in rabbits, and in the hind

pads of guinea-pigs). (2) On the failure of this to produce any sign of a lesion in 3–4 days, the inoculated area is cut out, ground and suspended in a little diluent and reinoculated in another animal. (3) The process is repeated with a third. This treatment should serve to reactivate, or to allow to grow, any virus left in the vaccine that is either weakened in virulence or too small in amount to form a lesion on a single inoculation. That it will do this was shown by experiment (*vide* Exp. 1). In the present state of our knowledge an absolute proof of the death of the virus cannot be had.

(iii) *Immunisation of animals.* The details varied in every experiment.

(iv) *Test of immunity.* This was always done on a quantitative basis and took the form of a titration of falling dilutions of an active virus upon the immunised animals and upon normal controls. Scarification was used for skin strains and intracutaneous inoculation for neurovaccine. Readings were taken daily until all lesions had healed. In watching these titrations on immunised animals three separate types of abnormal response were seen. (1) The animal reacts to weaker dilutions of the virus than the control. (2) The lesions form and heal more rapidly than normal. (3) The animal only reacts to stronger dilutions than the control. In describing the experiments, animals which respond in one of these three ways will be called (1) hypersusceptible, (2) allergic, (3) partially immune. The use of the term "immune" is reserved for those cases in which no reaction occurred at any dilution. Where the term "generalisation" is used it means that pocks were formed in parts other than those inoculated. In describing the titrations in tables, +++ indicates confluent lesions, ++ semi-confluent, + discrete lesions more than six, ± discrete lesions three to six in number, ∓ one or two lesions only. Days after inoculation are reckoned thus: if an animal is inoculated on Monday, Tuesday is day one.

DESCRIPTION OF EXPERIMENTS.

Exp. 1. *Immunisation of rabbits with heated and formolised neurovaccine.*

(i) *Preparation of vaccines.* The supernatant of a 2 per cent. suspension of neurovaccine brain after 30 minutes centrifugation was treated as follows:

Heated vaccine. 5 c.c. mixed with 5 c.c. diluent was heated in a water bath at 56° C. for 1 hour. Virus 1 per cent.

Formol vaccine. 5 c.c. mixed with 5 c.c. diluent containing 0·2 per cent. of formalin. Virus 1 per cent. formalin 0·1 per cent.

The titre of the untreated suspension was 10^{-6}. Both it and the two vaccines were bacteriologically sterile. The vaccines were stored in the refrigerator in rubber-capped vaccine bottles.

(ii) *Test of vaccines for inactivity.* This was done 6 days later. The first and second rabbits did not respond, but in the third passage a medium-sized lesion resulted from the heated vaccine and a small one from the formolised. The heated vaccine was heated again for 70 minutes at 56° C., and it therefore received in all 130 minutes' exposure to this temperature. When both vaccines

were 25 days old they were again tested. This test was negative and the vaccines were considered killed.

(iii) *Immunisation of animals.* Three normal rabbits were inoculated with each vaccine subcutaneously. Three doses, 0·1, 0·5 and 1·0 c.c. were given at intervals of 3 days.

(iv) *Tests of immunity.* Eight days after the last dose of vaccine the animals, with one normal control, were inoculated with falling decimal dilutions 10^{-1} to 10^{-7} of calf lymph on the left flank and of neurovaccine on the right.

(v) *Results.* With the heated vaccine two rabbits were normally susceptible to calf lymph, one was hypersusceptible. One rabbit was normally susceptible to neurovaccine, two were hypersusceptible. With the formol vaccine two rabbits were immune to calf lymph and partially immune to neurovaccine. One rabbit was partially immune to calf lymph and hypersusceptible to neurovaccine. Generalisation occurred in three out of the six rabbits and bore no relation to the degree of cutaneous susceptibility or immunity.

Exp. 2. *Immunisation of rabbits with raw, heated, phenolised and formolised skin virus.*

(i) *Preparation of vaccines.* A 1 per cent. suspension made from dried fifth day rabbit pustules was put in the refrigerator for 24 hours. The supernatant was then centrifuged for 1 hour at medium speed and the supernatant removed. The titre of this was between 10^{-3} and 10^{-4}. It was used as follows:

A. *Raw virus.* 8 c.c. + 8 c.c. diluent.

B. *Heated virus.* 8 c.c. + 8 c.c. diluent heated at 56° C. for 3 hours.

C. *Phenolised virus.* 8 c.c. + 8 c.c. diluent containing 2 per cent. of phenol.

D. *Formolised virus.* 8 c.c. + 8 c.c. diluent containing 0·2 per cent. of formalin.

The vaccines therefore contained 0·5 per cent. virus in all cases and 1 per cent. of phenol and 0·1 per cent. of formalin in the case of C and D. The vaccines were kept in the refrigerator.

(ii) *Test of vaccines for inactivity.* Vaccines B, C and D all passed the test and could be considered killed. Vaccine A was of course not tested.

(iii) *Immunisation of animals.* Three normal rabbits were used for each vaccine. Doses of 0·5 c.c., 1·0 c.c. and 2·0 c.c. were given subcutaneously at intervals of 3 days. Inoculations with vaccine A were begun as soon as it was made, but with B, C and D not until the nineteenth day, to allow for the inactivity test.

(iv) *Test of immunity.* This was done 6 days after the last dose of vaccine with dilutions from 10^{-1} to 10^{-5} of a suspension of the same dried crusts that were used to make the vaccines. One normal control was used for the series treated with vaccine A and one for the series treated with B, C and D.

(v) *Results.* With the raw vaccine A, one rabbit was immune, while two were partially immune. With the heated vaccine B, one rabbit was partially immune, while two were hypersusceptible. With the phenol vaccine C, one

rabbit was immune, one partially immune, one hypersusceptible. With the formol vaccine D, one rabbit was hypersusceptible, one normally susceptible, one partially immune.

Exp. 3. *Comparison of intracutaneous and subcutaneous routes. Comparison of phenolised and formolised vaccines and of the efficacy of mixing sago with the vaccine before inoculation.*

(i) *Preparation of vaccines.* A 2 per cent. centrifuged suspension of fresh rabbit pulp, the titre of which was 10^{-4}, was used to make the following vaccines:

A. *Formolised virus.* 15 c.c. + 15 c.c. diluent containing 0·4 per cent. of formalin = virus 1 per cent., formalin 0·2 per cent.

B. *Phenolised virus.* 25 c.c. + 25 c.c. diluent containing phenol 4 per cent. = virus 1 per cent., phenol 2 per cent.

C. *Phenolised virus with sago.* Eight days after making B, 12·5 c.c. of it were mixed with 12·5 c.c. of a thick sterile emulsion of sago = virus 0·5 per cent., phenol 1 per cent.

(ii) *Test of vaccines for inactivity.* A and B were tested 8 days after preparation and were negative. There was obviously no need to test C.

(iii) *Immunisation of animals.* Three rabbits received three doses of 1, 2 and 2 c.c. of vaccine A subcutaneously at weekly intervals. All survived. Three rabbits received three doses of 1, 2 and 4 c.c. of vaccine B subcutaneously at weekly intervals. One died. Three rabbits received three doses of 0·5, 1 and 2 c.c. of vaccine B intracutaneously at weekly intervals. One died. Three rabbits received three doses of 1, 2 and 4 c.c. of vaccine C subcutaneously at weekly intervals. All survived.

(iv) *Test of immunity.* This was performed 14 days after the last dose of vaccine with an active virus in dilutions from 10^{-2} to 10^{-7}. Two normal controls were included in the series. Areas of skin away from the site of the immunising doses were used in all cases.

(v) *Results.* With the formolised vaccine one rabbit was hypersusceptible, one normally susceptible and one partially immune and allergic. With the phenol vaccine given subcutaneously, both rabbits were hypersusceptible and allergic. With this vaccine given intracutaneously both rabbits were hypersusceptible. With this vaccine mixed with sago all the rabbits were allergic, one was normally susceptible, two were hypersusceptible.

The results of these three rabbit experiments were very disappointing. They gave no reliable indication that anything but the weakest immunity was produced. In every experiment as many animals showed titres higher than controls as showed titres lower than controls. Such irregularities may be due to variations in the natural susceptibility of different rabbits. The one completely immune animal in Exp. 2 (phenol series) and the allergic animals in Exp. 3 are the only real indication of any immunity at all. The use of rabbits was discontinued and attention directed to guinea-pigs.

Exp. 4. *Immunisation of guinea-pigs with formolised virus.*

(i) *Preparation of vaccine.* Formalin 0·1 per cent. was added to a 10 per cent. suspension of guinea-pig pads. The suspension before treatment had a titre of 10^{-4}. The vaccine was kept in the refrigerator.

(ii) *Test of inactivity.* This was done 11 days later and was negative.

(iii) *Immunisation of animals.* Six guinea-pigs were given three doses of 1 c.c. subcutaneously at intervals of 4 days.

(iv) *Test for immunity.* Ten days after the last dose of vaccine the immunised guinea-pigs and two normal controls were inoculated with falling doses of a guinea-pig virus. This proved to be inactive. A doubtful papule appeared on one control guinea-pig on the third day which had disappeared on the fourth. The other control and the immunised animals were negative.

Seventeen days after the last dose of vaccine the guinea-pigs were reinoculated with a fresh virus at 10^{-3} to 10^{-6} and one more normal control was added to the series. Inoculations were done on the same areas of skin as were used before.

(v) *Results.* None of the immunised animals showed any lesion at any time, but one of them died of an intercurrent infection on the third day. The control animals all showed typical lesions. One gave three to six lesions at 10^{-4} and a large number of discrete lesions at 10^{-3}. Another gave one or two lesions at 10^{-4} and three to six lesions at 10^{-3}. The third gave a large number of discrete lesions at 10^{-3} but none at 10^{-4}. The immunised animals were therefore protected against some one to ten minimal infecting doses.

(vi) *Repetition of test.* The first test was a mild one and, as all the treated guinea-pigs resisted it, all the animals, controls as well, were reinoculated with stronger doses of virus at 10^{-1} and 10^{-2}. It was now 25 days since the last dose of vaccine. Inoculations were done on areas of skin not previously used. A titration of the virus was also made on one entirely normal animal.

(vii) *Results.* The readings of the titrations are given in Table I. Although all the immunised animals reacted to some extent in the 10^{-2} dilution, the lesions were much fewer than in the case of the control. They also differed greatly in their rate of formation and healing. This is shown in a schematic form in Chart 1. In drawing up this chart the following conventional stages in the progress of the lesions have been adopted:

(*a*) The formation of an erythematous flush over the whole scarified area.

(*b*) Papules.

(*c*) Vesicles with almost clear fluid content.

(*d*) Pustles with opaque content.

(*e*) Formation of completely dry yellow crusts by drying of the pustules.

(*f*) Formation of a shallow ulcer covered with a haemorrhagic scab, presumably by the animal tearing off the crust.

(*g*) Healing of this ulcer and covering with new pink epithelium.

Chart 1 records the state of the lesions at the strongest dilution 1/10. Days

on which the guinea-pigs were seen are marked ●, days on which they were not seen ⊖.

Summarising the results of this experiment we find that 17 days after the last dose of vaccine, all the immunised animals were immune to between one

Table I. Exp. 4.

Days after inoculation	Control titration 363 10^{-1}	363 10^{-2}	363 10^{-3}	363 10^{-4}	Previous controls 349 10^{-1}	349 10^{-2}	350 10^{-1}	350 10^{-2}	353 10^{-1}	353 10^{-2}
1	–	–	–	–	–	–	–	–	–	–
2	+ + +	+ +	∓	–	–	–	+ + +	+	+ + +	+
3	+ + +	+ +	+	–	–	–	–	–	–	–
4	+ + +	+ +	+	–	–	–	–	–	–	–

Days after inoculation	Immunised animals 331 10^{-1}	331 10^{-2}	332 10^{-1}	332 10^{-2}	333 10^{-1}	333 10^{-2}	334 10^{-1}	334 10^{-2}	335 10^{-1}	335 10^{-2}
1	–	–	–	–	–	–	–	–	–	–
2	+ + +	+	+ + +	∓	+ + +	∓	+ +	∓	+ + +	∓
3	+ + +	+	–	–	–	–	+ +	+	–	–
4	+ + +	+	–	–	–	–	+ +	+	–	–

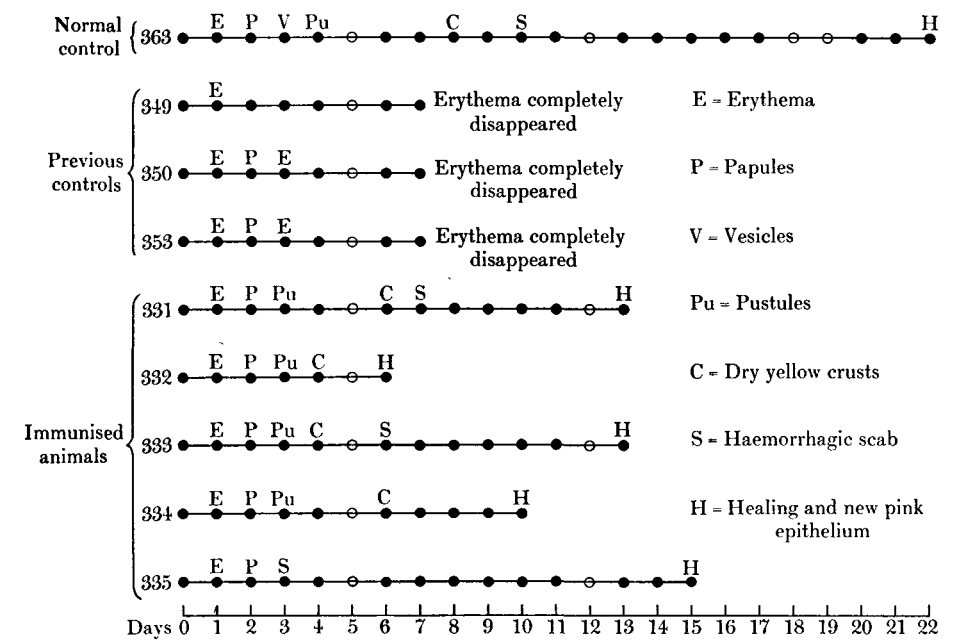

Chart 1.

and ten minimal infecting doses. Twenty-five days after the last dose of vaccine they were all partially immune to doses of ten and a hundred minimal infecting doses. This immunity is shown by the decrease in the number and size of the lesions and by the greater speed with which they formed and healed.

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The degree of immunity yielded by formolised vaccine lies between that derived from scarification with a small dose of living virus (349, 350 and 353) and the natural state of the normal guinea-pig (363). Scarification with a small dose of living virus did not produce a solid immunity (350 and 351). This experiment and the following one are both open to the objection that the immunity shown in the second test may not have been exclusively the result of the killed vaccine but may have been reinforced by the scarification with live virus that constituted the first test. This may be so, but it seems unlikely that an amount of living virus unable to cause even a single vesicle should produce an immunity when merely rubbed into the skin.

These results were encouraging and a second, more elaborate experiment was carried out.

Exp. 5. *Immunisation of guinea-pigs with formolised, phenolised and heated vaccines.*

(i) *Preparation of vaccines.* A centrifuged 10 per cent. suspension of guinea-pig pads was divided into three parts of 10 c.c. and treated thus:

A. *Formol vaccine.* 0·11 c.c. of 10 per cent. formalin added.

B. *Phenol vaccine.* 0·11 c.c. of liquefied pure phenol added.

C. *Heated vaccine.* Heated in a water bath at 56° C. for 2 hours.

The vaccines were kept in the refrigerator. The titre of the suspension before treatment was 10^{-3}.

(ii) *Test of inactivity.* This was done a week after making the vaccines and was negative.

(iii) *Immunisation of animals.* Batches of three guinea-pigs were inoculated with each vaccine receiving three doses of 1 c.c. at 4-day intervals. The animals were small and in poor condition before inoculation. Two died during inoculation from the formol series and one each from the phenol and heated series.

(iv) *Test of immunity.* This was done a week after the last dose of vaccine together with two normal controls. Dilutions 1/500, 10^{-3}, 1/5000, 10^{-4}. The titration on the normal controls was unsatisfactory: one of them gave numerous discrete lesions at 1/500 and one or two lesions at 10^{-3}, 1/5000 and 10^{-4}. The other gave one or two lesions only at 1/500. The dose received by the immunised animals cannot be given with accuracy therefore; it probably lay between one and ten minimal infecting doses. None of the immunised animals showed any lesions except 376 of the formol series, which developed one doubtful papule at 1/500 and one at 1/5000, and 378 of the heated series which developed one doubtful papule at 1/500. Since all the animals were immune to this mild test, it was repeated and 14 days after the last dose of vaccine they were reinoculated with a fresh and stronger virus at 10^{-2} and 10^{-3}. Three normal controls were similarly inoculated and also at 10^{-4}.

(v) *Results.* These are shown in Table II which refers only to the second test. The titre of the test virus was between 10^{-3} and 10^{-4}, and the immunised

animals were all immune to one minimal infecting dose and partially immune to ten minimal infecting doses. Only one guinea-pig, C 376, was completely immune to the test. This was one which produced two doubtful papules on the first test. The accelerated nature of the lesions on the immunised animals was again noticed and is shown in Chart 2. This was drawn up in the same way as Chart 1. Table II and Chart 2 (p. 64) contain some information relative to the next experiment also. This experiment gave no indication that any one of the ways of killing the virus was better than another.

Table II. Exps. 5 and 6.

Immunised animals. Exp. 5

Days after inoculation	Formalin				Heated		Phenol			
	375		376*		378*		379		381	
	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}
1	−	−	−	−	−	−	−	−	−	−
2	+	−	−	−	+	−	−	−	+	−
3	±	−	−	−	+	−	±	−	∓	−
4	±	−	−	−	±	−	±	−	−	−
5	−	−	−	−	−	−	±	−	−	−

Normal controls to both experiments

Days after inoculation	402			403			404		
	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
1	−	−	−	−	−	−	−	−	−
2	+ +	∓	−	+ +	∓	−	+ +	−	−
3	+ + +	±	∓	+ + +	±	−	+ + +	∓	∓
4	+ + +	±	∓	+ + +	±	−	+ + +	∓	∓
5	+ + +	±	∓	+ + +	±	−	+ + +	∓	∓

Immunised animals. Exp. 6

Days after inoculation	392			393			394		
	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
1	−	−	−	−	−	−	−	−	−
2	+ +	?	∓	+ +	∓	−	+ +	−	−
3	+ +	±	∓	+ +	±	−	+ + +	−	−
4	+ +	∓	−	+ +	∓	−	+ + +	−	−
5	+ +	∓	−	+ +	∓	−	+ + +	−	−

* These were the animals which gave the doubtful papules in response to the first test.

Exp. 6. *Immunisation of guinea-pigs by subcutaneous, intracutaneous and intracardiac routes with formolised virus.*

(i) *Preparation of vaccine.* To a 10 per cent. centrifuged suspension of guinea-pig pads 0·1 per cent. of formalin was added. The titre before treatment was 10^{-3}.

(ii) *Test of inactivity.* Performed 1 week later was negative.

(iii) *Immunisation of animals.* Three doses were given at 3-day intervals, 392 receiving 1 c.c. doses subcutaneously, 393 0·5 c.c. doses intracutaneously and 394 1 c.c. doses intracardially.

(iv) *Test of immunity.* Thirteen days later the animals and three normal controls were inoculated with living virus.

(v) *Results.* The results of this experiment are shown in Table II and Chart 2. The test was done at the same time as that of Exp. 5 and with the same test virus, and the control animals 402, 403 and 404 were the same for both experiments. Only slight immunity was produced in this experiment. The lesions on the immunised animals were less than on the controls and their formation and healing were slightly more rapid. There was no indication that one route of inoculation was better than another.

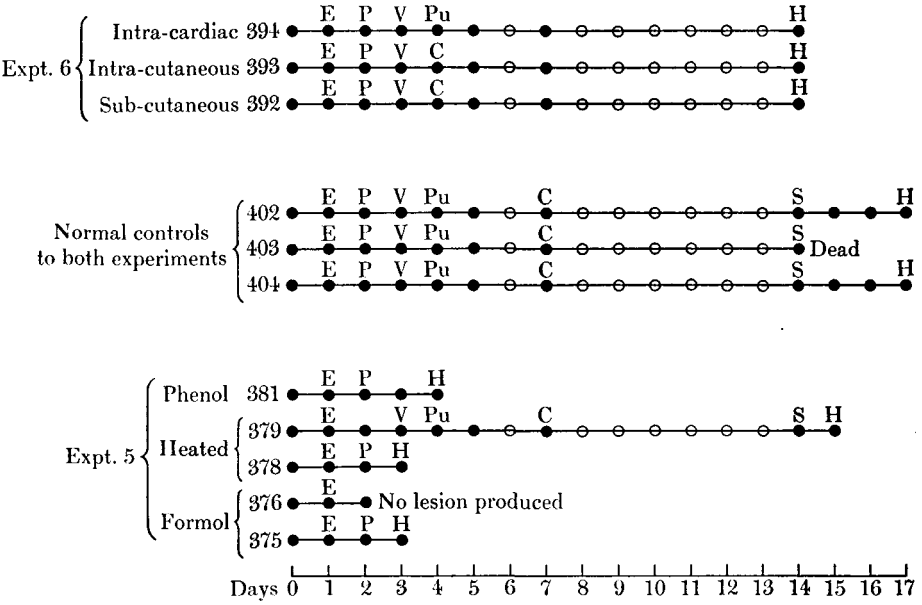

Chart 2.

The guinea-pig experiments were more encouraging than those done with rabbits but their success was not striking. An experiment was accordingly done with monkeys to see if they would prove any easier to immunise.

Exp. 7. *Immunisation of* Rhesus *monkeys with formolised virus.*

(i) *Preparation of virus.* Lesions were produced in a monkey with calf lymph and these were used to make a 10 per cent. centrifuged suspension to which 0·1 per cent. of formalin was added. Before treatment the titre of the suspension was 10^{-4} for the rabbit but only 10^{-2} for the monkey. This vaccine was not tested for inactivity after treatment: it was felt that previous experiments had sufficiently shown the destructive effect of formalin on the virus and the expense of monkeys prohibited their use for the test. The vaccine was not used till it had been formolised 29 days.

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(ii) *Immunisation of animals.* Three monkeys were given subcutaneous doses of 0·5 c.c., 0·7 c.c. and 0·75 c.c. at 6-day intervals. At the time of the first dose 0·2 c.c. of the vaccine was also given intracutaneously.

(iii) *Tests of immunity.* This was done 4 days after the last dose of vaccine, with dilutions from 10^{-1} to 10^{-6} of calf lymph. A control monkey was inoculated at the same time.

(iv) *Results.* One monkey was partially immune, the other two gave the same titre as the control but the lesions were larger. There was no difference in the time of appearance, rate of development or time of healing of the lesions in any of the animals and there was no indication that any degree of immunity had been produced. Admittedly the vaccine used was a weak one, but the sole purpose of this experiment was to see if monkeys were much better than guinea-pigs for this type of work and for such a purpose it was thought to be adequate.

DISCUSSION.

This work was done in the hope that it would lead to some method that could be used for the protection of man from small-pox. It must be recognised that in this it has failed; the experiments agree only too well with the experience of previous workers that immunity is very hard to produce with killed vaccinia virus. Why this should be when other viruses yield good killed vaccines it is difficult to see. It may be that the amount of antigenic material in such suspensions of vaccinia as we can make is too small to arouse a response when the virus is prevented from multiplying. Yet there seems no good reason for thinking so.

It seems more likely that delicacy of antigenic structure is the true explanation. Probably the means taken to kill the virus also ruin its antigenic qualities and it may be we should seek some less drastic method than those yet used. In any case the difficulty of immunising with dead material is not confined to the filterable viruses and is paralleled among the visible bacteria by *Brucella abortus* and by members of the genus *Pasteurella*. It is therefore possible that the solution of the difficulty may come as much from the study of bacterial immunity as from direct investigation of the viruses themselves.

At the same time there is no reason for abandoning the study of vaccinial immunity. There are many ways of killing the virus which may still be tried and there is the method of combined virus and antiserum inoculation which has already shown promise at the hands of Rhoads (1931). Furthermore, failures as they are in the main, the experiments reported here have yet brought to light two interesting points. In the first place they suggest that guinea-pigs are better than rabbits for this kind of work and direct attention to their use for future investigations. Secondly, it is of great interest that even a small degree of immunity can be got in guinea-pigs by the use of a killed vaccine. This is important because it confirms the conclusion that is to be drawn from the flocculation test of Burgess, Craigie and Tulloch (1929) and from the complement-fixation work of Gordon (1925) and of Bedson and

Bland (1929) that at bottom no distinction can be made between the immunity reactions of this virus and those of visible bacteria.

An even more practical point is raised by this successful immunisation of guinea-pigs. It might be that a killed vaccinial vaccine would give sufficient immunity to stop the generalisation of live virus used afterwards to convert the partial immunity into a solid one, and by this means we might remove the risk of post-vaccinal encephalitis. In the case of foot-and-mouth disease the formolised vaccine prevents the generalisation of the virus although it does not suppress local lesions at the site of inoculation, and further the administration of living virus converts the weak immunity given by the formolised virus into a solid one (Bedson, Maitland and Burbury, 1927). Such a method of immunisation has been shown to be of practical value in the case of distemper (Laidlaw and Dunkin, 1928), and if we could achieve no more than this with vaccinia it would be a definite advance for we should have a method of protection against small-pox little more complicated than the present one and devoid of its risks.

SUMMARY.

Experiments were carried out on rabbits, guinea-pigs and monkeys to test the immunity produced by vaccinia virus killed with heat, phenol or formalin. Before use, the vaccines were rigorously tested for their inactivity by a method of inoculation and triple passage in series. The test of immunity was quantitative.

The rabbit experiments gave equivocal results but indicated that a slight immunity was produced in some cases. In guinea-pigs more success was obtained: they could be protected against one to ten minimal infecting doses and showed a partial immunity to stronger tests. Monkeys were only tried in one experiment in which a weak vaccine was used. This did not protect them.

The superiority of guinea-pigs for this kind of work is discussed as also the bearing of the results obtained with them on the nature of immunity to vaccinia virus.

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