SOME EXPERIMENTS ON IMMUNITY AGAINST VACCINIA IN ANIMALS.

BY ALAN B. GREEN, M.A., M.D., B.C. CANTAB. Bacteriologist-in-charge, Calf Vaccine Department, Lister Institute of Preventive Medicine.

HITHERTO all efforts to set up immunity against vaccinia in animals have been restricted to the vaccination of animals in the customary manner, or to the injection of vaccine lymph into them.

In the present series of experiments an attempt was made to derive from calf vaccine an unorganised body capable of causing specific immunity against vaccinia in animals.

The method of production of such an immunisator has been :---

- 1. Collection of vaccine pulp from a calf.
- 2. Trituration of this pulp with normal saline solution.
- 3. Heating this mixture at 60° C. for 1 hour, in some instances as noted below.
- 4. Storage of this mixture.
- 5. Filtration of this mixture.

In the first experiment guinea-pigs were used. Into these animals filtrates of autolysed lymph were injected subcutaneously as detailed in the following Table I. Six varieties of filtrates were prepared from the pulp of one calf as follows:—

(a) Unheated portion of mixture.

1. Passed through a Berkefeld filter.

2. " " Chamberland filter.

3. " " Martin's gelatin filter.

(b) Heated portion of mixture.

- 4. Passed through a Berkefeld filter.
 - " " Chamberland filter.
- 6. " " Martin's gelatin filter.

5.

522 On Immunity against Vaccinia in Animals

Each of these six filtrates was injected subcutaneously at intervals into a series of eight pigs. Subsequently these pigs were vaccinated in the usual manner with one strain of lymph, as were eight control pigs which had received no injection; and the result of such vaccination was noted daily for a week.

The following Table I shows the results obtained.

TABLE I. (8 guinea-pigs in each cage.)

+ = Specific vesiculation. ? = Specific reaction not amounting to vesiculation. - = No specific reaction.

No. of cage			Injections		D-4	Describe of
	Material injected	April 4	April 22	April 29	vaccination	vaccination
23	Nil (control pigs)	nil	nil	nil	May 6	8+
25	Berkefeld V filtrate of heated autolysed lymph	1 c.c.	1 c.c.	4 c.c.	**	7 1 ?
26	Chamberland filtrate of heated autolysed lymph	**	**	,,	,,	5 - 3 ?
27	Martin's gelatin filtrate of heated autolysed lymph	"	••	••	**	6 + 2 -
28	Berkefeld V filtrate of unheated autolysed lymph	"	"	1,	,,	6 - 2 +
12	Chamberland filtrate of unheated autolysed lymph	"	,,	,,	53	6 - 2 + 2
22	Martin's gelatin filtrate of un- heated autolysed lymph	,,	,,	,,	,,	6 + 2

The results obtained with the control pigs differed markedly from those which had received subcutaneous injections of Berkefeld and Chamberland filtrates in that while the controls all developed good vesicles, the others consistently failed to develop vesicles, and only occasionally showed some modified specific reaction. Again, the pigs injected with Martin's gelatin filtrates as a rule showed vesiculation though possibly some immunisation had been set up by these filtrates. The immunisation set up by heated autolysed filtrates seemed on the whole to be better than that set up by unheated ones.

Experiment 2 was made on the same lines as the foregoing with the results detailed in Table II.

The results obtained by this second experiment bring out, if anything, more clearly, the points shown by experiment 1.

Experiment 3. A calf was injected subcutaneously with a Berkefeld filtrate of a heated autolysed vaccine. On July 10th, 10 c.c. were

A. B. GREEN

injected; on July 23rd, 10 c.c.; and on July 30th, 15 c.c. On August 7th the calf was vaccinated. No vesiculation resulted. Good vesiculation occurred on a control calf vaccinated with the same lymph.

TABLE II. (8 guinea-pigs in each cage.)

+ = Specific vesiculation. ?= Specific reaction not amounting to vesiculation. - = No specific reaction.

No. of cage		Injections			D. (Described
	Material injected	May 13	June 4	June 11	vaccination	vaccination
23	Nil (control pigs)	nil	nil	nil	July 8	8+
25	Berkefeld V filtrate of heated autolysed lymph	2 c.c.	2 c.c.	2 c.c.	"	7 - 1 ?
26	Chamberland filtrate of heated autolysed lymph	"	,,	,,	**	6 - 2 ?
27	Martin's gelatin filtrate of heated autolysed lymph	۰,	,,	13	,,	5+ 1? 2-
28	Berkefeld V filtrate of unheated autolysed lymph	**	**	,,	**	7 – 1 ?
12	Chamberland filtrate of unheated autolysed lymph	,,	"	,,	,,	7 – 1 +
22	Martin's gelatin filtrate of un- heated autolysed lymph	*1	"	"	**	4 + 2 ? 2 -

Experiment 4. A monkey was injected with a Berkefeld filtrate of heated autolysed vaccine. Subcutaneous injections were given:— November 25th, 10 c.c.; November 27th, 10 c.c.; November 30th, 10 c.c. On December 6th the monkey was vaccinated on a shaved area over the scapulae in linear incisions. On December 13th, the eighth day after vaccination when vesiculation should be at its height, two or three small round abortive vesicles, each about the size of a split pea, were visible at intervals along the lines of incision. On December 10th these vesicles had dried up. Proliferation of tissue forming a heaped up crust along each incision followed, but no further vesiculation.

Experiment 5. A monkey was injected subcutaneously with a Berkefeld filtrate of autolysed heated vaccine. On Jan. 20th, 15 c.c. were injected; on Jan. 23rd, 30 c.c.; on Jan. 28th, 15 c.c.; and on Jan. 31st, 30 c.c. On Feb. 13th the monkey was vaccinated. On Feb. 20th a few small abortive vesicles were visible as in experiment 4, along the lines of incision, but these were even less marked than those in experiment 4.

523

524 On Immunity against Vaccinia in Animals

The evidence afforded by this limited number of experiments points to the facts :---

1. That it is possible to produce from calf vaccine a specific unorganised immunisator.

2. That this immunisator is of such a nature as to be capable of filtration through a Berkefeld V or a Chamberland filter, and possibly in some small degree through a Martin's gelatin filter.

The fact that such an immunisator can be obtained, naturally suggests that it might be capable of setting up immunity against smallpox. It has not vet been found possible, however, owing to the scarcity of small-pox material, to carry out any work on these lines. Experiments will be made at the first opportunity. It is evident that if an immunisator against small-pox could be prepared from calf-vaccine its use would lead, in all probability, to an appreciable gain in time in dealing with small-pox contacts. Usually, of course, such contacts are vaccinated, and when the vaccination is successful an immunisator is manufactured in the vaccinated area of the patient, which immunisator is absorbed and gives rise to protection. If, however, the immunisator could be given directly to the contact, the time necessary for him to manufacture his own immunisator would be saved, and such gain in time might be important. Furthermore, the injection of the immunisator could, if desired, be used in addition to, and not instead of, vaccination in these cases. The immunisator might also possibly be injected advantageously during the course of small-pox. Until investigation on these lines is possible, other points in connection with the behaviour of the immunisator are being worked at.