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THE PRODUCTION OF A BASIC IMMUNITY AGAINST PULPY KIDNEY DISEASE

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Although the factors predisposing to the development of pulpy kidney disease are insufficiently known, the disease can be prevented by reducing the quantity or quality of food eaten by sheep. But such measures are inconsistent with optimal production and reproduction with the result that concerted efforts were made at developing effective vaccines. Bennetts (1932, 1936) was probably the first to introduce vaccination with *Cl. welchii*, Type D, formolized culture. It is known, however, that breakdowns have occurred in flocks injected with this type of vaccine, possibly because the level of circulating epsilon antitoxin produced was not sufficiently high or durable. Jansen (1960) showed that an epsilon antitoxin level of at least 0·15 IU per ml of serum is required to protect a sheep against pulpy kidney disease.

In an extensive trial Thomson & Batty (1953) compared the antigenic efficiency of formolized whole culture, alum-precipitated toxoid, formolized whole culture plus one per cent potash-alum and trypsin-activated alum-precipitated toxoid. They concluded that the immunity produced by two doses of trypsin-activated alum-precipitated toxoid given at an interval of not less than four weeks is greater both in degree and duration than that resulting from the same procedure but using anaculture, anaculture plus alum, or alum-precipitated toxoid. Although a higher level of immunity resulted from two 5 ml doses of activated alum-precipitated toxoid, a very satisfactory immunity was obtained with two doses of 2 ml. A high level of circulating antitoxin was still present 33 weeks after immunizing a naturally immune flock.

Hepple, Chodnik & Price (1959) described the preparation of a purified toxoid, aluminium-treated. They showed that a satisfactory immunity can be obtained in a flock of ewes by the use of this vaccine.

Although the findings summarized above prove unequivocally that a protective level of epsilon antitoxin can be produced by the injection of epsilon toxoid treated in different ways, no information could be found about the response of sheep to graded doses of antigen and to the spacing of the primary and secondary stimuli. For the production of a pulpy kidney disease vaccine it is essential to know the quantity of antigen required for optimal response; and for the benefit of farmers it is equally necessary to know the permissible variation in the interval between the primary and secondary injections. In an attempt to supply this information a series of experiments was undertaken.

Materials and Methods

Production of Antigen

Meat extract was prepared by boiling 0·45 Kg minced horse flesh in 1 litre distilled water for $1\frac{1}{2}$ hours; to every 10 litres of extract 100 gm peptone, 9·0 gm $Na_2HPO_4.12H_2O$ and 14·2 gm KH_2PO_4 were added.

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This medium was dispensed in 35 litre quantities in 40 litre flasks containing the boiled meat particle residue (about one-fifth by volume) and then sterilized at 120° C for three hours. After cooling, the flasks were transferred to an incubator at 37° C for the medium to attain the desired temperature.

Before the medium was inoculated, its pH was adjusted to 7·6 with sterile N NaOH solution, after which sterile 40 per cent commercial dextrin suspension was added to give a final concentration of 1·0 per cent.

Each flask of medium was inoculated with about 200 ml of an actively growing culture of *Cl. welchii*, Type D, and incubated at 37° C. After three hours growth the pH of the culture was adjusted to 8·5. After a total period of incubation of 21 hours, the liquid portion of the culture was siphoned off, filtered through a Seitz clarifying pad and its Lf value determined. Subsequently the pH of the toxic liquid was adjusted to 7·0 with N NaOH solution and trypsin (Merck) added to give a final concentration of 0·005 per cent. This mixture was put in the incubator for two hours and then formalin was added to a final concentration of 0·8 per cent. At pH 7 and at 37° C this concentration of formalin detoxicated culture fluids containing about 250 Lf toxin per ml in about six days. Higher concentrations of formalin caused quicker detoxication but greater destruction of the antigen whereas lower concentrations required longer incubation which also resulted in greater destruction of antigen. The 0·8 per cent formalin allowed for a slight excess which served as a preservative. Toxoiding was effected at pH 7·0 and 37° C and was regarded as complete when 0·2 ml of the liquid injected intravenously failed to kill mice weighing 18 to 20 gm.

The final product was precipitated with potassium alum by adding enough of a 10 per cent solution to give a final concentration of 1·5 per cent. The pH was adjusted to 6·0 and the alum-precipitated antigen tested for sterility and safety. This was called alum-precipitated toxoid (APT).

Experimental Animals

To obviate the difficulty, and possible source of inaccuracy, of having to relate results obtained in laboratory animals to sheep, it was decided to conduct all immuinty experiments in sheep. Experimental sheep were consequently bought from farmers who could guarantee that the particular animals had never been vaccinated against enterotoxaemia. They were further tested for the presence of circulating epsilon antitoxin by injecting a mixture of 0·5 ml of the serum from each sheep and 3 MLD toxin intravenously into mice. Control tests were done with known negative serum. Only sheep whose serum contained no antibody were used.

Serological Tests

A laboratory standard antitoxin of sufficient avidity was obtained by immunizing a horse with trypsin-activated epsilon toxoid. It was given a primary and a secondary dose (each of 1,000 Lf) of alum-precipitated toxoid at an interval of one month. It was rested for nine months and then given an intensive course of increasing quantities of toxoid (1,000, 2,000, 4,000 and 8,000 Lf at weekly intervals). Four days after the last injection it was bled and the serum refined and concentrated by the method of Pope (1939). The refined end-product, standardized in terms of international units (IU), contained 625 IU epsilon antitoxin per ml.

For determining the antitoxin content of the serum of experimental subjects, toxin neutralization tests were done in mice weighing 18 to 20 gm. In order to detect very low levels of antitoxin, a test dose of 3 MLD of dried, trypsin-activated,

ammonium-sulphate-precipitated toxin was used. At first the titrations were done with wide increments of serum and then repeated with smaller increments to arrive at a figure as close as possible to the neutral point. The 3 MLD of toxin corresponded to L + /50 doses.

To answer the possible objection that the L+ /50 dose was too low to produce reliable results, comparative tests were done on the same sera by the use of both the L+ /50 and the L+ /10 doses of toxin. No significant difference could be detected.

In accordance with the practice of Hepple *et al.* (1959) and Thomson *et al.* (1953) the quantity of antigen was expressed in Lf. For determining this value the flocculation test as described by Jansen (1961) for the beta antigenic fraction of *Cl. welchii*, Type B, was applied *mutatis mutandis* to the epsilon antigen. One Lf was equivalent to 1 IU of antitoxin.

To ensure that the Lf as determined was a reliable measure of the antigen content of a solution, Lf and total antitoxin combining power units (Mason, 1935) were determined in a series of 18 fresh toxoid solutions. The results obtained are summarized in Table 1.

TABLE 1.—*Lf and total antitoxin combining power units in a series of* 18 *fresh toxoid solutions*

Toxoid No.	Lf per ml	Total combining power units per ml
1	100	54
2	125	90
3	75	54
4	31	36
5	112	54
6	112	54
7	100	54
8	87	36
9	87	36
10	112	54
11	75	36
12	75	36
13	125	72
14	162	126
15	200	162
16	212	162
17	187	144
18	200	144

The correlation coefficient for these two sets of figures was calculated and found to be 0·91, proving tnat the flocculation test is an acceptable substitute for the total combining power test. The flocculation test is more easily and quickly performed and requires no experimental animals.

RESULTS

The determination of the optimal dose of antigen

The sheep of one group received 5 Lf of toxoid and those of another 45 Lf, in 2·5 ml APT. Samples of blood were taken at weekly intervals for five consecutive weeks, starting one week after the injection. The antibody titre of the serum obtained from each sample was determined and the results are recorded in Table 2.

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TABLE 2.—*The antibody titre (in units/ml) after a single injection of antigen*

5 Lf Group

Sampling Times (Weeks)				
1	2	3	4	5
0·0	0·10	0·20	0·0	0·0
0·0	0·24	0·44	0·24	0·20
0·0	2·00	2·90	1·30	1·30
0·20	0·57	0·30	2·90	0·24
0·20	1·30	0·80	0·80	0·67
0·20	1·00	0·80	0·80	0·57
0·0	0·30	0·30	0·10	0·10
0·10	2·00	0·80	0·80	0·57
0·10	0·67	2·90	1·30	1·30
0·20	0·30	0·80	0·50	0·50
0·20	5·00	6·70	2·20	2·20
0·07	0·27	0·20	0·10	0·10
0·0	0·10	0·20	0·10	0·0
0·0	0·67	0·20	0·20	0·20
0·10	0·30	0·30	0·20	0·10
0·0	0·25	0·20	0·10	0·20
0·0	0·20	0·20	0·20	0·10
0·0	2·00	2·00	0·57	0·57

45 Lf Group

Sampling Times (Weeks)				
1	2	3	4	5
0·0	2·00	2·90	2·00	1·30
0·0	0·29	1·30	2·50	2·00
0·0	0·29	2·00	2·00	1·00
0·10	5·00	10·00	6·70	4·00
0·0	0·40	2·00	2·00	2·00
0·0	0·29	0·40	0·40	0·33
0·0	0·29	2·00	1·30	1·00
0·0	0·67	2·00	3·30	2·20
0·0	5·00	10·00	6·70	5·00
0·0	0·50	1·00	0·80	0·80
0·0	1·00	2·20	2·50	2·90
0·0	4·00	2·50	2·00	1·00
0·0	3·30	6·70	5·00	5·00
0·0	0·40	0·57	1·30	1·00
0·0	5·00	2·90	2·50	2·50
0·0	5·00	2·90	2·90	2·90
0·0	1·00	2·00	1·30	1·30
3·30	6·70	4·00	2·90	2·00
0·0	0·25	0·57	0·50	0·50
0·0	1·30	2·00	1·00	0·80

From the results it can be seen that at the 5 Lf dose level the titre never rose very high and at the fifth sampling time two of the sheep had no detectable antibody while several had as little as 0·1 unit/ml. At the 45 Lf dose level the position is slightly better in the sense that all sheep were protected at the fifth sampling time but the titre never rose above 10 units and fell rather steeply. It is clear that a single injection given to fully susceptible sheep can be expected to protect them only for a short period.

When it was clear that both a primary and a secondary stimulus would have to be given, an experiment was planned to provide information on the dose level to be used and the optimal interval between the first and the second injection. The following dosages were selected: 5, 25, 45, 90 and 135 Lf each contained in 2·5 ml of APT. At each level the primary and secondary stimuli were separated by intervals of 1, 2, 3, 4, 5 and 6 weeks respectively in separate groups of sheep. Samples of blood were taken at weekly intervals for five consecutive weeks beginning one week after the second injection. The antibody titre of the serum obtained from each sample was determined and the results, recorded in detail in Table 3, provide information about the variation in the response of different sheep and the antibody levels attained. For each sampling time the geometric mean value of the titres of the different groups is entered on the table to give an indication of the average level of immunity.

TABLE 3.—*Antibody response (units/ml of serum) of sheep to two injections of antigen spaced at varying intervals*

Group I—1 week interval Group IV—4 weeks interval.

Group II—2 weeks interval Group V—5 weeks interval

Group III—3 weeks interval Group VI—6 weeks interval

Dose = 2 × 5 *Lf in APT*

Sampling Times (Weeks)	1	2	3	4	5
Group I.....	0·24	0·20	—	—	—
	0·57	1·00	0·36	0·40	0·40
	0·40	0·33	0·20	0·20	0·10
	10·00	6·70	2·50	2·00	1·30
	0·30	0·80	0·67	0·50	0·36
	10·00	10·00	5·00	2·90	2·00
	1·30	2·00	1·30	1·30	0·80
	0·24	0·33	0·20	0·20	0·10
GEOM. MEAN.....	0·92	1·11	0·55	0·48	0·33
Group II.....	5·00	2·20	1·30	1·30	1·00
	2·00	0·57	0·57	0·57	0·36
	4·00	3·30	2·50	1·00	1·00
	10·00	2·20	2·00	1·30	1·30
	2·00	0·57	0·40	0·29	0·22
	10·00	5·00	2·50	2·00	2·00
	2·00	0·57	0·67	0·36	0·27
	20·00	10·00	4·00	4·00	3·30
	10·00	10·00	4·00	3·30	2·50
GEOM. MEAN.....	5·28	2·25	1·48	1·11	0·92

TABLE 3 (*continued*)

Dose = 2 × 5 *Lf in APT*

Sampling Times (Weeks)	1	2	3	4	5
Group III.....	2·00	1·30	1·30	0·80	0·67
	2·00	1·00	0·80	0·44	0·33
	2·00	1·30	1·30	1·00	0·67
	2·00	1·30	1·00	0·50	0·40
	0·22	0·07	—	—	—
	3·30	2·50	1·00	1·00	0·67
	2·00	1·00	0·80	0·44	0·36
	2·00	5·00	4·00	4·00	2·20
	0·33	0·33	0·40	0·40	0·27
	5·00	4·00	3·30	2·50	2·00
GEOM. MEAN.....	1·54	1·10	0·88	0·83	0·49
Group IV.....	10·00	6·70	4·00	2·90	2·20
	1·30	1·30	1·30	0·67	0·67
	1·30	1·00	0·44	0·33	0·25
	2·00	2·00	2·00	1·30	1·30
	4·00	5·00	4·00	2·50	2·50
	0·57	0·67	0·57	0·57	0·44
	1·30	0·80	0·57	0·33	0·27
	0·80	0·80	0·44	0·33	0·25
	1·30	0·57	0·36	0·27	0·22
	1·30	2·00	0·80	0·67	0·50
GEOM. MEAN.....	1·63	1·46	0·97	0·69	0·57
Group V.....	1·30	1·00	0·80	0·67	0·40
	6·70	4·00	2·90	2·90	2·00
	0·22	0·25	0·27	0·20	0·10
	6·70	3·30	2·00	1·30	1·30
	2·20	2·00	1·30	1·30	0·67
	1·30	1·00	0·80	0·50	0·36
	4·00	3·30	2·50	2·00	1·30
	10·00	6·70	4·00	4·00	2·90
	2·00	1·30	0·80	0·57	0·36
	1·30	1·00	0·80	0·67	0·40
GEOM. MEAN.....	2·28	1·69	1·00	1·00	0·65
Group VI.....	3·00	2·20	2·00	1·00	0·57
	1·00	1·00	1·00	0·44	0·36
	3·30	2·20	2·20	1·30	1·00
	3·30	2·20	2·00	1·30	1·00
	1·30	1·00	1·00	0·67	0·36
	0·80	0·80	0·80	0·50	0·36
	3·30	4·00	4·00	3·30	2·50
	10·00	6·70	6·70	3·30	2·90
	2·00	1·00	1·00	0·57	0·57
	0·20	0·27	0·27	0·27	0·20
GEOM. MEAN.....	1·86	1·51	1·48	0·92	0·68

TABLE 3 (*continued*)

Dose = 2 × 25 *Lf in APT*

Sampling Times (Weeks)	1	2	3	4	5
Group Ia.....	2·00	1·30	0·67	0·67	0·50
	1·00	0·67	0·50	0·36	0·30
	6·70	5·00	2·50	1·30	1·00
	0·80	0·80	0·67	0·44	0·30
	0·29	3·30	1·30	1·00	0·67
	20·00	6·70	5·00	2·50	1·30
	2·90	3·30	2·20	0·80	0·57
	5·00	3·30	2·20	0·80	0·50
	0·80	1·00	0·57	0·44	0·30
	1·00	0·67	0·57	0·50	0·30
GEOM. MEAN.....	1·93	1·88	1·19	0·74	0·50
Group IIa.....	10·00	10·00	5·00	5·00	3·30
	20·00	10·00	5·00	5·00	3·30
	10·00	10·00	5·00	4·00	2·20
	10·00	10·00	5·00	2·90	2·00
	3·30	2·50	2·00	2·00	1·30
	5·00	3·30	2·20	2·00	2·00
	4·00	3·30	2·00	1·30	1·00
	10·00	6·70	4·00	2·50	2·00
	10·00	6·70	3·30	2·90	2·00
	1·30	0·67	0·50	0·30	0·25
GEOM. MEAN.....	6·66	4·90	2·86	2·24	1·62
Group IIIa.....	3·30	2·50	2·00	1·30	1·30
	3·30	3·30	2·90	2·00	1·30
	2·20	1·30	1·00	0·80	0·44
	3·30	2·00	1·30	1·30	0·80
	5·00	4·00	2·90	1·30	1·30
	6·70	4·00	2·50	1·30	1·30
	2·90	2·00	1·30	1·00	0·57
	20·00	10·00	10·00	6·70	4·00
	2·00	2·00	2·00	2·00	1·30
	10·00	6·70	2·90	1·30	0·80
GEOM. MEAN.....	4·45	3·14	2·30	1·55	1·09
Group IVa.....	1·00	1·00	1·00	0·44	0·33
	25·00	20·00	10·00	6·70	5·00
	6·70	6·70	4·00	3·30	2·50
	4·00	3·30	2·00	1·30	1·00
	5·00	5·00	3·30	2·50	2·00
	2·00	1·00	1·30	0·67	0·44
	2·50	2·00	2·00	2·00	1·00
	2·00	2·00	1·00	0·44	0·40
	10·00	6·70	4·00	2·20	2·00
	10·00	10·00	4·00	4·00	2·50
GEOM. MEAN.....	4·36	3·67	2·54	1·67	1·22

TABLE 3 (*continued*)

Dose = 2 × 25 *Lf in APT*

Sampling Times (Weeks)	1	2	3	4	5
Group Va.....	2·00	1·00	0·67	0·67	0·50
	0·33	0·30	0·24	0·24	0·10
	20·00	10·00	6·70	4·00	2·90
	10·00	10·00	5·00	3·30	2·90
	2·00	2·90	2·20	1·30	1·30
	2·90	2·90	2·20	1·30	1·30
	0·40	0·50	0·33	0·24	0·10
	1·30	1·00	0·50	0·44	0·36
	4·00	2·20	2·00	1·30	1·00
	0·80	0·57	0·33	0·29	0·25
GEOM. MEAN.....	2·04	1·66	1·11	0·82	0·60
Group VIa.....	10·00	6·70	4·00	2·50	2·00
	10·00	10·00	6·70	4·00	2·90
	33·00	20·00	20·00	10·00	5·00
	6·70	2·90	1·30	1·30	0·80
	0·80	0·44	0·33	0·33	0·10
	2·50	2·00	1·30	1·00	0·57
	0·80	0·44	0·30	0·24	0·10
	2·90	2·00	1·00	0·57	0·30
	6·70	4·00	2·50	2·00	0·80
GEOM. MEAN.....	4·45	2·84	1·83	1·31	0·68

Dose = 2 × 45 *Lf in APT*

Group Ib.....	0·40	0·80	2·50	2·90	2·50
	0·50	1·30	2·00	1·00	1·00
	2·00	5·00	5·00	5·00	5·00
	4·00	3·30	2·20	1·30	0·57
	10·00	10·00	5·00	4·00	4·00
	1·30	2·50	2·00	1·00	0·67
	2·50	4·00	2·20	1·30	1·30
	0·80	2·00	2·00	1·30	1·30
	0·44	2·50	2·20	2·00	2·00
	10·00	10·00	10·00	10·00	6·70
GEOM. MEAN.....	1·68	3·11	2·97	2·19	1·84
Group IIb.....	20·00	6·70	4·00	2·50	2·00
	4·00	4·00	4·00	2·50	2·00
	20·00	10·00	5·00	5·00	5·00
	25·00	30·00	10·00	6·70	5·00
	5·00	5·00	4·00	2·90	2·20
	20·00	10·00	10·00	6·70	5·00
	2·20	2·50	2·20	1·00	0·80
	10·00	6·70	4·00	2·90	2·00
	33·00	20·00	20·00	20·00	10·00
	20·00	20·00	10·00	6·70	6·70
GEOM. MEAN.....	11·92	8·77	4·73	4·17	3·22

TABLE 3 (*continued*)

Dose = 2 × 45 *Lf in APT*

	1	2	3	4	5
Group IIIb.....	0·67	0·80	0·44	0·33	0·24
	2·20	2·00	2·00	2·00	2·00
	1·30	1·30	1·00	1·00	0·67
	20·00	20·00	20·00	10·00	6·70
	20·00	20·00	10·00	6·70	5·00
	2·20	1·30	0·57	0·57	0·44
	10·00	10·00	10·00	5·00	4·00
	20·00	10·00	5·00	4·00	2·50
	3·30	4·00	2·50	2·00	1·30
	4·00	4·00	2·50	2·00	1·30
GEOM. MEAN.....	4·62	4·21	2·24	2·14	1·55
Group IVb.....	5·00	5·00	5·00	4·00	3·30
	33·00	20·00	20·00	20·00	10·00
	20·00	20·00	10·00	4·00	4·00
	20·00	5·00	4·00	2·20	2·00
	2·00	1·30	0·80	0·50	0·44
	2·90	2·50	2·50	2·00	2·00
	2·20	1·30	2·00	2·00	2·00
	2·00	1·30	1·30	1·30	1·30
	2·00	1·30	1·00	0·67	0·50
	5·00	4·00	2·90	2·20	2·00
GEOM. MEAN.....	5·28	3·51	3·01	2·20	1·90
Group Vb.....	5·00	6·70	6·70	5·00	2·90
	6·70	6·70	6·70	6·70	4·00
	33·00	33·00	33·00	25·00	20·00
	2·20	2·20	2·20	2·20	1·30
	33·00	33·00	20·00	20·00	10·00
	20·00	10·00	5·00	5·00	2·50
	4·00	6·70	5·00	2·90	2·00
	4·00	6·70	5·00	4·00	2·50
	5·00	6·70	6·70	6·70	5·00
	5·00	6·70	3·30	3·30	2·20
GEOM. MEAN.....	7·43	8·58	6·69	5·86	3·65
Group VIb.....	10·00	10·00	10·00	5·00	2·50
	25·00	20·00	10·00	4·00	2·50
	2·20	2·20	2·20	2·00	1·00
	6·70	6·70	6·70	6·70	4·00
	0·57	0·57	0·67	2·00	0·67
	10·00	10·00	10·00	6·70	3·30
	20·00	20·00	20·00	6·70	6·70
	10·00	10·00	10·00	4·00	2·50
	20·00	20·00	20·00	6·70	4·00
	2·20	1·30	1·00	1·00	0·57
GEOM. MEAN.....	6·71	6·22	5·75	3·81	2·15

TABLE 3 (*continued*)

Dose = 2 × 90 *Lf in APT*

Sampling Times (Weeks)	1	2	3	4	5
Group Ic.....	20·00	10·00	6·70	6·70	6·70
	20·00	20·00	20·00	10·00	10·00
	0·40	1·00	1·30	1·30	2·00
	0·40	2·00	1·30	1·00	1·00
	2·20	1·30	1·30	1·00	1·00
	2·50	2·50	2·20	1·30	1·30
	25·00	20·00	10·00	10·00	6·70
	20·00	20·00	10·00	10·00	10·00
	10·00	3·30	3·30	2·20	2·20
	20·00	20·00	20·00	10·00	6·70
GEOM. MEAN.....	5·68	5·67	4·64	3·46	3·34
Group IIc.....	10·00	10·00	6·70	4·00	2·90
	5·00	3·30	1·30	1·30	1·30
	100·00	100·00	33·00	20·00	20·00
	6·70	6·70	2·90	2·90	2·90
	10·00	10·00	6·70	6·70	6·70
	6·70	5·00	5·00	5·00	3·30
	2·00	2·00	2·00	2·00	2·00
	2·50	2·20	1·30	1·30	1·30
	2·90	3·30	1·30	1·30	1·30
	20·00	20·00	10·00	10·00	10·00
GEOM. MEAN.....	7·61	7·09	3·96	3·58	3·32
Group IIIc.....	100·00	33·00	20·00	20·00	10·00
	50·00	33·00	20·00	20·00	10·00
	50·00	25·00	20·00	20·00	20·00
	10·00	5·00	5·00	4·00	2·50
	2·90	2·20	2·20	2·00	1·00
	100·00	33·00	25·00	10·00	10·00
	33·00	33·00	33·00	20·00	10·00
	6·70	6·70	5·00	3·30	2·00
	10·00	10·00	10·00	5·00	3·30
	100·00	50·00	50·00	20·00	20·00
GEOM. MEAN.....	26·33	16·00	13·82	9·18	6·05
Group IVc.....	20·00	20·00	10·00	10·00	6·70
	33·00	20·00	10·00	10·00	4·00
	33·00	20·00	10·00	6·70	3·30
	50·00	33·00	20·00	10·00	5·00
	20·00	10·00	10·00	10·00	3·30
	10·00	6·70	3·30	2·00	1·00
	20·00	20·00	10·00	5·00	3·30
	20·00	20·00	10·00	5·00	2·90
	33·00	20·00	20·00	10·00	4·00
	100·00	33·00	20·00	10·00	6·70
GEOM. MEAN.....	27·92	18·49	11·03	7·12	3·61

TABLE 3 (*continued*)

Dose = 2 × 90 *Lf in APT*

Sampling Times (Weeks)	1	2	3	4	5
Group Vc.....	20·00	10·00	6·70	3·30	2·90
	10·00	10·00	6·70	4·00	2·90
	20·00	10·00	6·70	3·30	2·00
	20·00	10·00	10·00	5·00	3·30
	20·00	10·00	6·70	3·30	2·50
	10·00	6·70	4·00	2·90	2·00
	33·00	20·00	10·00	6·70	5·00
	20·00	20·00	10·00	10·00	6·70
	20·00	20·00	20·00	10·00	5·00
	20·00	10·00	10·00	4·00	3·30
GEOM. MEAN.....	18·31	11·82	8·33	4·73	3·30
Group VIc.....	20·00	10·00	10·00	5·00	5·00
	2·50	2·20	1·30	0·57	0·36
	20·00	20·00	6·70	4·00	4·00
	10·00	10·00	5·00	4·00	3·30
	50·00	25·00	20·00	10·00	10·00
	20·00	20·00	6·70	4·00	2·90
	20·00	20·00	10·00	5·00	5·00
	10·00	5·00	2·90	2·00	1·30
	33·00	20·00	10·00	6·70	4·00
	10·00	10·00	4·00	2·20	1·30
GEOM. MEAN.....	16·29	11·60	6·07	3·49	2·73
	Dose = 2 × 135 *Lf in APT*				
Group Id.....	10·00	3·30	2·20	2·00	0·80
	25·00	10·00	10·00	4·00	4·00
	4·00	2·50	2·00	1·00	0·67
	10·00	3·30	2·90	2·00	2·00
	6·70	2·00	1·30	0·86	0·57
	20·00	10·00	10·00	6·70	5·00
	20·00	5·00	5·00	5·00	2·50
	25·00	20·00	10·00	6·70	3·30
	2·00	1·30	0·67	0·44	0·30
	33·00	20·00	20·00	10·00	6·70
GEOM. MEAN.....	9·22	5·19	4·02	2·59	1·70
Group IId.....	20·00	10·00	6·70	3·30	3·30
	5·00	2·90	2·00	1·00	0·67
	25·00	10·00	10·00	4·00	4·00
	20·00	10·00	10·00	6·70	4·00
	10·00	10·00	4·00	2·50	2·00
	20·00	20·00	10·00	5·00	4·00
	20·00	20·00	6·70	4·00	2·90
	20·00	20·00	10·00	10·00	10·00
	33·00	20·00	10·00	5·00	5·00
GEOM. MEAN.....	17·20	11·86	6·91	3·93	3·26

TABLE 3 (*continued*)

Dose = 2 × 135 *Lf in APT*

Sampling Times (Weeks)	1	2	3	4	5
Group IIId.....	20·00	20·00	10·00	5·00	5·00
	10·00	10·00	6·70	5·00	4·00
	20·00	20·00	10·00	5·00	4·00
	10·00	10·00	5·00	5·00	4·00
	6·70	3·30	2·50	1·30	1·00
	5·00	4·00	3·30	2·50	1·00
	5·00	3·30	2·00	1·30	1·30
	6·70	6·70	3·30	3·30	2·00
	2·90	2·50	2·00	1·30	1·00
	100·00	50·00	20·00	20·00	10·00
GEOM. MEAN.....	10·27	8·25	4·86	3·43	2·47
Group IVd.....	10·00	6·70	3·30	3·30	2·20
	2·90	2·20	2·00	1·00	0·67
	5·00	4·00	2·50	2·00	1·00
	20·00	20·00	10·00	10·00	6·70
	3·30	3·30	2·20	1·30	1·00
	10·00	5·00	2·90	2·90	1·30
	10·00	4·00	3·30	2·90	1·30
	6·70	5·00	2·50	2·00	1·00
	25·00	20·00	10·00	10·00	5·00
	25·00	20·00	20·00	20·00	10·00
GEOM. MEAN.....	9·13	6·60	4·21	3·52	1·96
Group Vd.....	33·00	33·00	20·00	10·00	5·00
	50·00	50·00	33·00	20·00	10·00
	10·00	10·00	10·00	5·00	3·30
	20·00	20·00	20·00	10·00	5·00
	20·00	20·00	10·00	6·70	3·30
	20·00	20·00	10·00	6·70	2·90
	10·00	10·00	6·70	2·90	2·00
	20·00	20·00	10·00	6·70	2·50
	20·00	20·00	6·70	6·70	5·00
GEOM. MEAN.....	21·67	21·67	12·18	7·30	3·88
Group VId.....	3·30	2·20	1·30	0·57	0·40
	33·00	25·00	20·00	6·70	6·70
	10·00	6·70	6·70	2·90	2·50
	33·00	20·00	20·00	20·00	6·70
	20·00	10·00	6·70	2·90	2·00
	10·00	6·70	4·00	2·50	2·00
	6·70	6·70	2·50	2·00	1·30
	20·00	10·00	5·00	2·90	2·50
	10·00	5·00	3·30	1·30	1·30
	20·00	10·00	10·00	6·70	6·70
GEOM. MEAN.....	13·45	8·35	5·74	3·10	2·34

Thus, the detailed data in Table 3 show the response in individual sheep to five different doses of toxoid ranging from 5 Lf to 135 Lf, and for each dose the sheep are divided into six groups depending on the interval, from one to six weeks, between the primary and secondary stimulus.

These data were examined in the following way: for each group of sheep receiving a given dose of vaccine, the mean logarithmic response was calculated for each sampling time and the values obtained were plotted in a graph against the sampling times. The resulting figure is not shown here, but it was seen that a straight line with a negative slope of about 45 degrees could be fitted to each of these 30 individual sets of observations. Furthermore, it was clear that at each dose level, the line for Group I fell significantly below the lines for the other groups which were lying closely together. Whatever the dose, the immune response is obviously inadequate after a dose interval of one week, whereas it is immaterial whether two, three, four, five or six weeks are allowed to lapse between the two doses. The correctness of this conclusion was confirmed by an analysis of variance.

In order to determine the dose of toxoid required for maximal immune response, the mean logarithmic value of the pooled data for Groups II to VI was calculated for each sampling time at each dose level and the resulting values were plotted against the corresponding sampling times as shown in Fig. 1. It seems quite clear that the response increases significantly as the dose is increased from 5 Lf to 90 Lf and that a further dose increase is without effect. The significance of this interpretation was confirmed by an analysis of variance.

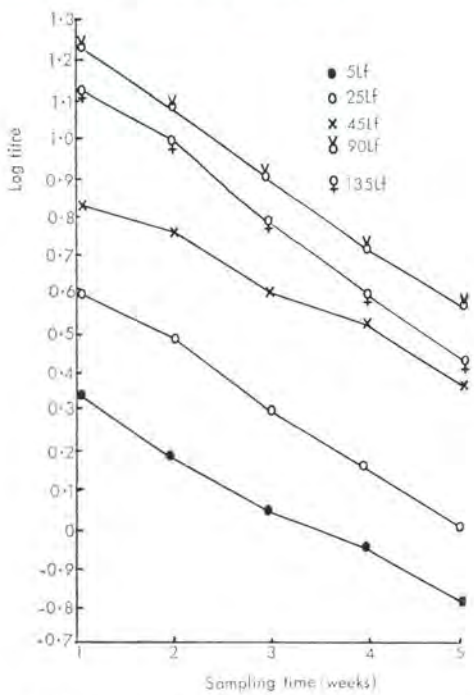

FIG. 1.—The mean log value of the pooled data at each dose level in relation to the corresponding sampling times

These results lead to the conclusion that a maximal immunity response to APT is produced by two injections each containing 90 Lf epsilon toxoid. The interval between these injections may vary from two to six weeks.

Discussion

The results obtained serve as a basis for interpreting the response to an alum-precipitated toxoid in relation to its antigen content. The response should always be judged on a group basis, since it may vary substantially among sheep receiving the same treatment. For instance, in the group injected with 90 Lf per dose with an interval of three weeks between the primary and secondary injections, some sheep had a titre of 100 units/ml at the first sampling time while one had only 2·9 units. It should also be noted that the toxoid solutions used for the preparation of APT were fresh, since it was proved in a previous series of experiments that in old preparations the flocculation test overestimates the antigen content as determined by the total combining power test.

It is clear that when sheep are vaccinated against pulpy kidney disease for the first time in their lives, they have to receive two injections. A single dose of vaccine produces a transitory protection in fully susceptible animals. From a practical point of view it is important to note that the interval between the primary and secondary stimuli may vary from two to six weeks without affecting the results. This allows a stockowner sufficient latitude in arranging the injection of his flock to suit his farming practice.

The response to two doses of vaccine containing 90 Lf epsilon toxoid per dose is maximal while the response to a vaccine with 45 Lf per dose is significantly less. This does not imply that the sheep receiving 45 Lf per dose were not satisfactorily protected, but it does provide the assurance that, if a vaccine containing 90 Lf per dose loses half of its immunizing capacity on storage, it will still be effective in protecting sheep. But when a flock of sheep is injected with a vaccine containing too low a quantity of antigen per dose, some animals may be susceptible to the disease by about the fifth week after the secondary injection, e.g. No. 2 in Group Va, 25 Lf.

For the production of a vaccine against pulpy kidney disease it is important to know that a single dose need not contain more than 90 Lf of antigen. A vaccine containing more antigen per dose cannot stimulate an increased response but costs more to produce.

Summary

A single dose of alum-precipitated epsilon toxoid (APT) produces only a transitory immunity when given to fully susceptible sheep. A maximal immunity response is brought about by two injections each containing 90 Lf toxoid. The interval between the primary and secondary stimuli may vary from two to six weeks without influencing the end result.

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