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CALF PARATYPHOID II.—ARTIFICIAL IMMUNIZATION.

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CONTENTS.

A.—THE IMMUNIZATION OF MICE AGAINST "SALMONELLA DUBLIN".

The object of this experiment was to determine the degree of immunity conferred on mice against virulent cultures of *S. dublin* when these mice had been previously inoculated with *S. dublin* vaccine prepared in different ways. Except where otherwise stated, the vaccine was prepared from a 24 hours agar culture, the density of the emulsion used corresponding to Brown's tube No. 3. The degree of protection conferred by other Salmonella species, e.g. *S. typhi* and *S. typhi-murium,* was also studied. When formalin was used for killing the bacteria the concentration was 0·15 per cent. (see Table I).

The immunity conferred was challenged by means of an intraperitoneal inoculation of approximately 62 to 125 million live organisms suspended in 0·25 c.c. saline. This dose probably corresponded to several lethal doses of bacteria, but it was found that smaller doses, even though lethal, often required a longer incubation period and caused death at irregular intervals.

The mice were inoculated subcutaneously with 0·25 c.c. of the vaccine and again seven days later with the same dose. Two weeks after the second injection the challenge dose of 62 to 125 million live bacteria was given.

The results of challenging the immunity conferred on mice by twelve different types of vaccine are given in Table I. The protection afforded is compared with the resistance possessed by unimmunized control mice when challenged in the same way. The results haven been statistically analysed by Mr. D. van der Reyden and have been graphically plotted in Figure I.

It will be noticed that an alcohol-treated *S. dublin* vaccine, composed of pure "O" antigen, gives less protection than a formalin-treated *S. dublin* vaccine, and much less protection than an $Al(OH)_3$ adsorbed formalin-treated vaccine. These results seem to indicate that a *S. dublin* vaccine composed of "H" plus "O" antigen has greater immunizing powers against *S. dublin* infection than one composed of "O" antigen alone. The agglutinogenic response of immunized,

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infected and carrier animals reported elsewhere in this paper (section B, C, and D), seems to support this observation. In these animals the "O" agglutinin titre of the serum remains unaltered or very low, whereas the "H" titre generally rises considerably. There is very little difference between the results obtained with an alcohol-treated and a formalin-treated $Al(OH)_3$ *S. typhi* vaccine. The results of only one of these, viz. the alcohol-treated vaccine, is therefore given in Figure I. The protection afforded by either of these is not appreciably higher than that possessed by unimmunized mice. It appears, therefore, that the "O" antigen of *S. typhi* which resembles that of *S. dublin* has very little, if any, immunizing value for mice.

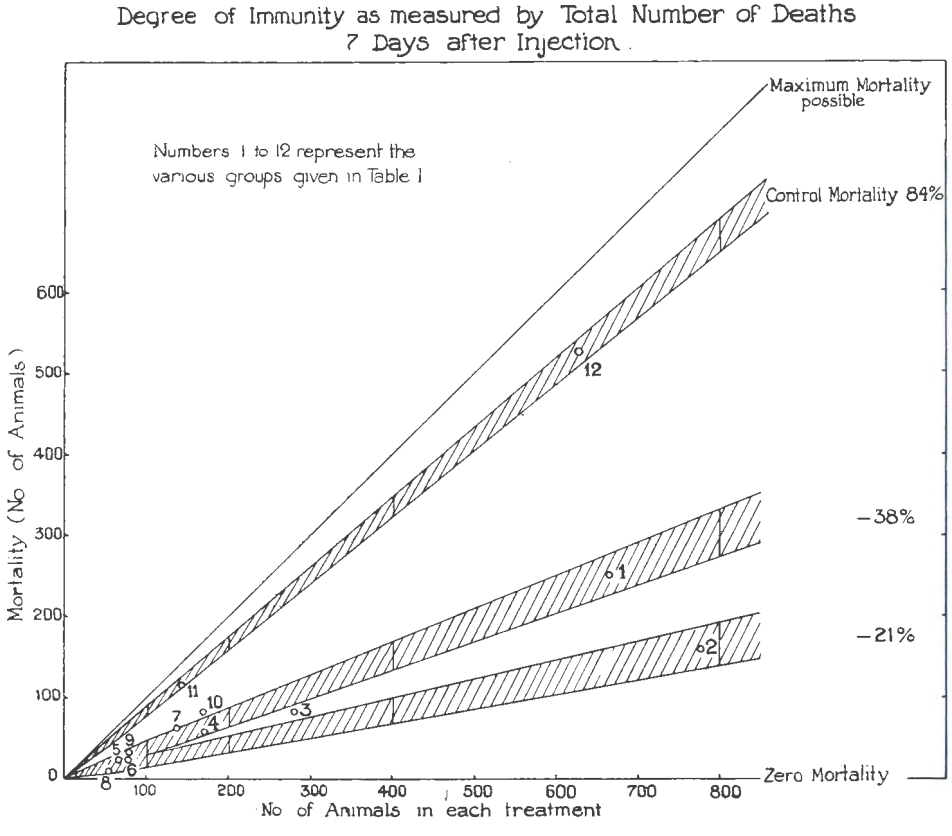

Fig. I

Since the total number of mice used in a treatment varied from 54 to 777, the percentage mortality will have unequal precision, a fact which must be taken into account when we evaluate the data. (See Table I and Figure I).

If p be the proportion killed, r the actual number killed and n the total number of mice used in each treatment,

$$r = p.n.$$

If the number killed (r) is plotted against the total number (n) the twelve points representing the twelve treatments will fall along three distinct lines, where the tangent of the line is given by p.

TABLE 1.

The results of challenging the immunity conferred on mice by twelve different types of vaccine.

Number of Group.	Type of Vaccine used.	Total Number of Mice. Tested.	Total Number of Deaths 4 Days after Injection.	Percentage of Deaths 4 Days after Injection.	Total Number of Deaths 7 Days after Injection.	Percentage of Deaths 7 Days after Injection.
1	Formalin-treated *S. dublin*	668	94	14·1	251	37·7
2	Formalin-treated *S. dublin* plus 0·017% $Al(OH)_3$	777	41	5·27	160	20·59
3	Chloroform-treated *S. dublin* plus 0·017% $Al(OH)_3$	280	19	6·78	82	29·3
4	Crystal-violet-treated *S. dublin*	173	15	8·67	63	30·6
5	†Merthiolate-treated *S. dublin*	66	2	3	23	36·37
6	Merthiolate-treated *S. dublin* plus 0·017% $Al(OH)_3$	77	9	11·68	23	29·8
7	Alcohol-treated *S. dublin*...	137	28	20·43	63	45·9
8	Formalin-treated *S. dublin* plus 0·1% saponin	54	5	9·25	10	18·5
9	*Formalin-treated *S. dublin* endotoxoid* plus 0·017% $Al(OH)_3$	79	17	21·5	33	41·77
10	Formalin-treated *S. typhimurium* plus 0·017% $Al(OH)_3$	170	40	23·5	83	48·8
11	Alcohol-treated *S. typhi*	144	68	47·15	115	79·8
11 (*a*)	Formalin-treated *S. typhi* plus 0·017% $Al(OH)_3$	76	34	44·7	59	77·6
12	Controls.....	628	378	52·2	528	87·2

* Prepared according to the method of Grasset (1933) and Grasset and Gory (1929).

† Merthiolate=Sodium ethyl mercuri thiosalicylate, Eli Lilly & Co.

Calculate for constant p the standard deviation of r per different n, by means of the formula np (1-p). This yields the shaded bands in the graph, and it means that all treatments falling within such a shaded band have the same percentage result although standard deviations are unequal.

If we regard a ratio of two standard deviations equal to 1·41 as significant, the vertical lines in the graph are obtained, and this means that all treatment points falling in the same shaded band within a block do *not* differ significantly.

A study of the graphs reveals that the treatments giving the best immunization are respectively numbers 2, 3 and 1, viz.—

(2) Formalin plus $Al(OH)_3$.

(3) Chloroform plus $Al(OH)_3$.

(1) Formalin.

According to these results formalinized suspensions of *S. dublin* containing 0·017 per cent. aluminium hydroxide gives by far the best results. It was, therefore, decided to adopt these suspensions for the routine immunization of calves.

B.—The Active Immunization of Calves Against Paratyphoid.

As nearly 100 per cent. of the epizootics of calf paratyphoid in South Africa are caused by *Salmonella dublin* (Henning 1939, 1952a), immunization was carried out exclusively with vaccines prepared with this organism. Freshly-isolated strains that proved to be virulent for both mice and calves were used for vaccine production. As the best protection, in mouse immunization tests, was produced by a vaccine composed of formalinized suspensions of *S. dublin* containing aluminium hydroxide, this vaccine was used exclusively.

The method of vaccine production.

The selected strain or strains of *S. dublin* cultivated over-night in beef-infusion broth is employed as seed-material. Ordinary nutrient agar in Roux flasks is used as culture medium; but before the sowing the agar flasks are kept on the bench at room temperature for about a week, so that the water of condensation in the flasks can evaporate off and the surface of the medium can become dry. When the flasks are ready the seed-material is sown. After 24 hours cultivation at 37·5° C. the growth is harvested by washing it off with sterile physiological saline. It is centrifuged, washed, and resuspended twice in physiological saline. The washed organisms are then suspended in flasks containing physiological saline to which 0·15 per cent. formalin had been added. The final density of the suspension corresponds to that of Brown's tube No. 3; i.e. approximately 2,000 million bacteria per cubic centimetre. In order to employ vaccines composed of more or less a uniform concentration of bacteria the density of the suspension is adjusted by means of a colorimeter. After the formalinized suspension of bacteria has been incubated at 37·5° C. for 48 hours 10 c.c. of a 10 per cent. solution of alum and 5 c.c. of a 7·4 per cent. solution of potassium hydroxide are added to every litre of fluid. The $AlK(SO_4)_2$ is first added, and after the mixture has been well shaken the KOH is introduced. A very fine floccular precipitate of aluminium hydroxide is formed, which adsorbs the bacteria in the suspension. On standing, this precipitate together with the bacteria readily settles at the bottom of the flask, leaving a clear supernatant fluid. The concentration of $Al(OH)_3$ formed is 0·017 per cent.

After 24 hours the clear supernatant fluid is carefully siphoned off, leaving the floccular precipitate at the bottom of the flask. An amount of physiological saline containing 0·1 per cent. formalin, and equal to the amount of supernatant fluid removed, is then added to the precipitate.

After shaking the flasks are allowed to stand on the bench at room temperature for 48 hours, and the vaccine is tested for sterility, safety, and potency.

One of the drawbacks of the vaccine is its property of occasionally producing serious shock soon after inoculation. This property has been largely eliminated by allowing the water of condensation to evaporate before seeding the flasks, by washing the harvested organisms in physiological saline, by adsorbing the bacteria to $Al(OH)_3$ particles, and by removing the clear supernatant fluid from the precipitate. If the vaccine has been found to be satisfactory it is issued in bottles containing two, five and ten doses of 5 c.c. The farmer is advised to inoculate his calves as soon as possible after birth and again seven days later. On badly infected farms a third or boosting injection is recommended 30 days after the second.

Symptoms of shock may sometimes appear soon after the calf has been inoculated. These symptoms, when present, may be alarming. Death is a frequent sequel. But the incidence of shock has been so rare that it has never yet been a serious drawback to the use of paratyphoid vaccine.

The vaccine is usually made in batches of 30,000 doses. Sometimes several different batches of vaccine have been issued to farmers without a single case of shock being reported, whereas one or two cases of shock may occur after the use of another batch. Moreover, several tests carried out by us with shock-producing batches of vaccine failed to set up shock in calves kept under our observation. When shock does occur, it appears to be limited to individuals in a group of calves that have been kept under identical conditions, and it seems likely that the shock-susceptible calves have been sensitized by some local environmental factor or by some constituent of the feed.

In a small percentage of inoculated calves a hard swelling is formed at the site of injection, but this is not more severe than that produced by other vaccines containing aluminium salts. In the vast majority of cases the swelling soon disappears, but in rare cases the swelling persists for about a month before it disappears.

Although the immunity produced by this vaccine, as shown by the mouse tests (Table I and Figure I), is only a partial immunity which cannot be compared in reliability with that conferred by some other bacterial vaccines, it is generally sufficient to protect the animal against natural exposure.

In uncontrolled field tests, extending over a period of more than 15 years, involving the use of about half a million doses of vaccine per annum, very few unfavourable results have been reported. In the great majority of outbreaks losses from paratyphoid ceased soon after the inoculation of the exposed calves. It is only in calves kept in grossly infected premises and in extremely unhygienic surroundings that breakdowns are liable to occur after the use of a routine vaccine. But even in these cases any further losses can generally be prevented by the use of a special vaccine prepared from local strains of *S. dublin*.

Several cases have been reported to us where farmers have had such excellent results with the vaccine after a few years that they decided to discontinue its further use. They believed that the infection had disappeared and that the inoculation of calves was no longer necessary, only to find that the disease reappeared in a far more virulent form than before. Only when the vaccination of all calves was re-instituted, preferably with a vaccine prepared from a local strain of *S. dublin*, did they succeed again in preventing losses.

The agglutinogenic response of calves immunized with S. dublin *vaccine.*

Several groups of calves from unimmunized cows were immunized with *S. dublin* vaccine and the agglutinin titre of the sera was determined at different periods. The majority of the calves used were less than one week old when they received the first inoculation. Before the inoculation, the "H" and "O" titres were determined. The "O" titre was invariably found to be less than 1:10, and in the majority of calves the "H" titre was 1:10 or less; in a small percentage the "H" titre was 1:25 or even 1:50.

Ninety-six calves were inoculated with routine formalinized *S. dublin* vaccine containing 0·017 per cent. aluminium hydroxide. (See Tables II and III). Two inoculations were given and the interval between the injections was seven days. Thirty days after the second inoculation a boosting injection was given to 33 of the calves. The dose of vaccine on each occasion was 5 c.c.

The "H" titre showed a definite rise seven days after the first inoculation, but there was seldom any evidence of "O" agglutination at this stage. After seven days the "H" agglutinin titre of the great majority of animals varied from

1:200 to 1:3,200. In approximately 7 per cent. the titre exceeded 1:3,200 and in about 25 per cent. it was less than 1:200. A week after the second inoculation, the "H" titre showed a further rise, attaining an end-point of 1:6,400 or more in the majority of cases. The titre remained more or less at this level for about four weeks. It was only in a small percentage of the calves that the titre dropped much during this period. Hardly any calves developed "O" agglutinins in appreciable amounts as a result of the inoculations.

The agglutinogenic response of 29 of the calves divided into six groups, each of which was inoculated with a vaccine made from a different strain of *S. dublin,* did not deviate appreciably from that of calves inoculated with routine vaccine made from another strain of *S. dublin* (see Table IV).

When the immunity produced by a routine vaccine was boosted by giving the animals a third inoculation, approximately 30 days after the second injection, the response was marked (see Table III). The "H" titre was usually increased several times by the boosting injection. For example, an "H" titre of calf No. 152 was found to rise from 3,200 to 1:51,200, and the titre of calf 1,185 rose from 1,600 to 102,400. The "H" titre was maintained at this high level for several weeks; and in some cases it remained fairly high for longer than four months.

In about 30 to 40 per cent. of the animals an "O" agglutinogenic response was observed after the boosting injection, but this seldom led to the production of an "O" titre of more than 1:100.

The extent of the agglutinogenic response after the boosting injection did not depend on the type of *S. dublin* vaccine used for the boosting. In calves inoculated with routine vaccine, the response to an alcohol-treated vaccine, when used for the boosting, was just as striking as the reaction to an aluminium hydroxide precipitated formalin-treated vaccine. (See Tables III, V.)

Calves inoculated with alcohol-treated (pure "O") vaccine. (Table V.)

Twenty-four calves were used in the experiment. Two inoculations were given and the interval between the injections was seven days. Thirty days after the second inoculation a third or boosting injection was given. The dose of vaccine given during each injection was 5 c.c. of the standard emulsion. There was no increase in the "O" agglutinogenic response when calves were inoculated with alcohol-treated instead of formalinized *S. dublin* vaccine. No evidence of "O" agglutination could be obtained even after the second injection, but a distinct, though slight, rise of "H" agglutinins was noticeable. The "H" titre obtained varied from less than 1:10 to 1:800. When the immunity was boosted 30 days after the second injection, a further rise in the "H" titre was obtained, but the "O" titre did not rise above the normal level. There was no difference in the response, whether alcohol-treated or formalinized aluminium hydroxide precipitated vaccine was used for the boosting.

C.—The Artificial Infection of Calves with Fresh Virulent Cultures of "Salmonella dublin."

As fresh cultures of recently-isolated strains of *S. dublin* appeared to be more pathogenic for young calves than old laboratory strains, only recently-isolated strains of the organism were used in these tests. Parenteral infection of the calves was not found to be a satisfactory method of producing symptoms of the disease. When fresh cultures were given either by the intravenous, the intraperitoneal or

the subcutaneous route the calves often succumbed from a septicaemia before typical symptoms of paratyphoid had time to develop. On the other hand, the administration of fresh cultures with the milk generally set up the typical syndrome of paratyphoid. The selected strain or strains of *S. dublin* were cultivated in broth overnight at 37·5° C., and on the following morning the milk ration of the calf was heated to 38° C., seeded with the broth culture, and incubated at 37·5 for two hours before it was fed to the calf.

Only young calves under ten days of age were used in the experiment, but as sufficient very young calves were not always readily available at the same time the calves were introduced into the experiment as they became available. The different calves were, therefore, infected at different periods.

TABLE II.

Immunization of calves with routine Alum-precipitated S. dublin *vaccine.*

Young calves from un-immunized cows were inoculated twice during a period of 7 to 14 days and the agglutinin titre of their sera was determined at different times.

Number of Cow.	Titre of Cow's Serum from 2–7 Days after Calving.		Date of Calving	Titre of Calf's Serum 2–7 Days after Birth and when 1st Injection was made.		Titre of Calf's Serum ± 1 Week after 2nd Injection.		Titre of Calf's Serum 3–4 Weeks after 2nd Injection.	
	O.	H.		O.	H.	O.	H.	O.	H.
51	0	50	2/11	0	50	0	12,800	0	800
54	0	25	8/11	0	100	0	12,800	0	3,200
55	0	50	2/12	0	50	0	6,400	0	6,400
59	0	25	8/11	0	25	0	204,800	0	1,600
60	0	25	9/10	0	50	0	800	0	3,200
61	0	100	24/11	0	100	0	51,200	0	51,200
64	0	50	25/11	0	100	—	—	0	51,200
68	0	0	22/10	0	0	0	1,600	0	800
69	0	50	19/10	0	25	0	204,800	0	3,200
70	0	50	8/11	0	50	0	800	0	800
71	0	25	28/10	0	50	0	800	0	3,200
73	0	0	15/11	0	50	0	400	—	—
74	0	25	29/10	0	50	0	800	0	3,200
76	0	0	24/11	0	100	—	—	0	6,400
78	0	0	16/11	0	0	0	800	—	—
80	0	25	27/11	—	—	0	1,600	0	800
81	0	25	19/10	0	25	0	51,200	0	25,600
86	0	400	8/10	0	100	0	25,600	0	25,600
87	0	200	12/11	0	25	0	800	—	—
88	0	0	6/11	0	50	0	800	0	6,400
89	0	25	1/11	0	200	0	3,200	0	3,200

COMMENTS.—The agglutinogenic response of the calves to inoculations of *S. dublin* was marked in some cases but weak in others; yet the response resulted in the production of " H " agglutinins exclusively and no evidence of the presence of appreciable amounts of " O " agglutinins could be found in the inoculated calves.

TABLE III.—*Boosting of immunity of calves immunized with routine* S. dublin *vaccine.*

Young calves from un-immunized cows were inoculated with routine alum-precipitated *S. dublin* vaccine, the intervals between the injections being 7 days. Some were inoculated twice, others three times. Approximately 30 days after the last injection a boosting injection was given with either routine vaccine or with an alcohol-treated vaccine. The agglutinin titres of the sera of the calves were recorded at different periods. At the time of the first injection the "O" titre of the calves was less than 1:10 and the "H" was not more than 1:50.

Number of Calf.	Titre of Calf's Serum 7 Days after first Injection.		Titre of Calf's Serum 7 Days after second Injection.		Nature of Vaccine used for Boosting.	Titre of Calf's Serum at Time of Boosting Injection.		Titre of Calf's Serum± 14 Days after Boosting.		Titre of Calf's Serum ± 30 Days after Boosting.		Titre of Calf's Serum ± 60 Days after Boosting.		Titre of Calf's Serum ± 120 Days after Boosting.	
	O.	H.	O.	H.		O.	H.	O.	H.	O.	H.	O.	H.	O.	H.
132*	0	1,600	0	51,200	R	0	25,600	100	409,600	100	163,400	50	163,400	25	819,200
136*	0	1,600	0	6,400	A	0	12,800	100	102,400	100	204,800	25	51,200	25	6,400
137*	0	400	0	6,400	A	0	6,400	0	102,400	0	409,800	0	51,200	25	3,200
144	0	1,600	—	—	A	0	6,400	0	102,400	0	204,800	—	—	25	409,600
151*	0	800	0	3,200	R	0	12,800	0	409,600	0	1,638,400	0	204,800	0	102,400
152	0	800	—	—	R	0	3,200	100	51,200	0	819,200	—	—	25	51,200
154*	0	0	0	6,400	R	0	12,800	0	204,800	0	409,600	—	—	0	25,600
155*	0	100	0	25,600	A	0	25,600	200	409,600	200	1,638,400	200	409,600	100	51,600
153*	0	100	0	3,200	A	0	25,600	400	102,400	100	102,400	—	—	25	102,400
156*	0	100	0	51,200	R	0	12,800	0	1,638,400	0	1,638,400	0	1,638,400	0	25,600
1155	0	800	0	3,200	R	0	3,200	0	102,400	—	—	0	6,400	—	—
1156	0	400	0	6,400	A	0	1,600	100	51,200	—	—	0	1,600	—	—
1158	0	51,200	0	102,400	R	0	51,200	50	102,400	—	—	0	12,800	—	—
1159	0	6,400	25	6,400	A	0	1,600	0	51,200	—	—	0	6,400	—	—
1161	0	3,200	0	51,200	R	0	6,400	0	51,200	—	—	0	3,200	—	—
1171	0	3,200	0	6,400	A	0	400	100	51,200	—	—	0	3,200	—	—
1179	0	400	0	1,600	R	0	1,600	0	51,200	—	—	0	12,800	—	—
1180	0	25	0	400	A	0	400	25	51,200	—	—	0	800	—	—
1182	0	1,600	0	51,200	R	0	3,200	0	51,200	—	—	0	25,600	—	—
1183	0	200	0	3,200	A	0	1,600	25	51,200	—	—	0	6,400	—	—
1185	0	50	0	12,800	R	0	1,600	0	102,400	—	—	0	3,200	—	—
1186	0	100	0	6,400	A	0	3,200	100	51,200	—	—	0	1,600	—	—
1187	0	400	0	12,800	R	0	6,400	0	102,400	—	—	0	25,600	—	—
1194	0	100	0	3,200	A	0	3,200	0	51,200	—	—	0	12,800	—	—
1197	0	50	0	6,400	R	0	3,200	0	12,800	—	—	0	800	—	—
1199	0	100	0	100	A	0	200	0	800	—	—	0	100	—	—

* Calf received a third injection one week after the second.
A—Alcohol-treated vaccine. R—Routine *S. dublin* vaccine.

TABLE III.—(Continued).

Number of Calf.	Titre of Calf's Serum 7 Days after first Injection.		Titre of Calf's Serum 7 Days after second Injection.		Nature of Vaccine used for Boosting.	Titre of Calf's Serum at Time of Boosting Injection.		Titre of Calf's Serum± 14 Days after Boosting.		Titre of Calf's Serum ± 30 Days after Boosting.		Titre of Calf's Serum ± 60 Days after Boosting.		Titre of Calf's Serum ± 120 Days after Boosting.	
	O.	H.	O.	H.		O.	H.	O.	H.	O.	H.	O.	H.	O.	H.
1202	0	3,200	0	25,600	R	0	12,800	0	51,200	—	—	0	3,200	—	—
1201	0	100	0	6,400	A	0	6,400	25	51,200	—	—	0	12,800	—	—
1235	0	400	0	51,200	R	0	6,400	0	102,400	—	—	0	51,200	—	—
1217	0	12,800	0	12,800	A	0	3,200	0	6,400	—	—	0	12,800	—	—
1234	0	200	0	51,200	A	0	800	25	6,400	—	—	0	800	—	—
1236	0	6,400	0	6,400	R	0	3,200	0	51,200	—	—	0	25,600	—	—
1237	0	400	0	25,600	A	0	3,200	50	25,600	—	—	0	12,800	—	—

COMMENTS.—Judging from the production of agglutinins, calves immunized with a routine alum-precipitated formalin-treated vaccine responded ver strongly to a boosting injection with either the same vaccine or with an alcohol-treated vaccine given approximately 30 days after the previous injection. The agglutinins persisted in large amounts for more than four months after the boosting. Calves which received 3 injections did not respond any better to the boosting injection than those which were inoculated twice.

It is significant that the immunogenic response, either after ordinary immunization or after boosting, resulted in the production of " H " agglutinins almost exclusively. Barely any " O " agglutinins were formed. This result corresponded with our observations under both field and experimental conditions where the immunogenic response of naturally infected animals, artificially-infected animals and vaccinated animals resulted in the production of practically only " H " agglutinins. Even animals that occurred in the carrier state reacted only to " H " agglutination tests. They rarely exhibited an " O " titre of as much as 1 : 10.

TABLE IV. *Immunization of calves with vaccine prepared from different strains of* S. dublin.

Six groups of calves were inoculated twice during a period of eight days with an alum-precipitated vaccine prepared from different strains of *S. dublin,* and the agglutination titre of the calves' sera was determined at different periods.

Group. No.	No. of Calf.	Number of Strain of *S. dublin* used for Antigen.	Titre of Serum before first Injection.		Titre of Serum 8 Days after first Injection and at Time of second Injection.		Titre of Serum 8 Days after second Injection.		Titre of Serum 30 Days after second Injection.	
			O.	H.	O.	H.	O.	H.	O.	H.
I	4276	Routine	0	0	0	100	0	3,200	0	400
	4297	Vaccine composed of 3 pooled Strains	0	25	0	200	0	6,400	0	400
	4289	Vaccine composed of 3 Pooled Strains	0	0	0	100	0	800	0	200
	4298	Vaccine composed of 3 Pooled Strains	0	10	0	400	0	3,200	0	400
	4299	Vaccine composed of 3 Pooled Strains	0	10	0	200	0	800	0	200
II	4275	1588	0	0	—	—	0	3,200	0	1,600
	4283	1588	0	0	0	1,600	0	6,400	0	800
	4284	1588	0	0	0	800	0	6,400	0	1,600
	4286	1588	0	0	0	800	0	6,400	0	800
	4288	1588	0	10	0	200	0	3,200	0	800
III	4280	1591	0	0	0	200	0	200	0	400
	4281	1591	0	0	0	200	0	3,200	0	1,600
	4287	1591	0	0	0	1,600	0	3,200	0	800
	4319	1591	0	40	0	200	0	400	0	100
IV	4317	1596	0	40	0	200	0	800	0	200
	4318	1596	0	0	0	100	0	400	0	50
	4320	1596	0	40	0	200	0	800	0	200
	4321	1596	0	0	0	400	0	800	0	400
	4322	1596	0	20	0	100	0	400	0	200
V	4312	1604	0	20	0	800	0	1,600	0	400
	4339	1604	0	20	0	400	0	1,600	0	800
	4340	1604	0	10	0	400	0	1,600	0	800
	4341	1604	0	10	0	100	0	200	0	100
	4343	1604	0	20	0	200	0	1,600	0	800
VI	4342	1616	0	10	0	400	0	800	0	400
	4344	1616	0	20	0	400	0	200	0	400
	4353	1616	0	0	0	1,600	0	800	0	200
	4377	1616	0	10	0	200	0	400	0	400
	7371	1616	0	0	0	400	0	800	0	800

COMMENTS.—The agglutinogenic response of these calves was greatest in groups I and II, but on the whole the response was not marked. Moreover, the response resulted in the production of "H" agglutinins exclusively. There was no evidence of appreciable amounts of "O" agglutinin in the sera of the inoculated calves. After 30 days there was hardly any.

TABLE V.

The immunization of calves with alcohol-treated S. dublin antigen, and the effect of boosting the immunity.

Young calves of un-immunized cows were inoculated twice with an alcohol treated (Pure "O") vaccine during a period of 8 days. The agglutinin titre of the sera of these calves was determined at various periods. A third or boosting injection was given approximately 30 days after the second injection, the vaccine used being either an alcohol-treated or a routine vaccine. The effect of the boosting injection on the agglutinin titre of the sera was observed 7 days after the inoculation. The "O" titre of the calves before the first injection was less than 1:10 and the "H" titre not more than 0:25.

Number of Calf.	Titre of Calf's Serum 7 Days after first Injection.		Titre of Calf's Serum ± 7 Days after second Injection.		Titre of Calf's Serum ± 14 Days after second Injection.		Titre of Calf's Serum ± 30 Days after second Injection.		Nature of Boosting Injection.	Titre of Calf's Serum 7 Days after Boosting.	
	O.	H.	O.	H.	O.	H.	O.	H.		O.	H.
1153.....	0	0	25	100	0	0	0	0	A	0	200
1157.....	0	0	0	50	0	0	0	0	R	0	200
1160.....	0	25	0	25	0	0	0	0	A	0	400
1162.....	0	0	0	100	0	800	0	0	R	0	800
1163.....	0	100	25	100	—	—	—	—	—	—	—
1167.....	0	0	0	400	0	100	0	50	R	0	200
1172.....	0	0	0	200	0	50	0	0	A	0	50
1173.....	0	25	0	0	0	0	0	0	R	0	400
1177.....	0	0	0	25	0	0	0	0	A	0	800
1181.....	0	0	0	100	0	0	0	50	R	0	3,200
1184.....	0	0	0	25	0	25	0	50	A	0	100
1189.....	0	0	0	100	0	25	—	—	—	—	—
1195.....	0	0	0	50	0	0	0	0	A	0	200
1196.....	0	0	0	0	0	0	0	0	R	0	400
1198.....	0	0	0	0	0	0	0	25	A	0	100
1200.....	0	0	0	0	0	0	0	0	R	0	400
1203.....	0	0	0	100	0	25	0	200	A	0	400
1204.....	0	0	0	0	0	0	0	0	R	0	1,600
1215.....	0	25	0	0	0	50	0	0	A	0	100
1213.....	0	0	0	0	0	25	0	200	R	0	800
1233.....	0	50	0	800	0	400	0	25	A	0	400
1231.....	0	25	0	200	0	100	0	25	R	0	6,400
1241.....	0	50	0	800	0	320	0	50	A	25	100
1240.....	0	0	0	400	0	200	0	50	R	0	1,600

A—Alcohol-treated vaccine. R—Routine vaccine.

Four calves, numbers 4482, 5002, 5003 and 5008, were introduced between the period February 9th and March 8th. All four were infected by means of two-hour milk cultures and all four developed typical symptoms of calf paratyphoid in approximately 48 hours. Calf No. 5008 died on the seventh day and calf No. 5003 on the eleventh day after infection. *S. dublin* was isolated from the internal organs of both calves and a histological study* of their organs revealed lesions that were regarded as typical of calf paratyphoid (see below). The other two calves, numbers 4482 and 5002, recovered completely. On April 18th, i.e. 68 and 62 days respectively after infection, the two were slaughtered. A bacteriological examination showed that the internal organs of the calves were entirely free from *S. dublin,* and a histological study of the organs did not reveal any lesions that could be associated with paratyphoid. Faeces cultures made from these calves at various periods during the course of the disease were also negative.

As milk cultures of *S. dublin* appeared to be a reliable method of infecting calves, nine more calves were introduced into the experiment. During the course of this study the development of symptoms and the production of agglutinins in the sera of the calves were observed; the presence of *S. dublin* in blood and faeces cultures was investigated, not only during the course of the disease, but also after apparent clinical recovery; and the effect of the treatment of sick calves with phthalylsulphathiazole was noted.

All nine calves were infected by means of two-hour milk culture. Six of these calves (numbers 5026, 5047, 5074, 5075, 5079 and 5080) which showed the worst clinical symptoms were selected and treated with large doses of phthalylsulphathiazole, and three (numbers 5029, 5077 and 5081) which appeared to suffer the least were not treated, but were left as controls. At times the condition of some of the treated calves appeared to be extremely critical and they were not expected to live. Nevertheless, improvement invariably followed soon after treatment was commenced. All six treated calves survived, whereas all three untreated ones succumbed from paratyphoid. Numbers 5029 and 5077 died 7 days after infection and 5081 after 16 days.

Several blood and faeces cultures were periodically made from the three control calves (numbers 5029, 5077 and 5081) during the course of the disease. MacConkey's bile salt agar as well as an enrichment medium, tetrathionate broth, was used for culturing. *S. dublin* was recovered only once from a blood culture from each of numbers 5077 and 5081. All the other blood cultures and all the faeces cultures were negative. After death, however, *S. dublin* was readily recovered from the liver, the spleen, and the mesenteric lymph glands of all three calves. A histological study of the internal organs revealed lesions which were indistinguishable from those of calf paratyphoid. The "H" agglutinin titre rose in seven days from less than 1:10 to 1:25 in numbers 5077 and 5081, and to 1:50 in No. 5029. As these calves soon died from paratyphoid no further readings were available.

There was no evidence of the production of any "O" agglutinins. In another calf (No. 5003), which was used in a preliminary experiment and which died eleven days after infection, the "H" titre rose from 1:10 to 1:200 in seven days.

Several blood and faeces cultures were also made at irregular intervals from the other six calves (viz. numbers 5026, 5047, 5074, 5075, 5079 and 5080).

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* This study was carried out by Dr. K. Schulz to whom the author wishes to express his thanks.

Two faeces cultures from calf 5026 were positive and one was negative, whereas all three blood cultures were negative. The first positive faeces culture was obtained 26 days after infection and the second 42 days after infection.

Two faeces cultures from No. 5047 were positive and five were negative, whereas all four blood cultures made were negative. The positive faeces cultures were made on the second and third days after infection.

One of the 13 blood cultures and three of the 14 faeces cultures from No. 5074 were positive; the others were negative. The positive blood culture was made four days after infection; the first positive faeces culture was made 22 days after infection and the last 33 days after infection.

Calf 5075 yielded only one positive culture out of twelve blood cultures. This was made five days after infection. The first positive faeces culture was obtained 17 days after infection. All subsequent faeces cultures yielded *S. dublin,* and the calf had become a permanent carrier in spite of apparent clinical recovery.

Eleven blood and 14 faeces cultures were made from No. 5079. Of these only one faeces culture was positive, this culture having been made 40 days after infection and when the calf appeared to be in perfect health. All the other cultures were negative.

Several blood and faeces cultures were made from No. 5080; but only one faeces culture, which was made 64 days after infection, was positive. The calf had meanwhile recovered apparently completely from clinical symptoms. The other cultures were all negative.

Positive blood cultures were usually obtained during the early and acute stages of the disease, whereas faeces cultures were more often positive later in the course of the disease, frequently after complete clinical recovery had apparently supervened.

A histological study made of the internal organs of some of the recovered calves (viz. numbers 4482, 5002, 5026, 5047) that were slaughtered showed only regenerative changes. A bacteriological examination of the liver, spleen, bile, mesenteric lymph glands, and intestinal scrapings failed to reveal *S. dublin.*

The fact that one calf excreted *S. dublin* with the faeces 40 days after infection, and another 64 days after infection, and also after clinical recovery, and the fact that a third calf discharged the organisms continuously after apparent recovery, shows that clinically "recovered" calves may play an important rôle in the dissemination of the infection.

The agglutinogenic response of calves which had been infected with *S. dublin* by the mouth did not appear to be very strong. The "H" agglutinin titre usually rose from less than 1:10 to 1:100 or to 1:200 in seven days. If the calf survived the titre generally rose to 1:800 or 1:1,600 in another week or two, and remained at this level for a few weeks. Then it gradually fell, but it rarely dropped below 1:100 during the succeeding two or three months. After the first three or four weeks the titre of calf No. 5075, which had become a permanent carrier, fluctuated between 1:100 and 1:800. There was no evidence of the production of any "O" agglutinins in any of the calves, and in no instance did the "O" agglutinin titre of the calves rise above the pre-infection level. The "O" agglutinin titre even of calves that remained inconstant or permanent carriers remained unaltered. There was no apparent difference in the agglutinin titres of the sera of the permanent carrier calf and of the calves that had apparently ceased to shed *S. dublin* in the faeces.

It is evident, therefore, that for the detection of the carrier state in calves infected with *S. dublin* more reliance should be placed on "H" than "O" agglutination. This observation does not agree with the previous studies of Henning and Haig (1939) and of Henning (1946) on *S. typhi-murium* and *S abortus-equi*. These authors have found that for the detection of the carrier state in birds infected with *S. typhi-murium* and of equines infected with *S. abortus-equi* "O" agglutination was far more important than "H" agglutination, and that "H" agglutinins might sometimes be absent in birds and animals with a high "O" titre.

When susceptible equines, however, were inoculated with an *abortus-equi* vaccine both "H" and "O" agglutinins might be formed in large amounts (Henning, 1946) in the sera of the inoculated animals.

The agglutinogenic response of adult cattle and calves to inoculations of *S. dublin* vaccine was also very marked. Both "H" and "O" agglutinins could be readily demonstrated in the sera soon after the inoculation, and the titre of both these antibodies in the sera of inoculated animals was usually considerably higher than in carrier animals (see Henning, 1952b, Tables I and II).

According to these results it is evident that the agglutination test as a method of diagnosing calf paratyphoid is of very little value until about 14 days after infection or 12 days after the beginning of symptoms. As death usually supervenes in about 7 days after infection or 5 days after symptoms have become apparent, the value of the agglutination test as a method of diagnosis is limited. Our observations have shown that blood cultures also have a limited diagnostic value as *S. dublin* can be recovered only in a small percentage of the cultures made by us. In the majority of our animals *S. dublin* could not be recovered from the faeces before two or three weeks had elapsed after the infection, so that faeces cultures can be of value only in carriers and other animals that have passed through the acute stage of the disease. Even in carriers faeces cultures do not invariably yield *S. dublin*, and the "H" agglutinin titre may not be very high. In our study it varied from 1:200 to 1:1,600, and "O" agglutination was of little value.

These results have also shown that phthalylsulphathiazole is an effective therapeutic agent for the treatment of calves infected with *S. dublin*. It is effective even when marked clinical symptoms are manifested. But calves which have recovered after treatment may still excrete *S. dublin* regularly or intermittently with the faeces. All the recovered calves have received very large doses of the drug, and so far no attempt has yet been made to determine the minimal therapeutic dose for a calf.

APPENDIX.—PROTOCOLS.

Calf No. 4482.—The calf was infected on February 9th, 1950, and showed a marked rise in temperature on the 11th. A severe diarrhoea had set in, the faeces being yellow or dirty-yellow and stinking. The calf was very sick and listless but drank its milk.

After about a week the temperature gradually fell; the diarrhoea became less marked, the faeces more solid, and the condition of the calf improved.

On April 18th the calf appeared to have recovered completely and it was slaughtered.

During the course of the disease four faeces cultures were made and all four were negative.

The "H" agglutinin titre of the calf's serum rose from less than 1:10 to 1:800 seven days after infection. 14 days after infection it was 1:1,600, and then it gradually fell to 1:200 a week before the calf was slaughtered. The "O" titre was less than 1:10 at the time of infection and remained at this level.

A bacteriological examination of the liver, spleen, mesenteric lymph glands, bile, and scrapings of the intestinal mucosa was negative for *S. dublin.*

A histological study of the internal organs showed no changes indicative of paratyphoid, but there was evidence of regeneration.

Calf No. 5002 was infected on February 15th, 1950. After 48 hours it showed a marked rise in temperature and a severe diarrhoea with dirty-yellow stinking faeces. The calf seemed to be very sick, but took its milk readily. The hyperthermia and diarrhoea continued for about a week before evidence of improvement could be noticed.

The improvement continued until April 18th, when the calf appeared to be normal and was slaughtered.

Faeces cultures were made twice; both with negative results.

The "H" agglutinin titre of the calf's serum rose from 1:10 to 1:200 in seven days and to 1:400 a week later. It then fell to 1:200, at which level it remained until the calf was slaughtered. The "O" titre never reached 1:10.

A bacteriological study of the liver, spleen, bile, mesenteric lymph glands, and scrapings from the mucosa of the small intestine was negative. A histological examination of the internal organs did not reveal any lesions which could be regarded as indicative for paratyphoid. The only changes observed were of a regenerative nature.

Calf No. 5003 was infected on February 20th, 1950. Two days later it showed a marked hyperthermia and a severe diarrhoea with dirty-yellow, stinking faeces. The calf appeared to be very ill. After a week the temperature fell and the consistency of the faeces was more solid.

On March 3rd the calf was found dead.

The "H" agglutinin titre rose from less than 1:10 to 1:200 in seven days.

A bacteriological examination of the liver and spleen yielded *S. dublin.*

A histological examination of the internal organs revealed lesions resembling those of a typical case of calf paratyphoid.

Calf No. 5008 was infected on March 8th, 1950. On the following day the temperature rose and diarrhoea set in, the faeces having an extremely unpleasant smell. The temperature remained high for a few days and the diarrhoea persisted. After five days the temperature gradually fell but the diarrhoea continued and soiled the hind-quarters.

On March 15th the calf died.

A bacteriological study of the liver and spleen was positive for *S. dublin,* and a histological examination of the internal organs revealed lesions which were regarded as typical for paratyphoid.

Calf No. 5026 was infected on March 28th, 1950. Two days later the temperature had risen markedly and flakes of bloody mucus were found in the faeces. The temperature continued to rise, a severe diarrhoea had set in, and the calf appeared to be very sick. On April 3rd treatment with phthalylsulphathiazole was commenced and carried on for ten days, after which time the calf showed a marked improvement and treatment was discontinued. Ten days later the condition of the calf had deteriorated and treatment was resumed. On May 9th a *B. necrophorus* ulcer was observed on the gums, and the calf was given 16 grams of sulphamezathine intravenously on two successive days. After a few days the ulcer had completely healed and the calf appeared to be in normal condition. On May 19th the calf was slaughtered. Cultures made from the liver, spleen, mesenteric lymph glands, bile and scrapings from the intestinal mucosa were all negative. A histological study of the internal organs revealed changes which were generally observed in a recovered animal. These were chiefly of a regenerative nature.

During the course of the disease the calf received 92 grams phthalylsulphathiazole and 32 grams sulphamezathine. Three blood and three faeces cultures were made. All three blood cultures and one of the faeces cultures were negative. The other two faeces cultures were positive, viz. the cultures made on the 26th day and the 42nd day respectively after infection.

The "H" agglutinin titre of the calf's serum rose from less than 1:10 to 1:25 and remained at this level. The "O" titre never reached the level of 1:10.

Calf No. 5029 was infected on March 30th, 1950. After 24 hours the calf looked sick the temperature was above normal, and diarrhoea had set in. During the succeeding days the temperature rose further, the diarhoea continued, and the calf remained sick. The faeces were dirty-yellow and stinking.

On April 7th the calf was dead. Cultures from the liver, spleen, and mesenteric lymph glands were all positive for *S. dublin.* A histological study of the internal organs revealed lesions which were typical for paratyphoid.

The "H" agglutinin titre rose from less than 1:10 to 1:50 in seven days, but the "O" titre remained less than 1:10.

Calf No. 5046 was introduced into the experiment on April 14th. It was kept in intimate contact with calves numbers 5026, 5047, 5074, 5075, 5077, 5079, 5080 and 5081, in order to observe whether close contact with the infected calves would cause it also to pick up the infection. Food was often given on the floor which was usually badly soiled with faeces.

Blood cultures were made on nine occasions and faeces cultures on 18 occasions at varying intervals during a period of over four months. The calf was discharged on September 2nd in an apparently healthy condition. *S. dublin* was obtained only once from a faeces culture, viz. the culture made on May 27th, i.e. 43 days after the beginning of the exposure. All the other cultures were negative. Apart from a short period from June 1st to 6th, when the calf suffered from diarrhoea, there was never any serious disturbance of the calf's health.

The "H" agglutination titre never rose above 1:25 and the "O" titre remained less than 1:10.

Calf No. 5047 was infected on April 20th, 1950, and on the following day a rise of the temperature was noticed. After 48 hours the hyperthermia was marked and a severe diarrhoea had set in. The symptoms remained unaltered until April 25th, when treatment with phthalylsulphathiazole was commenced.

On April 30th a marked improvement was apparent and treatment was discontinued.

On May 23rd the calf appeared to be in good health and was slaughtered. A bacteriological examination of the liver, spleen, mesenteric lymph glands, and scrapings from the intestinal mucosa was negative. A histological examination revealed lesions that were generally associated with a recovered case of paratyphoid. These were chiefly of a regenerative nature.

During the course of the disease four blood cultures and seven faeces cultures were made. All four blood cultures and five of the faeces cultures were negative. Two of the faeces cultures were positive, viz. those made on the second and the third day after infection. The "H" agglutinin titre of the calf rose from less than 1:10 to 1:200 seven days after infection and to 1:800 three weeks after infection. It remained at this level until the calf was slaughtered. The "O" titre remained less than 1:10.

Calf No. 5074 was infected on May 10th, 1950. After 24 hours a temperature reaction and a severe diarrhoea had set in. On the following few days the temperature rose further, the diarrhoea continued, and the calf looked very sick but kept on drinking its milk. On May 13th treatment with phthalylsulphathiazole was commenced. After two days' treatment there was evidence of improvement.

The treatment was continued until May 23rd when a definite improvement had set in. A total of 90 grams of the drug was given. This improvement was maintained and the calf remained in an apparently healthy condition until August 2nd, when it suddenly developed a fit of convulsions and died. Heartwater was suspected as an intercurrent disease, but no Rickettsias could be found in the brain, and sheep inoculated with heart blood did not develop symptons of heartwater.

A bacteriological study of the liver, spleen, mesenteric lymph glands, bile and scrapings of the intestinal mucosa was negative.

A histological study was negative for heartwater. The lesions found were hyperaemia and oedema of the lungs, marked hyperaemia of the brain, with minute focal and perivascular haemorrhages, a focal lymphocytic nephritis and a focal necrobiotic hepatitis (necrobiotic foci with proliferation of epithelioid cells in the liver).

During the course of the disease 13 blood and 14 faeces cultures were made. Of these one blood culture (May 14th) and three faeces cultures (May 22nd, May 27th and June 12th) were positive. Three other cultures were negative. The positive blood culture was taken 4 days after infection and the positive faeces cultures 12, 17 and 33 days respectively after infection.

The "H" agglutinin titre of the calf's serum rose from less than 1:10 to 1:800 seven days after infection and to 1:1600 in 14 days. The titre remained at this level for a few weeks and then gradually fell to 1:200, at which it remained until the calf died. There was no evidence of "O" agglutination at any time.

Calf No. 5075 was infected on May 10th, 1950. Within 24 hours the calf exhibited a rise in temperature. During the following few days the temperature rose further, a severe diarrhoea set in, and the faeces were bloody, mucous and stinking.

Treatment with phthalylsulphathiazole was commenced on May 13th and continued for nine days, by which time a total of 90 grams had been given. The treatment was discontinued when a marked improvement had become apparent, but was resumed when the diarrhoea returned, and a further 100 grams were given. On June 7th the calf appeared to be normal and healthy, and the treatment was again discontinued. The improvement was maintained and no further relapse occurred, but the calf, in spite of its apparent complete recovery, continued to discharge *S. dublin* continuously in the faeces. The calf had become a permanent carrier.

In all, twelve blood cultures and several faeces cultures were made. The blood culture made five days after infection was positive; all the others were negative. Faeces cultures made on May 12th, 14th, 19th and 22nd were negative. The first positive faeces culture was obtained on May 27th, i.e. 17 days after infection. All subsequent faeces cultures, up to the time of writing, were positive.

The "H" agglutinin titre rose from less than 1:10 before infection to 1:100 seven days later and to 1:1,600 in two weeks. It then fluctuated between 1:100 and 1:800. There was no evidence of any "O" agglutination.

Calf No. 5077 was infected on May 16th, 1950. During the following 24 to 48 hours the temperature rose and diarrhoea set in. But the calf did not appear to be very sick and readily drank its milk. The temperature remained high and the diarrhoea persisted until May 23rd, when the calf died.

A bacteriological study of the liver, spleen, mesenteric lymph glands, and scrapings of the intestinal mucosa was positive for *S. dublin.* A histological examination of the internal organs revealed lesions which were regarded as typical for paratyphoid.

During the course of the disease three blood cultures and three faeces cultures were made. Of these only one blood culture was positive (i.e. the one made on May 20th). All the other cultures were negative.

The "H" agglutinin titre of the serum rose from less than 1:10 to 1:25 just before the calf died. No "O" agglutinins could be detected.

Calf No. 5079 was infected on May 17th, 1950. Within 48 hours the calf showed a marked hyperthermia and a severe diarrhoea. The faeces were dirty-yellow and stinking.

On May 21st treatment with phthalylsulphathiazole was commenced. When improvement was apparent on June 4th the treatment was discontinued. But when the diarrhoea returned on June 26th the treatment was resumed. Soon afterwards a marked improvement set in again and the treatment was discontinued. In all, 180 grams of the drug had been given.

On September 16th the calf was discharged as apparently healthy.

During the course of the disease eleven blood cultures and 14 faeces cultures were made. All the blood cultures and 13 of the faeces cultures were negative. The only positive culture was the faeces culture made on June 26th when there was a relapse of diarrhoea, i.e. 40 days after infection.

The "H" agglutinin titre of the blood rose from less than 1:10 at the time of infection to 1:100 seven days later. It then fluctuated between 1:50 and 1:400 . It was 1:200 when the calf was discharged on September 16th. No "O" agglutination could be detected.

Calf No. 5080 was infected on May 18th. Two days later the calf was very sick; the temperature was high and severe diarrhoea had set in. The faeces were dirty-yellow and stinking. The symptoms persisted until June 22nd, when treatment with phthalyl-sulphathiazole was commenced and continued until June 3rd. Soon after the treatment was begun a marked improvement was evident, and this was maintained uninterruptedly. During this period 155 grams of the drug was given.

Several blood and faeces cultures were made during the course of the disease. Of these only one faeces culture, which was made on July 21st, i.e. 64 days after infection, was positive. All the others were negative.

The agglutinogenic response of this calf was poor, and it took 105 days for the "H" titre of the serum to rise from less than 1:10 at the time of infection to 1:200. The "O" titre remained less than 1:10.

Calf No. 5081 was infected on May 18th, 1950. After two days the temperature was high and diarrhoea had set in. The faeces had an unpleasant odour. The calf continued to take its milk readily until June 1st, when it was found in a recumbent state and very sick. It remained in this position until June 3rd, when it died.

Cultures made from the liver, spleen, mesenteric glands, and bile yielded *S. dublin,* but scrapings from the intestinal mucosa were negative. A histological study of the liver, spleen, kidneys, and mesenteric lymph glands revealed lesions resembling those of an acute natural case of paratyphoid.

Six blood cultures and four faeces cultures were made during the course of the disease. Of these only one blood culture, made 13 days after infection, was positive. The others were negative.

Before the experiment the "H" agglutinin titre of the calf's serum was less than 1:10 and rose to 1:25 seven days later. It remained at this level until the calf died. No "O" agglutinins could be detected.

D.—Challenging the Immunity Produced by the Routine Inoculation of Calves with "S. dublin" Vaccine.

After the excellent results obtained with milk-cultures of *S. dublin* as a means of producing calf paratyphoid (Section C) it was thought that this would be a reliable method for use in further experiments. Subsequent tests, however, have shown that the pathogenicity of milk-cultures of the same strain of *S. dublin* grown under apparently identical conditions may vary considerably at different times. It has not yet been possible to determine the cause of this variation, but it is believed that it may be connected in some way or other with the medium used. Whereas the milk-cultures used in section C invariably induced acute symptoms of paratyphoid which frequently resulted in death of the calves other tests performed set up less distinct clinical symptoms and caused comparatively fewer deaths.

In an attempt to challenge the immunity induced by routine inoculation, twelve young calves from 2 to 7 days old were obtained. Six were immunized with aluminium hydroxide adsorbed *S. dublin* vaccine and six were kept as controls. Two weeks after the second inoculation the six control calves, as well as the six immunized ones, were fed on fresh milk-cultures prepared in the same way as in Section C.

From 24 to 72 hours after the calves had received the milk-cultures symptoms of pyrexia and diarrhoea were observed in both groups. The fever reaction usually lasted for a few days, and in the majority of cases the temperature was

normal in about 7 days after the first rise. The diarrhoea continued in some calves for a week or two from the onset, but in others it was less marked and disappeared sooner. In some of the calves, however, exacerbations of fever and diarrhoea were observed intermittently. Most of the calves continued to take their milk more or less eagerly throughout the course of the disease.

The clinical symptoms manifested by the immunized calves, although resembling those shown by the controls, were generally much milder. In four of them, apart from the rise in temperature and a mild diarrhoea, no other disturbance of health could be detected, and the calves appeared to be lively and comfortable throughout the test. In the other two the pyrexia and the diarrhoea were more marked and the calves apparently suffered more. The symptoms manifested could not be differentiated from those exhibited by the control group.

On the whole more severe symptoms were observed in the control group; but in two of them the symptoms were so mild that the calves did not appear to suffer much inconvenience.

One of the control calves died on the tenth day after the infective feed, and *S. dublin* was isolated from its internal organs. The other five improved and were discharged as apparently healthy four weeks after infection. One of the immunized calves died on the seventh day but as *S. dublin* could not be cultured from its internal organs the cause of death could not be attributed to this organism. The other five calves never showed any symptoms which could be regarded as serious and were discharged in an apparently healthy state four weeks after the infective feed.

S. dublin was isolated from the faeces of most of the calves during the course of the gastro-intestinal disturbances, but at the time of their discharge four weeks after infection, all the faeces cultures were negative.

Comment:

Although the immunized calves undoubtedly suffered less from the challenge infection than the controls the test cannot be regarded as satisfactory, and no definite conclusions can be drawn from the results obtained. Arrangements are being made for a repetition of the experiment, when, it is hoped, more conclusive results will be obtained.

Summary and Conclusions.

1. Immunization experiments performed on mice with various vaccines prepared from *S. dublin* and other Salmonella species showed that a formalinized aluminium hydroxide precipitated vaccine gave the best protection against virulent cultures of *S. dublin*. The immunity produced gave only a partial or incomplete protection, yet when this vaccine was used for the routine immunization of calves on badly infected premises in different parts of the country a marked reduction in deaths from paratyphoid was effected. In a few outbreaks where the routine vaccine failed to prevent losses a vaccine made from a local strain of *S. dublin* invariably gave complete protection against natural exposure. But unless the vaccine was employed regularly and all the calves born on the farm were inoculated soon after birth, losses continued to occur. In a few cases where the use of the vaccine was discontinued fresh outbreaks of paratyphoid usually occurred.

In spite of the excellent results obtained with vaccine in the field, it is admitted that the immunity produced is of a low grade and that it cannot be relied upon entirely to prevent symptoms or even death from paratyphoid. When the immunity in calves was challenged with fresh milk-cultures given by the mouth the majority of them reacted and developed symptoms of paratyphoid, although much less severely than the control calves.

2. The immunization of calves with routine *S. dublin* vaccine resulted in the production of "H" agglutinins almost exclusively. Whereas the "H" agglutinin titre of the serum of the vaccinated animals rose from 1:25 or less to 1:3,200 or more after immunization, there was hardly any perceptible "O" agglutinogenic response in the majority of these animals, and the "O" titre remained extremely low in all of them.

When the immunity was boosted by means of a third injection of vaccine 30 days after the second a very marked rise in the "H" agglutinin titre resulted, but there was only a slight "O" agglutinogenic response in some of the animals and hardly any "O" agglutinins could be detected in the others.

3. An easy method of artificially infecting calves with fresh milk cultures of *S. dublin* is described. By the utilization of this method the pathogenesis and the course of the disease could be observed and treatment instituted. It was found that when calves manifesting typical symptoms of paratyphoid were treated with large doses of phthalylsulphathiazole by the mouth recovery supervened, whereas untreated calves, infected in the same way and left as controls, died.

Phthalylsulphathiazole can, therefore, be regarded as an effective therapeutic agent for calf paratyphoid, and its employment can be recommended for the treatment of this disease.

It was observed subsequently, however, that fresh milk-cultures of the same strain of *S. dublin,* grown under apparently identical conditions, might not always be equally pathogenic for calves. Sometimes an acute and fatal disease was set up, whereas, at other times, very much milder symptoms were produced. Although the cause of this variation is not known it is believed that some factor in the media is responsible.

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