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The Lethal Dose of the Toxins of Some Anaerobes for Sheep.

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A COMMON method of determining the immunity produced in sheep by the injection of antigens prepared from the sporulating anaerobes is the intramuscular or subcutaneous injection of living culture. Provided that one has had considerable experience with the antigen and the organism in question, little objection can be raised—a rough answer, usually sufficient for practical purposes, is obtained. However, not infrequently the test culture, prepared in the same manner as on previous occasions, fails to kill the controls or test animals that should die, and thus the experiment is rendered valueless.

With the exception of *Cl. chauvoei* (causing black quarter), the anaerobes responsible for diseases in sheep are toxin producers. These are *Cl. septique* (braxy), the lamb dysentery bacillus or *Cl. welchii*, Type B, Wilsdon (lamb dysentery, bloedpens), *Cl. oedematiens* (black disease), and *Cl. ovitoxicus* or *Cl. welchii*, Type D, Wilsdon (enterotoxaemia and pulpy kidney disease).

Since the toxins of the anaerobes are readily stabilized by precipitating toxic filtrates with ammonium sulphate and drying, and, further, since the injection of toxin is a more accurate method of testing antitoxic immunity than is the injection of culture, the writer decided to compare the toxicity of the various toxins in sheep and in mice and guinea-pigs. The results are presented here, in the hope that they may serve as a guide to others who have not had a sufficient number of sheep at their disposal to establish the ratio between toxicities in mice and sheep.

All the organisms were " single-celled " prior to the commencement of the test. Morphologically, culturally, biochemically, and as regards their pathogenic effect on guinea-pigs, they behaved in the manner described for typical strains. The *Cl. septique* and *Cl. oedematiens* cultures were originally obtained from the National Collection of Type Cultures, Lister Institute, the *Cl. ovitoxicus* strain (R_2) from Dr. Bennetts, and the lamb dysentery organism was a sub-culture of that originally isolated by Major Dalling. It should be noted that this Type B strain was of the " 1930 variety " (Montgomerie and Rowlands, 1934; Dalling, 1934; Mason, 1934), and thus did not produce the *epsilon* toxic fraction of Glenny *et alia* (1933).

All cultures were grown at 37° C. in Robertson's meat broth (horse flesh) for the following periods: Type B, 20 hours; *Cl. septique*, 36 hours; *Cl. oedematiens*, 48 hours; and *Cl. ovitoxicus*, 5 days. After filtration, first through pulp and then through a

Berkefeld candle, the filtrates were saturated with ammonium sulphate, the precipitate blotted and finally dried *in vacuo* over H_2SO_4. For use 100 mgm. were dissolved in 5·0 c.c. of saline solution.

The minimum lethal dose for mice and guinea-pigs was established in hundreds of animals. The *Cl. septique* and Type B toxins had been prepared seven years previously, their toxicities remaining constant over this period. The oedematiens toxin (toxicity worked out by the intramuscular injection of mice) and that of ovitoxicus were of recent preparation, their values being established over a period of months on approximately 70 mice.

The Sheep.—All were Merinos, mostly hammels and of different weights and ages. Nearly all had been through a louping-ill experiment; some through blue-tongue, black quarter, or various plant-feeding experiments. After the injection (always intravenous) of toxin, at least a week was allowed to elapse before the result was recorded. In some cases, sheep received three different toxins at different times. However, sheep which survived the injection of *Cl. welchii*, Type B toxin never received that of *Cl. welchii*, Type D and *vice versa*.

When experimentation indicated the approximate fatal dose, further tests were put up in which so much toxin per kilo-body-weight was given.

Results.

Cl. septique.

Number of sheep used: 38.

Weights varied between 20·4 and 32·0 kilograms.

TOXICITY.

Irrespective of Weight.		Per Kilogram Body weight.		Effect of injecting 2·6–3·3 mgm. per kilogram.
Mgm.	Result.	Mgm.	Result.	Weight (Kg.).
120	†	4·4	†	Less than 22 †††////
110	†	3·8	†	22–25 ††††////
100	†/	3·5	†/	25·3–27·5 †††††††//
90	†††/	3·3	††	28 or more ††††††//
80	††††/	3·0	††††//	
72, 74, 76	†††//	2·8	†//	
70	†††/	2·6	†††††††//	
66	††/	2·3	/	
60, 62	††/	2·2	†††//	
54	†/	1·7	///	
44, 46, 48, 50	†/////			
40	†/			

M.L.D. mice (15–18 gms.) 0·1–0·2 mgm. † = death.
M.L.D. guinea pigs (250–300 gms.) 3·0 mgm. / = live.

To be certain of killing a mouse, 0·2 mgm. was necessary, although 0·1 mgm. frequently did so. The same variation occurred in sheep, some being killed with 2·2 mgm. per kilogram and some living after having received 3·0-3·5 mgm. per kilogram. It would appear that more than 3·0 mgm. per kilogram was necessary to be reasonably certain of a lethal effect. Taking the lethal doses at 0·2 mgm. and 3·5 mgm. per kilogram for the mouse and sheep respectively, the ratio of mouse to kilogram sheep-body-weight is 1 : 17·5.

The time elapsing between injection and death varied between four hours (3·5 mgm. per kilogram) and 30 hours (2·6 mgm. per kilogram). However, as a rule, sheep injected during the day with a lethal dose were dead next morning.

Cl. welchii, Type B.

Number of sheep used: 18.
Weights varied between 12 and 32 kilograms.

TOXICITY.

Irrespective of weight.		Per kilogram body weight.	
Mgm.	Result.	Mgm.	Result.
20–24	**	1·1–1·55	***
16–17	*****	0·75–0·9	***
15		0·5–0·65	****
10–12	***	0·35–0·45	
7			

M.L.D. mice (15–18 gms.) 0·02 mgm. * death.
M.L.D. guinea pigs (250–300 gms.) 0·14–0·2 mgm. live.

Taking the sheep M.L.D. to be 1·2 mgm. per kilogram, the mouse to sheep ratio is 1 : 60. The interval between injection and death varied between one hour (1·1 mgm. per kilogram) and four days (0·66 mgm. per kilogram). Four sheep died within four hours, three in from 18 to 36 hours, and three in from 3 to 4 days.

Cl. welchii, Type D.

Number of sheep used: 23.
Weights varied between 14 and 35 kilograms.

TOXICITY.

Irrespective of weight.		Per kilogram body weight.	
Mgm.	Result.	Mgm.	Result.
100	*	6·6	*
10, 15, 20, 40	****	2·5	*
7, 8	**	0·7, 0·75, 1·0	***
4, 5	*	0·25	**
2·0–2·5	*****	0·14, 0·17	**
1·8		0·09–0·1	****
1·0		0·05, 0·065	
0·5, 0·7		0·04	
		0·025	

M.L.D. mice (15–18 gms.) 0·008 mgm. * death. live.

Cl. welchii, Type D toxin gave a sharper end point in sheep than either *Cl. septique* or *Cl. welchii*, Type B, because, with the exception of one sheep, all which received 0·1 mgm. (or more) per kilogram died, whereas those injected with 0·065 mgm. (or less) per kilogram lived. Taking 0·1 mgm. per kilogram as the lethal dose, the mouse : sheep ratio is 1 : 12·5.

The toxin killed quickly, 0·25 mgm. (or more) per kilogram killing in 1-4 hours. Doses around the M.L.D. killed in less than 24 hours, i.e. overnight the animals were dead, only one sheep (0·14 mgm. per kilogram) surviving this period, to die, however, within 48 hours. However, even after 24 hours this animal was moribund.

Cl. oedematiens.

Number of sheep used : 6.

Weights varied between 19 and 24·5 kilograms.

TOXICITY.

Irrespective of weight.		Per kilogram body weight.	
Mgm.	Result.	Mgm.	Result.
200	†	8·5	†
150	†	8·0	†
150	†	7·5	†
150	/	4·0	/
50	/	2·0	/
10	/	1·0	/

M.L.D. mice (15–18 gms.) intramuscular injection—0·06–0·08 mgm.
† = death. / = live.

The small number of sheep used does not allow the drawing of conclusions, but assuming 7·0 mgm. per kilogram to be a lethal dose for sheep, the mouse to sheep kilogram ratio is in the region of 1 : 100.

The three animals which succumbed, died within 24 hours.

Summary.

The lethal dose of the toxins of four anaerobes has been ascertained in mice, sheep, and in two instances in guinea-pigs also. The mouse : kilogram-body-weight-sheep ratio is as follows :—

Cl. septique 1:17·5, *Cl. welchii*, Type B 1:60, *Cl. welchii*, Type D 1:12·5 and *Cl. oedematiens* 1:100. The last ratio was arrived at by the use of only six sheep and is thus approximate.

References.

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