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THE TOXIGENIC FEATURES OF STRAINS OF THE DIPHTHERIA BACILLUS ISOLATED FROM HORSES AND FROM A MULE.

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IN December, 1920, Capt. F. C. Minett, R.A.V.C., of the Royal Army Veterinary School, Aldershot, published an account of diphtheria bacilli isolated by him from eleven horses and one mule. He states that during the last year of the War and for some months after the Armistice the laboratory at the Army Veterinary School received for examination numerous specimens of pus derived chiefly from suspected cases of ulcerative lymphangitis; a condition in horses in which swelling of the lower parts of a limb or limbs is accompanied by abscess formation followed by ulceration. From this material he collected and investigated a number of diphtheroid strains including the bacillus of Preisz-Nocard and in the course of his inquiry discovered twelve strains of the diphtheria bacillus.

Nine of the strains were cultivated from lesions of the type named; the source of the remaining three is given below.

(1) A swab from a trephine opening in the cheek of a horse. A trephining operation had been practised prior to February, 1920, to relieve a nasal discharge. Although the discharge lessened and the animal remained in excellent condition the operation wound proved obstinate in healing and was still open on 17 October, 1920, when *B. diphtheriae* and streptococci were isolated from it. (Culture D 34 referred to below in the text and in the tables.)

(2) Pus from acneiform skin lesions. These consisted of a dozen superficial suppurating areas covered by scabs and mostly confined to the region of the withers; each was about the size of a threepenny bit.

(3) Pus from a contused fetlock. The lesion was not regarded as a lymphangitis.

Capt. Minett gives a detailed description of the characters of the twelve strains and states that five of them proved to be toxic; the remainder yielded no demonstrable toxin. He kindly sent six of the cultures to this laboratory; our thanks are due to him for the opportunity of examining them.

Particulars of the source and date of isolation of the six cultures forming the basis of the present communication are given in Table I; the symbols chosen by Capt. Minett to designate the cultures have been retained.

Microscopically the cultures are in no way distinguishable from the diphtheria bacillus. When grown for toxin production in Erlenmeyer flasks all give a typical surface film and in the case of "L" culture—the one mostly worked with—the "curtains" characteristic of a heavy, coherent, rapidly growing pellicle of the *B. diphtheriae* are in some batches exceptionally well developed.

Daily estimations of the hydrogen-ion concentration during the period of growth of a number of batches inoculated with cultures "L" and "D 34" were made; the resulting curves resembled those of standard toxin-producing strains of the *B. diphtheriae*.

All the strains formed acid from glucose and maltose but not from mannite and saccharose.

The data concerning toxin production are arranged in Tables II to VIII; and the results obtained on the first attempt at toxin production may be briefly summarized.

(1) Each of the six strains yielded a filtrate of which 0·1 c.c. killed a "250 gramme" guinea-pig within 48 hours when injected under the skin. Later tests showed that the M.L.D. of the various filtrates approximated to 1/100 c.c. more or less and that a mixture consisting of equal volumes of each gave an M.L.D. of 1/100 c.c. (Table II).

(2) 200 M.L.D.'s of the pooled filtrates when mixed with five units of diphtheria antitoxin and injected subcutaneously produced neither local nor general symptoms in a guinea-pig; a result demonstrating complete neutralization of the toxin by diphtheria antitoxin (Table IV).

(3) The L + dose of the several toxins from the six strains varied from 0·5 c.c. to 1 c.c. (Table V).

(4) Intracutaneous tests of the individual toxins gave skin reactions with amounts corresponding with the relation known to exist between the subcutaneous minimal lethal dose and the intracutaneous minimal reacting dose of diphtheria toxin (1 : 1/500) (Tables III and VI).

These results confirm Capt. Minett's conclusion that the cultures are veritable strains of the diphtheria bacillus. Recent experience indicates that two of them, namely "L" and "D 34," are equal from the point of view of toxigenic ability to the routine diphtheria strains—most of them derivatives of the No. 8 bacillus of Park and Williams—that are used in this laboratory. Thus, later batches of toxin made from strains "L" and "D 34" each gave an M.L.D. of 1/450 c.c. and an L + dose of 0·13 c.c. (Tables VII and VIII).

Cobbett (1900) reported an instance of horse diphtheria which in the following circumstances apparently conveyed the infection to a child: a little girl having fallen ill of diphtheria, Dr A. Mearns Fraser, M.O.H. of Portsmouth, while seeking the source of infection, found that a pony belonging to the child's father was ill with a purulent and sanguineous discharge from its nose. From the nasal mucus Dr Fraser isolated a bacillus morphologically indistinguishable from the diphtheria bacillus; Cobbett to whom the culture was sent proved that it was a true diphtheria bacillus.

Capt. Minett's findings strengthen the views put forward by Cobbett that horse diphtheria is of practical importance in relation to the Public Health and that the occurrence of the disease in horses throws light on the comparative frequency of "normal" antitoxin in their blood.

The discovery of the diphtheria bacillus in superficial septic lesions in the horse apart from specific infection of the nasal mucosa is paralleled by recent

observations on the human subject. Thus, there is growing evidence that in Man the *B. diphtheriae* is apt to be associated with a variety of chronic skin lesions or may become implanted upon cutaneous or subcutaneous lesions already infected with pyogenic bacteria, for example, war wounds and friction sores. Martin (1917) isolated virulent diphtheria bacilli from sores of this kind which were refractory to the usual methods of treatment. It is significant that they occurred in men of the Australian Light Horse. Dr Martin informs me that similar observations were made afterwards by others in Egypt and Palestine.

Capt. Minett's observations suggest that reciprocal contagion of horse and human diphtheria happens more frequently than has been hitherto suspected. Inquiries undertaken with this probability in mind when the circumstances of infection are obscure, may lead to the detection and prevention of cases of diphtheritic infection; and may indicate effective treatment with antitoxic serum.

Table I.

Designation, source and date of isolation of 6 out of 12 cultures obtained from septic lesions in horses by Capt. F. C. Minett, R.A.V.C.

Designation	Source	Date of isolation
L	Pus from suspected case of ulcerative lymphangitis in horse at A.	2. vi. 1919
G	,, ,, ,, ,, ,, ,, at M.H.	Prior to 24. vi. 1918
H	,, ,, ,, ,, ,, ,, at C.	6. ii. 1919
O	,, ,, ,, ,, ,, mule at L.	6. ix. 1919
D 16	,, ,, ,, ,, ,, horse at O.	28. x. 1919
D 34	Swab from trephine opening in cheek of horse at W.	17. x. 1920

Tables II, III, IV, V, VI.

Tests on guinea-pigs weighing *circa* 250 grammes with toxins derived from the 6 strains; cultures inoculated on 21. i. 21 and filtered on 28. i. 21.

Table II. Subcutaneous M.L.D.

Date of test	Culture	Dose	Day of death
4. ii. 1921	L	1/100 c.c.	3rd
,,	G	,,	8th
,,	H	,,	6th
,,	O	,,	9th
,,	D 16	,,	—
,,	D 34	,,	6th
10. ii. 1921	Mixture of equal volumes of 6 toxins	,,	4th

NOTE. The animals that died from the subcutaneous inoculation of filtered cultures and of which the deaths are recorded in this and the following tables were examined postmortem and were found to present the appearances characteristic of diphtheria toxaemia in guinea-pigs.

Table III. *Intracutaneous test.*

Date of test	Culture	Dose	Cutaneous reaction on 4th day	Dose	Cutaneous reaction on 4th day
3. ii. 1921	L	1/25,000 c.c.	necrosis + +	1/50,000 c.c.	necrosis +
,,	G	,,	,, + +	,,	,, tr
,,	H	,,	,, + +	,,	,, + +
,,	O	,,	,, +	,,	,, tr
,,	D 16	,,	,, +	,,	,, tr
,,	D 34	,,	,, +	,,	,, tr

NOTE. tr = trace: + = slight: + + = definite.

Table IV. *Neutralization of mixture of toxins of 6 strains by diphtheria antitoxin.*

Date of test	Dose	Result
10. ii. 1921	2 c.c. of mixed toxins = 200 M.L.D.'s + 5 units of diphtheria antitoxin	No local reaction nor general symptoms; progressive increase in weight

Table V. *Subcutaneous test of L+ dose of toxins.*

Date of test	Culture	L + dose
14. ii. 1921	L	0·75 c.c.
19. ii. 1921	G	0·75 c.c.
,,	H	0·75 c.c. nearly*
,,	O	0·75 c.c. nearly†
17. ii. 1921	D 16	1·0 c.c.
21. ii. 1921	D 34	0·5 c.c.

* Death between 5th and 6th day.
† Death between 6th and 7th day.

Table VI. *Intracutaneous test of L+ dose of toxin* "L."

Date of test	Culture	Dose	Result
9. ii. 1921	L	1/500 A.U. + 1/2000 c.c. toxin	0
,,	,,	,, 1/1000 c.c. toxin	0
,,	,,	,, 1/666 c.c. toxin	? tr necrosis
,,	,,	,, 1/500 c.c. toxin	necrosis + +

NOTE. A.U. = antitoxin unit.

Tables VII and VIII.

Tests with later toxins derived from strains "L" and "D 34." "L" culture inoculated on 1. iii. 1921 and filtered on 10. iii. 1921; "D 34" culture inoculated on 2. iii. 1921 and filtered on 11. iii. 1921.

Table VII. *Subcutaneous M.L.D.*

Date of test	Culture	M.L.D. of toxin
21. iii. 1921	L	1/450 c.c.
,,	D 34	1/450 c.c.

Table VIII. *Tests of L+ dose.*

Date of test	Dose	Result with "L" toxin	Result with "D 34" toxin
2. iv. 1921	1 A.U. + 0·2 c.c. toxin	Death within 48 hours	Death within 48 hours
6. iv. 1921	,, + 0·15 ,, ,,	,, ,,	,, ,,
9. iv. 1921	,, + 0·13 ,, ,,	,, ,,	Death on 4th day
4. iv. 1921	,, + 0·10 ,, ,,	No local reaction: g.-pig gained weight	No local reaction: g.-pig gained weight

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