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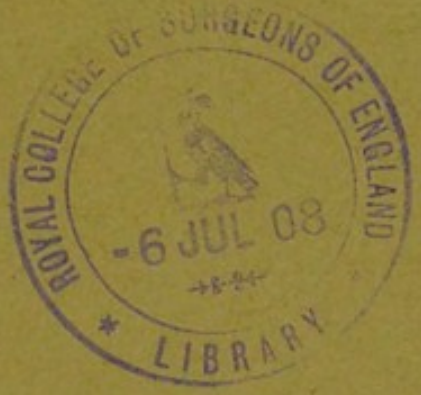

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Upon the Properties of an Antityphoid Serum obtained from the Goat.

By ALLAN MACFADYEN, M.D.

(Communicated by Dr. C. J. Martin, F.R.S. Received March 2,—Read March 8, 1906.)

(From the Bacteriological Department, Lister Institute.)

In this communication I propose to give a brief account of the results obtained by the immunisation of the goat with the cell juices of the *Bacillus typhosus*, in continuation of researches already published which had for their main object the production of an antibody for the endotoxin of the typhoid organism.* These experiments met with partial success and failure. It will be sufficient on the present occasion to say that the goat proved a more suitable animal than the horse for arriving at a solution of the theoretical and practical considerations involved. The object was to arrive at the best method of producing with the material employed an antiendotoxin in adequate amount, with a view to its reapplication to the horse.

During the progress of these researches experiments on analogous lines have been published by Dr. Besredka,† and to these I will, in the first instance, refer. Dr. Besredka describes the results following the intravenous injection into a horse of the intact dead and living typhoid bacilli. The injections of the bacilli were carried out for a period of two years. The serum was tested for antiendotoxic properties against (1) killed and dried cultures of the *B. typhosus*, of which 0·01 gramme killed guinea pigs on intraperitoneal injection; (2) a soluble endotoxin extracted from the dead and dried bacilli, of which 1/8 c.c. killed guinea pigs. The results were as follows: 10 or 15 centigrammes of the dried serum (about 1 c.c. and 1·5 c.c. before drying) neutralised 10 or 15 lethal doses of the killed typhoid culture respectively on intraperitoneal injection into the guinea pig, and the 10 centigrammes of the dried serum neutralised 16 lethal doses of the soluble

* "Upon the Immunising Effects of the Intracellular Contents of the Typhoid Bacillus, as obtained by the Disintegration of the Organism at the Temperature of Liquid Air," by Allan Macfadyen, 'Roy. Soc. Proc.,' March 12, 1903; "Upon the Intracellular Constituents of the Typhoid Bacillus," by Allan Macfadyen and Sydney Rowland, 'Centralblatt für Bakteriologie,' Abth. 1, vol. 30, 1901, No. 20; and vol. 34, 1903, Nos. 7 and 8.

† "Etudes sur le bacille typhique et le bacille de Peste," par Dr. Besredka, 'Annales de l'Institut Pasteur,' July 25, 1905: "De l'Antiendotoxine Typhique," par Dr. Besredka, *Ibid.*, February, 1906.

endotoxin. To obtain this low antitoxic value in the horse a period of two years was apparently necessary.

The typhoid cell juices employed in my experiments were prepared as follows: Virulent typhoid bacilli were cultivated on nutrient agar in Roux bottles for 18 hours at blood heat. The growth was brushed off and washed with distilled water in a centrifuge for half an hour. The bacilli were then triturated at the temperature of liquid air in the grinding pot already described by Rowland and myself.* The time allowed was 30 minutes per gramme of bacilli. The mass was taken up in 1/1000 solution of caustic potash, and centrifuged for two hours. The supernatant fluid was pipetted off, and represented a 10-per-cent. extract of the ground mass. This was treated with chloroform vapour for half an hour. The juices obtained under these conditions were sterile and toxic on intravenous injection into the test animals employed. The endocellular toxins obtained in this manner are unstable bodies, and the juices rapidly decrease in toxicity. The experiments with kept juices did not indicate that their injection would lead to any marked tolerance for fresh and markedly toxic juices; the probabilities were against this easier and less risky method of procedure. Resort was accordingly made to the use of fresh and acutely toxic juices, which contained on an average 10 to 12 milligrammes of solid matter per cubic centimetre. The fresh juices on intravenous injection were acutely toxic for the goat. The first goat died after the injection of 1 c.c., whilst 1/10 c.c. killed several animals. In two instances 1/20 c.c. killed within 12 hours. Death was preceded by profuse diarrhœa and collapse. Where death did not occur, the injection of 1/20 c.c. was followed by illness and diarrhœa, and 1/30 c.c. rendered certain animals ill, but with less acute symptoms. It was obvious that the intravenous injections would have to be carefully carried out to avoid unduly depressing or killing the animals.

A goat after receiving 1/20 and 1/10 c.c. cell juice died, whilst another, after the injection of doses of 1/20, 1/10, 1/2, and 1 c.c. at intervals of seven days, died within four hours after receiving the last injection. The indication, it appeared to me, was to start with small sublethal doses, and to raise them very gradually at duly spaced intervals. One injection weekly proved to be the safest procedure, and in the later experiments the same dose was repeated until it failed to produce toxic symptoms in the animal. A higher dose was then given and the process repeated. This method proved successful, as the animals became tolerant to otherwise fatal doses of the toxins.

In the experiments here recorded the antiendotoxic action of the serum

* 'Centralblatt f. Bakter.,' vol. 34, No. 7, 1903.

was tested by intravenous injection in rabbits. The endotoxin likewise killed rabbits acutely, *e.g.*, 1/10 c.c. killed with diarrhœa and collapse, and at times within two hours, whilst 1/20 c.c. was not infrequently a lethal dose. The onset of the toxic symptoms usually occurred in from one to two hours. The conditions were therefore sufficiently stringent in respect of any antitoxic action of the treated goats' serum. I satisfied myself that neither the goats' nor rabbits' serum possessed any appreciable antitoxic power for the toxins employed. Controls were subsequently found to be unnecessary. Three cubic centimetres of normal goat and rabbit serum did not neutralise two or ten lethal doses of the cell juices. The serum of the treated goats was tested against multiple lethal doses of the endotoxin. An estimate was likewise made of the agglutinative and bacteriolytic properties of the serum. I now proceed to an account of the first successful immunising experiment.

Billy Goat I.—Received following intravenous injections of toxic cell juices of *B. typhosus*:—

1905.			
May	16	1/20 c.c.	Ill.
„	24	1/10 „	Ill.
June	2	1/5 „	Ill.
„	9	1/2 „	Ill.
„	16	1 „	Ill.
„	23	1 „	Ill.
„	30	1 „	Ill.

1905.			
July	7	1 c.c.	No symptoms.
„	14	1·5 „	No symptoms.
„	21	1·5 „	No symptoms.
„	28	2 „	Ill.
Aug.	4	2 „	No symptoms.
„	11	2·5 „	Dead next day.

It was evident that with succeeding animals an even more careful system of dosage would have to be adopted. The goat was bled at intervals and its serum tested. In all instances the serum-toxin mixture was kept at 37° C. for 30 minutes previous to intravenous injection in the rabbit. The results are given in Table II.

I.—Test of Normal Goat's Serum.

Rabbit	I	3 c.c. serum + 1 c.c. toxin.	Dead.
„	II	2 „ + 1 „ „	Dead.
„	III	Control: 1/10 „ „	Dead.
„	IV	„ 1/20 „ „	Diarrhœa.

Three cubic centimetres of normal serum did not neutralise 1 c.c. of a toxic cell juice of which 1/10 killed and 1/20 c.c. produced acute diarrhœa in the rabbit.

II.—Tests of Serum of Goat I.

Date.	Test animal.	Amounts injected.	Results.
June 23.....	Rabbit 1	3 c.c. serum + 1 c.c. toxin.	Alive.
„	„ 2	Control, 1 c.c. toxin.	Dead.
July 7	„ 1	3 c.c. serum + 2 c.c. toxin.	Alive.
„	„ 2	3 „ + 1 „	„
„	„ 3	2 „ + 1 „	„
„	„ 4	Control, 2 c.c. toxin.	Dead, 4 hours.
„	„ 5	„ 1/20 „	„ 18 „
July 14	„ 1	3 c.c. serum + 2 c.c. toxin.	Alive.
„	„ 2	2 „ + 2 „	„
„	„ 3	1 „ + 1 „	„
„	„ 4	1/10 „ + 1 „	„
„	„ 5	Control, 1/10 c.c. toxin.	Dead.
„	„ 6	„ 1/20 „	Diarrhœa and collapse.
July 21	„ 1	1 c.c. serum + 1 c.c. toxin.	Alive.
„	„ 2	1/2 „ + 1 „	„
„	„ 3	1/10 „ + 1 „	„
„	„ 4	Control, 1 c.c. toxin.	Dead.
„	„ 5	„ 1/10 „	Diarrhœa and collapse.
„	„ 6	„ 1/15 „	„ „ „
August 4	„ 1	1/20 c.c. serum + 1 c.c. toxin.	Alive.
„	„ 2	1/50 „ + 1 „	„
„	„ 3	1/100 „ + 1 „	„
		Control not made.	
August 11.....	„ 1	1/20 c.c. serum + 1 c.c. toxin.	„
„	„ 2	1/50 „ + 1 „	„
„	„ 3	1/100 „ + 1 „	Dead.
„	„ 4	Control, 1 c.c. toxin.	„ 2 hours.
„	„ 5	„ 3/10 „	„ 18 „
„	„ 6	„ 1/10 „	„ 18 „

The death of the goat prevented further injections and tests being made. It will be seen that demonstrable antiendotoxins had developed in the goat and that the results were encouraging. After 12 injections of typhoid cell juice, 1/50 c.c. of the goat's serum protected a rabbit against 10 lethal doses of the typhoid endotoxin. This property was not present in 3 c.c. of normal goat's serum. It is not my intention in this paper to refer in detail to the production of agglutinins and bacteriolysins. It will be sufficient to state that whilst the amount of agglutinins present in the serum varied, the highest titrate obtained was in a dilution of 1/1,000,000. After 12 injections of typhoid cell juice, 1/10000 c.c. of the goat's serum protected guinea-pigs on intraperitoneal injection against 10 lethal doses of the *B. typhosus*. The serum was, therefore, proved to possess marked anti-endotoxic, agglutinative and bacteriolytic properties.

The next step was to control these results by the immunisation of fresh

goats, and to carry out the process in a still more careful manner. The method adopted is given in the following schedule :—

Nanny Goat II.—Received following intravenous injections of toxic cell juices of *B. typhosus*—

1905.				1905.		
Oct. 13	1/20 c.c.	Ill.		Dec. 22	1/8 c.c.	No symptoms.
„ 20	1/20 „	No symptoms.		„ 29	1/6 „	Ill.
„ 27	1/15 „	Ill.		1906.		
Nov. 3	1/15 „	No symptoms.		Jan. 5	1/3 „	No symptoms.
„ 10	1/10 „	No symptoms.		„ 12	1/6 „	No symptoms.
„ 17	1/10 „	Ill.		„ 19	1/4 „	No symptoms.
„ 24	1/10 „	Ill.		„ 26	1/4 „	No symptoms.
Dec. 1	1/10 „	No symptoms.		Feb. 2	1/3 „	Ill.
„ 8	1/8 „	Ill.		„ 9	1/3 „	No symptoms.
„ 15	1/8 „	No symptoms.				

III.—Tests of Serum of Goat II.

Date.	Test animal.	Amounts injected.	Results.
December 29	Rabbit 1	1/20 c.c. serum + 1 c.c. toxin.	Alive.
„	„ 2	1/50 „ + 1 „	Dead.
„	„ 3	1/100 „ + 1 „	Alive.
„	„ 4	Control, 1/5 c.c. toxin.	Dead, 2 hours.
„	„ 5	„ 1/10 „	Diarrhœa and collapse.
January 19, 1906...	„ 1	1/50 c.c. serum + 1 c.c. toxin.	Alive.
„ ...	„ 2	1/100 „ + 1 „	Dead.
„ ...	„ 3	Control, 1/10 c.c. toxin.	„
January 26	„ 1	1/50 c.c. serum + 1 c.c. toxin.	Alive.
„	„ 2	1/100 „ + 1 „	Dead.
„	„ 3	Control, 1/10 c.c. toxin.	„
February 2	„ 1	1/10 c.c. serum + 1 c.c. toxin.	Alive.
„	„ 2	1/20 „ + 1 „	„
„	„ 3	1/50 „ + 1 „	„
„	„ 4	1/50 „ + 1/2 „	„
„	„ 5	Control, 1/10 c.c. toxin.	Dead, 2½ hours.
„	„ 6	„ 1/20 „	„ 2 „
„	„ 7	„ 1/30 „	„ 18 „
February 9	„ 1	1/50 c.c. serum + 1 c.c. toxin.	Alive.
„	„ 2	1/50 „ + 1 „	„
„	„ 3	1/100 „ + 1 „	Dead.
„	„ 4	1/100 „ + 1/2 „	Alive.
„	„ 5	Control, 1/10 c.c. toxin.	Dead.
„	„ 6	„ 1/20 „	Diarrhœa and collapse.

Three goats are in process of immunisation by this method of small and gentle dosage and have not up to the present succumbed. It will be observed that whilst in the case of Goat I the dosage was raised from

1/20 to 2½ c.c., the dose reached in Goat II when the final test was made only amounted to 1/3 c.c. of juices containing 10 to 12 milligrammes of solid matter. The figures demonstrate the production in considerable amount of an antiendotoxin. The highest titrate obtained was a neutralisation of 30 ascertained lethal doses of endotoxin by 1/50 c.c. of the serum of Goat II. The results confirmed those obtained in the case of Goat I, and were equivalent despite the injection of a much smaller gross amount of typhoid cell juice. The raising of the antiendotoxic value of the serum had likewise been accomplished without any serious disturbance in the health of the animal. This was the difficulty which had retarded the progress of the initial experiments.

Some tests were made as to the action of the serum on the endotoxin when each was injected separately. The results were as follows:—

Rabbit I.—Fifteen lethal doses of typhoid cell juice were injected into a vein of the right ear and 1 c.c. serum into a vein of the left ear. A second injection of 1 c.c. serum was given 20 minutes later. The animal survived.

Rabbit II.—Received five lethal doses of the toxic juice in the right ear and 1 c.c. serum in the left ear. The animal survived.

Rabbit III.—Received five lethal doses intravenous and ¾ hour later at the onset of toxic systems 2 c.c. of serum. The rabbit survived. The serum, therefore, acted on separate injection into the blood stream.

The serum of Goat II was also tested against the endotoxin of the cholera organism. One-half cubic centimetre of typhoid serum was added to three lethal doses of cholera cell juice, and the mixture, after incubation for 30 minutes at blood heat, was injected into a rabbit. The animal died 2½ hours after the injection. One-half cubic centimetre of a typhoid serum, which had been found to protect against 30 lethal doses of typhoid endotoxin, did not protect a rabbit against three doses of cholera endotoxin, and was to this extent specific. The agglutinative power of the serum rose to 1/1,000,000, and 1/10000 c.c. protected the guinea-pig against 10 lethal doses of the typhoid bacillus.

The serum was finally tested in dilutions of 1/10, 1/100, and 1/500 c.c. for any evidence of a precipitin reaction on the fresh typhoid cell juices. The result was negative. There had been no appreciable development of precipitins in a serum containing at the time, when it was tested, marked antiendotoxic properties.

Conclusions.

1. The intravenous injection of the goat with the toxic cell juices of the *B. typhosus* (obtained under the conditions described) in small and carefully regulated doses resulted in the production of an antiendotoxin.

2. The antiendotoxic value, as so far tested, reached a point at which 1/50 c.c. of the serum neutralised 30 lethal doses of the toxic typhoid cell juice. This action was not demonstrable in 3 c.c. of normal goat's serum, and was obtained after about four months' treatment of the goat. The results, after a more rapid method of immunisation, are better *qua* goat and rabbit than those obtained by Dr. Besredka in the course of two years with dead and living bacilli *qua* horse and guinea-pig.

3. The serum was also agglutinative for the *B. typhosus*, the titrate rising to 1/1,000,000.

4. The serum was also bacteriolytic, 1/10000 c.c. neutralising 10 lethal doses of the *B. typhosus*.

5. The serum did not give a precipitin reaction with typhoid cell juices.

6. The serum whilst neutralising the typhoid did not neutralise the cholera endotoxin.

My next step will be to test in how far it is possible to obtain analogous results in the horse.

Analogous results have been obtained indicating the production of an antibody for the endotoxin of the cholera organism.

I am greatly indebted to Mr. E. T. Thompson for invaluable aid as well as for an important modification which has rendered the grinding process void of danger.

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