SOME IMMUNITY EXPERIMENTS ON HYPOPHYSECTOMISED ANIMALS

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It is well known that removal of the pituitary gland is followed by alterations in a number of metabolic processes. It was therefore thought of interest to determine whether these alterations involved any changes in certain immune processes, since very little is known at present of the factors controlling them. Our immunity experiments were made on rabbits and consisted in determining the complement content, the bactericidal power and the opsonic activity of the rabbits' serum.

Technique

Eleven mature animals of both sexes and various breeds weighing about 2·5–3·5 kg. each were used. Hypophysectomy was performed by the orbital route (Firor, 1933). The completeness of the operation was checked by macroscopic examination of the sella, which is probably very reliable, since the sella is an almost completely closed cavity. Removal was complete in six animals. In four animals, small fragments were found embedded in fibrous tissue, while in one animal the greater part of the gland was left in the cavity.

The rabbits were bled (using aseptic precautions) from the marginal ear vein a fortnight before operation, and at weekly intervals up to eight weeks after operation, when the nine animals which had still survived were killed. Two of the animals died at periods of four and five weeks respectively after operation.

The serum was separated from the blood and the complement titre determined as follows: the serum was diluted 1 in 5 with normal saline and 0·1, 0·2, 0·3, 0·4 and 0·5 c.c. quantities of the dilute serum were added to 0·5 c.c. of a 4 per cent. suspension of sensitised ox corpuscles; the tubes were incubated at 37° C. for two hours and the degree of haemolysis recorded.

The bactericidal power of the serum was determined by adding 0·05 c.c. of a very light bacterial suspension (100 millions per c.c. in bouillon) to 0·5 c.c. of sterile serum in a series of tubes; the tubes were incubated at 37° C. and loopfuls of the serum-bacterial mixtures were withdrawn (*a*) immediately and (*b*) after varying periods of incubation and inoculated on heated blood-agar plates; the plates were then incubated at 37° C. and the amount of growth was

noted on the following day; the time periods for subculture were four, eight and thirteen hours. The test organisms were *B. dysenteriae* (Flexner), *B. enteritidis* (Gaertner), *B. typhosus*, *V. cholerae*, and *B. paratyphosus B*, these organisms being normally susceptible to the bactericidal action of rabbit serum. A set of tubes similar to the above was also inoculated except that the serum used was previously heated at 55° C. for half an hour. This heating destroys the serum complement and also removes the bactericidal power, and the last experiment therefore served as a control.

The bactericidal action of serum on *B. anthracis* is different in type from the activity shown towards the organisms mentioned above. Anthracidal action does not require the presence of complement since it is exerted by heated serum. In view of this it was decided to test the anthracidal activity of the rabbit serum, both heated and unheated, as affording a test of another immune mechanism.

The opsonic activity of the serum was tested by adding a few drops of a *Staphylococcus* suspension and a drop of a washed leucocytic cream to 0·25 c.c. of the serum, and incubating for three hours at 37° C. Films were then made and stained with Leishman's stain and the degree of phagocytosis of staphylococci by the leucocytes was estimated. Controls were put up using saline instead of serum, and these showed no phagocytosis.

RESULTS

The determination of the complement titre showed that 0·1 c.c. of diluted serum gave little or no haemolysis, 0·2 c.c. gave a trace, 0·3 c.c. partial haemolysis, and 0·4 and 0·5 c.c. gave complete haemolysis. This was the average finding (with minor variations) for all the eleven rabbits both before hypophysectomy and at all stages following the operation. No appreciable differences were noted at any particular stage of the experiment.

The results of the tests of opsonic activity showed that the sera from all the animals consistently promoted marked phagocytosis. When the bacteria were treated with serum the majority of leucocytes was found to have ingested large numbers of them, whilst untreated bacteria were not thus phagocyted by the leucocytes. Taking the experiments as a whole there was nothing to suggest that the opsonic activity had been affected in any way by hypophysectomy.

A typical protocol of experiments on the bactericidal power is given in Table I which shows that the serum both before and after the operation had a marked bactericidal effect, as revealed by the results obtained with all the organisms used for the test. There is a marked bactericidal action of both heated and unheated serum taken both before and after the operation when *B. anthracis* is used as test organism, this result showing that the heat-stable anthracidin undergoes no change after hypophysectomy. The table gives one experiment only, but in none of the experiments was there any evidence of appreciable change in the bactericidal power of the serum following the removal of the pituitary gland.

Discussion

The data from all the experiments described show consistently that removal of the pituitary gland in the rabbit does not cause any alteration in certain immune properties of the serum. It will be understood that the tests used, namely, complement titration, and bactericidal and opsonic activity, do not yield exact quantitative data which can be used for accurate comparisons. One great difficulty is that the tests on the sera from the hypophysectomised

Table I. *Typical protocol of experiments on bactericidal activity before and after hypophysectomy*

Organism	Growth after subculturing							
	At once	After 4 hours	After 8 hours	After 13 hours	At once	After 4 hours	After 8 hours	After 13 hours
	Before hypophysectomy				After hypophysectomy			
B. typhosus	+ +	14	1	—	+ +	10	—	—
B. enteritidis (Gaertner)	+ +	+/2	2	—	+ +	23	—	—
V. cholerae	+ +	6	—	—	+ +	2	1	1
B. dysenteriae (Flexner)	+ +	16	5	—	+ +	28	5	—
B. paratyphosus B	+ +	+	+/2	+/2	+ +	+	+/2	+/2
B. anthracis (using unheated serum)	+ +	4	—	—	+ +	7	2	—
B. anthracis (using serum heated 30 min. at 55° C.)	+ +	+/2	1	2	+ +	+/2	4	1

+ + = heavy growth. + = good growth. +/2 = slight growth.
The numbers are the numbers of colonies where it was possible to count them.

animals must necessarily be carried out two, four and even ten weeks after the results have been obtained for the sera before operation. For this and other reasons all that can be asserted is that hypophysectomy causes no apparent change in the immune properties that we have studied.

Furthermore since samples of blood were withdrawn from all the animals at weekly intervals during a period of eight weeks, and since each sample (15 c.c.) represents an appreciable fraction of the total blood content of the animal, the continued presence of immune properties in definitely demonstrable amounts indicates that not only are they retained, but that they are continuously renewed in the total absence of the hypophysis.

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REFERENCE

W. M. Firor (1933). *Amer. J. Physiol.* **104**, 204.

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