THE EFFECT OF DIFFERENT SPECIES' LENS ANTISERA ON PREGNANT MICE AND RATS AND THEIR PROGENY.*

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1. Introduction.

A SERIES of experiments were undertaken, which had for their object the testing of the effects of injections of various species' lens antisera into pregnant mice and rats. Guyer and Smith found that by injecting anti-rabbit lens serum into pregnant female rabbits some of the progeny born under such treatment had defective eyes, and that this defect became hereditary. Further, they found that one of a group of rabbits injected with rabbit lens gave birth to young with defective eyes.

According to Bordet, lens is organ specific as regards immunity reaction, that is, an immune serum prepared against lens from one species will react in a similar manner with the lens proteins of other species.

Anti-lens serum was prepared in various ways, different species' lens being used as antigens. Rat lens, ox lens, and sheep lens were each used as antigens, and the rabbit and the fowl used to provide the serum. The injection of antigen was continued until a definite precipitin reaction could be demonstrated. The animals were then bled, the clear serum drawn off, bottled, and used for injection into the pregnant animals as quickly as possible—that is, within a few weeks.

Active immunisation was also attempted with rats and mice, using various species' lens as antigens.

2. Passive Immunisation.

The injection of crystalline lens into the blood stream, peritoneal cavity, or subcutaneously, causes anti-crystalline lens to be formed, as can be demonstrated by the precipitin

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test. But in addition to these precipitins it is probable that there are other forms of antibodies generated. The common antibodies—cytolysins, precipitins, agglutins, and antitoxins—are not identical, and one would have to demonstrate their presence before concluding that they had been formed. Also when lens is used as an antigen, one is injecting at least a sclero-protein, two globulins and an albumin, and it is probable that specific antibodies are formed against each of these. The standard set in these experiments was to continue with the injection of the lens until a precipitin for the protein of the lens which was soluble in normal saline solution could be demonstrated.

The Precipitin Test.—When it was desirable to test the blood, a few cubic centimetres were drawn from the auricular vein in rabbits, and from the axillary vein in fowls. This was allowed to stand for twenty-four hours, and the clear serum pipetted off. The solution of the lens was prepared by thoroughly grinding up the lens, mixing with normal saline, and then passing the mixture several times through the one filter paper, until a water clear filtrate was obtained. Tubes of 1 c.c. capacity were used for the test. From 0.2 c.c. to 0.5 c.c. of the different lens solution were put in a row of these tubes, and the serum to be tested was then run down the sides of the tubes. It formed a layer at the bottom, and in most cases the line of demarcation between the two fluids was sharp.

TABLE I.

Precipitin Test of Anti-Lens Sera.

Antiserum from Rabbit.	Solution of Lens and Vitreous Humour.				Control.	Remarks.
	Sheep Lens 1 in 40 c.c.	Ox Lens 1 in 80 c.c.	Rat Lens 1 in 10 c.c.	Mouse Lens 3 in 10 c.c.	Sheep Vitreous Humour 1 c.c. in 10 c.c.	
a	+++	+++	+++	+++	–	Bled for serum.
b	++	++	++	+	–	,,
c	–	–	–	–	–	For further injection.
d	–	–	–	–	–	,,
e	+	+	+	+	–	Bled for serum.

When precipitin was present a cloudy ring developed within a few minutes at the junction of the fluids. Fowl serum, however, being usually of an orange colour, did not allow this ring to be readily seen, but after standing for over an hour the precipitin, if present, was easily demonstrable in the serum. It was found that undiluted serum gave the best results, and a strength of lens solution that was found to give satisfaction was used throughout.

Only serum that reacted to this precipitin test was used. Table I. gives the details of an actual test, such as was made with all the sera. Rabbits *a*, *b*, *c*, *d*, had received eight injections of sheep lens; *e* — ox lens. The plus signs indicate degree of density of the ring; minus = negative.

Preparation of Antisera.—The foregoing Tables, II., III., and IV., give the details of preparation of antisera. In all, seven different antisera were prepared and injected chiefly into mice. For Nos. 1, 2, 3 (Table II.) rat lens were used as antigen; for 4 and 5 (Table III.) sheep lens; for 6 (Table III.) ox lens; while for No. 7 (Table IV.)—the control serum—ox vitreous humour was the antigen. The rabbit was used as serum animal in all these cases except in No. 2, for which the fowl was used.

3. Injection of Antisera and Breeding Results.

The mice for injection were carefully selected. Only albinos were used, owing to the fact that the unpigmented eye is easily examined. It was found from extensive breeding operations with mice that if young virgin females that have just reached maturity are mated with a vigorous male, they are almost certain to become pregnant within a few days. In this experiment selected females were mated ten days before injections started—six females to one male in each mating cage. They were separated when the litters were due, so that each female had a separate cage in which to rear her litter. They were all related mice of approximately the same age, and they were all kept under similar conditions. When a litter was born the date and number of the young were noted, so that the exact time of injection in relation to

pregnancy could be determined. The duration of pregnancy in rats and mice is twenty-one days.

To determine the state of the eyes in the F_1 mice, these were left till about half grown, and then each mouse was placed on the hand with its head toward the light. With albino rodents the inner margin of the iris can be distinctly seen and the lens is clear and transparent. Examination after death is of no use, as the lens quickly becomes opaque in all cases.

The details of the injection of the antisera and the breeding results are all given in Table V. In each group No. 1 mouse received 0.5 c.c. for each of the three injections, No. 2 received 0.75 c.c., and so on, the dose being increased by 0.25 c.c. for the following number in the group. The last mouse in each group received whatever amount of serum was left in the ampoule.

The table shows that a number of the mice became pregnant after the injection. As most of these were mice that received anti-rat lens serum, it is quite probable that in the first place these aborted as a result of injection of a specifically toxic serum, and then became pregnant a second time. This was supported by the fact that embryonic fragments were found in these mating cages prior to separation but after injection.

No. 1 antiserum (anti-rat lens) was injected into two groups (*a*) and (*b*). In (*a*) six mice were mated, but as one died before injection, this was omitted from the records. Of the eleven mice injected in the two groups, five produced litters when due, one a litter from a delayed pregnancy, and five were infertile.

No. 2 antiserum (anti-rat lens), for the production of which the fowl was used, was evidently a toxic serum. Only one litter was produced within time, and that was from the mouse that received the minimum dose (0.5 c.c.). There were four litters from pregnancies after injection, and one infertile.

No. 3 antiserum (anti-rat) was even more toxic than No. 2. A living litter was produced by the mouse receiving the minimum dose, a dead litter from the next in the series, while the remainder were infertile.

TABLE V.

Injection of Antisera and Breeding Results.

Antiserum.	No. of Mouse.	No. c.c. Serum injected in each of 3 Doses.	No. Days Pregnant when injected.*	No. Born to ♀ ♀ injected during Pregnancy.	No. that lived till Examination.	No. from Pregnancies after Injection and Infertile (Inf.).
No. 1. (*a*) Anti-rat	1	0·5	9, 11, 13	5	5	...
	2	0·75	12, 14, 16	2	2	...
	3	1·0	11, 13, 15	3	3	...
	4	1·25	...	...	...	Inf.
	5	1·25, 0·75, 1·5	...	...	...	Inf.
No. 1. (*b*)	1	0·5	...	...	...	Inf.
	2	0·75	...	...	...	Inf.
	3	1·0	3, 6, 8	1	0	...
	4	1·25	−7, −4, −2	...	...	4
	5	1·5	7, 10, 12	3	3	...
	6	1·75, 0·75, 1·25	...	...	...	Inf.
No. 2. Anti-rat	1	0·5	7, 9, 11	5	4	...
	2	0·75	−17, −15, −13	...	...	6
	3	1·0	−11, −9, −7	...	...	2
	4	1·25	−21, −19, −17	...	...	4
	5	1·5	...	...	..	Inf.
	6	1·25, 1·75, 0·75	−6, −4, −2	...	...	4
No. 3. Anti-rat	1	0·5	4, 7, 9	7	3	...
	2	0·75	...	Dead litter	...	...
	3	1·0	...	...	...	Inf.
	4	1·25	...	...	...	Inf.
	5	1·5	...	...	...	Inf.
	6	2·4, 2·0, 2·0,	...	...	...	Inf.
No. 4. Anti-sheep	1	0·5	6, 8, 10	5	1	...
	2	0·75	−23, −21, −19	...	...	6
	3	1·0	6, 8, 10	5	5	...
	4	1·25	6, 8, 10	7	7	...
	5	1·5	6, 8, 10	4	4	...
	6	1·75	−1, 1, 3	1	1	...
No. 5. Anti-sheep	1	0·5	7, 10, 12	6	6	...
	2	0·75	−2, 1, 3	4	4	...
	3	1·0	−4, −1, 1	4	3	...
	4	1·25	3, 6, 8	3	0	...
	5	1·5	...	...	...	Inf.
	6	1·0, 1·0, 1·75	0, 3, 5	2	0	...
No. 6. Anti-ox	1	0·5	6, 8, 10	4	1	...
	2	0·75	...	...	...	Inf.
	3	1·0	6, 8, 10	4	1	...
	4	1·25	6, 8, 10	4	Dead litter	...
	5	1·5	6, 8, 10	3	2	...
	6	1·75, 1·75, 2·0	−5, −3, −1	...	...	4
Control. No. 7. Anti-vitreous humour (ox)	1	0·5	2, 5, 7	6	6	...
	2	0·75	4, 7, 9	6	4	...
	3	1·0	4, 7, 9	8	8	...
	4	1·25	3, 6, 8	5	5	...
	5	1·5	...	...	...	Inf.
	6	1·5, 1·75, 1·75	3, 6, 8	5	5	...

* When the injections were given prior to pregnancy, this is indicated by a *minus* sign.

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Nos. 4 and 5 antisera (anti-sheep) gave similar results. In each group of six five mice gave litters within time. In No. 4 group there was one delayed, and in No. 5 one infertile.

With No. 6 antiserum (anti-ox) there were four litters within time of which one was found dead. There was one infertile and one delayed.

With the control serum, No. 7 (anti-ox vitreous humour) normal breeding results were obtained. There were five litters within time and one infertile, and the litter average was considerably higher than with the others.

There seems to be a great difference in the toxicity of these sera. The anti-rat lens sera undoubtedly was very toxic, for in these groups out of twenty-three female mice which had been mated under conditions favourable to pregnancy only seven produced living litters, within time. With the anti-sheep and the anti-ox lens groups there were thirteen litters from eighteen females; but though the numbers of litters and the numbers of offspring were below the normal, this was not so to a significant degree. These results would indicate that the lenses of the different species are not so nearly identical as chemical analysis and precipitin tests would lead us to believe. The relation of the rat and the mouse would account for the greater toxicity of the anti-rat lens serum for mice than that of the sheep and the ox. The eyes of all the F_1 generation that lived till they could be examined were normal.

Results in the F_2 Generation.—The fact that the anti-rat serum, at least, was toxic made it desirable to interbreed the F_1 generation, making brother × sister matings, or $\frac{1}{2}$ B × $\frac{1}{2}$ S as far as possible. Thus if the germ plasm of any of the F_1 generation was affected, this defect would have every possible chance of showing itself. This also acted as a control test of the breeding capacity of the strain. From the F_1 whose mothers had received anti-rat serum 40 F_2 individuals were reared; from the anti-ox group none (only one mating possible); from the anti-sheep lens group 62 F_2; from the control group 39 F_2. The results of these matings are shown in Table VI.

TABLE VI.

Results of Interbreeding the F_1 Generation of Mice.

Antiserum.	Injection of Mothers in Relation to Pregnancy.	F_1 Matings (B—brother S—sister).	No. in Litter.	Total.	Average Litter.
No. 1. Anti-rat; groups (*a*) and (*b*) combined	During	B × S	3	24	3
	,,	B × S	4		
	,,	B × S	0		
	,,	B × S (2)	5		
	,,	B × ½ S (3)	12		
	Before	B × S	3	3	3
No. 2. Anti-rat . .	Before	B × S	5	9	3
	,,	B × S	4		
	,,	B × S	0		
No. 3. Anti-rat . .	During	1 ♂ × 1 ♀ from No. 1 group	4	4	4
No. 4. Anti-sheep . .	During	B × S	7	37	4·6
	,,	B × S	4		
	,,	B × S	3		
	,,	B × S	3		
	,,	B × S	8		
	,,	B × S	2		
	,,	½ B × ½ S	5		
	,,	½ B × ½ S	5		
No. 5. Anti-sheep . .	During	B × S	– (♀) died	25	4·2
	,,	B × S	7		
	,,	B × S	5		
	,,	B × S	0		
	,,	½ B × ½ S	6		
	,,	½ B × ½ S	7		
No. 6. Anti-ox . .	During	½ B × ½ S	0	0	0
No. 7. Control. Anti-Ox vitreous humour	During	B × S	4	39	4·3
	,,	B × S	7		
	,,	B × S	6		
	,,	B × S	3		
	,,	B × S	5		
	,,	B × S	3		
	,,	B × S	1		
	,,	½ B × ½ S	6		
	,,	½ B × ½ S	4		

All of the 102 mice whose grandmothers had received anti-lens serum had normal eyes, and they could not be distinguished from the 39 F_2 controls which were likewise normal in every respect. The anti-rat lens group had lower average litters than the others combined, but this is scarcely

sufficient to indicate that they had a weakened hereditary constitution.

The average breeding results in both the F_1 and F_2 generations for the species' antisera may be shown briefly in Table VII. :—

TABLE VII.

	All Groups injected Anti-rat Lens.	All Groups injected Anti-sheep or Ox Lens.	Group injected Anti-vitreous Humour.
Average number of living F_1 for all females. (Delayed pregnancies not included.)	1·1	2·9	5
Average number F_2 from all F_1 matings.	3·1	4·1	4·3

4. Passive Immunisation of Rats.

Rats proved to be unsatisfactory for testing the antisera owing to the great difficulty in regulating their breeding. Some of the antisera used in the foregoing experiments were injected into rats, but only one litter was born under treatment. Antiserum No. 4 (anti-sheep lens) was injected in a dose of 5 c.c. a few days before pregnancy (exact time in relation to pregnancy not determined); and six days later, that is during the first week of pregnancy, a dose of 8 c.c. was injected. This latter is about the maximum injection that can be given a rat. The resulting progeny were all normal.

5. Active Immunisation.

An attempt was made to effect active immunisation of female rats and mice to lens. No test of antibodies other than the breeding test was carried out. The procedure was to inject lens substance in saline suspension intraperitoneally at a few days' interval. After about five injections, at which time with ordinary antigens one could expect strong immunity reaction, the animals were mated, but the injections were continued.

For the first of the series sheep lens was used, with vitreous humour for their controls. Table VIII. gives details of the

injection of lens into four rats, the dosage varying with the individual. Nos. 1 and 4 became pregnant while under injection, and produced normal litters of 6 and 10 respectively. The others did not produce litters then, but the months of November and December are low breeding months for rats. The mice which received similar treatment bred much better. From four that received sheep lens, three litters of 2, 10, and 7 were raised, an average of 4.75 for the group. The two mice injected with vitreous humour produced litters of 6 and 5. These mice also gave good F_2 litters. All were quite normal. There was no indication from the series that any antibodies produced in the injected females were toxic for the embryo.

For the second series of experiments, rat lens was injected into five rats, as shown in Table IX. All were pregnant at a time when any immunity reaction would have been at its maximum, and all produced young, though one litter was killed by the mother. For the second group in this series receiving rat lens, the six female rats which had previously been injected with sheep lens and vitreous humour were used. The injections were continued until pregnancies were recorded. Three produced litters of 5, 6, and 12 respectively. With the other three the injections were kept up longer. Of these one killed its young, one produced the litter too late for examination, and one did not become pregnant. The progeny of these rats were carefully examined, and it can be confidently stated that their eyes were normal. Moreover, the litters were of fair average size.

From the previous work done on lens immunity, one would expect that sheep lens injected weekly for a considerable time would produce an immunity reaction against sheep lens, but this was evidently not harmful to the embryos. Whether a similar immunity reaction would be produced in rats by the continued injection of rat lens is another question.

These experiments were carried out in the Laboratories of the School of Agriculture, Cambridge.

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TABLE VIII.

Active Immunisation of ♀♀ Rats and Mice to Sheep's Lens and Vitreous Humour.

Animals injected.	Antigen.	No. of Animal.	Date, No. Lenses, and No. c.c. of Vitreous Humour injected.							Date of Litter.	No. Mature F_1 produced.	No. F_2 from B × S Mating.
			October.			November.						
			20.	24.	28.	2.	7.	16.	23.			
6 rats	Lens	1	0·06	0·1	0·2	0·25	0·3	0·3	0·3	Dec. 9	6	2 litters
		2	0·12	0·2	0·3	0·35	0·4	0·4	0·4	...	...	...
		3	0·3	0·3	0·4	0·4	0·5	0·5	0·5	...	...	...
		4	0·3	0·4	0·5	0·6	0·7	0·6	0·6	Nov. 30	10	2 litters
	Vitreous humour	5	0·2	0·4	0·53	0·75	0·9	1·0	1·0	...	...	...
		6	0·5	0·8	1·07	1·25	1·5	1·75	1·75	...	...	...
6 mice	Lens	1	0·015	0·025	0·05	0·05	0·06	0·07	...	Nov. 27	2	...
		2	0·03	0·05	0·075	0·075	0·09	0·01	...	...	...	...
		3	0·08	0·075	0·1	0·1	0·12	0·13	...	Dec. 1	10	19
		4	0·08	0·1	0·15	0·2	0·15	0·16	...	Dec. 1	7	12
	Vitreous humour	5	0·05	0·1	0·13	0·15	0·2	0·2	...	Nov. 30	6	5
		6	0·1	0·2	0·27	0·3	0·3	0·3	...	Nov. 30	5	13

Both the rats and mice were mated on 7th November.

TABLE IX.

Attempted Active Immunisation of ♀♀ Rats to Rat Lens.

Animals injected.	No. of Animal.	Date and No. of Lenses each Dose.												Date of Litter.	No. Mature F_1 Produced.
		January.			February.					March.	April.				
		27	29	31	5	8	13	20	27	6	6	13	17	24	
5 rats . . .	1	1	...	1	...	1·5	2	2	2	1	...	...	...	...	Mar. 29	5
	2	1	...	1	...	1·5	2	2	2	1	...	...	...	...	Mar. 29	11
	3	1	...	1	...	1·5	2	2	2	1	...	...	...	...	Apr. 6	4
	4	1	...	1	...	1·5	2	2	2	1	...	...	...	...	Mar. 20	6
	5	1	...	1	...	1·5	2	2	2	1	1	1·5	2	...	Apr. 19	Killed young.
6 rats. Previously injected with sheep lens and vitreous humour.	1	...	2	...	3	...	3	3	2	1	1	...	...	...	Apr. 7	5
	2	...	2	...	3	...	3	3	2	1	1	1·5	2	2	Apr. 24	Killed young.
	3	...	2	...	3	...	3	3	2	1	...	...	...	...	Mar. 22	6
	4	...	2	...	3	...	3	3	2	1	...	...	...	...	Mar. 20	12
	5	...	2	...	3	...	3	3	2	1	1	1·5	2	2	Apr. 27	Killed young.
	6	...	2	...	3	...	3	3	2	1	1	1·5	2	2	...	...

Both groups were mated 21st February.

6. Summary and Conclusions.

1. The passive immunisation of mice and rats to lens was undertaken to study the effects of the antibodies on embryos. Rat lens, sheep lens, ox lens, and ox vitreous humour were used as antigens and antisera prepared.

2. Only lens antisera that gave precipitin reactions were employed for injection. These different sera all gave almost equally definite precipitin reactions with a normal saline extract of either mouse, rat, sheep, or ox lens.

3. The antisera were injected chiefly into female mice, the majority of which could be expected to be pregnant. The results differed greatly. The anti-rat lens serum was very toxic and the mice which received injections of this gave an average litter of 1.1 within the expected time; the sheep and ox antiserum group averaged 2.9; while the control group injected with anti-vitreous humour produced an average of 5 per litter. It would therefore seem that, as antigens, these different species lens are not so organ specific as the precipitin test would indicate. The relation of the rat to the mouse could account for the toxicity of the anti-rat lens serum for embryonic mice.

4. The eyes of the surviving F_1 were normal. An F_2 generation by brother × sister matings was also normal.

5. The active immunisation of female rats and mice to sheep's lens was attempted, but the litters born under treatment were of fair average numbers and were normal in every respect. If antibodies against sheep lens had been generated in the bodies of the female rats, as one could expect, then these had no apparent effect on the embryo.

6. Female rats were injected with rat lens at intervals for several weeks, until they became pregnant. The F_1 were normal. From the breeding results there was nothing to indicate that antibodies toxic for embryos had been generated.

7. In these experiments it is the injection of anti-rat serum into mice that has the most bearing on the work done by Guyer and Smith with rabbits. They found that anti-rabbit lens serum was very toxic for embryo rabbits. Fortunately

they were enabled to rear a number of the affected young which had defective eyes due to the specific influence of the antiserum. But with mice it is difficult to rear defectives, hence it is quite possible that of the many embryos that died, some were effected in a specific manner by the anti-lens serum.

7. References.

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