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Is Guinea Pig Follicular Fluid Auto-Antigenic?*

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In reporting some anaphylactic studies with mammalian follicular fluid Lyons and Van de Carr[1] claimed (1) that the follicular fluids of 4 mammals studied (sow, cow, mare and ewe) were closely related immunologically; (2) that guinea pig follicular fluid was auto-antigenic and that fatal anaphylaxis could be produced at a definite time in the oestrous cycle (tenth-sixteenth days) in normal guinea pigs on primary injection of heterologous follicular fluid; (3) that guinea pigs artificially sensitized to heterologous follicular fluid proteins are temporarily desensitized during the early part of the oestrous cycle by the liberation of their own follicular fluid.

While the first of these claims seemed not improbable from what is known about certain non species-specific or relatively species specific antigens, the others seemed so remarkable and, if substantiated, of such biological importance that we were led to try to verify them.

A number of healthy female guinea pigs weighing 300-350 gm. were observed to determine the length of the oestrous cycles. Only those animals having a fairly regular cycle, 15 to 17 days were used. The test injections were made into a large leg vein rather than intracardially, to avoid the danger of a pericardial hemorrhage. The beef and hog follicular fluid used in these experiments was collected as soon as possible after the animals were slaughtered. It was centrifuged and either used immediately or kept frozen until needed, at which time it was thawed slowly at room temperature.

Twenty female guinea pigs were injected intravenously (Table I) with varying amounts of one of 3 different lots of beef follicular fluid. Fifteen of these animals were injected at periods later than the tenth day after the beginning of the estrous cycle, 5 at earlier periods. Eight additional female guinea pigs were similarly injected with one of 2 lots of hog follicular fluid, 5 of these on days later

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[1] Lyons, W. R., and Van de Carr, F. R., *Arch. Path.*, 1930, **9**, 1.

than the tenth of the estrous cycle, and 3 earlier in the cycle. The results of the injections with hog follicular fluid are recorded in Table II.

TABLE I.
Reactions of Normal Guinea Pig to Beef Follicular Fluid.

Amt. of fluid used cc.	Lot	Day of cycle	No. animals (20)	Results
¼	A	15	1	0
½	A	14	1	0
½	A	15	2	0
¾	A	13	1	0
1	A	15	1	0
1	B	2	1	0
1	B	14	2	0
2	B	3	1	Died in about 1 hr.
2	B	14	2	0
2	B	15	1	Lay quietly on side; recovered
3	B	6	1	Lay on side; recovered
3	B	9	1	Died
3	B	14	1	"
3	C	6	1	0
3	C	15	3	0

TABLE II.
Reactions of Normal Female Guinea Pig to Hog Follicular Fluid.

Amt. of fluid used cc.	Lot	Day of cycle	No. animals (8)	Results
3	A	3	1	0
3	A	9	1	0
3	A	13	1	0
3	A	14	1	0
3	B	4	1	0
3	B	12	1	0
3	B	15	2	0

With the beef follicular fluid no difference was seen in the reactions of animals in the early and those in the late stages of the estrous cycle, although Lyons and Van de Carr produced fatal anaphylaxis with as little as ½ cc. of hog follicular fluid during the later part of the cycle. With one lot of the beef follicular fluid, lot B, amounts of 2 cc. and 3 cc. seemed toxic to both groups of animals. However, none of the reactions were characteristic of typical anaphylaxis. Trembling, roughening of the hair, and collapse, in some animals, and death in 3 cases, were seen but not the sneezing, gasping and convulsive movements of anaphylaxis. With lot A and C no toxic effects were noted on intravenous injection.

Likewise, with the hog follicular fluid no difference was seen between the reactions of the animals injected early and those in-

jected late in the estrous cycle. With neither of the 2 lots of hog follicular fluid were any toxic reactions seen with doses as great as 3 cc.

To test the possibility of cross immunization between follicular fluids from different species, each of 13 young male guinea pigs weighing approximately 350 gm. was given a sensitizing dose of 2 cc. of hog follicular fluid intraperitoneally, and each of 18 young male guinea pigs the same amount of beef follicular fluid intraperitoneally. Twenty days later 6 animals in each group were tested with the homologous fluid. The remaining 19 sensitized animals were tested with the heterologous fluid. All those animals which received homologous fluid in the test injection showed very definite and characteristic anaphylactic reactions, even with amounts of fluid as small as 1/8 cc.

Animals sensitized to hog follicular fluid, receiving beef follicular fluid showed characteristic anaphylactic reactions only if the amounts injected were as great as 1½ cc. Two non-sensitized male guinea pigs used as control showed no reaction to the intravenous injection of 2 cc. of the same beef follicular fluid.

In the group of animals sensitized to beef follicular fluid and receiving test doses of hog follicular fluid, 4 tested with 3 cc. amounts all showed marked anaphylactic reactions. Three of 6 animals receiving 2 cc. amounts showed marked reactions; the 3 others showed little or no reactions. The 2 animals receiving 1 cc. showed no reaction. Of 5 non-sensitized male controls one, receiving 3 cc. of hog follicular fluid, died; the others receiving as much as 2 or 3 cc. showed no change.

Thus there was demonstrated a definite cross anaphylaxis between cattle and hog follicular fluids, although rather large amounts of fluid (1½ cc. or more) were necessary for this purpose. On the other hand anaphylactic reactions were obtained with as little as 1/8 cc. of the homologous fluid.

Four female animals sensitized to hog follicular fluid all gave, after 20 days, characteristic anaphylactic reactions resulting in death when 2/5 cc. of hog follicular fluid was injected intravenously, although 3 of these animals received the test injection on the second day of the oestrous cycle, a time at which Lyon and Van de Carr claim that the female guinea pig is desensitized by the liberation of its own follicular fluid.

While we have thus been able to demonstrate a certain degree of relationship, as manifested by means of the anaphylactic reaction, between the follicular fluids of cattle and hogs, there was no indi-

cation of an auto-antigenic character of guinea pig follicular fluid, as claimed by Lyons and Van de Carr.

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An Attempt to Determine the Blood Groups of Mummies.

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The authors have shown previously[1] that dried human muscle may be used to determine the blood group of the person from which it came. The extension of the study to mummified tissue suggested itself, as information which might be thus obtained would be of interest in connection with the problem of the origin of the human blood groups and might be of value to anthropologists and archeologists. The present paper is a preliminary report of this work.

The technic was as described in the above reference. Anti-A and anti-B testing fluids, prepared by specific adsorption of immune rabbit sera, and also human sera of types B and A were used. These were adjusted in strength so that they agglutinated "+" at a dilution of 1:4 and "±" at 1:8. About 0.3 ml. was placed on each sample of about 0.05 gm. of finely ground mummified tissue. After thorough mixing and standing overnight in the icebox, the supernatants were allowed to come to room temperature and tested against A and B erythrocytes. The results are given in Table I.

It is evident that, in contrast to the American material, some of

TABLE I.
Tests for Adsorption of Agglutinins by Mummified Tissue.

I. American Indian Specimens										
Test Fluid	Test Cells	Specimen No. 1	2	3	4	5	6	7	8	9
Anti-A	A cells	+	+	+	+	+	+	—	+	+
Anti-B	B "	+	+	+	+	+	+	+	+	+
Type B serum	A "	+	+	+	+	+	+	—	+	+
" A "	B "	+	+	+	+	+	+	+	+	+

II. Egyptian Specimens							
Test Fluid	Test Cells	Specimen No. 1	2	3	4	5
Anti-A	A cells	+	+	+	+	+
Anti-B	B "	—	+	—	—	+
Type B serum	A cells	+	+	+	+	+
" A "	B "	—	+	—	—	+

[1] Boyd, W. C., and Boyd, L. G., *Science*, 1933, **78**, 578; *J. Immunol.*, in press.