one-treated pigeons (*7*) that were 6 weeks old when hormone treatment was begun.

The nitrocellulose-trapped RNA, dissolved in saline solution, was placed into the oviducts of ether-anesthetized chicks or pigeons by means of a 27-gauge needle and a Hamilton syringe. The volume was 100 μl. The recipient birds were of the same age, and had the same schedule of stilbestrol priming as described above. The birds were killed 24 hours later, and the avidin content of each treated oviduct, as well as that of various controls, was determined by a sensitive and specific assay based on binding of avidin to [^{14}C-carbonyl]biotin (*8*). Tissue was homogenized in 6 ml of 0.02*M* phosphate buffer, *p*H 7.1, containing 0.07*M* KCl, 0.004*M* $MgCl_2$, and 0.07*M* NaCl, and centrifuged at 5000*g* for 30 minutes. To 2 ml of supernatant was added 0.5 ml of [^{14}C]biotin (0.01 μc, 20 mc/mmole; Radiochemical Centre, Amersham) in 0.2*M* ammonium carbonate solution. The mixture was stirred and held at room temperature for 15 minutes. One milliliter of bentonite solution (10 mg of bentonite per milliliter of 0.2*M* ammonium carbonate) was added. The mixture was centrifuged at 2000*g* for 10 minutes. The precipitate was dissolved in 2 ml of 0.2*M* ammonium carbonate, and the centrifugation and purification procedure was repeated until the supernatant showed no significant radioactivity above the background when dissolved in Aquasol. The precipitate was then dissolved in 10 ml of Aquasol and the radioactivity was counted. Computation of the number of micrograms of avidin per gram of oviduct was based on a standard curve, with the use of avidin obtained from Sigma Chemical Company.

The response of the chick oviduct is shown in Table 1. Earlier results revealed that an intraoviductal dose of 500 μg of total RNA from stilbestrol- and progesterone-treated chick oviducts was required to stimulate avidin synthesis in estrogen-primed chicks (*3*). Nitrocellulose trapping effects a purification of approximately 50-fold. The RNA recovered after nitrocellulose chromatography stimulates avidin synthesis at a dose of 10 μg. This activity is lost when the poly(A) RNA is first treated with pancreatic ribonuclease. The oviduct of the stilbestrol-primed chick responds to poly(A) RNA extracted from the oviducts of pigeons treated with stilbestrol and progesterone. There is no avidin present in the oviducts of stilbestrol-primed chicks that served as controls (intraoviductal instillation of saline).

Avidin synthesis by the pigeon oviduct is shown in Table 2. Like the chick, and several other species reported (*9*), the oviduct of the estrogen-primed pigeon synthesizes avidin in response to progesterone. This response is brought about also by the intraoviductal administration of homologous oviductal RNA, prepared from hormone-treated pigeons. A dose of 10 μg of poly(A) RNA elicits a significant level of avidin synthesis. The pigeon oviduct responds, also, to poly(A) RNA recovered from hormone-treated chicks. Prior treatment of the chick RNA preparation with pancreatic ribonuclease eliminates the activity. A similar hydrolysis experiment with pigeon RNA has not been done.

The heterospecific transfer of hormonal stimulation, through the mediation of hormone-induced RNA in vivo, has not been described previously, although in vitro systems have been described (*10*). This finding is of interest with respect to the specificity of RNA coding in the vertebrate classes, as well as in relation to the evolution of hormonal control mechanisms. Evidently, the progesterone-receptor complex (*11*) interacts with highly similar or identical chromatin acceptor sites in the nuclei of both the chick and the pigeon, and does not discriminate between the two. Thus, once the hormone stimulates the production of RNA coded for avidin synthesis in the oviductal nuclei of either species, the hormone molecule itself is no longer required; and the newly synthesized RNA can complete the steps leading to avidin synthesis, interchangeably between the oviductal cells of the two species. This may be the physiological basis for the interspecies cross-reaction of many hormones. Most likely, the active component is a form of messenger RNA, since it is highly purified by nitrocellulose trapping (*4*). It has been concluded (*3*) that a contaminating residue of progesterone cannot account for the avidin synthesis observed after RNA administration. This conclusion is further strengthened by our observation of avidin induction with the purified RNA preparation that is active at a dose as low as 10 μg. As in earlier experiments with either progesterone-induced RNA or estrogen-induced RNA (*12*), enzymatic degradation with pancreatic ribonuclease eliminates the activity.

Our experiments do not reveal whether the exogenous RNA—homologous or heterologous—is translated directly or acts in some manner at the nuclear level to influence transcription of avidin-coded RNA. A lack of absolute antigenic identity between the avidin molecules produced by the two species, if found to exist, would provide an excellent experimental basis to resolve this question.

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References and Notes

1. B. W. O'Malley, W. L. McGuire, P. O. Kohler, S. G. Korenman, *Recent Progr. Horm. Res.* **25**, 105 (1969).
2. R. Hertz, R. M. Fraps, W. E. Sebrell, *Proc. Soc. Exp. Biol. Med.* **52**, 142 (1943).
3. P. Tuohimaa, S. J. Segal, S. S. Koide, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 2814 (1972).
4. G. Braverman, J. Mendecki, S. Y. Lee, *Biochemistry* **11**, 637 (1972).
5. G. C. Rosenfeld, J. P. Comstock, A. R. Means, B. W. O'Malley, *Biochem. Biophys. Res. Commun.* **47**, 387 (1972).
6. The chicks were Rhode Island Reds (Hall Brothers Hatchery, Wallingford, Conn. 06492).
7. The pigeons were white Carneaux (Palmetto Pigeon Plant, Sumter, S.C. 29150).
8. S. G. Korenman and B. W. O'Malley, *Biochim. Biophys. Acta* **140**, 174 (1967).
9. R. Hertz and W. E. Sebrell, *Science* **96**, 257 (1942).
10. D. Villee, *ibid.* **158**, 652 (1967).
11. B. W. O'Malley and D. O. Toft, *J. Biol. Chem.* **246**, 1117 (1971).
12. S. J. Segal, O. W. Davidson, K. Wada, *Proc. Nat. Acad. Sci. U.S.A.* **54**, 782 (1965).
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Male Specific Antigen: Homology in Mice and Rats

Abstract. *Male lymphoid cells from some strains of rats can sensitize C57BL/6 female mice against C57BL/6 male skin grafts; this indicates that the male specific antigen of these two species is homologous.*

In both mice (*1*) and rats (*2, 3*) there is one exception to the rule that skin grafts are uniformly accepted between members of an isogenic strain. This occurs when females are challenged with grafts from male donors. Since the only genetic difference between male and female members of an isogenic strain is that males have the Y chromosome, of which there is no homolog in females, the simplest explanation for this incompatibility is the

presence of a histocompatibility antigen or antigens determined by a locus on this chromosome. Moreover, the numerous studies conducted to analyze the behavior of the Y antigen (or H-Y antigen or Y factor, as it has come to be called) are all consistent with this interpretation (*4*).

Because of the similarities in the behavior of the Y factor in mice and rats, attempts have been made to determine whether they are antigenically similar (*3*). These included repeatedly injecting newborn and very young C57BL/6 females with BN male rat spleen or marrow cells, and subsequently challenging them with C57BL/6 male skin grafts to determine whether a state of tolerance of the Y factor had been induced; and inoculating adult C57BL/6 females with suspensions of spleen cells from male BN donors in the hope of demonstrating sensitization in respect to subsequent grafts of C57BL/6 male skin. No evidence was forthcoming from these experiments to indicate any overlap between the antigens determined by the Y chromosome of the two species.

However, these initial attempts to demonstrate homology between the Y factor in mice and rats were all conducted with BN rats because, of the rats available, the incidence of females that rejected male skin grafts was the highest in this strain. In retrospect this rationale was not a good one; as shown by later studies, the capacity of females of different strains to reject intrastrain male skin grafts is not necessarily a function of the immunogenicity of the Y antigen (*4*). Because different combinations of rat and mouse strains might yield different results, we reinvestigated the possibility of an H-Y homology in mice and rats by testing the capacity of male cells from various inbred strains of rats to sensitize C57BL/6 females against subsequent C57BL/6 male skin grafts.

Nine rat strains were surveyed. These animals represented six different *Ag-B* genotypes, the major histocompatibility locus of the species. The Lewis (LE), Fischer (FI), and BH donors, all of which are $Ag\text{-}B^1/Ag\text{-}B^1$, originated from domestically maintained stocks, as did rats of the BN ($Ag\text{-}B^3/Ag\text{-}B^3$), DA ($Ag\text{-}B^4/Ag\text{-}B^4$), and August (AUG) ($Ag\text{-}B^5/Ag\text{-}B^5$) strains. Wistar-Furth (WF) ($Ag\text{-}B^2/Ag\text{-}B^2$), Buffalo (BUF) ($Ag\text{-}B^6/Ag\text{-}B^6$), and ACI ($Ag\text{-}B^4/Ag\text{-}B^4$) animals were obtained from Microbiological Associates, Bethesda, Maryland. Cells from BN/FI ($Ag\text{-}B^3/Ag\text{-}B^1$) F1 hybrid males were also analyzed.

Virgin C57BL/6 females, 8 to 12 weeks of age, were inoculated with 10×10^6 male rat lymph node cells from each of these strains and were challenged 18 to 22 days later with an adult C57BL/6 male skin graft. The survival of these grafts, as compared with similar grafts on females inoculated with female rat lymph node cells, indicated whether the xenogeneic male cell inoculum had sensitized the recipients with respect to H-Y antigen. To obtain information on the degree of immunity induced, the survival of C57BL/6 male skin grafts on C57BL/6 female mice treated with rat cells was also compared with the survival of similar grafts on C57BL/6 females that had received 10×10^6 allogeneic CBA/Ss male or female lymph node cells 3 weeks earlier.

The C57BL/6 recipients originated either from our colony or from the

Table 1. Number (*N*) and percentage of surviving C57BL/6 male skin grafts after transplantation on C57BL/6 females that had been inoculated with lymph node cells from various strains of rats or from CBA mice. Abbreviations: MST, median survival time; S.D., standard deviation.

Donor	*Ag-B* genotype	Data	Time after transplantation (days)														MST (days)	S.D. (days)	*P**
			0	10	12	14	16	18	20	22	24	26	28	30	50	>100			
LE ♂	1/1	*N*	41	34	27	21	18	15	10	5	3		2		0		14.5	1.45	
		%		83	66	50	44	37	24	12	7		5						
LE ♀	1/1	*N*	26			25	22	21	15	11	8		7		0		22.2	1.27	.00003
		%				96	85	80	58	42	31		27						
FI ♂	1/1	*N*	36	33	31	27	21	17	8	4	3	2			1	0	16.5	1.26	
		%		92	86	75	58	47	22	11	8	6			2				
FI ♀	1/1	*N*	19				15			9	5	0					21.1	1.36	.0192
		%					79			47	26								
BH ♂	1/1	*N*	36		31	23	18	13	12	4		1			0		16.0	1.36	
		%			86	64	50	36	33	10		3							
BH ♀	1/1	*N*	19				15	14	11	9	6		3		0		21.1	1.32	.002
		%					79	74	58	47	32		16						
WF ♂	2/2	*N*	24					23	20	15	10	7	3	1	0		23.0	1.14	
		%						96	83	63	42	29	13	4					
BN ♂	3/3	*N*	32				31	26	19	13	11	10		5	0		22.5	1.31	
		%					97	81	59	41	34	31		16					
BN ♀	3/3	*N*	9				7		5	3		2		0			20.2	1.31	.2981
		%					78		56	33		22							
DA ♂	4/4	*N*	26				24	22	14	8	7	4			2	0	21.0	1.20	
		%					92	85	54	30	27	15			8				
DA ♀	4/4	*N*	17				15	14	9	7	3	1	0				20.4	1.19	.29
		%					88	82	53	41	18	6							
ACI ♂	4/4	*N*	24				23	22	14	10	6		3	2	0		21.0	1.27	
		%					96	92	58	42	25		12	8					
AUG ♂	5/5	*N*	36		35	34	29	19	11	5	4	3	2	1		1	18.4	1.20	
		%			97	94	81	53	31	14	11	8	6	3		3			
AUG ♀	5/5	*N*	25			24	21	20	16	12	7		5	3	0		21.9	1.35	.0314
		%				96	84	80	64	48	28		20	12					
BUF ♂	6/6	*N*	24				23	20	14	6	4	3	2	1	0		21.0	1.17	
		%					96	83	58	25	17	12	8	4					
BN/FI ♂†	3/1	*N*	23			21		18	15	12	10	7	5	2	0		22.2	1.33	
		%				91		78	65	52	43	30	22	9					
CBA ♂		*N*	22		19	13	5	2		1			0				14.6	1.19	
		%			86	59	23	9		5									
CBA ♀		*N*	21				20	19	17	12	8	4	3	2	0		23.0	1.17	.00003
		%					95	90	81	57	38	19	14	10					

* *P* value obtained in the Mann-Whitney U test when survival times of male grafts on females inoculated with male and female cells of the same strain were compared. † Y chromosome from FI.

Jackson Laboratory, Bar Harbor, Maine. Although these two sublines have been separated since 1957, female-female and male-male skin transplants exchanged between them are uniformly accepted, and females of both sublines behave similarly toward male skin grafts. Nevertheless, in all cases females were challenged with male skin from the same subline.

Viable cell suspensions were prepared from the axillary, brachial, cervical, and mesenteric nodes of adult rats and mice as described (*5*). The cells were administered intraperitoneally in a standard volume of 1 ml of Hanks balanced salt solution.

Skin grafting entailed the transfer of a full-thickness disk of nonactive trunk skin, about 1.0 to 1.2 cm in diameter, to the right side of the host's chest. The operative technique and method of appraisal of the well-being of the grafts have been described (*6*). In several experiments, grafts were scored without knowledge of the recipient's treatment.

Median survival times (MST's) of grafts were estimated by Litchfield's nomographic method (*7*). Statistical significance was determined by the Mann-Whitney U test (*8*).

The results are summarized in Table 1. Male cells from four (LE, FI, BH, and AUG) of the nine strains assayed succeeded in sensitizing C57BL/6 females against subsequent C57BL/6 male skin grafts. On the other hand, all female cells, as well as male cells from WF, BN, DA, ACI, and BUF donors, proved ineffective in sensitizing their female mouse recipients against male skin grafts. The results with BN cells are in accord with our previous findings (*3*).

It is undoubtedly significant that the rat cells most successful in inducing immunity to the mouse H-Y antigen all stemmed from *Ag-B*^{1}/*Ag-B*^{1} males. Indeed, the level of immunity induced by these cells, as indicated by the MST's of male skin grafts on their recipients, compared favorably with that observed after the inoculation of similar numbers of allogeneic CBA male lymphocytes.

The capacity of male cells from only some strains of rats to sensitize female C57BL/6 mice to the Y antigen could be due to differences in their survival in the host species. Thus, LE, FI, and BH cells may have survived significantly longer in the C57BL/6 hosts than did cells of other *Ag-B* genotypes; the latter, in most cases, might have been rejected before they were able to induce any immunity at all to the relatively weak H-Y antigen. Indeed, the fact that not all recipients of *Ag-B*^{1}/*Ag-B*^{1} male cells gave a demonstrable immune response when challenged with a male skin graft suggests that even these cells may not have persisted long enough in some animals to induce immunity. If the survival of rat cells in mice is a function of their *Ag-B* genotype, this would certainly not be surprising, since there is ample evidence of serologic cross-reactivity between the histocompatibility antigens of different species (*9*). Most pertinent is the report by Sachs *et al.* (*10*) that mice challenged with rat xenografts produce antibodies that can detect the major histocompatibility alloantigens of the donor rats with remarkable specificity. Direct evidence for the longer survival of *Ag-B*^{1}/*Ag-B*^{1} cells, as opposed to those of other genotypes, could be obtained by establishing the fate of isotopically labeled rat cells, derived from various strains, in C57BL/6 recipients.

Alternatively, it might be argued that the better results obtained with LE, FI, and BH male cells stems from the fact that their Y antigen is more closely related to the Y factor of C57BL/6 mice than is the male antigen of other rat strains. This possibility is, however, ruled out by the fact that BN/FI (*Ag-B*^{3}/*Ag-B*^{1}) F_1 hybrid cells—that is, cells derived from an animal whose Y chromosome was of FI origin—were ineffective in inducing sensitization to H-Y in mice. Indeed, the fact that these cells behaved like BN cells lends further support to the thesis that the results obtained reflect differences in the capacity of rat cells of different *Ag-B* genotypes to survive in a mouse milieu.

Evidence that allelic differences at the *H-Y* locus were not responsible for the results obtained is also derived from previous grafting experiments that, with one exception (*11*), indicate that the Y factor is identical in male rats of all strains (*3*, *12*).

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References and Notes

1. E. J. Eichwald and C. R. Silmser, *Transplant. Bull.* **2**, 148 (1955).
2. R. E. Billingham and W. K. Silvers, *ibid.* **6**, 399 (1959); I. M. Zeiss, N. W. Nisbet, B. F. Heslop, *ibid.* **30**, 161 (1962); B. F. Heslop, *Proc. Univ. Otago Med. Sch.* **47**, 53 (1969); *ibid.*, p. 55.
3. R. E. Billingham, B. A. Hodge, W. K. Silvers, *Proc. Nat. Acad. Sci. U.S.A.* **48**, 138 (1962).
4. D. L. Gasser and W. K. Silvers, *Advan. Immunol.* **15**, 215 (1972).
5. R. E. Billingham, in *Transplantation of Tissues and Cells*, R. E. Billingham and W. K. Silvers, Eds. (Wistar Institute, Philadelphia, 1961), pp. 87–106.
6. ———, in *ibid.*, pp. 1–26.
7. J. T. Litchfield, *J. Pharmacol. Exp. Ther.* **97**, 399 (1949).
8. S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956), pp. 116–127.
9. E. Albert, K. Kano, C. J. Abeyounis, F. Milgrom, *Transplantation* **8**, 466 (1969); C. J. Abeyounis and F. Milgrom, *Transplant. Proc.* **1**, 556 (1969); J. J. van Rood, A. van Leeuwen, H. Balner, *ibid.* **4**, 55 (1972); F. E. Ward, H. F. Seigler, R. S. Metzgar, *ibid.* p. 63; M. E. Dorf, S. Y. Eguro, D. B. Amos, *ibid.* p. 71; D. Götze, S. Ferrone, R. A. Reisfeld, *J. Immunol.* **109**, 439 (1972).
10. D. H. Sachs, H. J. Winn, P. S. Russell, *Transplant. Proc.* **3**, 210 (1971).
11. Y. Mullen and W. H. Hildemann, *Transplantation* **13**, 521 (1972).
12. B. F. Heslop, *ibid.* **15**, 31 (1973).
13. Supported by PHS grant AI-09275. S.L.Y. was a Ford Foundation fellow in reproductive biology. The technical assistance of J. Johnson is acknowledged. We are also indebted to D. L. Gasser for helpful criticism and advice.

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Seasonal Changes in the Learning and Activity Patterns of Goldfish

Abstract. *Goldfish exhibit cyclic changes with an annual rhythm in their learning and activity patterns. Maximum learning ability and active behavior occurred during the months of January, February, and March. Poor learning was obtained in the summer months, after the onset of the spawning season. The results indicate that the annual periodic changes of the hormonal levels which govern spawning may also influence learning and activity patterns.*

In studies of the biochemical changes that occur in the brain during learning, the ability of goldfish to learn new swimming skills was found to vary with the season of the year (*1*). The animals learned well during the winter and very poorly in the summer months. A systematic study of this behavior