The highest level of differentiation was present in the fibrin clot in the chamber cavity, where three-dimensional interaction was possible (Fig. 3). This teratoma represented the end-stage of a process noted in other chambers, where embryonic cells often colonized various regions of the fibrin. In the presence of adequate cell numbers and contacts, the inherent tendency for embryonic cells to associate at a high level of integration was expressed here, even in an entirely abnormal fashion.

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References

1. D. W. Fawcett, G. B. Wislocki, C. W. Waldo, *Anat. Rec.* **108**, 71 (1947); D. R. S. Kirby, *Nature* **194**, 696 (1962).
2. A. McLaren and A. K. Tarkowski, *J. Reprod. Fertility* **6**, 385 (1963).
3. D. B. Amos, in *Transplantation of Tissues and Cells*, R. E. Billingham and W. K. Silvers, Eds. (Wistar Inst. Press, Philadelphia, 1961), p. 69.
4. J. Hammond, *Nature* **163**, 28 (1949).
5. G. D. Snell, *Biology of the Laboratory Mouse* (Blakiston, Philadelphia, 1941), pp. 1–54.
6. B. Mintz, *Science* **138**, 594 (1962).
7. M. Smithberg and M. N. Runner, *J. Exptl. Zool.* **143**, 21 (1960).
8. L. Levitow and H. Eagle, *Ann. Rev. Biochem.* **30**, 605 (1961).
9. G. Sato, L. Zaroff, S. Mills, *Proc. Natl. Acad. Sci. U.S.* **46**, 963 (1960).
10. M. Abercrombie and E. J. Ambrose, *Cancer Res.* **22**, 522 (1962).
11. P. Weiss, *Intern. Rev. Cytol.* **7**, 391 (1958).
12. I thank William U. Gardner of the Yale University School of Medicine for his constant encouragement and advice. This work represents part of a thesis, Yale University, 1963. It was supported by USPHS grants CA-5505 and C-343. I also thank Paul Weiss of the Rockefeller Institute for editorial suggestions.

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Lactate Dehydrogenase in Pigeon Testes: Genetic Control by Three Loci

Abstract. *Homogenates of pigeon testes show three types of lactate dehydrogenase isozyme patterns. Recombination of the polypeptide subunits of the dehydrogenase in mixtures of the different types, and the distribution of the three isozyme patterns in six different pigeon populations demonstrated that lactate dehydrogenase synthesis in pigeon testes is under the control of three distinct loci, A, B, and C.*

By the method of starch-gel electrophoresis five distinct types (isozymes) of lactate dehydrogenase (LDH) have been separated and identified in most mammalian and avian tissues (*1*). Information currently available (*2*) indicates that each isozyme is a tetramer formed by association of two polypeptides, A and B. Thus isozymes 1 to 5 have the following polypeptide compositions: A_0B_4, A_1B_3, A_2B_2, A_3B_1, A_4B_0. The synthesis of each of the polypeptides, A and B, appears to be under the control of two separate genetic loci (*3*), so that the isozyme composition of any tissue depends on the relative activity of the genes at these loci.

Electrophoresis of testicular homogenates from several mature animals revealed one or more unusual LDH isozymes ("band X"), suggesting that a third genetic locus contributed to the synthesis of LDH isozymes in mature testes (*4*). Further evidence for this additional genetic locus has been obtained by studying the LDH isozymes in homogenates of pigeon testes.

We used racing homer pigeons, White Carneau and Silver King pigeons, and wild park pigeons (*5*). All of the birds were delivered to the laboratory, killed by decapitation, and dissected to determine sex and maturity. Most of the racing homer pigeons were males, whereas the wild population comprised approximately equal numbers of males and females. Testes and several other tissues from the males were frozen immediately after dissection, and enzyme studies were carried out within 2 weeks. The electrophoresis patterns of fresh and frozen tissues were the same. Methods for preparation and electrophoresis of tissue homogenates, localization of LDH isozymes in starch gel, dissociation and recombination of LDH polypeptide subunits, and determination of total LDH activity were similar to those previously described (*4*).

As shown in Fig. 1, homogenates of pigeon testes exhibited three different patterns of isozymes when lactate was used as substrate. Type I showed seven isozymes, including two new ones (designated "X"), type II showed a total of eight, and type III showed four. The bimodal distribution of activity among the isozyme bands in the type II pattern suggested the presence of a second isozymic group whose first two members were superimposed on LDH-4 and LDH-5.

This interpretation was supported by studies with α-hydroxybutyrate as substrate (*4*, *6*). Two of the bands in type I, five in type II, and one in type III (all designated "X" in Fig. 1) exhibited higher catalytic activity with this substrate than did the usual five isozymes. When α-hydroxybutyrate was substituted for lactate in the reaction mixture, LDH-1, -2, and -3 in testes, as well as LDH-4 and -5 in tissues containing large amounts of these isozymes, were barely visible.

In order to investigate the polypeptide composition of the "band X" complex in pigeon testes, various combinations of types I, II, and III testicular homogenates were treated with 0.5*M* sodium chloride and 0.1*M* phosphate and frozen for 24 hours prior to electrophoresis. This method was first used by Markert (*7*) to dissociate the polypeptide subunits of the usual five isozymes. After dissociation and random

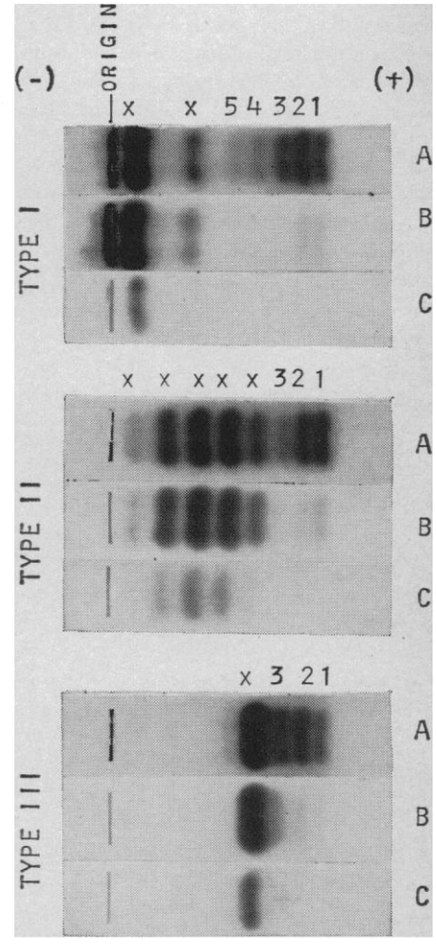

Fig. 1. Lactate dehydrogenase isozymes in mature testes from types I, II, and III pigeons. *A*, Enzymatic activity with 0.5*M* lactate; *B*, activity with 0.5*M* α-hydroxybutyrate; and *C*, activity with 0.5*M* α-hydroxyvalerate. The homogenates were electrophoresed simultaneously in the same starch gel, and the conditions for localizing LDH activity, except for substrate used, were identical.

reassociation of the polypeptides in a mixture of type I and III homogenates, the pattern which developed (Fig. 2) was identical to that found in type II homogenates treated in a similar manner. No new bands appeared if sodium chloride and phosphate were omitted from the mixture of type I and III homogenates during freezing.

Studies on recombination with type I revealed increased staining of the minor "band X" and a reduction of activity in the other isozymes. Two faint bands appeared in the region of LDH-4 and LDH-5 (Fig. 2). These changes were more pronounced after dissociation and reassociation of the polypeptides in a mixture containing homogenates of type I testes and heart, a tissue which has predominantly B subunits. The redistribution of isozymic activity observed in these experiments suggests that the minor "band X" is a result of the recombination of subunits from the usual five isozymes and the "band X" nearest the cathode. Further evidence for the presence of B (or A) subunits in the minor "band X" was obtained when α-hydroxybutyrate was the substrate. The reactivity of the minor "band X" was intermediate to that exhibited by the major "band X" and the usual five isozymes (Fig. 1). The minor "band X" generated in the recombination experiments with type I had similar catalytic properties. The recombination of polypeptides from the major "band X" and the usual isozymes indicates that the structure of the subunits is closely related. If this were not so, then functional molecular species would not be formed in mixtures of the polypeptide subunits of these isozymes.

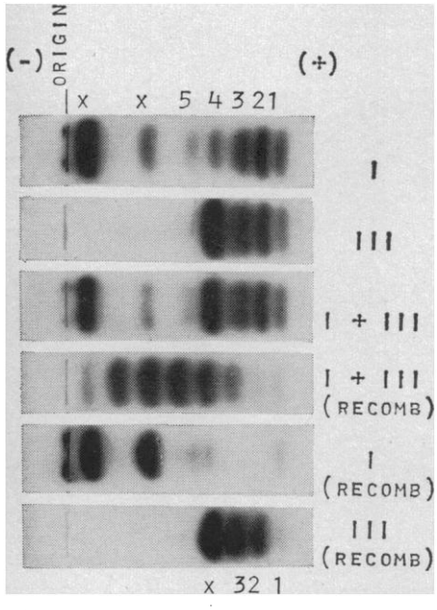

Fig. 2. Lactate dehydrogenase isozymes in homogenates of types I, III, and a mixture of equal parts of type I and type III pigeon testes. The patterns of testicular homogenates treated with 0.5*M* sodium chloride and 0.1*M* phosphate and frozen for 24 hours prior to electrophoresis are designated (*RECOMB*).

Table 1. Distribution of type I, II, and III in six different pigeon populations. The upper figures of each pair are the observed; the lower, the expected.

Number of pigeons			Allele frequencies		S.E.*
Type I	Type II	Type III	C	C′	
Wild Pigeons, Washington, D.C.					
143	63	4	0.83	0.17	0.02
145	59	6			
Wild Pigeons, Baltimore, Md.					
232	77	3	.87	.13	.01
235	72	5			
Wild Pigeons, Oshkosh, Wis.					
48	28	5	.76	.24	.03
47	29	5			
Racing Homer, Baltimore, Md.					
140	58	6	.83	.17	.02
140	58	6			
White Carneau					
4	40	56	.24	.76	.03
6	36	58			
Silver King					
12	24	14	.48	.52	.05
12	25	13			

* Standard error of allele frequency estimates.

The results of the experiments with recombination can be most easily interpreted by assuming that LDH synthesis in pigeon testes is controlled by three genetic loci: A, B, and C. Apparently all the pigeons examined have been homozygous at the A and B loci, whereas some were heterozygous at the C locus. Thus with reference to the C locus three genotypes are possible: CC, C′C, and C′C′. If the polypeptide composition of the major "X" bands in the type I and type III patterns are designated C_4 and C_4' respectively, then the isozymes of the "band X" complex in type II testes could be designated by the following formulas: C_4C_0', C_3C_1', C_2C_2', C_1C_3', and C_0C_4'.

Further evidence for the existence of allelic genes at the C locus was obtained by measuring the relative frequencies of the three types of LDH patterns in six different pigeon populations. As shown in Table 1, the distribution of the three types in each population agrees with that expected according to the Hardy-Weinberg law for a single pair of alleles. Also the distribution of the three types in the wild pigeons and the racing homers is remarkably constant. Observations on White Carneau and Silver King pigeons suggest that there might be considerable variation in the frequencies of the C and C′ alleles in different breeds.

An extensive analysis of the isozymes of many tissues from mature male and female pigeons revealed that the "band X" isozymes were detectable only in testes. If the location and the ontogeny of the pigeon "band X" complex resembles that of the "X" bands in other animals (*8*), then one would expect the "band X" isozymes first to appear in the differentiating germ cells at pubescence. Total LDH activity in homogenates of each testicular type were approximately the same. The physical features of the type I, II, and III birds were indistinguishable in the pure bred pigeons.

A conclusive demonstration of the mode of inheritance of the LDH isozyme patterns in pigeon testes with reference to the C locus will only be possible when appropriate mating experiments are carried out. The present findings, however, do conform with the hypothesis that LDH isozymes in pigeon testes are under the control of three genetic loci, A, B, and C.

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References and Notes

1. C. L. Markert and F. Møller, *Proc. Natl. Acad. Sci. U.S.* **6**, 753 (1959); P. G. Plagemann, K. F. Gregory, F. Wroblewski, *J. Biol. Chem.* **235**, 2288 (1960); R. D. Cahn, N. O. Kaplan, L. Levine, E. Zwilling, *Science* **136**, 962 (1962).
2. C. L. Markert, in *Hereditary, Developmental, and Immunologic Aspects of Kidney Diseases*, J. Metcoff, Ed. (Northwestern Univ. Press, Evanston, 1962), p. 54.
3. C. R. Shaw and E. Borto, *Proc. Natl. Acad. Sci. U.S.* **50**, 211 (1963); S. H. Boyer, D. C. Fainer, E. J. Watson-Williams, *Science* **141**, 642 (1963); W. E. Nance, A. Claflin, O. Smithies, *ibid.* **142**, 1075 (1963).
4. A. Blanco and W. H. Zinkham, *Science* **139**, 601 (1963); W. H. Zinkham, A. Blanco, L. Kupchyk, *ibid.* **142**, 1303 (1963).
5. The racing homer pigeons were obtained from several lofts in Baltimore, the White Carneau and Silver King pigeons from Palmetto Pigeon Plant, Sumter, S.C., and the wild pigeons were collected at single trapping sites in Washington, D.C., Baltimore, Md., and Oshkosh, Wis.
6. J. M. Allen, *Ann. N.Y. Acad. Sci.* **94**, 937 (1961).
7. C. L. Markert, *ibid.* **140**, 1329 (1963).
8. W. H. Zinkham, A. Blanco, L. J. Clowry, Jr., *Ann. N.Y. Acad. Sci.*, in press.

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