Figure 2 shows the different stages of oxidation and reduction at the 245-m layer; until August 1965, nitrate values increased, with corresponding decrease in ammonia and oxygen; the increase in nitrite reflects its appearance as an intermediate product of the nitrification process. The situation is reversed after August 1965. Considering the almost complete disappearance of dissolved oxygen (to less than 0.5 ml/liter) one may say that the process of denitrification started thereafter and that the decaying organisms started to utilize oxygen from the nitrate for their oxidation, wtih resultant formation of a reducing layer.

During December 1965 a significant increase in nitrite was associated with decrease in nitrate and an almost zero value for ammonia. Because ammonia, the end product of denitrification, did not increase, it is apparent that the process was not completed during the observation; it was in the intermediate or nitrite stage. After this observation the whole process proceeded systematically; the nitrate and nitrite decreased gradually and disappeared completely, along with oxygen, during August 1966, with the consequent formation of ammonia and appearance of stagnation.

During stagnation the ammonia content increases until new water flows in. In the absence of nitrite and nitrate one may assume that the inorganic nitrogen has been completely utilized and that the increase in ammonia is due to denitrification of organic nitrogen compounds, since some amino acids are known to produce ammonia by denitrifying bacteria under anoxic conditions. No observation was made between May and August 1966, and one may suppose that this process also may have contributed to the high concentration of ammonia, if the stagnation developed during this period.

During stagnation enormous amounts of phosphate and ammonia accumulate in the basin; when the water is displaced upward by new inflow, the ammonia, being gradually oxidized to nitrate, increases the nutrient contents of the overlying waters. When the nutrients reach the surface they increase productivity considerably. For example, during the big stagnation of 1956–61 about 1.1×10^5 tons of phosphate and 3×10^5 tons of mineralogenic nitrogen accumulated in Gotland Basin; when lifted to the surface they can be expected to have increased the normal concentrations of such nutrients in the productive layer of the whole Baltic Sea by about 50 percent. The result was increase in phytoplankton bloom and in fish.

By taking into consideration the volume of water below 60 m, the river discharge, and the inflow and outflow, the residence time of the deeper waters of the central Baltic has been calculated at about 5 years (*4*). The current spurt in fisheries in the Baltic may reflect the fact that the 1961 stagnant waters from Gotland Basin have reached the surface. Similar fertilizations of the Baltic may be expected about 1969 and 1972. The surface waters of the Baltic are very near the starvation point in nutrients, and it may be that only these periodic stagnations enable fishing to flourish there.

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Fetal Hemoglobin Variants in Mice

Abstract. *Two strains of mice, DBA and C3H, have a fetal globin polypeptide chain which differs in electrophoretic mobility from the corresponding fetal chain of the C57Bl strain. Mice of the DBA and C3H strains also differ from those of the C57Bl in adult hemoglobin type. Results of backcrossing the (DBA × C57Bl) hybrid to the C57Bl suggest that the fetal chain locus and the adult β-chain locus are closely linked.*

In the embryonic and fetal stages of life many animals have hemoglobins that differ from those they possess as adults. The human hemoglobin F (Hb F) is the best-studied fetal hemoglobin, and its structure is very similar to that of the two adult human hemoglobins Hb A and Hb A_2. Hemoglobin A has the structure $\alpha_2\beta_2$, whereas Hb A_2 is $\alpha_2\delta_2$ and Hb F is $\alpha_2\gamma_2$. The β-, δ-, and γ-polypeptide chains show a great deal of homology with each other (*1*). The genes controlling the structures of the adult β- and δ-chains are closely linked and are thought to be tandem duplications of a primordial β-chain gene (*2*). The linkage of the β and δ genes was discovered by studying families in which a parent was doubly heterozygous for variant β- and δ-chains and the corresponding normal chains (*1*). Because of the structural homology of the β- and γ-chain polypeptides, it has been suggested that the β- and γ-chain genes are similarly related to each other by duplication (*2*) and may also be closely linked (*3*), but there is so far no direct evidence for this close linkage. Although newborn infants who possess structural variants of the γ-chain have been described (*4*), family studies with individuals doubly heterozygous for β- and γ-chain variants have not yet been possible.

In mice, two variants of adult hemoglobin, "diffuse" and "single," are known (*5*); the difference between them is due to differences at the β-chain locus (*6*). We now describe a variation in a fetal hemoglobin between two strains of mice which also differ in adult hemoglobin type. These differences make it possible for the first time to analyze unambiguously the linkage between the loci for a fetal polypeptide chain and the adult β-chain.

The difference in fetal hemoglobins between strains of mice has been demonstrated by two-dimensional starch-gel electrophoresis. The first dimension at gel *p*H 8.4 is used to separate the various native hemoglobins (*7–9*), and it gives results similar to those reported by Craig and Russell (*10*). The second electrophoretic dimension at right angles to the first is in an acidic gel 8*M* in urea and 0.07*M* in mercaptoethanol (*11–14*); this electrophoresis at gel *p*H 4.0 separates the globin subunits of the native hemoglobins already separated by the first electrophoresis.

Figure 1a shows the two-dimensional pattern of a hemolyzate from 12-day-old embryos of the C57B1/10SNJ mouse (*15*); C57B1/6J gives an identical pattern. Figure 1b shows the two-dimensional pattern for the 12-day-old embryo hybrid, C3H/HeAu ♂ × C57B1/6Au ♀, and Fig. 1c shows the pattern for the 12-day-old embryo C3H/HeAu (*16*). The three patterns are clearly different. The C57B1 em-

bryo has a globin, Y^1, which migrates more rapidly at *p*H 4 than the corresponding globin, Y^2, of the C3H embryo; the hybrid has both Y^1 and Y^2. [The nomenclature used in Fig. 1 is primarily that of Fantoni *et al.* who have described the globin composition of the C57B1/6J embryonic hemoglobins (*17*). Our results are in agreement with theirs, and there is only one way in which their nomenclature could be applied to our results (*18*).]

The Y chain is common to two hemoglobins, EI and EII. Hemoglobin EII is similar to human fetal Hb F in its possession of α-chains; EII is also the embryonic hemoglobin which persists the longest during fetal life—it is still present at day 17 of gestation when the other embryonic hemoglobin components have disappeared (*19*). For these reasons we consider EII a fetal hemoglobin and refer to the Y chain as a fetal polypeptide chain. If future chemical data should show the Y chain to be analogous to the human γ-chain, then at that time it could usefully be renamed the γ-chain.

The simplest hypothesis concerning the genetic control of the variant fetal chains Y^1 and Y^2 would be that the two chains are controlled by alleles at a single locus. To test this and to find out whether the Y locus is linked to the β-chain locus, backcrosses were performed. The only hybrid readily available to us was the $B_6D_2F_1$, which is DBA/2J ♂ × C57B1/6J ♀ (*16*). The DBA, like the C3H, has the diffuse type of adult hemoglobin, while the C57B1 has a single adult hemoglobin (*20*). The DBA embryos also possess a Y fetal globin with the same electrophoretic mobility as that of the C3H. [C3H is related to the DBA, being derived from offspring of crosses of DBA mice and Bagg albinos made in 1920 (*21*).]

Embryos (14-day gestation) from the cross $B_6D_2F_1$ hybrid ♂ × C57B1/6J ♀ were individually bled, and enough hemoglobin was obtained to perform the necessary electrophoresis experiments independently for each embryo. In these experiments we ran only the part of the alkaline gel containing EII hemoglobin through the two-dimensional procedure, and used the rest of the alkaline gel for a benzidine test of the adult component in order to distinguish those fetuses containing the single adult hemoglobin from those with both the single and diffuse hemoglobins. (This was necessary because the β-chains of the two adult types of hemoglobins have the same mobility in the acid-urea gel.) In our tests, all 26 embryos heterozygous for the single and diffuse hemoglobins had both Y chains; all 30 embryos homozygous for the single adult hemoglobin also had only the single Y^1 chain. This result is consistent with the hypothesis that the genes controlling the Y^1 and Y^2 fetal polypeptides are alleles, and it suggests that the Y locus is closely linked to the β-chain locus. No crossovers have been observed in 56 cases; this is compatible with a recombination frequency no greater than 5.4 percent at the 95 percent confidence level (*22*).

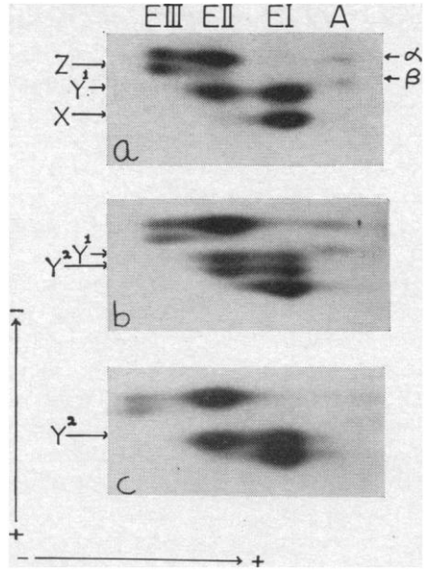

Fig. 1. Two-dimensional starch-gel electrophoresis of hemolyzates from embryo mice (12-day gestation). (a) C57B1/10SNJ with Y^1 fetal polypeptide chain. (b) C3H/HeAu ♀ × C57B1/6Au ♂ hybrid with Y^1 and Y^2. (c) C3H/HeAu with Y^2. The electrophoretic origins are not shown; a typical migration in the alkaline gel is 5.7 cm for EI and 4.2 cm for EII, and in the acid-urea gel is 7.6 cm for Y^1 and 7.1 cm for X.

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11. The acid-urea gel was made with 300 ml of a barium lactate buffer with a final concentration in barium of 0.02*M* and a buffer *p*H of 3.2 (*12*), 240 g urea, 2.5 ml of 2-mercaptoethanol, and 63 g of Starch-Hydrolyzed lot No. 241-1 (from Connaught Medical Research Laboratories, Toronto, Canada). Urea gels were made by the method of Smithies *et al.* (*13*). The procedure for performing two-dimensional starch-gel electrophoresis is that of Smithies *et al.* (*14*). Electrophoresis was carried out in the cold for 40 to 48 hours at 4 volt/cm.
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15. Care was used in taking the embryonic blood to minimize contamination from the mother; each embryo was washed twice in its membranes before it was bled into saline. Packed cells were stored in the frozen state. Hemolyzates with distilled water were centrifuged before use.
16. The C57B1/10SNJ, C57B1/6J, and $B_6D_2F_1$ hybrid mice were obtained from Jackson Laboratory, Bar Harbor, Maine. The C3H/HeAu and C57B1/6Au were from the colony of Dr. R. Auerbach, Department of Zoology, University of Wisconsin, Madison, and had originally come from Jackson Laboratory. The animals were mated naturally, and day zero of gestation was counted as the day vaginal plugs were observed.
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18. The embryonic component A was not present in the 11-day-old yolk-sac hemolyzate of Fantoni *et al.* (*17*), but it is generally observed in whole-blood hemolyzates of embryos of 12 days or more of gestation (*10*). This component has the same mobility in alkaline starch gel as adult hemoglobin; it gives rise to three bands in the acid-urea gel, the X band and two bands identical with the globin bands of adult hemoglobin. We established directly that the faster moving of the adult globin bands is the α-chain. Purified C57B1 α-globin (a gift of Dr. D. Rifkin, Rockefeller University, New York) and freeze-dried C57B1/10SNJ adult hemoglobin were run side by side in an acid-urea gel; the faster of the globin components had a mobility identical with that of the α-globin.
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