(*4*) found a high frequency of antibodies to Gm and Inv in children aged 6 months to 5 years, and serums from young pigs may similarly have a high frequency of isoantibodies to γ-globulins without deliberate immunization. There were antibodies to Gl_a in 2 out of 20 serum samples from pigs which were approximately 4 months old, and in another there were antibodies to a complementary serum factor, designated Gl_b (*5*). One of the serums with antibodies to Gl_a and the single serum with antibodies to Gl_b were used as agglutinators in all of the tests reported here.

For the test for Gl_a, one volume of a 2 percent suspension of red cells (washed seven times) from a K_b-positive (*6*) pig of group O (*7*) was mixed with two volumes of an antiserum (diluted 1:16) which contained incomplete antibodies to K_b. Cells from a group-O pig were used since naturally occurring antibodies to pig O are rare (*7*) and would not be likely to interfere with the tests for Gl factors. The mixture was incubated for 2½ hours at room temperature (24°C) to coat the red cells with antibody. The coated cells were then washed four times, and saline (0.91 percent NaCl) was added to make a 1-percent suspension. In each of two tubes one drop (1/30 ml) of the suspended red cells was added to a mixture of equal parts of antiserum to Gl_a (diluted 1:16) obtained from a pig, aged 4 months, and the diluted serum to be tested (1:4 and 1:8, respectively). The agglutinator and test serum were previously incubated at room temperature for 2 hours. The mixture of test serum, agglutinator, and coated cells was incubated at room temperature for 5 minutes, centrifuged gently (270*g*) for 1 minute, and examined macroscopically for agglutination. After an additional 2 hours incubation the tubes were again examined for agglutination, before and after centrifuging. The Gl(a+) test serums strongly inhibited agglutination of the coated cells by antibody to Gl_a; Gl(a−) serums inhibited weakly or not at all. For the test for Gl_b, the serum (diluted 1:8) obtained from a pig aged 4½ months was used as the agglutinator, and incomplete antibodies to L_c were used for coating the L_c-positive (*6*) red cells of the same group-O pig used as a source of cells in the test for Gl_a. Controls consisted of: known Gl(a+b−) and Gl(a−b+) serums tested with each agglutinator and appropriate coated cells; diluted test serum, saline, and coated cells; saline, agglutinator, and coated cells; and saline plus coated cells. The red cells coated with incomplete antibodies to K_b or L_c can be agglutinated by the addition of rabbit antiserum to pig globulin as well as by the appropriate isoantibodies.

Table 1. Inheritance of Gl types in pigs.

Mating types	Matings (No.)	Offspring		
		a+b−	a+b+	a−b+
a+b− × a+b+	6	20	22	0
a+b− × a−b+	2	0	14	0
a+b+ × a+b+	5	7	26	8
a+b+ × a−b+	4	0	14	20
a−b+ × a−b+	4	0	0	30

Porcine γ-globulins (Cohn Fraction II) and albumin (Cohn Fraction V) (*8*) were checked for freedom from other serum proteins by immunoelectrophoresis, and saline solutions of each fraction (20 mg/ml) were used in inhibition tests. There was no inhibition of agglutination by albumin, whereas the γ-globulin (diluted to 20 mg/ml) inhibited antibody to Gl_a up to a further dilution of 1:8, and inhibited antibody to Gl_b up to 1:4096. These data suggest that most of the pigs from which the pool for preparation of the γ-globulin was obtained were Gl(a−b+).

The distribution of Gl_a and Gl_b in serums from adult males and females (chosen to avoid including samples from pairs of full sibs) was: Duroc pigs, 2 Gl(a+b−), 10 Gl(a+b+), 2 Gl(a−b+); Yorkshire pigs, 1 Gl(a+b−), 3 Gl(a+b+), 12 Gl(a−b+).

No serums have been found to be Gl(a−b−), and it appears likely that allelic genes are responsible for factors Gl_a and Gl_b. The globulin types of 161 offspring, tested when 3 months old or older, from 21 matings are given in Table 1. These data are in accord with the hypothesis that Gl^a and Gl^b are codominant alleles, so that genotypes Gl^a Gl^a, Gl^a Gl^b, and Gl^b Gl^b determine phenotypes Gl(a+b−), Gl(a+b+), and Gl(a−b+). The existence of additional alleles, including Gl^- and Gl^{ab}, is not excluded by these limited data from two breeds of pigs.

Specific Gl types are not associated with specific haptoglobin, transferrin, prealbumin or amylase (*9*) types, nor are they associated with specific red-cell antigens in the A-O, B, C, E, F, G, H, I, J, K, or L (*6, 7, 10*) systems. Results of segregation in offspring from matings of animals of known Gl and red-cell types have excluded sex linkage and close linkage between the *Gl* locus and the *A, B, C, E, F, G, H, J, K,* and *L* loci controlling red-cell antigens.

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13 May 1965

Degeneration of the Eyes of Tyrosine-Deficient Chick Embryos

Abstract. *Subjecting 4-day-old chick embryos to a yolk-sac perfusion medium lacking tyrosine resulted in arrest of retinal pigmentation and in degeneration of the neural retina. Phenylalanine was ineffective in replacing tyrosine. Possibly retinal tyrosinase played a part in initiating the degenerative changes.*

The first successful study of amino acid deficiencies in chick embryos was reported by Klein, *et al.* (*1*), who applied the explantation techniques of Spratt (*2*) and of Hayashi and Herrmann (*3*) in studies with defined

media. Klein *et al.* (*1*) found that omission of lysine or leucine from the medium reduced growth, as measured by protein nitrogen in the embryo, but they observed no gross abnormalities.

We were not able to show a definite response to amino acids in embryos subjected to yolk-sac perfusion (*4*) until improvements in our techniques permitted more effective studies of various amino acid requirements (*5*). We now report that a deficiency of the amino acid tyrosine results in profound changes in the development of the eye.

The basic techniques of yolk-sac perfusion (*4*) were used in these studies. After 3 days of incubation, a canal through a coagulum of the egg white was formed to make a passage connecting the interior of the yolk-sac with the outside of the shell. Through this passage most of the yolk was flushed out and replaced by a solution of glucose, salts, and antibiotics. On the fourth day, the egg was attached to the perfusion apparatus, and 30 ml of defined medium were given at 8-hour intervals. From the 4th day on, the eggs were kept in an atmosphere of 50 percent oxygen plus 50 percent nitrogen. Eggs which were prepared with the canal of coagulated egg white, but from which the yolk was not removed, were used as controls.

The composition of the solution used for flushing out the yolk, in milligrams per liter of demineralized water, was as follows: NaCl, 4460; KCl, 1700; $NaHCO_3$, 1100; NaH_2PO_4, 280; $MgCl_2 \cdot 6H_2O$, 340; $MgSO_4 \cdot 7H_2O$, 200; $CaCl_2$, 20; glucose, 1500; erythromycin (as glucoheptonate), 95; and dihydrostreptomycin (as sulfate), 660. The defined medium contained, in addition, the following (mg/lit.): L-alanine, 120; L-arginine HCl, 280; L-cystine, 60; glycine, 120; L-glutamic acid, 380; L-histidine, 80; L-isoleucine, 200; L-leucine, 300; L-lysine HCl, 280; L-methionine, 80; L-phenylalanine, 160; L-proline, 120; DL-serine, 440; L-threonine, 180; L-tryptophan, 60; L-tyrosine, 160; L-valine, 220; pyridoxine HCl, 1.0; biotin, 0.1; folic acid, 0.1; niacin, 1.0; thiamine HCl, 1.0; calcium pantothenate (dextrorotatory), 2.0; riboflavin, 2.0; vitamin B_{12}, 0.002; inositol, 10.0; choline chloride, 300. The concentration of amino acids in the medium is approximately twice that used by Klein *et al.* (*1*) and four times the amounts usually used in tissue-culture media.

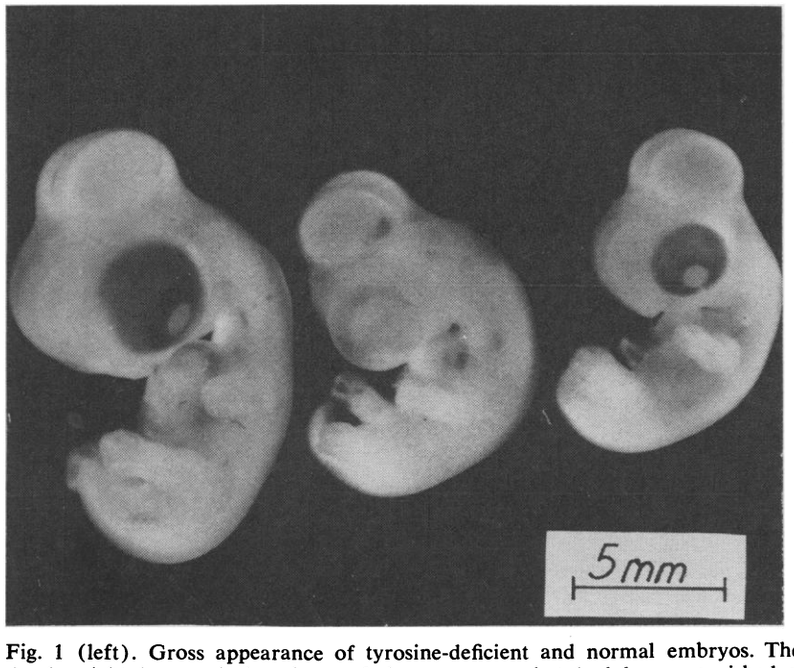
Table 1. Death times of embryos nourished by defined media with varying phenylalanine and tyrosine concentrations. The median time of death (fourth death occurring in a group of six embryos) is shown, together with the range.

L-Tyrosine (mg/lit.)	L-Phenylalanine (mg/lit.)	Death time (hr)
0	160	64 (40–64)
40	160	56 (32–72)
160	160	56 (48–72)
160	320	48 (24–72)
0	320	48 (32–64)

The defined medium afforded good survival for about 40 hours of perfusion beginning at 4 days of incubation; by 72 hours, however, all embryos were dead. Death of the embryo was determined by inspection, at 8-hour intervals, of the appearance of the yolk-sac circulatory system, which was viewed by a candling procedure.

In the first experiment embryos were perfused with media containing varying concentrations of phenylalanine and tyrosine (Table 1). Survival was not affected by raising the tyrosine concentration from 0 to 160 mg/lit., or by providing 160 or 320 mg of phenylalanine per liter, but pigmentation of the retina was greatly affected (Fig. 1). Scorings of 0 (no grossly visible pigment) to 3 (normal pigment) were made of each embryo (Fig. 2). When the tyrosine concentration was 40 mg/lit., some pigment was formed, but less than at higher concentrations. Examination of sections of the eyes of tyrosine-deficient embryos revealed that after 40 hours of perfusion there was almost no pigmentation and the neural retina had degenerated completely. Degenerative changes had also occurred in the lens.

In the second experiment, perfusion was again begun at 4 days with defined media containing varying concentrations of phenylalanine and tyrosine.



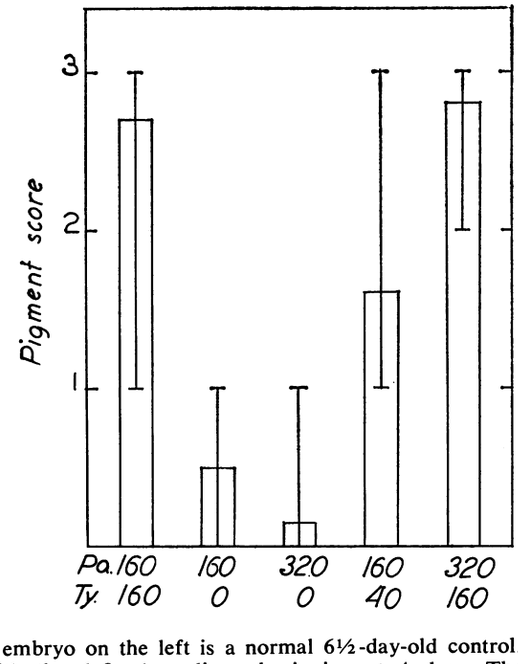


Fig. 1 (left). Gross appearance of tyrosine-deficient and normal embryos. The embryo on the left is a normal 6½-day-old control. At the right is a typical embryo of the same age that had been nourished with the defined medium, beginning at 4 days. The middle embryo received the defined medium without tyrosine. Fig. 2 (right). The scores of retinal pigmentation of embryos subjected to various yolk-sac concentrations (milligrams per liter) of phenylalanine (*Pa*) and tyrosine (*Ty*); 3, normal pigment; 0, no pigment.

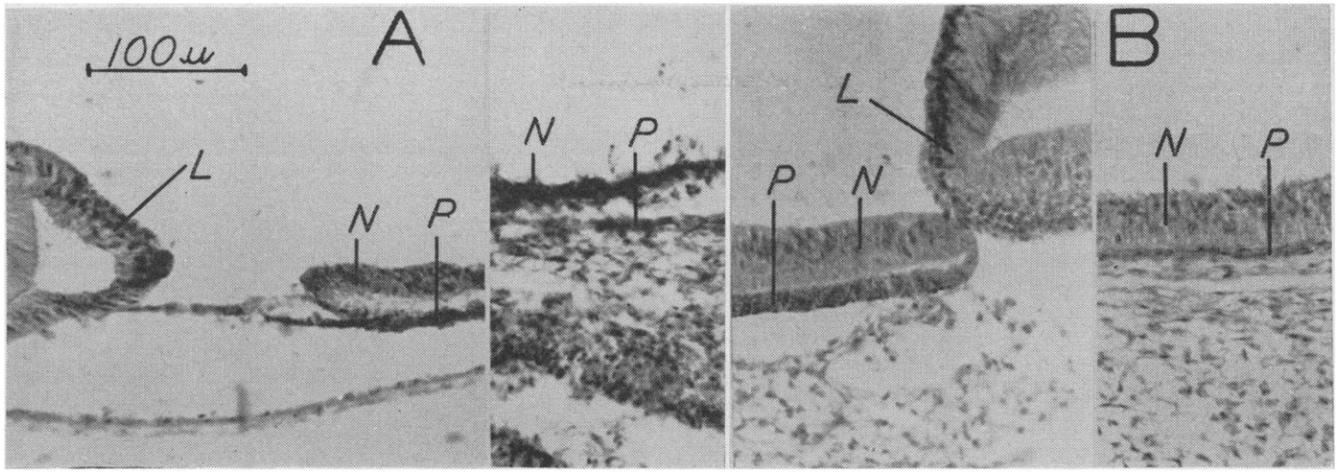


Fig. 3. Sections of eyes of chick embryos subjected to yolk-sac perfusion for a 24-hour period beginning at 4 days of incubation. The perfusion fluids contained 160 mg of L-phenylalanine per liter. Embryo *A* received no tyrosine; Embryo *B* received 160 mg of L-tyrosine per liter of perfusion medium. Left photomicrograph of each pair shows part of the lens and ciliary junction. The lumen of the eye is at the top. The right photomicrograph is of the fundus region. *L*, lens; *N*, neural retina; *P*, pigmented epithelium.

Representative embryos were sacrificed at 5 or 6 days of incubation, and sections were compared with control embryos. The results at 5 days, after only 24 hours of perfusion, were striking. Without tyrosine in the medium, there was extensive degeneration of the neural retina, especially in the fundus region (Fig. 3*A*). By 48 hours of perfusion (6 days of incubation), the neural retina had disappeared and only scattered and degenerating cells remained in the vitreous chamber. The pigmented layer appeared to be degenerating. Also, there was a deficiency of melanin which was especially obvious in unstained preparations. The perfused controls which had received tyrosine were almost normal after 24 hours (Fig. 3*B*), but after 48 hours showed retarded development of the eye. Nevertheless, the retina in these controls was heavily pigmented. The lens was flattened and vacuolated and had become separated from the ciliary zone of the retina. The brain and spinal cord contained small areas of degeneration, and mitotic figures were scarce. Other tissues appeared to be affected relatively little.

From these experiments we have concluded that the young chick embryo's total requirement for tyrosine cannot be met by phenylalanine. In contrast, the tyrosine requirement of the young chick can be met entirely by the conversion of phenylalanine to tyrosine in the liver (*5*, *6*). In mammals this does not occur until late in embryonic development or early in postnatal life, when significant amounts of the phenylalanine hydroxylase enzyme appear (*7*). The activity of this enzyme in the avian embryo has not been determined.

It is possible that the embryos, which were perfused with defined media lacking tyrosine, survived and grew as well as the controls because the tyrosine requirement for protein synthesis in the embryo is low, and therefore it could be met by the synthesis of a minimal amount of tyrosine from phenylalanine, or by effective utilization of small amounts of tyrosine from intracellular yolk-sac contents, or by albumen proteins which entered the yolk-sac. When both phenylalanine and tyrosine were omitted, development was greatly retarded, and except for the presence of some dead cells, the retina appeared to have changed relatively little in the course of the experiment.

During the 5th day of incubation, the eye normally increases greatly in size. Coulombre (*8*) has pointed out the dynamic effects of this expansion on the surface area of the pigment layer in relation to cell division in the neural layer and the dependence of the neural layer on the pigment layer for transport of nutrients, gases, and metabolites from and to the blood vessels, which lie outside the pigmented layer of the retina.

The following statement is offered to explain the effects of tyrosine deficiency on retinal pigment formation by the young embryo. The tyrosine requirement for general protein synthesis can probably be met from residual yolk or white, or possibly even by limited synthesis from phenylalanine. Thus, omitting tyrosine from the defined medium does not significantly affect survival and growth. In the eye, however, the active tyrosinase system of the pigment layer of the retina (*9*) may oxidize to melanin the limited supply of tyrosine absorbed from the blood by the pigment cells, and thereby deprive the neural retina of the tyrosine needed for tissue growth. As the eye increases in size, the pigment layer becomes stretched in the normal way, but in the neural layer cell division and tissue synthesis are halted by the tyrosine deficiency, and the integrity of the connections between the layers is lost. Thus the neural layer, separated even further from the blood supply, rapidly degenerates.

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8 February 1965