TABLE I.
The Detection of Digitoxin in (1) Tyrode's Solution, (2) Rat Serum, and (3) Human Serum by the Embryonic Duck Heart Preparation.

	Tyrode's solution			Rat serum			Human serum		
Conc. of digitoxin (μg/cc)	Avg. time "dig. effect" (min.)	Stand. error of mean (min.)	No. hts.	Avg. time "dig. effect" (min.)	Stand. error of mean (min.)	No. hts.	Avg. time "dig. effect" (min.)	Stand. error of mean (min.)	No. hts.
.001	ND		10	ND		12	ND		10
.005	48	3.0	15	ND		10	ND		10
.010	41	1.6	21	ND		10	ND		10
.05	23	1.5	33	ND		10	ND		10
.10	12	0.8	20	ND		10	ND		10
.20	—	—	—	62	1.3	18	ND		15
.40	—	—	—	38	1.7	18	ND		15
.60	6	0.2	20	24	0.5	36	49	2.0	23
.80	—	—	—	21	0.8	20	34	1.6	24
1.00	4	0.2	29	11	0.4	22	21	1.1	28

ND indicates that no glycoside could be detected by occurrence of "digitalis effect" in embryonic heart preparation.

ever, was not found[3] to be inhibited similarly by serum.

Conclusions. 1. Quantitative assay of extremely minute amounts of digitoxin in Tyrode's solution and in rat and human serum was found to be possible by means of the embryonic duck heart preparation.

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Peculiar Enlargement of Eyeballs in Chicks Caused by Feeding a High Level of Glycine.*

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Previous reports[1,2] from this laboratory have shown that glycine is highly "pellagragenic" when fed to chicks receiving a nicotinic acid-low diet. On the other hand, the chick tolerated unusually high levels of glycine (4 to 6%), provided a sufficient amount of nicotinic acid was contained in the diet.[2] The latter observation was somewhat surprising for it had been reported earlier that more than 2% of glycine was toxic to the young chicken[3] and that 4 g of glycine per day were harmful to hens.[4]

Because of this discrepancy, an investigation was undertaken to determine the minimum toxic level of glycine and the extent to which this level could be influenced by nicotinic acid feeding. The present report describes a peculiar overgrowth of the eyeballs and other conditions observed in chicks resulting from feeding a highly purified diet con-

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TABLE I.
Composition of Basal Diet 113GN.

	%
Cerelose	71.4
Casein (crude)	18.0
Mineral mixture 1M	6.0
Soybean oil	4.0
dl-Methionine	0.3
Choline chloride	0.2
i-Inositol	0.1
Vitamins (mg/100 g).	
Thiamine HCl	0.4
Riboflavin	0.8
Ca pantothenate	2.0
Pyridoxine HCl	0.6
Biotin	0.02
Pteroylglutamic acid	0.2
Para-aminobenzoic acid	0.2
2-Methyl-1,4-naphthoquinone	0.1
Alpha-tocopherol	0.5
Vit. A and D_3, 1200 U.S.P. and 170 A.O.A.C. units, respectively, by dropper weekly.	

taining 8% of glycine.

Experimental. Day-old New Hampshire chicks of mixed sexes were used. The chicks were distributed according to weight, confined in electrically heated wooden cages with wire screen floors and given feed and water *ad libitum*. Weighings and other observations were made weekly. All substitutions in the basal diet 113GN were made at the expense of cerelose (Table I).

Results. Table II summarizes the results of feeding 8% of glycine to chicks in combination with various levels of nicotinic acid. It is quite evident that glycine at this level inhibits growth and that the growth depression is not overcome by feeding nicotinic acid in amounts as high as 100 mg per 100 g of diet. A few cases of perosis occurred in the groups receiving glycine and feathering was inferior. No "chick black-tongue" was seen, as might be expected with the high level of glycine fed.

During the first week of the experiment the chicks in the 3 groups receiving glycine exhibited periods of extreme prostration accompanied by tremors. This condition seemed to be especially severe after eating. Sometimes the chicks would become so comatose they would appear to be at the point of death at which time they would gradually rally. In spite of these symptoms, constant weight gains were made.

After the first week the chicks appeared to outgrow the tendency toward prostration, but slight tremors existed in the birds throughout the whole experiment. They seemed to be constantly fatigued and would stretch their wings and legs at frequent intervals.

At about the third week a peculiar puffiness about the eyes was noticed. By the fourth week it was evident that either the eyeballs were becoming enlarged or that an edemic condition existed in the optic vesicle which was causing the eye to be forced out, as occurs in exophthalmic goiter in the guinea pig. The eye condition at this point was severe enough to interfere with the normal movements of the nictitating membrane.

At the end of 4 weeks, 3 typical chicks from each group receiving glycine were selected and the total of 9 chicks were given the diet containing 8% of glycine and 10 mg of nicotinic acid per 100 g of diet. They were continued on experiment to 7 weeks of age along with the 6 control chicks. From the fourth week to the seventh week, the "popped" eye condition became worse. From about the fifth week on, the eyes were so bulged that normal vision was impaired to the point where it was evident that the eyes could not be focused on objects close at hand. Consequently, the feed hoppers had to be kept quite full so that the chicks could eat properly.

At 7 weeks of age all the chicks were killed by severing the jugular vein. All had been fasted for a period of 6 hours prior to killing. The heart, liver with bile sac, spleen, thyroids, and one eye from each chick were removed and weighed immediately. All adhering muscular tissue was cut away from the eyes before they were weighed. In Table III the average values obtained for the various organs are given expressed in terms of percent of body weight. The data show that on this basis the values of the 2 groups for the thyroids, heart, liver plus bile sac, and spleen were not appreciably different. However, a marked difference existed in the weight of the eyeballs. The eyes from the chicks which received 8% of glycine were almost twice as great on a percent-of-body-weight

TABLE II.
Effect of 8% of Glycine on Chicks Receiving Various Levels of Nicotinic Acid.

Group No.	Supplement of basal diet 113GN containing 5% gelatin	No. chicks	No. dead	Avg wt 4 wk, g	No. perosis	Feed efficiency*
1	10 mg % nicotinic acid	6	0	235	0	.510
2	10 " " " " + 8% glycine	6	1	157	2	.501
3	50 " " " " + " "	6	1	152	1	.551
4	100 " " " " + " "	6	1	158	1	.504

* $\frac{\text{Total weight gained}}{\text{Total feed consumed}}$ = feed efficiency.

TABLE III.
Effect of 8% of Glycine in the Diet of Chicks on the Size of Various Organs of the Body.

Diet	No. chicks	Avg body wt 7 wk, g	% of body weight (avg)				
			Eye*	Thyroid†	Heart	Liver and bile sac	Spleen
A	6	532	0.343 (0.289-0.396)	0.008	0.503	2.776	0.210
B	9	399	0.638 (0.565-0.762)	0.007	0.537	2.893	0.232

Diet A. 113GN + 5% gelatin, 10 mg % nicotinic acid (control group).
Diet B. 113GN + 5% gelatin, 10 mg % nicotinic acid, 8% glycine.
* Values given are for one eye.
† Values given are for both thyroids.

basis as were the eyes from the controls. This striking eye condition may be seen in Fig. 1 which shows the difference in size of 2 excised eyeballs, one from a control chick and the other from a chick which received 8% of glycine.

The production of an apparent overgrowth of the eyeballs seems to be related to the form in which the amino acid is fed. The same basal diet supplemented with 10% of gelatin, 25% of bone ossein, and 10 mg of nicotinic acid per 100 g did not cause an enlargement of the eyeballs, tremors, or marked growth depression, although this diet contained approximately 8% of glycine but in the peptide form. (Bone ossein was used in place of gelatin because the diet tended to stick to the beaks of chicks when it contained more than 10% of gelatin. It is assumed that bone ossein and gelatin are equally digestible because both depressed growth in about the same order of magnitude when fed to chicks receiving nicotinic acid-low diets. No explanation for the protective action of protein is offered.)

Furthermore, the effect of free glycine under the conditions of these experiments is confined to the growing chick. In experiments with laying hens, 4, 8, and 12% of glycine in a semi-purified diet containing 50 mg of nicotinic acid per 100 g did not cause any eye abnormalities during successive feeding periods of 5, 3, and 4 weeks, respectively. Feed consumption and egg production were only slightly lowered during the latter 4-week treatment.

The underlying physiological cause of the observed biological anomaly is as yet unknown. Whether the enlargement of the eyeballs is the result of actual tissue growth, or increased optic fluid caused by disturbed osmotic relationships, or other conditions must be determined through additional experimentation. The fact that the condition was observed in young growing chickens and not in laying hens would lend support to the belief that the enlargement is the result of actual overgrowth of tissue and not to increased ocular fluid caused by osmotic disturbances. Whether or not other amino acids will cause this same condition has not been studied.

Summary. Growth depression and tremors were observed in chicks fed 8% of glycine as part of a highly purified diet. The depressed growth rate was not overcome by feeding unusually high levels of nicotinic acid. In addi-

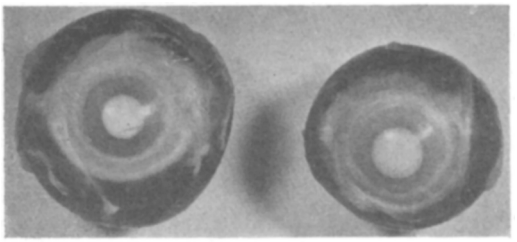

FIG. 1.
Contrast in size of eyeballs. The eyeball on the left is from a 7-week-old chick which received a diet containing 8% of glycine; its body weight was 395 g. The eyeball on the right is from a normal 7-week-old chick; its body weight was 574 g.

tion, a peculiar enlargement of the eyeballs developed which interfered with normal vision and functioning of the nictitating membrane. Eight percent of glycine as the peptide produced no such effect. The action of glycine appeared to be confined to the growing chicken. The feeding of 12% of glycine to hens over an extended period produced no apparent ocular abnormalities.

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Effect of Dicumarol on Ac-Globulin and Prothrombin Activity.*

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(Introduced by Walter H. Seegers.)

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The administration of dicumarol to prevent the occurrence or extension of intravascular thromboses has given encouraging results.[1-3] The rationale of this treatment is based upon the ability of dicumarol to lower the prothrombin concentration in the circulating plasma.[3] Dicumarol has been thought to act principally upon the prothrombin portion of the coagulation mechanism and thus to depress the clotting activity of whole blood. It is now apparent that another factor, Ac-globulin, warrants study in connection with dicumarol therapy. Ac-globulin[4] is a plasma protein normally found in the circulating plasma. It acts to assure normal physiological conversion of prothrombin to thrombin during the process of blood coagulation. A deficiency of this factor impairs thrombin formation and a bleeding tendency may develop.[5]

The existence of Ac-globulin was unknown until recently, and previous reports on prothrombin measurements by both the one- and two-stage tests have not considered the possible role of this factor. With accurate and specific methods now available, a study was undertaken of both prothrombin and Ac-globulin levels as they are influenced by dicumarol administration.

Methods. Blood was obtained by venipuncture. In order to reduce contamination by tissue fluids the initial 3 cc of blood drawn into the syringe were discarded. The sample to be analyzed was received into a second syringe containing the anticoagulant. In this

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