pheral vascular system in this nutritional deficiency, and its possible significance has been reported previously.[1]

Discussion. Although the mucosal blood vessels were not observed directly in this study, the complete cessation of blood flow found frequently in the small arteries and arterioles supplying the mesentery and traversing it to supply the intestine support the assumption that accompanying the "flight reaction" there is often a complete stagnation of blood flow in this viscus. This is apparently maintained throughout the period of response to the stimulus, and subsequently returns slowly. Of particular interest is the fact that even at the height of response, continuous blood flow through the arterial arcades and the larger venules and veins in the mesentery affords a means of draining much of the residual capillary blood in this region and returning it, along with the blood shunted to the arcade system, back to the general circulation. One can assume that this would considerably increase the volume of blood available for other somatic areas during the period of active vascular response.

The absence of vasospasm in the mesentery and serosa during the flight response in scorbutic animals is associated with a general depression of arteriolar and precapillary tone, and impaired venular flow.[1] It remains to be determined whether these manifestations of ascorbic acid deficiency are a result of general tissue disfunction or are perhaps a specific defect in some vasotonic mechanism.

Summary and conclusions. 1. Technics were devised for viewing the mesenteric and serosal capillary bed of guinea pigs not under a general anesthetic agent.

2. The degree of emotional response to auditory stimuli could be correlated with the magnitude of changes in the visualized splanchnic vessels.

3. (a) A "startle response" was occasionally accompanied by transient slight vasoconstriction and slowing of blood flow.

(b) A "flight response" was frequently characterized by intense vasoconstriction of arterioles, complete stagnation of capillary blood flow, and a drainage of residual capillary blood into patent venules.

3. Certain "key sites" along the arteriolar tree have a lower threshold of response than remaining areas, and exert much control of arteriolar flow by sphincter-like activity.

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17272. Reversal of Insulin-Induced Hypoglycemia in Chick Embryos by Nicotinamide and α-Ketoglutaric Acid.*

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In a series of papers Landauer and his collaborators[1-4] have described the teratogenic effects which insulin produces when it is injected into the yolk sac of chick embryos. When the insulin is applied in this manner to early embryos (0-72 hrs) caudal defects (rumplessness) are the most frequently encountered anomalies. When injected into older embryos (96-168 hrs) the insulin causes a high incidence of disproportionate shortening of the legs (micromelia). These effects of insulin disappear when the insulin is inactivated and reappear when inactivated insulin is reactivated. Landauer[5] demonstrated that when

* This investigation was aided, in part, by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

1 Landauer, W., *J. Exp. Zool.*, 1945, **98**, 65.

2 Landauer, W., and Lang, E. H., *J. Exp. Zool.*, 1946, **101**, 41.

3 Landauer, W., and Bliss, C. I., *J. Exp. Zool.*, 1946, **102**, 1.

4 Landauer, W., *J. Exp. Zool.*, 1947, **105**, 145.

5 Landauer, W., *J. Exp. Zool.*, 1948, **109**, 283.

nicotinamide is injected into the yolk sac either shortly before, after or simultaneously with insulin the incidence of micromelia is reduced from 41 - 53% to 3 - 9% depending on individual experiments. When the same experiments were repeated on earlier stages it was found that nicotinamide also reduces the incidence of rumplessness. In like manner α-ketoglutaric acid reduces the incidence of micromelia, though not as effectively as nicotinamide, but does not, however, exert much of an inhibiting effect on the rumpless-inducing properties of the insulin.

Zwilling[6] showed that the injection of insulin into the yolk sac of 120 hr embryos is followed by an hypoglycemia which, in some cases, persists for 8 days. The blood sugar in all embryos is restored to normal levels by the fourteenth day of development. In the course of this investigation it was found that a very good correlation exists between the degree and persistence of the hypoglycemia and the degree and incidence of micromelia. Factors which increase the micromelic effects (such as adrenal cortical extracts, Landauer[7]) also exaggerate the hypoglycemic effects.

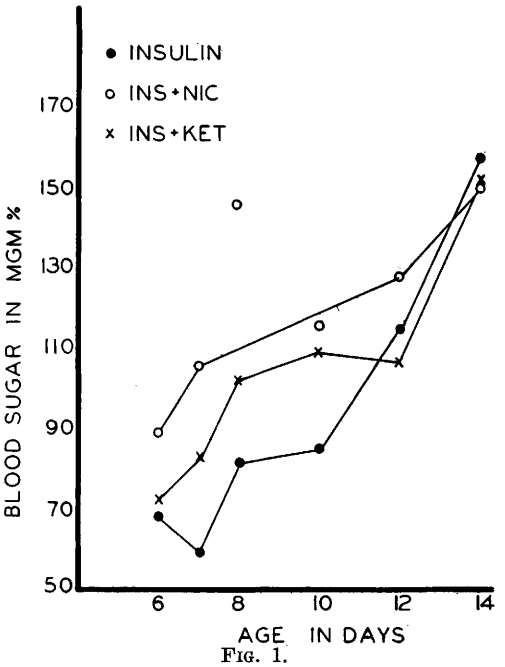

FIG. 1.

Course of blood sugar in chick embryos treated with: insulin alone (2 units), insulin + nicotinamide (18.9 mg), and insulin + α-ketoglutaric acid (20.2 mg) at 5 days. Each point represents the average of blood sugar values for all embryos assayed at a given stage.

It was of some interest to us to determine whether nicotinamide and α-ketoglutaric acid have any effect on the insulin-induced hypoglycemia. We found that these substances do decrease the incidence of hypoglycemia and that this decrease follows the same order that it does for their micromelia-alleviating capacities; *i.e.* the effect is more pronounced with nicotinamide.

Material and method. Eggs from unselected White Leghorn hens were used in this work. They were all injected at 120 hours as described by Landauer.[1] When two substances were used they were injected separately, but in sufficiently rapid succession so that they may be considered simultaneous injections. Blood was obtained and assayed for reducing substances by the technic described previously.[6] For each stage blood samples were taken from embryos which had been injected with insulin alone (2 units), nicotinamide alone (18.9 mg) or α-ketoglutaric acid alone (20.2 mg) and both insulin and one of the other two substances. The sequence of obtaining blood from the embryos was altered sufficiently so that it could not influence the results.

Results. The data from these experiments are presented in Fig. 1 and in Table I. Since neither nicotinamide alone nor α-ketoglutaric acid alone cause any marked deviation from normal blood sugar levels the data obtained for these substances have been omitted. All of the points on these curves represent averages for all blood sugar values obtained for a given stage. This explains the difference between the curve for 2 units of insulin presented here and that presented in the previous paper.[6] In the latter the points represented the average values for all hypoglycemic embryos at any given stage. However, these data for insulin alone duplicate those of last year in all essential respects. It can be seen that both nicotinamide and α-ketoglutaric

[6] Zwilling, E., *J. Exp. Zool.*, 1948, **109**, 197.
[7] Landauer, W., *Endocrinol.*, 1947, **41**, 489.

TABLE I.

Summary of Blood Sugar Data from Embryos Injected with Insulin (2 Units), Insulin + Nicotinamide (18.9 mg) and Insulin + α-ketoglutaric Acid (20.2 mg). All injections were into the yolk sac of 5 day embryos. In the last column under each substance the range of blood sugar values is given for only the hypoglycemic embryos. Values in the normal range have been omitted.

	2 units insulin			Insulin + nicotinamide			Insulin + α-ketoglutaric acid		
Age in days	No. of embryos	No. hypoglycemic	Range of hypoglycemia, mg %	No. of embryos	No. hypoglycemic	Range of hypoglycemia, mg %	No. embryos	No. hypoglycemic	Range of hypoglycemia, mg %
6	13	13	43.4- 97.2	10	9	62.5-108.6	10	9	42.7- 83.9
7	13	13	38.0- 85.3	9	4	67.8- 90.0	10	9	59.4-103.0
8	16	15	49.8-101.2	10	2	100.5-105.6	18	11	61.3-105.0
10	16	13	40.0-105.1	11	4	66.9- 96.1	18	10	43.1-108.2
12	13	6	34.7- 95.8	9	2	42.4- 94.0	8	4	32.1- 88.2
14	7	0		6	0		7	0	

acid reduce the average hypoglycemia and that this effect of the nicotinamide is evident within twenty-four hours of the time that it is administered. From the data presented in Table I it is evident that this is due, in large part, to a reduction in the number of embryos which are hypoglycemic at any one time. However there is also a decrease in the degree of hypoglycemia of the individual embryos. Nicotinamide is more effective than α-ketoglutaric acid in producing both of these results. This correlates well with the fact that cases of extreme micromelia are not found following injection of insulin + nicotinamide, while they do occur after injection of insulin + α-ketoglutaric acid. The one discordant point (insulin + nicotinamide at 8 days) may be due to a sampling error. However, there is a possibility that it may, in part, represent an actual increase. Not only were there fewer cases of hypoglycemia but the range of "normal" values was higher than usual. A similar phenomenon has been observed in our other work; it seems as though recovery to normal levels may be accompanied by an over-compensation.

Discussion. Again we have an instance in which the level of blood sugar varies as does the degree of micromelia. In this instance nicotinamide and α-ketoglutaric acid are instrumental both in decreasing the incidence of micromelia and in restoring the blood sugar of the embryos to more normal levels. It might be pointed out that the curve for insulin + nicotinamide described here is very similar to the one which describes the course followed by the blood sugar in embryos which receive only insulin but which show none of the teratogenic effects of that substance.[6]

The causal relationship between the morphological effects of insulin and its hypoglycemic action in the chick embryo is still obscure. It is not yet certain that these effects are *directly* due to a decrease in available carbohydrate energy, though it is evident that the carbohydrate metabolism is involved. In like manner the mechanism of the alleviating action of the nicotinamide is not clear. It may either be a result of its restoring the blood sugar to normal levels or due to a more direct action on the embryonic tissues.

We have found that sulfanilamide, which produces essentially the same micromelia as insulin,[8] does not alter the blood sugar (unpublished). However its micromelic effects are also reversed by nicotinamide (unpublished). There is a possibility that the low concentration of reducing sugars in the insulin-treated embryos may result in a higher nicotinamide requirement, while the sulfonamide may affect the nicotinamide content in another fashion. Levy and Young[9] have shown that virtually all of the nicotinamide (or nicotinic acid) in the chick embryo is bound in the diphosphopyridine nucleotide (coenzyme I). They also indicate (as has been found in other animal tissues[10]) that excess nicotinic acid (or amide) does not result in an increase in the coenzyme beyond its maximum levels. If these same conditions obtain under the abnormal circumstances induced by insulin (and sulfanilamide), then it seems likely that these substances may interfere, in some manner, with the synthesis of the coenzyme.

The hypoglycemia-alleviating effect of nicotinamide demonstrated here is not without precedent. Burke and McIntyre[11] have shown that this substance increases the tolerance of rats to insulin; a decrease in the duration of hypoglycemia was noted following the injection of a standard dose of insulin into nicotinamide-treated animals. These are essentially the same results which we have obtained with chick embryos.

That α-ketoglutaric acid also prevents insulin abnormalities as well as decreasing the duration of the hypoglycemia may indicate that pyruvate metabolism is, in some way, inhibited (possibly via coenzyme lack) and that this alternate pathway may be utilized as an energy source. At any rate these data indicate that carbohydrate metabolism is of considerable importance for normal limb formation in chick embryos.

Summary. The duration of insulin-induced hypoglycemia is reduced following the administration of nicotinamide and α-ketoglutaric acid to insulin treated chick embryos. Nicotinamide, which is the more effective in preventing insulin-induced abnormalities, is also more effective in decreasing the duration of the hypoglycemia.

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17273. Influence of Methionine and Thiouracil on Nitrogen Balance Index and Organ Weights of Adult Rat.*

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Allison, Anderson, and Seeley[1] have demonstrated that small quantities of methionine conserve body nitrogen when added to the diet of the dog. Earle, Small, and Victor[2] and Reisen, *et al.*[3] have found, however, that larger amounts of the amino acid will cause a marked loss in body weight in rats. This is in apparent contradiction to Harrison and Long[4] who reported that a diet containing 2% of methionine would increase the liver nitrogen of rats. Lea-

* Presented as partial fulfillment of the degree of Doctor of Philosophy at Rutgers University.

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[2] Earle, D. J., Jr., Small, K., and Victor, J., *J. Exp. Med.*, 1942, **76**, 317.

[3] Reisen, W. H., Schweigert, B. S., and Elvehjem, C. A., *Arch. Biochem.*, 1946, **10**, 307.

[4] Harrison, H. C., and Long, C. N. H., *J. Biol. Chem.*, 1945, **161**, 547.