around the cannula tip into the aorta has been demonstrable. With this cannula it has been possible to measure total left coronary inflow in vessels impossible to cannulate by other techniques.

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Developmental Abnormalities in Chick Embryos Treated with Sugar

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As a part of a problem concerning the carbohydrate metabolism of early embryo chicks, a series of eggs was treated by injecting 1 ml of a 2-*M* solution of sucrose into the albumen. Although this treatment was carried out at various ages of incubation, the present report concerns only eggs injected prior to incubation. These eggs were opened after 72, 96, or 120 hr of incubation, and whole-mount slides or serial sections prepared of all living embryos. Another series of eggs was injected with a like amount of normal saline, incubated, and prepared along with the experimental material, as controls.

In the sucrose-treated series 195 eggs had living embryos when opened. Of these 44.5% ± 3.6% showed definite morphological abnormalities, compared with 8.3% ± 1.8% of 234 living embryos in the saline-treated series. The great variety of abnormal morphological types produced by this treatment is of interest. These malformed embryos resemble each other broadly, in that the most usual affected areas are the nervous and circulatory systems. This might be expected, since, as Weiss (*1*) has discussed, any deleterious agent seemingly will affect the more sensitive areas of an organism first, and more severely than less susceptible parts. The stages treated here—0–72 hr, 0–96 hr, and 0–120 hr—cover the period when these two systems are particularly active in growth and differentiation. However, the specific syndromes of abnormality found within this broad general pattern show extreme variation, ranging from suppression or atypical development of a single region, such as the eye, to a completely amorphous mass, or to complete absence of an embryo from the blastoderm. No one specific syndrome of abnormality occurs in a significant percentage of the material; rather a large number of abnormal conditions reported in the literature of being produced by experimental treatment of various types seem to have been closely duplicated here.

To cite a few such examples: The "rumplessness" produced by Landauer (*2*) by injection of insulin or other chemicals, and described by Moseley (*3*) appears in 8 embryos. The various abnormalities of the central nervous system, particularly the "sinuous nervous system" described by Catizone and Gray (*4*) after treatment with lead salts, and by Hansborough (*5*) as being produced by nicotinic acid, appear to be duplicated in 13 embryos. The presence of a double heart, shown by Szepsenwol (*6*) and by Waddington (*7*) to be brought about by operative injury, occurs in two examples. The author believes, however, that he has ruled out mechanical injury as a factor in the present work. Gray and his co-workers (*8, 9*), in two interesting abstracts, have shown that the injection of certain optically active compounds will significantly change the percentage of occurrence of heterotaxic embryos. In the present experiment, 9.7% ± 2.1% of the treated embryos were heterotaxic compared with 2.8% ± 1.1% in the saline-treated controls. These heterotaxic embryos often show other abnormalities in varying degrees, but only embryos that were nearly enough "normal" to determine their essential morphological pattern have been included in the above figures. Gray did not comment on the occurrence of other abnormal conditions in his material.

Particularly interesting is a comparison of the present results with the recent report by Eakin (*10*) on amphibian embryos that had been immersed in solutions of sucrose. His results also show variation in the abnormalities produced. Eakin has specifically described failure of pituitary development. Upon examination of sectioned embryos in the present series, a broadly comparable condition was found; i.e., the formation of Rathke's pouch was atypical. In 13 of the embryos examined this structure was considerably smaller (in two instances completely absent) than in control embryos of comparable age. This condition is typically accompanied by various other abnormalities, but there seems to be no correlation between the suppression of this structure and any other specific abnormal condition. In the material examined thus far the infundibulum seems to be normally developed. Examination of more, and of older, specimens will be necessary before commenting further on this particular condition.

Eakin (*10*) has offered several possible suggestions as to the cause of the anomalies produced in his experiments, without specifically attempting an analysis of those causes. The present author can do little better. According to Needham (*11*), the chick embryo during the stages under discussion is largely dependent on a carbohydrate metabolism as a source of energy for growth and differentiation. Landauer (*12*) believes that the various abnormalities produced in his work result from interference with this metabolism. It would seem possible that sucrose reacts with the embryonic system in some such fashion, disturbing the basic developmental patterns, and being remarkably nonspecific in its effects. When any material is injected into an egg, as in this work, there is obviously no control over its subsequent distribution. Time and spatial factors may enter in, so that the embryo or a region of the embryo may well be exposed to dif-

ferent concentrations of the material at different stages in development. If sucrose is capable of affecting development during these stages, as seems evident (whatever the specific effect may be), this might well explain the great variety of anomalies produced. As Weiss (*1*, 483) has pointed out, "Not only may the same type of malformation arise in various ways, but various kinds of malformations may also be caused by the same disturbance." The present results seem to be another illustration of this point.

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Quantitative Microdetermination of Amino Acids after Paper Chromatography[1]

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Paper chromatography (*1*) and microbiological determination are the two most useful modern methods of protein analysis. Paper partition chromatography was initially only a qualitative or semiquantitative method, which several workers soon tried to improve so as to meet quantitative standards. Generally this has been attempted by employing the ninhydrin reaction and measuring the color and area on the paper (*2–4*) or by extracting the amino acids from the paper spots and performing the ninhydrin reaction or a copper amino nitrogen determination (*5–9*). Even with these refinements, however, the estimation of the amino acids is only approximately quantitative, or the process is tedious, especially when it is necessary to extract the amino acid and use special reagents.

We have been using a method which results in the accurate determination of quantities of amino acids of about 10 γ; only rarely (in the case of histidine) is it necessary to use a greater amount of the compound (Table 1) to obtain a proportionality between the color produced and the quantity of amino acid present in the paper spot. Our procedure consists in (1) localizing the spots on the paper and marking lightly their limits with a pencil, under an ultraviolet lamp; (2) cutting the spots, introducing them in a volumetric 10-ml flask and developing a color reaction with ninhydrin; and (3) measuring the color intensity at the end of the reaction. Localization of the spots by ultraviolet fluorescence is possible only after the paper is dried at room temperature for some days or heated at 100°–105° C for about 5 min. We prefer the heating. According to Patton *et al.* (*10*), this fluorescence is essentially due to the paper surface being modified by deposition of the amino acid.

TABLE 1

Quantity of amino acid (in γ)	Optical density (2-cm cell)					
	Glycine	Alanine	Valine	Glutamic acid	Phenylalanine	Histidine monohydrochloride
3	0.13					
5	0.17	0.16	0.14	0.09	0.09	
7	0.20					
10	0.29	0.25	0.21	0.15	0.12	
15		0.34	0.29	0.22	0.16	
20						0.16
30						0.21
40						0.27

The marked spots are cut, introduced into a 10-ml dried volumetric flask, and 0.5 ml of an appropriate ninhydrin solution is added. The volume of ninhydrin solution must be less than 1 ml, otherwise the reaction becomes less sensitive. The flasks are heated for 15 min in a bath of vigorously boiling water, with occasional shaking, and are then immersed in water at room temperature for about 5 min. Distilled water is added, and the color intensity is measured in a colorimeter. Meanwhile, the piece of paper is allowed to remain in the volumetric flask, the error due to it being negligible. For the colorimetry we use a Lumetron colorimeter with a 550-mμ filter and a microcell 2 cm thick.

A blank with a piece of filter paper, of about the same size as that with the spot, and ninhydrin reagent are prepared in the same way. We found that the blanks gave the same values for widely different pieces of paper and therefore may be neglected when comparative results are being obtained—that is, when pure amino acids are used as the standard. Of course, after the use of ammonia in paper chromatography, it is necessary to eliminate this compound; otherwise the blank may be variable.

The ninhydrin solution must be almost neutral, since the optimum pH for the reaction, without the use of reducing agents, is about 7. For the small volume in which the reaction is performed the contribution of the piece of paper to changing the pH may be very important. We noticed that different papers (Whatman No. 1, No. 2, and others), when immersed in 0.5 ml of ninhydrin solution, may more or less strongly modify the pH. This is especially true after developing the chromatogram. The contribution of the amino acid to alteration of the pH is negligible. It is necessary, therefore, to correct the pH of the acid aqueous ninhydrin solution with a buffer to obtain a final pH

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