Antagonistic Effects of Cortisone and Growth-Hormone on the Developing Chick Embryo.* (23785)

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In a study of effects of various agents on the fate of grafts to the chorio-allantoic membrane of the chick embryo, it was noted that cortisone acetate when applied to the chorio-allantoic membrane at maximum dose permitting the embryo to survive, resulted in a striking inhibition of embryonic growth and development. These observations corresponded to the findings of Karnofsky *et al.*(4), who fully described the specific "cortisone effect" which, in addition to growth inhibiting action, also involved characteristic developmental modifications. As these effects were produced by relatively small doses of the steroid and as the effective dose of cortisone (1 mg/egg) resulted in very high mortality of the treated embryos, we searched for an agent to alleviate the deleterious systemic action of the steroid and to increase viability of the treated embryos, and possibly tolerance of larger doses of cortisone. On grounds of the type of certain histological changes in tissues of cortisone treated embryos the attempt was made to counteract the action of cortisone by administration of growth hormone (somatotrophic hormone, STH). It has been shown that in cortisone-treated chick embryos there is an excessive excretion of sodium chloride and reducing sugars into the allantoic fluid (Danowski, *et al.*(2). It could be assumed that the negative balance of these substances in the embryo might play a part in producing the symptoms of the "cortisone effect." Therefore experiments were also made in which saline with glucose, *i.e.* Tyrode's solution, was administered to cortisone treated embryos.

Material and methods. Fertile white Leghorn eggs were incubated at 38°C. Injections of the various agents were made under sterile conditions onto the chorio-allantoic membrane through a small window cut out in the shell directly over the membrane. After injection, the opening was covered by a coverslip and sealed with paraffin. Three series of injections were run concurrently: a) one in which embryos of 8 days of incubation were injected with a single dose of 1 mg of cortisone, b) a second in which embryos injected with the same dose of cortisone at the same age were, in addition, treated with growth hormone. In this series the embryos were injected 24 hours previous to cortisone treatment with 250 γ of growth hormone dissolved in Tyrode's solution and these injections were repeated afterwards daily; and c) a third series in which embryos were treated in a similar way as in series b) except that Tyrode's solution was substituted for growth hormone solution.‡ The embryos which died before the 13th day of incubation were discarded, even though the "cortisone effect" could be first clearly seen at 10th to 11th day. The embryos surviving to the 15th day were sacrificed, weighed, measured and examined.

Results. Effects of cortisone on chick embryo. The effect of cortisone on the developing embryo expressed itself in the high mor-

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‡ Growth hormone, in lyophilized form, was kindly supplied by Armour Laboratories, Chicago, through the courtesy of Dr. J. Bunding. Cortisone (11-dehydro-17-hydroxy-corticosterone acetate, "Cortone," Merck & Co.) was available in aqueous suspension, preserved in 1.5% benzyl alcohol necessary to produce appreciable injury to the chick embryo was 10 mg (Karnofsky(4)), whereas the amount of only 0.5 mg was injected with 1 mg of cortisone. It was found that Tyrode's solution when injected daily to otherwise untreated eggs did not affect detectably the development and growth of the embryos.

tality, in inhibition of growth and various developmental changes of surviving embryos. Our results corroborate in general the findings of Karnofsky *et al.*(4). The mortality of embryos injected with 1 mg of cortisone amounted to 40% (Table I); surviving em-

TABLE I. Mortality in Chick Embryos Injected with Cortisone and STH. Dose of cortisone, 1 mg per egg; age at injection, 8 days.

Additional agent	No. of eggs inj./No. dying (% mortality)
None	20/8 (40%)
Tyrode	27/6 (22)
STH	32/5 (17)

bryos were very small and showed characteristic curling of the body, the beak being pressed tightly against the left foot; feather formation was markedly deficient, the body being pale and somewhat edematous, the eyes were protruded and disproportionally large (Fig. 1). All embryos had exteriorized viscera, while the heart was greatly enlarged, the liver yellow and relatively very small, especially its left lobe. Embryonic membranes were deficient in development.

Administration of Tyrode's solution to the cortisone-injected embryos brought about a small but significant drop in mortality rate of the embryos (as shown in Table I). The probable explanation for this result will be discussed later This increase in survival rate was, however, not accompanied by a significant increase in growth and failed to prevent occurrence of the characteristic cortisone syndrome in the embryo.

FIG. 1.

Action of growth hormone on cortisone-treated embryos. When injected as described above, growth hormone showed a marked counteracting action on the effects brought about by cortisone in the chick embryo. The high mortality was lowered to 17%, reaching approximately the level of that occurring spontaneously during incubation. The slightly higher than usual percentage of mortality of treated embryos (about 2%) may probably be attributed to trauma of the injection procedure. Since the high mortality rate, caused obviously by cortisone, was also lessened, although in a much lower degree, by administration of saline with glucose, the most striking effects of growth hormone are those concerning growth and development of the embryo and the embryonic membranes. Embryos treated with cortisone and growth hormone showed a marked increase in body length and weight, when compared with those treated with cortisone only (Table II). Organs and various regions of the body attained almost normal proportions, the neck being relatively long, the eyes of usual size and shape. The occipital region of the head showed the usual curvature, the legs and wings were properly developed; the liver was of normal shape and of usual size. In many cases the abdominal wall was closed over the viscera as usual at this age. On the pteryle regions of the skin, which was pinkish in colour, feather papillae were formed and growing feathers were distinct (Fig. 1).

Concerning the embryonic membranes the yolk-sac seemed normally developed, enclosing the yolk to the usual degree. The chorio-allantoic membrane, however, showed a specific, repeatedly occurring thickening and a parchment-like texture, especially noticeable at the site of injections and around it. This thickening of the chorio-allantoic membrane,

TABLE II. Weight and Length of Chick Embryos Treated with Cortisone and STH.

	No. of embryos	Avg wt at 15 days, g	Avg length at 15 days, cm
Cortisone	15	3.1	4.3
" + Tyrode	24	3.6	4.4
" + STH	30	5.6	6.2

which in some cases attained the diameter of 2-3 mm interfered with the spatial position of the embryo in the egg and probably also with the functions of the extra-embryonic blood system.

Some other modifications caused by cortisone were not alleviated by the growth hormone. Embryos treated with both hormones retained the curling of the body, with the foot pressed against the head, and in some cases the viscera remained exteriorized and the heart was disproportionally enlarged.

Discussion. The present experiments demonstrate that the deleterious effects of cortisone on growth, development and survival of the chick embryo can be partially counteracted by simultaneous administration of growth hormone. This counteraction is expressed not only in an increased rate of survival, but also in the amelioration of many of the typical symptoms of cortisone treatment (Karnofsky *et al.*(4)), resulting in formation of nearly normal embryos. It seems, therefore, safe to suggest that the actions of these 2 hormones might be antagonistic in many respects.

The mode of action of cortisone in the embryo is unquestionably complex and comprises mainly two types of effects: a general inhibition of development of the embryo, expressed by retardation of growth and increase in body weight and length; and secondly, a disturbance of various physiological and metabolic processes as indicated by enlargement of heart, fatty degeneration of liver, changes in elimination of electrolytes and sugar. All these effects contribute, in extreme cases, to an increase in mortality of the embryos. In addition a local inhibitory effect of cortisone at site of its application to the chorio-allantoic membrane is observable in early embryos, where it prevents normal growth of the membranes.

The effect of cortisone on growth and development of embryos may, at least partly, be attributed to the known growth inhibiting action of this steroid on actively proliferating tissues, *e.g.* granulation tissue, newly forming cartilage and bone (Ragan *et al.*(6); Ragan (7)) and various tumors (Ingle *et al.*(3)); Stock(10) and others). This action affects principally the connective tissues and their derivatives, causing a delay in the formation of all elements of these tissues.

The general growth-stimulating effect of growth hormone (STH) on intact and hypophysectomized animals can also be traced, at least in part, to the action of this hormone upon connective tissues and their derivatives. Thus, for example, in juvenile rats growth hormone promotes proliferation of epiphyseal cartilages and stimulates osteogenesis (Simpson *et al.*(9)), while in full grown animals increased growth hormone stimulation results in appositional bone growth and in proliferation of soft tissues (acromegaly, Selye(8)).

The distinctly opposite effects of the 2 hormones under consideration suggest strongly that their actions are actually antagonistic. Recent experiments have indeed shown that growth hormone counteracted almost completely the inhibiting effects of cortisone upon formation of granulation tissues (Taubenhaus *et al.*(11)). The present observations on the developing embryos demonstrate that the antagonism is effective also at the level of systemic actions of these hormones, thus supporting the conclusion that growth hormone may act as a true antagonist to cortisone.

The development of feathers in cortisone and growth hormone treated embryos seems of interest. Cortisone almost completely inhibits formation of feather follicles, acting apparently selectively on dermal papillae. Embryos treated simultaneously with cortisone and growth hormone show considerably improved feather formation and consequently a richer plumage. This may be interpreted as due to abolition of the inhibiting effect of cortisone upon development of dermal papilla, the latter subsequently stimulating growth and differentiation of the ectodermal elements of the feather (Lillie & Hsi Wang(5)).

There remains to be mentioned the effect of Tyrode's solution on cortisone-treated embryos. The changes in chemical composition of the allantoic fluid in the cortisone treated embryos described by Danowski *et al.*(2) may provide a clue to the mechanism of influence of Tyrode's solution on survival of embryos.

In cortisone-treated embryos a marked increase in sodium, chloride and sugar (reduc-

ing substance) concentration in the allantoic fluid can be observed, while the potassium level is noticeably lowered. These alternations in electrolyte and sugar elimination may have some bearing upon the high mortality rate of such embryos. Addition of Tyrode's solution, consisting of physiological saline and glucose, to cortisone injected embryos raises slightly their survival rate. The results indicate a compensation therapy effect of the solution on embryos under conditions of increased sodium, chloride and sugar excretion.

Summary. 1) Single injections of 1 mg egg of cortisone on the chorio-allantoic membrane of chick embryos produce a striking and specific syndrome: mortality of the embryos is highly increased, growth of surviving embryos is markedly retarded and characteristic developmental modifications are produced: electrolytes and sugar metabolisms are disturbed. 2) Simultaneous administration of Tyrode's solution, consisting of saline and glucose, to such embryos brings about a slight rise in survival rate of embryos, owing probably to its compensation therapy effect upon loss of sodium chloride and sugar (reducing substance). 3) Treatment of cortisone-injected embryos with growth hormone (STH) results in striking alleviation of most of the cortisone caused changes. Mortality rate is lowered almost to the level of that occurring spontaneously during incubation; inhibition of growth as well as many deleterious modifications are successfully counteracted. 4) Various aspects of the interaction of hormones applied are discussed and the conclusion is reached that the action of growth hormone is antagonistic to that of cortisone.

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Metabolism of Lactic Acid and Glycerol in C^{14}-Glyceryl Lactopalmitate.* (23786)

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The metabolism of acylated glycerol to carbon dioxide and water, in the light of current knowledge depends upon the intermediate formation of free or deacylated glycerol. Similarly, metabolism of fatty acids esterified with glycerol is dependent upon hydrolysis. The hydrolysis rates of glyceryl esters thus become a variable, along with absorption rates and possible transesterifications, determining the catabolism of glycerol. Karnovsky and Gidez(1,2) compared the appearance of CO_2 from the metabolism, in rats, of C^{14}-glycerol esterified with various acids. Esterification with oleic acid decreased the rate at

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