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Effect of Salicylic Acid on the Early Development of the Chick Limb-Bud

SULEIMAN A. SULEIMAN* and JOHN R. BAKER*

Mitochondria are organelles found in all aerobic organisms in which the oxidation of pyruvic acid to carbon dioxide and water takes place.

Several workers have isolated mitochondria to study their properties and find their role in embryogenesis. Carey and Greville (1959a,b) found that the mitochondria obtained from embryonic preparations appeared to be more fragile than their adult counter parts. McNally (1960) reported a marked increase in phosphate activity (which is related to mitochondria) in the ectodermal tip of the developing limb-buds of rat embryos. These observations and others (Bosund, 1957; Charnock, and Opit 1962a) show an essential mitochondrial activity during early development.

Salicylates are thought to be inhibitors of mitochondrial activity, which may act by disrupting or uncoupling the link between oxidation and phosphorylation. Brody (1956) was the first to show that salicylates in a concentration above 3 mg/100 ml. can decrease the P:O (phosphorylation: oxidation) ratio of rat liver and kidney mitochondria oxidizing a variety of substrates. The actual site of salicylate activity has not yet been found; but Charnock and Opit (1962b) have suggested that the locus of the action of salicylate in uncoupling oxidative phosphorylation is at the level of the mitochondrial membrane. It was therefore considered desirable to observe the effect of Salicylic acid on the early development of the chick limb-buds and to find if Salicylic acid has another effect in addition to its suggested uncoupling factor.

METHODS AND MATERIALS

The hind limb-buds of white leghorn chick embryos at stage 19 of Hamilton and Hamburger, were extirpated and transplanted singly onto the chorioallantoic (CA) membrane of host embryos of stage 35 of Hamilton and Hamburger. Immediately after transplantation, 0.08 ml of salicylic acid were dropped on top of the transplanted buds with a microdropper. There were seven groups of transplantis corresponding to seven concentrations. The host eggs were again incubated at 38°C. The transplanted limb-buds were recovered at intervals of 6 hours, 1, 2, 3, 4, and 5 days after operation.

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Some of them were then fixed in 4 per cent neutral formalin and others in Halley's fluid, washed in tap water, dehydrated, and embedded in paraffin. The sections were cut at 4 u by AP-121 Microtome, and stained either by Novelli's method for demonstration of mitochondria or with eosine and haematoxylin (E and H). Controls were done with each group of experimental concentrations in which 0.05 ml of Pannek-Compton solution were added to the transplant.

For thin sectioning, limb-buds were fixed in chilled gluteraldehyde (4°C) for 4 hours, and post fixed in osmium tetroxide (in phosphate buffer) for 45 minutes. After dehydration with graded alcohols (35, 70, 90 per cent and three changes of absolute alcohol) the tissues were transferred to the Araldite mixture, the Araldite was changed twice or thrice during this time. The sections (5 - 10 u thick) were cut by glass knives on LKB ultra-microtome, and were stained with acid fuschin.

OBSERVATIONS

The group of limb-buds treated with 250 ug/ml and 200 ug/ml concentration showed a pronounced growth inhibition during the whole time of transplantation, and especially during the first 4 days of transplantation, compared to the controls (Figure 1). The groups of limb-buds treated with the concentrations of 170, 150, and 135 ug/ml also showed growth inhibition but to a lesser degree and in the period of the first 3 days of transplantation (Figure 2). The groups of limb-buds treated with the concentration of 125 and 100 ug/ml did not show any significant growth inhibition; (Figure 3) and the growth of the group treated with 100 ug/ml was very similar to the controls. The only difference between the groups of limb-buds treated with the last two concentrations and the control was less growth in the first day (Figure 3).

In all groups of transplanted limb-buds (except for those treated with the first two concentrations) the growth (length, weight, and volume) tends to become normal after the third day of treatment. In the groups treated with the last two concentrations the growth is very close to the normal. In all the transplanted limb-buds there was no significant difference in growth during the first 5 hours of transplantation compared to the controls. After this the growth behavior differed according to the concentration.
Limb Bud Development

In the untreated limb-buds (Figure 4) the basal side of the ectoderm is sharply demarcated from the mesoderm. It was observed that in Salicylic acid treatment with the higher concentrations (250 µgm/ml and 200 µgm/ml) the basal membrane looked loose under the light microscope, and it was easy to separate an almost intact ectoderm from the examined transplanted limb-buds in the period of the third day of transplantation.

In the case of the lower concentrations the loosening was less significant and it was more difficult to separate the ectoderm (Figure 6). In the groups receiving 150 µgm/ml and 100 µgm/ml concentrations there was no loosening except after six hours treatment. Whether any change has occurred in the chemical constitution of the basal membrane is not known.

DISCUSSION

During the past twenty years, much experimental work has been directed to the study of ectoderm-mesoderm interaction in limb morphogenesis in avian embryos. It has been debated whether the ectoderm or the mesoderm of the limb-bud is the site of developmental factors. Balinsky (1956) suggested that both mesoderm and ectoderm take an active part in the development of an induced as well as normal limb.

Recently two different hypotheses have been formulated to account for the development of limbs in tetrapod vertebrates. In both hypotheses the mesoderm is claimed to be the essential site for limb development. The area of disagreement is in the role played by limb-bud ectoderm, particularly the apical ridge. An increasing body of evidence has accumulated which...
loosening in the basal membrane, special attention must be
given to this seemingly important intermediate zone between
the ectoderm and mesoderm in the limb-bud of the chick.
It is observed that any effect on this is reflected on the limb-
bud as growth inhibition. The above observation would also
stress the importance of ectoderm (not just a covering), since
the loosening of the basal membrane and its tendency to stay
with the ectoderm reflects inhibition of growth.

In various publications the term basal membrane is
applied to various structures, in this paper the basal membrane
is defined as the zone present at the dermo-epidermal jun-
ction. In electron microscopy the term is usually applied to a
continuous membrane covering the basal membrane cells
(Sjostrand, 1953; Jurand, 1965).

It has been suggested that the basal membrane composed
mainly of tropocollagen fine filaments which are either em-
bedded in an amorphous matrix of the same density (pro-
ably mucopoly-saccharides), or are very closely compacted
so that the individual fiber can not be resolved (Fawcett, 1966).
Although the exact function of the basal membrane is still
uncertain, it is believed to be a diffusion barrier (Balinsky,
1956, 1957). Caesar and Edward (1957) suggested a protec-
tion function from too rapid ion concentration changes.

It is not known if salicylic acid acts on the tropocollagen
in the basal membrane, but it interferes with the metabolism
of collagen (Bellamy, 1963). It affects the biosynthesis of mu-
copoly saccharides in connective tissues (Bostrum, 1955,
1963). The loosening of this membrane observed in our ex-
periments may be attributed to this effect, or it might sug-
gest an effect of salicylic acid on other components not yet
known. The inhibition of growth by salicylic acid disappears
especially in the groups treated with 100-170 µgm/ml con-
centrations (Figures 1, 2, 3). This disappearance may be
explained by diffusion of salicylic acid into the surround-
ing tissue. It is also likely that it affects an interaction be-
tween the ectoderm and mesoderm through this membrane,
ing tissue. It is also likely that it affects an interaction be-
tween the ectoderm and mesoderm through this membrane.

More biochemical studies must be done to determine the
precise make-up of the basal membrane, and the exact mode
of action of salicylic acid on this membrane.
LITERATURE CITED


