Effect of Hormones on the Chondroitin Sulfate Metabolism of Chick Embryo Femora Growing in Vitro. 3. Effect of Sex Steroids on Chondroitin Sulfate Synthesis in the Cartilagenous Bones Growing in the Natural Medium*

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Synopsis

The femora from 9-day old chick embryos were cultivated for 4 days by the roller-tube method in 35S-labeled natural medium, consisting of 1 volume of chick embryo extract, 5 volumes of horse serum and 4 volumes of balanced salt solution to permit physiologic ossification and chondrification of the bones. By measuring radiosulfate uptake, sex steroids were examined for their effects on the chondroitin sulfate synthesis of the cartilagenous bones growing in vitro. With increasing concentration in the medium, testosterone and 19-nortestosterone stimulated the 35S-sulfate incorporating activity per unit dry weight increase of the bones, while the reverse was the case with estradiol and progesterone. However, dehydroepiandrosterone exerted no appreciable effect.

Despite the fact that sex steroid hormones control the growth of cartilage in living animals (Silberberg and Silberberg, 1956), only sporadic reports are so far available on in vitro experiments so that conclusive evidence as to whether sex steroids act directly or indirectly on bone in vivo (Biggers, 1965) has not been obtained. For studying such direct hormonal effects on isolated organs or tissues, the organ culture method is believed to be the best among many in vitro experimental techniques, because the method can permit the explants to retain an active state of intercellular organization that is essential for manifestation of hormonal effects of physiological significance.

On the other hand, chondroitin sulfate is one of the principal components of cartilage and fixation of radiosulfate into the acid mucopolysaccharide of cartilage matrix has been adopted as one of the useful indices for studying the basic metabolism and its control in cartilagenous tissues. Murota and Endo (1969) have shown the active incorporation of 35S-sulfate by chick embryo femora growing in vitro. The radioactivities of the cultured bones reflected in close approximation the amount of chondroitin sulfate synthesized during cultivation. Thus, Murota et al., (1969) have studied the effects of cortisol on chondroitin sulfate synthesis by the cultured bones.

The present communication deals with the effects of sex steroids on the biosynthesis of chondroitin sulfate in the cartilagenous chick embryonic bones growing in a natural medium,
which has been confirmed to be the best for ossification and chondrification of the bones (Endo, 1960; Ito et al., 1963, Murota and Endo, 1969).

**Materials and Methods**

The femora of 9-day old chick embryos were cultivated by the roller-tube method (Endo, 1960) for 4 days with no renewal of medium. The medium used was a natural one composed of 11-day chick embryo extract, horse serum and Gey’s saline solution in the proportion of 1:5:4 by volume and contained tracer dose of $^{35}$S-sulfate. In order to take accurate measurement of dry weight increase of the bones during cultivation, femora from one side of an embryo were used to determine the initial dry weight of the bones and those from opposite side of the same embryo for measuring the final dry weight and incorporated radioactivities of the bones after cultivation. Biological and biochemical characteristics of the culture medium (Endo, 1960; Ito et al., 1963; Murota and Endo, 1969) and procedures for extracting and measuring the labeled chondroitin sulfate in the cultured bones (Ito et al., 1960) have been described in detail.

Testosterone, 19-nortestosterone, estradiol, progesterone and dehydroepiandrosterone were tested, to be added into the medium as ethanol solution. Final concentrations of the steroids in the medium ranged from $10^{-6}$ to $10^{-4}$ M, while alcohol concentration in the medium was settled on as 0.2 % (v/v) all through the experiments.

**Results**

As for androgenic steroids, testosterone suppressed consistently the increase in dry weight of the bones during cultivation and 19-nortestosterone inhibited the increase progressively with concentration (Fig. 1), whereas radiosulfate fixation of the bones was remarkably affected by neither of the steroids at all concentrations tested (Fig. 2).

In contrast, estradiol and progesterone affected the increase in dry weight of the bones very little (Fig. 1), while a progressive inhibition was observed on $^{35}$S uptake by the bones with increasing concentration of the steroids (Fig. 2).

However, dehydroepiandrosterone affected...
Fig. 2. Effect of sex steroids on $^{35}$S-sulfate uptake of 9-day old chick embryo femora cultivated in natural medium for 4 days. See legend to Figure 1 for presentation of results.

Fig. 3. Effect of sex steroids on $^{35}$S-sulfate uptake per unit dry weight of 9-day chick embryo femora cultivated in natural medium for 4 days. See legend to Figure 1 for presentation of results.
neither the increase in dry weight nor the
radiosulfate incorporation (Figs. 1 and 2) sig-
nificantly.

On employing an index of the amount of
radioactivity per unit dry weight increase of the
bone, therefore, these steroids could be more
readily distinguished from each other by qualita-
tively different effect on radiosulfate uptake of
the bones (Fig. 3). Testosterone and 19-
nortestosterone rather showed a stimulatory
effect, estradiol and progesterone gave progres-
sive inhibition with increasing concentration
and dehydroepiandrosterone exerted no signifi-
cant effect at all.

Discussion

Steroid hormones have long been known to
influence growth and development of the
cartilage and bone. On the other hand, skeletal
growth and development also occurs in the
absence of the hormones. Steroids have thus
been considered to act in living animals as
accelerators or inhibitors to modify the chemi-
cal and histological development of cartilage
and bone.

As to the effects of sex steroids on
cartilage metabolism, however, only a few
sporadic works have been done. Priest and
Koplitz (1962) have observed reduced activity
of $^{35}$S-sulfate uptake of costal cartilage from
estradiol treated rats, and Kowalewski (1958)
has reported that anabolic androgen 17a-ethyl-
19-nortestosterone produced a significant rise
of $^{35}$S-sulfate fixation in fractured bone of rats
while testosterone propionate showed no appre-
ciable effect. From these in vivo experiments,
however, it is difficult to determine the direct
actions of steroids on cartilage precisely.

For the purpose of evaluating the direct
actions of sex steroids on a given tissue, tissue
culture technique should be very fruitful. On
examining the effects of steroids on cartilage
metabolism along this line, Lash and White-
house (1961) have found that progesterone and
testosterone showed no effect on $^{35}$S uptake in
cultures of chick embryonic somites to dif-
ferentiate into cartilage, and Goyena (1955) has
reported no significant effect of estradiol on
the femora from 7- and 8-day old chick
embryos in culture. Unfortunately, however,
those results are difficult to interpret since far
too little attention has been paid to possible
relationship between responses and doses of the
hormones or characteristics of the culture
condition.

Murota et al., (1969) have observed a fact
that effect of cortisol on $^{35}$S incorporation
into 9-day chick embryo femora in culture
varies with both concentration of the steroid
and the type of culture medium. Considering
the importance of establishing physiologic cul-
ture environment in hormone research, on the
other hand, characters of natural media have
been studied in detail through histological and
chemical examination of the cultured bones.
The natural medium used in this work has thus
been confirmed to permit nearly physiologic
ossification and chondrification of the bone
rudiments (Endo, 1960; Ito et al., 1963). By
using such natural media and examining the
test materials at wide range of concentrations
the authors have been able to demonstrate the
direct stimulatory or inhibitory effect upon
bone formation in culture of pituitary growth
hormone (Ito et al., 1960), biologically active
proteins of salivary glands (Ito and Endo,
1956) and saliva (Ito et al., 1961), and several
chemical substances supposedly affecting ossi-
fication (Miyazaki et al., 1957).

The results of the present experiment,
considered together with those mentioned
above, indicate that each of the sex steroids can
produce characteristic direct effect on cartilage
growth while dehydroepiandrosterone with no
hormonal activity in itself exhibits no appreci-
ciable effect at all, though the evaluation of the
physiological significance of these findings will
not be possible until detailed systematic in vivo
studies are carried out.

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References


