EFFECT OF OESTRADIOL CYCLOPENTYLPROPIONATE ON SERUM AND LIVER LIPIDS AND AORTIC $^{35}$S-SULPHATED MUCOPOLYSACCHARIDES IN COCKERELS FED NORMAL OR ATEROGENIC DIET

By

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ABSTRACT

Effect of a single dose of 5 mg oestradiol cyclopentylpropionate on lipids of serum and liver and on $^{35}$S-sulphated mucopolysaccharides of aorta in 3 week old cockerels was studied. Birds were fed normal or cholesterol rich diet. Significant rise of serum and liver lipids was observed in birds on the 7th day after the dosage of hormone and this effect of oestradiol was not modified by the quality of diet. Significant increase of $^{35}$S-uptake by sulphated mucopolysaccharides of the aorta was observed in cockerels treated with oestradiol, or given cholesterol diet alone.

Atheromatous vascular lesions associated with post-oestrogen hyperlipaemia have been described by Lindsay et al. (1946), Horlick & Katz (1948) and Chaikoff et al. (1948).

Atherogenic effect of oestradiol cyclopentylpropionate in young cockerels has been studied recently by Caldwell & Suydam (1959) and Kowalewski (1960 b). We were able to show that even a single injection of this hormone results in significant changes in serum, liver and aortic lipids in cockerels fed normal diet (Kowalewski 1960 c).

It is apparent that arterial lesions observed in birds fed atherogenic diet are associated with alteration in mucopolysaccharides of vascular connective tissue (Kowalewski 1959, 1960 a). It is also apparent that oestrogen induced atherosclerosis may produce changes in serum polysaccharides (Caldwell & Suydam 1959). Because both atherogenic diet and oestrogen produce alterations in lipids
METHODS

Cockerels of White-Rock Broiler strain used for the present study were obtained from a commercial hatchery when 8 days old and were kept in thermostatic brooders. They received a routine chicken starter with tap water as desired.

Oestradiol cyclopentylpropionate (Upjohn Co.) was given intramuscularly as a single dose of 5 mg, dissolved in 0.2 ml of corn oil, per bird. Cholesterol rich diet contained 2% of cholesterol and 5% of olive oil in chicken starter. Birds were divided into the following 4 groups:

1. No treatment, normal diet; 2. Oestradiol, normal diet; 3. No treatment, cholesterol diet; 4. Oestradiol, cholesterol diet. Treatment and experimental diet were both initiated when birds were 3 weeks old and this study lasted 7 days.

Forty-eight hours before the end of experiment radiosulphate (35S in H2SO4, Atomic Energy, Canada) was injected subcutaneously in dosage of 1 mc/kg of bird's weight. The dose was dissolved in 5 ml of distilled water together with 40 mg of sodium sulphate (Kowalewski 1958a, b). The cockerels were killed by decapitation and the blood collected. Serum and livers were used for the determination of lipids. Aorta and femur of each bird were carefully dissected. Bone was studied to compare 35S uptake of the aorta with that of a tissue (bone) which has specifically high sulphate-fixing capacity. Aortas were studied for 35S radioactive mucopolysaccharides following the method previously described (Kowalewski & Williams 1958). Bones were dissolved by the nitric-perchloric acid wet-digestion procedure (Osborne & Kowalewski 1956) and the final precipitate of barium sulphate obtained after processing of the tissues was transferred to planchettes for counting, using the method and equipment previously described (Osborne & Kowalewski 1956).

Results were expressed in counts per minute (c. p. m.). Total cholesterol was determined following the method of Abell et al. (1952). For study of lipid phosphate the samples were extracted with alcohol ether and analysis continued by the procedure of Zilversmit & Davis (1950).

Mean values for treated and control groups were compared by the Rank Sum Test and significance levels were expressed in per cent (Wilcoxon 1945).

RESULTS

Average body weight of cockerels in the 4 groups were respectively 214, 205, 221 and 224 g at day of injection and 320, 313, 355 and 337 g at the end of experiment. Injection and diet were well tolerated and no inhibition of appetite or growth resulted. The data on study of lipids in serum and liver are presented in Tables 1 and 2. Significant rise of serum and liver cholesterol was noted in cockerels treated with oestradiol and fed normal or cholesterol diet. Cholesterol
Table 1.
Effect of oestradiol on serum and liver cholesterol in cockerels fed normal or cholesterol rich diet. Mean ± S.E. Number of birds in brackets.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum (mg %)</th>
<th>Liver (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet (control)</td>
<td>129 ± 4 (19)</td>
<td>343 ± 7 (18)</td>
</tr>
<tr>
<td>Oestradiol + normal diet</td>
<td>*560 ± 47 (20)</td>
<td>*608 ± 81 (18)</td>
</tr>
<tr>
<td>Oestradiol + cholesterol diet</td>
<td>*878 ± 65 (19)</td>
<td>*2904 ± 184 (15)</td>
</tr>
<tr>
<td>Cholesterol diet</td>
<td>*1405 ± 88 (20)</td>
<td>*3742 ± 61 (20)</td>
</tr>
</tbody>
</table>

* Significance level, treated versus control, less than 1 %.

Table 2.
Effect of oestradiol on serum and liver phospholipids in cockerels fed normal or cholesterol rich diet. Mean ± S.E. Number of birds in brackets.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum (mg %)</th>
<th>Liver (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet (control)</td>
<td>251 ± 11 (19)</td>
<td>2329 ± 61 (14)</td>
</tr>
<tr>
<td>Oestradiol + normal diet</td>
<td>*2555 ± 188 (17)</td>
<td>*3064 ± 118 (18)</td>
</tr>
<tr>
<td>Oestradiol + cholesterol diet</td>
<td>*1718 ± 154 (19)</td>
<td>*2823 ± 157 (15)</td>
</tr>
<tr>
<td>Cholesterol diet</td>
<td>266 ± 14 (20)</td>
<td>*3031 ± 62 (20)</td>
</tr>
</tbody>
</table>

* Significance level, treated versus control, less than 1 %.

diet alone had comparable effect. Phospholipids of serum were significantly increased after the injection of oestradiol to birds fed normal or cholesterol diet. Diet by itself did not affect serum phospholipids. Oestradiol treatment resulted, however, in significant rise of liver phospholipids, which were also increased in cockerels fed cholesterol diet alone.

Table 3 gives the results of measurement of $^{35}$S uptake by the fraction of aortic tissues, considered to contain sulphated mucopolysaccharides. Total $^{35}$S uptake by femora of cockerels is also recorded. Increased $^{35}$S uptake by the sulphated mucopolysaccharides of the aorta was observed in birds treated with oestradiol and given normal or cholesterol diet. In agreement with our previous work (Kowalewski 1959 a), cholesterol diet alone resulted also in a significant rise of $^{35}$S sulphated mucopolysaccharides of aorta. Cholesterol diet and oestradiol did not affect radioactivity of bones.
Table 3.
Uptake of $^{35}$S by bones and radioactivity of $^{35}$S sulphated mucopolysaccharides of aortas in cockerels (c. p. m./100 mg dr. wt). Mean ± S. E. Number of birds in brackets.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bone</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c. p. m./100 mg</td>
<td>mg dr. wt.</td>
</tr>
<tr>
<td>Normal diet (control)</td>
<td>918 ± 35 (16)</td>
<td>44 ± 4 (16)</td>
</tr>
<tr>
<td>Oestradiol + normal diet</td>
<td>1112 ± 27 (16)</td>
<td>45 ± 3 (16)</td>
</tr>
<tr>
<td>Oestradiol + cholesterol diet</td>
<td>1109 ± 188 (16)</td>
<td>49 ± 3 (16)</td>
</tr>
<tr>
<td>Cholesterol diet</td>
<td>1171 ± 36 (12)</td>
<td>47 ± 4 (12)</td>
</tr>
</tbody>
</table>

* Significance level, treated versus control, less than 1%.

COMMENT

Significant rise of lipids in serum of young cockerels was found on the 7th day after a dose of an oestrogen. This occurred in the birds fed normal or cholesterol rich diet during the same 7 day period. Cholesterol diet alone had comparable effect, as far as total cholesterol is concerned. An interesting observation was that a single dose of a hormone or such a short period of feeding the atherogenic diet may influence serum lipids. Atherogenic diet influenced liver phospholipids but did not raise serum phospholipids. Oestradiol resulted in significant rise of serum and liver phospholipids. The observation that both atherogenic diet and oestradiol influence the uptake of $^{35}$S by mucopolysaccharides of aorta seems interesting. The possibility exists that the changes in aortic connective tissues, which may be studied by $^{35}$S uptake procedure, are comparable in various types of experimental atherosclerosis. This hypothesis needs further investigation to be verified.

Many important aspects of mobilization and biosynthesis of lipids remain unexplained. It is known, however, that some hormones influence these processes and oestradiol cyclopentylpropionate seems to be a potent and rapidly active stimulant of lipid metabolism. Exact function of oestrogens in the metabolism of lipids is not explained. This hormone may affect the enzyme system involved in absorption and transformation of exogenous fats into tissue lipids. It may also play a role in post-absorption phase of lipid synthesis in the tissue and in the mobilization of endogenous fats. Possible effect of oestrogen on cellular permeability and on transport of lipids may also be considered. Effect of these hormones on synthesis and distribution of connective tissue mucopolysaccharides shall be further investigated.
ACKNOWLEDGEMENTS

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REFERENCES