COLLAGEN CONTENT OF BARREN AND PREVIOUSLY PREGNANT UTERINE HORNS IN OLD MICE

C. A. FINN,* S. M. FITCH AND R. D. HARKNESS

Department of Physiology, University College, Gower Street, London, W.C.1 and Department of Physiology, Royal Veterinary College, London, N.W.1

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Summary. The collagen content of the uterine horns of young (2 months old) virgin mice and 2-year-old mice which had ceased breeding was determined by estimation of hydroxyproline. Both total collagen content and collagen concentration were greater ($\times 3$ and $\times 1.5$ respectively) in the old mice but no difference was found between horns which had contained foetuses, and others which, as a result of ovariectomy on one side before sexual maturity, had not.

INTRODUCTION

It is established that the litter size of mice declines as the mother gets older (Bittner, 1936; Biggers, Finn & McLaren, 1962) mainly because of an increasing incidence of early embryonic death (Finn, 1962). This loss of foetuses is thought to be caused by the inability of the uterus to maintain the implanted blastocysts, thus implicating the uterus as the failing organ causing reduced reproductive output in aged mothers. If this is so, it is important to determine what factor or factors in the uterus change with age and reduce its ability to maintain pregnancy. It has been suggested that increased deposition of collagen might impair uterine function, possibly by interfering with vascularization (Biggers et al., 1962). This suggestion is based on the finding of increased ‘collagen’ staining material in histological sections of the uterus and other tissues of old animals, when the common ‘collagen’ stains are used (Loeb, Suntzeff & Burns, 1939; Burack, Wolfe, Lansing & Wright, 1941; Biggers et al., 1962). However, conclusions based on the appearance of sections of the uterus can be criticized on the grounds of lack of objectivity and specificity of staining and the impossibility of obtaining an accurate quantitative assessment of the amount of collagen present. Chemical determination of the quantity of hydroxyproline present in a tissue gives an estimation of the collagen content which is not open to such criticism. We have, therefore, used this technique to get quantitative data on changes in the collagen content of the uterus of mice with age.

*Present address: Department of Biological Sciences, Wye College, Kent.

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MATERIALS AND METHODS

ANIMALS

Mice of the TO strain randomly bred in the Animal House of the Royal Veterinary College were used.

Three groups of animals were compared:

Group 1. Virgin mice aged 2 months.

Group 2. Normal mice allowed to breed continuously until they ceased to produce living young (at about 15 months of age) and killed at 2 years of age. The young were removed immediately after birth in all cases, so that none of the animals went through the process of lactation.

Group 3. Mice ovariectomized on one side to prevent pregnancy on that side but otherwise treated as the second group. They ceased to produce young a little earlier than these (at about 11 months of age) but were killed at the same time.

The animals were killed by dislocation of the cervical vertebrae and the whole uterus was removed. One horn or, in the third group above, each horn separately, was sealed with 5 ml of 6 N-HCl in a test tube, and hydrolysed for 4 hr at 40 lb/sq in. pressure in an autoclave. The hydroxyproline content of the hydrolysate was estimated by the method of Neuman & Logan (1950) and from it the collagen content, by multiplying by 7.46. Horns in which the collagen content was not estimated in this way were fixed in Bouin's fluid, embedded in paraffin wax, sectioned and stained by Van Gieson's method.

RESULTS

The collagen contents of the uterine horns of the three groups of mice are given in Table 1. The collagen content of the uteri of the old mice was higher than that of the young, both in absolute amount and as a proportion of the tissue present (concentration). Surprisingly, there was no difference between the barren and fertile horns in the old mice ovariectomized on one side (Group 3).

There was great variation between animals in the weights and total collagen

Table 1

COLLAGEN CONTENT OF SINGLE UTERINE HORMS OF MICE OF DIFFERENT AGES AND REPRODUCTIVE EXPERIENCE

<table>
<thead>
<tr>
<th></th>
<th>Weight of uterine horn (mg)</th>
<th>Total collagen (mg)</th>
<th>Concentration of collagen (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young virgin mice</td>
<td>48.9 ± 16.4</td>
<td>0.94 ± 0.33</td>
<td>1.91 ± 0.14</td>
</tr>
<tr>
<td>Old mice</td>
<td>143.1 ± 56.1</td>
<td>3.75 ± 1.51</td>
<td>2.60 ± 0.11</td>
</tr>
<tr>
<td>Old mice, normal horn</td>
<td>93.5 ± 22.7</td>
<td>3.01 ± 0.71</td>
<td>3.19 ± 0.20</td>
</tr>
<tr>
<td>Old mice, ovariectomized horn</td>
<td>99.2 ± 25.2</td>
<td>3.13 ± 0.81</td>
<td>3.29 ± 0.23</td>
</tr>
</tbody>
</table>

There were five mice in each group. The young mice (line 1) were virgin and aged about 2 months. The old mice had all been bred until reproduction ceased (lines 2, 3 and 4). Lines 3 and 4 refer to mice which had the ovary removed from one side before they were bred so that the horn on this side never contained foetuses. The estimate of variation is the standard error of the mean.
contents of the horns, but a close correlation between the horns of the two sides in individual animals of Group 3. The variation was greatest in the ‘old mice’ not ovariectomized on one side (Table 1, line 2). The high figure for weight and total collagen is due mainly to one animal which had a uterine weight of 360 mg and collagen content of 9.6 mg.

DISCUSSION

It is clear from the absence of any difference in collagen content between the barren and repeatedly pregnant horns of old mice that the difference between these and the virgin uterus is not the result of the burden of carrying embryos. The barren horn is, however, subject to the changes in hormonal levels in blood which take place in pregnancy, and in the absence of a group of old virgin mice it is not possible to decide whether difference between it and the virgin horn is the result of this. There are, however, some indications that it is not. Earlier histological work (Loeb et al., 1939; Burack et al., 1941) indicates an increase in collagen content with age independently of pregnancy. After the first pregnancy, at least in rats (Harkness, Harkness & Moralee, 1956), the involutionary process reduces the collagen content of the horns below the original non-pregnant level so that there is no question of a residual increase. It is interesting too that in earlier work it was found that the litter size in old mice was about the same, whether the mother had bred continuously, or had not bred, or had been kept continuously pseudopregnant in earlier life (Finn, 1963), indicating that the litter-size reduction in the old mice was independent of previous breeding history. However, in contrast to this evidence is that of Woessner (1962) which indicates an increase in collagen content of the human uterus with successive pregnancies independently of any effect of age per se.

REFERENCES