VOLUME, pH AND PROTEIN CONTENT OF FLUIDS FROM LIGATED UTERI OF OESTROUS RABBITS

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(Received 8th October 1963)

Summary. The pH, protein concentration, and amount of oestrous uterine fluid were determined in mature Dutch type rabbits from 1 to 20 weeks after ligation of the uterus. Uterine fluid accumulated at the rate of 2-10 to 4-48 ml per cornu per week. These fluids were serous, slightly turbid, and colourless or slightly yellow. The protein concentration decreased from 5-13 mg/ml after the first week to 1-82 mg/ml after the 20th week of accumulation. The high initial protein concentration may have been due to contamination of the uterine fluid with blood at the time the uterus was ligated. The in-utero pH of fluids was 7-64. A rapid increase in pH with time and handling after removal from the uterus indicated the importance of carbon dioxide in buffering uterine fluids.

INTRODUCTION

Woskressensky (1891) noted fluid accumulation in rabbit uteri 6 weeks after double ligation of the uterus. Bond (1898) reported that these fluids could also be obtained when only the cervical ends of the uteri were ligated and, in 1906, observed that uterine fluids were colourless, watery and slightly alkaline, and that negligible amounts of fluid accumulated in the uterus of pregnant or ovariectomized rabbits. However, Lutwak-Mann (1962) recovered from 0-1 to 2-0 ml of rabbit uterine fluid with high bicarbonate content at 5 to 24 hr after ovulation, without uterine ligation.

The pH values reported for rabbit uterine fluids have ranged from 7-6 (Zimmermann, 1961) to 8-5 (Aasland, 1932). The protein concentration was estimated to be 2-70 mg/ml by Shih, Kennedy & Huggins (1940). Gregoire, Gongsakdi & Rakoff (1961) reported an average accumulation of 4-62 ml of uterine fluid per uterine cornu per week from oestral New Zealand rabbits. The occurrence, characteristics, and possible significance of uterine fluid in mammalia have recently been reviewed by Stevens (1963), and the present research was initiated to re-evaluate the pH, protein content, and accumulation of uterine fluid in ligated uteri of oestrous rabbits.

MATERIALS AND METHODS

Dutch-type rabbits were anaesthetized with ether or sodium pentobarbital. The lower abdominal region was shaved and sterilized with 70% ethanol and a 5 cm
incision was made beginning 5 cm anterior to the pelvis. If the ovaries were seen to contain normal oestrous follicles, the uteri were exposed and tightly ligated adjacent to each cervix with synthetic suture (Supramid suture, Jensen-Salsbery Laboratories, Kansas City, Mo.) or with silk suture. The tubo-uterine junction was similarly ligated in certain rabbits. The uteri were then returned to their normal position and the incision was closed. Special post-operative care was not necessary.

One to 20 weeks later, rabbits with ligated uteri were anaesthetized and the uteri were exposed as described above. Accumulated uterine fluid was aspirated with a 26 gauge needle attached to a syringe. Thereafter, most rabbits were arbitrarily assigned to a second accumulation interval. The volume and pH of the aspirated fluid was recorded at the time of collection. The fluids collected were centrifuged at 2000 g for 10 min at 5° C and then stored at −20° C until subsequently thawed for protein analysis by the method of Gornall, Bardawill & David (1949).

The cellular material precipitated by centrifugation was smeared on to clean microscope slides, air dried, and stained with the pyronin Y-methyl green procedure of Paape, Hafs & Snyder (1963). The percentages of epithelial cells, lymphocytes, neutrophils and monocytes were determined by direct microscopic count at magnification ×960.

RESULTS

A serous, slightly turbid, and colourless or slightly yellow fluid accumulated in most ligated uteri as illustrated in Pl. 1, Figs. 1 and 2. Whereas the great majority of uteri ligated with synthetic suture accumulated fluid, only a small proportion of the uteri ligated with silk suture accumulated fluid. Normal oestrous follicles were always present on the ovaries of rabbits in which uterine fluids were found. Occasionally, amber to brown samples of uterine fluid were obtained.

The average volume of fluid obtained from each uterine cornu after 1 to 20 weeks of accumulation is listed in Table 1. The volume of fluid was approximately proportional to the duration of accumulation, 2·10 to 4·48 ml accumulating per cornu per week. Whether the fluid was obtained from right or left uterine cornu had no significant effect on the volume (P > 0·10). Similarly, the average volumes of fluid obtained from cornua ligated only at the cervix and from cornua ligated at both extremities did not differ significantly (P > 0·10). Some uteri failed to accumulate an obvious quantity of fluid. However, when a second ligature was placed adjacent to the first, such uteri usually began to accumulate fluid, indicating that the first ligature was not sufficiently tight. For this reason, only cornua which obviously contained fluid are included in Table 1.

Initially, the pH of the uterine fluid was measured after storage for about 15 hr at 5° C. Under these conditions, the average pH was 8·44 ± 0·03. However, when uterine fluid was dispensed into a one drop electrode immediately after aspiration, the average pH was 7·86 ± 0·03. This observation prompted the measurement of intra-uterine pH, which averaged 7·64 ± 0·01. When fluid
Fig. 1. Exposed uterine cornua (u) and vagina (v) of control oestrous rabbit. c = cervix.
Fig. 2. Cornua (u) of oestrous rabbit 16 weeks after cervical ligation. Note extensive vascularity.

(Facing p. 332)
was slowly aspirated and measured in the syringe after a minimum of handling, the average pH was 7.62 ± 0.01. The pH was unaffected by the accumulation interval \((P > 0.10)\).

Protein concentrations of the uterine fluid are tabulated in Table 2. The protein concentration declined from 5.13 mg/ml after the 1st week to 1.82 mg/ml after the 20th week \((P < 0.01)\). However, the total protein content per cornu increased with accumulation time as a result of the increasing volumes of fluid \((P < 0.01)\). The concentration of protein in the amber or brown samples was always well above the averages shown in Table 2.

The cellular material from uterine fluids was 76% epithelial cells, 14% lymphocytes, 8% monocytes and 2% neutrophils.

**Table 1**

<table>
<thead>
<tr>
<th>Weeks of accumulation</th>
<th>No. cornu</th>
<th>Fluid per cornu (ml)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Average</td>
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<tr>
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<td>2.48</td>
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<td>116</td>
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<td>4</td>
<td>6</td>
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<td>5</td>
<td>9</td>
<td>10.48</td>
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<td>7</td>
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<td>31.36</td>
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<tr>
<td>16</td>
<td>6</td>
<td>35.17</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>71.00</td>
</tr>
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</table>

**DISCUSSION**

The total volume of uterine fluid collected was somewhat less than that reported by Gregoire *et al.* (1961), but this difference probably reflected the difference in the size of animals used in the two experiments. Apparently, uterine fluids did not escape through the tubo-uterine junction, since there was no difference in the volume of fluid which accumulated when either the cervical end or both ends of the uterus were ligated. This confirmed the observations of Bond (1898).

At the outset of this research, it was frequently observed at the time of recovery of fluid from a ligated uterus that the fluid was forced through the cervical ligature into the vagina by uterine contractions. Ligating the uterus almost to the point of severing it minimized, but did not eliminate, this loss of uterine fluid. Loss of fluid through the cervical ligature could account for the fact that fluid did not accumulate in some uteri. In some rabbits only one cornu accumulated fluid, and re-ligating the cornu with no fluid usually resulted in fluid accumulation. The evidence therefore indicated that all normal oestrous uteri would accumulate fluid provided the ligature was sufficiently tight.

The average uterine fluid pH measured immediately after collection was 7.62, a value in good agreement with that of 7.6 reported by Zimmermann (1961). The average value after storage at 5° C was 8.44, which agrees with
the values reported by Aasland (1932). The increase in pH of the uterine fluid with time after collection has also been observed in the rat (Blandau, Jensen & Rumery, 1958) and the cow (Gupta, 1962); it is probably due to a loss of CO2 as postulated by Bishop (1961). The high in-vivo pH of rabbit uterine fluids, relative to blood serum, was probably due to their high bicarbonate content, as described by Vishwakarma (1962).

Collection of rabbit uterine fluids from ligated uteri has been criticized by Heap (1962) on the basis that ligation altered the proportion of components in the fluid. Similarly, Ringler (1961) indicated that ligation of rat uteri increased the total protein concentration and altered the concentration of the protein components. On the other hand, Homburger, Grossman & Tregier (1955) reported that the concentration of mouse uterine fluid proteins decreased with ligation and that the protein to carbohydrate ratio was unaffected by ligation. The data presented here agree with the mouse data, but do not agree with those of Ringler (1961) for the rat, since the protein concentration decreased with accumulation time in the present study.

The majority of the amber coloured samples were collected during the first 2 weeks of accumulation, suggesting that the amber colour may have been caused by an initial contamination with blood. Red blood cells were not found in all of the amber-coloured samples, and the colour could have been a normal characteristic of some of these fluids; on the other hand, the red blood cells may have been haemolysed. Introduction of some blood into the lumen of the uterus at the time of ligation may have been responsible for the higher protein concentrations in uterine fluid collected after 1 or 2 weeks. The data suggest that an initial blood contamination may have been progressively diluted with accumulating uterine fluid.

The average protein content of rabbit uterine fluids was estimated by Shih et al. (1940) to be 2-7 mg/ml as calculated from total nitrogen content, an average which is in general agreement with the values after the 1st week of accumulation in the present study.

The relatively high proportion of epithelial cells in uterine fluid indicated

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### Table 2

**Protein Content of Uterine Fluid at Varying Intervals After Ligation**

<table>
<thead>
<tr>
<th>Weeks of accumulation</th>
<th>No. cornua</th>
<th>Protein per ml (mg) Average</th>
<th>Protein per ml (mg) S.E.</th>
<th>Protein per cornu (mg) Average</th>
<th>Protein per cornu (mg) S.E.</th>
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<td>1</td>
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<tr>
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</tr>
<tr>
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<td>7</td>
<td>2.24</td>
<td>0.12</td>
<td>70.53</td>
<td>15.32</td>
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<tr>
<td>16</td>
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<td>2.05</td>
<td>0.02</td>
<td>69.69</td>
<td>22.28</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>1.82</td>
<td>0.03</td>
<td>126.76</td>
<td>16.85</td>
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</tbody>
</table>

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that these fluids may provide a method of removing degenerate epithelial cells originating from the oestrous endometrium. This hypothesis is in general agreement with Bond's (1899) statement that "it appears that the uterine secretion is associated with the destructive processes which ordinarily go on in the generative canal in the female, and not with the constructive processes which take the form of increased growth of tissue and which are associated with pregnancy".

ACKNOWLEDGMENTS

This paper is Journal Article No. 3242 from the Michigan Agricultural Experiment Station. The work was supported in part by a Ralston Purina Research Fellowship and by National Institutes of Health grant No. GM 10584-01.

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