METABOLIC CLEARANCE AND PRODUCTION RATES OF CORTICOSTERONE IN MALE AND FEMALE VIRGIN AND BREEDER RATS

By

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ABSTRACT

Repeatedly bred male and female rats spontaneously develop arteriosclerosis. In order to evaluate the endocrine aspects of this condition volumes of distribution, metabolic clearance (MCR) and production rates (PR) of corticosterone (B) were measured in ether-stressed virgin male and female rats initially and after a significant gain in weight for comparison with heavier breeders. After the iv injection of [1,2-3H]B values were calculated from the disappearance of total radioactivity and [1,2-3H]B from plasma employing a single- and double compartment model. The latter was used for final comparisons. There was a significant difference in the half-lives of the initial and slower disappearance curves between males (3.7 and 14 min) and females (2.4 and 10 min), respectively. For both sexes with an increase in size there was a corresponding increase in the volumes of distribution, MCR and PR of B. In female breeders there was a significant decrease in these same parameters: MCR dropped from 8.5 to 5.7 l/day, PR from 6.4 to 3.5 mg/day. Circulating plasma levels decreased from 76.4 to 61.3 μg/100 ml. The results indicate that physiological factors in addition to the adrenal are responsible for the decreased PR in arteriosclerotic breeder female rats.

Previous reports from our laboratory have described the spontaneous development of arteriosclerosis in repeatedly bred male and female rats (Wexler &
True 1963; Wexler 1964a,b; Wexler et al. 1964). Results obtained from incubation studies with adrenal glands stimulated in vitro with ACTH demonstrated a significant decrease in total steroids, and corticosterone production capacity in breeder female rats (Wexler & Kittinger 1965; Kittinger & Wexler 1965). In addition, there is histological evidence that adrenal function is altered in the arteriosclerotic breeder rats (Wexler 1964c; Wexler & Kittinger 1967). Although adrenal activity is reasonably well-reflected by in vitro incubation studies (van der Vies 1960), we wished to extend our investigations of the endocrine aspects of arteriosclerosis by an in vivo evaluation of those factors which might contribute to altered adrenal steroid production. In addition to comparative metabolic rate constants, volumes of distribution, metabolic clearance and production rates of corticosterone, preliminary results obtained with tissue distribution of labelled corticosterone in virgin rats with normal arteries and arteriosclerotic breeder rats are presented in this report.

MATERIALS AND METHODS

Rats were obtained from the Sprague-Dawley Farms, Madison, Wisconsin, and then housed in air-conditioned, humidity controlled quarters for at least one week prior to the study. They were fed a regular commercial rat chow (Teklad) and tap water to drink ad libitum. Subsequently they were divided into six groups for study consisting of: male and female virgin rats, initially, and after a suitable rest period -- which allowed for a significant gain in weight -- and repeatedly bred male and female rats. At autopsy, the degree of macroscopic arteriosclerosis in each animal was noted, but the groups were not subdivided according to degrees of severity of arteriosclerosis.

[1,2-3H]Corticosterone ([1,2-3H]B) (57.2 Ci/m mole) and [4-14C]corticosterone ([4-14C]B) (55.9 mCi/m mole) were purchased from New England Nuclear Corp. Standard recommended precautions were followed to minimize decomposition of the steroid isotopes. The isotopes were chromatographed on Whatman No. 1 paper for 18 h in a benzene:formamide system (Zaffaroni 1953). The peak areas corresponding to corticosterone were located with a Nuclear Chicago (Model 1002) radiochromatogram scanner and eluted. Prior to use, [1,2-3H]B was diluted to the injection dose which contained 6 ng or 1.05 μCi/0.5 ml of 9% ethanol in normal saline.

Animals were lightly anaesthetized with Nembutal (3 mg sodium pentobarbital per 100 g body weight) followed by ether for one-half hour prior to, and during the exsanguination period. Blood samples were collected from 8:30 a.m. to 10:00 a.m. at intervals indicated below. Plasma concentrations of corticosterone in these samples were elevated in response to the procedural stress, but remained constant during the experimental period.

[1,2-3H]B was administered as a single, rapid injection into the external jugular vein; blood samples (0.4 to 0.5 ml) were collected from the opposite vein in a 1 ml heparinized tuberculin syringe 2, 4, 15, 20, 25, 30 and 35 min later. In animals to be sacrificed the final blood sample was collected by decapitation. Within minutes after collection, the blood samples were centrifuged and 0.1 or 0.5 ml (decapitation sample) plasma aliquots removed and added to known quantities of [4-14C]B. After dilution with 1 ml of water the samples were extracted with 6 ml of methylene chloride. Part
of the extract was counted without further purification and the remainder added to 50 mg of corticosterone and chromatographed on paper (vide infra). The corticosterone area located by UV absorption and strip counter scanning was eluted for counting. Corrections were made for extraction and chromatographic losses. Excess plasma obtained from decapitation samples were pooled and extracted. An aliquot of the extract was chromatographed as above and part was acetylated with pyridine-acetic anhydride and chromatographed in hexane:benzene:formamide on Whatman No. 1 paper (Zaffaroni 1953). Sample purity was indicated by the observation that the $^{14}$C/$^{3}$H ratios obtained from three different segments of the free or acetylated radioactive peaks, were the same.

Samples were dissolved in 15 ml of phosphor solution ($5 \text{ g POPOP} + 100 \text{ mg POPOP/l of toluene}$) and counted in a three-channel Nuclear Chicago Model 6860 scintillation counter at an approximate efficiency of 40 $\%_0$ for $^3$H and 55 $\%_0$ for $^{14}$C. Quench correction was made by the external standardization method using $^{133}$Ba.

At the time the animals were sacrificed the heart, both adrenals, right kidney, and segments of the liver and aorta were removed, freed of fat and other extraneous tissue. Rinsed with normal saline, and weighed. Tissues were stored at $-20^\circ\text{C}$ for no longer than two weeks prior to extraction. Tissues were homogenized with 5 ml of water in a Ten Broeck glass homogenizer to which had been added a known amount of [4-$^{14}$C]B to correct for recoveries. After centrifugation small aliquots of the aqueous phase from the adrenal homogenates were saved for corticosterone determinations and the remainder extracted similar to other tissues. The aqueous phase from the tissue homogenates were extracted with an equal volume of iso-octane. The latter was discarded and the aqueous phase extracted with 6 ml of methylene chloride. The extract was dried in scintillation vials and the radioactive concentration determined as above.

Plasma corticosterone levels were determined on 0.05 to 0.1 ml sample aliquots using essentially the combined methods of Guillemin et al. (1959), and Solem & Brinch-Johnsen (1965), but with a reduced volume modification. Similarly, adrenal corticosterone was determined from the aqueous phase of the adrenal homogenates.

Metabolic clearance rates (MCR), volumes of distribution, and production rates (PR) of corticosterone were calculated on the basis of a single and double compartment model both as dpm of $^3$H, and dpm of tritiated corticosterone as indicated in the section on Results.

The mathematical treatment and discussion of the subject of steroid dynamics that is relative to this investigation has been fully reviewed (Peterson 1959; Yates & Urquhart 1962; Tait 1963; Tait & Burststein 1964; Yates 1965). In particular, the presentation of data was modelled after the reports by Tait et al. (1961, 1962). The more pertinent equations are presented in Fig. 1 and as follows:

\[
\text{MCR (single compartment)} = \frac{\beta}{B}
\]

\[
\text{MCR (double compartment)} = V_1 K_2 = \frac{\alpha \beta}{A \beta + B \alpha}
\]

\[
\text{PR} = \text{MCR} \times \text{plasma concentration}
\]

\[
\text{Transport between pools} = k_1 = V_2 (A \beta + B \alpha)
\]

\[
\text{Metabolism in inner pool} (V_1) = K_2 = \frac{\alpha \beta (A + B)}{A \beta + B \alpha}
\]

\[
\text{Volumes of distribution, } V_1 + V_2 = \frac{\beta^2 A + B \alpha^2}{(A \beta + B \alpha)^2}
\]
Disappearance of radioactivity from the plasma of male virgins. Figure constructed from the mean values in Table 1 for 357 g rats. The curve has the algebraic form \( X' = A'e^{-at} + B'e^{-bt} \). Primes are used to indicate fractions. The \( \alpha \) slope is obtained by subtracting the extrapolated region of the \( \beta \) slope from the plotted values of the initial, rapid disappearance curve.

where \( V_0 \) is the volume of the outer pool. Calculations are performed with \( A \) and \( B \) expressed as fractions.

The first part of the disappearance curve was obtained by the plotting of determined values, whereas the later part of the curve was derived by plotting the regression equation obtained by the method of least squares (Snedecor 1956). With the latter method there was generally good agreement between plotted and calculated values.

Statistical evaluation of the data was performed according to Snedecor (1956). The probabilities given in the data were obtained from the \( t \) test.

RESULTS

The initial measurements of metabolic clearance and production rates of corticosterone in male and female virgin rats were made on the basis of total methylene chloride extractable dpm of \( ^3\text{H} \) in plasma. Subsequently these parameters were measured as above, and as plasma dpm of \( ^3\text{H} \) present as corticosterone. During the first 4 min following the injection of [1,2-\( ^3\text{H} \)]B there was no discernible difference in radioactivity attributable to metabolism. The average radioactive concentrations were used to plot the first part of the disappearance curves. In samples collected 35 min after the administration dose, 80–86\% of the plasma radioactivity was unmetabolized corticosterone (Table 6).
There was no apparent sex difference in these values. Metabolic clearance and production rates were calculated according to a single and double compartment model. Consistently higher values were obtained with a one compartment system. This is exemplified by the data presented in Table 2.

**Male and female virgins**

The mean metabolic rate constants, volumes of distribution, plasma levels of corticosterone, MCR and PR for male and female virgin rats before and after a significant \( (P < 0.01) \) increase in body weight are shown in Tables 1 and 2. In both sexes the increase in size was reflected by an increase in the volumes of distribution, a corresponding elevated rate of transfer of the injected dose between compartments \((K_1)\) and an increased rate of metabolism \((K_2)\) in the inner pool. Although the slopes of the slower disappearance curve \((\beta)\) differed significantly between sexes, they remained essentially unchanged with an increase in size. Further, statistical evaluation of the two compartment model showed that the increased MCR and PR with increased size in both sexes was significant \( (P < 0.01) \). The slight decrease in circulating levels of corticosterone with increased size was not significant \( (P > 0.05) \). A similar comparison between males and females before and after a gain in weight showed that the MCR differences were not significant \( (P > 0.05) \), whereas the PR values differed \( (\text{before } P < 0.01, \text{ after } P < 0.05) \). The sex difference in circulating levels of corticosterone was significant \( (P < 0.01) \). The values for larger rats may be directly compared with values for virgins (same animals).

**Table 1.**

Mean metabolic rate constants and volumes of distribution of corticosterone in male and female virgin rats before and after a gain in weight.*

<table>
<thead>
<tr>
<th>Animal</th>
<th>A</th>
<th>B</th>
<th>(\alpha)</th>
<th>(\beta)</th>
<th>(K_1)</th>
<th>(K_2)</th>
<th>(V_1)</th>
<th>(V_1+V_2)</th>
<th>(\overline{V})</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt., g</td>
<td>%dose/ml</td>
<td>units/day</td>
<td>ml/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>357 (9)**</td>
<td>1.6</td>
<td>1.3</td>
<td>322</td>
<td>72</td>
<td>84</td>
<td>128</td>
<td>104</td>
<td>151</td>
<td>235</td>
</tr>
<tr>
<td>447 (8)</td>
<td>1.3</td>
<td>0.69</td>
<td>436</td>
<td>71</td>
<td>151</td>
<td>153</td>
<td>128</td>
<td>219</td>
<td>342</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>253 (9)</td>
<td>4.5</td>
<td>1.0</td>
<td>480</td>
<td>97</td>
<td>134</td>
<td>272</td>
<td>75</td>
<td>130</td>
<td>887</td>
</tr>
<tr>
<td>296 (7)</td>
<td>3.1</td>
<td>0.75</td>
<td>522</td>
<td>96</td>
<td>156</td>
<td>286</td>
<td>91</td>
<td>176</td>
<td>530</td>
</tr>
</tbody>
</table>

* Values calculated on the basis of the disappearance of total radioactivity from plasma.

** Numbers of animals in a group are indicated in parentheses.
Table 2.
Mean metabolic clearance and production rates of corticosterone on a single and double compartmental basis for male and female virgin rats before and after a gain in weight.*

<table>
<thead>
<tr>
<th>Animal wt., g</th>
<th>MCR&lt;sub&gt;1&lt;/sub&gt;↑ ml/day</th>
<th>PR&lt;sub&gt;1&lt;/sub&gt; mg/day</th>
<th>MCR&lt;sub&gt;2&lt;/sub&gt; ml/day</th>
<th>PR&lt;sub&gt;2&lt;/sub&gt; mg/day</th>
<th>Plasma corticosterone μg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>357 (9)**</td>
<td>6120</td>
<td>3.30</td>
<td>4729</td>
<td>2.56</td>
<td>54.3</td>
</tr>
<tr>
<td>447 (8)</td>
<td>10773</td>
<td>5.30</td>
<td>8397</td>
<td>4.12</td>
<td>49.6</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>253 (9)</td>
<td>9281</td>
<td>7.29</td>
<td>5004</td>
<td>3.95</td>
<td>79.4</td>
</tr>
<tr>
<td>296 (7)</td>
<td>14548</td>
<td>10.85</td>
<td>7822</td>
<td>5.88</td>
<td>76.4</td>
</tr>
</tbody>
</table>

* Values calculated on the basis of the disappearance of total radioactivity from plasma.
** Numbers of animals in a group are indicated in parentheses.
† Subscript numbers refer to single and double compartment model.

in Tables 3 and 4 where the data was compiled from the disappearance curves of [1,2-<sup>3</sup>H]<sub>B</sub>.

Table 3.
Mean metabolic rate constants and volumes of distribution of corticosterone in male and female virgin and breeder rats.*

<table>
<thead>
<tr>
<th>Animal</th>
<th>A</th>
<th>B</th>
<th>α</th>
<th>β</th>
<th>K&lt;sub&gt;1&lt;/sub&gt;</th>
<th>K&lt;sub&gt;2&lt;/sub&gt;</th>
<th>V&lt;sub&gt;1&lt;/sub&gt;</th>
<th>V&lt;sub&gt;1&lt;/sub&gt;+V&lt;sub&gt;2&lt;/sub&gt;</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%/dose/ml</td>
<td>units/day</td>
<td>ml/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>virgins (8)**</td>
<td>1.3</td>
<td>0.71</td>
<td>424</td>
<td>82</td>
<td>128</td>
<td>166</td>
<td>128</td>
<td>204</td>
<td>331</td>
</tr>
<tr>
<td>breeders 11)</td>
<td>1.1</td>
<td>0.81</td>
<td>470</td>
<td>84</td>
<td>143</td>
<td>159</td>
<td>120</td>
<td>185</td>
<td>278</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>virgins (7)</td>
<td>3.2</td>
<td>0.67</td>
<td>512</td>
<td>103</td>
<td>134</td>
<td>310</td>
<td>101</td>
<td>166</td>
<td>608</td>
</tr>
<tr>
<td>breeders (9)</td>
<td>4.3</td>
<td>0.96</td>
<td>485</td>
<td>101</td>
<td>127</td>
<td>289</td>
<td>59</td>
<td>104</td>
<td>338</td>
</tr>
</tbody>
</table>

Virgins are rats previously described in Tables 1 and 2 after a gain in weight. The mean weights of male and female breeders are 460 and 337 g, respectively.

* Values calculated on the basis of the disappearance of radioactive corticosterone from the plasma.
** Numbers of animals in a group are indicated in parentheses.
Table 4.
Mean metabolic clearance and production rates of corticosterone in male and female virgin and breeder rats.*

<table>
<thead>
<tr>
<th>Animal</th>
<th>MCR ml/day</th>
<th>PR mg/day</th>
<th>Plasma corticosterone μg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>virgins (8)**</td>
<td>9216</td>
<td>4.52</td>
<td>49.6</td>
</tr>
<tr>
<td>breeders (11)</td>
<td>8519</td>
<td>4.08</td>
<td>48.8</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>virgins (7)</td>
<td>8488</td>
<td>6.36</td>
<td>76.4</td>
</tr>
<tr>
<td>breeders (9)</td>
<td>5731</td>
<td>3.53</td>
<td>61.3</td>
</tr>
</tbody>
</table>

* Values calculated on the basis of the disappearance of radioactive corticosterone from plasma using a double compartment model.

** Numbers of animals in a group are indicated in parentheses.

Non-arteriosclerotic virgin rats vs arteriosclerotic breeder rats

The mean metabolic rate constants, volumes of distribution, plasma levels of corticosterone, MCR and PR for male and female virgins and breeders are shown in Tables 3 and 4. The disappearance of radioactive corticosterone from the plasma of these animals is shown plotted in Figs. 2 and 3. Male

![Disappearance of 3H-corticosterone from the plasma of male virgin and breeder rats.](image)

Fig. 2.

Disappearance of 3H-corticosterone from the plasma of male virgin and breeder rats.
breeders tended to weigh more than the male virgins, but the difference was not significant ($P > 0.05$). By contrast, the female virgins weighed considerably less than the female breeders ($P < 0.01$). Whereas in the previous study it was found that the heavier rats had an increased rate of metabolism ($K_2$) in the inner pool ($V_1$), the heavier breeders had a slight decrease in the $K_2$ values, and a decrease in the volumes of distribution. The latter is more pronounced in the female group. The slight decrease in MCR, PR and circulating levels of corticosterone in male breeders was not significant ($P > 0.05$). Among the female rats, the decreased MCR in the breeders was significant ($P < 0.05$) and the decreased PR and circulating levels of corticosterone, highly significant ($P < 0.01$).

**Tissue radioactivity and adrenal concentration of corticosterone**

The mean paired adrenal weights, and adrenal concentrations of corticosterone and radioactivity are shown in Table 5. The sex difference in adrenal weights was significant ($P < 0.01$), but there was no statistical difference between virgins and breeders. Surprisingly, there were no statistical differences in the concentrations of corticosterone calculated either on the basis of per pair of adrenals or per unit weight of tissue. Between virgin subjects, the radioactive concentrations were significantly ($P < 0.01$) different using either manner of expressing the results. There were no statistical differences in the radioactive concentrations between breeders and between female virgin and
Table 5.
Mean concentration of corticosterone and radioactivity in adrenals from male and female virgins and breeders.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Adrenal wt., mg</th>
<th>Corticosterone µg per adrenals</th>
<th>µgB*</th>
<th>DPM per adrenals</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>virgins (8)**</td>
<td>50.6</td>
<td>2.7</td>
<td>52.7</td>
<td>130</td>
<td>323</td>
</tr>
<tr>
<td>breeders (10)</td>
<td>53.6</td>
<td>2.6</td>
<td>47.9</td>
<td>92</td>
<td>228</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>virgins (7)</td>
<td>63.6</td>
<td>3.5</td>
<td>56.0</td>
<td>72</td>
<td>251</td>
</tr>
<tr>
<td>breeders (8)</td>
<td>65.2</td>
<td>3.2</td>
<td>48.9</td>
<td>73</td>
<td>218</td>
</tr>
</tbody>
</table>

* µgB = µg corticosterone.
** Numbers of animals in a group are indicated in parentheses.

breeders. The differences in radioactive concentrations between male virgins and breeders were significant when expressed either as »per adrenals« ($P < 0.05$), or »per g of tissue« ($P < 0.01$). In reviewing the individual values comprising the means in Table 5, it was apparent that most of the variation in the male virgin group was attributable to one male whose adrenals weighed within the female range of weights and whose concentration of corticosterone per adrenals was twice the level of the other males. If this animal is eliminated from the group, the mean concentration of corticosterone is lowered to 2.4 µg/adrenals, and the DPM/µg corticosterone raised from 130 to 140. These changes would raise to significance ($P < 0.01$) the difference in concentration of corticosterone per adrenals between virgins of both sexes, and the male difference in DPM per µg corticosterone without appreciably altering other values.

The radioactive concentrations of tritium found in the different tissues 35 min after the administration of labelled corticosterone are shown in Table 6. A comparison may be made with the plasma levels expressed both as total extractable radioactivity and radioactivity as corticosterone. For each tissue analyzed, the $^3$H levels were higher in the virgins than in the breeders, as opposed to the plasma concentrations. With the exception of the aorta the tissues from female rats had a lower radioactive concentration than those from male rats. In two female breeders sclerotic and non-arteriosclerotic segments of the aorta were separately extracted; the former had a much higher concentration of radioactivity.
Table 6.
Tissue radioactivity per gram expressed as a per cent of injection dose.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Tissues</th>
<th>Plasma* DPM</th>
<th>DPM as B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>virgins</td>
<td>0.10</td>
<td>0.11</td>
<td>0.21</td>
</tr>
<tr>
<td>breeders</td>
<td>0.08</td>
<td>0.08</td>
<td>0.15</td>
</tr>
<tr>
<td>Females</td>
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<tr>
<td>virgins</td>
<td>0.04</td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>breeders</td>
<td>0.03</td>
<td>0.02</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Plasma radioactivity per ml (% of dose) as total extractable (DPM), and after isolation of corticosterone (DPM as B).

DISCUSSION

The present study was undertaken in order to compare the MCR and PR of corticosterone between virgins and arteriosclerotic breeders. Because of the larger size of the breeders, a more meaningful interpretation of the results could be obtained by comparing breeders with virgins initially, and after the latter had gained in weight.

Our results, and those of Glenister and Yates (1961) show that 35 min after injection of labelled corticosterone at least 80% of the circulating radioactivity is present as unmetabolized corticosterone; sex differences with this regard are insignificant. Although our final observations were drawn from the disappearance of radioactive corticosterone, a similar interpretation of the results could be made if based on the disappearance of total radioactivity from the plasma.

The significant sex difference between virgins in the half-life (t 1/2) of the slower disappearance curve (β, Fig. 1 and Table 1) is in general agreement with the results reported by other investigators (Ulrich & Long 1956; Glenister & Yates 1961; Kitay 1961), i.e., approximately 20 min for males and 13 min for females. This differs from our findings of approximately 14 and 10 min for males and females, respectively. In the present study a total of 6 ng of tritiated corticosterone was injected per rat, in contrast to a minimum injected dose of 50 µg in other studies. However, similar differences (t 1/2) in the β slope have been observed after the injection of tracer and »pharmacologic« doses of cortisol in man (Peterson 1959). The results (Table 1), in contrast to those in a previous study (Glenister & Yates 1961), show an increase in the volume of

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distribution with an increase in animal size. Although, we had not considered the larger virgin rats «clinically» obese it is of comparative interest that there is an increased »relative distribution space« in obese patients (Dunkelman et al. 1964).

The sex difference, and increase in PR₂ of corticosterone with an increase in body size (Table 2) is in general agreement with that which may be predicted from the secretion rates calculated by Glenister & Yates (1961). Although circulating levels of corticosterone were significantly higher in the smaller females than in the larger males, the opposite was true for the metabolic clearance rates (MCR₂). As a result the actual difference in PR₂ is insignificant.

In males and females the concomitant increased MCR and rate of metabolism (K₂) with increased body weight (see Tables 1 and 2) is consistent with increased hepatic enzyme activity in proportion to an increase in liver and body size (Urquhart et al. 1959; Glenister & Yates 1961). The larger K₂ values for females may indicate a greater amount of A⁴-steroid hydrogenase activity in their livers (Glenister & Yates 1961).

The sex difference in corticosterone secretion in rats has intrigued investigators for more than a decade. In rats selected for equivalent adrenal weights a much higher concentration of corticosterone was found in the adrenal venous blood of females (Kitay 1961). Under the experimental conditions described by this investigator, the immediate regulatory effect of the liver was apparently minimal and the sex difference in secretion rate was attributed to a greater quantity of ACTH release in the female rat in response to stress. The results (Table 5), and those reported earlier by us (Kittinger 1960) show that there was no sex difference in the concentration of corticosterone per unit mass of adrenals. The apparent differences in results described above may be entirely methodological.

In contrast to the insignificant differences between male virgins and breeders, in parallel with reproductive activity, there is a significant reduction in the volumes of distribution, MCR and PR of corticosterone in females. This observation is more marked considering that the breeders weighed more than the virgins and we had previously observed an increase in these values with an increase in size. These results tend to confirm and extend the in vitro results previously reported (Wexler & Kittinger 1965; Kittinger & Wexler 1965). Repeatedly bred rats spontaneously develop arteriosclerosis. Even on a relatively low fat diet breeders develop fatty livers as well as elevated serum cholesterol levels (Wexler et al. 1964; Wexler & Kittinger 1967). The pivotal role of the liver in steroid metabolism and the maintenance of endocrine balance has been described in a number of reports (Urquhart et al. 1959; Peterson 1960; Berliner & Dougherty 1961; Coppage et al. 1962; Yates 1965). In view of the fatty liver infiltration in breeder rats, which is more severe in the female, it seemed particularly pertinent to estimate the MCR of corticosterone
in these animals. Unstressed serum levels of corticosterone in virgin and breeder female rats are not significantly different (14 μg vs 18 μg/100 ml). However, in response to the procedural stress in the present study the virgins attain a significantly higher level of circulating corticosterone than do the breeders (Table 4). Indications are that this does not necessarily reflect a decrease in corticotrophin secretion in the breeders since adrenal weight is maintained (Table 5), and histological evidence suggests that the pituitary glands of repeatedly bred female rats may contain extra adrenocorticotrophin activity (Wexler & Saroff 1968).

Apparently the female breeder rats have a decreased capacity for the biosynthesis of corticosterone. However, the decreased MCR and volumes of distribution indicate that physiological factors in addition to adrenal function per se have been altered. The breeder rat, in particular the female, undergoes degenerative changes which may be described as either premature or accelerated aging (Wexler & Saroff 1968).

In some of the earlier work on the subject of aging and steroid metabolism (Samuels 1956; Samuels et al. 1957) it was observed that following the infusion of cortisol in elderly subjects there was a significant decrease in the rate and volume of distribution to the tissues, and in the rate of metabolic removal. It was suggested that the decreased »apparent volume of distribution« and slower attainment of equilibrium could be attributed to a greater density of extravesicular tissue and a slower penetration into the tissues, whereas the decreased rate of metabolism was attributed to either reduced hepatic cellular activity or blood flow. More recently Flood et al. (1967), observed a decrease in the MCR, secretion rate and plasma concentration of aldosterone in elderly subjects. The lowered MCR was attributed to both reduced splanchnic extraction and blood flow.

Although there are definite similarities between the results observed in breeder rats and in elderly subjects identical mechanisms may not be operative. The tissue radioactivity (Table 6) viewed in conjunction with the concentration of radioactive corticosterone in serum indicates that there is a slower diffusion of radioactivity into the tissues of breeder rats. However, in spite of this, there is no marked diminution in the volumes of distribution or MCR in the male breeder rats. Whether this reflects a compensatory hormonal mechanism in the latter group, or is mediated by the degenerative pathologic changes common to breeders of both sexes but most pronounced in the female, is uncertain. Studies have been initiated to measure the protein binding of testosterone, corticosterone and 17β-oestradiol in serum from virgin and breeder rats. The preliminary results indicate that with increasing severity of arteriosclerosis in female breeder rats there is a progressive decrease in the protein binding of testosterone and corticosterone.

The significant decrease in the rates of corticosterone metabolism and pro-
duction in female breeder rats indicates that the reproductive status of an animal should also be taken into account when assessing adrenal function, as well as considerations of age (Flood et al. 1967) and circadian rhythm.

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REFERENCES

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