Renin Content of Systemic Blood of Rats with Desoxycorticosterone and Metacorticoid Hypertension

By J. L. Prado, Zuleika P. Picarelli, Regina Kupper, Eline S. Prado, and J. R. Valle

No increased renin concentration was detected in the systemic blood of rats with desoxycorticosterone and metacorticoid hypertension, using the guinea-pig ileum method of renin estimation. Re-establishment of the circulation through totally ischemic rat kidney is followed by the liberation of large amounts of renin.

It has been demonstrated in different laboratories that chronic treatment with desoxycorticosterone acetate (DCA) induces in rats a particular type of hypertensive disease. Observations by Friedman and Friedman, Friedman, Friedman and Nakashima, Prado and Herbert-Carrington, Magaldi and Prado showed that following abrupt interruption of the desoxycorticosterone treatment, from 30 to 40 per cent of the animals become permanently hypertensive. These results, with a still higher incidence of permanent hypertension, were confirmed by Green and colleagues. The later phase of desoxycorticosterone hypertension has been named self-sustained, residual and postdesoxycorticosterone; Selye and Horváth have lately suggested the name melahormonal or metacorticoid hypertension.

Although an extremely informative study of desoxycorticosterone and metacorticoid hypertension has been recently reported by Green and co-workers, the participation of the renal pressor system in both phases of desoxycorticosterone hypertension has not yet been directly investigated. It was thought that by using the previously described sensitive method of renin detection and estimation at different times during the developmental and the permanent phase of hypertension such an objective would be attained.

EXPERIMENTAL

Preliminary Experiments: Renin Assay in Rat Plasma. Plasma obtained from heparinized blood was treated as already described, its hypertensinase being destroyed before the incubation period with hypertensinogen by lowering the pH to 3.9 during 20 minutes at 25°C. In general, 2 to 4 ml. of plasma were used, half of which was kept acid in the refrigerator to serve as unincubated control (acid control), the other half being incubated with excess hypertensinogen. Both filtrates were made up to 4 to 5 ml. from which 0.5 ml. aliquots were tested on the gut. In a few instances, in order to test the whole incubated plasma, proteins were removed by adding three volumes of ethanol, heating in a boiling water bath for 10 minutes, filtering, washing the precipitate with three small portions of alcohol and then evaporating to dryness under reduced pressure. The residue was taken in a small volume of saline and tested.

In order to verify that no interfering substance would be liberated from renin-free plasma by incubation with hypertensinogen, blood plasma from 24-hour nephrectomized...
RENIN ASSAY IN DCA HYPERTENSION

Determination of added renin in rat plasma by the guinea-pig ileum method. Following incubation with excess hypertensinogen, filtrates corresponding to different amounts of renin were tested on the gut. 

\[ T_i, T_3, T_5 \text{ correspond respectively to } 0.075, 0.037 \text{ and } 0.015 \text{ Indianapolis renin units. } KaT_i, KaT_3, \text{ and } KaT_5 \text{ are the unincubated acid controls. } P_i \text{ and } P^P_i \text{ represent } 0.125 \text{ ml. of incubated and unincubated plasma from 24-hour nephrectomized rats.} \]

rats was so incubated and found to elicit no contraction of the guinea-pig ileum. If to such renin-free plasma one adds increasing amounts of renin, it may be as easily assayed as when only renin is incubated with excess hypertensinogen (fig. 1).

The next step was to determine which would be the smallest amount of renin detectable in rat's blood. Twenty-four-hour nephrectomized rats, of approximately 300 Gm. body weight, previously heated during 10 minutes in a 40 C chamber, were injected with 1.57 units of renin through a tail vein. One, 2, 5, 10, 20 and 60 minutes later, 6 to 8 ml. of blood were drawn from the aorta* for renin determination. The.

* When the blood collection had to be done one and two minutes after the renin injection, the aorta was first isolated and held on a thread; otherwise it would have been difficult to draw blood within such short periods of time.

fig. 1. **Determination of added renin in rat plasma by the guinea-pig ileum method.** Following incubation with excess hypertensinogen, filtrates corresponding to different amounts of renin were tested on the gut. 

\[ T_i, T_3, T_5 \text{ correspond respectively to } 0.075, 0.037 \text{ and } 0.015 \text{ Indianapolis renin units. } KaT_i, KaT_3, \text{ and } KaT_5 \text{ are the unincubated acid controls. } P_i \text{ and } P^P_i \text{ represent } 0.125 \text{ ml. of incubated and unincubated plasma from 24-hour nephrectomized rats.} \]

fig. 2. **Disappearance of injected renin from the circulation of nephrectomized rats.** Following intravenous injection of 1.57 renin units into a 24-hour nephrectomized rat blood renin was assayed at 60 (\(T_6\)), 20 (\(T_2\)), 10 (\(T_{10}\)), 5 (\(T_5\)), 2 (\(T_2\)) and 1 minute (\(T_1\)). Control tubes are marked b. TB = rat plasma plus 1.57 renin units. Only one-eighth of the incubated amounts were tested on the ileum.

fig. 3. **Blood renin assay in 24-hour nephrectomized rats injected with renin.** Following intravenous injection of 0.5, 1.0, 4.0 and 8.0 renin units into 24-hour nephrectomized rats, blood renin was assayed five minutes later. One eighth of the 2 ml. incubated sample tested on the gut. \(EPiNa\): Standard hypertensin, assaying 2.5 Indianapolis units per milligram. \(Plasma\): incubated plasma from 24-hour nephrectomized rats.
TABLE 1.—Data Related to Rats Used for Renin Assays in the Developmental Phase of DCA Hypertension

<table>
<thead>
<tr>
<th></th>
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<td>F1</td>
<td>220A</td>
<td>55</td>
<td>DCA 10 days</td>
<td>(7)100 (10)122</td>
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<td>4.0</td>
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<tr>
<td></td>
<td>220B</td>
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<td>(7)100 (10)125</td>
<td></td>
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<tr>
<td>F2</td>
<td>220C</td>
<td>60</td>
<td>DCA 10 days</td>
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<td>1.5</td>
<td>4.0</td>
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<tr>
<td></td>
<td>220D</td>
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<td></td>
<td>(7)130</td>
<td></td>
<td></td>
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<tr>
<td>F3</td>
<td>222A</td>
<td>47</td>
<td>control 10 days</td>
<td>(10)75</td>
<td>1.0</td>
<td>4.0</td>
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<td>222B</td>
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<td>(10)70</td>
<td></td>
<td></td>
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<tr>
<td>F4</td>
<td>221A</td>
<td>62</td>
<td>DCA 18 days</td>
<td>(11)150 (13)150 (17)170</td>
<td>1.5</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>221B</td>
<td>56</td>
<td></td>
<td>(11)153 (13)170 (17)178</td>
<td></td>
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</tr>
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<td>F5</td>
<td>221F</td>
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<td>DCA 18 days</td>
<td>(13)150 (17)172</td>
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<td>4.0</td>
</tr>
<tr>
<td></td>
<td>221G</td>
<td>62</td>
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</tr>
<tr>
<td>F6</td>
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<td>67</td>
<td>DCA 18 days</td>
<td>(11)130 (13)130 (17)165</td>
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<td>4.0</td>
</tr>
<tr>
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<tr>
<td>F7</td>
<td>222A</td>
<td>71</td>
<td>control 18 days</td>
<td>(10)105 (13)110 (17)130</td>
<td>1.5</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>222B</td>
<td>44</td>
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<td>(10)60 (13)120 (17)125</td>
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<td>F8</td>
<td>220G</td>
<td>70</td>
<td>DCA 32 days</td>
<td>(11)110 (13)140 (17)105 (25)190 (31)210</td>
<td>2.0</td>
<td>5.0</td>
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<tr>
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<td>66</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F9</td>
<td>221A</td>
<td>50</td>
<td>DCA 32 days</td>
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<td>1.5</td>
<td>5.0</td>
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<tr>
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<td>221B</td>
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<td></td>
<td>(13)140 (17)192 (25)170 (31)205</td>
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</tr>
<tr>
<td>F10</td>
<td>222A</td>
<td>50</td>
<td>control 32 days</td>
<td>(13)110 (17)120 (26)110 (31)100</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>222B</td>
<td>61</td>
<td></td>
<td>(7)77 (13)115 (17)95 (26)115 (31)105</td>
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<tr>
<td>F11</td>
<td>222C</td>
<td>50</td>
<td>control 32 days</td>
<td>(7)80 (13)128 (17)120 (26)125 (31)80</td>
<td>1.5</td>
<td>5.0</td>
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<tr>
<td></td>
<td>222D</td>
<td>73</td>
<td></td>
<td>(7)85 (13)115 (17)115 (25)130 (31)130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F12</td>
<td>222E</td>
<td>50</td>
<td>DCA, 38 days</td>
<td>(13)125 (25)155 (38)180</td>
<td>1.2</td>
<td>6.0</td>
</tr>
<tr>
<td>F13</td>
<td>221E</td>
<td>67</td>
<td>DCA, 38 days</td>
<td>(13)105 (25)170 (38)220</td>
<td>1.2</td>
<td>6.0</td>
</tr>
<tr>
<td>F14</td>
<td>222F</td>
<td>41</td>
<td>control, 38 days</td>
<td>(7)75 (13)90 (38)135</td>
<td>1.8</td>
<td>6.0</td>
</tr>
<tr>
<td>F15</td>
<td>222G</td>
<td>44</td>
<td>control, 38 days</td>
<td>(10)50 (13)125 (17)115 (25)130 (38)120</td>
<td>1.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* Filtrates F1 through F11 were prepared from a pooling of equal parts of blood from two rats.
† All uninephrectomized and given 1% saline to drink.
‡ Figures in parentheses refer to days after implantation.

results from one experiment are shown in figure 2. It is clear that from one to five minutes renin was found in approximately the same amounts; in 10 to 20 minutes there were smaller concentrations, and in one hour no renin was detectable.

To determine how small the amounts of injected renin might be and still be assayed, two series of 24-hour nephrectomized rats, ranging from 215 to 240 Gm. of body weight, were injected with 0.5, 1.0, 4.0 and 8.0 renin units and their blood plasma renin concentration determined five minutes later. Within this time interval the injected renin would have been well mixed with all the rat's blood and none of it would probably have disappeared from the circulation.

Figure 3 shows that injection of even 0.5 unit of renin could be detected, although only one eighth (0.25 ml.) of the incubated plasma...
### Table 2: Data Related to Rats with Melanocortoid Hypertension Used for Renin Assays

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Body Wt. (Gm.)</th>
<th>Treatment*</th>
<th>Observations!</th>
<th>Vol. incubated plasma</th>
<th>Vol. filtrate</th>
</tr>
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<tbody>
<tr>
<td>F16 216H</td>
<td>80</td>
<td>210</td>
<td>10 mo. DCA</td>
<td>At 3 mo. two pellets removed. Remained hypertensive. Kidney +++</td>
<td>2.0</td>
</tr>
<tr>
<td>F17 213C</td>
<td>71</td>
<td>235</td>
<td>15 mo. DCA</td>
<td>At 6 mo. one small pellet removed, other not found. Remained hypertensive.</td>
<td>2.0</td>
</tr>
<tr>
<td>F18 213F</td>
<td>70</td>
<td>220</td>
<td>15 mo. DCA</td>
<td>At 6 mo. pellets were not found. Remained hypertensive.</td>
<td>2.0</td>
</tr>
<tr>
<td>F19 216G</td>
<td>95</td>
<td>235</td>
<td>10 mo. DCA</td>
<td>At 3 mo. two pellets removed. Remained hypertensive.</td>
<td>2.0</td>
</tr>
<tr>
<td>F20 214H</td>
<td>79</td>
<td>332</td>
<td>15 mo. K</td>
<td>Normotensive. Kidney 0</td>
<td>2.0</td>
</tr>
<tr>
<td>F22 217A</td>
<td>90</td>
<td>300</td>
<td>10 mo. DCA</td>
<td>At 4 mo. no pellets found. Remained hypertensive. Kidney +++</td>
<td>2.0</td>
</tr>
<tr>
<td>F23 217I</td>
<td>95</td>
<td>215</td>
<td>10 mo. DCA</td>
<td>At 4 mo. one pellet removed; other not found. Remained hypertensive. Kidney +++</td>
<td>1.5</td>
</tr>
<tr>
<td>F24 213A</td>
<td>79</td>
<td>303</td>
<td>15 mo. DCA</td>
<td>At 6 mo. small pellet removed; other not found. B.P. between normo and hypertensive. Kidney +++</td>
<td>2.0</td>
</tr>
<tr>
<td>F25 213E</td>
<td>75</td>
<td>225</td>
<td>15 mo. DCA</td>
<td>At 6 mo. no pellets found. B.P. between normo and hypertensive. Kidney +++</td>
<td>2.0</td>
</tr>
<tr>
<td>F26 214J</td>
<td>76</td>
<td>310</td>
<td>15 mo. K</td>
<td>Normotensive. Kidney 0</td>
<td>2.0</td>
</tr>
<tr>
<td>F27 217B</td>
<td>85</td>
<td>325</td>
<td>10 mo. DCA</td>
<td>At 4 mo. no pellets found. Remained hypertensive. Kidney +++</td>
<td>2.0</td>
</tr>
<tr>
<td>F28 218H</td>
<td>115</td>
<td>245</td>
<td>10 mo. DCA</td>
<td>At 4 mo. no pellets found. Remained hypertensive. Kidney +++</td>
<td>2.0</td>
</tr>
<tr>
<td>F29 218F</td>
<td>85</td>
<td>325</td>
<td>10 mo. DCA</td>
<td>At 4 mo. one pellet removed, other not found. B.P. between normo and hypertensive. Kidney +++</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Rats were castrated, uninephrectomized, given 1 per cent saline to drink (K) or also implanted with two 20 mg. DCA pellets (DCA).  
† A three-plus kidney was enlarged and pale with characteristic intense roughness of its surface, small hemorrhagic areas and sometimes large depressions; the capsule was usually very adherent.

was added to the muscle chamber. In the rats injected with 8 renin units, assuming that it was distributed only in the plasma (calculated volume 12 ml.), and taking the responses obtained on the ileum as equivalent to one eighth (50 micrograms of EP11a) of a hypertensin unit, then, the total amount of liberated hypertensin would correspond to 6 renin units. The recovery obtained is considered satisfactory for this kind of experiment.

There is also the possibility that the difference is real and represents renin that actually disappeared from circulation. A second experiment gave identical results and lead us to the conclusion that from 0.5 to 1.0 renin unit might be detected when injected a few minutes previously into a nephrectomized rat.

**Renin Assay in the Systemic Blood of Desoxycorticosterone Hypertensive Rats.** Thirty albino rats were unilaterally nephrectomized and their drinking water substituted by one per cent sodium chloride solution; 20 of them were implanted subcutaneously with two 20 mg. desoxycorticosterone pellets, while the remainder were kept as controls. From the date of implantation, blood pressure was repeatedly determined by Magaldi's plethysmographic apparatus. As may be deduced from the values shown in table 1, where other pertinent data are also given, the implanted survivors became markedly hypertensive or not, depending on the time they were kept alive. Control animals remained normotensive. At the tenth, eighteenth, thirty-second and thirty-eighth days after implantation, some of the desoxycorticosterone and control rats were used for renin...
assays. After pentobarbital anesthesia blood was drawn from the aorta, using a heparinized syringe; before exsanguinat ing the animal, the remaining kidney was always excluded from the circulation to avoid any possible liberation of renin as a result of the acute hypoten sion.

Filtrates F1 through F11 were obtained from pooled blood (two rats each), while filtrates F12 to F15 were obtained from single animals. Equally small amounts of renin were, in general, present in control, desoxycorticosterone-implanted but still normotensive, and desoxycorticosterone-implanted hypertensive rats.

Renin Assays in the Systemic Blood of Metacorticoid-Hypertensive Rats. A group of albino rats with metacorticoid hypertension and three unimplanted controls were used in this experiment. With exclusion of rat 213A (see table 2) the implanted animals became highly hypertensive, the average peak blood pressure during the implantation period being 216 mm. Hg. From three to six months following the desoxycorticosterone implantation, a large incision was made on the back and the pellets thoroughly searched for and removed when found. Had any residue of desoxycorticosterone pellets then remained it would probably have been completely absorbed around the sixth month from implantation. Those 10 animals which had become hypertensive remained so during many months, some of them up to nine months, the average peak blood pressure in the metacorticoid phase of hypertension being 197 mm. Hg. Rat 213A, which had been normotensive all the time previously, presented two hypertensive readings during the last month of observation.

At the day of the renin assay the pedicle of the remaining kidney was ligated and approximately 8 ml. of blood were removed from the aorta. The kidney of each rat was then macroscopically inspected and the lesions graduated.

Fig. 4. Blood renin assays in the metacorticoid phase of DCA-hypertension in rats. The assays were done in three different guinea-pig ileum preparations. EP11a: Standard hypertensin. F16 to F29 and bF16 to bF29 are the filtrates mentioned in table 2 and the respective unincubated acid controls.
from zero to three plus (table 2). It is worth
mentioning that animal 213A also had a dam-
aged kidney. An examination of figure 4 dem-
onstrates that the small amounts of renin some-
times found in hypertensive rats are comparable
to those present in normotensive control ani-
mals.

**Liberation of Renin from Ischemic Rat Kid-
neys.** An attempt was made to determine the
total amount of renin liberated by ischemic
kidneys upon re-establishment of their circu-
lation. Under ether anesthesia a renal pedicle
was clamped so as to induce complete ischemia,
and the contralateral kidney removed. Follow-
ing a variable period the animal was again
anesthetized, the clamp removed, and 4 to 10
minutes later, when it was noticeable that the
circulation was partially re-established, the
animal was exsanguinated through the aorta.
Plasma renin was then assayed and the total
amount set free calculated from the total
plasma volume of the rat (fig. 5 and table 3).
In one experiment a two-hour ischemia period
was not followed by renin release. In six exper-
iments, in which ischemia lasted five to seven
hours, variable amounts were detected. This
variability might be due in part to the degree
of the re-establishment of the renal circu-
lation. As an average, 290 Gm. of rat, corre-
sponding approximately to 0.87 Gm. of kidney

<table>
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<tr>
<th>Rat</th>
<th>Body Wt. Gm.</th>
<th>Period of ischemia</th>
<th>Time between clamp release and blood collection</th>
<th>Total amt. liberated renin (Indianapolis units)</th>
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<td>1</td>
<td>330</td>
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<td>4-5 min.</td>
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<tr>
<td>2</td>
<td>385</td>
<td>5 hr. 10 min.</td>
<td>4.5-5.5 min.</td>
<td>13.0</td>
</tr>
<tr>
<td>3</td>
<td>360</td>
<td>2 hr.</td>
<td>9-10 min.</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>6 hr. 10 min.</td>
<td>5-8 min.</td>
<td>5.5</td>
</tr>
<tr>
<td>5*</td>
<td>[270] [250]</td>
<td>6 hr. 20 min.</td>
<td>4-5 min.</td>
<td>9.7</td>
</tr>
<tr>
<td>6</td>
<td>260</td>
<td>5 hr. 45 min.</td>
<td>5 min.</td>
<td>24.0</td>
</tr>
<tr>
<td>7</td>
<td>215</td>
<td>7 hr. 15 min.</td>
<td>4 min.</td>
<td>15.8</td>
</tr>
</tbody>
</table>

* Pooled blood from two rats.

mass, released 13.8 Indianapolis units of renin.

**DISCUSSION**

No increased amounts of renin were found
in the systemic blood of rats either in the de-
velopmental or in the metacorticoid phase of
desoxycorticosterone hypertension. The sensi-
tivity of the guinea-pig ileum method of renin
estimation used is such as to detect 0.5 to 1.0
Indianapolis renin unit injected a few minutes
before into a nephrectomized rat. Such a de-
gree of sensitivity is considered high enough to
show that the renal pressor system does not
operate as the mechanism through which both
phases of desoxycorticosterone hypertension
are either induced or maintained.

The results here presented complete observa-
tions derived from kidney-grafting experi-
ments in which it was shown that the grafting of
desoxycorticosterone-hypertensive kidneys
was not followed by blood pressure increases
of receptor rats. They are also in keeping with
the observations of Söve and Wakerlin and
of Haynes, Forsham and Hume who verified
that administration of desoxycorticosterone to
dogs did not increase their plasma renin
concentration. Our results seem to be even more
significant because desoxycorticosterone-hyper-
tensive disease in rats is definitely more in-
tense than that in dogs and because we also
included the metacorticoid phase of hyper-
tension.
This is not the first time in which a sensitive method of renin assay has been used to study the rôle played by the renin-hypertensinogen-hypertensin system in particular types of experimental hypertension. Even recently Braun-Meneadez, Covian and Rapela,17 employing the vasoconstriction produced by hypertensin in the toad vascular preparation, showed that the hypertension produced by unilateral perinephritis is not due to an increased amount of circulating renin. Of course, as far as desoxycorticosterone hypertension is concerned, one might also adopt the opposing view that the guinea-pig ileum is not sensitive enough and that better methods of renin assay would have to be devised before participation of the renal pressor system can be disregarded. It would probably, however, be more fruitful to search for other mechanisms to explain desoxycorticosterone and metacorticoid hypertension.

The amounts of renin set free by the ischemic kidney following re-establishment of the circulation were large. Taquini and Braun-Meneadez18 calculated that the amount of renin liberated by 70 Gm. of dog's kidney was equivalent to 840 Buenos Aires units of hypertensin, which corresponds to approximately 3300 Indianapolis units. In the present experiments, the observed average of 13.8 renin units unloaded per 0.87 Gm. of rat kidney mass would indicate that 1110 renin units would be discharged from 70 Gm. of rat kidney. If one takes the particular case in which 24 renin units were set free, one would reach a total amount of renin which would be comparable to the enormous quantities which were released from dog's kidney.

SUMMARY

Assay of the systemic blood of desoxycorticosterone-hypertensive rats and rats with metacorticoid hypertension demonstrated that renin concentration is no greater than in control animals. It was concluded that the renal pressor system does not participate in either phase of desoxycorticosterone hypertension.

Re-establishment of the circulation through totally ischemic rat's kidneys is followed by release of renin. As an average, 0.87 Gm. of rat kidney, totally ischemic for five to seven hours, discharged 13.8 Indianapolis units of renin. This amount is comparable to that set free from ischemic dog's kidney if one takes into consideration the renal mass.

ACKNOWLEDGMENT

We are especially indebted to Dr. Florencio Russo, from Laboroté rá picas S. A., São Paulo, without whose cooperation the hypertensin and renin preparations used would have been very difficult to obtain.

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