THE CLEARANCE OF VASOPRESSIN FROM THE SPLANCHNIC VASCULAR AREA AND THE KIDNEYS

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SUMMARY

The recoveries from portal blood of Pitressin and Azovan blue injected into the superior mesenteric artery in neurohypophysectomized rats were determined. The ratio of the recoveries of Pitressin and Azovan blue was 1.00 ± 0.15. In rats with intact neurohypophyses and anaesthetized with urethane, the concentration of antidiuretic hormone in arterial blood was not different from that in portal blood. It is therefore concluded that vasopressin and endogenous antidiuretic hormone are not cleared rapidly from the circulation in the intestines. The pressor responses to 20 mu. Pitressin given intraperitoneally were smaller than those obtained with 10 mu. given intravenously, suggesting rapid clearance of Pitressin from the circulation in the liver.

A technique is described for determining the renal clearance of test substances infused intravenously into conscious rats followed by sampling of the arterial blood. In six rats the urinary clearance of Pitressin was 1.24 ± 0.06 times the clearance of inulin. In two rats in which urine flow was unusually low, clearance of Pitressin was respectively 0.161 and 0.325 times the inulin clearance. It is calculated that the rate of clearance of Pitressin from the circulation in conscious rats corresponds to a half-life of 42 sec.

The fate of vasopressin in the body is determined, in the first place, by rapid clearance from the circulation involving the kidneys and organs in the splanchnic vascular area [Ginsburg & Heller, 1953a; Crawford & Pinkham, 1954; Dicker, 1954]. With regard to the splanchnic vascular area; while it is known that both liver and intestinal preparations can inactivate vasopressin in vitro [Larsen, 1938; Christlieb, 1940; Dicker & Greenbaum, 1956], it has not been possible to specify whether vasopressin is removed from the circulation in the intestine or in the liver of the living animal. In the present work an attempt has been made to determine the relative contributions by the liver and intestines towards the clearance of vasopressin in the splanchnic vascular area.

The rapidity with which injected vasopressin is excreted in the urine has been noted by Heller [1937], who suggested that a process of renal tubular secretion might be involved. This suggestion has been further investigated in conscious rats by determining the renal clearance of vasopressin.
MATERIALS AND METHODS

Male albino rats (weight 200–350 g) were used in all experiments.

Perfusion of intestinal vessels. The rats were anaesthetized with urethane (2.0 g/kg subcutaneously) or pentobarbitone sodium (65 mg/kg intraperitoneally). An external jugular vein and a common carotid artery were cannulated. The abdominal aorta was tied below the renal arteries; the renal vessels were then ligated and both kidneys removed. A polythene cannula was placed in the aorta at the level of the left renal artery with the tip immediately below the origin of the superior mesenteric artery. Most (but not all) of the material injected through this cannula was driven by the arterial pressure into the superior mesenteric artery, provided that the arterial pressure exceeded 90 mm Hg and the rate of injection <0.2 ml./10 sec. The portal vein was cannulated close to the liver; immediately thereafter heparin (100 i.u./100 g i.v.) was given and the cannula in the portal vein was connected to that in the jugular vein. The portal outflow was thus carried to the jugular vein and blood which had accumulated in the intestine during cannulation of the portal vein cleared rapidly. To collect samples of portal blood, the cannulae in the portal and jugular vein were detached from each other and the portal outflow was allowed to run into tubes moving in a rotating sample collector. To avoid a reduction of blood volume and blood pressure changes during the collection of portal blood, the cannulated carotid artery was connected to a reservoir of heparinized rat blood in a Delorme [1951] compensator at a pressure of 100 mm Hg.

Intraportal injections. Following the technique described by Johnson [1954], a no. 17 serum needle was placed in the portal vein in rats anaesthetized with urethane, permitting intraportal injection, and measurements of portal pressure were made without obstructing the flow of blood.

Determination of renal clearance. In previous determinations of renal clearance in rats it has not been possible to fulfil certain experimental conditions as in other animals. Thus, test substances have been given by subcutaneous injection and it has been necessary to assume (on the basis of the experiments of Friedman, Polley & Friedman [1947]) that as a result of, for example, the slow absorption of inulin, there would be a period following the injection during which the concentration of inulin in plasma was more or less constant. Washing-out the bladder at the beginning and end of the clearance period has been omitted. Again, blood has been obtained from the tail or by heart puncture, which may affect the animal and often does not yield satisfactory blood samples [Lippman, 1948]; alternatively, blood has been taken from anaesthetized animals only at the end of the clearance period when the animal was killed.

In the present study such disadvantages have been avoided by the use of conscious rats in which, under ether anaesthesia, polythene cannulae had been placed in one common carotid artery, an external jugular vein and in the bladder on the day preceding the experiment.

The cannulae in the carotid artery and the jugular vein were sealed, the arterial cannula being filled with a heparin solution (100 i.u./ml.). They were then exteriorized through the skin at the back of the neck. The bladder cannula (about 2 cm long) was tied in such a way that the bladder dead-space was minimal. The cannula was then
brought through the abdominal wall and the skin incision closed by purse-string sutures around the cannula. After the operation, food was withheld from, but free access to water was permitted.

The experimental procedure was started 18 hr later on conscious animals as follows:

0 hr 00 min: 5 ml. water/100 g body weight, orally. 1 ml. 5% inulin/100 g body weight, s.c.
1 hr 00 min: Start of inulin infusion: 5 mg/ml. at rate of 15–20 mg/100 g/hr, i.v.
1 hr 50 min: Heparin 100 i.u./100 g, i.v.
1 hr 58 min: 1st blood sample withdrawn from carotid artery. Cannula opened and blood collected in a syringe after displacing heparin in cannula by blood; 0-7–1-0 ml. of blood was taken and the cannula was refilled with heparin solution and sealed.
2 hr 00 min: Narrow-bore polythene tubing placed in the bladder via the bladder cannula and bladder washed out 3 times through it with 0-5 ml. warm 0-9% NaCl solution.
2 hr 28 min: 2nd blood sample withdrawn from carotid artery.
2 hr 30 min: Bladder washed out and washings added to urine secreted since 2 hr 00 min.

Pitressin, when used, was given by intravenous infusion, starting at 1 hr 00 min.

_Biological assay and chemical methods._ The method of Ginsburg & Heller [1953b] was used for antidiuretic assays. Azovan blue was estimated in a photo-electric absorptiometer and inulin was estimated by the method of Schreiner [1950].

**RESULTS**

*Intestinal and hepatic clearance of Pitressin*

The ultimate intention in these experiments was to compare the recoveries in portal blood after injection into the superior mesenteric artery of Pitressin and of Azovan blue, a dye which does not easily pass out of the circulation.

In the first experiments the rate at which Azovan blue appeared in the portal blood after injection into the superior mesenteric artery was determined. The dye (600 µg in 0-2 ml.) was injected into the cannulated aorta in 20 sec. Portal vein blood was collected simultaneously with the beginning of the injection. Nine or ten samples of portal blood were taken, each collected over a period of 9 sec. The rate of blood flow from the portal vein varied between 0-1 and 0-2 ml./100 g/sec and usually did not fall during the experiment.

Azovan blue appeared first in the portal blood collected 9–18 sec after the beginning of the injection and the concentration increased to a maximum after 18–27 sec (Fig. 1a). Up to 81 sec after the injection the total recovery of dye from the portal vein was only 45–60% of the dose. At that time (81 sec) the concentration of the dye found in arterial blood indicated that almost 25% of the injection escaped into the systemic circulation without passing into the portal vein. The concentration of Azovan blue in the portal outflow in the last sample collected was higher than that in arterial blood, showing that not all the dye which had entered the superior mesenteric
artery had passed into the portal vein by that time. Fig. 1B and C shows the recoveries of Azovan blue in portal blood after the injection of the dye, together with 1-0 and 3-0 mu. Pitressin. In both cases, the first appearance of the dye in portal blood was delayed until 18–27 sec after injection. With the lower dose of Pitressin the concentration of dye reached a maximum 63–72 sec after injection (compared with 18–27 sec in the absence of Pitressin), and with 3-0 mu. the maximum had not been reached 90 sec after injection. When the dye was injected with Pitressin the total amounts of Azovan blue found in portal blood were between 20 and 25 % of the dose. The concentration of dye in the arterial blood indicated that up to 50 % of the dye had passed into the general circulation, but even 90 sec after injection the concentration of the dye in portal blood was 3–4 times greater than in arterial blood, showing that some dye was still trapped in the bed of the superior mesenteric artery.

Fig. 1. Recovery of Azovan blue in portal blood in rats after injection into the superior mesenteric artery via the cannulated aorta (see Methods). A, injection of 600 μg Azovan blue in 0-2 ml. 0-9 % NaCl; B, injection of 600 μg Azovan blue + 1-0 mu. Pitressin in 0-2 ml. 0-9 % NaCl; C, injection of 600 μg Azovan blue + 3-0 mu. Pitressin in 0-2 ml. 0-9 % NaCl.

The results described above showed that after 3-0 mu. Pitressin the injected dye appeared slowly in the portal blood and was thus distributed in a relatively large volume of blood. On the other hand, the concentration of endogenous antidiuretic activity in portal blood under the conditions of these experiments was 0-3–1-0 mu./ml. blood, and hence >3-0 mu. Pitressin might be needed to produce a significant increase in the antidiuretic activity of portal blood. To overcome these difficulties rats which had been neurohypophysectomized 4–7 days previously were used. In five animals in which no neurohypophysial tissue was present at autopsy, no antidiuretic activity could be detected in portal blood (< 0-04 mu./ml.). In two animals in which fragments of neurohypophysis were found, the activities of portal blood were 0-09 and 0-30 mu./ml. Fig. 2 shows the results of an experiment on a neurohypophysectomized rat. 1-0 mu. Pitressin and 600 μg Azovan blue in 0-3 ml. were
injected into the cannulated aorta in 30 sec. The dye appeared in the portal blood 18–36 sec after the injection started, the greatest amounts of dye being found in those samples collected 36–72 and 72–108 sec after injection, containing 13·9 and 11·0 % of the injected dye respectively. The antidiuretic activities of these samples were 123 and 88 mu. respectively, representing 12·3 and 8·8 % of the amount injected. Thus, in this experiment the amounts of Pitressin recovered in portal blood were 88 and 80 % of the recoveries of Azovan blue.

In similar experiments on five neurohypophysectomized rats the ratio of Pitressin recoveries to those of Azovan blue was 1·00 ± 0·15 (mean and s.e. of eight observations). This result indicates that the uptake of Pitressin from the circulation in the bed of the superior mesenteric artery was not greater than that of Azovan blue.

**Fig. 2.** Recoveries of Azovan blue and Pitressin in portal blood in a neurohypophysectomized rat after injection of 600 μg Azovan blue + 1·0 mu. Pitressin in 0·2 ml. 0·9 % NaCl into the superior mesenteric artery via the cannulated aorta (see Methods). □ Azovan blue; ■ Pitressin.

**Fate of endogenous antidiuretic hormone in the intestinal circulation**

Endogenous antidiuretic activity in portal blood of rats prepared for the experiments described in the previous section was found only in animals with neurohypophysial tissue, indicating that the activity is attributable to the antidiuretic hormone and not to some substance secreted into the blood during its circulation in the intestine. Thus, less antidiuretic activity in blood drawn from the portal vein than in blood drawn simultaneously from an artery would indicate clearance of the endogenous hormone in the intestine. Table 1 shows the results of determinations of antidiuretic activity in portal and arterial blood. The activity in portal blood is clearly not less than in arterial blood.

**Pressor effects of intraportal injection of Pitressin**

The clearance of Pitressin from the circulation by the liver was investigated by the comparison of pressor effects following intraportal and intravenous injection of Pitressin. The results of a typical experiment are shown in Fig. 3. The pressor effects of 10 or 20 mu. Pitressin given intraportally were considerably less than the response
to 10 μu. given intravenously. Portal pressure was not affected by the intraportal injection; the difference between the effects of intraportal and intravenous injection was not due therefore to intrahepatic vasoconstriction delaying the distribution of Pitressin to other parts of the cardiovascular system. Thus under the conditions of the experiments (the blood pressure and the portal pressure were low) substantially more than half of the Pitressin injected into the portal vein was cleared from the blood in a single circulation through the liver.

Table 1. Antidiuretic hormone content in arterial and portal blood in rats anaesthetized with pentobarbitone

<table>
<thead>
<tr>
<th>No.</th>
<th>Arterial blood</th>
<th>Portal blood</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>0.30</td>
<td>0.32</td>
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<tr>
<td>3</td>
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<tr>
<td>4</td>
<td>0.09*</td>
<td>0.13</td>
</tr>
<tr>
<td>5</td>
<td>&lt;0.04†</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>

* Incomplete neurohypophysectomy.
† Complete neurohypophysectomy.

Fig. 3. Pressor effects of Pitressin injected into a femoral vein and the portal vein in a rat anaesthetized with urethane.

Renal clearance of Pitressin

Inulin clearance determinations, using constant intravenous infusion of inulin and arterial blood sampling as described above (see Methods), gave a mean inulin clearance of 0.81 ± 0.07 ml. plasma/100 g/min (in eight normal unanaesthetized rats). This was in good agreement with experiments using subcutaneous injection of inulin and single blood samples taken under ether anaesthesia at the end of the clearance period following the method of Dicker & Heller [1945], which gave a mean inulin clearance of 0.83 ± 0.11 ml./100 g/min (seven rats).

Table 2 shows the results obtained from simultaneous determinations of the renal clearance of Pitressin and inulin by the constant intravenous infusion technique. The rats received infusions of Pitressin at rates varying between 3–0 and 5–9 μu./100 g/min and the clearance period started 1 hr after the infusion of Pitressin was begun. The usual pattern of change in urine flow during Pitressin infusion was an initial inhibition of diuresis lasting 20–30 min, followed by a recovery of urine flow. In all but two of the rats used, urine flow was 0.020–0.047 ml./100 g/min during the clearance period;
in the two exceptions, inhibition of diuresis was prolonged and urine flow was only $<0.008 \text{ ml./100 g/min}$ during the clearance period.

The inulin clearance was depressed by Pitressin infusion, the mean clearance being reduced from $0.81 \pm 0.07 \text{ ml./100 g/min}$ in eight control animals to $0.49 \pm 0.05 \text{ ml./100 g/min}$ in eight animals receiving Pitressin. In the two rats in which diuresis was inhibited during the whole period of the Pitressin infusion, the inulin clearances were within the range of values found for the other rats given similar treatment.

In applying renal clearance techniques to substances known to be destroyed in the kidneys, slight modification of the conventional terms used is necessary. In such cases the ‘true renal clearance’, which is the volume of plasma cleared of the substance in unit time by the kidneys, must be greater than the ‘urinary clearance’ ($C_U$) given by $UV/P$ (i.e. the amount of the substance excreted in urine per min divided by the concentration of the substance in plasma).

### Table 2. The clearance of Pitressin and inulin in conscious rats

<table>
<thead>
<tr>
<th>No.</th>
<th>Weight (g)</th>
<th>Rate of Pitressin infusion (ml./100 g/min)</th>
<th>Urine flow (ml./100 g/min)</th>
<th>Urinary clearance of inulin (ml./100 g/min)</th>
<th>Urinary clearance of Pitressin (ml./100 g/min)</th>
<th>$C_PIT^*$</th>
<th>Pitressin concentration in plasma (µ/ml.)</th>
<th>$C_TOT^*$</th>
<th>Pitressin/Cv (ml./100 g/min)</th>
<th>Urinary excretion of Pitressin (% of infusion rate)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>209</td>
<td>$3.0$</td>
<td>$25$</td>
<td>$0.51$</td>
<td>$0.53$</td>
<td>$1.04$</td>
<td>$0.63$</td>
<td>$4.8$</td>
<td>$11.7$</td>
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<tr>
<td>2</td>
<td>240</td>
<td>$3.4$</td>
<td>$24$</td>
<td>$0.56$</td>
<td>$0.75$</td>
<td>$1.34$</td>
<td>$0.90$</td>
<td>$4.25$</td>
<td>$16.0$</td>
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</tr>
<tr>
<td>3</td>
<td>300</td>
<td>$3.3$</td>
<td>$20$</td>
<td>$0.57$</td>
<td>$0.69$</td>
<td>$1.20$</td>
<td>$1.8$</td>
<td>$1.8$</td>
<td>$37.8$</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>170</td>
<td>$5.9$</td>
<td>$23$</td>
<td>$0.30$</td>
<td>$0.43$</td>
<td>$1.47$</td>
<td>$3.2$</td>
<td>$1.9$</td>
<td>$23.3$</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>340</td>
<td>$4.1$</td>
<td>$47$</td>
<td>$0.74$</td>
<td>$0.86$</td>
<td>$1.16$</td>
<td>$1.0$</td>
<td>$4.1$</td>
<td>$21.3$</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>320</td>
<td>$4.3$</td>
<td>$24$</td>
<td>$0.41$</td>
<td>$0.60$</td>
<td>$1.32$</td>
<td>$1.2$</td>
<td>$3.6$</td>
<td>$14.9$</td>
<td></td>
</tr>
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<td>7</td>
<td>260</td>
<td>$3.4$</td>
<td>$&lt;8$</td>
<td>$0.43$</td>
<td>$0.14$</td>
<td>$0.32$</td>
<td>$4.6$</td>
<td>$0.74$</td>
<td>$8.9$</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>290</td>
<td>$3.2$</td>
<td>$&lt;8$</td>
<td>$0.32$</td>
<td>$0.07$</td>
<td>$0.16$</td>
<td>$3.2$</td>
<td>$1.0$</td>
<td>$15.1$</td>
<td></td>
</tr>
</tbody>
</table>

* $C_PIT$ = urinary clearance of Pitressin; $C_{IV}$ = urinary clearance of inulin; $C_{TOT}$ = total plasma clearance.

The mean urinary clearance of Pitressin in those animals with relatively high urine flows was $0.63 \pm 0.07 \text{ ml./100 g/min}$. In all cases this value was greater than the inulin clearance, the mean ratio $C_U$ Pitressin/$C_U$ inulin being $1.24 \pm 0.06$ (six rats). The results obtained in the two rats with low urine flows are quite different and must be treated separately. In these animals the urinary clearances of Pitressin were $0.07$ and $0.14 \text{ ml./100 g/min}$, and the ratios $C_U$ Pitressin/$C_U$ inulin were $0.161$ and $0.325$. However, the rate of Pitressin excretion in the urine, when expressed as a percentage of the rate of Pitressin infusion, was not less in these animals than in the animals with high urine flows in which $C_U$ Pitressin/$C_U$ inulin was $>1.0$.

During clearance periods the concentration of Pitressin in the plasma had reached a steady level; the difference between the two plasma samples was never greater than the error of the assay method. Under equilibrium conditions, the disappearance of Pitressin from the plasma must equal the rate at which it is infused into the animal.

$$C_{TOT} = R_{inf} \times P,$$

where $C_{TOT}$ is the total clearance of Pitressin from the circulation in ml. plasma/100 g/min, $R_{inf} = \text{rate of infusion of Pitressin (µ/ml./100 g/min)}$, $P = \text{concentration of Pitressin in plasma µ/mL}$. $C_{TOT}$ for Pitressin was less in the two animals with low urine flows than in those with high urine flows. In the latter group, the mean $C_{TOT}$ was $3.4 \pm 0.5 \text{ ml./100 g/min}$. This is in excellent agreement with previous work [Ginsburg & Heller, 1953].
in which the estimate of $C_{TOT}$ by analysis of the exponential disappearance of Pitressin from the circulation in anaesthetized rats was 2.7 ml./100 g/min. Since Pitressin given in a single injection disappears from the circulation exponentially, the half-life of Pitressin in the circulation can be calculated from $C_{TOT}$; in un-anaesthetized rats it is 42 sec, compared with 51 sec in rats anaesthetized with ether [Ginsburg & Heller, 1953a].

**DISCUSSION**

The results described in this paper raise the question whether Pitressin is excreted by glomerular filtration or by tubular secretion. From previous work [Ginsburg & Heller, 1953a; Crawford & Pinkham, 1954] it has been deduced that, in a single circulation, the kidneys in normal rats extract nearly all the Pitressin from the blood which passes through them and that only a portion of the Pitressin taken up by the kidneys is excreted in the urine. Inactivation or destruction could account for the difference between the amount of Pitressin taken up by the kidneys from the circulation and that appearing in the urine. Pitressin is rapidly inactivated by kidney preparations *in vitro*, and Heller & Zaidi [1957] have shown that in animals killed only 3 min after intravenous injection of Pitressin, no activity could be detected in the renal tissues, although substantial amounts were found in the bladder, ureters and kidney dead space. It has been shown that the capacity of tubular cells to inactivate Pitressin is greater than that of glomerular cells [Heller & Zaidi, 1957], and it may be suggested that Pitressin is rapidly taken up by tubular cells and at least partly destroyed there. However, the question remains whether Pitressin excreted in urine is filtered, thus avoiding inactivation in the tubular cells, or is secreted into the tubules before inactivation is complete. Unfortunately, the determinations of renal clearance described in this paper do not permit an unequivocal answer. The animals seem to fall into two distinct groups. In rats with high urine flow rates, the urinary clearance of Pitressin was slightly greater than the inulin clearance. This difference, though significant and found in every animal in that group, is too small to allow the assertion that Pitressin was secreted. From the observations that Pitressin is partially bound by plasma proteins [Heller, 1937; Heller & Lederis, 1957] it is, however, unlikely that it could be filtered by the glomeruli as freely as is inulin. In the two animals with low urinary clearances of Pitressin, the inulin clearances were not different from those in the larger group, but total clearance of Pitressin was low, that is to say the low urinary clearance of Pitressin was associated with a general impairment of the ability to remove Pitressin from the circulation and was not related to changes in glomerular filtration rate. Thus, the evidence supports, but does not prove, the view that urinary excretion of Pitressin is by tubular secretion. In terms of the quantities of antidiuretic hormone which may be expected to occur in the blood of a normal animal, the doses of Pitressin used were very high. Although the biggest doses infused were approximately equivalent to only 10-0 ng/100 g/min of active peptide, those doses are nevertheless so great on a physiological scale that it is possible that the kidneys were presented with amounts of Pitressin beyond their secretory capacity. Technical limitations, such as the volume of blood which can be taken from an animal and the sensitivity of antidiuretic assays, have prevented the use of smaller doses of Pitressin.
For reasons given below it may be assumed that the animals with low urinary clearance of Pitressin were abnormal. Within the normal group, the urinary clearance of Pitressin was correlated with the inulin clearances and the ratio of these values is remarkably constant. By contrast, the percentage of the dose of Pitressin which was excreted in urine and total clearances of Pitressin vary over a wide range. The variability of the percentage excretion, with urinary clearance remaining constant, seems contradictory but may be explained by the inverse correlation between percentage excretion and $C_{TOT}$ ($r = -0.80$, $t = 2.76$; $t = 2.77$ at $P = 0.05$). Thus, when the disappearance of Pitressin from the circulation is relatively slow and the concentration in plasma therefore tends to be high, the percentage of the dose excreted in urine is great without alteration of the urinary clearance. From this it can be concluded that the proportion of the dose of Pitressin infused which is excreted in urine is not necessarily related to renal function but could be dependent upon the clearance of Pitressin by other organs, principally the liver. These considerations apply also to the two ‘abnormal’ rats: although clearance of Pitressin was very low in them, the percentage of the dose excreted was not greatly different from that in the normal animals, because the concentration of Pitressin in blood was exceptionally high.

The amounts of Pitressin used were sufficient to increase the blood pressure of unanaesthetized rats by 40–50 mm Hg and to maintain it at that level. The reduction of the glomerular filtration rate observed would be expected as a result of the vascular effects, but the restoration of urine flow during constant infusion of Pitressin was surprising. The two animals in which antidiuresis was sustained and the urinary clearances of Pitressin only one-third to one-tenth of those in the normal group did not appear to be in discomfort during the experiments and were as active and alert as the other animals. In the course of other experiments similar amounts of Pitressin have been infused into eighteen rats and in all cases the antidiuresis was not maintained throughout the period of the infusion. The prolonged antidiuresis and low urinary clearance of Pitressin in the two ‘abnormal’ rats may have been due to a degree of adrenocortical insufficiency [Lockett, 1952; Ginsburg, 1954].

The diminished pressor effect of Pitressin when given by intraportal injection agrees with the observations of Eversole, Birnie & Gaunt [1949] and Møller-Christensen [1951] who have shown that after intrasplenic injection of vasopressin in rats and rabbits, antidiuretic and vasopressor responses were diminished. The conclusions of Eversole et al. and Møller-Christensen that their findings indicated clearance of vasopressin by the liver could be criticized on the grounds that, although material injected into the spleen enters the portal vein very rapidly [Milnes Walker, Middlemiss & Nanson, 1953], there may be some inactivation of vasopressin in the spleen itself [Jones & Schlapp, 1936; Dicker & Greenbaum, 1956].

The experiments on the extraction of Pitressin from the circulation in the splanchnic vascular area were not entirely satisfactory because a sizeable proportion of the injection found its way into the general circulation; it is possible that during injection the column of dye solution in the aorta extended as far as the coeliac artery and hence a proportion of the amount injected could have by-passed the portal circulation. The amounts of Azovan blue diverted into the systemic circulation were greater when it was given with Pitressin. This might have been a result of the vasoconstrictor action of Pitressin on the vessels in the bed of the superior mesenteric artery since as little
as 1 mu. Pitressin caused a very pronounced delay in the appearance of Azovan blue in the portal blood.

The results show clearly that the extraction of Pitressin from the circulation in the bed of the superior mesenteric artery is not significantly greater than that of Azovan blue. This and the finding of diminished pressor responses to Pitressin when given intraportally suggest that the rapid clearance of Pitressin in the splanchnic vascular area is attributable to the liver and not the intestine. There was no difference between the antidiuretic activities in portal and arterial blood in animals with intact neurohypophyses, suggesting a similar fate for the endogenous antidiuretic hormone. The inactivation of vasopressin by intestinal tissue in vitro is as great as that by liver tissue [Larson, 1938; Christlieb, 1940; Dicker & Greenbaum, 1956], but evidently the significance of these observations depends upon the efficient clearance during life of the substance from the circulation by the organ in question.

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