Observations on the Vasodilator Properties of Urine

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III. Comparison of Vasodilator Activity in the Urine of Normal Individuals and Patients with Orthostatic Hypotension

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These experiments trace the recent interest in the vasodilator properties of urine. This interest was triggered by the incidental observation of a potent coronary dilator influence when urine was introduced into the reservoir of the isolated supported heart preparation. A comparison of human urine with nitroglycerin then revealed that 10 ml. of urine had 2 to 3 times the dilator potency of 0.6 mg. nitroglycerin. Neither agent altered myocardial oxygen consumption or efficiency at the peak of their effect.

The development of a simple, rapid and reproducible bioassay technic which would permit the comparative examination of numerous samples in the same preparation was a necessary precursor to realizing the broader objective of ascertaining whether the vasodilator substance in urine has physiologic significance for circulatory regulation. Accordingly, a re-examination was made of the limitations of the femoral arterial flow technic. The irregularity in the amplitude and contour of sequential responses to the same test substance in spite of an apparently steady hemodynamic state suggested the possibility of inadequate mixing after injections into the external circuit. Model studies with blue dye confirmed this impression and a magnetic mixer was therefore introduced into the external circuit in order to thoroughly mix the injected material. This resulted in substantially improved reproducibility and the obtaining of satisfactory dose-response curves.

This technic was then used to assay the 24 hour output of vasodilator activity in the urine of 5 hypotensive patients and 5 normal individuals. The hypotensive group exhibited something less than 15 per cent of that secreted by the control group. The substance under examination is non-dialyzable and, chemically, resembles callicrein. The urines from 9 mammalian species all exhibited high activity. These observations were construed as being compatible with the hypothesis that the vasodilator agent under examination is, in some way, associated with autonomic function and/or the regulation of arterial pressure. This view was supported by the observation of a diminished vasodilator output in the sheep while under the influence of ganglionic blockade with hexamethonium.

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I. Comparison of Effect of Human Urine and Nitroglycerin on Coronary Resistance and Myocardial Oxygen Consumption in the Isolated Supported Heart Preparation

The aims of these experiments were (a) to ascertain the consistency with which direct coronary vasodilator activity is present in human urine, (b) to compare the direct coronary vasodilator effect of urine with that of nitroglycerin, and (c) to ascertain whether either nitroglycerin or urine increases myocardial oxygen consumption or decreases myocardial efficiency in the canine heart.1 Previous experience with those hemodynamic parameters which influence myocardial oxygen consumption2 and the availability of a preparation in which these parameters could be independently controlled3 made possible the design of experiments in such a manner as to exclude indirect effects on the coronary vascular bed resulting from changing myocardial oxygen requirements.

METHODS

The isolated supported heart preparation was used in these studies. In it, central nervous system influences are eliminated by the isolation procedure, but the metabolic environment of the heart under study is kept normal or almost so by circulating the metered, total coronary venous flow through another dog before returning it to the reservoir of the isolated heart circuit.4 Aortic pressure, cardiac output and heart rate are independently controlled.5 The volume of the reservoir-heart system into which the urine or nitroglycerin is introduced is between 1,750 and 2,000 ml. The concentration of the introduced agent diminishes with time since blood leaves the reservoir-heart system through the coronary vascular bed and is continuously diluted with arterial blood from the support dog. Other things being equal (rate of destruction, etc.), the greater the coronary venous blood flow from the support dog, the more rapid will be the decline of the concentration of the substance introduced into the reservoir. In some experiments arterial and coronary venous blood were continuously metered by densitometers6,7 which were calibrated with 5 to 10 sets of duplicate gasometric determinations.8 Myocardial oxygen consumption was calculated from coronary flow and the arteriovenous oxygen difference. Left ventricular minute work in kilogram meters was calculated as the ratio of work in kilogram meters to the product of oxygen consumption in milliliters per minute and 2.06.7 Coronary vascular resistance was calculated as the ratio of mean aortic pressure in milliliters of mercury to the total coronary flow in milliliters per minute. Pressures were recorded with strain gages, aortic flow with a Potter electroturbimeter,5 coronary flow with a rotameter7 and heart rate with a Waters cardiotachometer. Heart rate was frequently but not always controlled with right atrial stimulation. All values were continuously recorded. The isolated supported heart preparation, its performance characteristics and stability are described in detail elsewhere.9

RESULTS

Twenty-six voided urine specimens from nine adult persons (including 1 female) and 1 specimen of canine urine were introduced into the reservoir of 8 isolated supported heart preparations in amounts varying from 2 to 20 ml. A rise in coronary flow was always observed although the response varied considerably and was slight after the smaller amounts. The twelve 10 ml. injections were followed by peak increments in flow above control of 32 to 210 per cent with an average of 95 per cent.

FIG. 1. Dose-response relationship of urine in the coronary vascular bed of the isolated supported heart preparation. Dog weighed 21.4 Kg.; total heart weight 188 Gm.; combined ventricle weight 146 Gm.; left ventricle weight 100 Gm. Blood volume of isolated heart-reservoir system 1,800 ml. ± 50 ml. Aortic pressure remained at 133 systolic, 69 diastolic and mean 97 mm. Hg ± 2 mm. Hg throughout all runs. Cardiac output remained at 2,100 ± 100 ml., and heart rate was controlled at 165/min. throughout all runs. Urine was injected at time "2 min." in each instance. Solid dots, response after a 5 ml. injection into the blood reservoir; open circles, response after 10 ml., triangles, response after 20 ml.
Figure 2. Tracings from an isolated supported heart preparation. Dog weighed 33 Kg., heart 352 Gm. (greyhound), right and left ventricles combined weighed 303.5 Gm., and left ventricle 229 Gm. The blood volume of the isolated heart-reservoir system was 1,950 ± 60 ml. (Top to bottom) mean left atrial pressure, aortic pressure (full pulse pressure alternating with mean pressure), aortic flow (cardiac output minus coronary flow) and total coronary flow. Ten milliliters of urine were introduced into the reservoir of the isolated heart system at arrow. Heart rate controlled at 162/min. throughout. One minute time indicator is at the right in the fourth channel.

**Figure 3** shows the results of an experiment in which cardiac output was elevated and aortic resistance lowered so as to produce a low diastolic pressure. The effect of 0.6 mg. of nitroglycerin in 10 ml. of saline was then compared with the effect of 10 ml. of human urine on coronary flow, arteriovenous oxygen difference, left atrial pressure and myocardial efficiency. Aortic pressure, heart rate and cardiac output were held constant throughout both procedures. The mean level of coronary flow above baseline in the first 10 min. after the injection of urine was 2.7 times as great as with the nitroglycerin (fig. 3). The difference in the effect of the 2 agents was actually somewhat greater than that shown in figure 3 since the increase in coronary flow was greater with the urine and hence the rate of reservoir replacement and agent dilution proceeded at a greater rate in the 10 min. observation period after the urine injection than was the case after the injection of nitro-
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glycerin. Alternating the sequence of the administration of these agents did not influence the results. The mean increase of coronary flow above the control level in the first 10 min. after the injection of 10 ml. of urine was an average of 2.3 times greater than that observed after 0.6 mg. of nitroglycerin in the 5 such experiments in which this comparison was made. This figure must, however, be regarded only as a qualitative comparison since no attempt was made to adjust urine concentration on the basis of the relative 24-hour volumes.

In the 4 experiments in which gasometric determinations were done, coronary venous oxygen saturation rose and the arteriovenous oxygen difference narrowed after the administration of both agents but changes in either myocardial oxygen consumption or cardiac efficiency were not observed at the peak of the response after the use of either agent as shown in figure 3.

DISCUSSION

The coronary vasodilator influence of the nitrates has, of course, long been known although the rationale of their use in angina pectoris was not originally predicated on this aspect of their action. However, data concerning the effects of this agent on coronary flow while controlling aortic pressure, cardiac output and heart rate were not apparent in previous studies; indirect effects on coronary vascular resistance because of changing myocardial oxygen requirements could thus not be excluded. Moreover, from the above data, it would appear that when this agent is administered under conditions wherein it is possible to exclude such acute changes, as was the case in these studies, no change takes place in the myocardial oxygen consumption or myocardial efficiency in the isolated dog heart. These findings apparently differ from the results of studies utilizing the nitrous oxide technic in man.

In regard to the coronary vasodilator action of urine, it is clear from the above that similar considerations apply, namely, that the effect is a direct one of the agent on the coronary vascular bed and it not secondary either to changes in hemodynamically engendered increases in myocardial oxygen requirements or to the type of hypoxating effect induced by sodium cyanide, since no evidence of failure was apparent even after repeated and closely spaced injections. On the contrary, left atrial pressure frequently fell slightly (fig. 2). The accumulation of past and recent evidence from dialysis, precipitation, and column adsorption studies makes it increasingly apparent that the primary significant vasodilator in urine is a callicrein or a substance closely akin to it (see III). In this regard it is noteworthy that callicrein has been demonstrated by Krayer and Ruhl to be a potent coronary vasodilator.
That the dilator effect of human urine is not restricted to the coronary bed is suggested from the early observations by Abelous and Bardier and the observations by Wollheim of the hypotensive response after its intravenous administration. That an actual fall in peripheral resistance is induced by human urine was established by the experiments of Green et al.

**SUMMARY**

The direct dilator effect of nitroglycerin and human urine was observed in the isolated supported heart preparation. Ten milliliters of human urine was observed to have an effect at least comparable to that of 0.6 mg. of nitroglycerin. Neither agent caused a change in myocardial oxygen consumption or efficiency under the experimental conditions studied.

**II. Problem of Reproducibility in Blood Flow Bioassay Techniques for Vasoactive Substances—Studies With Human Urine**

This phase of study was prompted by the need for a simple and rapid vascular bioassay technic which would permit the satisfactory comparison of numerous samples in the same preparation. It was felt that the essential component of a useful bioassay technic is the reproducibility of response when the same biochemical or pharmacologic intervention is repeatedly imposed, as emphasized by Bliss in his excellent recent analysis of the principles of bioassay. In the absence of this, little meaning can be attached to the varying responses obtained. Conversely, the extent to which reproducibility can be achieved with the same intervention will determine the extent to which significance can be attributed to the varying responses following upon varied interventions. Reproducibility will, in turn, depend not only upon the constancy of reactivity of the tissue being studied but will also require that the intervention imposed is brought about in the same manner each time. It was with the emphasis on the latter factor that this phase of the study was carried out.

Although the femoral venous outflow technic of Green et al. was at first considered for use, it appeared to have several disadvantages. Because of the lack of reproducibility, the separate injection of a reference agent (methacholine) was required as a standard along with each test substance injected. With this aspect in mind, a preliminary re-examination of the simpler technic of direct, continuous metering of femoral arterial flow was made and fair reproducibility was sometimes obtained. This, however, was usually not adequate for satisfactory dose-response curves. Since an attempt was made to keep the level of anesthesia and blood pressure constant, as described below, it was suspected that the observed variability in response might be attributable to the inconstancy of the manner in which the biochemical intervention was imposed. That is to say, the irregularity of the contour of the flow tracing after some injections (fig. 7, Middle) suggested inadequate mixing as a possible cause. This section presents the results of attempts to arrive at a solution to these problems.
Fig. 5. Multiple-injection, magnetic mixing cuvette. Blood enters cuvette through stainless steel tube at left (arrow), is agitated by the rotating, plastic covered magnet in the cuvette and exits from the steel tube at the right (arrow). An agent injected through any one of the 10 tuberculin syringes and stopcocks arranged peripherally around the cuvette is thoroughly mixed with the cuvette content. Magnet activator is mounted below. Stopcock on top of cuvette provides for bubble removal. The stopcocks into which each of the 10 tuberculin syringes are inserted have a reduced bore (0.25 mm.) on the outflow limb and their ends are flush with the inside of the mixing cuvette. It is nevertheless necessary to forcefully flush each stopcock with 1 ml. of saline before reloading with the next substance to be tested in order to completely avoid contamination with the previous test substance even though the dead space is only 0.06 ml.

METHODS

Two methods of study were employed. The first included the use of a densitometer* through which saline was made to flow at a constant rate. A known amount of the blue dye, T-1824, was then rapidly introduced into the tubing just proximal to a mixing cuvette which contained a small bar magnet. Repeated observations were made both with and without agitation of the contents of the mixing cuvette by the magnetic stirrer.

The second method employed mongrel dogs weighing 14 to 29 Kg. which were anesthetized with 30 mg./Kg. of sodium pentobarbital given intravenously. Although the sacrum and lower extremities were in the supine position, the thorax, head and upper extremities were placed in the right lateral decubitus position since, in the longer experiments, the dogs appeared to fare better if this was done. A drawing of the preparation is shown in figure 4. A centrally directed cannula in the left femoral artery conducts blood flow through siliconized tygon tubing to a Shipley-Wilson rotameter, then through the cuvette of a multiple injection, magnetic mixer (fig. 5) and finally back to the femoral artery through a cannula directed distally. Femoral arterial pressure was measured with a Statham strain gage distal to the rotameter and proximal to the re-entry point into the femoral artery. Mepesulfate* (1.0 Gm. initially and 0.5 Gm. each hour thereafter)

*Kindly supplied by Hoffmann-La Roche, Inc., Nutley, New Jersey.
was given as anticoagulant. Pressure and flow recording was done on a multi-channel, direct-writing oscillograph. Since experiments frequently lasted for 12 hours or longer, an intravenous infusion of 5 per cent dextrose in saline was administered at the rate of approximately 1 to 1.5 ml./min. After the preparation was completed a continuous infusion of sodium pentobarbital (6 mg./ml.) was administered with a Braun infusion pump at that rate which would just suppress shivering and maintain arterial pressure at a constant level. From experiments on more than 80 dogs it was clear that the long term stability of the preparation was substantially enhanced by close attention to the required rate of administration of the anesthetic agent. The average preparation yielded over 100 individual assays. It was thus possible to obtain reliable comparisons of vasoactive potency within each of the 10 groups of 10 test specimens. Substantial falls in rectal temperature were avoided by the use of a heating pad. Lightly spraying the float and body of the rotameter with Dow-Corning Anti-foam A and then wiping them free of observable droplets usually inhibited the troublesome clumping of microscopically verified platelets on the rotameter float. Plasma hemoglobin levels were determined by the method of Bing and Baker. The volume of the injected test substances was kept low (0.4 ml.) so as to avoid or diminish the Bayliss constrictor responses to sudden vascular distention observed when larger volumes were used.

RESULTS

Model Studies. The results of representative model studies with blue dye are shown in figure 6. The series of 4 sequentially obtained curves at the left, when the cuvette magnet was stationary, show inadequate mixing and poor reproducibility. The 4 sequentially obtained curves at the right, obtained with the magnet in motion, are smoother and more reproducible.

In Vivo Studies. Figure 7 shows 3 sets of curves with 4 curves sequentially obtained at 5 min. intervals in each set. Each curve was the response to the injection of 0.4 ml. of the same specimen of dialyzed human urine. The top set shows the fair reproducibility sometimes obtained without agitation by the mixer; the middle set shows the poor reproducibility sometimes obtained without agitation by the mixer. The bottom set shows the good
Figure 8. Top. Five 0.4 ml. injections of urine from a hypertensive patient diluted (from left to right) 1:8, 1:4, 1:2, 3:4, 1:1 made at 5 min. intervals. Duration of each segment of the tracing, 2.7 min.; interruptions of tracing (white space), time interval; F.A.F., femoral artery flow in milliliters per minute. Bottom. Plot of peak increment of flow in milliliters against dilution. Dog weight, 19 Kg.

reproducibility obtained with agitation by the mixer. Results similar to those shown in figure 7 (Top and Middle) were also obtained when repeated injections were made directly into the tubing without the mixer in the circuit. It was, in fact, such findings in the early experiments which prompted the use of the mixing cuvette.

An example of the type of dose-response relationship obtainable with this preparation is shown in figure 8 Top. Injections of 0.4 ml. of varying dilutions of urine from a hypertensive patient were made at 5 min. intervals. A plot of the peak increment in flow against relative concentration is shown in figure 8 Bottom. The semi-log plot was linear.

The circular manner of construction of the multi-injection, magnetic mixer (fig. 5) was found to confer 2 main advantages. The first, as mentioned above, is that more thorough mixing of the agent to be tested is possible because of the agitation produced in the mixing cuvette. The second is that, at any given baseline flow, there is a constant interval during which the injected agent is in contact with the blood before arrival at the reactive blood vessel site. This would not be the case were the multiple injection manifold arranged linearly.

In three experiments, plasma hemoglobin determinations done in duplicate on carefully drawn blood samples obtained upstream and downstream to the mixer failed to reveal evidence of hemolysis.

It must be firmly emphasized that, in spite of the limited dead space (0.06 ml.) in each of the 10 injection ports of the mixing cuvette, it is necessary to forcefully flush each port with 1 ml. of saline in order to avoid contamination by the previously injected active substance.

SUMMARY

An attempt was made to increase the value and reliability of the femoral artery flow technic as a bioassay method for locally vasoactive substances. Model studies suggested the possibility that inadequate mixing was a limiting factor in previous technics. In vivo studies revealed that the use of a circular, multiple-injection, magnetic mixing cuvette yielded substantially improved reproducibility and provided the means for obtaining more satisfactory dose-response curves.

III. Comparison of Vasodilator Activity in the Urine of Normal Individuals and Patients With Orthostatic Hypotension

This section will deal with the results of attempts to differentiate the amount of vasodilator activity present in the urine of patients with orthostatic hypotension from that present in the urine of normal individuals. These experiments were undertaken in the anticipation that they might throw some light on the possible physiologic significance for circulatory regulation of a potent vasodilator substance present in urine.

METHODS

Five normal volunteer individuals and 4 patients with orthostatic hypotension were simultaneously hospitalized for study. These patients had previously been extensively investigated,* and the details of their individual cases described else-

*The authors are grateful to Drs. H. N. Wagner, E. Braunwald and L. Terry for the availability of background information concerning these patients and the opportunity to study them.
TABLE 1.—Data on Subjects

<table>
<thead>
<tr>
<th>Initials</th>
<th>Legend number</th>
<th>Age (yr.)</th>
<th>Weight (kg)</th>
<th>24-hour urine vol. (ml.)</th>
</tr>
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<tr>
<td>Normal controls</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P.S.</td>
<td>1</td>
<td>27</td>
<td>62.6</td>
<td>2140</td>
</tr>
<tr>
<td>R.D.*</td>
<td>3</td>
<td>22</td>
<td>66.8</td>
<td>1510</td>
</tr>
<tr>
<td>G.G.</td>
<td>5</td>
<td>22</td>
<td>75.0</td>
<td>2045</td>
</tr>
<tr>
<td>B.S.</td>
<td>7</td>
<td>23</td>
<td>76.4</td>
<td>2615</td>
</tr>
<tr>
<td>D.V.</td>
<td>9</td>
<td>19</td>
<td>64.0</td>
<td>1460</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td>22.8</td>
<td>68.9</td>
<td>1954</td>
</tr>
<tr>
<td>Hypotensive patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W.A.</td>
<td>2</td>
<td>68</td>
<td>53.2</td>
<td>2055</td>
</tr>
<tr>
<td>B.B.</td>
<td>4</td>
<td>68</td>
<td>54.1</td>
<td>1110</td>
</tr>
<tr>
<td>H.C.</td>
<td>6</td>
<td>57</td>
<td>71.5</td>
<td>1872</td>
</tr>
<tr>
<td>M.H.</td>
<td>8</td>
<td>59</td>
<td>56.0</td>
<td>1240</td>
</tr>
<tr>
<td>C.W.</td>
<td>10</td>
<td>58</td>
<td>79.4</td>
<td>1100</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td>56.6</td>
<td>62.8</td>
<td>1475</td>
</tr>
</tbody>
</table>

* The only female in the series.

There was little doubt about either the diagnosis or the concomitant generalized autonomic defect characteristic of that syndrome. A fifth patient, C. W., without apparent orthostatic hypotension but with a surgically repaired tetralogy of Fallot, was also included in the hypotensive group. This patient was of particular interest because, although he has no radial pulse or blood pressure recordable in either arm, he is clearly not a classical orthostatic hypotensive and sustains normal activity as a technician in this laboratory.* His directly recorded aortic pressure under sedation was 90/60 mm. Hg with a similar systolic pressure in the right ventricle.

All subjects were on the same standard hospital diet and received no medication during the period of study. Two sequential 24-hour collections of urine were obtained in all 10 subjects simultaneously and the volume of each adjusted with distilled water to the highest 24-hour output of the group (table 1). Aliquots of the 2 collections from each of the 10 subjects were then used for bioassay both before and after dialysis. The remainder was frozen for later study. Dialysis against water was performed at a temperature of 32 C. with continuous agitation of the urine in standard dialysis tubing (Visking) against 200 times the content of the bag and with 3 changes of the outside fluid in a 10-hour period. The nondialyzable fraction was used for assay.

The details of the bioassay technic used are set forth above (section II). Dose response curves were obtained by making sequential 0.4 ml. urine injections varying from full strength to a one-eighth concentration. Dose-response curves were also obtained by injecting urine of the same concentration in amounts varying from 0.1 to 0.6 ml. With commercially prepared calliercin (Paldutin*) as a reference standard, a variety of chemical technics were used in an attempt to ascertain whether the active substance of interest in these experiments is a calliercin, a type of protein known to occur normally in urine.*

*Laboratory of Cardiovascular Physiology, National Heart Institute.

*Supplied through the courtesy of Dr. M. L. Tainter, Sterling-Winthrop Co.
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RESULTS

Comparison of Vasodilator Activity in the Urine of Control and Hypotensive Groups. Figure 9 (A to D) depicts the results of 4 sets of urine assays. Each set consists of one assay of each of the 10 urine samples studied. The top set (A) shows the flow responses after injection of the raw nondialyzed samples from the first day's collection. The second set (B), done in the same preparation but later in the day, shows the flow responses after dialysis had been performed on these urine samples. The third set (C) shows the flow responses in a subsequent preparation. The bottom set (D) shows the response to the injections of the raw nondialyzed urine samples of the second day's urine collections in still another preparation. It will be noted that although there was, as must be expected in any bioassay technic, some variability in the absolute response when comparing set to set and preparation to preparation, there was a gratifying consistency in the relative activity of the various specimens within any given set. The urines from the control group (odd numbers in fig. 9 and table 1) consistently showed greater activity than those of the hypotensive group (even numbers). Further, the highest activity in the hypotensive group was always substantially lower than the lowest activity seen in the control group in that set.

An attempt was made to quantitate the relative difference in the vasodilator activity of the urine from these 2 groups. For this purpose, the dose-response relationship of the most active urine in the hypotensive group (no. 4) was determined simultaneously with the dose-response relationship of the second most active urine in the control group (no. 1). The results are shown in figure 10. The difference between these 2 curves was the smallest difference observed in any comparison made between the hypotensive and the control groups. It would appear that about 4 times the concentration of the most active urine in the hypotensive group was required to give the same peak increment in flow as the second most active urine in the control group. A similar set of curves was obtained to compare the urine activity of patient no. 2, the third most active hypotensive urine, with the activity in the urine of control no. 3, usually the fourth most active in the control group. These data showed that 6 times the concentration of hypotensive patient no. 2's urine was required to give the same peak increment of flow as that from control no. 3. Dose-response curves were also done on a volumetric rather than a concentration basis, that is, by injecting increasing volumes rather than increasing concentrations. The results were the same. Matched dose-response curves were not attempted using the urine of the two lowest activity hypotensives (no. 8), (no. 10), since their undiluted urine showed little discernible activity and would result in an ap-
Fig. 11. Top set of 10 tracings show the results of an assay set from the 10 urine samples previously assayed (fig. 9) after storage at -15 C. for 2½ months and after alumina column adsorption and elution. Note similarity of relative activity to those observed in figure 9. The duration of each tracing is 2.7 min. The interval between tracings is 3.3 min.

Middle tracings show the femoral artery flow response to 0.4 ml. of dialyzed urine from man (M), elephant (E), horse (H), monkey (Mk), sheep (S) and Scottish Highland cow (C). The sheep urine was diluted 1:7. The duration of each tracing is 3.3 min. The interval is 2.7 min.

Bottom set of 4 tracings represents the change in activity induced by hexamethonium bromide over 4 successive days. Twenty-four hour urine output collected by means of an indwelling catheter, sheep harness and attached bottle. Each day's urine volume was adjusted to the volume of the highest output per day. All urine samples frozen until use and then dialyzed. First tracing shows flow response to the control day urine; second and third tracings during course of hexamethonium; fourth tracing is again a control day. Each injection was 0.4 ml. of a 1:6 dilution of the volume adjusted, dialyzed urine. Duration of each tracing is 2.7 min. Interval between is 3.3 min.

A general idea of the mammalian species prevalence of vasodilator potency in urine was obtained by assaying 0.4 ml. of catheterized urine from the rabbit, weights 69 and 63 Kg., respectively. That urinary pH was of little significance in accounting for the differences observed in the 2 groups was apparent from the following: (a) the average pH of the control group was 6.10 (S.D. = 0.23) and the average pH of the hypotensives was 6.17 (S.D. = 0.80), (b) that the relative activity was consistently retained after dialysis, and (c) that the amount of urine tested is small (0.4 ml.) and is injected into an environment with a high buffer capacity. There was, however, a substantial discrepancy in the average ages. Accordingly, an examination for dilator activity in the urine specimens from 5 normotensive individuals between the ages of 51 and 66 years (average 60 years) was made. The results of these observations revealed that the results (fig. 9) were not age dependent, a view supported by the finding that the youngest patient by far in the hypotensive group (no. 10) had one of the 2 least active urines in that group.

In Vitro Stability. The in vitro stability of the active vasodilator substance under examination was indicated by the results of repeated comparative dose-response curves after an interval of 2½ months during which time aliquots of each specimen had been frozen at -15 C. There was no apparent change in the relative activity of these specimens. These data indicated that the active substance deteriorates little if at all in the frozen state, a view confirmed by the data shown in figure 11 Top.
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cat, dog, man, sheep, monkey, horse, elephant
and Scottish Highland cow.* All showed
high activity. The flow responses observed
after the injection of dialyzed specimens from
the last 6 of these were sequentially assayed
in the same preparation and are shown in
figure 11 Middle. Although no reasonable
means presented itself for making volume ad-
justments of urine output on a realistic basis,
the results of these assays nevertheless sug-
gest that these species excrete a vasodilator
at least comparable in effect to that observed
in man. The 5 sheep urine samples assayed
were the most potent, by far, of any examined
(fig. 11 Middle). The effect of 225 mg.
hexamethonium bromide (given over an 8
hour period) on the 24 hour output of vasod-
dilator activity in one of these sheep is shown
in figure 11 Bottom. This response, although
not consistently observed, occurred during 3
successive courses of the agent in 1 of the 2
sheep examined.

Effect of Diphenhydramine on Vasodilator
Response to Urine. That the active substance
of interest was neither acetylcholine nor his-
tamine was suggested by its nondialyzability.
Further, the dilator response to 0.4 ml.
of normal dialyzed human urine was almost un-
influenced by diphenhydramine in the same
preparation in which the response to his-
tamine was nearly abolished (fig. 12 Top).

Comparison of Effects of Epinephrine and
Urine. Figure 12 Middle shows the arterial
flow response to an injection of 1 μg. epineph-
rine in 0.4 ml. saline, the response to 0.4
ml. of dialyzed human urine and lastly the
response to the simultaneous injection of both.
This tracing is of interest in that (a) the
urine was capable of more than counteracting
the constrictor effect of the epinephrine and
(b) that the onset of action of the urine was
at least as rapid as that of epinephrine. This
was also observed when comparing the rapid-
ity of onset of urine with norepinephrine,

histamine and methacholine. Lastly, no in-
hibition of the urine dilator response was
noted after inducing an augmented-peripher-
al arteriolar resistance with bilateral occlu-
sion of the common carotid arteries.

Properties of the Vasodilator Substance. A
variety of chemical isolation technics was em-
ployed to ascertain the extent of the chemical
similarity between callierein and the active
urinary substance under examination. The
vasodilator activity of urine was retained af-
ter dialysis and precipitation by ammonium
sulfate, lead subacetate and uranium acetate,
properties characteristic of callierein. 25, 26 It
was, as also reported by these authors, irre-
versibly adsorbed to charcoal. The stability
of callierein and of the vasodilator substance
in urine was similarly influenced by lowering the hydrogen ion concentration to below pH 4.0. Between pH 5.0 and 8.0 both were stable. It was also found that a salt free, active urine concentrate and callicrein behaved similarly on alumina columns under the conditions described by Werle and Marcus.27

An exploration of the optimal range of pH and temperature conditions for adsorption to alumina resulted in the development of a method which made it possible to adsorb the activity directly from raw urine and to recover from 90 to 100 per cent in the eluate. This will be described in detail elsewhere.13 Figure 11 Top shows the results of assay after the application of this alumina column method to the same urine specimens studied in figure 9. These specimens had been preserved in the frozen state (−15 C.) for 2½ months after which alumina column exposure, dialysis and bioassay were performed. The assay on these 10 specimens was done in the same manner as those shown in figure 9 A-D and in the same order. A similarity in the relative activities of these 10 alumina column fractions to those of the urine samples previously assayed is evident. It is worthy of note that, with 90 to 100 per cent of the activity retained, the total nondialyzable solids present in the alumina column eluates were only one-fourth of the total nondialyzable solids in the starting urine samples.

**Estimate of the Order of Magnitude of Potency.** Although there is normally some variation in dilator activity from individual to individual in the control group (fig. 9), it was possible to estimate the general order of magnitude of the normal range by comparison with a callicrein standard. The injection of 0.4 ml. of normal human urine (no. 1) produced approximately the same vasodilator response as 10 μg. of commercially prepared hog pancreas callicrein (fig. 12 Bottom). Analysis of 3 of the 5 control urines assayed in figure 9 revealed that samples of urine which originally contained an average of 30 mg./ml. of total solids contained 0.22 mg./ml. of nondialyzable solids. More recently, using the technic of ion exchange chromatography, it was found that all the dilator activity occurred in one fraction which was 16 per cent of these nondialyzable solids or 0.035 mg./ml. after adjustment to the initial urine concentration. Since 0.4 ml. of urine is used in the assay, the observed dilator activity was induced by approximately 14 μg. of total solids. In view of the fact that the above mentioned commercially prepared callicrein has been reported to be susceptible of fifty-fold purification,28 the possibility suggests itself that, if callicrein is the active principle being studied, it exhibits the activity shown above in amounts approximating 0.2 to 0.3 μg., which, on a weight basis, is an order of activity comparable to epinephrine and norepinephrine. Since the molecular weight of callicrein is reported to be about 45,000 and that of epinephrine 183, on a molar basis callicrein would appear to be 200 to 300 times more potent than epinephrine. While these considerations are, of course, approximate and do involve important assumptions, it is nevertheless clear that the potency of the active vasodilator substance under examination is of a high order of magnitude. In the studies shown above (1) the coronary dilator effect of 10 ml. of human urine was found to be more than twice that of 0.6 mg. of nitroglycerin.

**DISCUSSION**

The initial studies described above were undertaken with the limited objective of investigating the relative potency of the coronary vasodilator action of urine by comparing it to that of nitroglycerin while the working conditions of the heart were controlled. However, the high potency observed, the mammalian species prevalence, and the rapid onset of activity subsequently gave rise to speculation concerning the physiologic role which this activity might possibly play in circulatory regulation. It was reasoned that, in many of its aspects, autonomic control is dual in nature as is, for example, the control of heart rate. Even the long debated problem of the existence of active vasodilator fibers seems at last to have been reasonably well established even if their function is not clearly understood.30 One functional component which still appears to be lacking to
complete the general balance of arterial pressure regulation is the demonstration of the activity of a generally acting, vasodilator substance. Acetylcholine does not fit in as the reciprocal analogue of the catechol amines since its activity is not general but rather local under physiologic circumstances. It was largely on the basis of this type of "reasoning" that it was thought desirable to formulate a working hypothesis as a point of departure. This is schematically represented in figure 13.

This view attributes physiologic significance to the presence of the vasodilator substance in urine insofar as this may be a reflection of the blood activity thereof; further, that its general effect is directionally opposite to that of the catechol amines and helps to dampen elevations of arterial pressure much as the catechol amines serve to limit the lowering of arterial pressure. It was also postulated that such a regulatory influence might be more leisurely in character than is the case with the sympathetic responses to "fight or flight" situations.

As shown above, in the 4 patients with the classical syndrome of orthostatic hypotension and in the 1 patient with no observable autonomic defect but with systemic arterial hypertensive tetralogy of Fallot, the 24 hour output of urinary vasodilator substance amounted to less than 15 per cent of that observed in 5 simultaneously studied normal individuals. The amount of stable, potent, rapidly acting, species prevalent vasodilator substance excreted in 24 hours may be a reflection of the 24 hour amount produced by the organism. These data appear compatible with the view that this substance, presumably callicrein, is in some way associated with autonomic function and/or the regulation of arterial pressure. Observations in the sheep showing a reduction of the 24 hour output of vasodilator under partial ganglionic blockade were construed as being in support of this view.

It does not as yet seem appropriate to attempt to integrate the findings described above with the voluminous work that has taken place in this field since Abelous and Bardier first described the hypotensive effect of intravenous injection of urine in 1909. The reader is, however, referred to the recent symposium on polypeptides edited by Gaddum, the monograph on callicrein by Frey, Kraut and Werle, and the publications of Wollheim. Although certain of the theoretical aspects of the work described above partially coincide with the earlier views of Wollheim, the authors are not inclined to agree with Green et al. that the vasodilator substance of interest under examination is Wollheim's detin rather than callicrein. Preliminary observations on the femoral vascular dilator activity of urine from hypertensive patients revealed that while such activity was diminished in some of them, in others it was quite high and by no means was it uniformly absent in essential hypertensive patients as was indicated by Wollheim for detin.

SUMMARY

The 24 hour output of vasodilator activity in the urine of 4 patients with classical orthostatic hypotension and 1 hypertensive patient without the autonomic features of the syndrome was, as a group, less than 15 per cent of that observed in 5 simultaneously studied normal individuals. The active substance is nondialyzable and has certain chemical characteristics in common with callicrein. Urine specimens from the 9 mammalian species studied all contained high activity. These observations were construed as being compatible with the hypothesis that the vasodilator agent under examination is in some way associated with autonomic function and/or the regulation of arterial pressure.
ADDENDUM

Subsequent to the studies reported above, observations have been made on the vasodilator properties of human serum with the flow technic described. Relatively little activity was observed when serum at normal pH was injected. Lowering the pH of normal serum, however, conferred upon it marked dilator activity which reached its peak effect at pH 5.5 to 6.0 and then rapidly diminished with further lowering of its pH. These observations are consonant with those of Werle concerning the pH dependence of dissociation in the calliherein-inactivator complex.

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SUMMARY IN INTERLINGUA

Parte I
Le directe effecto dilatatori de nitroglycerina e de urina human esseva observate in le isolate e supportate preparato cardiace. Esseva observate que 10 ml de urina human habeva un effecto al minus comparabile al effecto de 0,6 mg de nitroglycerina. Ni le un ni le altere del due agentes causava un alteration del consumption myocardial de oxygeno sub le conditiones experimental usate.

Parte II
Esseva interprendite le tentativa de augmentar le valor e le fidelitate del technica a fluxo del arteria femoral como bio-essayo de substantias localmente vaso-active. Experimentos critic in modellos suggereva le possibilitate que mismimento inadequate esseva un factor limitante in previe technicas. Studios in vivo demonstrava que le uso de un magnetique cuvette circular resultava in un melioration marecato del reproductibilitate e provdeva le base de plus satisfacente curvas del responsa como function de dosage.

Parte III
Le rendimento del activitate vasodilatatori mesurate pro 24 horas in le urina de 4 pacientes con classic hypotension orthostatic e de 1 paciente hypotensive sin le characteristics autonome del syndrome, considerate como un gruppo, esseva minus que 15% del rendimento correspondentemente observate in 5 individuos normal qui esseva studiate simultaneemente. Le substantia active es non-dialysable e ha certe characteristics chimic in commun con callihereina. Specimens de urina ab 9 species de mammales studiate contineva omnes alte grados de activitate.

Esseva concluidi que le datos esseva compatibile con le hypothesi que le agente vasodilatatori sub investigation es associate in un maniera o un altere con le function autonome e/o le regulation del pression arterial.

REFERENCES
10. BRUNTON 1867. QUOTED BY GOODMAN, L. S., AND GILMAN, A.: The Pharmacological
VASODILATOR PROPERTIES OF URINE

Observations on the Vasodilator Properties of Urine

S. J. SARNOFF, R. B. CASE, R. MACRUZ, L. C. SARNOFF, K. E. SUSSMAN and J. V. PIERCE

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