± 47.5 ml·min^{-1}. These increases in the ventilation were seen at 30 min of the distension. In contrast, exteriorized bladder distension for the same period of time (i.e., to 30 min) did not result in an appreciable increase of the ventilation (Fig. 34).

During the release of the pressure of the exteriorized bladder there was an increase of ventilation to 514 ± 71.1 ml·min^{-1} in comparison to the predistension value of 413 ± 67.3 ml·min^{-1}. Whereas, with bladder in situ even 5 min after releasing the bladder pressure the minute ventilation was 572.4 ± 65.8 ml·min^{-1} which is +17.7 % above the predistension value of 486 ± 47.5 ml·min^{-1}.

4. DISCUSSION

4.1. Criticism of the methods

4.1.1. Methods of distension of the urinary bladder

4.1.1.1. Pneumatic distension of the urinary bladder

In the preliminary experiments on dogs the urinary bladder was distended by introducing a balloon into it and was distended pneumatically with varying intraluminal pressures. This method of bladder distension was previously used by SHAH et al. (1965) in dogs. The advantage of this method of distension is that maximum tension can be exerted on the bladder wall and that thereby impressive cardiovascular and respiratory changes are obtained. On the other hand it has the following disavantages:

1. Normally the urinary bladder is filled with urine. So distending the bladder with a balloon is far from the natural way of distending it.
2. Due to high intraluminal pressures (e.g., above 120 Torr)

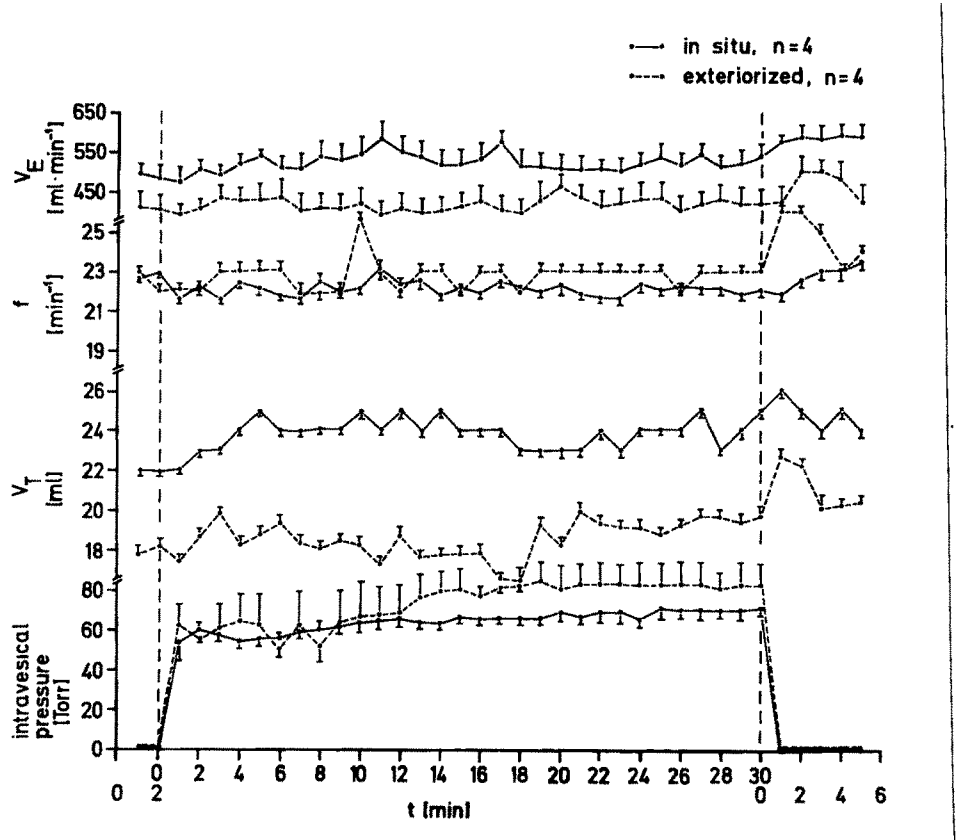

Figure 34: Average changes in the tidal volume (V_T ml), respiratory frequency ($f \cdot min^{-1}$), and minute ventilation $\dot{V}_E$ $ml \cdot min^{-1}$, during prolonged distension of the urinary bladder in situ and exteriorized bladder distension. The data presented are the average values ($\bar{X}$) with the standard error of the mean (Sm). n is the number of observations obtained from four cats.

Experimental group	$\bar{P}a$ Torr	Systolic Pa Torr	Diastolic Pa Torr	$HF_{min^{-1}}$	V_T ml	$f \cdot min^{-1}$	$\dot{V}_E$ $l \cdot min^{-1}$
Distension of the bladder by saline (dogs)	increase P=0.0005	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Distension of the bladder in cats	n.i.	increase P<0.0002	increase P<0.0002	n.s.	n.s.	n.s.	increase P=0.0003
Electrical stimulation of pelvic nerve	n.i.	increase P<0.0002	increase P<0.0002	n.i.	increase P<0.0002	n.s.	increase P=0.0027
Electrical stimulation of hypogastric nerve	n.i.	increase P<0.0002	increase P<0.0002	n.i.	increase P=0.0027	n.s.	increase P=0.016
Hypogastric vs pelvic nerve stim. arterial pressure	n.i.	increase hypogastric>pelvic P<0.0002	P<0.0002	n.i.	n.i.	n.i.	n.i.
Distension of the bladder with intact ureters vs cannulated ureter	n.i.	increase intact uret.>cannul.uret. P = 0.04	P = 0.02	n.i.	n.s.	n.s.	n.s.
Distension of the bladder; intact sino-aortic nerve vs after sino-aortic denervation	n.i.	increase int.buff.n.< buff. den. P = 0.018	P=0.0027	n.i.	n.s.	n.s.	increase intact buff. nerve > buff. denervation P = 0.01
Electrical stimulation before and after sino-aortic denervation		increase int.buff.n. <buff. den.					
Pelvic nerve	n.i.	P<0.0002	P<0.0002	n.i.	n.s.	n.s.	n.s.
Hypogastric nerve		P<0.0002.	P<0.0002	n.i.		increase int.buff.n.< buff. den. P<0.0002	P=0.0010
Electrical stimulation before and after renal denervation							
Pelvic nerve	n.i.	n.s.	n.s.	n.i.	n.s.	n.s.	n.s.
Hypogastric nerve	n.i.	increase int.ren.n.> bil.r.den. P=0.006	n.s.	n.i.	n.s.	n.s.	n.s.
Bladder distension control vs renal denervation	n.i.	increase control > renal denerv. P=0.035	P=0.035	n.i.	n.s.	n.s.	n.s.
control vs clamping renal vessels	n.i.	increase control> clamping ren.vess P=0.0027	P=0.024	n.i.	n.s.	n.s.	n.s.
Bladder distension control vs ang.II antagonist	n.i.	increase control> AT II antagonist P=0.0027	P=0.001	n.s.	n.s.	increase control AT> II antagonist P = 0.03	n.s.
Electrical stimulation control vs angiotensin II antagonist		increase control> AT II antagonist					
Pelvic nerve	n.i.	P=0.005	P=0.0005	n.i.	n.s.	n.s.	n.s.
Hypogastric nerve	n.i.	P<0.0002	P<0.0002				

Table 12: Summary of the direction and significance of the results. n.i. means not investigated; n.s. indicates not statistically significant i.e. P values above 0.05.

used in this study, the occurrence of haematoma and damage to the bladder mucosa was high, therefore the reproducibility of the responses was less.

4.1.1.2. Distension of the urinary bladder with saline solution

For the reasons mentioned above, in all the later experiments on dogs as well as cats the urinary bladder was distended with urethral infusion of saline. In the experiments with short term distension of the bladder in cats (2 - 3 min) the rate of infusion of the saline was about 80 $ml \cdot min^{-1}$ with a pressure head of 80 Torr. Undoubtedly this rate of saline infusion is exceedingly high compared to the normal rate of urine inflow to the bladder. But the purpose of using these high flow rates was to obtain an intravesical pressure of about 50 Torr within a period of 30 s. It was suggested by WHITTERIDGE (1960) that vesico-vascular reflexes can be obtained only when the intravesical pressure was above 50 Torr. With this method it is also possible not only to achieve the desired high intravesical pressure but also to repeat the distension of the bladder in the same experiment without damaging it.

4.1.2. Renal denervation

In the experiments involving the bilateral renal denervation, the renal nerves around the renal artery were isolated and were cut. One can doubt about the completeness of the renal denervation. It is likely that not all the many renal nerve branches have been cut. But the aim of these experiments is only to compare the cardiovascular and respiratory parameters before and after renal denervation. If the responses in these parameters were significantly altered after denervation of the kidneys, it can be assumed that the extent of renal denervation was sufficient in serving the purpose of these experiments.

4.1.3. Specificity of the angiotensin antagonists

The main conclusions regarding the participation of angiotensin in the observed pressor responses during the urinary bladder distension were based on the results obtained by the use of 1-sarcosine-8-alanine angiotensin II and 1-sarcosine-8-leucine angiotensin II. The efficacy of these two antagonists was tested by recording the pressor responses with intravenous injection of 0.5 $\mu g.ml^{-1}$ of angiotensin II and 2 $\mu g \cdot ml^{-1}$ of norepinephrine. If the pressor response of 0.5 $\mu g \cdot ml^{-1}$ of angiotensin II was reduced after infusion of the antagonist without affecting the pressor response to 2 $\mu g \cdot ml^{-1}$ of norepinephrine it was assumed that the antagonist had specifically blocked the pressor action of angiotensin II. Similar criteria for testing the angiotensin antagonists were used by other workers (STEELE and LOWENSTEIN, 1974; TRIPPODO, et al., 1977). The pharmacological specificity of angiotensin antagonists was also reviewed at length recently by REGOLI et al. (1974). Angiotensin II antagonists may also have effects on the release of other vasoactive hormones. Since the aim of these experiments besides observing the reflexes involved is to test the contribution of the renin-angiotensin system to the pressor responses during the bladder distension. The use of these antagonists could thus be justified.

4.2. Discussion of the results

The aim of the present experiments is to evaluate not only the changes in cardiovascular system but also the missing information about the minute ventilation in response to bladder distension in anaesthetized cats and dogs. The mechanisms responsible for the alterations in the cardiovascular system have been mostly attributed to the neurogenic factors. The present study demonstrates that humoral factors could also play an important role in addition to the neurogenic factors. The results shall be discussed under the following main points.

1. Changes in the cardiovascular system during distension of the urinary bladder.
2. Ventilatory changes during the distension of the bladder.

4.2.1. Changes in the cardiovascular system with distension of the urinary bladder

4.2.1.1. Arterial pressure changes

An increase in the mean arterial pressure was seen in the majority of the experiments on dogs with pneumatic distension of the bladder. These results are in agreement with those reported by SHAH et al. (1965). As it was thought that the pneumatic distension of the bladder was not a natural way of distension, in all the later experiments urethral infusion of saline was used for distending the bladder. Increase in the arterial pressure was consistently seen in both dogs and in cats also,with this method of distension; confirming the results reported in anaesthetized dogs (HOROWITZ et al., 1966; TULIN, 1964; RUBINSTEIN and CARREA, 1961) and also in cats (WATKINS, 1938; MUKHERJEE, 1957b; TAYLOR, 1963).

The increase in the arterial pressure seen in dogs was not as much marked as it was in cats (Fig. 4). The rate of urethral infusion of saline in dogs was between 40 - 45 $ml \cdot min^{-1}$, while in cats it was almost kept constant at 80 $ml \cdot min^{-1}$ which is approximately double the flow rate used in the dogs. So the less marked response in dogs could be attributed to the flow rates used rather than the species difference. Otherwise, the pressor responses obtained with distension of the bladder were almost the same in cats and dogs.

In response to the bladder distension in cats, the arterial pressure began to increase, with a latency of 3 to 20 s; These values differ slightly from the values reported by others. WATKINS (1938) reported a latency of 2 - 5 s, whereas MUKHERJEE (1957b) obtained

latencies of 2 - 40 s. This difference may be due to the fact that in their experiments the flow rates used for distending the bladder varied from 60 to 120 $ml \cdot min^{-1}$ whereas in the present study it was kept close to 80 $ml \cdot min^{-1}$.

The bladder pressure at which the blood pressure began to increase was about 58.1 ± 1.9 Torr in cats, and 41.1 ± 3.9 Torr in dogs. WHITTERIDGE (1960) suggested that for obtaining an increase in the arterial pressure by bladder distension, the intravesical pressure should be increased above 50 Torr. The results obtained in the experiments on cats confirm experimentally this suggestion and also explain to certain extent why other workers (BERMAN and ROSE, 1958; MAY and BARELARE, 1958) were unable to observe any increase or a negligible increase only in the arterial pressure during the bladder distension.

WATKINS (1938) and SZASZ and WHYTE (1967) reported a linear relationship between the bladder pressure and arterial pressure increases. In the present study no such relationship in these parameters was noticed. On the contrary, though the bladder pressure was still increasing the arterial pressure after reaching a maximum did not show any further increase (Fig. 4) in dogs or it showed a slight decrease from the maximal increase (Fig. 7) in cats. According to TAYLOR (1968) the tension in the bladder wall is the effective stimulus for the increase of the blood pressure, rather than the absolute intravesical pressure, the volume or ischemia of the bladder. TANG (1955) calculated the tension on the bladder wall by using the equation $T = (P/2) \cdot \sqrt[3]{(3/4\,\pi)V} = 0.31 \cdot P \cdot \sqrt[3]{V}$, where T is the tension in the bladder wall, P is the intravesical pressure and V is the volume of the fluid in the bladder. From his calculations he could show that at higher volumes of fluid in the bladder, the relation between the intravesical pressure and the tension in the bladder wall are closely related. If that is so, one should expect a linear relation between the intravesical pressure (considering it as an approximate index of the tension) and the arterial pressure increase. Since, in these experiments such a linear relationship was not seen, it is difficult to reconcile this result with the suggestion that tension on the bladder wall

is an effective stimulus for eliciting an increase in the arterial pressure.

4.2.1.2. Arterial pressure changes with electrical stimulation of the pelvic and the hypogastric nerves with reference to the afferent pathway

It has been suggested that the main afferent pathway for the vesico-vascular reflex is the pelvic nerve (TAYLOR, 1968). This suggestion was based mainly on the results obtained from the selective denervation of the bladder afferents during vesical distension, while simultaneously observing the changes in the arterial pressure. (TALAAT, 1937; WATKINS, 1938; TAYLOR, 1968). This method is a conventional way of studying the afferent pathway of a reflex. Application of this method to the study of the vesico-vascular reflex, however, has got its own limitations. For example, in the experimental animals the urinary bladder has to be distended abnormally for obtaining an increase in the arterial pressure. With this abnormal distension there is a possibility of other pelvic organs of getting distorted. The distortion of these pelvic organs might itself result in a change of the arterial pressure. So cutting one of the bladder afferents may not be able to abolish or reduce the magnitude of the pressor response. Moreover, the information obtained from cutting the posterior roots of the different segments of the spinal cord need not necessarily indicate that these afferents are arising from the bladder.

Because of these limitations of denervation studies, in the present experiments electrical stimulation of the cut central ends of the pelvic and the hypogastric nerves was chosen. By electrically stimulating separately the cut central ends of the pelvic and the hypogastric nerves it is possible 1) to study the relative contribution of these nerves in the pressor response seen during the bladder distension, 2) the possible afferent pathway for the vesico-vascular reflex. The results obtained in these experiments have been as follows: a) with identical stimulus parameters, the

increase in the arterial pressure (both the systolic and the diastolic pressures) was always more pronounced with hypogastric nerve stimulation b) similarly the changes in the heart frequency (initial tachycardia followed by bradycardia) was also more marked with the hypogastric nerve stimulation as compared with the pelvic nerve stimulation.

In these experiments no attempt was made to find out the type of sensory nerve fiber being stimulated. So, one possible explanation of the difference in the response would be that a higher number of sensory fibers was stimulated in the hypogastric nerve for a given stimulus parameter than in the pelvic nerve. However, such an explanation seems to be unlikely in view of the electrophysiological investigations reported on the bladder afferents on cats. IGGO (1955, 1966) reported that the pelvic nerve contains a slowly adapting type of sensory receptors, which would indicate that they belong to the small myelinated or non-myelinated nerve fibers. Recently FLOYD et al. (1976) have shown that the hypogastric nerve of cats contains sensory receptor fibers of the slowly adapting type arising from the urinary bladder and their conduction velocities range from 0.5 to 12 $m.s^{-1}$. Considering this evidence it is reasonable to assume that with identical stimulus parameters the same type of nerve fibers might be getting excited in both nerves. A more logical interpretation of the results would then be that the hypogastric nerve is the main afferent pathway for the hypertensive response obtained by bladder distension. This interpretation can be further supported from the histological data reported on the bladder afferents. Histologically it was shown that the submucosa of the bladder neck of the cat contains mainly a population of afferent axons belonging to the hypogastric nerve, whereas the pelvic nerve afferents were equally distributed in all the regions of the muscular wall of the bladder (UEMURA et al., 1975). Furthermore, it was demonstrated that the maximal increase in the arterial pressure was obtained when the tension was applied to the bladder neck and trigone, rather than to the other regions of the bladder (TAYLOR, 1968).

4.2.2. Neural and mechanical factors influencing the arterial pressure response during the distension of the urinary bladder

4.2.2.1. Role of the sino-aortic nerves

GUTTMANN and WHITTERIDGE (1947) observed an especially marked increase in the arterial pressure with bladder distension in paraplegic patients having lesions between C_8 - T_5. They suggested that the pronounced elevation of the arterial pressure was due to the partially blocked buffering effect of the baroreceptors of the sino-aortic region. In view of this suggestion, in the present series the systolic and the diastolic pressures were analysed before and after sino-aortic denervation with distension of the urinary bladder and also during the electrical stimulation of the cut central ends of the pelvic and the hypogastric nerves. The main observations are as follows: 1) After the sino-aortic denervation the increase in the systolic pressure was enhanced throughout the distension period of the bladder, while the increase in the diastolic pressure was enhanced only in the initial phase of the distension (i.e., up to 120 s). 2) Similar results were obtained with electrical stimulation of the hypogastric nerve, and also with pelvic nerve stimulation.

The enhanced increase of the arterial pressure, especially the systolic pressure indicates a tonic inhibitory influence of the baroreceptor nerves on the vasomotor tone during bladder distension. It is known that the baroreceptors exert a constant inhibitory influence on the spinal efferent sympathetic neurones. After denervation of the baroreceptors this inhibitory influence does no longer exist. Thereby the bladder distension in this situation results in a larger change of the arterial pressure.

In the buffer denervated animals, interestingly enough the enhancement of the systolic pressure increase was seen throughout the distension phase of the bladder. The diastolic pressure, however, was increased only during the initial phase of the distension. The simultaneous tachycardia which was seen shows that the systolic

pressure increase is partly due to a reflex on the heart. Repeating the experiments of GUTTMANN and WHITTERIDGE (1947), WURSTER and RANDALL (1975) observed marked increase of the systolic pressure during bladder distension in paraplegics with lesions situated above T_5 where the major part of the efferent pathway of the baroreceptor reflex is interrupted.

4.2.2.2. The role of the ureters

It is not clear from the available literature what is the role of the ureters during the distension of the bladder. MUKHERJEE (1957b) studied the vesico-vascular reflex in animals with ureters cannulated, whereas in the reports from others it was not mentioned whether the ureters were cannulated or left intact. In human studies it can be expected that during the extreme phases of the bladder distension the ureteral orifices would be closed. This would result in an accumulation of the urine in the ureter and also in an increase of the renal pelvic pressure. These changes might modify the pressor responses observed during the bladder distension. In the present series, comparison of the arterial pressure before and after cannulating the ureters have shown that in ureter cannulated cats the increase in the arterial pressure was considerably less ($P = 0.02$ to 0.04 for $n = 9$ to 10) especially in the later phases of the bladder distension (i.e., 0 - 120 to 150 s). VANDER and MILLER (1965) reported that elevation of the ureteral pressure results in an increase of renin secretion from the kidney and also an increase of the arterial pressure. So, the possible explanation for the diminution of the arterial pressure reaction in the ureter cannulated animals would be the lesser renin secretion followed by a lesser angiotensin formation. This could explain the smaller arterial pressure response seen in the ureter cannulated animals. It is noteworthy that the pressor response is less mainly in the later phases of the distension, suggesting a slow building up of pressure in the ureters during the distension of the bladder if ureters are left intact. Furthermore these results also point out the importance of the ureters in cardiovascular alterations observed

during long term distension of the urinary bladder as is commonly seen in patients with urologic disorders and also in paraplegics.

4.2.2.3. Influence of intraabdominal pressure

Distension of the urinary bladder for 30 min with a flow rate of about 17 to 20 ml·min^{-1} in anaesthetized cats resulted in an initial increase of arterial pressure (both systolic and diastolic) which reached its maximum within 120 to 150 s. Such increases were also seen with short term distension of the bladder (for 2 to 3 min) in the present study. In the later phases of distension inspite of the elevated intravesical pressure of about 71.4 ± 3.8 Torr, the arterial pressure started to decline and almost reached the predistension control value. Similarly in the initial phases of exteriorized bladder distension the arterial pressure responded with an increase, but from 2 min onwards it started to fall. However, the fall of the arterial pressure was not so much pronounced. It always remained above the control value. Similarly in dogs, the urinary bladder distension in situ for 2 hours resulted in a hypotension, while in the same animals exteriorized distension lead to an increase of the arterial pressure. This time related fall of the arterial pressure with prolonged distension of the bladder occurred inspite of maintained elevated bladder pressures (49.6 ± 7.6). The decrease of the arterial pressure could possibly be due to the mechanical compression of the neighbouring blood vessels during the distension in situ. This assumption is supported experimentally by measuring the venous pressure below and above the bladder during the long term distension in dogs. The venous pressure below the bladder increased, while above the bladder it decreased. Furthermore also with the short term distension of the bladder in cats a marked increase of the venous pressure below the bladder was seen with distension in situ. Since such changes were not seen in the exteriorized distension, it is likely that embarassment of the venous circulation to a considerable extent is the main reason for the late fall of the arterial pressure during the distension in situ. These results would also explain the massive oedema of

the lower extremities often reported in patients with chronically distended bladder (CARLSON and GARSTON, 1960; STOUTZ, 1961; SMITH et al., 1963).

4.2.3. Adaptation of the arterial pressure increase during the distension of the bladder and the possible mechanisms involved

During the short term distension of the urinary bladder, the increase in the arterial pressure (both systolic and diastolic) reached its maximum value at about 90 - 120 s. Thereafter, the pressures are slightly decreasing from the maximum value inspite of the elevated intravesical pressure. This adaptation of the arterial pressure could be due to the a) intact baroreceptors, b) adaptation of the bladder sensory receptors, c) the mechanical disturbances arising from the bladder distension in situ like increase of venous resistance, d) adaptation of the efferent (neural/humoral) mechanisms.

Bilateral denervation of the sino-aortic nerves did not abolish the adaptation of the arterial pressure, suggesting that the baroreceptors are not responsible. Since such adaptation of arterial pressure also occurred with electrical stimulation of the afferents from the bladder (the hypogastric and the pelvic nerves), this would preclude the role of the bladder sensory receptors.

For testing the possibility of mechanical disturbances arising from the bladder distension in situ the bladder was exteriorized keeping the nervous and vascular supply intact and was distended for 30 minutes. The arterial pressure after attaining a maximum value at about 120 s started to decline from the maximum value. This decrease of the arterial pressure, despite maintaining the elevated intravesical pressure occurred at a level of 62.6 $\pm$ 10.6 Torr. This implies that adaptation of the arterial pressure is not solely due to the mechanical disturbances. Thus leaving the possibility that some arterial pressure adaptation is occurring at the efferent mechanisms, which may be either neurogenic or neuroge-

nically mediated humoral mechanisms.

4.2.4. Are humoral factors involved in the increase of the arterial pressure?

4.2.4.1. Role of the Kidney

In the sino-aortic denervated and ureter cannulated cats, distension of the urinary bladder still resulted in an increase of the arterial pressure. After bilateral renal denervation the magnitude of the pressor response was reduced after 90 s of the bladder distension. Electrical stimulation of the hypogastric nerve in sino-aortic denervated cats resulted in marked elevation of the systolic and diastolic pressures. Bilateral renal denervation also reduced the systolic pressure increase during the hypogastric nerve stimulation. Furthermore, there was an increase in the efferent renal nerve activity before the increase of the arterial pressure under hypogastric nerve stimulation. In contrast, the increase of the arterial pressure observed with pelvic nerve stimulation was not affected by renal denervation and there was only a very slight increase in the efferent renal activity during the stimulation. In other words, these results suggest that increased efferent renal nerve activity may lead to renal vasoconstriction and in fact, such a renal vasoconstriction during the bladder distension was reported by MUKHERJEE (1957b). According to LANGLEY and KIMURA (1959) the afferent pathway for the 'vesico-renal reflex' is the pelvic nerve. But the results of the present study strongly suggest that the hypogastric nerve is the main afferent pathway rather than the pelvic nerve.

Electrical stimulation of the renal nerves is known to release renin from the kidney (VANDER, 1965, 1967; COOTE et al., 1972). Recently ZANCHETTI et al. (1976) pointed out the role of the renal nerves in the release of renin. Considering these evidences it seems that the renin-angiotensin system may be involved in the pressor reactions observed during the bladder distension. In order to test this hypothesis both the renal artery and the vein were clamped during the

distension of the bladder. Bilateral clamping of the renal vessels itself resulted in a reduction of the predistension arterial pressure, and also reduced the pressor response induced by the bladder distension. It is noteworthy that these responses were reversible after removal of the clamp from the renal vessels. These results would implicate that a pressor substance of renal origin is released during the distension of the bladder.

One of the established pressor substances is angiotensin II which may be activated by renin. By using specific angiotensin II antagonists (1-sarcosine-8-leucine angiotensin II and 1-sarcosine-8-alanine angiotensin II) the role of renin-angiotensin was studied.

After the infusion of the angiotensin II antagonists the important observations of the present study are as follows: a) the predistension arterial pressure was markedly reduced, b) the pressor responses induced by the bladder distension were much reduced and in some cases almost abolished after 60 s of distension, c) the increase in the arterial pressure obtained by electrical stimulation of the hypogastric nerves and interestingly enough also of the pelvic nerve were appreciably decreased in their magnitude. The results obtained with the pelvic nerve are not necessarily contradicting the explanations offered in the preceding paragraphs. These results with pelvic nerve stimulation would be discussed at a later stage of the discussion. A decrease of the arterial pressure after the angiotensin II antagonists was also observed by TRIPPODO et al. (1977) in sino-aortic denervated rabbits.

It is important to note that the pressor responses during the initial phase (up to 60 s) of the bladder distension were not appreciably affected by the antagonist. These observations could possibly be explained on the basis of the activation of the sympathetic system. An increased blood catecholamine level and also increased excretion of norepinephrine during the veiscal distension were reported (GARNIER et al., 1963; GILMORE and VANE, 1971). In the present study adrenalectomy did not affect the increase of the arterial pressure induced by the electrical stimulation of the bladder afferents suggesting that the role of the adrenals in the pressor responses

is negligible. The pressor responses of 2 $\mu g \cdot ml^{-1}$ of norepinephrine were unaltered by the angiotensin II antagonists. These results suggest that the initial pressor response during bladder distension is due to catecholamines mainly norepinephrine. This may be partly the case by release of catecholamines from the sympathetic nerve endings and to a small part only by release from the adrenals.

As it was observed in this study the pressor reactions in the later phases of the bladder distension (from 60 s onwards) were almost abolished by the angiotensin II antagonist; This indicates the involvement of the renin-angiotensin system in the hyptertensive responses of the late phase of the reaction.

Since it has been suggested from the present results that the renin-angiotensin system is playing an important role in the arterial pressure elevations observed with the bladder distension, the question arises whether the renin-angiotensin system is solely responsible for these hypertensive responses or is it acting by influencing the adrenergic system? It has been shown that angiotensin II apart from its direct action on the cardiovascular system (REGOLI et al., 1974), also acts as a 'modulator' of the sympathetic outflow by enhancing the release of norepinephrine (HUGHES and ROTH, 1969, 1971). Furthermore FELDBERG and LEWIS (1964, 1965) have shown that release of adrenalin from the adrenals was increased after angiotensin II infusion. So the pressor responses during the later phases of the bladder distension could be either a direct action of angiotensin II or to some extent an action of it on the adrenergic system.

It has already been demonstrated in the present study that bilateral renal denervation resulted in a diminution of the pressor response (especially the systolic pressure) to electrical stimulation of the hypogastric nerve. Such a diminution of the pressor response after renal denervation was not seen with pelvic nerve stimulation. However, after the angiotensin II antagonist the pressor response obtained with electrical stimulation of both the hypogastric and the pelvic nerve was reduced. VANDER (1965) has shown that electrical stimulation of renal nerves and also

infusion of catecholamines into the renal artery are capable of increasing the release of renin from the kidney. So, the possible explanation of the present results would be that during the hypogastric nerve stimulation the participation of the renin-angiotensin system is primarly due to the increased renal nerve activity, whereas with the pelvic nerve it is due to the increased amount of catecholamines released into the circulation. Furthermore these results would provide additional support for the suggestion that the renin-angiotensin system is involved in the pressor reactions observed during the bladder distension. Quantitative estimation of the renin release during the bladder distension is however undoubtedly a prerequisite for substantiating this hypothesis.

4.2.5. Changes in the heart frequency during the distension of the bladder

In the present study pneumatic distension of the urinary bladder resulted in an increase of the heart frequency in dogs. Upon releasing the bladder pressure there was a bradycardia. In the experiments performed on cats, distension of the bladder for 2 or 3 min with urethral infusion of saline solution resulted in an initial bradycardia followed by a return towards the control value. In some experiments extrasystoles were also seen during the period of distension. The tachycardia which was seen with pneumatic distension of the bladder in dogs was in accord with the previous reports (HOROWITZ et al., 1966). Severe distension of the bladder is known to increase the heart frequency (TAYLOR, 1968). Distending the bladder with a balloon is expected to exert more tension on the bladder wall which would explain the increase of the heart frequency seen in these experiments.

The initial bradycardia during the bladder distension in the experiments performed on cats seems to be due to the activation of the

baroreceptors, because in the sino-aortic denervated cats such bradycardia was not seen. Instead of bradycardia an increase in the heart frequency was observed during the distension as well as during the post distension phase. This would mean that during the bladder distension the primary response of the heart frequency seems to be tachycardia. With intact sino-aortic nerves such an increase in the heart frequency is being masked by the secondary counteraction by the baroreceptors. The extrasystoles which were occasionally seen in the present study may be due to the abnormal distension of the bladder.

During the short term distension of the bladder mechanical factors like the increased abdominal pressure and the ureteral pressure seem to have little influence onthe heart frequency responses. However, during the long term distension of the bladder for 30 min. in the in situ situation there was a bradycardia, whereas during exteriorized distension of the bladder for the same period of time the decrease of the heart frequency was not so marked. In the present experiments distension of the bladder in situ resulted in an increase of the peripheral venous pressure distal to bladder. So it seems that during the long term distension increase in the intraabdominal pressure might be affecting the venous return. The decreased venous return seems to be responsible for the bradycardia observed in prolonged distension.

Changes in the heart frequency obtained by electrical stimulation of the afferents from the bladder namely the hypogastric and the pelvic nerve are as follows: 1) An initial tachycardia which lasted for about 5 to 7 s of stimulation, 2) following this tachycardia there appeared a progressive bradycardia during the later phases of stimulation (i.e., 10 s onwards), 3) this bradycardia persisted during the post stimulation period.

Electrical stimulation of the pelvic and hypogastric nerves is a strong way of mimicking the excitation of the bladder sensory nerve endings as compared to their excitation by bladder distension. This

may be one of the reasons why a consistent change in the heart rate could better be seen with the electrical stimulation as compared with the bladder distension. The initial tachycardia seems to be due to the increased sympathetic activity. The bradycardia which was seen from 10 s of stimulation could be due to the activation of the baroreceptors. However, some bradycardia was also seen after the bilateral sino-aortic denervation, suggests that baroreceptor activation is not solely responsible for the observed response.

It is interesting that the bradycardia persisted even 30 s after stopping the electrical stimulus. If the decrease of the heart frequency is attributed purely to a neurogenic mechanism it should be expected that after releasing the stimulus the heart rate should return to the prestimulus value. Because such a return of the response was not seen in these experiments it seems plausible that a humoral substance might be released into the circulation. As the vagus nerves are intact in these experiments this substance could be acting on the vagal sensory receptors (of the thoracic region), thus reflexly resulting in a bradycardia. The inability of the heart frequency to return to the prestimulus value after stopping the stimulus can be explained on the basis of slow degradation of this substance in the circulation.

From the results obtained in the present study it is difficult to come to a definite conclusion about the possible nature of this substance. However, from the observations after the renal denervation and adrenalectomy some suggestions regarding the possible nature of this substance can be made. The magnitude of the bradycardia with the bladder afferents stimulation as well as in the post stimulation period was reduced after bilateral renal denervation. Similar results were also obtained after bilateral adrenalectomy. Adrenaline has a positive chronotropic effect. It therefore cannot directly cause bradycardia. Nevertheless it is also capable of increasing the renin secretion from the kidney

(VANDER, 1964, 1965). Because renal denervation reduced the magnitude of the bradycardic response it is likely that the renin-angiotensin system may be partly responsible for the bradycardia observed during the later phases of the stimulation of the afferents from the bladder. Further support for this suggestion can be gained from the established fact that intravenous infusion of angiotensin results in a bradycardia (GOODMAN and GILMAN, 1970). In the present study infusion of angiotensin II (0.5 $\mu g \cdot ml^{-1}$) was found to cause bradycardia in sino-aortic denervated cats provided the vagus nerves were intact. This leeds to the suggestion that the renin-angiotensin system is involved in the bradycardia during the electrical stimulation of the bladder afferents. This suggestion, however, has yet to be confirmed in future experiments.

4.2.6. Ventilatory changes during the distension of the bladder

In the present study an increase of the respiratory frequency was seen in response to the pneumatic distension of the urinary bladder in dogs. SHAH et al. (1965) also reported an increase of respiratory frequency with pneumatic distension of the bladder in one dog. Distending the bladder with urethral infusion of saline in cats resulted in an increase of the ventilation in the majority of the experiments. This increase of the minute ventilation is due to the increase of both the tidal volume and the respiratory frequency. Occasionally, however, a decrease of the tidal volume and the respiratory frequency were also encountered (Fig. 6). WATKINS (1938) reported an increase in the rate of respiration and sometimes a decrease with bladder distension by saline. Since no quantitative data of the ventilatory parameters are available from the study of WATKINS (1938) the present study could thus demonstrate that bladder distension in fact results in an average increase of minute ventilation. HOROWITZ et al. (1966) observed a decrease of the thoracic compliance with severe degree of bladder disten-

sion in dogs. So it is possible that the decrease of the ventilation which was occasionally encountered in the present experiments could be due to the decrease of the thoracic compliance.

During the short term distension of the bladder there was no appreciable difference in the minute ventilation with exteriorized bladder distension as compared with the bladder distension in situ. Furthermore cannulating the ureters did not affect the ventilatory responses to bladder distension.These results suggest that at least during the short term distension of the bladder factors like increased abdominal pressure and ureteral pressure are not influencing the respiratory response.

The prolonged distension of the bladder in situ resulted in an increase of the tidal volume, respiratory frequency and the minute ventilation. Such changes were less apparent with exteriorized bladder distension. These results indicate that respiratory excitation observed with long term bladder distension in situ could be (1) secondary to the haemodynamic alterations like decrease of arterial pressure and venous return, 2) and could also be due to the stretching of the abdominal muscles resulting from the overdistended bladder. Furthermore, some degree of respiratory excitation is still present with exteriorized bladder distension which indicates a direct nervous influence from the bladder receptors.

Interestingly enough, there was a diminution of the magnitude of the ventilatory increase in the sino-aortic denervated animals with bladder distension. MUKHERJEE (1957b) reported no change in the respiratory frequency with bladder distension in presence of the buffer nerves. Whereas after bilateral denervation of the carotid sinus and vagus nerves he observed irregularities of respiration during bladder distension. The present results are not in agreement with these observations. Denervation of the sino-aortic nerves would mean not only the denervation of the baroreceptors but also the chemoreceptors. Since there was a marked increase of the arterial pressure during the bladder distension the activity of the baroreceptors is expected to increase. Baroreceptor activation is known to inhibit the ventilation. So the

diminished response of the ventilation in the sino-aortic denervated animals cannot be explained on the basis of the baroreceptors. Bladder distension is known to result in an increase of the sympathetic activity. MILLS and SAMPSON (1969) reported that electrical stimulation of the cervical sympathetic trunk resulted in an increase of the tidal volume. Denervation of the carotid sinus nerves abolished the increase of the ventilation. So possibly during the bladder distension there might have been an increased chemoreceptor activity due to the increased sympathetic activity. This increased chemoreceptor activity appears to be partly responsible for the increased ventilation observed with the bladder distension. The elimination of the chemoreceptor activity after bilateral sino-aortic denervation would explain the diminution of the ventilatory increase during bladder distension in buffer denervated cats.

Electrical stimulation of the pelvic nerve with intact sino-aortic nerves resulted in a marked increase of the ventilation much more than after stimulation of the hypogastric nerve. However, after bilateral sino-aortic denervation the hypogastric nerve stimulation resulted in an increase of the minute ventilation. With identical stimulus parameters the increase of the arterial pressure was always more pronounced in the hypogastric nerve stimulation than in the pelvic nerve stimulation. It is plausible that increased activity of the baroreceptors might mask the ventilatory responses. This would explain the less marked increase of the respiration with the hypogastric nerve stimulation in presence of the buffer nerves. The increase of ventilation during the electrical stimulation of the afferents from the bladder indicate that part of the respiratory responses during the bladder distension are due to the increased activity of the bladder receptors.

After the angiotensin II antagonist there was an increase in the respiratory frequency. This increase of the respiratory frequency was accompanied by a simultaneous reduction of the arterial pressure. Possibly the increased respiratory frequency may be secondary to the fall of the arterial pressure. Bilateral renal denervation, clamping of the renal vessels and angiotensin II antagonist did not affect the ventilatory responses resulting from the bladder distension.