STEPWISE CYSTOMETRY

A NEW METHOD TO INVESTIGATE
PROPERTIES OF THE URINARY BLADDER

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
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BOUDEWIJN LOUIS RENE ANDRE COOLSAET
GEBOREN TE KRUISHOUTEM — BELGIE

«The first process in the effectual study of the science must be one of simplification».

James Clark Maxwell.

PROMOTOR : Professor DR. N. J. BAKKER
CO-REFERENTEN : Professor DR. G. van den BRINK
Professor DR. M.W. van HOF

LIST OF ABBREVIATIONS

P	pressure
V	volume
t	time
NaCN	cyanide
l	length
l_0	unstrained length
V_0	stretch volume of the bladder
S	stress
f	function
E	strain
m	meter
N	Newton
m^2	square meter
E	elastic modulus
$\dot{\varepsilon}$	change of strain
η	viscosity modulus
V_t	tissue volume
C	constant
a	elongation
e	base of natural logarithm (2.72)
log	logarithmic
lin	linear
A	coefficient
B	coefficient
C	coefficient
α	relaxation constant
β	relaxation constant
γ	relaxation constant
F	force
d	area perpendicular to the stress
G_a	weight in air
P_t	density of the tissue
P_a	density of the air
g	acceleration due to gravity
G_w	weight in water
P_w	density of water
τ	time constant
C	Centigrade
sec	second
msec	millisecond
μg	microgram
ml	milliliter
$\|E\|$	elastic coefficient
$E\|\dot{\varepsilon}\|$	elastic modulus dependent on the amplitude of strain
$\|\dot{\varepsilon}\|$	amplitude of the applied strain
δ	elastic exponent
C	contractile element
R	radius
T	tension
cm	centimeter
min	minute
e	relative elastic modulus
p	percentage error

CONTENTS

Introduction

The urinary bladder has a twofold function :

1. to store urine and
2. to expel it if necessary under complete voluntary control.

The bladder can store various volumes of urine at a low and approximately constant intravesical pressure until capacity is reached.

In the literature, this characteristic of the bladder was originally ascribed to reflex mechanisms (Mosso and Pellacani, 1882; Ausems, 1957). However, experimental evidence to show that inherent properties of the detrusor muscle, independent of neurogenic control, play an important role, has been put forward by some authors (Nesbit and Lapides, 1948; Tang and Ruch, 1955). Remington and Alexander (1955) have distinguished active and passive properties of the detrusor muscle. They demonstrated that viscoelastic properties of smooth muscular organs are determined primarily by passive components of the tissue.

The objectives of the studies presented here can be summarized as follows :

1. To demonstrate again that the urinary bladder has viscoelastic properties.
2. To describe quantitatively the physical properties of the bladder wall in the collection phase by means of a passive model.
3. To study the influence of the active elements on passive behaviour.
4. To compare the present method with the classical cystometry with regard to its value in representing physical characteristics of the urinary bladder.
5. To propose a new cystometry method by which the physical properties of bladders can be quantitatively analysed. These properties are represented in parameters, based on passive features.

I. Cystometry

One of the methods of studying the possible pathologic behaviour of the bladder is cystometry. This technique measures the pressure-volume relationship of the bladder.

Changes of volume are realized by a liquid medium (Lewis, 1938) or a gas medium (Golji, 1956; Bradley et al, 1968; Oliver and Young, 1973). The bladder is filled continuously (Rose, 1927; 1947; Serralach, 1954) or by incremental filling (Denny-Brown and Robertson, 1933; Bauer, 1956; Simons, 1938; Nesbit and Baum, 1954; Ausems, 1957). The rate of filling is different from one center to the other. Therefore, the International Continence Society (1976) proposed the following terms for the rate of filling :

a) up to 10 ml per minute : slow fill cystometry,
b) 10-100 ml per minute : medium fill cystometry,
c) over 100 ml per minute : rapid fill cystometry.

Other authors (Alexander, 1971) measure volume changes under controled pressure.

I. 1. Some historical remarks

Cystometry was introduced by Mosso and Pellacani (1882). The method has been further developed during this century by Schwarz (1920), who introduced a cystometer for simultaneous recording of intravesical and intra-abdominal pressure. Denny-Brown and Robertson (1933) used an improved version for simultaneous and continuous recording of intravesical pressure, intrarectal pressure, movements of the abdominal wall and movements of the perineum. Catheters connected with pressure chambers, the pressure within which was transmitted by air to the membrane manometer. The device was too complicated for clinical use. Further modifications of the cystometry method have been described by Lewis and Langworthy (1938). The Lewis cystometer had one membrane manometer in connection with an intravesical catheter. The fluid was introduced via a Y-connection. Pressure was registered against time. In more recently developed urodynamic devices, the cystometer is incorporated in an apparatus with which the whole bladder-urethral complex is investigated (Miller, 1971). During filling, voiding and cessation of voiding, the following information is presented :

— size, shape and movement of the bladder and urethra;

— vesical pressure and «intra-abdominal pressure»;
— difference between these two pressures (detrusor pressure);
— intra-urethral pressure;
— difference between vesical and urethral pressure (closure pressure);
— perineal muscular activity.

Pressure and volume are registered against time. The shape of the classical cystometrogram (pressure-volume curve) is generally divided into three segments (Fig. 1) :

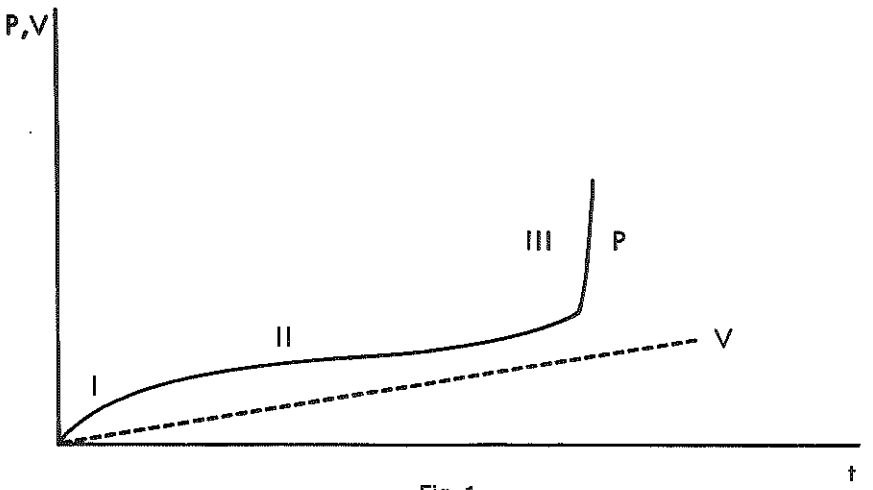


Fig. 1
Pressure and volume curves are registered against time. The pressure curve is divided into three segments : initial pressure rise (I), initial limb (II) and ascending limb (III).

Segment I : initial pressure rise from zero level to the first inflection,

Segment II : initial limb, begins at the first inflection and ends at micturition contraction or, in the absence of micturition, continues into segment III,

Segment III : ascending limb during micturition or, in the absence of micturition, during terminal pressure rise.

I. 2. Objectives of cystometry

The objectives of cystometry can be divided into two major groups :

a) the investigation of neurophysiological properties of the bladder,

b) the investigation of intrinsic properties of the bladder wall. Cystometry has become a useful test in determining the presence of a neurogenic bladder. Variations of the shape of the curve, combined with the presence or absence of spontaneous detrusor contractions, perception disturbances and bladder capacity lead to several classifications of neurogenic bladders (Munro, 1936).

Other authors developed tests to investigate various reactions of the bladder to drugs, the betanechol chloride test for example (Lapides et al, 1962; 1963). In clinical urology, however, cystometry in itself seldom supplies a diagnosis of neuromuscular detrusor dysfunction.

The degree of reproducibility is low, even in normal persons. This is particularly true in these patients who have myogenic disorders of the bladder wall. To determine intrinsic bladder wall properties, the shape of the cystometrogram is often used. Some authors (Emmett and Love, 1968) have noticed the difficulty in interpreting classical cystometrograms in this context, for example in individuals with urinary retention without neurologic symptoms. Many authors, however, refer to the forms of the cystometrogram as a representative for tone (Tang and Ruch, 1955; Ausems, 1957). The confusion in the literature concerning the usage of the term «tone» will be discussed in the following section.

I. 3. Tone and adaptation

The prolonged, nearly flat segment II of the cystometrogram is often referred to as the tonus limb (Sabetian, 1965; Oliver and Young, 1973). The conception of tone as applied to the bladder was introduced with the observations of Mosso and Pellacani (1882). They were able to record pressure-volume curves in both humans and dogs and observed :

a) that the bladder held different volumes under one and the same pressure;
b) that it had the ability to accommodate to increasing volumes of fluid with little rise in pressure.

This characteristic was ascribed to a change in tone. This implied an active mechanism, the same as that which produces bladder contractions. Denny-Brown and Robertson (1933) extended this interpretation. They filled the bladder with 100 ml successive increments and observed a slight fall of pressure during the

intervals. This pressure-decay under isometric conditions indicates the existence of a process of adaptation, which increases with increasing volume. Increase of the filling rate results in an increase of the «reactionary» rise of pressure. The authors stated that the cystometrogram reflects an interplay between an excitatory reflex (tone) and an inhibitory reflex (adaptation). Since the bladders of patients with lesions of the cauda equina also show the adaptation phenomena, they concluded that tone is the result of neural activity in the plexus of the bladder wall. The neurogenic origin of bladder tone was further advocated by Langworthy and Kolb, 1933; Serralach, 1954; Ausems, 1957; Grasset, 1961 and Rose, 1961.

However, Nesbit and Lapides (1948) stated that bladder tone is independent of neurogenic reflex control. They found that in both human subjects and dogs, the ability of the bladder to maintain an even low pressure at increasing volume remains unchanged after administration of tetra-ethyl-ammonium chloride, a ganglion blocking agent, the micturition reflex being suppressed.

This observation has been very important to better understand different interpretations of changes in tone as mentioned in the literature. A first distinction is to be made, indeed, between the shift to the right or the left of the micturition threshold, and a second one concerning the steepness of the second segment. A shift to the left of the micturition threshold has been interpreted as hypertonicity e.g. by Langworthy and Kolb (1933). They suggested that a reflex center for bladder tone is situated in the Barrington's pontine center. This center is normally under control of the cerebral cortex. Reflex micturition at a smaller volume than before, by removal of the central influence, was interpreted by them as increased bladder tone. Tang and Ruch (1955) clearly demonstrated that the steepness of the second segment was not influenced by sections of the neural axis at different levels. Supra spinal transsection, spinal transsection, sacral ganglionic blockade by tetra-ethyl-ammonium chloride, pentobarbital anaesthesia or even death cause no change in the steepness of the tonus limb. Tang and Ruch (1955) concluded that the nervous system has no influence of bladder tone as represented by the steepness of segment II of the cystometrogram. They postulated that, if bladder tone is non-neural in origin, the second segment may reflect the physical state of the bladder. Hypotonicity, then, is caused by overdistention of the bladder wall. The degree of hypotonicity being a function of the degree of stretch and the length of time the bladder is kept distended (Nesbit and Lapides, 1948). Hypertonicity, characterised by a steepness of the tonus limb, is also a consequence of the physical state of the bladder.

To illustrate this idea, Veenema et al (1952) performed a series of studies on empty bladders of dogs. In one batch of dogs the ureters were divided and exteriorized, and in another batch these workers carried out the same manoeuvre, in addition to the division of the preganglionic parasympathetic nerves. The volume-pressure curves of the empty innervated bladders were the same as those of the empty bladders in which the preganglionic parasympathetic pathways had been cut.

If tone represents intrinsic properties of the bladder wall, the question arises as to whether tone is caused by a sustained contraction of smooth muscle or a prolonged muscle relaxation, or by pure physical phenomena. The observations of Remington and Alexander (1955) produced some clear insights. When studying the stretch behaviour of kitten bladders, they observed that the pressure in the bladder lumen is dependent on the rate of stretch, and that a decrease of the pressure takes place in the course of time under constant volume. They stated that the behaviour of smooth muscle organs bears a definite but incomplete resemblance to the behaviour of some of the simpler mechanical models as springs and dashpots. The elastic properties of the bladder are qualitatively the same in the living animal and after the bladder has been killed by application NaCN. They concluded, therefore, that the elastic properties of the tissue are determined primarily by passive components which are still present in dead tissue. When measuring pressure-volume curves on bladder stretch, a great part of the recorded pressure will originate from passive elements. Tone is defined by Alexander (1957) as that portion of the pressure which is produced by sustained active contraction of the muscle, which leaves one with the problem of defining tone in mechanical terms. Since passive mechanisms at least play a role in the pressure-volume relationship, especially as seen in the initial limb (segment II) of the cystometrogram, further research was necessary to understand these passive properties better and to evaluate the influence of the active mechanisms on this passive behaviour.

The purpose of this study is to obtain more insight in the physical properties of the bladder. This may enable us to provide information leading to a more exact interpretation of pressure-volume relationship and thus to better understanding of disorders such as extrophia vesicae, overstretched bladders, fibrotic bladders and the consequences for the bladder of obstructive or neurogenic pathology.

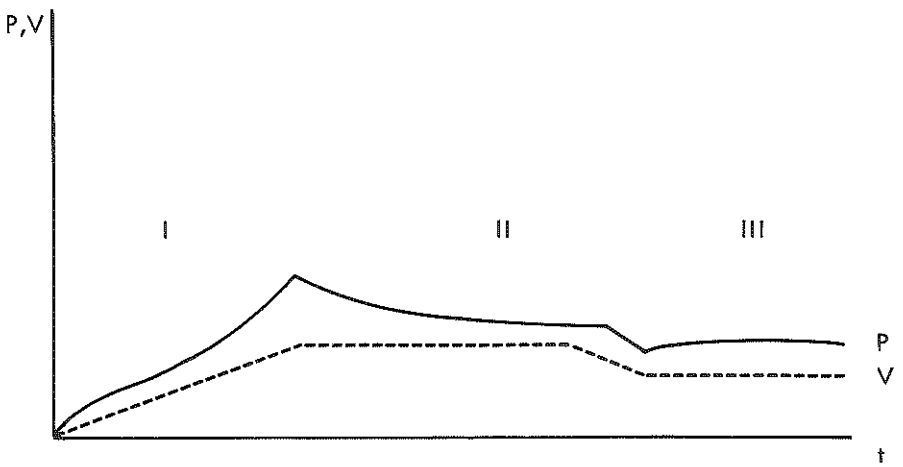
II. Qualitative mechanical properties of the urinary bladder

II. 1. Rate dependency of bladder pressure during stretch

When a certain amount of saline is infused into the lumen of the urinary bladder at a fast rate, the intraluminal pressure rises rapidly during filling. The height and form of the pressure curve are, among other things, dependent on the rate of filling, that is the rate with which the bladder wall is strained. This rate dependency can be illustrated by filling the same bladder at different infusion rates. At a slow infusion rate, the pressure rise will be very small. When the infusion rate is very fast, the pressure curve will be steep and the pressure peak will be markedly higher (Fig. 2). The shape will further be influenced by the speed of the registration.

It can be understood from this rate dependency that the slope of the tonus limb can become either flat («hypotonic») or steep («hypertonic»), depending on the infusion rate. For this reason it was proposed in the urodynamic literature that infusion rates be standardized. However, the strain rate dependency implies that when a small bladder and a large bladder are both filled at the same infusion rate, the pressure rise will be steeper and higher in the small bladder than in the large one.

So if the slope of the initial limb is to be representative for the physical properties of the bladder wall, one has to stretch the bladder wall with the same relative changes in length $\frac{l - l_o}{l_o}$ per unit of time, which is impossible in practice.



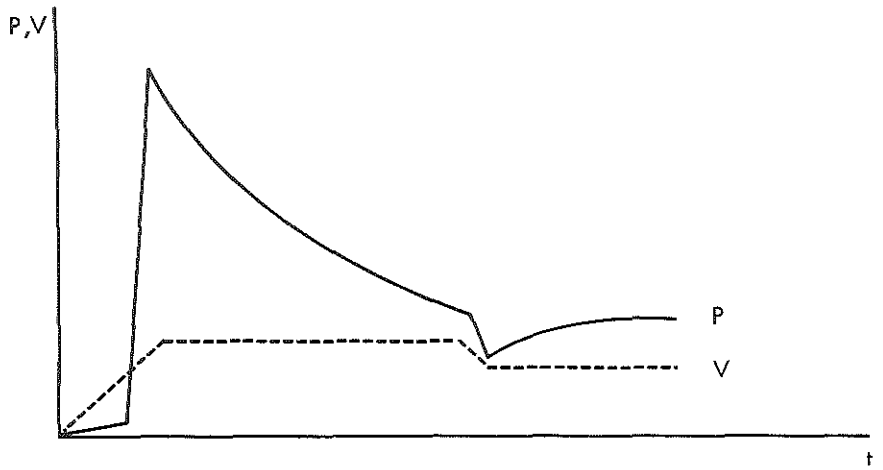


Fig. 2
The same volume is infused into the lumen of the urinary bladder with different rates of filling. The height and form of the pressure curve are dependent on the rate of filling (I). As soon as the filling is stopped the pressure decreases in relation to time (II). In withdrawing a certain amount of fluid, the intra-vesical pressure declines rapidly, which is followed by a rise of the pressure as soon as the withdrawal of fluid has been stopped (III).

The rate dependency has already been mentioned by Denny-Brown and Robertson (1933). They concluded that acceleration of the rate of filling increases the reactionary rise of pressure by means of an excitatory reflex. Whether this rate dependency is caused by an active contraction or by passive mechanisms can be investigated by examining the behaviour of polymers and dead tissue. In interpreting the gross behaviour of an organ, one comes to rely ultimately on the physical-chemical nature of its proteins and upon the nature of the mechanical behaviour of polymers in general. The rate dependency of polymers has been described by Alfrey and Gurnee (1957). The studies of Remington and Alexander (1955) imply that it is not necessary to accept neurogenic mechanisms or active muscular mechanisms to explain rate dependency qualitatively. Successive stretches were applied on kitten bladders during life and 18 hours after death by cyanide. The same general pattern of pressure curves was obtained.

However, more recent investigations on electrical activity of smooth muscle tissue during strain seems to point to the fact that active mechanisms are involved.

Bülbring (1955) described spontaneous electrical and mechanical activity in taenia coli. The slow depolarisation waves were correlated with a higher frequency of spike potentials and a higher tension.

Ursillo (1961) investigated the electrical activity of single cells in the smooth muscle of isolated pelvic nerve bladder strip preparations from rabbits. Spontaneous electrical activity was investigated under isometric conditions. The preparations exhibited spontaneous spike potentials associated with slower waves of depolarisation. When the bladder tissue is strained, tension rises, membrane potential decreases and frequency of the spike potentials increases.

Nevertheless, from the literature it can be concluded that membrane potential and tension are not always correlated. Calcium free conditions abolish mechanical responses in depolarized taenia coli (Axelsson and Bülbring, 1959). On the other hand, chemical stimuli such as acetylcholine (Edman and Schild, 1961) and carbachol (Durbin and Jenkinson, 1961) may cause changes in tension in depolarised tissues. So it is not known whether stretch generates an electrically induced contraction or not.

II. 2. Time dependency of the bladder pressure at constant stretch

The time dependent behaviour of materials can be examined under isometric (at constant length) or isotonic (at constant load) conditions. The most basic of the time dependent properties in isotonic conditions is that called «creep», i.e. the increase in deformation which occurs over the course of time under constant load (Fig. 3). On the other hand, in isometric conditions, the

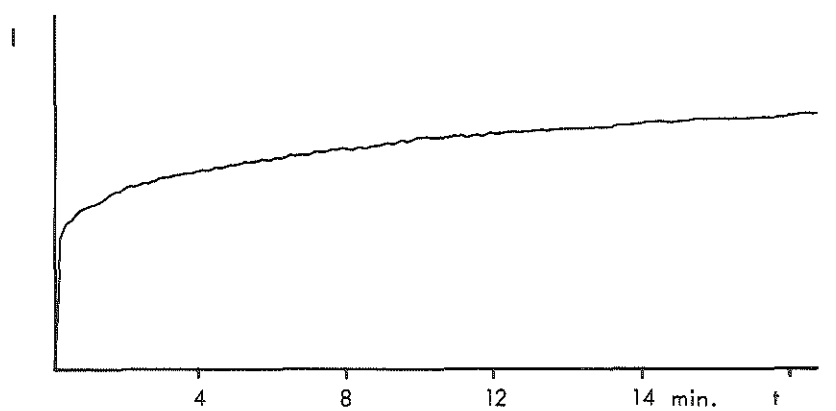


Fig. 3
Time dependent behaviour of a bladder wall strip at constant load. The increase in deformation which occurs over the course of time in isotonic conditions is that called «creep».

decrease in stress which takes place in the course of time under constant strain will be called «stress decrease» in this context (Fig. 2).

Creep and stress decrease have been observed in almost every tissue of the body and in nearly all materials such as rubber and polymers in general (Alfrey and Gurnee, 1957).

Denny-Brown and Robertson (1933) filled bladders with successive increments of 100 ml and noted a slight fall of pressure during the intervals. This so-called adaptation was definitely greater at higher volumes. Since then, it has been shown repeatedly, by Remington and Alexander, 1955; Kondo et al, 1972 and Coolsaet et al, 1972; 1973, that when a volume has been introduced into the cavity of the bladder and then kept constant, the intraluminal pressure that has been built up decreases slowly. In the literature this process has been called «tension decay», «stress relaxation», «stress decay» and «stress decrease».

Some authors (Ausems, 1957; Grasset, 1961; Rose, 1961) consider pressure decay after small increments of fluid as an expression of «tone».

Pressure decrease is slow, in the «hypertonic» bladder, in the «hypotonic» bladder it is fast, which is interpreted as maximal adaptation. Other observations point to the influence on stress decrease of smooth muscle. Zatzman et al, (1954) performed experiments to study the time course of stress decrease in arterial structures. The amount of stress decrease was dependent on the amount of smooth muscle in the tissue.

The electrical changes during stress decrease have been examined as well. Ursillo (1961) recorded intracellular electrical changes and changes of force at constant strain during 45 minutes. While tension decreased as a function of time, the membrane potential rose just to the original prestretched value. Bozler (1963; 1975) stretched smooth muscle tissue (retractor muscle of the snail) to 15 % of its length (undefined). The stress produced by stretching was followed by a stress decrease at constant length. When the muscle was subsequently electrically stimulated to produce an isometric contraction, the time-course of stress decrease agreed with that of stress decrease after stretch. Remington and Alexander (1955) demonstrated on kitten bladders that the pressure decrease was similar in the living and dead bladders. They emphasized that the time-dependent behaviour may not be related to the functional active contractile elements, but rather to the passive structures still present in dead tissue.

Jordan Utrecht (1938) and in more recent literature Apter and Graessley (1970) and Coolsaet et al (1972; 1975) described stress decrease in terms of viscoelasticity. This will be discussed in section 4.

II. 3. Time dependency of the bladder pressure after stretch release

When a certain amount of fluid is withdrawn from the strained bladder, the intravesical pressure declines rapidly. This is followed by a rise of the intravesical pressure as soon as the withdrawal of fluid has been stopped (Fig. 2). This «recovery» process is seen even if only a small amount of the volume is reduced, so that a positive strain continues.

This phenomenon is also observed in bladder wall strips, dead bladders in toto and rubber balloons.

Alexander (1956) observed this recovery process of pressure in experiments with mesenteric veins. He called this aspect of the recovery process «reversal of delayed compliance». The process turned out to be quantitatively dependent on the contractile state of the veins. The more contracted the veins, the more recovery of the pressure was observed.

Since this recovering process was also observed in dead tissue, devoid of active processes, it can be inherent in passive structures, influenced by contractile elements.

II. 4. Conclusions

The shape of the pressure curve depends on the rate with which the bladder is strained, i.e. the relative changes in length which occurs per unit of time. At constant strain the pressure decreases in relation to time. After strain release, the pressure rises in relation to time (Fig. 2). This behaviour of the urinary bladder can be understood qualitatively by passive processes.

From experiments performed by Alexander (1957) there is evidence that smooth muscle activity is capable to alter the passive behaviour of smooth muscular organs. The interaction between passive and active processes has important patho-physiological importance, particularly in regard to changes of tone as defined by Alexander (1957). Quantitative determination of this interaction will be discussed in section IV. 3. 6.

Factors such as rate- and time- dependency seriously interfere with the interpretation and comparison of classical pressure-volume measurements.

III. Complexity of problems in trying to make pressure curves of bladders comparable

III. 1. A certain volume does not correspond always to the same pressure in the same bladder

Several factors influence the pressure-volume relationship.

III. 1.1. Hysteresis

Hysteresis between stress and strain is found when the response of an element is not only defined by the actual starting situation, but also by the preceding conditions. When a certain volume is injected into the lumen of the urinary bladder with a constant rate and then withdrawn at the same rate, the pressure-volume curve fails to follow identical paths. The result of this failure to retrace the same path on withdrawal as on injection is the formation of a hysteresis loop (Fig. 4). When the same volume

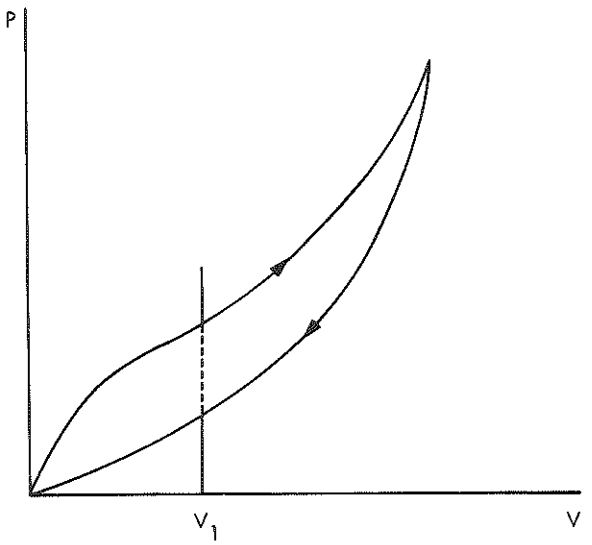


Fig. 4
Pressure-volume curve during volume injection and withdrawal : hysteresis loop. The pressure at volume V_1 is higher during filling as during withdrawal.

inputs and withdrawals are made successively, the pressure rise and the surface of the obtained loops decrease during the four

first volume changes until a relative stability is obtained for the following volume changes (Fig. 5).

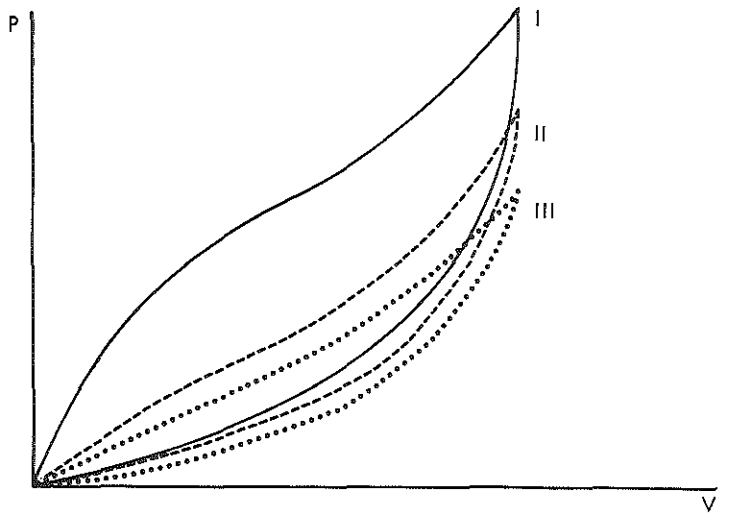


Fig. 5
Pressure-volume curves obtained during successive injections and withdrawals of the same volume. The pressure rise and the area of the obtained loops decrease during the first volume changes.

During the infusion, the pressure response depends on the rate at which the volume is infused, that is, the rate at which the bladder wall is strained. So, when a large and a small bladder are filled at the same infusion rate, the cystometrogram of the small bladder will show a steeper rise of the pressure curve in segment II than that of the large one, since the strain, i.e. the relative change of length per unit of time, will be higher in the small bladder than in the larger one. In spite of possible equal wall properties, the small bladder may demonstrate a «hypertonic» cystometrogram and the large bladder a «hypotonic» one. The differences in both curves, however, can be sufficiently understood by the influence of passive mechanisms and, so far, have no relationship with tone as related to active properties.

It can be noticed that, when a certain volume is brought into the bladder and kept constant, the pressure will increase or decrease depending on the preceding condition of volume (Fig. 6). When a volume level «a» is preceded by a lower volume «o»

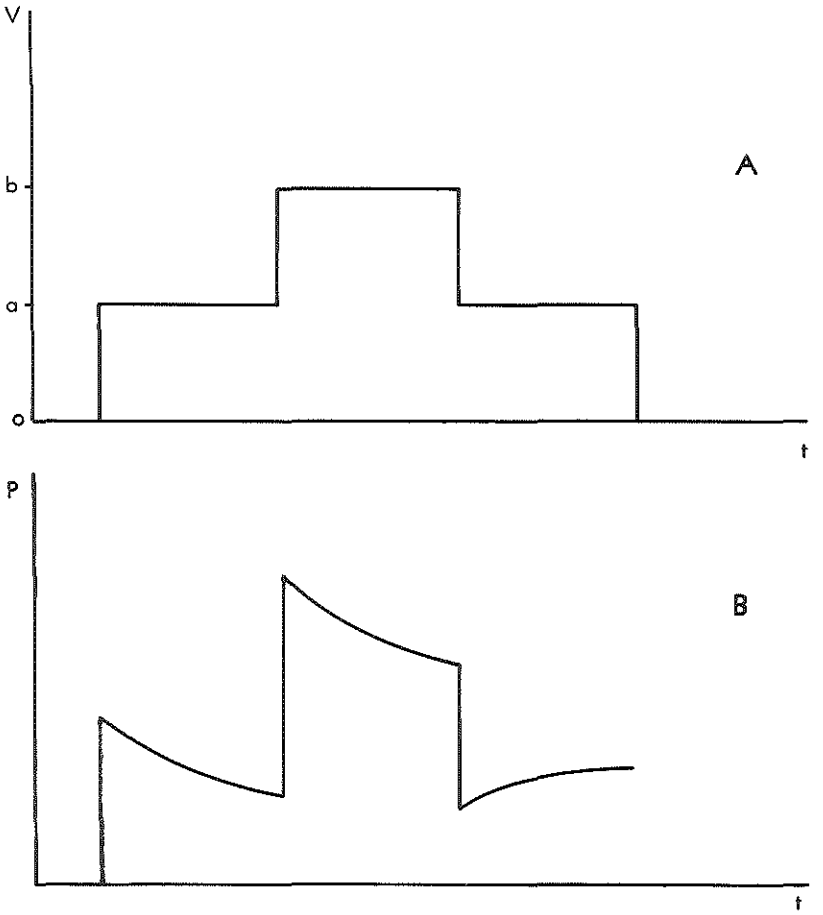


Fig. 6
The pressure (B) increases or decreases depending on the preceding condition of volume (A). When a volume level a is preceded by a lower volume 0, pressure decreases during time at a constant volume a. When this volume a however, is preceded by a higher volume b, pressure increases at the volume level a.

the pressure wil decrease with time at a constant volume «a». When this volume «a», however, is preceded by a higher volume «b», the pressure will increase at the volume level «a».

III. 1.2. The state of the contractile elements

Muscle activity can produce changes in length of the tissue (Alexander, 1957) which influence the capacity of the bladder. Also, it makes the tissue stiffer and it stress-relaxes more slowly (Apter et al, 1972).

III. 2. The stretch volume (V_0) of the bladder

The stretch volume of the bladder is defined as the maximum volume that a bladder can contain without strain.

The problem can be illustrated by comparing the pressure curves obtained while filling a rubber balloon and a urinary bladder. When a balloon is filled, while hanging in a water reservoir, there is a sharp mark on the pressure curve where the wall of the balloon changes from a stretched into a strained situation (Fig. 7). The volume at which this sharp change in the pressure curve

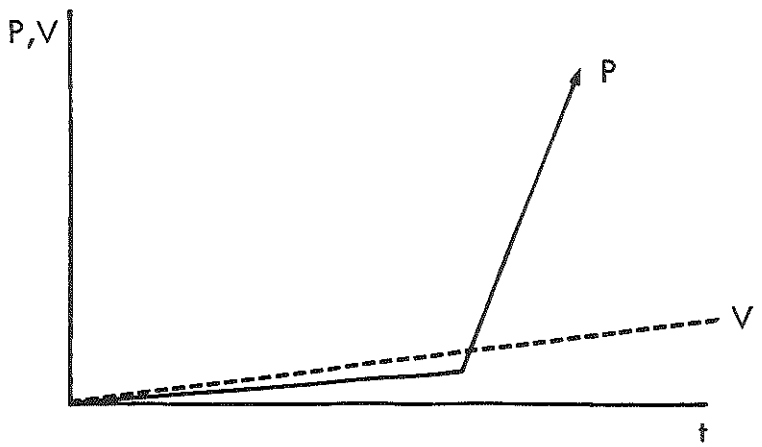


Fig. 7
Pressure and volume curves obtained in a rubber balloon. There is a sharp mark on the pressure curve where the wall of the balloon changes from a stretched into a strained situation.

takes place can be considered as the stretch volume (V_0). When two balloons with the same degree of vulcanisation are equally strained from this volume, equal curves will be obtained. Filling the bladder in the same way as a rubber balloon, there is no mark in the pressure curve where the bladder wall starts to strain (Fig. 8). Even in dead bladders of bladder wall strips, the curve shows a smoothly increasing rise. The fact that it is impossible to determine the stretch volume of the bladder makes it impossible to strain different bladders in proportionally the same way. This is one of the reasons why classical cystometrograms are not comparable. In the beginning of the filling, the small bladder will be strained by an intraluminal volume, by which the wall of the large bladder is not even stretched. So the curves of a strained and a flaccid bladder are wrongly compared. Furthermore, when the stretch volume is unknown, it is impossible to calculate the infusion rate needed for each bladder to realize the same rate of

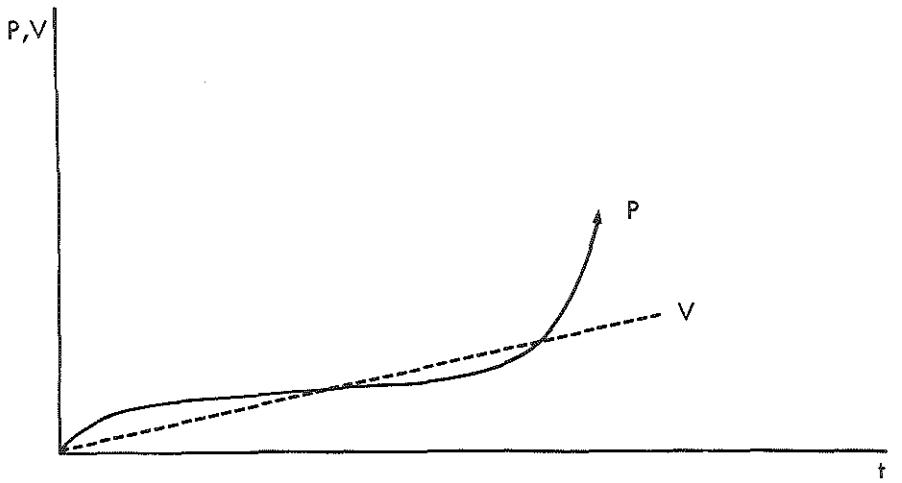


Fig. 8
Pressure and volume curves obtained in a urinary bladder. Filling the bladder in the same way as the rubber balloon in Fig. 7, the curve shows a smoothly increasing rise.

strain on bladders which differ in size. Also for these reasons it is insufficient to standardize infusion rates.

The reason why the pressure curve is rising smoothly instead of showing an angular curve, is not completely understood. From the passive properties which will be analysed in section 4, it may be concluded that it is possible to strain the bladder wall very slowly with a small rise of the pressure. In addition, when a bladder is observed during filling, it may be ascertained that some parts of the bladder are stained before others, which may contribute to the smooth transition of the pressure curve.

The sharp mark in the pressure curve obtained on rubber balloons can also become indistinct, by placing the balloon directly on the table for example, or even more so, by fixing an elastic strip over the balloon (Fig. 9). In this manner it can be shown that small changes in the circumstances can make it impossible to determine the stretch volume even in rubber balloons.

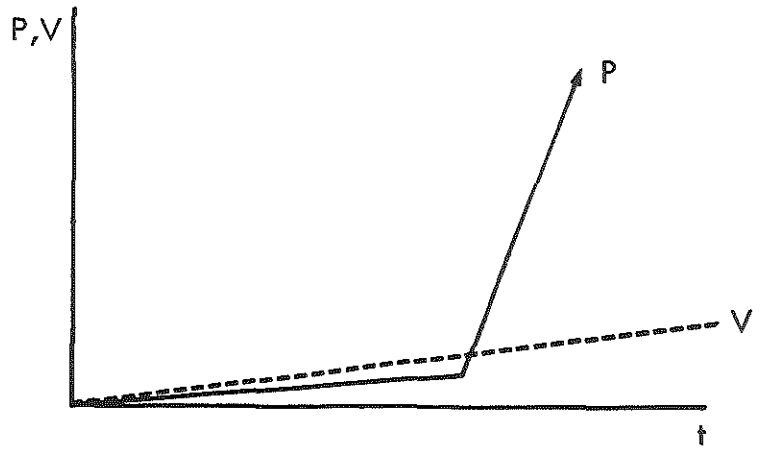


Fig. 9

A

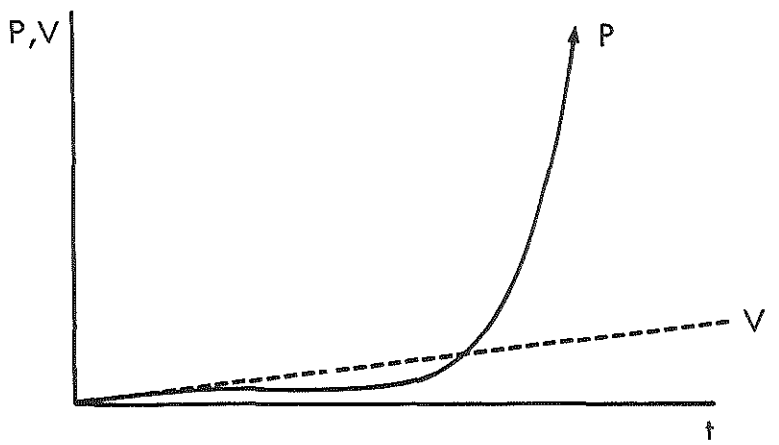


Fig. 9

B

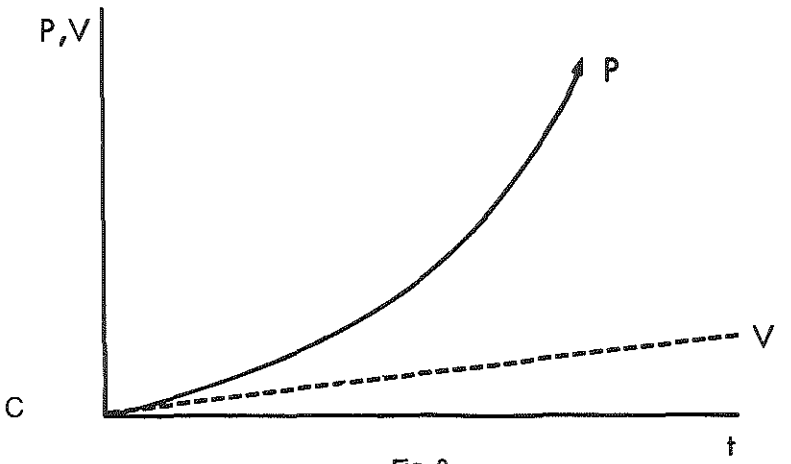


Fig. 9
Pressure and volume curves on rubber balloons under different conditions.
A : balloon hanging in a water reservoir.
B : Balloon placed on the table.
C : Elastic strip fixed over the balloon.

III. 3. Influences of surrounding organs

The urinary bladder is surrounded by the intra-abdominal organs. The intra-abdominal pressure may be transmitted to intravesical lumen. This difficulty may be avoided by subtracting the intra-abdominal from the intravesical pressure. So, the detrusor pressure is obtained. In experimental situations, the intra-abdominal organs can be retracted.

III. 4. Psychogenic influences

Mosso and Peilacani (1882) stated that emotion and intellectual work are associated with bladder contractions. Straub et al (1949) studied pressure changes with isometric cystometry during psychiatric interviews. Emotions such as repression were associated with bladder hypoactivity, while anxiety or agression caused hyperactivity. Psychogenic bladder disturbances can be avoided by performing the cystometry under general anaesthesia, by which also reflex mechanisms are eliminated.

IV. Quantitative viscoelastic properties of the urinary bladder

From the study of the qualitative physical properties of the bladder it may be concluded that it is very difficult to make classical cystograms sufficiently comparable in view of the different strain which is applied to bladders differing in size, and also because of the great many other influences, as a result of which intraluminal pressure is built up. In order to describe the physical properties of the bladder, a different cystometry method is needed, whereby more reliable quantitative parameters independent on the stretch volume are obtained. For this purpose an approach based on the physical properties of the system has been applied.

IV. 1. Definitions

Elasticity is that property of a material which determines the tendency of the stressed material to return to its unstressed geometrical configuration (Landowne and Stacy, 1957). For the deformation of an elastic material a stress is needed, depending on the kind of material. The size of the stress depends only on the size of deformation, which disappears immediately on removal of the stress. Generally, this relationship between stress and strain can be expressed in a formula :

$$S = f(\varepsilon) \qquad (1)$$

where S means stress (force per unit area in N/m^2) and ε is strain, i.e. the relative deformation produced by the application of stress :

$$\varepsilon = \frac{l - l_0}{l_0} \qquad (2)$$

l is the length of the stretched material in m and l_0 the unstretched length of the material in m.
Lineair elasticity is described by Hooke's law :

$$S = E \,.\, \varepsilon \qquad (3)$$

where E is the elastic modulus [N/m^2], defined as the ratio of stress and strain.

Mechanically a lineair elastic material can be represented by a coiled ideal spring.

Viscosity, is that property of a material which tends to retard deformation of the stressed material (Landowne and Stacy,

1957). For the deformation of a viscous material a stress is needed, depending on the kind of the material, the size of which depends on the rate of deformation. Deformation continues during the application of stress and remains unchanged when the stress disappears.

This behaviour can be represented by the following formula :

$$S = f(\dot{\varepsilon}) \quad (4)$$

$$\dot{\varepsilon} = \frac{d\varepsilon}{dt} (sec^{-1}) \quad (5)$$

A linear form of viscosity is described by Newton's law :

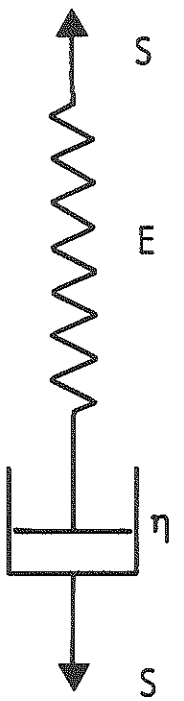
$$S = \eta \dot{\varepsilon} \quad (6)$$

Where η is the viscosity modulus, defined as the ratio of stress and the rate of strain.

Mechanically, a viscous material can be represented by an ideal dashpot.

Viscoelasticity is a combination of both, it means that the energy which is applied to strain such a material is partially conserved (elastic) and partially dissipated (viscous). Viscoelasticity can be represented by a combination of a spring (E) and a dashpot (η) in series (Maxwell element) or in parallel (Voigt element) (Fig. 10).

A



B.

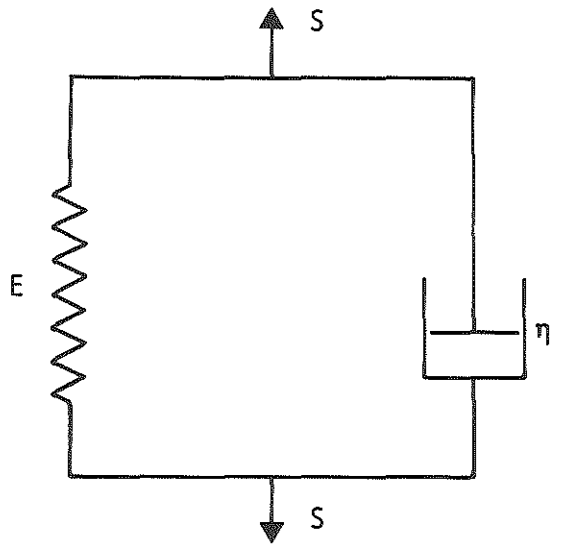


Fig. 10
A - Maxwell element : spring and dashpot in series.
B - Voigt element : spring and dashpot in parallel.

All body tissues show time-dependent behaviour. Some tissues possess relatively high elastic moduli (such as tendon) and others possess low elastic moduli and usually contain contractile elements (for example the bladder).

The bladder wall contains :

— collagen, which has a high elastic modulus and shows hardly any rate dependency of stress during strain;
— elastin, which is viscoelastic and has a more rate dependent behaviour;
— smooth muscle, which may creep to several times its unloaded length, while the stress developed in it during constant strain may entirely disappear (Remington, 1957).

The physiologic background of these time dependent phenomena is not yet clear. Stress applied to a tissue brings about a change in length of the molecular chains (Stacy, 1957). The histological architecture of the elements plays an important role. Bull (1957) illustrated the importance of the microscopic structure of the tissue considering the mechanical features of a nylon tissue. A nylon thread at room temperature is not significantly extensible. When, however, nylon thread is woven into a tissue, the stress-strain curve becomes rate dependent and exhibits a hysteris loop.

IV. 2. Systems approach

IV. 2.1. The «black-box»

Since the intraluminal pressure depends on many factors (section 3), it is shown to be useful to apply a systems approach (Coolsaet et al, 1975). The bladder is considered as a «black-box» (Fig. 11) with an input and an output. The «black-box»

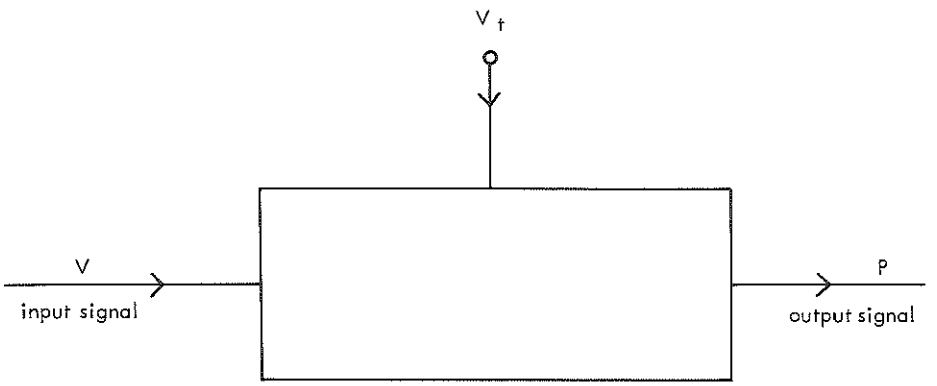


Fig. 11

The «black-box».

approach is often used in the study of complex processes. In attempting to understand and describe the behaviour of the «black-box», in this case the bladder, one is not interested in the structure of the box and what happens inside. A signal, in this case a volume signal, is introduced into the «black-box» and another signal, in this case a pressure signal comes out of it. Without considering either the amount of collagen, elastin and smooth muscle or the architecture of these elements, or the influence of active processes on these elements, the relationship between in- and output can be studied. In the course of further description, the «black-box», will be split up into other different «black-boxes», and will become more «white».

IV. 2.2. The input signal

The change in volume has to be known quantitatively. The strain of the wall can be calculated from the volume change. This can be realized in different ways, for example as a step- or a ramp function (Fig. 12).

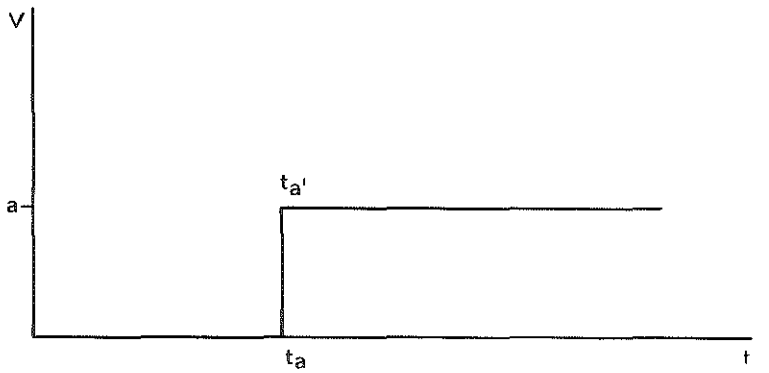


Fig. 12 - A
The volume is infused in an infinitely short time ($t_a = t_{a'}$) : mathematical step function.

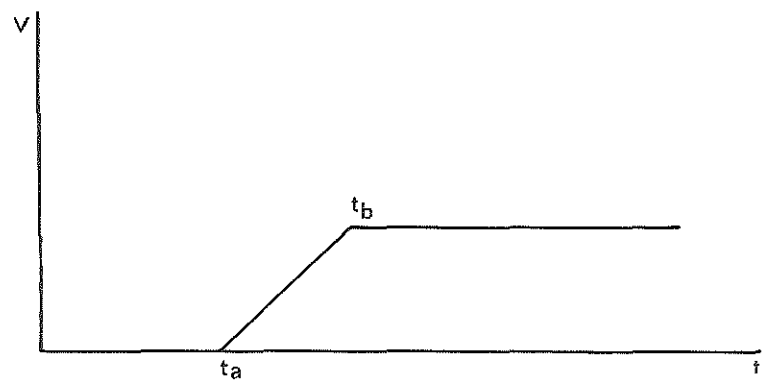


Fig. 12 - B
The volume is infused with a known constant rate during a time ($t_b - t_a$) : ramp function.

A mathematical step function implies that the volume is infused in an infinitely short time ($t_a = t_{a'}$). In practice, such infusion rates are not realisable. There is always a certain time needed to infuse the fluid.

When one is interested in the processes during strain, the fluid can be infused at a known rate during, for example, a time $t_b - t_a$ (Fig. 12). This is called a **ramp function.**

Since we are interested in what happens after the fluid has been introduced at a fast rate, a steep ramp is used. This is called a **physical step function.** When the ramp function is known, a correction can be applied in the theoretical model for correction to a mathematical step.

IV. 2.3. The output signal

As a response to a physical step function of the volume change, the pressure in the lumen of the urinary bladder will rise very fast during strain. As soon as filling is stopped, pressure begins to decrease. The pressure decrease curve can be analysed into two parts, a part where the pressure is a function of time, and a part where there is almost no change in pressure (Fig. 13).

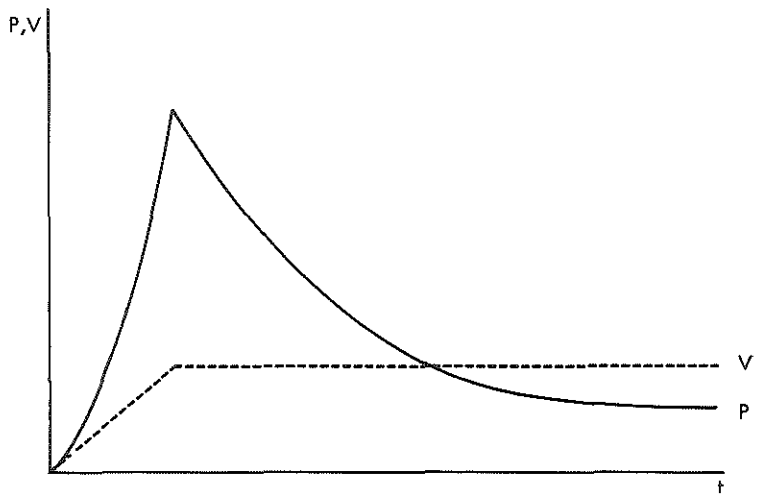


Fig. 13
Pressure curve as a response to a physical step function of the volume change. The pressure decrease curve can be divided into a part where the pressure is a function of time and a part where there is almost no change in pressure.

For this stepwise straining method, the rate dependent properties already described are used to evoke a dynamic and analysable response in that part of the cystometrogram where pressure is nearly constant during the classical cystometry (Fig. 14). A pressure P_1 is registered during slow filling of the bladder at an intraluminal volume V_1. The pressure P_2 registered when the same volume V_1 is infused stepwise will be much higher. The high peak pressure P_2 will be followed by an analysable pressure decrease curve to the P_1 level.

In the following chapter, we will try to analyse quantitatively the stress decrease curve after a stepwise strain has been applied.

Two main questions are to be answered :

a) Can the stress decrease curve be described in a quantitative way which is clinically applicable, and

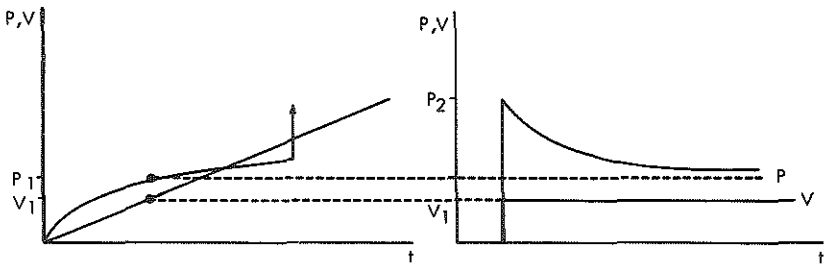


Fig. 14
The pressure registered in the bladder lumen is dependent on the speed with which the fluid is infused.
Following rapid infusion of a volume V_1 there occurs first a rapid increase in pressure and then an analysable pressure decrease curve (right) the same volume V_1, however, when infused slowly, produces only a slight pressure increase (left).

b) Since it is impossible to strain several bladders to comparable volume levels, are there any parameters which are independent of the strain level.

We shall first describe the analysis of the results of experiments performed in bladder wall strips.

IV. 3. Stepwise straining of bladder wall strips

The investigation of the quantitative viscoelastic properties of the bladder was started with studies on bladder wall strips. For the following reasons we choose for bladder wall strips instead of whole bladders :

a) The stress in the wall of bladders in toto cannot be measured directly, but must be calculated from pressure data. The relation between stress and pressure is determined by the bladder geometry, which is more complicated (Coolsaet et al, 1973).
b) It is easier to influence the contractile state of the tissue by means of locally applied drugs.
c) The measuring equipment can be kept more simple, while at the same time possibly disturbing effects such as originating from anaesthetics are avoided.

The traumatic effect caused by dissection of the tissue, however, results in a contracture, while the nonphysiologic environment may also effect the properties of the tissue.

IV. 3. 1. A mechanical and a mathematical model

To describe the viscoelastic behaviour of tissues, mechanical models have been used as analogous models, because of the ease with which such models were described and the apparent

similarity of the models with tissues (Apter and Graessley, 1970). The most simple mechanical model which produces a stress decrease curve after a stepwise elongation consists of a coiled spring and dashpot in series (Maxwell element, Fig. 10). When this element is elongated stepwise from l_0 to l and is kept at the length l, the stress increases very fast to a certain level (Fig. 15), after which a stress decrease follows.

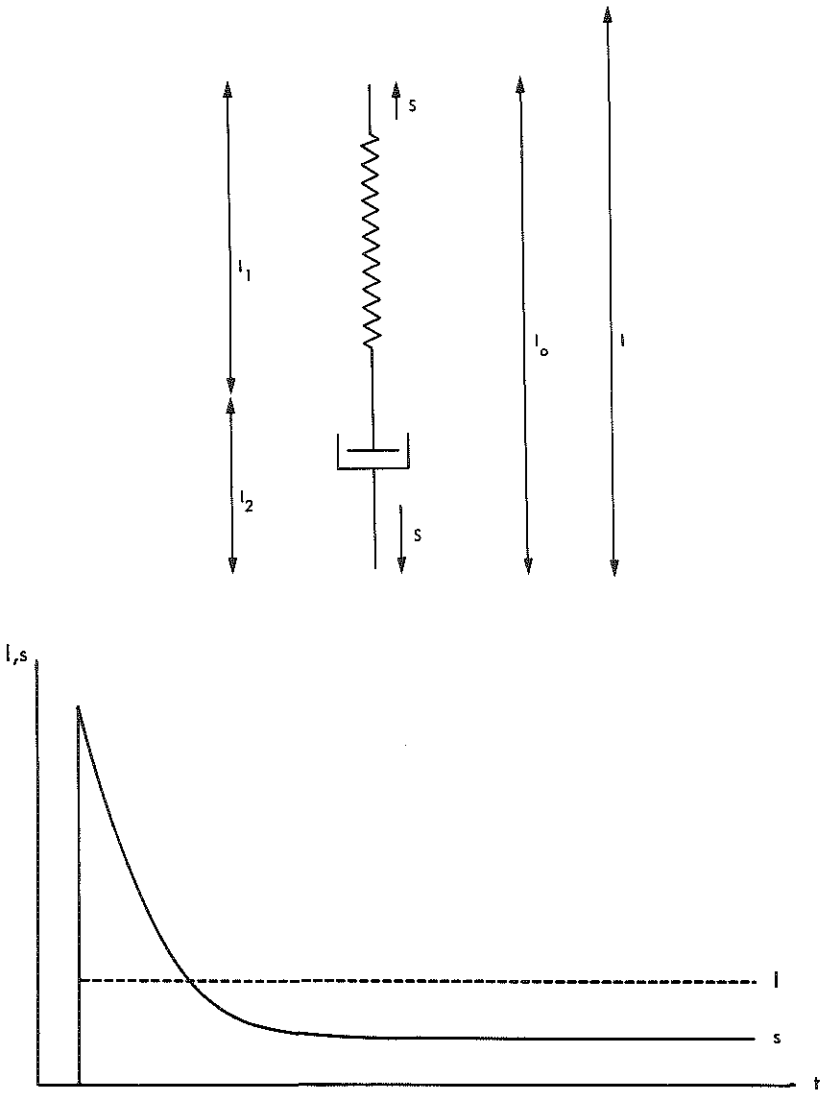


Fig. 15

Above : Maxwell element.
Below : Stress decrease curve following a stepwise increase in length by a Maxwell element (mono-exponential function).

The increase of the stress results from the elongation of the coiled spring. During rapid elongation the plunger of the dashpot follows slowly. When the maximal length l is reached, the plunger is pulled out by the force in the spring. As the dashpot elongates, the force in the spring decreases accordingly. In the case of bladder tissue, the stress decreases to a static value > 0. In the model, therefore, an extra spring in parallel to the Maxwell element is necessary. Hence, the simplest mechanical model which qualitatively represents stress decrease of the bladder after stepwise straining is a Maxwell element in parallel with a spring (Fig. 16). The stress decrease of a Maxwell element can be

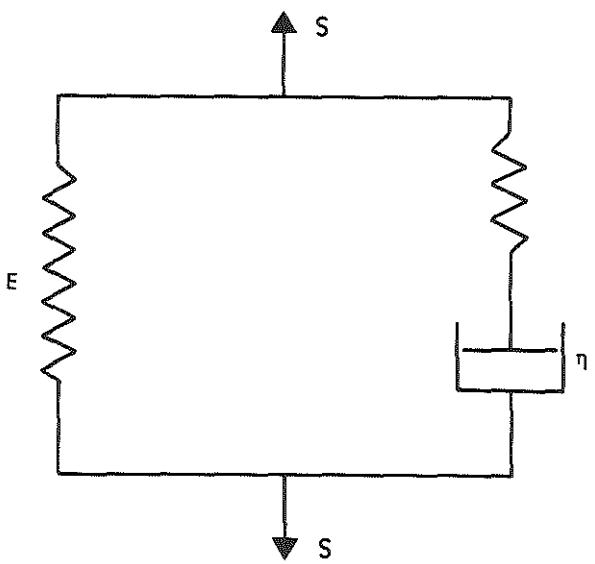


Fig. 16

Apter model.

described by one exponential function, which means that there is a constant relative percentage rate of the stress decrease.

For the spring we have :

$$S = E \cdot l_1 \tag{7}$$

S being the stress, l_1 the length of the spring and E the elastic modulus.

For the dashpot we have :

$$S = \eta \cdot \frac{d\, l_2}{d\, t} \tag{8}$$

S being the stress, l_2 the length of the dashpot and η the viscosity modulus.

Since :

$$l = l_1 + l_2 \tag{9}$$

We obtain :

$$\frac{dl}{dt} = \frac{dl_1}{dt} + \frac{dl_2}{dt} = \frac{dS}{dt} \cdot \frac{1}{E} + \frac{S}{\eta}$$

When l is increased stepwise and $t > 0$ we have :

$$\frac{dl}{dt} = 0 \tag{11}$$

It follows that :

$$\frac{dS}{dt} \cdot \frac{1}{E} = -\frac{S}{\eta} \tag{12}$$

Or :

$$\frac{dS}{S} = -\frac{E}{\eta} \, .dt \tag{13}$$

After integration we find :

$$S = C \, . \, e \tag{14}$$

C being a constant and e the base of the natural logarithm (2.72)

For t approaching O infinitely we have :

$$e^{-\frac{E}{\eta}.t} = 1 \tag{15}$$

So :

S = C, and since S = a.E (where a is the elongation, Fig. 12), we obtain :

$$C = a.E \tag{16}$$

This leads to :

$$S = a.E.e^{-\frac{E}{\eta}.t} \tag{17}$$

An exponential function ($S = A.e^{-\alpha t}$) plotted on a semilogarithmic scale results in a straight line (Fig. 17).

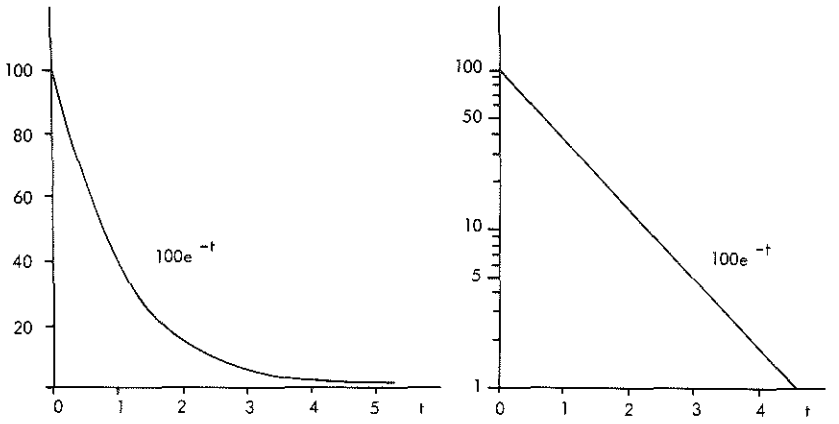


Fig. 17
One exponential function (left) displayed semi-logarithmically produces a straight line (right).

The stress decrease curve of bladder tissue can be plotted on a logarithmic ordinate for pressure, versus linear abcissa for time (Fig. 18). Since this curve is not a straight line, a single

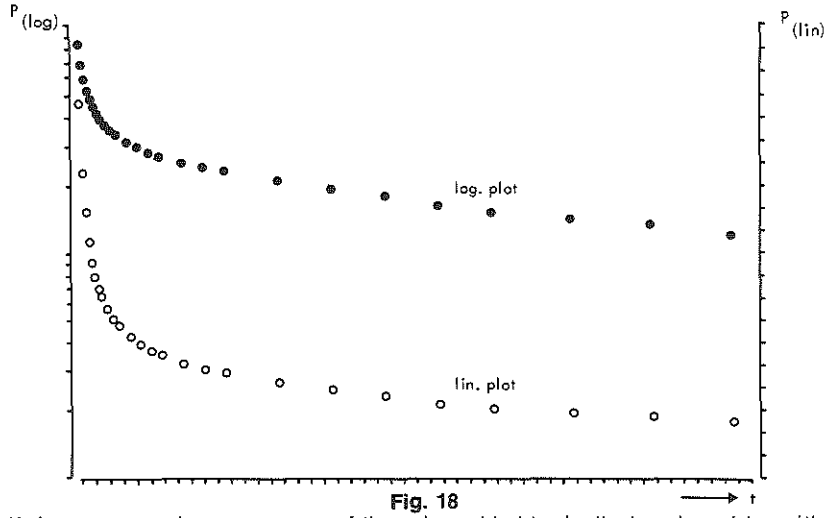


Fig. 18
If the pressure decrease curve of the urinary bladder is displayed semi-logarithmically, no straight line is obtained.

exponential function resulting from the elongation of one Maxwell element, cannot account for the stress decrease of bladder tissue.

In order to obtain an adequate quantitative description of the dynamic behaviour, we therefore have to extend the model by a combination of two more Maxwell elements coupled in parallel to obtain a multi-exponential model (Fig. 19).

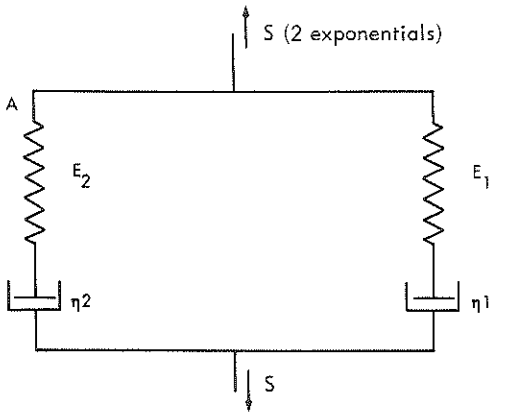


Fig. 19 - A
Two-exponential model (Two parallel Maxwell elements).

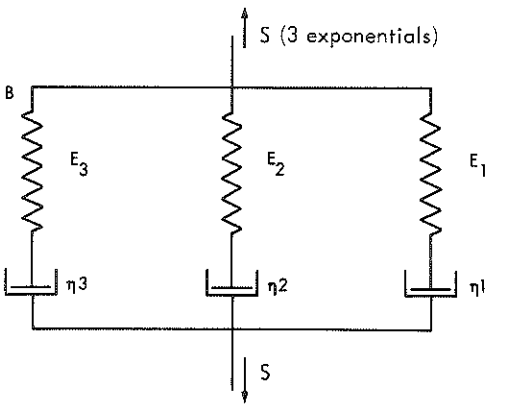


Fig. 19 - B
Three-exponential model (Three parallel Maxwell elements).

For two-exponential functions, the mathematical model is expressed by the following formula :

$$S = A.e^{-\alpha t} + B.e^{-\beta t} \qquad (18)$$

and for three-exponential functions we have :

$$S = A.e^{-\alpha t} + B.e^{-\beta t} + C.e^{-\gamma t} \qquad (19)$$

S being stress [N]; A, B and C the coefficients [N], α, β and γ the relaxation constants [sec^{-1}], t the time [sec] and e the base of natural logarithm (2.72).

The coefficients are determined by the immediate reactions of the system resulting from stretching the springs. The plungers of the dashpots will move out at constant length so that the force will decrease. The stress decrease rate is characterized by the relaxation constants. Since the stress decrease curve of bladder tissue does not reach zero level, an extra spring in parallel is still necessary to express the resting constant stress when all movements in the Maxwell elements are damped out and only the force over the parallel spring E_0 is measured (Fig. 20).

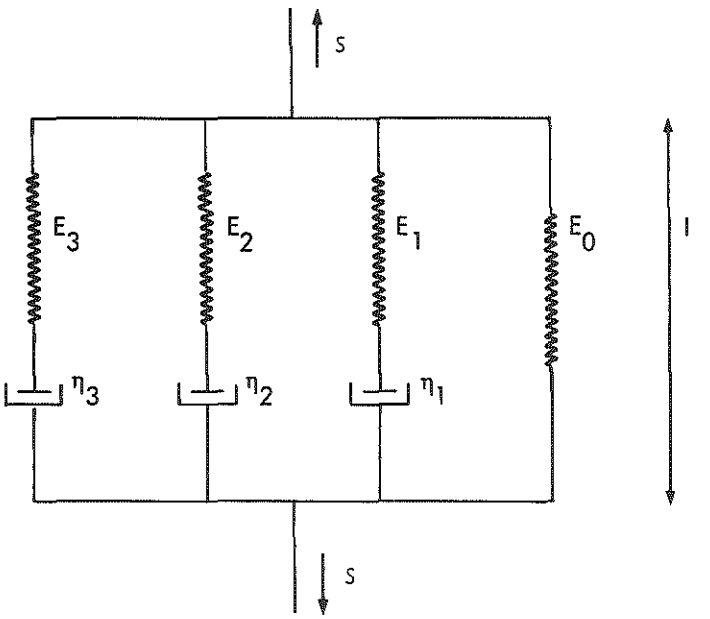


Fig. 20
Three Maxwell elements and a spring parallel.

The mathematical model including this static behaviour is represented by the following formula :

for the two exponential model :

$$S = A.e^{-\alpha t} + B.e^{-\beta t} + K \qquad (20)$$

and for the three exponential model :

$$S = A.e^{-\alpha t} + B.e^{-\beta t} C.e^{-\gamma t} + K \qquad (21)$$

The exponential function can be obtained from a stress decrease curve in various ways :

a) Extrapolation and subtraction.

The stress decrease curve is plotted on a logarithmic ordinate for stress and on a linear abcissa for time. Exponential functions are found by a manual method of extrapolation and subtraction starting with the constant and proceeding from the slowest to the fastest exponentials (Kondo et al, 1972 and Kondo and Susset, 1974). The slowest component is obtained from the slope of the linear tail portion of the semilogarithmic plotted stress decrease curve.

A first straight line through the straight portions is prolonged to the ordinate, which gives the slowest exponential ($Ce^{-\gamma t}$). Substraction of data corresponding to each point of this line from that of the original curve gives the second curve. The later portion of this curve again gives a straight line which may be prolonged to the ordinate to give the second exponential ($B.e^{-\beta t}$). By repeating the same subtraction, the third one ($A.e^{-\alpha t}$) is obtained.

We did not use this method. It is obvious that plotting a straight line through the measuring points cannot be quite exact because of the limited measuring accuracy. The method is time consuming, too.

b) Computer analysis.
One of the most advanced iterative methods has been published by Kirkegaard (1970). The measured stress decrease curves are fitted to an equation with three or two exponential terms and a constant (Coolsaet et al, 1973, 1975). The fitting is done by a digital computer using a least square approximation method.

With this method, as used by us, results are less good in analysis of viscoelastic stress decrease curves. Therefore an other method, called expostep, has been developed by Van Mastrigt (1977).

c) Simulation.
An electronic simulator to analyse exponential stress decrease curves was developed by Van der Zwart (1973) for clinical purposes. With the apparatus, the parameters could be adjusted mutually independently.

The measured curve is represented on a monitor. The signal of the model is also projected on the monitor so that by turning the knobs the adjusted curve fits the measured one as good as possible.
A measure of the fit is built in the device and can be read out of it. The measure of fit correlates with that used in the computer analysis. The value of the parameters can be read directly. The advantage of the device is that spontaneous contractions can be easily eliminated, a process which is

more difficult with the computer analysis. For clinical application further refinements are required.

IV. 3.2. Calculation of E and η

The coefficients A, B, C in the mathematical model represent the initial values of the exponential terms. They form the initial peak values of stress at the end of the stepwise straining and are mechanically represented by the force in the springs since the viscous elements (dashpots) cannot elongate infinitely fast, as would be necessary to follow the input step function.

From these coefficients the elastic moduli E_1, E_2 and E_3 can be computed. The constant K, which represents the static value of the signal, is related to the static elastic modulus E_o.

Since the initially evoked stress is caused by equal straining of all the elastic elements only, we may write :

$$S(0) = \varepsilon\ (E_o + E_1 + E_2 + E_3) : \tag{22}$$

where S (0) is the initial evoked stress for t = 0

Now since :

$$K + A + B + C = S(0) \cdot d \tag{23}$$

where d represents the area [m^2] perpendicular to the stress we can write :

$$K + A + B + C = d \cdot \varepsilon\ (E_o + E_1 + E_2 + E_3) \tag{24}$$

Since for bladder wall strips :

$$V_t = l \cdot d \tag{25}$$

where V_t is the tissue volume [m^3] and l the stretched length of the tissue [m]

we find :

$$E_o = \frac{K.l}{\varepsilon.V_t} ; E_1 = \frac{A.l}{\varepsilon.V_t} ; E_2 = \frac{B.l}{\varepsilon.V_t} ; E_3 = \frac{C.l}{\varepsilon.V_t} \tag{26, a, b, c, d}$$

In experiments the tissue volume is measured by weighing the bladder wall strips in air and water.

Weighing the strip in air yields :

$$G_a = V_t (P_t - P_a) \cdot g \tag{27}$$

where G_a means the weight in air [N], V_t the tissue volume [m^3], P_t the denstity of the tissue [kg/m^3], P_a the density of the

air [kg/m^3] and g the acceleration due to gravity [m/sec^2].

The weight of the tissue strip submerged in water is :

$$G_W = V_t (P_t - P_W) . g \qquad (28)$$

where G_W means the weight in water [N] and P_W the density of water [kg/m^3].

By a combination of both formulas we obtain :

$$V_t = \frac{G_a - G_W}{g.(P_W - P_a)} \qquad (29)$$

The relaxation constants (α, β, γ) or the corresponding time constants (τ_1, τ_2 and τ_3) characterize the stress decrease curve. Using the calculated elastic moduli, we can derive the viscosity moduli from the relaxation constants as follows :

$$\eta_1 = \frac{E_1}{\alpha} \; ; \; \eta_2 = \frac{E_2}{\beta} \; ; \; \eta_3 = \frac{E_3}{\gamma} \qquad (30, a, b, c)$$

It turned out that the standard deviation of the elastic moduli and relaxation constants are too large to give reliable values for the viscosity moduli (see IV. 3.5.). We therefore use the relaxation constants (α, β, γ) and elastic moduli (E_0, E_1, E_2, E_3) and not the viscosity moduli to characterize the behaviour of bladder wall strips.

IV. 3.3. Determination of l_o

The value of the unstretched length l_o has to be determined to calculate the strain ($\frac{l - l_o}{l_o}$). The unstretched length l_o is differently defined by different authors. In studies on striated muscle, the in situ length of an inactive muscle, has been used as the length l_o. At that length striated muscle develops maximal tension in response to tetanic stimulation (Wilkie, 1956). Ramsey (1960) also defines l_o as the length at which the muscle develops maximal tension. Aberg and Axelsson (1965) defined l_o as the greatest length obtained by an inactive muscle when the force applied to straighten it did not exceed 50 dynes. In later studies Axelsson (1970) proposed a laborious pre-treatment. Pieces of taenia were dissected at 37 °C in a solution containing adrenaline. After determination of the inactive unstressed length of adrenaline relaxed pieces, the tissue was depolarized and allowed to

shorten. After recovering in saline, the pieces were once more relaxed by adrenaline. The value of l_0 was then determined. This pre-treatment improved the reproducibility of their results. The method, however, is time-consuming and has possibly far reaching influence on the physiological conditions of the tissue. Other authors do not standardize initial conditions (Kondo and Süsset, 1974). In our experiments the strips were fixed in a bath of Krebs solution at 37 °C and were left without strain for half an hour to allow them to recover from the dissectional contracture (Bath-Smith and Bendall, 1947). Next, the value of l_0 was determined as the maximal length to which the strip could be elongated by applying a force of maximally 0.1 Newton, during one second. This method may contribute to the extent of the spread in the experimental results. The value of l_0 is not defined in inactive muscles since we want to compare the results with active muscles in situ. A separate group will be investigated with inactive contractile elements.

IV. 3.4. Experiments on bladder wall strips with stepwise straining at increasing strain levels

IV. 3.4.1. Methods

These experiments (Coolsaet et al, 1975) were performed on bladder wall strips from mongrel dogs *. All strips in this series were prepared from the front wall of the bladder in a longitudinal

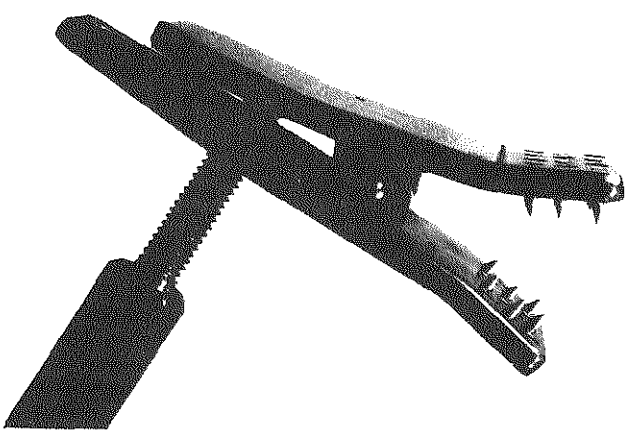


Fig. 21
The specially made clamp in which the bladder strips were fixed. Note the penetrating pins which avoid tearing and the deformation caused by single hooks.

* The dogs were sacrified for other experiments; some were exsanguinated, others received nembutal anaesthesia.

direction and were about 0.02 m long and 0.01 m wide. Both ends were fixed in specially made clamps (Fig. 21). These clamps had penetrating pins to avoid tearing. Fixation by a single hook (Apter and Graessley, 1970) was not used to avoid deformation of the strips.

A modified Krebs solution (Alexander, 1971) was used with the following composition : Na^+ 137.0; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 0.6; Cl^- 117.0; HCO_3^- 25.0; PO_4^{2-} 1.2; SO_4^{2-} 0.6; glucose 10.

The solution was aerated by a mixture of 95 % O_2 and 5 % CO_2 and kept at 37 C.

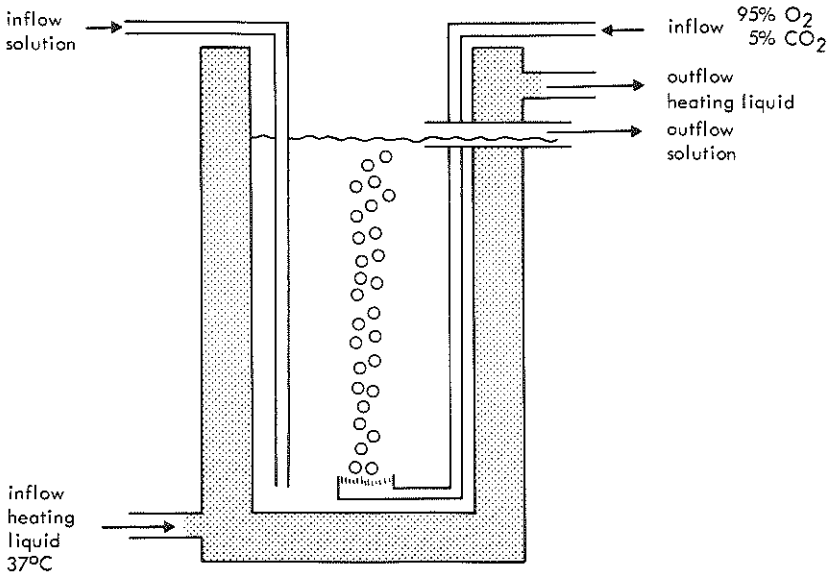


Fig. 22
Bath used for measurements made on bladder wall strips.

A diagram of the apparatus used in these studies is shown in Figure 22. One end of the strip was fixed to the bottom of a container, and the other was attached to a Grass FT.03 force transducer, which could be moved vertically (Fig. 23).

A rapid increase in length of the strips was produced by a cocked spring in a time not exceeding 50 msec.

The elongation was kept constant during 15 minutes. After that a recovery time of 15 minutes at l_0 was allowed between successive increasing steps (Fig. 24). The force was converted into electrical resistance variation by a force transducer (Fig. 25). A strain gauge amplifier converts resistance variation into voltage. The voltage is sampled at a rate of 1 per second during 900 seconds via an interface. The samples were digitized and

punched on paper tape. The cards obtained via the papertapes were fed to an IBM 360/65 computer and processed in this series

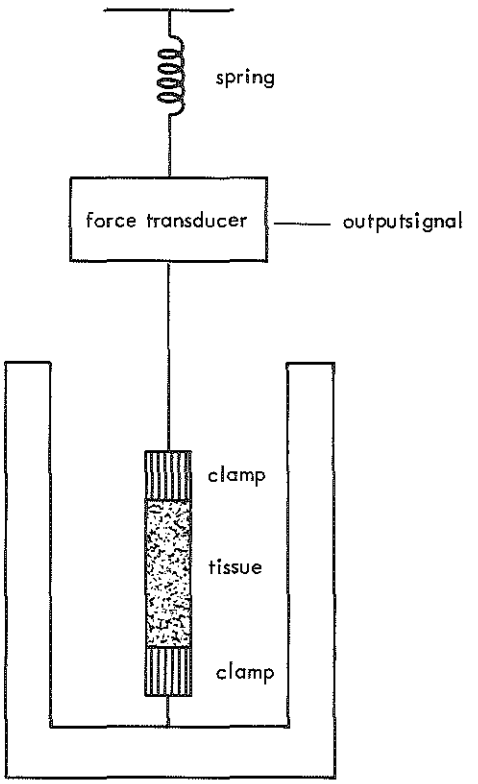


Fig. 23
Schematic representation of the monitoring system.

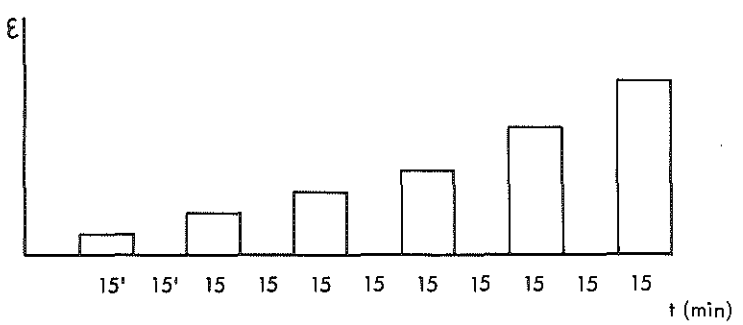


Fig. 24
Stepwise strains at increasing strain levels.

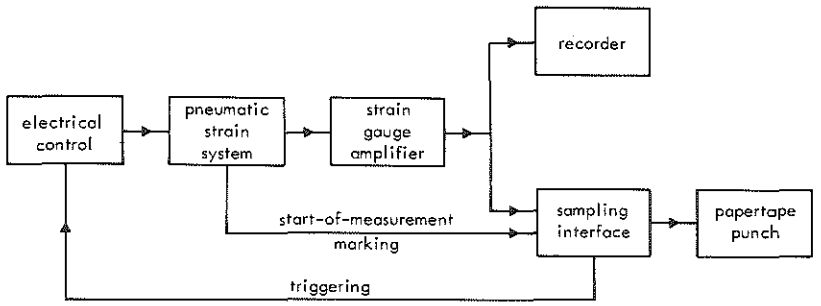


Fig. 25
Schematic representation of the measuring instrumentation with which the output signal was investigated.

of experiments by means of a programme discribed by Kirkegaard (1970).

Coefficients and relaxation constants are obtained from the analysis. Elastic moduli are computed from the coefficients.

The stress decrease curve was also written on a strip-chart recorder.

IV. 3.4.2. Results

Over two hundred stress decrease curves were recorded. More then half of these had to be rejected because they either showed too much spontaneous activity or too small a stress response caused by too small a strain. In this series the strain was not a constant fraction of l_o.

The 95 curves were analysed with the three exponential and the two exponential model using the Kirkegaard programme.

The elastic moduli were found to depend on the strain level in a way which differs for each strip (Fig. 26A and B). Even the static elastic modulus (E_0), which was expected to increase with increasing strain as stated by King and Lawton (1950) and Kondo and Susset (1974), fluctuated strongly (2 to 6 $N/_m2$).

The relaxation constants are represented in Table 1. The standard deviation is as much as 28 - 69 %.

The spread in the value of the relaxation constants of different strips is possibly due for the most part to different states of the

Model	α (sec^{-1})	Standard deviation	β (sec^{-1})	Standard deviation	γ (sec^{-1})	Standard deviation	Number of experiments
Two exponentials and a constant	- 0.14	0.06	- 0.0063	0.0018	--	--	95
Three exponentials an a constant	- 0.26	0.10	- 0.023	0.016	- 0.0035	0.0012	95

Table 1
Relaxation constants from bladder wall strips at increasing strain levels.

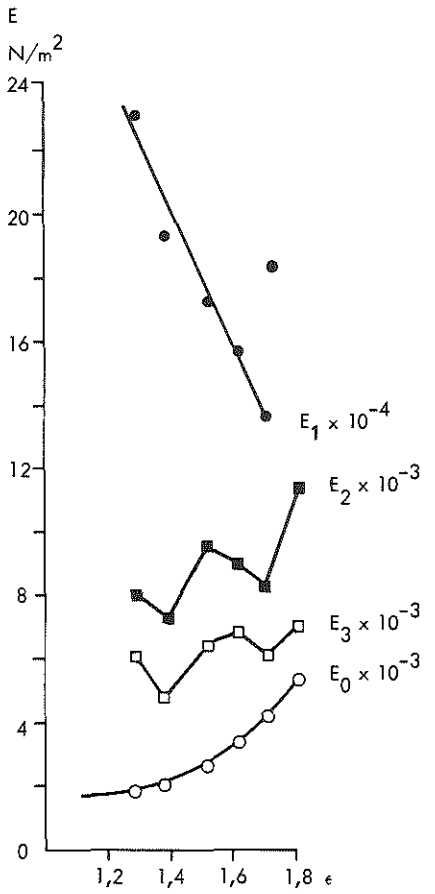


Fig. 26 - A
Elastic moduli obtained in bladder wall strips as a function of strain.

contractile elements. This problem will be discussed in section IV. 3.6. For each separate strip the standard deviation is significantly smaller, as can be seen in Figs. 27 and 28. No systematic trend could be found as a function of the strain.

From these experiments we conclude that the relaxation constants do not depent significantly on the strain level. For conversion of the method of stepwise straining to a clinical cystometry method, this observation is very important since the strain level can be chosen arbitrarely. Infusion of bladders to a same strain level is thus not necessary to make them comparable with regard to the relaxation constants.

The elastic moduli, however, depend on the strain level. It would not be justified, therefore, to draw conclusions concerning the physical state of the bladder wall from the values of the elastic moduli.

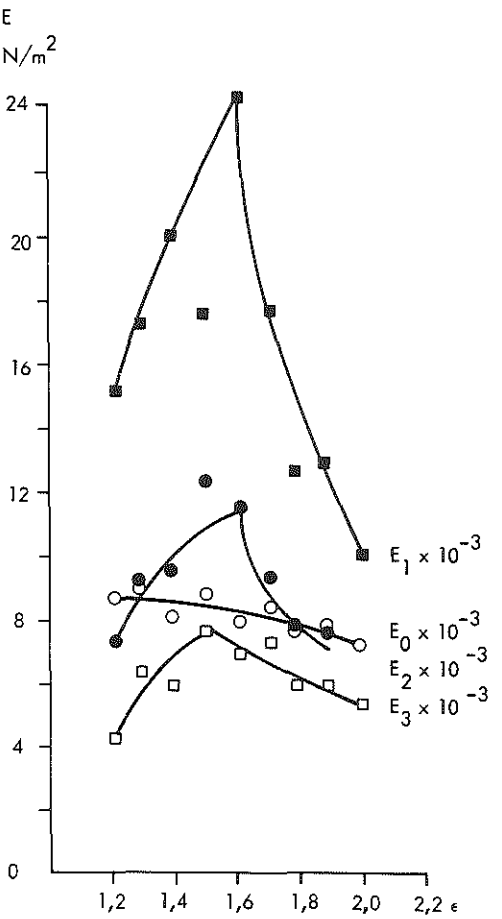


Fig. 26 - B
Elastic moduli obtained in bladder wall strips as a function of strain.

Comparing the results of the two exponential and three exponential models we see that the standard deviation of the relaxation constants is sometimes larger using the three-exponential model (for α the standard deviation is 28 % of the value; for ß 69 %; for γ 34 %) than the two-exponential model (for α 43 %; for ß 38 %). The three exponential model is more sensitive to distortion. The three-exponential model gives a better fit, as might be expected, since more parameters are fitted (Fig. 29). For the represented curves, the minimum of the sum of the least squares is three times less when the two-exponential model is used in comparison with that obtained when the three-exponential model is used. Therefore, we concluded that the two-exponential model is obviously inadequate to describe the stress decrease curves.

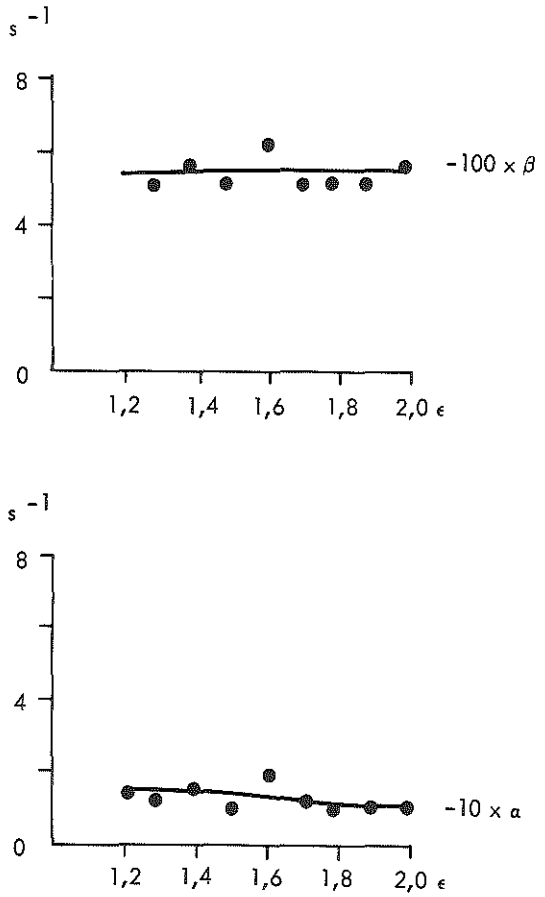


Fig. 27
Relaxation constants obtained in bladder wall strips as a function on strain after analysis with the two-exponential model.

IV. 3.5. Experiments on bladder wall strips with straining at constant strain levels

In this series (Coolsaet et al, 1976) the reproducibility of the parameters will be investigated by straining at constant strain levels and by introducing some modifications of the method of investigation and analysis.

IV. 3.4.1. Method

The step functions were now realized by a specially developed pneumatic strain device (Fig. 30). The force transducer was fixed by means of a rigid rod to avoid artificial changes by sudden

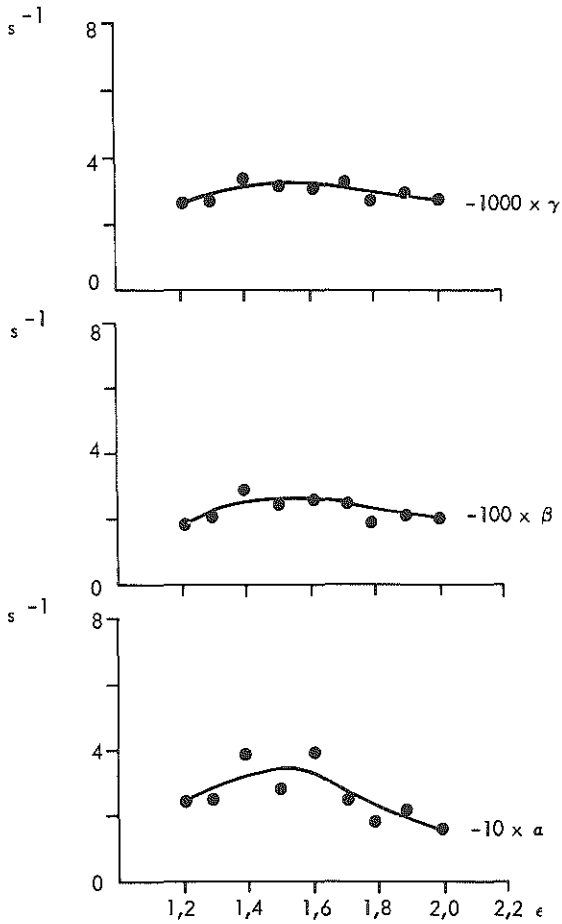


Fig. 28
Relaxation constants obtained in a bladder wall strip as a function of strain after analysis with the three-exponential model.

movements of the transducer. The lower clamp was moved in a vertical direction by a pneumatic system. The initial length and elongation levels could be adjusted mutually independently with micro screws. The tissue could be elongated up to 50 mm within 50 msec. The pneumatic system was electronically controlled. The electrical control of the system was triggered by the interface, so the first sample was always taken exactly 200 msec after the start of the stepwise straining. This is important for the analysis of the exponential term related to the fastest stress decrease. A new computer programme was developed by Van Mastrigt (1977) for the analysis of the stress decrease curves.

The minimum of the sum of the least squares of the deviations turned out to be significantly better using this procedure in com-

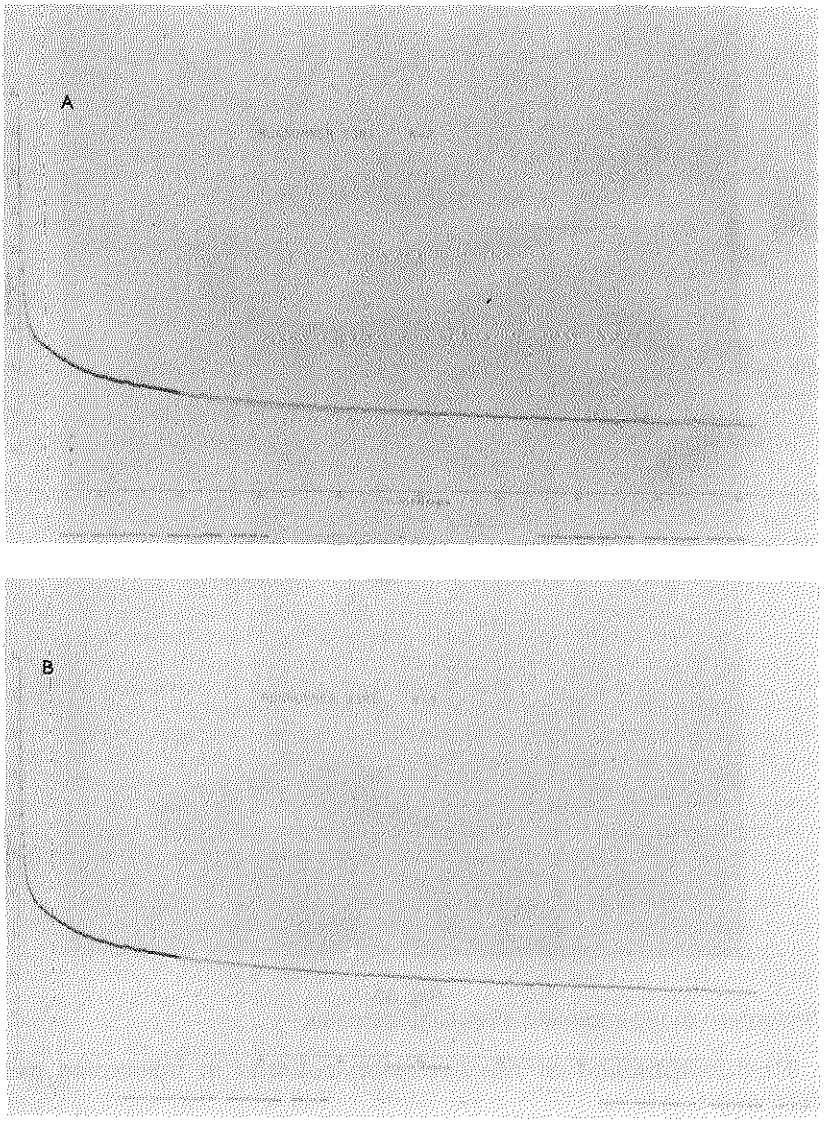


Fig. 29
Measured and fitted curves as obtained by the computer from the two-exponential (A) and three-exponential (B).

parison with the program described by Kirkegaard as used in the previous series. In this series of experiments a relative elongation (ε) of 0.30 was repeatedly applied for 15 min. (Fig. 31). A recovery time at the initial length during 15 min. was allowed between successive stretches.

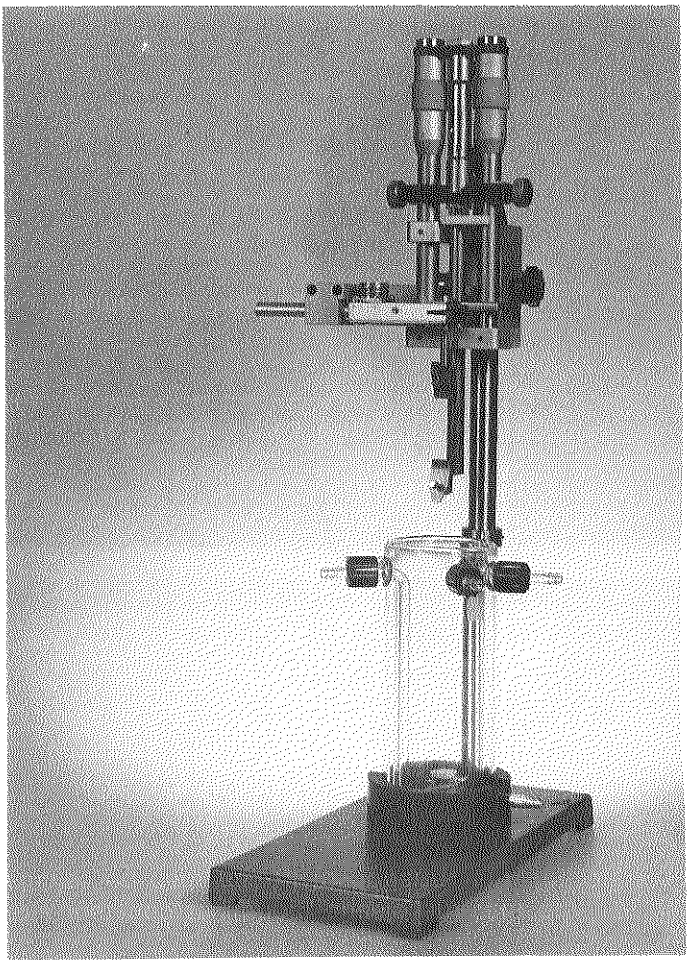


Fig. 30
Photograph of the pneumatic stretching apparatus.

The composition of the perfusion solution in millimoles per liter was :

Na^+ 137.0; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 134.0 HCO_3^- 15.1; $H_2PO_4^-$ 1.2; glucose 11.5.

The solution was continuously perfused to maintain a constant composition in the bath.

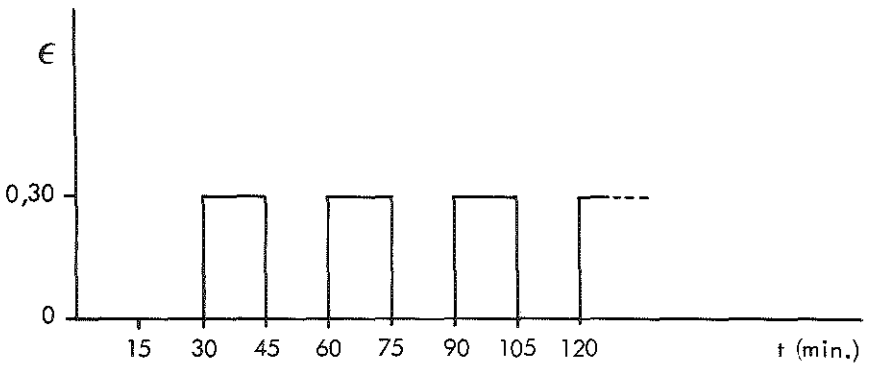


Fig. 31
Stepwise strains at constant strain level.

IV. 3.5.2. Results

We recorded 122 curves from which 118 could be fitted to the model. Two curves were incorrectly punched due to a failure in the interface, one was distorted by a wrong adjustment of the strain gauge amplifier and one could not be fitted by the computer programme since the curve showed an increasing shape.

The mean values and standard deviations of the relaxation constants are presented in Table 2. They differ appreciably (factor 2 for the largest relaxation constant) from those in Table 1. This difference can be ascribed to the new method of analysis. The standard deviations are even larger than those in the previous series. They are 65 % of the value of α , 71 % of ß and 50 % of γ using the three-exponential model; 44 % of α and 38 % of ß using the two-exponential model. Seven curves had relaxation constants which differed markedly from the mean values. They are however, included in the overall mean values.

Because of the use of another analytical model (Expostep), which gives a better fit, differences between the various strips are more pronounced and more curves could be analysed.

The value of the relaxation constants with standard deviations of each strip are presented in Table 3. The standard deviations are significantly less than those of the mean values of all strips as represented in Table 2.

Model	α (sec $^{-1}$)	Standard deviation	β (sec $^{-1}$)	Standard deviation	γ (sec $^{-1}$)	Standard deviation	Number of experiments
Two exponentials and a constant	- 0.25	0.11	-0.0083	0.0032	--	--	124
Three exponentials and a constant	- 0.47	0.31	-0.045	0.032	-.0050	0.0025	118

Table 2
Relaxation constants from bladder wall strips at constant strain levels.

The elastic moduli showed a decreasing trend as a function of time in spite of the nearly constant strain level (Fig. 32).

This decrease in the elastic moduli can be interpreted to be due to an increase in the effective initial length (l_o). Such an increase was observed experimentally. It has not been measured quantitatively. Exact measurement turned out to be unreliable because of curling of the strip after a stretch release.

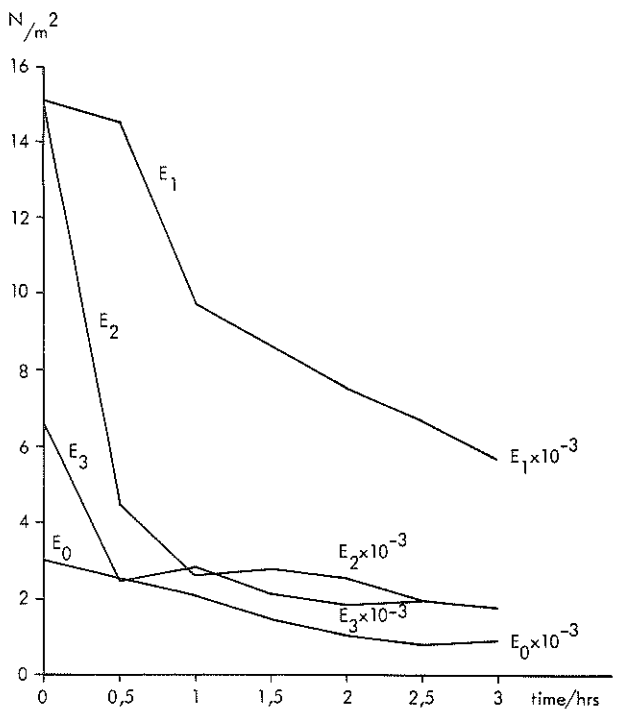


Fig. 32 - A
Elastic moduli as a function of time at constant elongation level.

These possible changes of l_0 made it necessary to add two elements to the model, a viscous element (η_o) and an active one (C). The viscous element (η_o) has been included to account for the increase of the unstretched length l_0 under stress. This element increases in length when the model is strained and has to

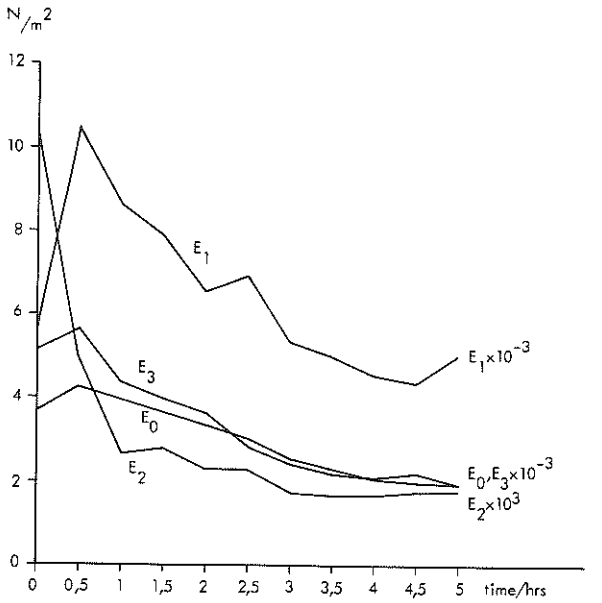


Fig. 32 - B
Elastic moduli as a function of time at constant elongation level.

be actively reset by the active element (C) and must have a very large time constant.

Alexander (1957) already stated that the contractile elements can change the unstretched length l_0 of the tissue and have an influence on the rate of recovery after stretch.

The influence of the active elements on the passive behaviour will be discussed in the following section.

Number of the segment	$-\alpha$ (sec^{-1})	Standard deviation	$-\beta$ (sec^{-1})	Standard deviation	$-\gamma$ (sec^{-1})	Standard deviation	Number of experiments
38	0.34	0.36	0.038	0.014	0.0045	0.0006	11
39	0.30	0.01	0.032	0.003	0.0057	0.0017	6
40	0.18	0.05	0.022	0.004	0.0041	0.0010	8
41	0.19	0.07	0.030	0.014	0.0050	0.0008	8
42	0.29	0.04	0.030	0.011	0.0028	0.0012	6
43	0.35	0.07	0.031	0.004	0.0055	0.0018	5
46A	0.72	- -	0.051	- -	0.0037	- -	1
46B	0.55	0.60	0.035	0.024	0.0028	0.0012	4
47	0.30	0.01	0.028	0.007	0.0067	0.0018	6
48	0.35	0.04	0.029	0.006	0.0034	0.0011	6
49	0.77	0.33	0.058	0.036	0.0054	0.0020	6
50	0.72	0.17	0.054	0.031	0.0046	0.0023	4
51	0.72	0.13	0.051	0.017	0.0027	0.0034	5
52	0.52	0.21	0.045	0.022	0.0046	0.0013	4
53	0.47	0.10	0.041	0.014	0.0046	0.0022	8
54	0.85	0.25	0.091	0.033	0.0061	0.0016	7
55	0.36	0.04	0.031	0.004	0.0041	0.0004	10
56	0.59	0.26	0.052	0.016	0.0052	0.0007	6
							111 +

Table 3
Relaxation constants per bladder wall strip at constant strain levels.

The two additional elements influence the analysis in the following way. We assumed that traumatic contracture which is

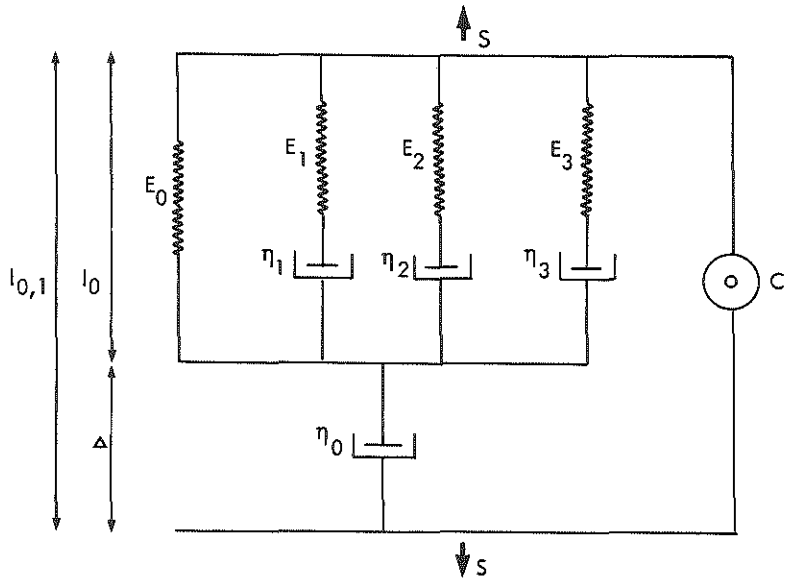


Fig. 33
Enlargement of the mechanical model with a contractile element (C) and a dashpot in series ( ).

generally observed after dissection reset the viscous element η_o completely. Hence, before the first strain l_O is equal to $l_{O.1}$ (Fig. 33). During strain, η_o will be elongated by an amount which is partly determined by the active element C. This elongation after the first stretch and release is represented by Δ.

So, $(l - \Delta)$ is the length, at which the model will be elongated during the second stretch. The strain for the first elongation is by definition :

$$\varepsilon_1 = \frac{l - l_{O.1}}{l_{O.1}} \tag{31}$$

When the strip is elongated again to the same level l as formerly, the strain will be less, since the model had become longer :

$$\frac{l - \Delta - l_{0,1}}{l_{0,1}} = \frac{l - (\Delta + l_{0,1})}{l_{0,1}} = \frac{l - l_{0,2}}{l_{0,1}} \tag{32}$$

Since, for measurements at a given elongation, the strain decreases by an elongation of η_o, it can be understood that the elastic moduli, calculated on the assumption that the strain is

constant, decrease during the series of measurements on one strip (Fig. 32).

If we assume that the elastic moduli at constant strain will remain constant, the variation of l_o can then be calculated as follows :

$$\varepsilon . (E_0 + E_1 + E_2 + E_3) = \frac{(K + A + B + C)}{V_t} . l \qquad (33)$$

For the first strain we have :

$$\varepsilon_1 . (E_0 + E_1 + E_2 + E_3) = \frac{(K_1 + A_1 + B_1 + C_1)}{V_t} . l = R_1 \qquad (34)$$

For the second strain we have :

$$(E_0 + E_1 + E_2 + E_3) = \frac{(K_2 + A_2 + B_2 + C_2)}{V_t} . l = R_2 \qquad (35)$$

$(E_0 + E_1 + E_2 + E_3)$ is assumed to remain constant.

So :

$$\frac{R_1}{\varepsilon_1} = \frac{R_2}{\varepsilon_2} \qquad (36)$$

Or :

$$\varepsilon_2 = \varepsilon_1 \frac{R_2}{R_1} \qquad (37)$$

Or :

$$\varepsilon_2 = \frac{l - \Delta - l_{0.1}}{l_{0.1}} = \frac{l - l_{0.2}}{l_{0.2}} = \varepsilon_1 \frac{R_2}{R_1} = \frac{l - l_{0.1}}{l_{0.1}} . \frac{R_2}{R_1} \qquad (38)$$

So that we can derive :

$$l_{0.2} = l - (l - l_{0.1}) . \frac{R_2}{R_1} \qquad (39)$$

The same procedure can be followed for ε_3 and $l_{0.3}$, ε_4 and $l_{0.4}$ etc. The changes of l_0 for one strip are presented in Fig. 34. From these results it may be concluded that, if we may assume that the elastic moduli remain constant in the small range within which the strain varies, the unstretched length l_0 will increase by applying successive strains. To account for this l_0 variation the model had to be enlarged by a viscous element (η_0) in series and a contractile element (C) in parallel. The influence of the contractile element on the passive behaviour will be investigated in the following section.

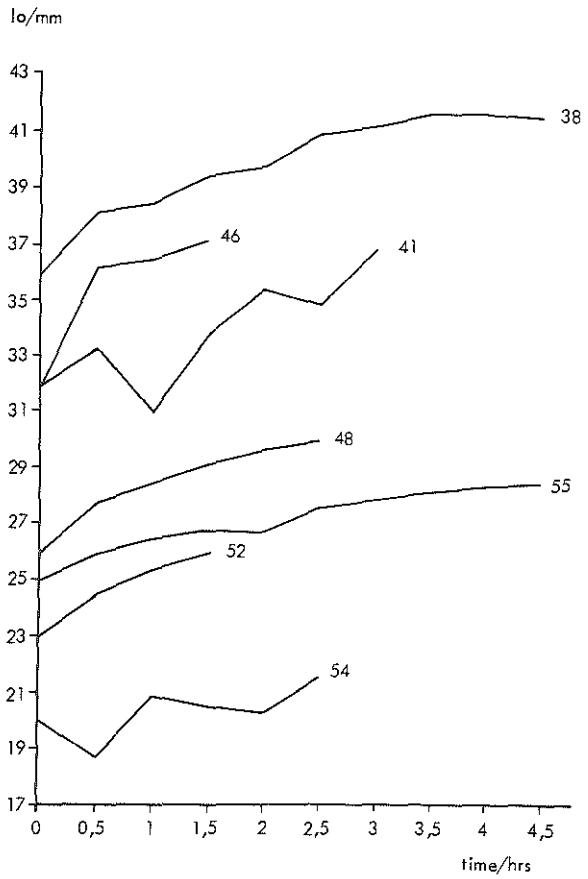


Fig. 34
The changes of l_0 as a function of time, applying successive strains at a constant elongation level.

IV. 3.6. Experiments with bladder wall strips applying stepwise straining at a constant strain level with the aim to investigate the influence of the contractile element on the passive behaviour

In the previous series of experiments the contractile element was not made inactive, as proposed by Aberg and Axelsson (1965), to avoid a selection of inactive muscles which would cause an artificial diminution of the spread. This series of experiments was performed in order to investigate the influence of the active element (C) on the passive behaviour.

IV. 3.6.1. Method

Since pig bladders have large spontaneous activity they are very suitable for this investigation. Segments of pig bladders * were taken from the dorsal side of the bladder, proximal to the trigone. The length of time between the death of the pig and the start of the experiments was about one hour. The technique for realizing the step functions and the method used for analysing the results of the measurements were the same as described in section IV. 3.5.

The strips were divided into three groups. In each group the response of the strips to a stepwise change in strain was investigated under specific conditions : the composition and temperature of the solution in which the strips were being kept varied from group to group. The outlines of the investigation were taken from Axelsson (1970). who considered the variables to be taken into account during mechanical measurements on taenia coli. No studies in this field were found in the literature concerning the bladder wall. So we have to assume that what holds for taenia coli, also holds for the bladder wall in this context. It was not our purpose to describe refined pharmacological experiments. Therefore bath solutions and changing variables are taken from literature.

1st group : **Control group in Krebs solution.**

For the sake of comparison a group of strips was investigated in the same way as the dog bladder strip described under IV. 3.5. No additions were made to the basic solution used by Aberg and Axelsson (1965). This solution had the following composition (in millimoles per liter) :

Na^+ 137.0; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 134.0; HCO_3^- 5.1; $H2PO4^-$ 1.2; glucose 11.5; aerated with a mixture of 95 % 02 and 5 % CO2.

2nd group : **Reduction of the influence of the contractile element**

* Pig bladders were obtained from the slaughter house.

The following methods were used :

a) Calcium-free EGTA solution [Ethylene-glycol-bis (ß-amino-ethyl-ether) n, n' tetra-acetic-acid]. A calcium-free solution was used with addition of 0.5 % EGTA. Calcium was replaced by sodium.

After excision, the tissue was rinsed in this solution with addition of acetylcholine (1 μg/ml) as advised by Casteels (personal communication). Free calcium is bound by EGTA and the efflux of calcium is increased by acetylcholine (Durbin and Jenkison, 1961; Schatzmann, 1961).

Stretch neither induces spike potentials nor phasic tension in a calcium-free EGTA solution (Tomita, 1970). The results of the measurements were compared with those of a series of measurements on dog bladder strips where all spontaneous spike discharges and contractile phenomena had been inhibited by D 600 [-isopropyl- (N-methyl, N, homoveratryl) - amino-propyl - 3,4,5, -trimethophenyl-acetonitril] (Mayer et al, 1972). D 600 was first used for our purpose but was more difficult to obtain.

b) Calcium-free EGTA solution with depolarisation of the cell membrane Axelsson (1970) claims that the cell membrane may still depolarize by stretching in a calcium-free EGTA solution. In order to investigate this possibility, measurements were also performed on strips in a bath were K^+ (148.1 millimoles per liter) had been substituted for Na^+.

c) Metabolic inhibitors.

The binding of calcium by EGTA may, by itself, introduce changes in the physical properties (Alexander, 1957). Therefore this series with metabolic inhibitors was performed to diminish active processes. Alternations of the glucose concentration in the bath solution soon bring about a change in the glucose concentration in the muscle tissue. In glucose-free solution, the membrane response falls with the tissue glycogen level (Axelsson et al, 1965).

Measurements were carried out in a bath where sorbitol (11.5 millimoles per liter) had been substituted for glucose and nitrogen for oxygen.

d) Decreased temperature.

Gordon and Siegman (1971) have investigated the mechanical properties of smooth muscle tissue and claim that the spontaneous activity disappears at 22 C. A number of experiments was done at 22 C to study the stress decrease curves after spontaneous activity had been eliminated by the decrease in temperature.

3rd group : **Stimulation of the contractile element**

Measurements were performed also with stimulation of the contractile elements by means of additional calcium (10 millimoles per liter). This leads to depolarisation of the membrane and a reduction in its electrical resistance (Bülbring and Kuriyama, 1963).

IV. 3.6.2. Results

To facilitate the comparison between the various groups, the results are presented in tables as well as by means of histograms showing the frequency with which different values of the relaxation constants (the exponents of the exponential terms) occur in the experimental material.

The variation in the elastic moduli in each group was so great that no significant alterations in these moduli could be detected under the different sets of experimental conditions used.

1st group : **Control group in Krebs solution.**

Pig bladder strips show more spontaneous activity as compared with those of dogs (Fig. 35). One hundred and eighteen curves were analysed. The results of the analysis with two and three exponential terms and a constant are shown in Table 4 and 5 and Fig. 36 and 37. In the course of the analysis with the three-exponential model, it was found that the largest relaxation constants had a bimodal distribution defining two subgroups with an appreciable spread of the relaxation constants. The relaxation constants constituting the subgroups with the largest average value are unreliable as a consequence of too low a sample rate,

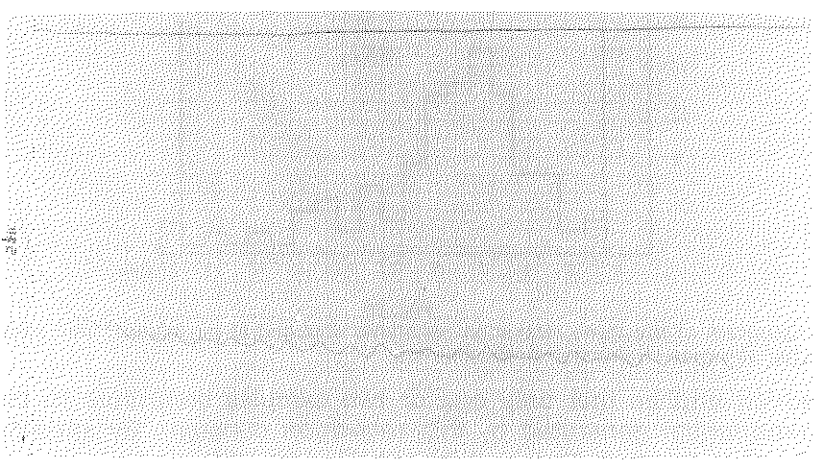


Fig. 35
A strip of pig bladder showing much spontaneous activity during stress decrease.

Model	α (sec^{-1})	Standard deviation	β (sec^{-1})	Standard deviation	γ (sec^{-1})	Standard deviation	Number of experiments
Two exponentials and a constant	-0.37	0.23	- 0.014	0.0085	--	--	101
Three exponentials and a constant	-5.32	5.3	- 0.14	0.04	- 0.0090	0.0041	99

Table 4
A group of relaxation constants from bladder wall strips in Krebs solution.

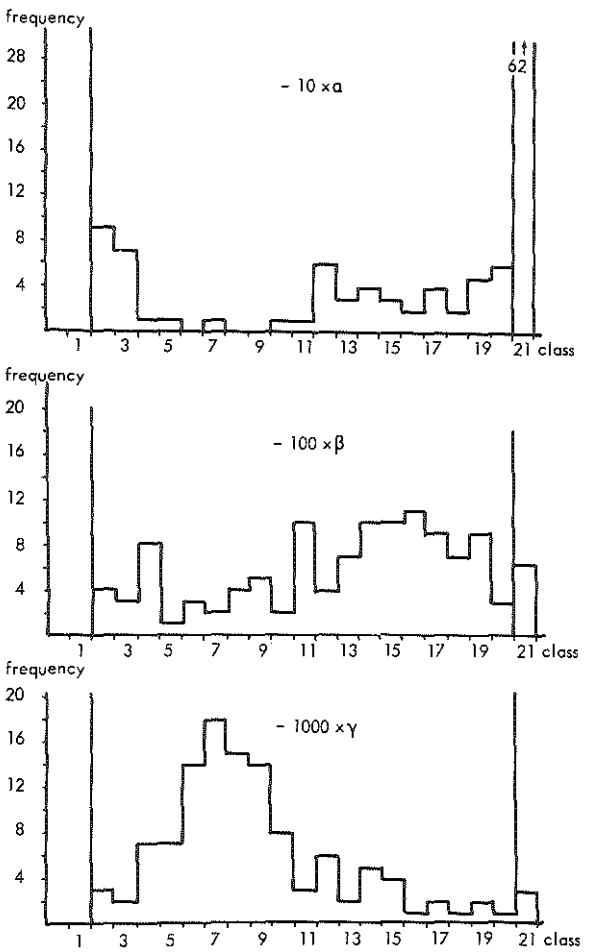


Fig. 36
Histogram of the three relaxation constants obtained in pig bladder wall strips after stepwise straining in Krebs solution.

which implies that only one or two samples contribute to the exponential generated by this relaxation constant. By ignoring the first two samples in the analysis and refitting these curves with two exponentials, it was shown that these two relaxation constants are equal to the smaller two found with the previous

Model	α (sec^{-1})	Standard deviation	β (sec^{-1})	Standard deviation	γ (sec^{-1})	Standard deviation	Number of experiments
Two expenentials and a constant	- 0.18	0.05	- 0.0084	0.0035	--	--	19
Three expontent-ials and a constant	- 0.23	0.13	- 0.033	0.015	- 0.0044	0.0028	19

Table 5
A group of relaxation constants from bladder wall strips in Krebs solution.

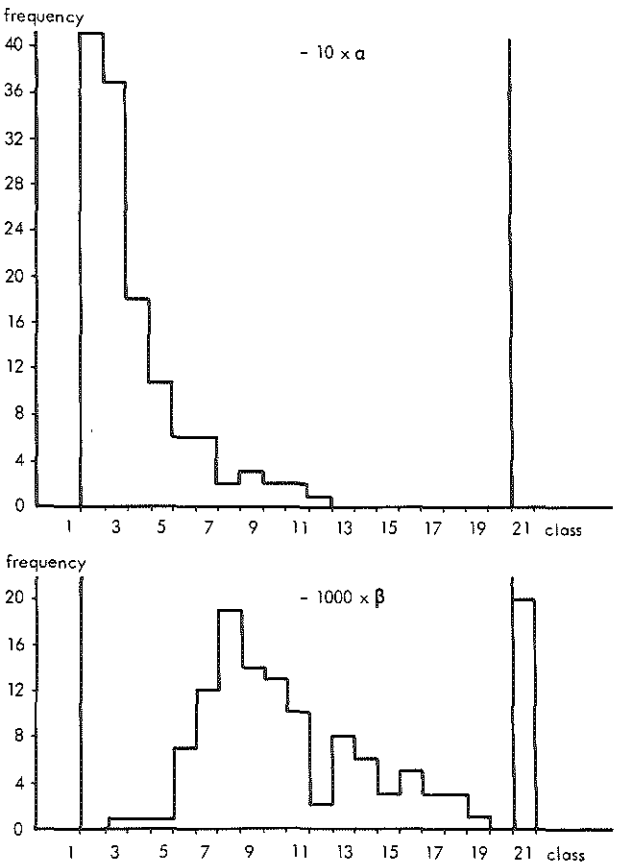


Fig. 37
Histogram of the two relaxation constants obtained in pig bladder wall strips after stepwise straining in Krebs solution.

analysis. From this it may be concluded that the two smaller relaxation constants formed in the three-exponential analysis are reliable in spite of the unreliability of the largest relaxation constants.

The spread in the smaller two constants might be related to variations in the contractile apparatus. This conjecture is corroborated by the above mentioned finding of a large

spontaneous activity. It will appear from the results of the following groups that the spread is also reduced when the activity of the contractile system is reduced.

2nd group : **Reduction of the influence on the contractile elements**

a) Calcium-free EGTA solution.

One hundred and nine stress decrease curves were recorded; one hundred and four of these were used for analysis. Four curves had to be rejected because of technical defects in the measurement and one curve showed an abnormal form with increasing stress which could not be analysed using our model.

The results are shown in Table 6 and Fig. 38. The standard deviations of the various relaxation constants are roughly the same for the entire group as for each separate

Model	α (sec $^{-1}$)	Standard deviation	β (sec $^{-1}$)	Standard deviation	γ (sec $^{-1}$)	Standard deviation	Number of experiments
Two exponentials and a constant	- 0.58	0.23	- 0.010	0.0048	--	--	103
Three exponentials and a constant	- 1.05	0.24	- 0.077	0.039	- 0.0061	0.0038	104

Table 6

Relaxation constants from bladder wall strips in a calcium-free EGTA solution.

strip (Table 7). The reproducibility is good. Using the three-exponential model, the standard deviation of the values of the relaxation constants is 23 % for α , 50 % for ß, 62 % for γ ; using the two-exponential model, 39 % for α and 48 % for ß. The values for the two larger relaxation constants are situated in a range where hardly any values were found in group 1. The smallest relaxation constants, however, appeared to be in the same range as in the first group. The results for group 2a agree quite well with those for dog bladder wall strips in a D 600 solution, as can be seen in Table 8.

b) Calcium-free EGTA solution with depolarisation of the cell membrane.

A hundred and thirty-one stress decrease curves were analysed. The results are shown in Table 8 and Fig. 39. The values found are roughly equal to those for group 2a

Model	α (sec $^{-1}$)	Standard deviation	β (sec $^{-1}$)	Standard deviation	γ (sec $^{-1}$)	Standard deviation	Number of experiments
Two exponentials and a constant	- 0.25	0.049	- 0.0060	0.0013	--	--	26
Three exponentials and a constant	- 0.82	0.27	- 0.062	0.023	- 0.0037	0.0011	26

Table 7

Relaxation constants from bladder wall strips treated with D 600.

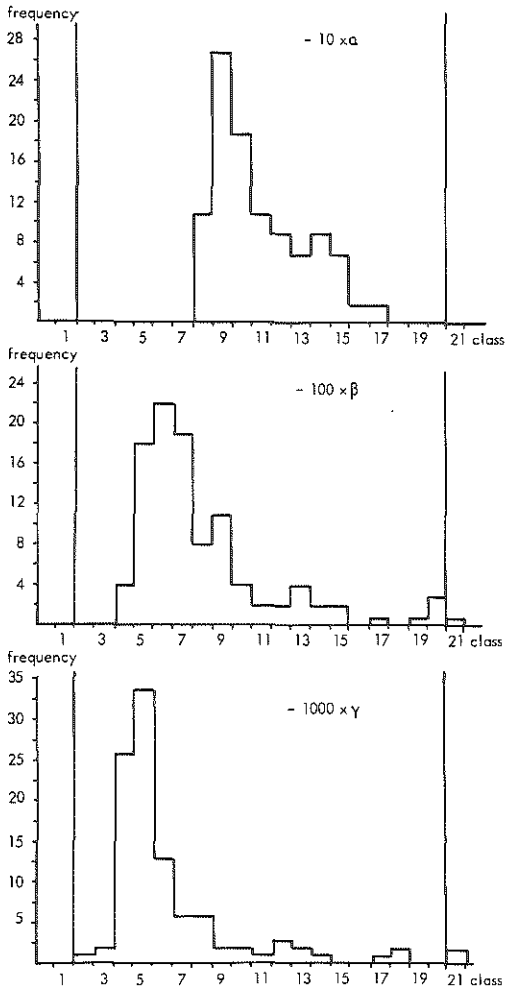


Fig. 38
Histogram of the three relaxation constants obtained in pig bladder wall strips after stepwise straining in a calcium-free EGTA solution.

Model	α (sec^{-1})	Standard deviation	β (sec^{-1})	Standard deviation	γ (sec^{-1})	Standard deviation	Number of experiments
Two exponentials and a constant	- 0.41	0.17	- 0.0075	0.0049	- -	- -	133
Three exponentials and a constant	- 1.00	0.21	- 0.054	0.010	- 0.0038	0.0008	131

Table 8
Relaxation constants from bladder wall strips in a calcium-free EGTA solution with depolarisation of the cell membrane.

but the spread is even smaller when the three-exponential model is used. The standard deviation is 20 % of the mean

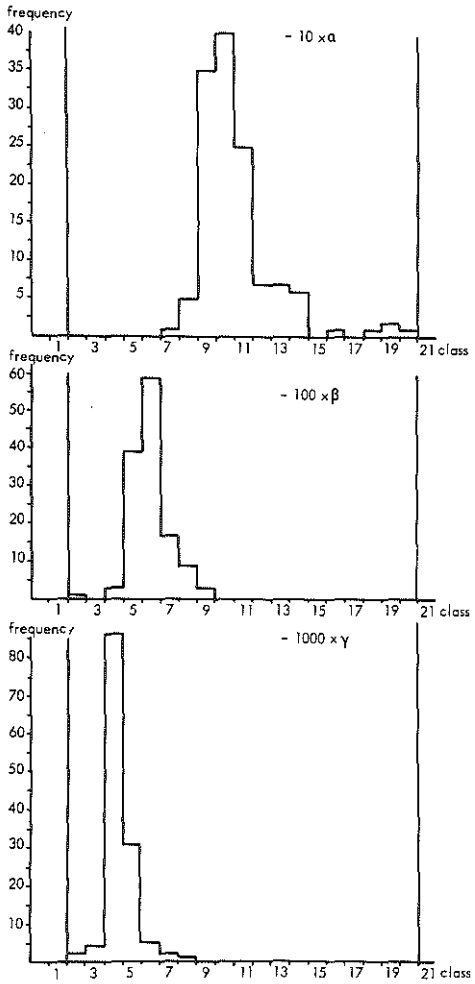


Fig. 39
Histogram of the three relaxation constants obtained on pig bladder wall strips after stepwise straining in a calcium-free EGTA solution with depolarisation of the cell membrane.

values of the smallest relaxation constant, 18 % of the middle and 23 % of the largest one. This may imply that depolarisation in a calcium free EGTA solution may still be effective (Axelsson, 1970).

Model	α (sec^{-1})	Standard deviation	β (sec^{-1})	Standard deviation	γ (sec^{-1})	Standard deviation	Number of experiments
Two exponentials and a constant	- 0.30	0.28	- 0.0099	0.012	--	--	26
Three exponentials and a constant	- 0.82	0.29	- 0.047	0.019	- 0.0036	0.0017	26

Table 9
Relaxation constants from bladder wall strips treated with metabolic inhibitors.

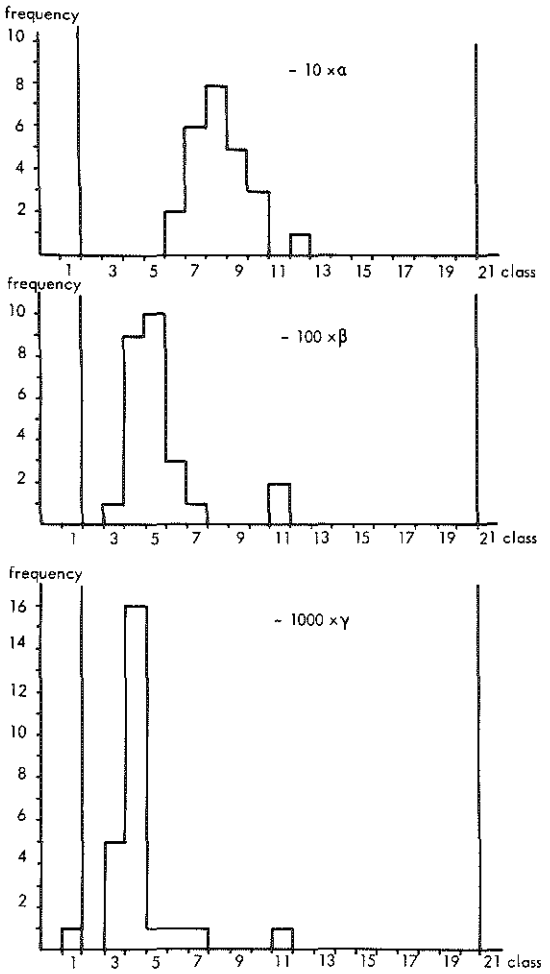


Fig. 40
Histogram of the three relaxation constants obtained on pig bladder wall strips after stepwise straining in solution with metabolic inhibitors.

c) Metabolic inhibitors.

We analysed twenty six curves. The results are shown in Table 9 and Fig. 40. The values are about the same as those for group 2a. Apparently, there is no significant influence of a calcium-free EGTA solution on the passive behaviour.

d) Decreased temperature.

During stress decrease no spontaneous contractions were observed. However, the form of the curves, while reproducible, was so different under these conditions (Fig. 41) that it is impossible to analyse them in terms of our present model. Similar curves have been described by

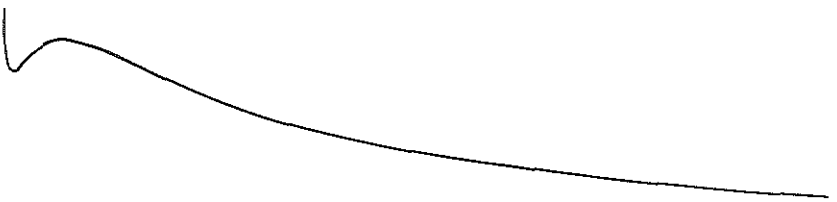


Fig. 41
Stress curve in the course of time after stepwise straining of pig bladder wall tissue at 22 C.

Apter and Graessley (1970). Their conclusion was that these alterations depend on the extent to which the bladder wall is strained. We could add that the active behaviour in experiments of this kind depends on temperature.

3rd group : **Stimulation of the contractile element**

Ninety-one curves were recorded and analysed with stimulation of the active element with calcium. The spontaneous contractions increased considerably, making the results much less reproducible. The results are shown in Table 10 and 11 and Fig. 42.

It will be seen that the experimental material also has a bimodal distribution. By and large, the histogram is comparable with that for group 1, but the spread in the relaxation constants is even larger using the three-exponential model (the standard deviation is even 100 % of the mean value of ß).

Model	α (sec $^{-1}$)	Standard deviation	β (sec $^{-1}$)	Standard deviation	γ (sec $^{-1}$)	Standard deviation	Number of experiments
Two exponentials and a constant	- 0.21	0.12	- 0.013	0.0054	--	--	82
Three exponentials and a constant	- 8	5	- 0.12	0.05	- 0.10	0.0041	82

Table 10
A group of relaxation constants from bladder wall strips in which the contractile elements are stimulated.

Model	α (sec $^{-1}$)	Standard deviation	β (sec $^{-1}$)	Standard deviation	γ (sec $^{-1}$)	Standard deviation	Number of experiments
Two exponentials and a constant	- 0.29	0.17	- 0.013	0.0030	--	--	8
Three exponentials and a constant	- 0.55	0.31	- 0.089	0.089	- 0.10	0.0047	9

Table 11
A group of relaxation constants from bladder wall strips in which the contractile elements are stimulated.

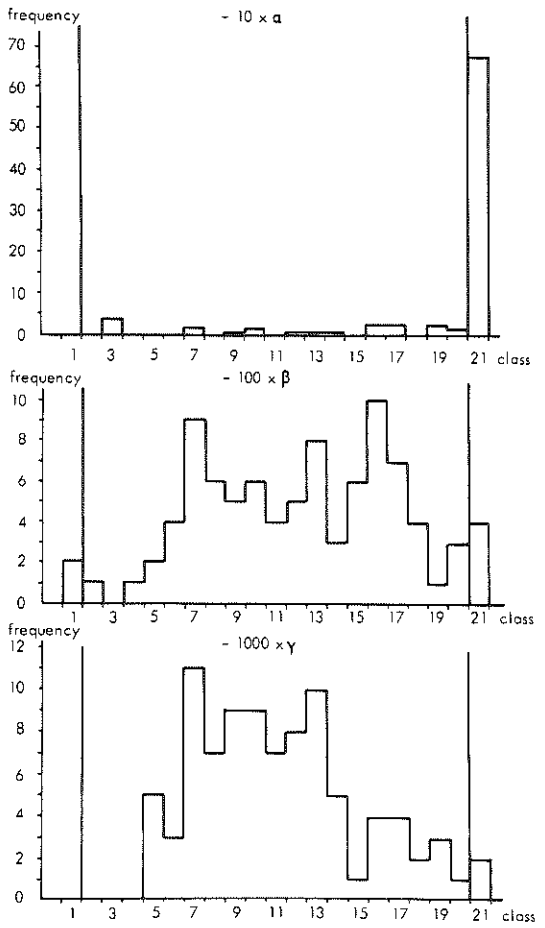


Fig. 42
Histogram of the three relaxation constants obtained in pig bladder wall strips after stepwise straining with stimulation of the contractile elements with extra calcium.

IV. 3.6.3. Conclusion

The most important conclusion which may be drawn from the results of these experiments is that the spread in the relaxation constants is significantly reduced when the influence of active elements is reduced. Active elements obviously have a variable influence on the overall behaviour. No reliable statement can be made concerning the systematic influence of active elements on the average value of the relaxation constants, because of the very large spread of the measuring results under normal circumstances.

Reducing spontaneous activity by a decrease in temperature

led to a large qualitative change in the curves. Pharmacological stimulation of the active element leads to an increase in the spread of the results, which is in agreement with the previous reasoning. It is noteworthy that the smallest relaxation constants are equal in groups 1 to 3 (0.0036 to 0.010). The largest relaxation constants (α) differ appreciably (0.18 to 8). We can conclude from this, that the active element has no influence on the smallest relaxation constants (γ). The influence of the active element on the second relaxation constant (ß) appears to be small.

In terms of tone, defined as that part of the stress caused by the active element, the results suggest that tone, in relation with the stress decrease after a stepwise strain, changes the largest relaxation constant.

IV. 3.7. Experiments on bladder wall strips with pulsewise straining

The length dependence of the stress was investigated by applying a series of pulsewise strainings on bladder wall strips. The pneumatic strain device described in section IV. 3.5.1. was used. Since we are interested in the passive length dependent properties, the influence of the contractile element was reduced by a calcium-free EGTA solution as used in section IV. 3.6.1.

In section IV. 3.5.2. we demonstrated that during constant strain, the dashpot η_0 elongates with time. This elongation results in a change of the unstretched length l_0, which influences the length dependent parameters (E_0, E_1, E_2, E_3). To avoid as much as possible these time-dependent phenomena, pulsewise strainings were performed (Van Mastrigt et al, 1976). In this way, the time during which the strip is strained is kept very short; 625 msec. The strip was strained every 62.5 sec with increasing strain levels.

The length dependence of the stress is represented in Fig. 43. By computing the sum of the elastic moduli as a function of strain, a mono-exponential function can be fitted to the measured values. This mono-exponential function can be described by the following formula (Van Mastrigt, 1977) :

$$E(|\varepsilon|) = |E|\, e^{\delta|\varepsilon|} \qquad (40)$$

where $E(|\varepsilon|)$ means the elastic modulus dependent on the amplitude of ε, $|E|$ the elastic coefficient, δ the elastic exponent and $|\varepsilon|$ the amplitude of the applied strain.

IV. 3.8. General conclusion

The stress decrease curve obtained by stepwise straining of bladder wall strips can be described by a mathematical model.

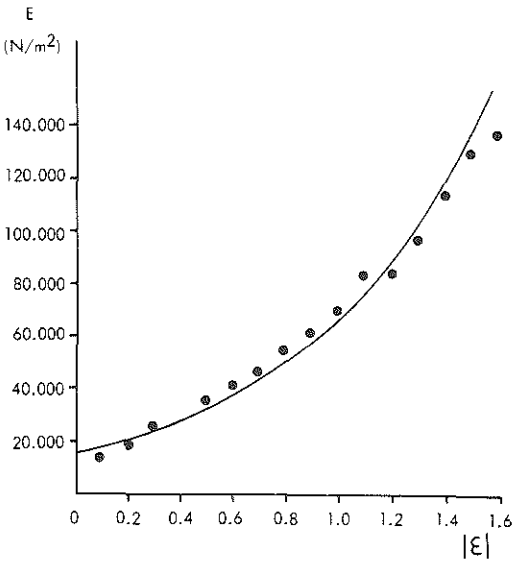


Fig. 43
Measured elastic modulus function (dots) and fitted mono-exponential function (line).

The model consisting of three exponential terms and a constant gives a good fit of the stress decrease curve. From the results obtained by computer analysis, parameters are obtained of which elastic moduli represent elastic behaviour (length dependent properties) and the relaxation constants represent viscoelastic behaviour (time dependent properties) :

The elastic moduli

a) The elastic moduli turn out to show significant variations when straining at increasing strain levels. Sometimes they increase, sometimes they decrease and frequently a combination of both is seen (section IV. 3. 4.).
b) When straining takes place at constant strain levels the elastic moduli show in general a decreasing trend as a function of time. This is interpreted as a change of the unstretched length (l_0) during straining. To explain the l_0 variation as a function of the time during which the model is strained, the mechanical model, which consists of three Maxwell elements and a spring in parallel, had to be enlarged by a dashpot (η_0) in series with the previous model and an active element (C) in parallel. This element is able to reset the dashpot (η_0), which is elongated after straining, and determines the length of the model.

c) The sum of the elastic moduli of the model shows a mono-exponential trend as a function of strain when applying a series of strain pulses of very short duration.

The active element

The active element (C) influences the length of the tissue. From the results of the experiments with reduction and stimulation of the influence of the active element, we can conclude that the largest relaxation constants are influenced by the active element. This element causes a considerable spread in the values of the largest relaxation constants. The smallest relaxation constants are not changed by the active element.

The relaxation constants

The relaxation constants are rather independent of strain and show a good reproducibility. Only the largest relaxation constants are influenced by the active elements.

With this knowledge obtained on bladder wall strips, we can investigate whether stepwise straining of the bladder in toto will give us useful parameters which represent the physical properties of the bladder.

IV. 4. Stepwise straining of the bladder in toto

From the experiments on bladder wall strips, a mathematical model has been obtained which enables us to describe the stress decrease curve after stepwise straining. In order to investigate whether this model is also applicable to stress decrease curves of whole bladders, the geometry being different from that of strips, experiments on bladders in toto had to be undertaken (Coolsaet et al, 1975). If these turned out to be realizable, a comparison had to be made with the parameters obtained by way of the classical cystometry method in view of the physical properties of the bladder.

IV. 4. 1. Methods of investigation

Experiments were done on female mongrel dogs (15 - 25 kg) during continuous intravenous pentobartibal anaesthesia. The dogs were fixed, while lying on their backs. To avoid disturbances from bowel movements, the abdomen was opened and intestines fixed so that the bladder could be filled without resistance from the intraabdominal organs. The ureters were ligated and diverted to bags to avoid reflux and efflux of urine. The pelvic nerves were connected to a Grass stimulator. A double

lumen catheter was introduced via the urethra. Saline at body temperature was infused through the outer canal with an electronically controled pump. Intraluminal pressure was measured through the inner canal by a pressure transducer (Fig. 44).

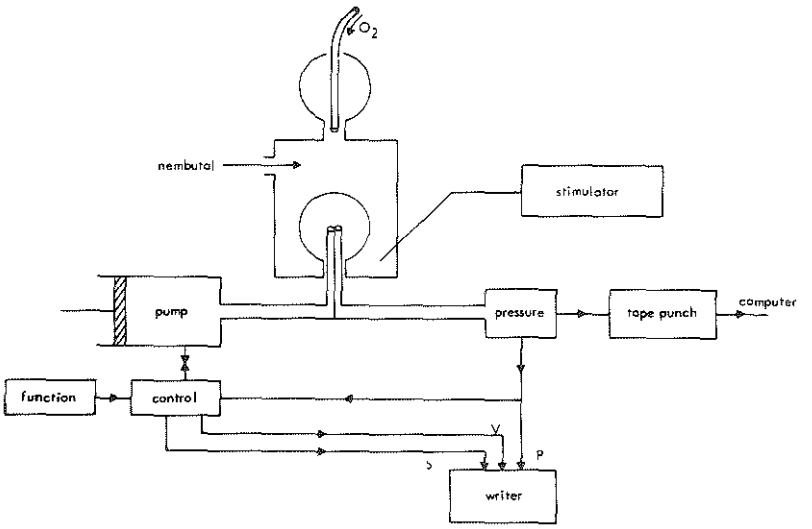
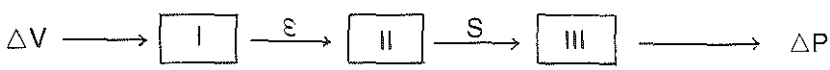


Fig. 44
Schematic representation of the measuring system.

Intravesical pressure was recorded simultaneously with the intraluminal volume on a recorder and also punched on paper tape. These paper tapes were converted to punch cards, which were fed to an IBM 360/65 computer for further analysis. The volume was infused at an infusion rate of 9.16 ml per second to a volume level which was supposed to give an analysable stress decrease curve during fifteen minutes. The sample time was one second. Models of two and three exponential terms were used for the analysis of the pressure decrease curves. The models were fitted to the measurements according to a least-square fitting procedure. The programme used was the one published by Kirkegaard (1970).

IV. 4. 2. Geometry of the bladder

Using the systems analysis approach, the bladder can be considered as built up by several blocks which are connected to form a block scheme :



A volume signal ($\triangle V$) will cause a strain (ε)through the geometry of the bladder. The resulting change of stress will cause a change of pressure, through the geometry of the bladder.

From the results of experiments on bladder wall strips we can replace block II by a mathematical model consisting of two or three exponentials and a constant.

For block I some difficulties arise. The first problem is the determination of V_0 (stretch volume) which we need to calculate the strain :

$$\varepsilon = \frac{l - l_0}{l_0} = \frac{l}{l_0} - 1 = \left(\frac{V}{V_0}\right)^{1/3} - 1 \qquad (41)$$

The volume (V) which is infused is known. V_0 can only be estimated but not exactly be measured as argued in section III. 2.

In our experiments, V_0 was defined as that volume which stretched the bladder to a spherical shape without straining the wall. With the abdomen closed the bladder was filled with a syringe until the pressure started to rise significantly. Although rough, this method could be used since the relaxation constants turned out to be rather independent of the volume.

In block III, a relation has to be formed between pressure (intraluminal) and stress (in the wall).

Laplace's law (Ruch and Patton, 1965) gives us a way of describing the relation between pressure and stress. For a thin-walled sphere it expresses the balance between the forces arising from the pressure (P) in the lumen of the sphere, and the forces arising from the tension in the wall of the sphere. These two forces, one pushing the halves of the sphere apart ($P.\pi R^2$) and one holding the two halves together ($T.2\pi R$), must be equal (Fig. 45). In a formula this means :

$$P.\pi R^2 = T.2\pi R \qquad (42)$$

hence :

$$T = \frac{P.R}{2} \qquad (43)$$

where T is the tension [N/m], P is the pressure [N/m^2] and R the radius [m], which is calculated from the volume [$V = 4/3\pi R^3$]. Laplace's law however is not applicable to the bladder, since the bladder is not thin-walled. Because we have to consider wall thickness and the changes therein and because we need «stress» in stead of «tension» as a variable, the tension defined by the

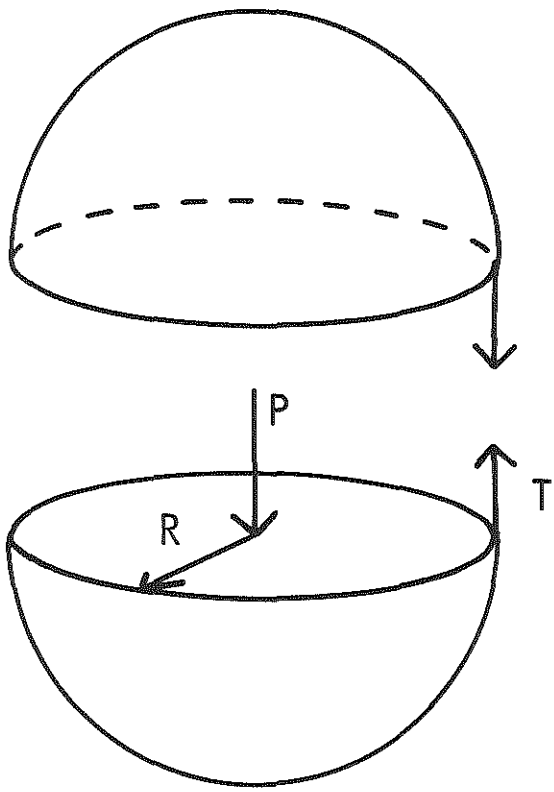


Fig. 45
Representation of Laplace's formula - the halves of a sphere are forced apart by a force ($P.\pi R^2$) and held together by a force ($T.2\pi R$).

Laplace formula must be divided by wall thickness to obtain stress (Coolsaet et al, 1973) :

$$S = \frac{P.R}{2\ d} \qquad (44)$$

where S is stress in N/m^2 and d the wall thickness in m. In the Laplace formula it is assumed that the wall thickness is negligible. A better assumption however is that the tissue volume (V_t) remains constant.

$$d.4\pi R^2 = V_t = \text{constant} \qquad (45)$$

So :

$$d = \frac{V_t}{4\pi R^2} \qquad (46)$$

where V_t means tissue volume [m^3].

Substitution of equation 46 into equation 44 yields :

$$S = \frac{3.P.V}{2.V_t} \qquad (47)$$

where V is the intraluminal volume.

The tissue volume (V_t) can be measured after an experiment as described above (section IV.3.2.).

From the measured intraluminal pressure (P), the coefficients A, B, C and K can be obtained by means of the mathematical model (see formula 23). By means of the following formula, E_1, E_2, E_3, and E_0 can be derived from the coefficients :

Applying Hooke's law :

$$S = 2.\varepsilon.E\,, \qquad (48)$$

where the factor 2 is used because of the straining of the tissue in all directions (Van Mastrigt, 1977) and using the formula's 47 and 54.

we can write :

$$\frac{3PV}{2V_t} = S = 2\left[\left(\frac{V}{V_0}\right)^{1/3} - 1\right].E \qquad (49)$$

A correction is to be made to obtain the E-values from mathematical stepfunctions instead of the physical stepfunctions actually used (see Van Mastrigt, 1977). The relaxation constants, however, are not corrected since there is no difference in using a rampfunction or a stepfunction (Van Mastrigt, 1977). The correction was performed assuming a constant strain rate instead of a constant change of volume rate (see formula 47). So the correction is only approximate. Especially for the fastest exponential the error will be important since the slower the infusion is performed, the less the fastest exponential is measured in the pressure decrease curve. Measuring on total bladders, we therefore prefered the use of the two exponential model.

IV. 4. 3. Results.

IV. 4. 3. 1. Experiments with the abdomen open at constant volume level

The reproducibility of the elastic moduli and relaxation constants was tested by applying a series of stepwise volume changes with the volume held constant for 15 minutes and with 15 minutes rest between the stepfunctions (Fig. 46).

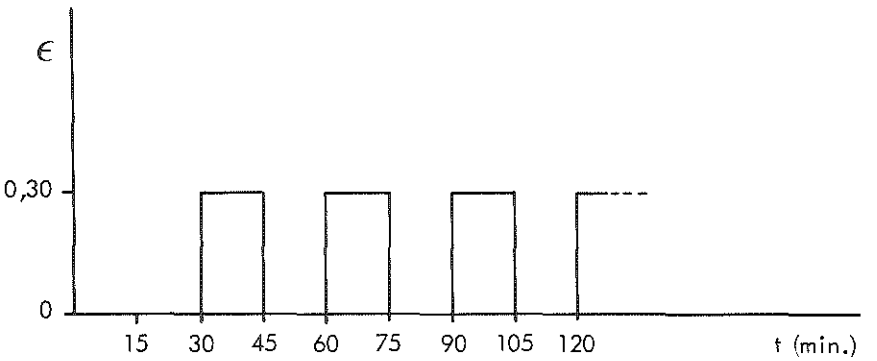


Fig. 46
Stepwise strains at a constant volume level.

The elastic moduli

The elastic moduli are represented in Fig. 47. At the end of the experiment on the total bladder, a tissue strip of this bladder was investigated in vitro to compare the elastic moduli (Fig. 48). The elastic moduli, which represent the strain-dependent properties, show a remarkable spread when straining at a constant strain level (from 12 000 to 30 000 N/m^2 for E_1). In the mechanical model the values of the elastic moduli change by length-dependency and by the changes of η_0, a time-dependent factor. These two factors may influence the values of the elastic moduli in different manners, which results in a remarkable spread as demonstrated. Because of this great variability in the trends of the elastic moduli, relative elastic moduli were calculated.

Since the trends in all elastic moduli (E_0, E_1, E_2 E_3) appear to be the same, the value of the elastic moduli relative to the sum of the values of all elastic moduli, however, should be constant.

So we obtain :

$$e_0 = \frac{E_0}{E_0 + E_1 + E_2 + E_3} = \text{constant} \qquad (50)$$

where e_0 is the relative elastic modulus.

The same procedure yields for e_1, e_2 and e_3.

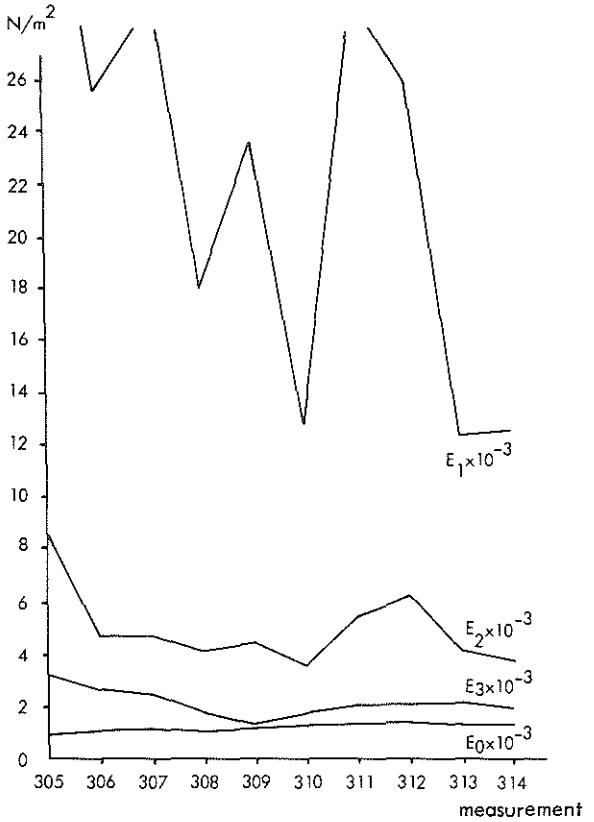


Fig. 47

Elastic moduli obtained in dog bladder in vivo after analysis with the three-exponential model of the pressure curves, following stepwise volume changes at a constant volume level.

The values of the relative elastic moduli are represented in Table 12. Denny-Brown and Robertson (1933) already noted that the fall of pressure at constant volume was definitely greater at higher volumes and was approaching a constant fraction of the pressure peak at the end of the infusion. This constant fraction represents the e_0 value which is constant indeed.

The relaxation constants

The relaxation constants are represented in Table 13. The values are comparable to those on bladder wall strips as represented in Table 2.

IV. 4. 3. 2. Experiments with closed abdomen at constant volume level.

Similar volume changes were performed with closed abdomen

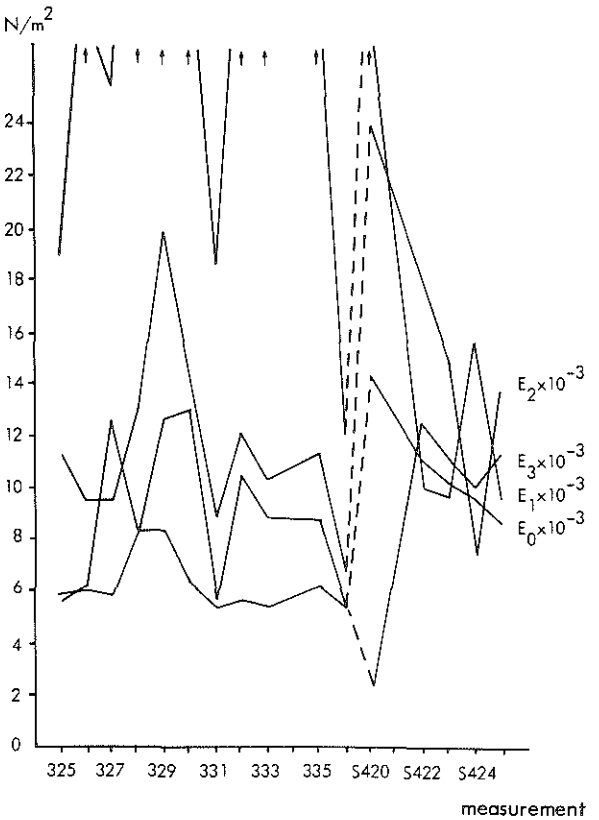


Fig. 48
Elastic moduli obtained in dog bladder in vivo (left) and in vitro (right) after analysis of the stress curves with a three-exponential model.

e_0	Standard deviation	e_1	Standard deviation	e_2	Standard deviation	e_3	Standard deviation	Number of measurements
0.17	0.11	0.36	0.12	0.29	0.08	0.18	0.05	44

Table 12
Average relative elastic moduli and standard deviations measured **in vivo.**

Model	α (sec $^{-1}$)	Standard deviation	β (sec $^{-1}$)	Standard deviation	γ (sec $^{-1}$)	Standard deviation	Number of experiments
Two exponentials and a constant	- 0.12	0.04	- 0.0066	0.0071	--	--	106
Three exponentials and a constant	- 0.25	0.21	- 0.038	0.032	- 0.0033	0.0019	85

Table 13
Relaxation constants from whole bladders at constant volume levels.

to investigate the influence of the abdominal wall and the intra-abdominal organs on the relaxation constants.

The values of the relaxation constants are represented in Table

Model	α (sec $^{-1}$)	Standard deviation	β (sec $^{-1}$)	Standard deviation	Number of experiments	Number experiments
Two exponential and a constant	- 0.039	0.006	- 0.0056	0.0006	4	15
	- 0.12	0.20	- 0.008	0.004	2	16
	- 0.16	0.05	- 0.004	0.002	2	17

Table 14
Relaxation constants from whole bladders in a closed abdomen.

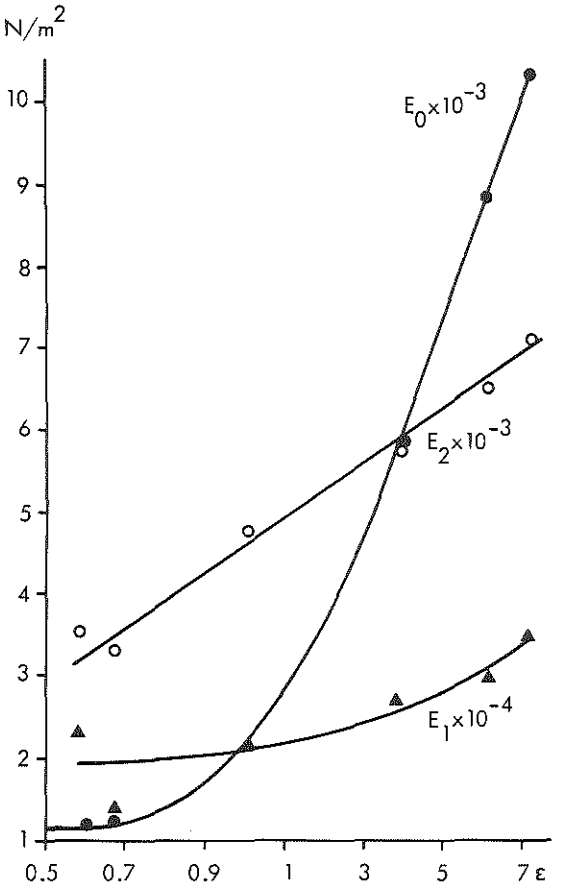


Fig. 49
Elastic moduli obtained in dog bladder in vivo after analysis of the pressure curves following stepwise volume changes at increasing volume levels, with the two-exponential model.

14. Where compared with the mean values obtained with opened abdomen (α = 0.12 sec^{-1} and β = 0.0066 sec^{-1}) the mean values obtained with closed abdomen are in the same order of magnitude (α = 0.12 sec^{-1} and β = 0.0058 sec^{-1}). From these results we may conclude that the surrounding organs have no

significant influence on the relaxation constants of the bladder in situ.

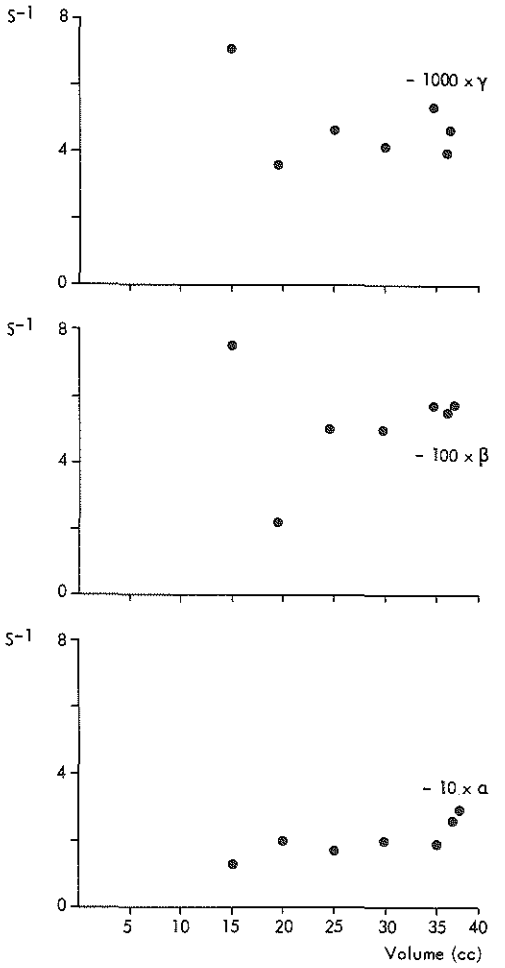


Fig. 50

Relaxation constants as a function of volume after analysis with the three-exponential model.

IV. 4. 3. 3. Experiments with increasing volume levels

To test the strain dependence of the parameters, a series of measurements was performed with increasing strain levels.

The elastic moduli, as represented in Fig. 49, show an increasing trend with increasing strain. All elastic moduli E_1, E_2, E_0 show the same trend.

The relaxation constants turned out to be independent of the

strain level (Fig. 50) which confirms our findings in bladder wall strips (Figs. 27, 28).

IV. 4. 3. 4. Experiments with active contractions

Active contractions induced during stress decrease by stimulation of the nervi pelvici (10 millisec, 20 volt, 30 pulses/sec), have no influence on the relaxation constants as is obvious from Table 15 and Fig. 51.

When the contractions are induced 5 min. before the infusion, the relaxation constants are not significantly altered. The values are represented in Table 16.

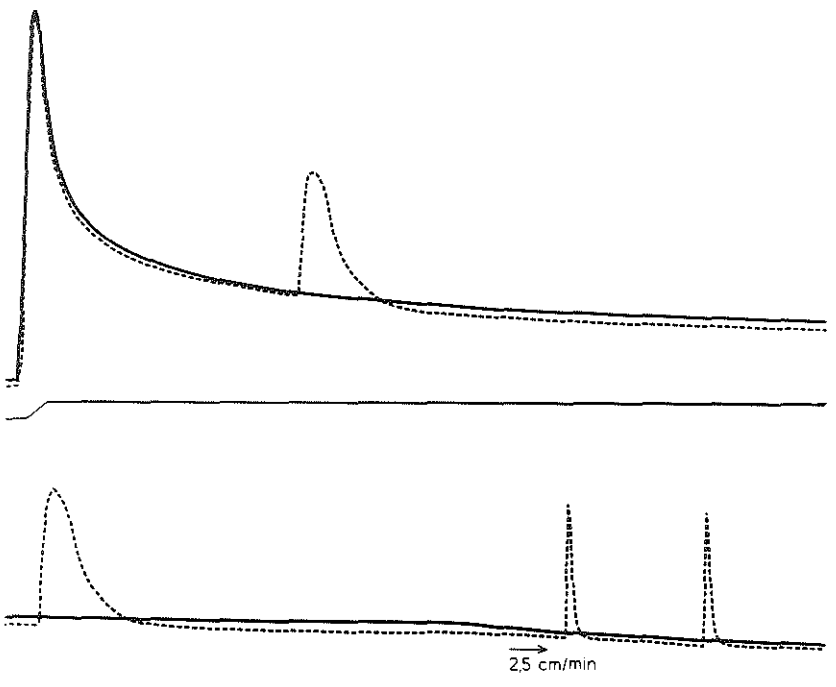


Fig. 51
Effect of superimposed contractions on pressure decrease.

Model	α (sec^{-1})	Standard deviation	β (sec^{-1})	Standard deviation	Number of experiments	Number experiments	
Two exponentials and a constant	- 0.012 - 0.14	0.007 0.008	- 0.0039 - 0.0085	0.0003 0.0005	2 5	15 16	without contractions
Three exponentials and a constant	- 0.14 - 0.16	0.01 0.04	- 0.0053 - 0.0194	0.0004 0.01	2 4	15 16	with contractions

Table 15
Comparison of relaxation constants from whole bladders with and without induced contractions.

Model	α (sec $^{-1}$)	Standard deviation	β (sec $^{-1}$)	Standard deviation	Number of experiments	Number experiments
Two exponentials and a constant	- 0.16	0	- 0.0045	0.0009	2	after 15min. rest
	- 0.11	0.01	- 0.0045	0.0003	3	
Two exponentials and a constant	- 0.12	0.006	- 0.0039	0.0001	3	5 min. after a contraction
	- 0.12	0.007	- 0.0039	0.0003	2	

Table 16
Relaxation constants from whole bladders after 15 minutes rest and 5 minutes after a contraction.

IV. 4. 4. Conclusion

From the experiments on bladders in toto we may conclude that the method by which the bladder wall is strained stepwise is feasible. The pressure decrease curve which follows a stepwise change of the intraluminal volume can be described by a mathematical model consisting of three or two exponentials and a constant.

The relaxation constants are independent of the volume level and agree very well with those obtained from bladder wall strips. This seems to indicate that the model of the bladder geometry which has been used is correct. Since the relaxation constants are independent of the strain, the time dependent properties (the parameters α, ß, and γ) of the bladder can be obtained by one stepwise infusion at an arbitrarily chosen volume level which gives an analysable pressure decrease curve. The intraabdominal organs do not influence these time-dependent properties. Induced contractions alter the pressure decrease curve, neither when they are given before the strain, nor during the pressure decrease. From the experiments on bladder wall strips, however, we concluded that changes of the influence of the contractile element result in changes in the values of the largest relaxation constant. These changes are not observed in whole bladders since the bladder cannot be strained as fast as bladder wall strips. The influence of the largest relaxation constant is therefore only partly present in the pressure decrease curve.

The elastic moduli (E_0, E_1, E_2, E_3) which represent the length dependent properties show a remarkable spread when straining at constant volume level. This spread can be explained by changes of the unstretched length l_0, which are caused by changes of the η_0 dashpot. To obtain parameters which are independent of the volume level, the relative elastic moduli were calculated. So, three or four (e_0, e_1, e_2, e_3) parameters are obtained which represent the length dependent properties. These can be obtained by one stepwise volume change.

V. Clinical Cystometry

V. 1. Some remarks with regard to the classical cystometry.

Classical cystometry is a valuable method for investigating neurogenic disturbances. It provides information on proprioreception, abnormal contractions during the collection phase, the micturition and the bladder capacity.

However, a great difficulty is that classical cystometrograms are not comparable. Apart from the fact that the methods used (infusion rates, registration method) are not uniform, which may cause apparent variations of the obtained pressure curve, the length- and time-dependency are not taken into account (see sections 2 and 3). Because of the time-dependency, it is difficult to make pressure curves comparable with the classical method, the bladder wall having to be strained in such a way, that the relative changes of length $(\frac{l - l_0}{l_0})$ per unit of time will be equal, which cannot be realized.

The time dependent properties also create difficulties with the interpretation of the second segment of the cystometrogram. Since «tone» has been thought to be represented by this second segment («tonus limb»), wrong conclusions have been attached to the shape of the cystometrogram. A long and flat «tonus limb» has been ascribed to a «hypotonic» bladder. However, tone is a theoretical definition and is defined as «that portion of the pressure which is caused by active mechanisms». Since one cannot distinguish quantitatively the active and passive portion of the measured pressure during the filling of the bladder, tone cannot be measured by classical cystometry. Therefore, the terms «hypotonic» and «hypertonic» should be avoided in this context. Using stepwise strains, we could only demonstrate that the active mechanisms cause a significant spread in the largest relaxation constants and that the smaller relaxation constants are not affected by changing the influence of the active elements (section IV. 3. 6.)

A possible method to make classical cystometrograms comparable and to adjust the obtained pressure curves to our mechanical model used in our experiments, is to base them on length dependent properties only. In order to do this, we have to make them independent of time, in other words static. The thus obtained cystometrogram will then be called a «static cystometrogram».

V. 2. The static and pseudo-static cystometrogram

Theoretically, the static cystometrogram represents that pressure-volume relationship of the bladder in which pressure is not dependent on time, the intraluminal volume remaining constant.

The mechanical model used (Fig. 34) helps us to understand what is static and what is dynamic. A fast strain changes the length of the springs E_0, E_1, E_2 and E_3 which results in a peak force. This force in the springs pulls out the plungers of the viscous dashpots. The movement of the plungers depends on time. The force in the springs disappears to the extent that the plungers are pulled out. The time needed for this dynamic behaviour depends on the viscous properties of the dashpots and the elastic properties of the springs.

When the force in the springs E_1, E_2, E_3 has disappeared, only the parallel spring E_0 will keep its force. This remaining force determines the static force in the system when all dynamic processes have been stopped.

In practice, this situation cannot be reached because of the viscoelastic components of the model, which will also affect the pressure. In fact, although the pressure decrease curve can be described by a passive model, active properties are involved also, as could be demonstrated on bladder wall strips (section IV. 3. 6.). So, the interaction between η_0 and the active component C will influence the pressure-volume relationship, even when the bladder is filled very slowly.

The E_0 values depending on strain can also be obtained by stepwise filling of the bladder. The pressure decrease curves can be analysed by a mathematical model (see formula 22). From the constant K, the E_0 value can be calculated (see formula 57). These E_0 values, thus obtained by straining at increasing strain levels, however, are dependent on the viscoelastic element (η_0) in the mechanical model (see Fig. 34). So a correction should be applied to obtain a static cystometrogram.

A theoretical static cystometrogram cannot be obtained by the classical cystometry. However, a pseudo-static cystometrogram can be obtained by filling the bladder very slowly. In terms of the model (Fig. 34), one has to fill slowly enough so that the plungers of the dashpots (η_1, η_2, η_3) can follow the strain. In that case almost no force will be generated in the springs and only the force in the parallel spring E_0 will be measured.

If a maximum error of 15 % is accepted for a bladder with a capacity of 300 ml, the maximum infusion rate can be obtained with the following formula :

$$f = \frac{C.a}{K.\gamma.V} \qquad (51)$$

where f is the percentage error, C the coefficient of the slowest exponential, a the infusion rate in ml/sec, K the constant, γ the exponent of the slowest exponential in sec^{-1} and V the volume in ml*.

Formula 51 is correct when the transfer from volume to pressure is linear. This assumption can be handled in the second segment of the cystometrogram.

If we estimate for $C/_K V$ a value of about 750, the infusion rate has to be about 3.6 ml/min., which for this bladder means a filling time of one hour and a half.

A second method in which a pseudo-static cystometrogram can be obtained consists of performing stepwise volume changes at increasing volume levels, noting the intraluminal pressure after 15 minutes pressure decrease. A pseudo-static cystometrogram is then constructed with the values obtained (Fig. 52). From the time dependent parameter, in this case the slowest exponential, the error with regard to the static cystometrogram, can be calculated as follows :

$$C.e - \gamma t + K \qquad (52)$$

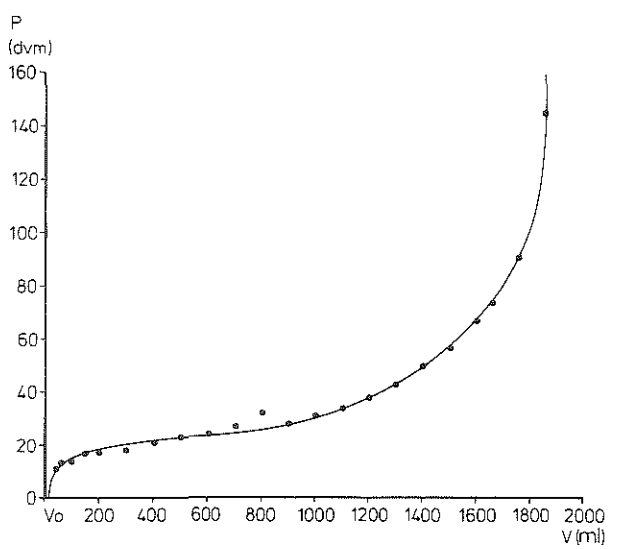


Fig. 52

Pseudo-static cystometrogram.

* The ml units have been used in this context since they are commonly used in clinical practice.

where C is the coefficient of the slowest exponential of the mathematical model (see formula 22), e the base of the natural logarithm, γ the relaxation constant, t the time and K the constant.

After 15 minutes (t = 900 sec) strain we obtain :

$$C.e^{-\gamma 900} + K \tag{53}$$

When all dynamic processes are damped (t = infinite) we only obtain the static pressure K.

The difference between static and dynamic is then :

$$C.e^{-\gamma 900} \tag{54}$$

when the value of γ is approximately 0.003 we obtain :

$$\frac{0.0672.\ C}{K} \tag{55}$$

for C/K = 2.5, an error of 15 % is obtained.

V. 3. Parameters obtained by the classical pseudo-static cystometrogram

If a cystometrogram is really measured pseudo-statically, it is possible to describe the pressure adequately by a mono-exponential curve of the elastic modulus function (E_0) (see Van Mastrigt, 1977). In this way we obtain from the classical cystometrogram the elastic exponent δ as a parameter. In order to obtain this parameter, V_0 has to be estimated in the way described in section IV. 4. 2.

V. 4. Stepwise cystometry

To describe the passive properties of the urinary bladder a stepwise infusion can be performed. The volume must be infused quickly (10 ml/sec) until a pressure peak is reached after which a pressure decrease curve should be registered during 15 minutes. To calculate the relative change of length of the wall which is caused by the volume change, we have to estimate the unstretched volume V_0. This is done by filling the lumen of the bladder by means of a syringe until a fast pressure rise is observed. At that volume the bladder wall is already strained, but no other method can be found. The pressure decrease curve is analysed by a multi-exponential model.

The stress decrease curve of bladder wall strips is analysed by a mathematical model consisting of three exponential terms and a constant. In total bladders, however, filling of the bladder to a convient volume level requires a certain time. The peak pressure, which is length- and rate-dependent, will be lower the slower the volume is infused. Therefore, a correction has to be performed for the elastic moduli (E_0, E_1, E_2), calculated from the coefficients (A, B, K) in the mathematical model (see formula 57). The correction, however, is only approximate, since a constant strain rate is assumed instead of a constant change of volume rate (see formula 47). The fastest exponential which determines the initial fast pressure decrease after stepwise filling, will only be measured for a very small part. So, the correction for the fastest exponential would be important. Therefore, the two-exponential model consisting of two exponential terms and a constant is prefered to describe the pressure decrease curve after straining of the total bladder with a mathematical step function. The obtained elastic moduli (E_0, E_1 and E_2) are strain-dependent. Since it is impossible to apply the same strain to bladders differing in size, the values of the elastic moduli are not comparable. Therefore, the relative elastic moduli are used : e_0, e_1 and e_2. These are independent of strain (see formula 58, 59) and describe **the length-dependent properties** of the bladder wall.

The time-dependent properties are also obtained from the mathematical model. The relaxation constants α , β, describe the pressure decrease curve. They are independent of the strain level (see Fig. 50).

When more stepwise fillings are performed at increasing volume levels, **the strain dependence of the elastic moduli** will be described by a mono-exponential term, (see formula 47) which gives two other parameters : the elastic coefficient |E| and the elastic exponent δ (section V. 1). For the calculation of these parameters V_0 and V_t are to be estimated. V_0 can only be determined approximately. The bladder is filled very fast with a syringe until a marked pressure rise is seen. V_t could only be determined in experimental conditions. It turned out that V_t is approximately 90 % of V_0.

Since the active components influence the fastest exponential term from the three exponential model which was used to describe stress decrease curves obtained on bladder wall strips, the influence of active mechanisms will hardly be observed on total bladders because of the slower strain rate.

The clinical relevance of the obtained parameters has to be evaluated in normal and pathological conditions.

Summary

The classical cystometry which so far has been used to investigate the function of the urinary bladder, discloses information on pain perception, temperature and occurence of contractions (Section I. 2). The cystometrogram patterns had to reveal pathological changes of the urinary bladder wall. The diagnosis was based on qualitative changes in the form of the cystometrogram, a procedure that caused confusion. A factor leading to confusion was the use of the term «tone» as it has been interpreted quite differently by various authors (Section I. 3.). Thus some regard the length of the second segment of the cystometrogram as representative of the tone, which would be influenced by neurogenic mechanisms. Tang and Ruch (1955) have shown that the length of this second segment is determined by the occurence of the micturition reflex which, though indeed of neurogenic origin, is not in any way related to the wall properties. Changes in the wall properties by overstretch or isolation of the bladder might then be reflected by a more or less steep pattern of the second segment during filling. However, experience has taught that even in healthy people the cystometrogram could not adequately be reproduced. Therefore, the reasons for this inadequate reproducibility had to be found out, and a reproducible quantitative assessment method for the wall properties had to be devised. The investigations by Remington and Alexander (1955) meant a major breakthrough. These authors found that the pressure volume curves are largely determined by viscoelastic properties, which are apparent in the form of hysteresis, among other phenomena. They established that pressure in the bladder depends on the rate of strain and that, after strain, a pressure decrease at constant volume is observed. Much earlier, Denny-Brown and Robertson (1933) had made these same observations, but had attributed them to neurogenic reflex mechanisms. Remington and Alexander came to the conclusion that qualitatively similar signs of hysteresis were found to occur in dead bladder tissue, in which neurogenic influences and active muscular processes can be ruled out.

In section II, these qualitative mechanical properties of the urinary bladder are discussed. During filling, pressure is determined, among other factors, by wall straining and the rate of straining. When the bladder is indeed filled slowly and later rapidly, the pressure curve will show a steeper course in the latter case, as a consequence of the passive viscoelastic

properties. If the intraluminal volume is kept at a constant level, pressure will decrease due to a stress decrease in the wall. A rise in pressure will occur, after part of the volume has been removed. These phenomena are also observed with rubber balloons. When comparing cystometrograms obtained by slow, continuous filling, these passive properties should be taken into account. Volume pressure curves can be compared only when the same relative change of length per time unit applies for the walls. Section III shows that this is impossible because of hysteresis and the impossibility of determining the so-called stretch volume of the bladder. The stretch volume (V_0) is determined as the volume by which the bladder wall, though stretched, is not strained. To avoid that the pressure curves of an unstrained and strained bladder would be compared, the stretch volume must be determined. Proceeding from this volume, the wall should have to be strained in an equal manner. This, however, seems to be impossible.

Therefore, a method had to be designed that would provide quantitative parameters not related to the volume. Because of the complexity of the problems, a systems approach was applied (Section IV). The bladder is thereby considered a «black-box» in which one signal is put in (here a volume signal), and another signal is obtained (here a pressure signal). This «black-box» can be distributed into various black-boxes. Indeed, a change in intraluminal volume results in a change of length of the bladder wall. The latter gives rise to a changed stress. And this, in turn, is measured geometrically as a pressure change of the bladder.

Through strain experiments with bladder wall strips, a mathematical model has been developed as outlined in section IV. 3. This model consists of the sum of three exponential functions and a constant. The stress decrease following a stepwise change in length can be described with the model. This mathematical model can be translated into a mechanical model, consisting of three Maxwell elements connected in parallel with a spring. From the model, parameters are obtained. The elastic moduli are calculated from the extent of stress attained at the termination of the stepwise straining. The values of these elastic moduli represent the length-dependent properties. It appeared from the investigation that at constant strainings for 15 minutes the elastic moduli show a decreasing trend. This trend was interpreted as a change in the initial length, l_0, of the strip. By applying a dashpot (η_0) connected in series and a contractile element (C) connected in parallel, the lengthening of the model under the influence of the strain, could be computed in the analysis (Section IV. 3. 5. 2). The elastic moduli seem to relate to the

extent of strain. This relationship was investigated by straining the wall for very brief periods (Section IV. 3. 7). With increasing strain, the sum of the elastic moduli indicate an increasing function that may be described by a mono-exponential function. Because of this length dependence, the elastic moduli cannot be used as parameters. Therefore, the relative elastic moduli were calculated, since the elastic moduli appeared to show a similar trend. A modulus relative to the sum of the other ones is a constant, unrelated to the strain (Section IV. 4. 3. 1). The relaxation constants representing the time-dependent characteristics are not related to the extent of straining. They were also assessed during changed influences of the contractile apparatus (Section IV. 3. 6). By stimulating the contractile apparatus, spreading of the higher relaxation constants increases.

The smaller relaxation constants are not influenced by the contractile element. Analysis of the pressure decrease curve was also investigated on bladders in toto under different circumstances (Section IV. 4). The relaxation constants obtained are of the same magnitude as in bladder wall strips (Section IV. 4. 3). Also, for the bladder in toto, they have no relation with the volume. The relaxation constants are not influenced by opening the abdominal wall or not doing so, nor by previous, actively induced contractions. Contractions during the pressure decrease at a constant volume do not interfere with the relaxation constants either.

The elastic moduli show varied patterns for bladders in toto : they may be upward or downward. This can be explained from the mechanical model as a consequence of the influence of the dashpot connected in series and the contractile elements. Therefore, the relative elastic moduli are used as parameters also here. Classical cystometrograms are not comparable because, apart from the fact that filling technique and method of recording differ widely, length- and time-dependent properties are not taken into account (See sections 2 and 3). The term «tone» has been wrongly used with regard to the shape of the classical cystometrogram. Classical cystometrogram can be made comparable by excluding time-dependent phenomena and by measuring almost only the length-dependent properties. This is the static cystometrogram (See section V. 2). In terms of the mechanical model, the force over the parallel spring E_0 represents the static cystometrogram. This force, however, will be influenced by the dashpot η_0, and the contractile element C.

A pseudo-static cystometrogram can be obtained, provided filling of bladder is done very slowly (± 5 ml per minute, accepting an error rate of 15 %). In that case, the shape of this mono-ex-

ponential curve provided one parameter, the elastic exponent (δ) which represents length-dependent properties. A pseudostatic cystometrogram can be obtained also by stepwise filling of the bladder. From the pressure decrease curve, E_0 can be calculated. By applying a correction for η_0, a static cystometrogram can be calculated.

When a stepwise change in volume is applied at a random volume level, a pressure decrease curve is thereafter obtained (Section VII). Analysis of this pressure decrease curve yields various comparative parameters representing length-dependence, i.e. the relative elastic moduli e_0, e_1 and e_2. This necessitates an estimation of the stretch volume of the bladder. At the same time, the relaxation constants representing the time-dependent properties are obtained (α and ß).

When at least two stepwise volume changes are applied, the elasticity coefficient ($|E|$) and elastic exponent (δ) are obtained as well, representing the length dependence of the elastic moduli. This necessitates an estimation of the tissue volume of the bladder (V_t).

The concept «tone» defined as the part of pressure that is produced by active elements is used improperly for the description of cystometrograms, as the tone cannot be measured quantitatively. Investigations have shown that the active elements primarily produce a spreading of the higher relaxation constants. Concepts deducted from the tone, such as «hypertony» and «hypotony» lead to confusion and are hence to be avoided in the literature.

To evaluate the physical properties of the urinary bladder, a stepwise cystometry is indicated. The clinical relevance of the obtained parameters has to be evaluated in normal and pathological conditions.

Samenvatting

De cystometriemethode die tot nu gebruikt wordt om de functie van de urineblaas te onderzoeken, verschaft informatie betreffende de perceptie van pijn, temperatuur en het voorkomen van contracties (sectie I. 2). De vorm van het cystometrogram is gebruikt voor het vaststellen van pathologische veranderingen van de wand van de urineblaas. De diagnose steunde op kwalitatieve veranderingen in de vorm van het cystometrogram, wat aanleiding gaf tot verwarring. De verwarring werd in de hand gewerkt door het gebruik van de term «tonus» die door de verschillende auteurs op een andere wijze werd gedefinieerd en bepaald (sectie I. 3). Sommige auteurs gebruikten de lengte van het tweede segment van het cystometrogram als representatief voor tonus, die dan beïnvloed zou worden door neurogene mechanismen. Door Tang en Ruch (1955) werd aangetoond dat de lengte van dit tweede segment bepaald wordt door het optreden van de mictiereflex, die inderdaad van neurogene oorsprong is, maar geen verband heeft met de wandeigenschappen. Veranderingen in de wandeigenschappen door overrekking of isolatie van de blaas, zouden dan teruggevonden worden in het min of meer steil verloop van het tweede segment tijdens de vulling. De ervaring leerde echter dat het cystometrogram zelfs bij gezonde mensen slecht reproduceerbaar is, zodat het noodzakelijk was de oorzaken voor het niet reproduceerbaar-zijn op te zoeken en een reproduceerbare kwantitatieve methode van onderzoek van de wandeigenschappen te ontwikkelen. Een belangrijke vooruitgang werd gebracht door de onderzoekingen van Remington en Alexander (1955). Deze auteurs toonden aan dat de volumen-drukcurven grotendeels bepaald worden door visco-elastische eigenschappen die zich onder meer uiten in hysteresis. Ze stelden vast dat de druk in de blaas afhankelijk is van de snelheid waarmee de wand wordt gerekt en dat er na rek een vermindering van de druk in het lumen optreedt bij constant volumen. Deze observaties werden overigens eerder gedaan door Denny-Brown en Robertson (1933) die ze echter verklaarden vanuit neurogene reflex mechanismen. Remington en Alexander (1955) stelden vast dat kwalitatief dezelfde hysteresis verschijnselen werden gevonden bij dood blaasweefsel, waarbij neurogene invloeden en actieve musculaire processen uitgesloten zijn.

In sectie II worden deze kwalitatieve mechanische eigenschappen van de urineblaas besproken. Tijdens de vulling wordt de

druk onder andere bepaald door de rek van de wand en de snelheid waarmee deze rek wordt aangebracht. Wanneer dezelfde blaas traag of snel gevuld wordt, zal als gevolg van de passieve visco-elastische eigenschappen, de drukcurve steiler oplopen bij snelle vulling. Wordt het ingebracht volumen constant gehouden, dan zal de druk dalen als gevolg van een spanningsvermindering in de wand. Wanneer een gedeelte van het volumen wordt verwijderd, treedt daarna een stijging van de druk op. Deze fenomenen worden ook vastgesteld bij rubber ballonnen. Bij het vergelijkbaar maken van cystometrogrammen, verkregen door een langzame continue vulling, moet met deze passieve eigenschappen rekening worden gehouden. Volumen-druk curven zijn alleen vergelijkbaar, wanneer de wand dezelfde relatieve lengteverandering per tijdseenheid ondergaat. In sectie III wordt aangetoond dat dit onmogelijk is, gezien de hysteresisverschijnselen en gezien de onmogelijkheid van het zogenaamde strekvolumen van de blaas te bepalen. Het strekvolumen (V_0) wordt gedefinieerd als dat volumen waarbij de blaaswand wordt gestrekt doch nog niet wordt gerekt. Om te vermijden dat de drukcurve van een nog niet gerekte en van een gerekte blaas worden vergeleken, zou de bepaling van het strekvolumen noodzakelijk zijn. Vanuit dit volumen zou de wand op een gelijke wijze moeten gerekt worden. Dit blijkt onmogelijk te zijn.

Om deze reden was het noodzakelijk een methode te ontwikkelen waaruit kwantitatieve parameters worden verkregen die onafhankelijk zijn van het volumen. Vanwege de complexiteit van de problemen werd gebruik gemaakt van een systeem-theoretische benadering (sectie IV). Hierbij wordt de blaas beschouwd als een «black-box» waar een signaal wordt ingestuurd (in dit onderzoek een volumen signaal) en aan de uitgang een signaal wordt verkregen (in dit onderzoek een druksignaal). De ene «black-box» kan verdeeld worden in verscheidene. Het is immers zo dat door een volumen-verandering een lengteverandering van de wand ontstaat. Door die verandering in de lengte ontstaat een verandering in de spanning van de wand. Deze verandering van de spanning wordt via de geometrie van de blaas gemeten als een drukverandering.

Op grond van rek-experimenten met segmenten blaasweefsel is in sectie IV. 3 een mathematisch model ontwikkeld bestaande uit de som van drie exponentiële functies en een constante. De spanningsvermindering die volgt op een stapvormige lengteverandering kan met dit model beschreven worden. Het mathematisch model kan vertaald worden in een mechanisch model dat bestaat uit drie Maxwell elementen parallel geschakeld met een veer. Uit het mathematisch model worden parameters verkregen. De elasticiteitsmoduli worden berekend uit de hoogte

van de spanning die bereikt wordt bij het beëindigen van de stapvormige verlenging. De waarden van deze elasticiteitsmoduli stellen de lengte-afhankelijke eigenschappen voor. Uit het onderzoek kwam naar voren dat de elasticiteitsmoduli bij constante verlengingen gedurende vijftien minuten een dalend verloop vertoonden. Deze daling werd geïnterpreteerd als een verandering van de initiële lengte l_0 van het weefselfragment. Door het inbrengen van een demper (η_0) in serie en van een contractiel element in parallel werd het mogelijk de verlenging van het model onder invloed van de rek te betrekken in de analyse (sectie IV. 3. 5. 2). De elasticiteitsmoduli blijken afhankelijk te zijn van de grootte van de rek. De rekafhankelijkheid van de elasticiteitsmoduli werd onderzocht door verlengingen aan te brengen gedurende zeer korte tijd (sectie IV. 3. 7). De som van de elasticiteitsmoduli vertoont bij toenemende rek een oplopende functie die beschreven kan worden door een mono-exponentiële functie. Door deze lengte-afhankelijkheid kunnen de elasticiteitsmoduli niet gebruikt worden als parameters. Daarom werden de relatieve elasticiteitsmoduli berekend. Het bleek namelijk dat de elasticiteitsmoduli een gelijke trend vertonen. Een modulus relatief tot de som van de anderen is een constante, onafhankelijk van de rek (sectie IV. 4. 3. 1). De relaxatieconstanten die de tijdsafhankelijke eigenschappen voorstellen zijn onafhankelijk van de grootte van de rek. Ze werden tevens onderzocht bij veranderde invloeden van het contractiel apparaat (sectie IV. 3. 6). Door stimulatie van het contractiel apparaat wordt de spreiding van de grootste relaxatieconstanten groter. Op de kleinere relaxatieconstanten heeft het contractiel element geen invloed.

De analyse van de drukverminderingscurve werd tevens onderzocht bij totale blazen in verschillende omstandigheden (sectie IV. 4). De verkregen relaxatieconstanten zijn van dezelfde grootte als bij segmenten blaasweefsel (sectie IV. 4. 3). Ook bij de totale blaas zijn ze onafhankelijk van het volumen. De relaxatieconstanten worden niet beïnvloed door het al dan niet openen van de abdominale wand noch door voorafgaande actief geïnduceerde contracties. Ook contracties tijdens de drukvermindering bij constant volumen veranderen de relaxatieconstanten niet.

De elasticiteitsmoduli vertonen bij de blazen in toto een wisselend verloop. Soms zijn ze stijgend, soms dalend, hetgeen aan de hand van het mechanisch model verklaard kan worden als gevolg van de invloed van de demper in serie en het contractiel element. Daarom worden ook hier de relatieve elasticiteitsmoduli als parameters gebuikt.

Klassieke cystometrogrammen zijn niet vergelijkbaar, omdat naast verschillen in de methode, geen rekening gehouden wordt met de lengte- en tijdsafhankelijke eigenschappen (secties 2 en 3).

Klassieke cystometrogrammen kunnen vergelijkbaar gemaakt worden door de tijdsafhankelijke fenomenen uit te schakelen en vrijwel alleen de lengte-afhankelijke eigenschappen te meten. Dit is het statisch cystometrogram (sectie V. 2). In termen van het mechanisch model stelt de kracht over de E_0 veer het statisch cystometrogram voor. Deze kracht zal echter in geringe mate beïnvloed worden door demper in serie (η_0) en het contractiel element (C). Het is echter mogelijk een pseudo-statisch cystometrogram te verkrijgen door heel traag te vullen (± 5 ml per minuut wanneer een fout van 15 % wordt aanvaard). De helling van een op deze wijze verkregen cystometrogram verschaft één parameter, de elasticiteitsexponent (δ) die de lengte-afhankelijke eigenschappen weergeeft. Een pseudo-statisch cystometrogram kan ook verkregen worden door stapvormig vullen van de blaas. Uit de drukverminderingscurve wordt E_0 berekend. Door een correctie aan te brengen voor de η_0 kan een statisch cystometrogram berekend worden. Wanneer een stapvormige volumen verandering wordt aangebracht op een willekeurig volumen niveau, wordt daarna een drukverminderingscurve verkregen (sectie VII). Uit de analyse van deze drukverminderingscurve volgen verscheidene vergelijkbare parameters die de lengte-afhankelijkheid voorstellen; de relatieve elasticiteitsmoduli e_0, e_1 en e_2. Hiervoor is het noodzakelijk een schatting te maken van het strekvolume (V_0) van de blaas. Tevens worden de relaxatieconstanten verkregen die de tijdsafhankelijke eigenschappen voorstellen (α and ß).

Wanneer ten minste twee stapvormige volumenveranderingen worden toegepast, worden tevens de elasticiteitscoefficient (|E|) en de elasticiteitsexponent (δ) verkregen die de lengte-afhankelijkheid van de elasticiteitsmoduli voorstellen. Hiervoor is het noodzakelijk tevens een schatting van het weefselvolume van de blaas te maken (V_t).

Het begrip tonus, gedefinieerd als dat deel van de druk dat door actieve elementen wordt veroorzaakt is ten onrechte gebruikt bij de beschrijving van cystometrogrammen. Tonus kan niet quantitatief gemeten worden. Uit het onderzoek is gebleken dat de actieve elementen vooral een spreiding van de grote relaxatieconstanten veroorzaakt.

Uit tonus afgeleide begrippen als hypertonie en hypotonie zijn alleen verwarrend en dienen in de literatuur te worden vermeden.

Voor het onderzoek van de fysische eigenschappen van de urineblaas is de stapvormige cystometriemethode aangewezen. De klinische relevantie van de verkregen parameters dient aan normale en pathologische blazen onderzocht te worden.

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STELLINGEN

1. Het klassieke cystometrogram biedt zelfs wanneer het pseudo-statisch is verkregen, slechts één parameter om de physische eigenschappen van de blaaswand te beschrijven.
2. Bij de stapvormige cystometrie, waarbij een drukvermin-deringscurve wordt onderzocht nadat een voldoend volumen stapvormig in het lumen van de urineblaas is gebracht, worden twee parameters verkregen die de tijdsafhankelijke physische eigenschappen voorstellen en drie parameters die de lengteafhankelijke physische eigenschappen voorstellen.
3. Het begrip «tonus» wordt op een verkeerde wijze verbonden aan het klassieke cystometrogram.
4. Het is nuttig de studie van ingewikkelde medische proble-men door middel van een systeem theoretische benadering aan te vatten.
5. Wanneer bij drukmetingen aan uitgezette hogere urine-wegen, de druk nauwelijks stijgt bij perfusie, is een obstructie ervan niet uigesloten.
6. Het «notekraker» fenomeen, waarbij de vena renalis geklemd is tussen de aorta en de arteria mesenterica superior, is een veel voorkomende oorzaak van periureterale varices en een omgekeerde bloedstroom in de vena gonadalis en uitzetting ervan.
7. De paradox van de leugenaar (van Eubulides, 4^{e} eeuw vóór Christus) waarin door Epimenides («de leugenaar van Kreta») wordt gesteld dat alle Kretensers altijd liegen, kan worden opgelost door onderscheid te maken tussen taal en metataal.
8. In het boek «Ambrunitië, het avondland in de morgen» stelt Mark Eyskens dat het geluk = $\frac{\text{bevredigingsmiddelen}}{\text{behoeften}}$. Deze definitie van geluk is zeker niet algemeen en kan zelfs verkeerd zijn.
9. Wanneer de mens meer met de realiteit van de dood zou geconfronteerd worden zou hij waarschijnlijk anders leven.
10. Daar het noodzakelijk is bij het onderwijs naast een rationale opvoeding ook emotionele factoren aan te wenden, zou het nuttig zijn bij de aanstelling van de lesgevers ook met andere dan rationele factoren rekening te houden.
11. Door een recent wetsvoorstel in België waarin wordt gesteld dat de belastingen op de verboden kansspelen zullen worden verhoogd, tracht de Belgische staat de belastingsbijdragen beneden die van de Nederlandse te houden.

Bo Coolsaet — Rotterdam, 2 februari 1977

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