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Clinical pharmacology, physiology and pathophysiology of superficial veins—1[1]

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1 Venous resistance contributes very little to total peripheral resistance; more than half of the total blood volume, however, is contained in the extrathoracic veins. Owing to marked differences between venous and arterial anatomy and physiology, studies on veins and arteries usually require different methodological approaches. Whereas for arteries the most relevant parameters are resistance, pressure and flow, for veins volume and compliance are most important. For studies of general aspects of the peripheral circulatory system, venous occlusion plethysmography is probably the most useful method. The determination of both the rate of rise in limb volume and the total volume rise after inflating a proximally applied occlusion cuff to a subdiastolic pressure permits the concomitant estimation of both arterial flow and venous compliance.

2 Studies of direct pharmacological or physiological effects on veins, interactions of various pharmacological or physiological stimuli, or pathophysiological changes in venous responsiveness have been facilitated by the development of investigational techniques relying on direct measurements of the compliance of single human veins *in vivo*. One of these, relying on the use of a linear variable differential transformer (LVDT) for determining changes in the compliance of superficial veins at a standardized congestion pressure, has been found very suitable for the practical application in both patients and healthy subjects.

3 Physiological studies were carried out on the effect of age, exercise, temperature, and the menstrual cycle on venous compliance and venous responsiveness to various stimuli. In addition, interindividual variability in venous responsiveness in monozygotic and dizygotic twins and in unrelated subjects was investigated, and studies on the function of the endothelium were carried out in man *in vivo*.

4 Pathophysiological studies using this technique were reported from patients with hypertension, orthostatic hypotension, myocardial infarction, varicosis, cystic fibrosis, asthma, diabetes, systemic sclerosis, and cluster headache.

5 Clinical pharmacological studies represent a most important field for the use of this method. Studies were carried out on the effects of a large number of constrictor and dilator agents, and also on drug interactions on human veins *in vivo*. Venoconstriction was observed after local administration of α-adrenoceptor and 5-HT-receptor agonists, ergot derivatives, angiotensinogen, angiotensin I and II, and several prostaglandins.

6 Owing to the low venous tone present under normal conditions, venodilator effects can usually be quantified only on veins that have been preconstricted by e.g. noradrenaline or 5-hydroxytryptamine. Under these conditions dilatation was observed after the administration of β-adrenoceptor agonists, cholinergic (muscarinic) agonists, nitrates, calcium antagonists, bradykinin, substance P and several prostaglandins.

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7 Studies on drug interactions on human veins were also reported, relating for example to the mode of action of ergot alkaloids, the effect of ACE inhibitors on the formation of angiotensin II, and the actions of α- and β-adrenoceptor antagonists. The technique appears very suitable for studies to investigate the mode of action of new drugs in man *in vivo* and to investigate potential species differences in the actions of such drugs.

Keywords venous compliance superficial veins venoconstriction venodilatation pharmacodynamics

Introduction

The veins are often neglected in studies on cardiovascular pharmacology and physiology. This probably reflects the fact that most of the experimental techniques used on the arterial side of the circulation are not suitable for studies on veins. This in turn is due to the anatomical and physiological differences between these two parts of the circulatory system. Most of the pressure gradient between the left ventricle and the right atrium is spent on the arterial sections of the vascular bed, and only little is left for the veins. It has, however, to be remembered that, although venous resistance contributes very little to total peripheral resistance, about two-thirds of the blood volume is contained in the extrathoracic veins but only about 15% in the arterial system and the rest in the intrathoracic organs (heart and lungs) [1–4].

Owing to their easy accessibility, their low intravascular pressure, their relatively thin vascular walls, and their distensibility, veins offer a unique opportunity for the direct study of actions and interactions of pharmacological and physiological stimuli on a human vascular bed *in vivo*, without the need for invasive procedures. Several methods have been developed for this purpose and are used to study direct effects of such stimuli and their interactions on superficial veins in man [5, 6]. One of the techniques used for the determination of changes in the compliance of human veins *in vivo* relies on a linear variable differential transformer (LVDT), a technical device that is relatively easy to use in human beings [7]. This technique has been found useful to study the effects of different constrictor and dilator agonists on venomotor receptors *in vivo*, as well as to investigate interactions between agonists and antagonists on a human vascular bed *in vivo*, to explore the effects of age and disease on the vascular actions of various agonists, and to study physiological and pathophysiological issues.

This review first briefly discusses the various methods that are available to study changes in venous compliance in man *in vivo*, then refers in more detail to the methods used for the direct measurement of effects on superficial human veins. It compares the technique using the LVDT with previous versions of the method and analyses its potential for clinical pharmacological, physiological and pathophysiological investigations. The main part of the article consists of a review of the studies carried out in man *in vivo* using the superficial hand vein methods.

In vivo methods to study venous compliance

Introduction

In view of the many physiological and pathophysiological differences between the venous and the arterial system, it is understandable that different techniques are usually required to evaluate the effects of physiological and pharmacological actions on these main parts of the circulatory system. On the arterial system, the determination of arterial pressure and flow is essential, whereas the important parameters relating to veins are venous volume and venous compliance.

The most widely used method for the *in vivo* assessment of changes in venous tone in a given peripheral region, usually an arm or a leg, is plethysmography. It exists in several modifications that are all based on the same principle. The volume increase of a limb or a segment of a limb is measured after the occlusion of venous outflow by means of a proximally applied occlusion cuff, which is inflated to a subdiastolic pressure. This permits the determination of venous volume, the changes of which, as long as the occlusion pressure remains constant, reflect changes in venous tone. This method, which will be discussed below in more detail, has the added advantage that it can, at the same time, be used to measure arterial blood flow in the limb investigated [8, 9].

A more global view of changes in blood volume in different areas of the body can be obtained by radioactive labelling of the plasma volume, e.g. with technetium-labelled albumin and measurement of the distribution of radioactivity by means of a gamma camera before and after administration of the stimulus to be investigated [10]. With this method it was possible to document on the one hand the shift of blood from the peripheral veins into the thoracic area occurring after physical (e.g. compression bandage of the legs) or pharmacological (e.g. dihydroergotamine) procedures constricting peripheral veins and on the other hand the opposite shift of blood towards the periphery occurring after the administration of nitroglycerine or the inhibition of venous outflow of the legs by means of an occlusion cuff [10, 11]. Such general studies on the global blood distribution in the human body remind us of an important fact: any acute reduction of venous volume in a given region of the body must necessarily lead to a corresponding increase in another area. Only in the long-term do adaptive changes in total blood volume occur.

None of the methods discussed above, however, permits to distinguish between direct actions of pharmacological or physiological stimuli on a given vein and reflex changes of venous tone secondary to effects occurring in other areas of the vascular bed. For studies of direct physiological or pharmacological effects on human veins and for investigations of interactions between different agents or stimuli on venomotor tone, experiments on single veins *in situ* are required. Three main techniques for studies on superficial human veins have been developed for this purpose (for details see below): 1) measurement of pressure changes in a venous segment isolated from the general circulation [12, 13]; 2) measurement of pressure changes in a vein not physically isolated after injection of constrictor agents [14, 15]; and, more recently, 3) measurement of changes in venous diameter at a standardized congestion pressure [7, 16, 17]. This last method, which is the most frequently used today, is based on the fact that, when the venous pressure remains constant, changes in venous diameter are proportional to changes in venous tone.

Venous tone is very dependent on temperature [18, 19], and marked shifts of regional blood volumes can occur on changing position [20]. It is therefore essential that all techniques used to measure changes in venous compliance be carried out at a constant room temperature and after an adequate period of acclimatization of the subject to the laboratory conditions. For repeated measurements the subject needs to be placed as far as is possible in the same position. Also, the timing of the measurements with respect to the time of the day and the time of meals can be important and should, therefore, be standardized if possible.

Plethysmography

As mentioned above, the method used most frequently for the assessment of the circulation in the human extremities is plethysmography. It permits the simultaneous determination of parameters related to the arterial and the venous side of the circulation. The basic principle of the many modifications of this technique consists of the measurement of changes in the volume of a limb or a segment of a limb after the abrupt inflation of a proximally applied occlusion cuff to a pressure which is higher than venous but lower than diastolic pressure. Hence this occlusion cuff temporarily inhibits venous outflow but does not interfere with arterial inflow. The rate of the volume increase immediately after starting the occlusion therefore reflects arterial flow. After a period of time (usually several minutes) venous pressure has risen to the occlusion pressure, and arterial inflow now equals again venous outflow. The difference in volume of the studied limb (or a segment of the limb) before and after inflation of the occlusion cuff consequently reflects the blood pooled in the limb (or the segment of the limb) at the occlusion pressure applied. It does, however, normally not represent the total blood volume of this segment, but only the volume increase from the basal volume after the occlusion. Volumes very near to total blood volume at the given pressure can, however, be determined by first draining the veins of the limb in question by elevating the foot or hand of the supine subject above heart level.

The very first apparatus used for determining changes in limb volume was the water-filled plethysmograph [21], in which an arm or a leg of the subject is placed. By measuring the increase in the volume of water displaced by the extremity after inflating the proximally applied occlusion cuff, very accurate measurements of changes in arterial flow and in venous volume can be obtained. It is, however, not easy to tighten the apparatus around the limb without interfering with venous outflow; in addition, to avoid thermal effects on venous tone it was essential to keep the temperature of the water in the apparatus exactly at the temperature of the limb. Several attempts have therefore been made to overcome these limitations.

One was the use of an air-filled so-called collection cuff instead of the water-filled device [22, 23]. This collection cuff is comparable with the occlusion cuff or a standard sphygmomanometer-type cuff. It is applied distally to the occlusion cuff around the section of the limb for which haemodynamic parameters have to be determined and inflated to a low pressure of around 10 mm Hg. Changes in the volume of the section of the limb encircled by the collection cuff now lead to minute changes in the pressure of this cuff. These can be recorded and used to calculate the changes in limb volume. A calibration is easily possible by adding or subtracting given volumes to the cuff.

Today, the most widely used version of the plethysmographic method is based on the application of a strain-gauge around the limb [8]. Such a strain-gauge consists of a rubber or silastic tube filled with mercury. Like the air-filled collection cuff just described, it is fixed around the limb distally to the occlusion cuff. Increases in the limb volume produce a lengthening of the tube and thus a thinning of the mercury column in this tube and consequently an increase of the electrical resistance of the strain-gauge. From the recordings of electrical resistance the changes of the volume of the segment of the limb can therefore easily be calculated [8]. An interesting variant of this technique uses plastic 'sliding-links' by means of which the mercury tube is held at a small distance from the skin of the limb to avoid a potential influence of temperature on the mercury column and an adhesion of the tubes to the skin [24]. This method has been used to assess normal values as well as the reproducibility of measurements of arterial and venous circulation in the extremities of healthy subjects [9].

Other, less commonly used variants of the plethysmographic technique have been developed, such as the combination of blood pool scintigraphy with plethysmography, in which volume changes after inflation of the occlusion cuff are measured by determining changes in radioactivity over the limb or the section of the limb to be investigated [25–27].

Single-vein studies

1 Isolated venous segment This method, which is based on pressure measurements in an isolated sec-

tion of a superficial human vein, was described by Burch & Murtadha [12]. A segment of a superficial forearm vein is isolated from the general circulation by means of externally applied wedges. A needle is then inserted into this segment so that venous pressure can be recorded. Pressure changes can be determined after the application of physiological stimuli or the direct injection of the drugs into this segment. This technique was one of the first *in vivo* methods used for studying direct drug effects on single human veins. It was found useful for evaluating dose-response curves of constrictor agents such as noradrenaline and angiotensin II and to study the effects of various physiological stimuli such as a deep inspiration on venous tone [12, 13]. The procedure with the wedges is, however, rather cumbersome, and any movement of the hand of course interferes with the measurement.

2 Venoconstriction test Sicuteri's group [14, 15] introduced a technique by which pressure in a superficial dorsal vein of the hand or wrist is measured before and after local infusion of a drug that produces venous spasm, i.e. a complete constriction of the vein investigated. Under these conditions the volume of blood contained between the spasm and the nearest venous valves is separated from the general circulation in a similar way as the 'isolated venous segment' used in the techniques described above. Pressure in this segment is recorded, and the area under the pressure-time curve [28] can be used to quantify the venoconstrictor activity of the constrictor agent administered. The method has been used to obtain dose-response curves for different agonists such as noradrenaline and 5-hydroxytryptamine and their interactions with antagonists [15, 28]. Relatively high doses of the constrictor agents have, however, to be used in this test, as complete constriction is required for a pressure rise.

3 Compliance of superficial veins Optical method: An attractive method to determine changes in the compliance of superficial human veins *in vivo* was described by Nachev *et al.* [16]. It relies on the measurement of changes of the diameter of a vein at a constant occlusion pressure. This technique is based on the fact that, when venous pressure remains constant, changes in venous diameter directly reflect changes in venous tone. Contrary to the techniques described above, however, this method can be used to detect very small effects on venous tone, e.g. after the local infusion of low concentrations of constrictor agonists.

The tone of superficial hand veins varies considerably, depending on the position of the hand with respect to heart level. A standardized position has therefore to be used in such studies in order to obtain meaningful results. In all versions of this technique, an arm of the subject resting flat supine is placed on a rigid support sloping upwards at an angle of 30°, so that the position of the hand is definitely above heart level. Consequently all superficial veins are completely drained. If a sphygmomanometer cuff is now placed on the upper arm and inflated to a subdiastolic pressure (45 mm Hg has most frequently been used) the veins are filled, distended and become visible. In the original version of the technique [16, 29], a stereomicroscope was used to measure the diameter of superficial hand veins. To permit this, a spot on the top of the distended vein is marked with black ink. The stereomicroscope is then positioned at exactly right angles to the skin surface. It is then focused on the marked spot on the summit of the vein once before and once after inflation of the sphygmomanometer cuff on the upper arm to a subdiastolic pressure of usually 45 mm Hg. The exact positions of the microscope are assessed before and after inflation of the cuff. The difference between the readings of these two positions is identical to the diameter of the vein at the congestion pressure applied. The method has been found useful to establish dose-response curves for various constrictor agonists [30, 31] and to study interactions between constrictor agonists and their antagonists on human veins [32]. Effects of drugs can be studied either during their direct local infusion through a small needle that is inserted into the vein distal to the point of measurement or, of course, after their systemic administration. Also dilator effects of drugs can be examined with this method, if the vein has been preconstricted with a constrictor agonist such as noradrenaline or 5-hydroxytryptamine [30, 33].

There are, however, two important technical drawbacks of this otherwise very useful original version of the method. One is that the diameter of the vein cannot be continuously recorded, and the other that even slight movements of the hand interfere with the measurement.

Several modifications of this technique have therefore been developed. In one variant, published by Aminu & Vere [34, 35], a small capacitor was placed on the dorsum of the hand. One of its plates was fixed on the skin directly over the vein to be investigated and the other on the skin beside the vein. Changes in venous diameter therefore produced relative movements of these plates and changes in the capacitance of the device, which could be recorded. Although the method was found useful for continuous recording of changes in venous diameter, problems were encountered with respect to the fixation of the plates on the skin.

In another modification, White & Udwadia [36] used a displacement transducer and placed the tip of its arm over the summit of the superficial hand vein to be studied. This again enabled continuous recording of diameter changes, but because the body of the transducer was fixed on a stand beside the hand there remained the problem of a great sensitivity to even slight hand movements.

The diverse problems with all the different variations of the optical method led to the development of a new technique relying on the use of a linear variable differential transformer (LVDT), which permits continuous recording of venous diameter and in addition is less dependent on hand movements, relatively easy to execute and not uncomfortable to the subjects studied. This is described in detail below.

Another modification has been suggested by Steen *et al.* [37], who used a photoelectric technique to

determine changes in the diameter of superficial veins and reported results on the effects of α-adrenoceptor agonists on human saphenous veins [37, 38].

Measuring compliance of superficial veins with a linear variable differential transformer (LVDT)

Description of the method

The optical method described above does not permit continuous recording of venous tone, and also its various modifications presented problems, with respect to either the positioning of the measuring device or the disturbing influence of hand movements. A new technique was therefore developed, which is based on the same basic principle as the optical technique, namely the measurement of changes in the diameter of a superficial human vein at a constant and standardized occlusion pressure. Because the measuring device is placed on the dorsum of the hand, small movements of the hand do not interfere with the measurements. This was made possible by using a linear variable differential transformer (LVDT) [39] for this purpose, which is a rather small and light device. With the aid of a small tripod, it can be placed directly astride a superficial vein on the dorsum of the hand or the foot and used to record changes in venous compliance [7, 40]. An LVDT consists of a linear array of three identical coils of which the central primary one is energized by an alternating current. At the outset, therefore, the voltages induced in both of the secondary coils are identical. The two secondary coils are connected in serial opposition, so that the phase of the voltage of one of them differs by 180° from that of the other. The resultant voltage at the output of the transformer is therefore zero. This voltage remains zero if an iron core is placed into the central aperture of the transformer, as long as this core remains in an exactly central position. The voltage induced in the secondary coils will, however, become different when the core is displaced from its central position. Thus there is now a resultant voltage, which is either positive or negative, depending on the direction of the movement of the core. The magnitude of this voltage reflects the distance by which the core has been moved from the central position. This device, which can be exactly calibrated, is therefore a displacement transducer and allows the continuous recording of the position of the central core of the transformer and, therefore, venous diameter.

The experimental procedures of this technique are essentially the same as those used for the optical method [16, 31]. The lower arm (or the lower leg or the foot if veins in other parts of the body are studied; see below) of the subject, who is lying flat in the supine position, is placed on a rigid support in such a way that the hand (or the foot) is situated above the level of the heart. This leads to complete emptying of all superficial veins in this area. The support used for the arm or the leg is preferably moulded in such a way that the subject feels comfortable even over longer periods of time (studies lasting for several hours have been found to be feasible), but at the same time is restricted as far as arm and hand movements are concerned. An occlusion cuff (standard sphygmomanometer cuff) is then placed on the upper arm (or on the leg) and inflated to 45 mm Hg (lower occlusion pressures have been used by some investigators). The superficial veins of the hand (or the foot) are thereby dilated and become visible. A vein suitable for measurements is then chosen. A vein is considered suitable if it has no tributaries over a segment of about 2–3 cm and if it is neither over- nor under-crossed by other veins within about the same distance. With the aid of a small tripod, the differential transformer (Schaevitz, Type 025 MHR) is mounted on the back of the hand (or the foot). This is done in such a way that a very light (0.5 g) core, which is inserted into the central aperture of the transformer, stands exactly on the summit of the vein to be studied. The core used in our experiments is composed of an iron part situated in the centre of the transformer (and responsible for the changes in the voltage induced in the secondary coils) and a non-magnetic part which connects the magnetic iron part with the surface of the vein. Vertical movements of this core reflect changes in the diameter of the vein. As described above, such displacements of the core produce changes of the voltage induced in the secondary coils of the transformer, and these changes are, within the range of diameter changes observed in such human investigations, linearly dependent on the displacement of the core, i.e. the changes in venous diameter. This device, therefore, enables a direct and continuous recording to be made of changes in venous diameter (Figure 1).

Since its first publication, this method has been employed by many investigators for studying the effects of various agents on venous compliance after direct local infusion [7, 41–49], intravenous or subcutaneous injection [43, 44, 49] and oral [7, 43] and other [50–52] routes of administration of drugs, to study miscellaneous physiological effects [49, 55, 56] and pathophysiological changes in various diseases [56–62], but also to investigate direct drug interactions on human veins *in situ* [43, 51, 57, 63–74]. Barthel & Koth [75] were the first to report the use of the method on superficial foot veins where they studied the venoconstrictor actions of noradrenaline, dihydroergotamine and etilefrine. Most of the studies discussed in the present review have been carried out with this technique.

Methodological details

Duration of cuff inflation Different schedules have been used, but in a comparative study in healthy subjects no difference in the diameter of superficial hand veins and their responses to the constrictor effect of noradrenaline was observed when the occlusion cuff was either inflated just for the last 2 min of each of the five infusion steps of the noradrenaline dose-response curve (each lasting for 5 min) or continuously during the 25 min required for the completion of the whole noradrenaline dose-response curve [7].

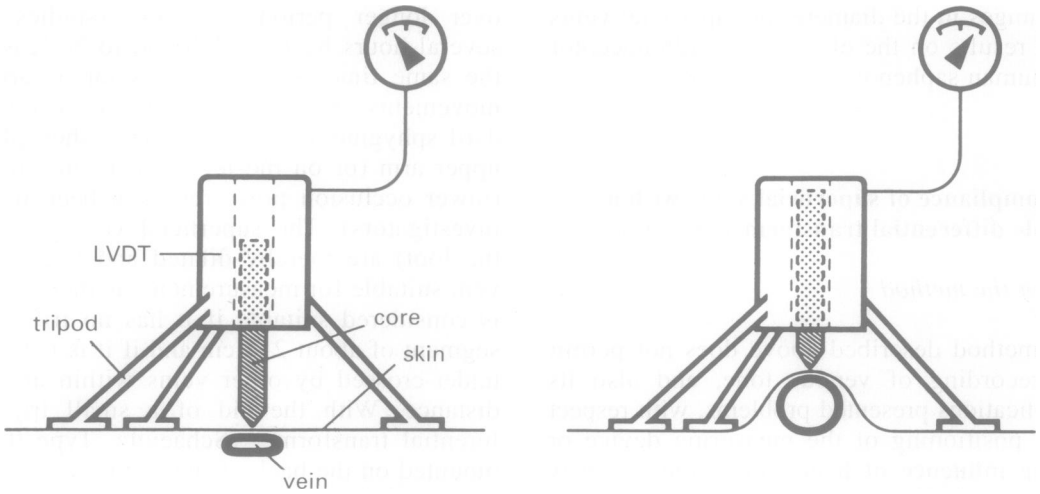

Figure 1 Diagram of the experimental set-up of the LVDT method. On the left the core stands over the empty vein and on the right over the vein dilated during inflation of the sphygmomanometer cuff placed on the upper arm to 45 mm Hg (reproduced from [7] with permission).

Temperature Because superficial human veins are involved in the regulation of body temperature, venous tone is highly dependent on ambient temperature. In experiments using the optical version of the technique on superficial hand veins, Nachev *et al.* [16] found a positive correlation between local skin temperature and environmental temperature on the one hand and venous diameter at a congestion pressure of 45 mm Hg on the other. Also the effects of constrictor agonists on superficial hand veins have been found to vary with temperature (see below). It is therefore preferable that the experiments be carried out in a temperature-controlled room (we usually employed a temperature of 22° C (range 21.5–22.5° C)) and the subjects are acclimatized to the room temperature for at least 45 min before the experiment proper. This should not be started until repeated inflation of the occlusion cuff to 45 mm Hg yields stable measurements of venous diameter.

Smoking, caffeine To avoid interference with the measurement, smoking and caffeine-containing drinks should not be permitted on the study days. In one of our studies (Aellig, unpublished), the smoking of a cigarette produced a marked reduction of the diameter of superficial hand veins.

Food and drinks In studies in which repeated tests are carried out in the same subjects, or in which prolonged observations of venous compliance are carried out after the administration of drugs or other stimuli, also diet has to be standardized, as digestion exerts marked effects on the circulation and specially blood distribution between the splanchnic area and the rest of the circulation. In our studies we usually allowed a light breakfast 2 h before each experiment. No other food is then taken during short experiments, whereas in longer-lasting studies a small sandwich is given about every 2 h, starting at 4 h after the beginning of the experiment. Food was always taken immediately after measurements in order to permit the longest possible interval before the next measurement. Also liquid intake was usually standardized in our studies, 100 ml of xanthine-free beverage was given hourly immediately after the end of a measurement.

Local drug administration

The direct effects of drugs can be investigated by local infusion into the vein to be studied. This is one of the most useful aspects of the methods used to determine changes in the compliance of single superficial human veins *in vivo*, because they permit the determination of drug effects after the local application of very low doses of these substances, thus avoiding effects on the systemic circulation and cardiovascular reflexes. This can be particularly useful, if the effects of new drugs have to be studied, where there is little experience in man.

Method for local drug infusion The drugs are in our own studies infused through a fine 27-gauge needle of a lymphangiography set which is inserted into the vein about 10 mm distal to the point of measurement. This needle is inserted at the beginning of the experiment, before the acclimatization period, to avoid any residual effect of venepuncture. It should, however, be mentioned, that the tiny needle used in these studies is usually subjectively and objectively (lack of effect on venous tone) very well tolerated.

Duration of local drug infusions For short-acting substances such as noradrenaline or adrenaline several dose-response curves can be established in one experimental session, therefore permitting the study of interactions between such agonists and other drugs. If several infusions of short-acting substances are given on the same day, they have to be separated by an interval long enough to permit recovery from the effects of the previous infusion. During this time physiological saline is infused in the same volume as the drug, in order to prevent blockage of the needle and to render the experimental conditions before, during, and after drug infusions as identical as possible.

Volume of drug infusion The infusion volume (saline or active drug) in most of our studies was 0.1 ml min^{-1} during the whole experiment. The active drug is usually diluted with physiological saline to obtain the concentrations required for infusion of the preset volume. Higher infusion volumes have, however, been given. In our first studies we used 0.1 and 0.2 ml min^{-1}, and Collier *et al.* [30] used 0.25 ml min^{-1}. Beermann [65] investigated the effects of various infusion volumes up to 4 ml min^{-1} on venous diameter at a congestion pressure of 45 mm Hg. They found no difference in venous diameter when measured at the different infusion rates. It should, however, be mentioned, that in this study only the effect of the infusion volume on venous diameter was determined. It may well be that by diluting venous blood high infusion volumes might influence physiological effects, such as the conversion of angiotensin I to angiotensin II. This suggests that, although variable infusion rates render the administration of increasing doses of drugs easier, as only one single solution in a given concentration has to be prepared, the use of different solutions administered in very low volumes is preferable, as in this way the blood flowing in the veins is not excessively diluted and normal physiological conditions are maintained.

For the construction of dose-response curves of short-acting drugs, each dose is usually administered for a period of about 5 min, and the plateau of venous diameter (at an occlusion pressure of 45 mm Hg) reached during the last 3 min of each infusion taken as the diameter during that dose.

The method was used for many investigations of the direct effects of locally infused drugs on superficial veins as well as for studies on direct local interactions of drugs. Dose-response curves demonstrating a marked constrictor effect of noradrenaline, adrenaline and 5-hydroxytryptamine were constructed after local infusion of doses ranging from 2 to 32 ng min^{-1}, i.e. in doses about 1000 times lower than those required for systemic effects [7]. A similar ratio of the doses required to produce an effect after local infusion directly into superficial hand veins and those necessary by systemic intravenous administration was also suggested by Goldberg *et al.* [76]. They found that the dose of phentolamine required for a given reduction of the noradrenaline dose producing a 50% constriction of superficial hand veins by local infusion was about 1000 times higher when phentolamine was administered intravenously than when it was given by direct local infusion.

Both the optical and the LVDT technique were used to study the constrictor effects of many substances such as locally infused catecholamines [7, 30, 44], phenylephrine [57], 5-hydroxytryptamine [7, 30], angiotensin I [65, 67, 77], angiotensin II [30, 77], prostaglandins A_1, A_2, B_1, E_2 and $F_{2\alpha}$ [78, 79], somatostatin [41], and several ergot derivatives [7, 31, 42–44, 80–83]. This is discussed in more detail in the section on clinical pharmacology (see part 2). Apart from the investigation of the effects of direct local drug infusions, the method described of course also permits the determination of changes in venous diameter after systemic, transdermal or intranasal administration of drugs but also the study of physiological stimuli and pathophysiological conditions (see below).

Reproducibility of measurements

Within- and between-subject variability The reproducibility of dose-response curves for the venoconstrictor effects of noradrenaline established with this method was assessed by Alradi & Carruthers [44]. Noradrenaline dose-response curves were constructed repeatedly in a group of healthy volunteers on different days, and the results showed that intrasubject variability even for data obtained on different days was low, whereas larger differences were observed between the values obtained in different subjects. Intrasubject variability was low for studies not only on the same vein but also on different veins of either hand of the same subject [44, 84]. A good reproducibility of dose-response curves for venoconstrictor agents was also found in our own studies. Both the intra- and the interindividual variability of the constrictor effects of noradrenaline (Figures 2 and 3) and of the interindividual variability of the constrictor effects of dihydroergotamine (Figure 4) on hand veins of healthy subjects were comparable with those reported by Carruthers *et al.* [120–122]. In addition, however, this study also showed that, with respect to the venoconstrictor activity of the agents tested, the inter- and intraindividual variabilities of results obtained on foot veins of the same subjects were of the same order of magnitude as those obtained on hand veins [49] (see also below). In another study, the influence of pizotifen and ergotamine on dose-response curves for the venoconstrictor effects of noradrenaline and 5-hydroxytryptamine were investigated [63]; because this study included a placebo control, the results also permit an estimation of the reproducibility of repeatedly established dose response curves for the constrictor agents tested. The results show that the mean results of the dose-response curves are almost superimposable; there was however, an interindividual variability as in the other studies mentioned.

Diurnal variations In a study carried out to measure changes in venous compliance after oral administration of dihydroergotamine [7], the subjects received in randomized double-blind order the active drug and placebo. The results showed that after placebo administration there was remarkably little fluctuation in venous diameter for the first 4 h after administration, and also after 6 h and after 8 h only small variations occurred. The subjects of this study did not, however, leave the temperature-controlled room throughout the experiment. They were lying flat supine on the couch the first 4 h, whereas after the 4 h and after the 6 h measurement they were allowed to rise for 1 h. A relatively steady behaviour of venous diameter over time was also observed in two other studies, in which the effects of locally infused or systemic intravenous dihydroergotamine and placebo were studied [42, 43]. Systemic intravenous placebo as well as locally infused placebo were without any relevant effect on

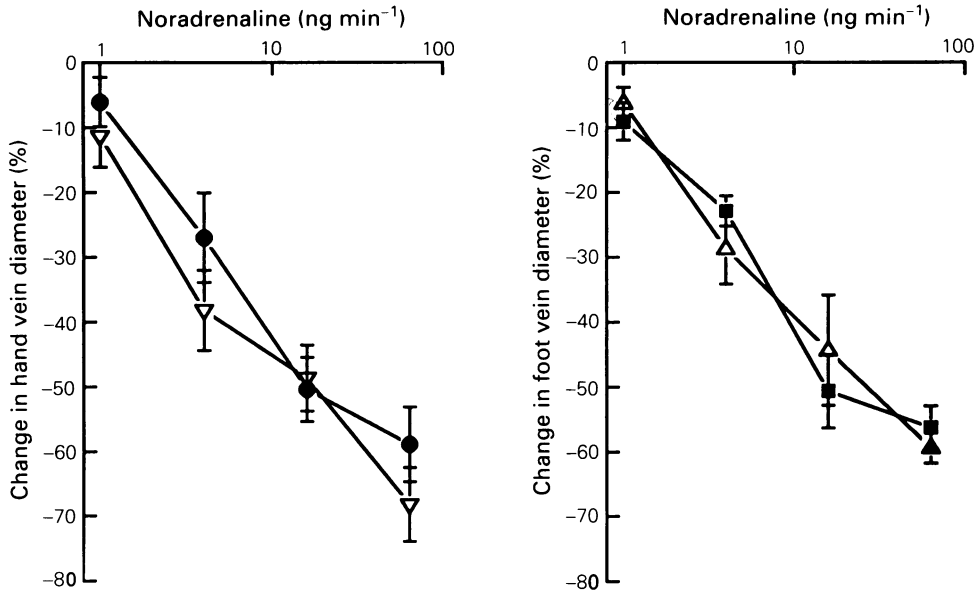

Figure 2 Reduction of hand and foot vein diameter at an occlusion pressure of 45 mm Hg after local infusion of noradrenaline; means ± s.e. mean (data from [49]).

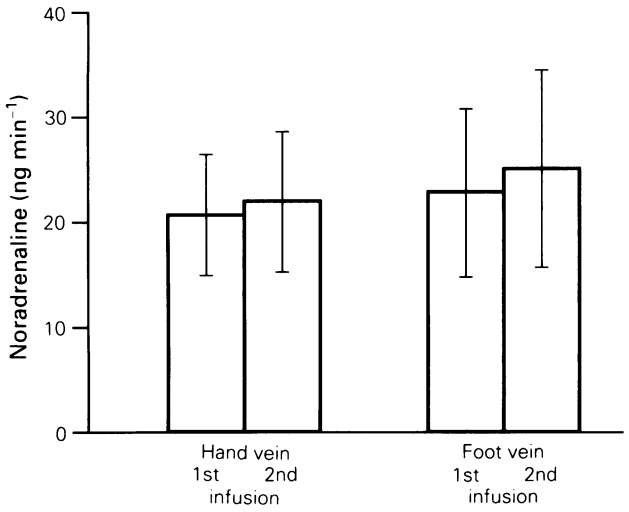

Figure 3 Dose of noradrenaline required to reduce the diameter of superficial hand or foot veins at an occlusion pressure of 45 mm Hg by 50%; means ± s.e. mean, $n = 5$ (data from [49]).

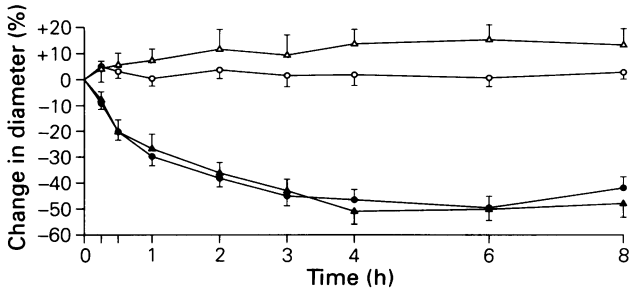

Figure 4 Changes in hand (open and solid circles) and foot (open and solid triangles) vein diameter at an occlusion pressure of 45 mm Hg after intramuscular administration of 0.5 mg dihydroergotamine (solid circles and solid triangles) and placebo (open circles and triangles); means ± s.e. mean, $n = 12$ (reproduced from [49] with permission).

venous tone in both of these studies with periods of observation of 120 min and 90 min, respectively.

Day-to-day variability The diameter of a given vein of a subject was found to show only little variability from day to day. This was shown previously with the optical technique [16] but also confirmed with the method using an LVDT [44]. The changes were of the same order as those found with repeated measurements taken on the same vein on the same day, discussed above. It is, however, important to recognize that in these studies measurements were always taken under standardized experimental conditions, including a constant room temperature and a sufficiently long acclimatization to the room temperature.

Systemic drug administration

The method, of course, also permits the investigation of drug effects after systemic administration. Only few studies have, however, been published on this topic. The venoconstrictor effect of dihydroergotamine was investigated both after intravenous [43] and after oral [7, 31] administration. In another parenteral study the inhibitory effect of phentolamine on the venoconstrictor actions of noradrenaline was investigated both after systemic intravenous and after direct local administration [76].

Other routes of drug administration

The LVDT method has been used in several investigations of the effects of drugs after less common routes of administration. In one such study the venoconstrictor effects of dihydroergotamine and its time course were compared after intramuscular and intranasal administration. The results showed that both the venoconstrictor effect of the drug and its time course were very similar after administration by either route [50]. In another study Hiremath *et al.* [51, 52] found

that intravenous nitroglycerine expectably reduced the venoconstrictor action of locally infused phenylephrine (see also below), whereas the topical application of nitroglycerine patches was without such an effect.

No difference in response between veins in different areas of the body

After publication of our first studies carried out on superficial hand veins, the question was often asked whether veins in other areas of the body would react in a similar way to the various drugs and other stimuli investigated as the superficial human hand veins studied in these experiments. An investigation was therefore carried out to investigate potential differences in the responsiveness of superficial human veins from different areas of the body [49, 80]. Two groups of healthy male volunteers were studied. One group received local infusions of noradrenaline both into hand and foot veins, the other group received dihydroergotamine by systemic intravenous administration. Changes in venous compliance (at an occlusion pressure of 45 mm Hg) were determined by means of the linear variable differential transformer. In the first group of five subjects, two dose-response curves each for the venoconstrictor effects of noradrenaline were established after direct local infusion into superificial dorsal hand and foot veins. The results showed that the dose-response curves in the two areas were parallel, and the doses required to reduce venous diameter by 50% were almost the same in both veins (Figures 2 and 3). Also the slopes of the regression lines fitted to the logarithmic noradrenaline dose-response curves were virtually identical. Similarly in the second group of subjects (n = 12), who received in double-blind fashion and randomized order 0.5 mg dihydroergotamine or placebo intramuscularly, the venoconstrictor effects of dihydroergotamine in hand and foot veins were almost identical with respect to both their intensity and their time course (Figure 4). This suggests that, despite the marked differences in hydrostatic pressure to which they are exposed during orthostasis, superficial human hand and foot veins react quantitatively and qualitatively similarly to the constrictor effects of the agents investigated. It has, however, to be noted, that in this study veins of similar calibre were used on the hands and the feet of the different subjects (diameters of the hand and foot veins selected for the investigation had to be about 0.5 to 2.0 mm at a congestion pressure of 45 mm Hg). This lack of large differences in the diameter of the veins may be of little importance in studies with oral drug administration, where local drug concentrations in all superficial veins of the body are probably identical. But it appears to be of special importance for the experiments with local drug infusions, as marked differences in local drug concentrations in the vein would otherwise occur. The comparable reaction of superficial hand and foot veins to constrictor agents was confirmed in a study by Luthra *et al.* [85], who, however, specifically mentioned the potential importance of differences in venous diameter if veins in different areas of the body are compared. In their study they determined drug concentrations in blood samples obtained directly from the veins investigated and found that EC_{50} values showed a better correlation between the effects observed on hand and foot veins than just ED_{50} values. As local drug concentrations are usually not easy to determine, the selection of veins of similar diameter, as done in the study mentioned above [49], may help to overcome this problem. The topic of drug concentrations in superficial hand veins was further investigated in a recent study by Borkowski *et al.* [86]. They reported a positive relationship between the dose of noradrenaline required to reduce venous diameter by 50% and the actual noradrenaline concentration determined in the blood sampled from the vein examined.

According to the data reported, results of studies carried out on superficial hand veins therefore appear to be representative for superficial veins in other areas of the body. Technically, measurements can be performed more easily on superficial hand veins than on other superficial human veins. The dorsum of the hand will therefore probably remain the site of choice for investigations on single human veins *in situ*. But it is reassuring to know that the data obtained in these studies are of a more general relevance.

The potential of the method to investigate veins in different parts of the body has, however, been used in a pathophysiological study reported by Streeten [62], who found, that in patients with hyperadrenergic orthostatic hypotension, signs of a denervation hypersensitivity were observed in veins of the feet, whereas the responses of hand veins to noradrenaline remained normal (see section on pathophysiology below).

The use of the method in animals

The method using the LVDT has also been found to be practicable in studies on animals. Müller-Schweinitzer [87] adapted the method described above for the measurement of changes in the diameter of saphenous veins of the dog *in vivo* and found it to be relatively easy to use for studying drug effects and drug interactions after local and systemic administration [87–92]. This may, at first sight, not be very interesting for the clinical researcher. It has, however, to be remembered that, when new drugs are first administered to man, dosages and expected effects are usually based on the results of pharmacological studies carried out in animals. It is therefore desirable to use as far as possible the same experimental methods both in animal studies and in man. If under such circumstances differences between the results of experiments carried out in animals and man are observed it can be concluded that these are not due to differences in the experimental methodology used but rather to species differences.

Physiology of superficial human veins

The effect of age

Studies on superficial hand veins represent an easily accessible tool for the investigation of age-related

changes in venous responsiveness. In one such study in healthy subjects aged between 19 and 79 years, Pan *et al.* [93, 94] found that the dilator effects of isoprenaline determined on hand veins preconstricted by the local infusion of phenylephrine were reduced with age. The dose of isoprenaline required for producing 50% of the maximum vasodilation as well as the maximum relaxation attained were markedly reduced in the older subjects. No age-related changes in the responsiveness of superficial hand veins were, however, observed for the dilator effects of nitroglycerine [94, 95] (but see below), prostaglandin E_1 [53, 96] or adenosine [97]. From the evidence for a progressive reduction of the venodilating effect subsequent to β-adrenoceptor stimulation, but not to the dilator or constrictor effects of other substances, Pan *et al.* [94] concluded that ageing is associated with a specific decrease in β-adrenoceptor-mediated vascular relaxation.

Ford *et al.* [98, 99] tested the hypothesis that desensitization of β-adrenoceptors due to increased sympathetic activity might be responsible for the reduced β-adrenoceptor responsiveness in the old. Both the cardiac chronotropic effects of intravenously administered isoprenaline and the direct venodilator effect on superficial hand veins were determined in old and in young subjects before, during, and after oral treatment with propranolol. The cardiac effects of isoprenaline showed the expected and previously reported (see review by Prichard & Walden [100]) hypersensitivity after propranolol withdrawal. There was, however, no difference in cardiac hypersensitivity between young and old subjects, and on the veins no withdrawal hypersensitivity at all was observed. The results, therefore, did not support the hypothesis, but indicate that a β-adrenoceptor hypersensitivity after the withdrawal of propranolol can be detected only on cardiac but not on vascular β-adrenoceptors. From *in vitro* studies in the rat, Tsujimoto *et al.* [101] suggested that the decrease in β-adrenoceptor mediated smooth muscle relaxation with age was not due to a reduction in the number of β-adrenoceptors but to a decrease in cAMP accumulation induced by β-adrenoceptor stimulation and probably another defect, not yet identified, more distal in the adenylcyclase pathway. To elucidate these *in vitro* data further, Hiremath *et al.* [53] compared the age-related changes in the dilator effects of PGE_1 and isoprenaline on preconstricted superficial hand veins *in vivo*. The results show that an age-related decline in vascular response occurs only with respect to the β-adrenoceptor agonist effect but not to that of PGE_1, thus suggesting that the reduced response to isoprenaline does not reflect a general loss in responsiveness of adenylcyclate-coupled receptors. Similar conclusions were reached by Ford *et al.* [97, 102], who found that also the venodilator effects of adenosine (locally infused into preconstricted hand veins), which like isoprenaline causes venodilation by activating the adenylate cyclase system but through separate receptors, is not diminished with age.

As discussed below (see section on the pathophysiology of hypertension), a reduced β-adrenoceptor response was also shown in hypertensive patients; this could be partly corrected by the use of a low sodium diet [60]. Feldman [103] therefore tested, whether this was also the case in normotensive elderly subjects. When he found that the maximal isoproterenol mediated dilatation of preconstricted hand veins, which was impaired on a high sodium diet, showed a significant increase, when they were placed on a low sodium diet, he suggested an important role for dietary modification in the adrenergic regulation of vascular tone in the elderly.

There is some controversy with respect to the presence or the absence of an age-dependent reduction of venous responsiveness to nitroglycerine. The studies mentioned above [94, 95] suggest no such reduction. They were, however, not confirmed by the results of a plethysmographic study on the forearm of healthy subjects carried out by Gascho *et al.* [104], who reported that not only venous compliance as such but also venous responsiveness to nitroglycerine was significantly diminished in the elderly.

Bedarida *et al.* [105] showed that histamine induced dilatation of preconstricted superficial hand veins is not impaired with age. They found, however, that histamine produces dilatation by both an H_1-mediated endothelium dependent pathway, which is preserved with aging and an H_2-receptor mediated cAMP dependent pathway, which is reduced in the aged (see also part 2, section on the clinical pharmacology of other vasodilators). Dachman *et al.* [106, 107] showed that also bradykinin induced venodilatation is not impaired with age and that there is also no difference in effect between the genders.

A series of experiments were carried out on superficial hand veins in order to investigate the potential age dependency of the venoconstrictor effects of various agonists. The results showed that the constrictor effects observed after α-adrenoceptor stimulation by noradrenaline [47] or phenylephrine [94] were of the same magnitude in old and young subjects. Martin *et al.* [47], however, pointed out that a very large number of subjects would be required for a definitive judgment because of the large interindividual variability in responsiveness in the individual subjects of both age groups. Klein *et al.* [108] confirmed the lack of age-related changes in α-adrenoceptor responsiveness for other organs in a study in which blood pressure and heart rate changes were meaured after systemic drug infusion. However, Docherty *et al.* [109–111] suggested, from studies on human saphenous veins *in vitro*, that, although there was no evidence for an age-related change in responsiveness of presynaptic α_2- and postsynaptic α_1-receptors, the responses of postsynaptic α_2-receptors was blunted in the elderly. O'Malley *et al.* [112] reviewed the effects of aging on the function of vascular and neuronal adrenoceptors and found no consistent evidence for an age-dependent change in α-adrenoceptor function, but confirmed the decrease in the response of the β-adrenoceptor system. In a recent study, Supiano *et al.* [113] tested the hypothesis that decreased adrenergic responsiveness with aging might reflect a down-regulation of receptors due to an age-related increase in sympathetic tone. They found an age-related decrease in α_2- but not

α_1-adrenergic venoconstriction on superficial hand veins, when they compared the data with those obtained in younger subjects studied previously [114]. Reduction of sympathetic tone by oral treatment with the adrenergic neuron blocker guanadrel reduced plasma noradrenaline levels and resulted in an increased venoconstrictor response both to α_2- and α_1-agonists in the young, but only to α_2-agonists in the elderly, suggestive of an upregulation of receptors. The authors suggested that the lack of an age-related decrease in α_1-adrenoceptor mediated venoconstriction and the lack of an upregulation of this response on guanadrel indicates that the regulation of α_1-adrenoceptor response is impaired in the elderly.

Blauw *et al.* [115], in a plethysmographic study on the forearm, found no age-related changes in the responsiveness of veins to the constrictor action of 5-hydroxytryptamine, a fact indicating that also constriction on veins mediated by stimulation of 5-HT receptors is not diminished with age.

Spontaneous rhythmic contractions of various vessels can occur in experiments *in vitro*. Schoeffter & Godfraind [116] investigated the potential influence of the age of the donor subjects on such effects and they discovered that rhythmic contractions occurred much more frequently in veins from older persons. They studied the mechanism of action of these contractions and found that they were inhibited by cyclooxygenase inhibitors (aspirin and indomethacin), but not by adrenergic or histamine antagonists, thus suggesting the involvement of endogenous prostaglandins. Benjamin *et al.* [117] showed that such effects may also occur on superficial human hand veins *in vivo* and that they could be induced by intermittent rises of the occlusion pressure, but also by a single deep breath [118]. They demonstrated that bradykinin, glyceryl trinitrate and the potassium channel opening drug cromakalim inhibited the phasic activity, whereas acetylcholine was not effective [117].

The effect of exercise

In their first publication reporting on the establishment and the evaluation of the optical method for measuring changes in the compliance of superficial hand veins, Nachev *et al.* [16] observed that physical exercise at a work-load of 100 to 175 watts for 2 to 3 min induced a clear reduction of venous compliance (measured immediately after the exercise). No further studies investigating exercise-induced changes in the compliance of superficial human veins have been reported, probably because of methodological difficulties related to the movements of the subjects during exercise and consequently the need to carry out the measurement after the end of the actual period of exercise. Such studies might, however, yield interesting insights, e.g. into the pathophysiology of heart failure and its treatment.

Robinson *et al.* [119], using blood pool scintigraphy, reported that a reduction in forearm venous compliance was also observed during a mental arithmetic stress test. Studies on superficial hand veins using the LVDT technique might help to simplify investigations in this area.

Studies in twins

Carruthers and his group [120–122] used the LVDT method to study whether the variability of the responsiveness of superficial hand veins to constrictor agents differs between unrelated subjects and twins. The results of their experiments show that the inter-individual variability in the venous responsiveness to locally infused noradrenaline was much smaller between monozygotic twins than between dizygotic twins or unrelated subjects.

The menstrual cycle

Variations in miscellaneous cardiovascular parameters have been reported during the menstrual cycle, and sexual hormones can exert vascular effects [123–125]. Only one study investigating potential changes of the compliance of superficial hand veins and its time course has, however, been reported. Walters & Shields [126], using the optical version of the method, found a significantly lower tone of superficial hand veins of healthy female subjects of childbearing age during the first 2 weeks after menstruation, compared with the second 2 weeks. This was suggested to be due to the dilator action of oestrogen. It would be worthwhile to study, besides the impact on the basal tone of superficial human veins, potential changes in the sensitivity to various constrictor agonists during the menstrual cycle.

Studies on the function of the endothelium

Results of *in vitro* studies performed by Furchgott & Zawadski [127] have shown that the dilator action of acetylcholine in rabbit aortic strips is dependent on the presence of the intact endothelium. It is mediated via the release of a substance from endothelial cells, which was later called EDRF (endothelium derived relaxing factor) and is now known to be nitric oxide. Studies carried out by Thom *et al.* [128] on human isolated arteries and veins confirmed this finding for human vessels, although in other *in vitro* investigation on human veins Thulesius *et al.* [129] suggested the absence of an endothelium-dependent relaxing action on veins. Lüscher *et al.* [130], in a comparative *in vitro* study on human internal mammary arteries and veins and human saphenous veins, demonstrated an endothelium-dependent relaxing effect of acetylcholine, thrombin, and adenosine diphosphate on both vessel types and confirmed that this endothelium-dependent relaxation was more marked in the arteries than in the veins. Yang *et al.* [131] reported that, although the endothelium-dependent relaxation was smaller on veins than on arteries, nitric oxide as such produced a comparable relaxation on both vessel types. Yang *et al.* [132] showed in further *in vitro* studies that in human internal mammary arteries but not saphenous veins the presence of the intact endothelium protects against contractions induced by histamine and serotonin.

In hand veins *in vitro* Arner & Högestätt [133] found that acetylcholine produced only a small relaxation, which was not affected by removal of the endothelium. Collier & Vallance, however, carrying out studies in superficial hand veins showed that in man *in vivo* the removal of the endothelium of the veins by perfusion with distilled water produced a vasoconstriction and a loss of the dilator action of acetylcholine, whereas the dilator action of glyceryl trinitrate was not impaired [134, 135]. This study on hand veins was the first investigation providing direct evidence for an endothelium-dependent relaxation of vascular smooth muscle in man *in vivo*. These results were further supported by another series of studies published by the same group [54], which showed that, when locally infused into superficial hand veins, L-NMMA (N^G-monomethyl L-arginine), a substance which suppresses the synthesis of EDRF or nitric oxide from L-arginine, inhibits the venodilator action of acetylcholine and bradykinin but not that of glyceryl trinitrate. The results of both of these studies on superficial hand veins are in good agreement with those of another, somewhat more invasive investigation of the same group [136], in which L-NMMA infused into a brachial artery attenuated the dilator response to acetylcholine but not that to glyceryl trinitrate. The administration of L-NMMA increases arteriolar [137], but not venous [54] tone. Collier & Vallance [137] therefore hypothesized, that basal sympathetic tone in arteries might stimulate basal release of nitric oxide, which then could be inhibited by L-NMMA or that nitric oxide could interfere with sympathetic neurotransmission. In a study on superficial hand veins, however, they found no evidence for an influence of L-NMMA on noradrenaline dose-response curves or sympathetically mediated venoconstriction. L-arginine is the physiological precursor for the formation of nitric oxide in the endothelium. Calver *et al.* [138] therefore compared the effects of local infusion of L- and D-arginine into preconstricted hand veins and found that both isomers lead to dilatation. Also on brachial arteries the local infusion of both isomers produced vasodilatation. From the absence of a stereoselectivity of the dilator effect of arginine the authors concluded that it was not caused by an activation of the L-arginine/nitric oxide pathway through the provision of excess substrate.

Lüscher *et al.* [139, 140] found that in human arteries and veins *in vitro* endothelin is a potent endothelium-derived vasoconstrictor. In human arteries the release of nitric oxide from the endothelium is able to suppress completely contractions induced by endothelin, whereas in human veins the maximum of this inhibitory effect is much lower. From these data the authors suggested that the ability of human veins to release nitric oxide is much smaller than that of arteries. These results are in good agreement with the finding that endothelium-derived relaxation is less in veins that in arteries [130]. As endothelin was reported to stimulate generation of nitric oxide and prostacyclin by vascular endothelial cells *in vitro* Haynes & Webb [141–143] studied this topic in man *in vivo*. Acetylsalicylic acid at a dose known to inhibit prostacyclin synthesis was found to potentiate the venoconstrictor effect of endothelin, thus suggesting, that on human veins *in vivo* prostacyclin formed after its administration attenuates its constrictor action. L-NMMA, however, which inhibits the formation of nitric oxide (see above), did not affect the venoconstrictor action of endothelin.

Studies on receptor distribution

In several *in vivo* studies on superficial hand veins the distribution of receptor types and subtypes was investigated. Schulte *et al.* [73] reported that the non-selective α-adrenoceptor agonist noradrenaline, the α_1-adrenoceptor agonist phenylephrine, and the α_2-adrenoceptor agonist azepexole all produced a constrictor action after local infusion into superficial hand veins. The maximum effect reached, however, was greater after phenylephrine than after azepexole, thus suggesting that, while both α-adrenoceptor subtypes were present on human veins, the α_1-subtype appeared to predominate. Noradrenaline was more active than either phenylephrine or azepexole alone. Similar conclusions were reached by Blöchl-Daum *et al.* [144] and by Kongpatanakul *et al.* [145, 146], although the latter group used a different approach. They found that, on superficial hand veins, the α_1-adrenoceptor antagonist labetalol was only slightly less effective in inhibiting the venoconstriction produced by local infusion of the non-selective α-adrenoceptor agonist noradrenaline than that produced by the α_1-adrenoceptor agonist phenylephrine. Subsequent infusion of the non-selective α-adrenoceptor antagonist phentolamine produced some further dilation of the veins preconstricted by noradrenaline. On the strength of these results the authors confirmed the existence of α_1- and α_2-adrenoceptors on human veins, previously reported in *in vitro* studies on human saphenous veins [147], but postulated that postsynaptic α_2-adrenoceptors on human veins had only a limited role in regulating venous tone. Barthel [148], who studied the effects of α_1- and α_2-adrenoceptor agonists on human dorsal foot veins reported, that also in this vascular bed α_1-adrenoceptors appear to predominate.

Kaiser *et al.* [149] carried out studies in the dog and postulated that veins and arteries possess both α- and β-adrenoceptors and that stimulation of α-adrenoceptors leads to constriction in both vessel types, whereas the stimulation of β-receptors dilates arteries but constricts veins. Sicuteri *et al.* [150] used the venoconstriction test (see above) to investigate this hypothesis and studied the direct effects of noradrenaline and adrenaline and their interaction with a β-adrenoceptor blocking drug on superficial human hand veins. Both adrenaline and noradrenaline produced a venoconstriction, and noradrenaline was the more active of the two. Administration of a β-adrenoceptor blocking drug markedly increased the constrictor action of adrenaline, but not that of noradrenaline. They concluded that administration of a β-adrenoceptor blocking drug inhibited the dilator component of the action of adrenaline but left the constrictor effect of α-adrenoceptor stimulation unchanged, thus showing that stimulation of β-adrenoceptors on veins pro-

duces dilatation, as on arteries. In later studies on β-adrenoceptors of human veins it was found that the β-adrenoceptor stimulant isoprenaline did not produce much effect on normal resting venous tone when locally infused into superficial hand veins, but that it induced a marked and dose-dependent dilatation of veins that had been preconstricted by the local infusion of noradrenaline or 5-hydroxytryptamine (see part 2, effects on β-adrenoceptors).

Local administration of 5-hydroxytryptamine produces a marked constriction of superficial veins *in vivo* [14, 15, 151]. This fact formed the basis for the development of the venoconstriction test (described above) by Sicuteri *et al.* [14]. From the results of their studies the existence of separate 5-HT receptors on human veins different from α-adrenoceptors was postulated [14, 15]. This subject was further investigated in a study on superficial hand veins using the LVDT. It was found that after local infusion 5-hydroxytryptamine and noradrenaline produced quantitatively and qualitatively similar constrictor effects, but that the local infusion of the 5-HT receptor antagonist pizotifen selectively inhibited the constrictor actions of 5-HT, but not those of noradrenaline [63] thus supporting the existence of separate 5-HT receptors and α-adrenoceptors on human veins. Borton *et al.* [152] reported *in vitro* experiments on human saphenous veins and showed that the 5-HT_2-receptor antagonist ketanserin inhibits the constrictor effect of 5-HT but that also selective 5-HT_1-receptor agonists produce venoconstriction. The existence of both 5-HT_1- and 5-HT_2-receptors on human veins was therefore postulated, the stimulation of each of them leading to venoconstriction. This was confirmed on human hand veins *in vitro* by Bodelsson *et al.* [153], who also investigated the effect of a 5-HT_3-receptor antagonist on 5-hydroxytryptamine mediated venous contractions and found no evidence for an involvement of 5-HT_3-receptors. In other *in vitro* studies on human saphenous veins, Molderings *et al.* [154] provided evidence for the presence of presynaptic 5-HT-receptors on sympathetic nerves similar to the 5-HT_{1D}-type and responsible for the inhibition of noradrenaline release from sympathetic nerve terminals.

The influence of temperature

The fact that the compliance of superficial human veins is temperature-dependent has been mentioned in the discussion of the methodology (see above). A direct correlation between the compliance of superficial human hand veins and skin or environmental temperature was described by Nachev *et al.* [16]. This is in good agreement with the fact that venoconstriction and venodilatation, respectively, were observed in a plethysmographic study in man both on normal and on varicose leg veins when immersed in cold or warm water [18]. This confirms the importance of superficial veins for the regulation of body temperature [1]. Environmental and skin temperature, however, influence not only venous compliance but also the responsiveness of the veins to the effects of constrictor agents. In a study on superficial hand veins using the optical technique, we found that the local infusion of pizotifen, a partial agonist at 5-HT receptors, produced a greater constriction of the vein when the study was carried out at a low (18° C) than at a higher (23° C) room temperature [32]. Interestingly, a temperature dependence of the responsiveness of superficial veins to constrictor agonists was observed not only in *in vivo* studies but also in *in vitro* experiments. An increased responsiveness of 5-HT receptors at low temperature was confirmed *in vitro* by Bodelsson *et al.* [155] on segments of subcutaneous hand veins. The authors therefore suggested a potential role of 5-HT receptors in cold-induced peripheral vasospasm. In studies on isolated ring segments of canine [156] and human [19] saphenous and human hand [157] veins, also an increased susceptibility to the constrictor effects of noradrenaline was observed when temperature was reduced. Bodelsson *et al.* [158] found that this increased responsiveness to noradrenaline was unaltered by prazosin, but abolished by yohimbine, thus suggesting that it was mediated by an increased responsiveness of α_2-adrenoceptors.

Sumner *et al.* [159] employed plethysmography to study the effect of ice application to the forehead and found that it produced a marked venoconstriction on the forearm. This effect was similar in magnitude when the veins had been dilated by the administration of nitroglycerine. The authors concluded that sympathetically mediated venoconstriction was not attenuated by nitroglycerine-induced venodilatation. They warned, however, that a general inference from this experimental situation in healthy volunteers to patients may not be warranted. Future studies on superficial hand veins are recommended to test whether sympathetically induced venoconstriction may be affected in patients taking nitrates for therapeutic purposes.

Pathophysiology of superficial human veins

Hypertension

Hypertension is one of the most common diseases, and many experimental studies have been carried out in the search for its cause(s). One of the important topics in this search is whether an increased responsiveness of the vasculature to constrictor stimuli contributes to the elevation of blood pressure in hypertensive patients. Walsh *et al.* [160] in a plethysmographic study found that venous distensibility in the forearm was reduced in hypertensives compared with normotensive controls. This finding indicated an increased venomotor tone in patients with essential hypertension, which was found to be reduced when these patients were treated with various antihypertensive drugs. Shkhvatsabaya *et al.* [161] reported differing results from studies carried out on the vessels of the finger: venous tone in this vascular bed was reduced in hypertensives and increased during therapy. Takeshita & Mark [162], however, confirmed the increase in venous tone in the forearm of hypertensive patients and showed that the acute administration of phentolamine leads to only partial normalization,

thus suggesting that the increase is only partly due to adrenergic mechanisms. Widgren *et al.* [163] found that also normotensive subjects with a family history of hypertension display a decreased venous compliance and an increased arterial pressure response to acute increases in vascular fluid volume. Fitzpatrick *et al.* [164] suggested that reduced venous distensibility leading to reduced blood pooling in the lower extremities on standing may be partly responsible for the blunted renin response to upright posture frequently observed in patients with hypertension. A reduced distensibility of veins taken from patients with essential hypertension was also found *in vitro* by Sudhir *et al.* [165], who also showed that this could not be attributed to an increase in the thickness of the media of these veins. Veins from hypertensive patients exhibited an increased responsiveness to angiotensin II and an increased responsiveness to neural stimulation, which was considered to be possibly related to a reduced prejunctional α_2-adrenoceptor-mediated autoinhibition and a diminished neuronal uptake of noradrenaline. Surprisingly α-adrenoceptor responsiveness was found to be reduced in these veins. This was suggested to be due to a downregulation of postsynaptic α-adrenoceptors consistent with enhanced neurally mediated sympathetic activity in these patients [165, 166].

Eichler and Ford *et al.* [57, 167] studied superficial hand veins and did not find any evidence of an increased venous responsiveness to the constrictor action of a local infusion of the α-adrenoceptor agonist phenylephrine in patients with essential hypertension when compared with normotensive subjects. This suggests no increased responsiveness of postsynaptic α-adrenoceptors but does also not support the hypothesis of a downregulation. Dose-response curves to phenylephrine were established in some of the same hypertensive patients again during oral treatment with the α_1-adrenoceptor antagonist prazosin in doses of 1–2 mg day^{-1}. The results showed the expected marked shift of the phenylephrine dose-response curves to the right typical for a competitive antagonism. Orally administered nifedipine was also reported to produce a rightwards shift of the dose-response curve for the venoconstrictor effect of phenylephrine and noradrenaline in therapeutic studies in patients with essential hypertension [168]. These studies illustrated the usefulness of the LVDT technique for investigating effects of drugs used for the treatment of cardiovascular diseases not only under experimental conditions but also in the therapeutic situation.

Not only the venoconstrictor effects of α-adrenoceptor stimulant drugs, but also those of 5-hydroxytryptamine or of concomitantly infused 5-hydroxytryptamine and noradrenaline did not differ between hypertensive patients and normotensive subjects [169].

Because essential hypertension is more prominent in blacks than in white subjects, Eichler *et al.* [55] compared the responsiveness of superficial hand veins of normotensive black and white subjects to the local infusion of phenylephrine. The results showed that the maximal venoconstriction was significantly greater in whites, whereas the maximal venodilatation (of preconstricted veins) obtained by the local infusion of isoprenaline was not significantly different in the two groups. The implications of this study in normotensive subjects are not clear at present, and further studies on black and white hypertensive subjects and/or subjects with hypertensive parents are required.

Studies by Takeshita *et al.* [170] showed that salt loading of hypertensive patients reduces venous distensibility even further and that phentolamine does not significantly influence this effect, thus suggesting that it results from non-adrenergic mechanisms.

In view of data showing that hypertensive patients have a reduced lymphocyte β-adrenoceptor responsiveness, which can be corrected by a low sodium diet, Feldman *et al.* [60] investigated superficial hand veins of borderline hypertensive patients to test whether lymphocyte β-adrenoceptors are representative for β-receptors on human veins. They found, that indeed, similar to the results obtained on lymphocytes, the β-adrenoceptor response of veins, measured by the dilator effect of isoprenaline on preconstricted veins was significantly reduced, whereas α-adrenoceptor-mediated contraction was unaltered. The authors therefore proposed the existence of a generalized defect of β-adrenoceptor responsiveness in human hypertension. This reduction in β-adrenoceptor responsiveness on human veins could be corrected by a low sodium diet, which also reduced blood pressure. In a further study the same group [171] found, that in contrast to the results obtained with a low sodium diet, treatment of hypertension with a calcium antagonist (verapamil) or a diuretic (hydrochlorothiazide) did not restore β-adrenoceptor sensitivity, although blood pressure was lowered. The authors concluded that vascular β-adrenoceptor response appears to be regulated by a specific mechanism, which is influenced by a low sodium diet, but not the antihypertensive drugs investigated.

An interesting study on superficial hand veins probably relevant to the pathophysiology of congestive heart failure, but possibly also to that of hypertension, was published by Benjamin *et al.* [172]. They reported that the constriction of hand veins that is observed after a single deep breath was markedly augmented by locally infused angiotensin II in a dose, that by itself did not reduce venous diameter. The same dose of angiotensin II did not, however, influence the venoconstriction produced by the local infusion of noradrenaline. In a further study the same group [173] found that also a sympathetically mediated arterioconstriction is augmented by low doses of angiotensin II. The results on superficial hand veins were suggested to indicate, that the inhibition of an indirect venoconstrictor effect of angiotensin II may contribute to the first-dose hypotension, that occurs in some patients with congestive heart failure (e.g. Cleland *et al.* [174]) or essential hypertension and that, in a study in hypertensive patients was shown to be positively correlated with preexisting angiotensin II levels [175]. The results available may also explain the venodilator effect, that has been reported to occur to a greater or lesser degree after the administration of ACE inhibitors to patients with congestive heart

failure [176–181], in whom venous tone was shown to be positively correlated with the severity of the disease [182, 183].

Since insulin resistance has been associated with hypertension, Feldman and Bierbrier [184] investigated whether insulin may exert a direct vasodilator effect and whether such an effect may be altered in hypertension. They found that insulin produces a dose-dependent dilatation of preconstricted hand veins and that this effect is impaired in hypertensive patients. This impairment was significantly correlated with blood pressure and body mass index. From this it was concluded that insulin may be an endogenous vasodilator and that impairment of insulin-mediated vasodilatation may contribute to the increase in peripheral resistance in hypertension.

Orthostatic hypotension

Orthostatic hypotension is associated with an excessive pooling of blood in the veins of the lower extremities. Miller & Streeten [61] studied superficial hand veins and compared the effects of local infusion of noradrenaline in subjects with sympathicotonic orthostatic hypotension with those observed in healthy volunteers. The results showed no difference between the healthy subjects and 9 of the 11 patients. However, one of the other two patients showed a marked hypersensitivity to noradrenaline while a lack of responsiveness was found in the other. The authors suggested that the hypersensitivity observed in one subject might have been due to venous denervation and the lack of responsiveness in the other to a defect in venous receptors or smooth muscle function.

The lack of an alteration of noradrenaline responsiveness in the other nine patients was suggested to indicate that some other mechanism was responsible for their disease; it might, however, also have been due to a difference in the responses of hand veins and veins of the leg. Such a difference is suggested by a study on the pathogenesis of hyperadrenergic orthostatic hypotension reported by Streeten [62]. He found that, similarly to the results of the study reported above, contractile responses of superficial hand veins of patients with this disease were not different from those observed in healthy subjects, whereas their foot veins showed hypersensitivity to noradrenaline. In patients with 'diffuse' autonomic failure he detected hypersensitivity of both hand and foot veins to the constrictor action of noradrenaline. It was suggested that these data indicated that anatomic or functional postganglionic denervation of lower limb veins caused excessive gravitational blood pooling and was thus leading to orthostatic hypotension.

In another investigation MacLeod *et al.* [185] studied 24 patients with reflex sympathetic dystrophy (RSD, a poorly understood disease with pain and vasomotor abnormalities), half of the cases resulting from trauma or diabetes and the other half from hemiplegia following a cerebrovascular accident. They found that, compared with healthy subjects, patients with RSD of less than 6 months duration (but not those with a longer lasting disease) showed a significant increase in the responsiveness of superficial hand veins to noradrenaline, consistent with upregulation of α-adrenoceptors and denervation hypersensitivity. These studies are good examples of the potential of the LVDT method to investigate pathophysiological changes.

Myocardial infarction

Robinson *et al.* [186] reported a reduced compliance of superficial hand veins in patients immediately after myocardial infarction, which they attributed to an increased sympathetic tone. During the first 7 days after the event venous compliance was gradually rising, in parallel with a fall in central venous pressure. The authors reported that there was no evidence that other factors, such as pain or anxiety, were contibuting to a major extent to the venoconstriction observed.

Varicose veins

Several causes have been suggested for the pathogenesis of varicose veins [187]. In a study using the LVDT technique the responsiveness of superficial veins to the constrictor action of locally infused noradrenaline was compared in healthy subjects and patients with varicose veins. In the patients not only normal superficial hand veins but also varicose veins of the legs were studied by this method. The results showed that patients with varicose veins required more than double the noradrenaline concentration for a half-maximal venoconstriction than normals, and this not only for their varicose veins of the leg, but also for the apparently normal superficial hand veins. The suggestion was made that in patients with varicose veins a constitutional decrease in venous α-adrenergic responsiveness exists, affecting not only varicose veins but also apparently healthy veins [56, 188].

Diabetes

Three studies on superficial hand veins were carried out in diabetic patients. Blöchl-Daum *et al.* [189] speculated that, as diabetes is associated with endothelial dysfunction and as diabetics have a higher prevalence of hypertension there might be an increased constrictor action of endothelin in diabetics caused by a facilitated acess of endothelin to vascular smooth muscle due to disintegration of the endothelial layer. In this study, carried out in patients with type 2 diabetes, however, no such change was found on superficial hand veins of insulin-dependent diabetics. Eichler *et al.* [190] showed, that in patients with diabetic autonomic neuropathy lower doses of noradrenaline were required for a 50% constriction of superficial hand veins, compared with asymptomatic diabetics, whose results did not differ from those of healthy subjects. Phenylephrine dose-response curves were not different in all three groups. The authors suggested, that since both vasopressor drugs act on vascular α-adrenoceptors, but only noradrenaline is taken up into sympathetic nerve end-

ings, vascular hypersensitivity to catecholamines in diabetic patients with autonomic neuropathy was primarily determined by a decreased neuronal catecholamine uptake. Bodmer *et al.* [191] found that also in insulin-dependent diabetics with microalbuminuria the sensitivity of superficial hand veins to the constrictor action of noradrenaline was increased compared with diabetics with no albuminuria and non-diabetic subjects. They proposed that such an exaggerated response to noradrenaline precedes the development of hypertension and could be responsible for the microalbuminuria attributed to intraglomerular hypertension. They therefore suggested, that such investigations might help to detect susceptible patients at an early stage of their disease and to start appropriate therapy. The impairment of the venodilator effect of insulin in patients with hypertension [184] has been discussed above in the section on hypertension.

Other diseases

Eichler *et al.* [58] found that in patients with cystic fibrosis higher doses of isoprenaline were required for the dilatation of superficial hand veins (preconstricted by phenylephrine) than in normal healthy controls and that also the extent of the maximal venodilatation was reduced. This decrease of β-adrenergic responsiveness was not correlated with the severity of the disease. It was considered to indicate a defect of the cAMP-dependent pathway in vascular smooth muscle cells. Such a defect has also been suggested to be involved in the general pathophysiology of the disease, including the main regulatory disturbances seen in secretory cells [192].

The same group found no difference in the responsiveness of superficial hand veins of healthy subjects and of patients with asthma to the constrictor effect of phenylephrine, or to the dilator effect of isoprenaline, and therefore suggested that there was no evidence of a generalized change in α- or β-adrenergic responsiveness on smooth muscle cells in this disease. Local infusions of prednisone or dexamethasone, and oral treatment with dexamethasone, produced a significant attenuation of the responsiveness to isoprenaline in asthmatic patients as well as in healthy subjects [59].

Matucci-Cerinic *et al.* [193] studied patients with systemic sclerosis, to test the hypothesis that it is a microcirculatory disorder with endothelial injury as one of its main features [194, 195]. They found that, in comparison with a group of healthy controls, the dilator response of (preconstricted) superficial hand veins to substance P (inducing endothelium mediated dilatation) was diminished at an early stage of the disease, whereas the dilator response to glyceryl trinitrate (direct effect) was reduced only at a more severe stage. They concluded that in systemic sclerosis an early functional deficit of the endothelium precedes the onset of extensive visceral and skin involvement.

Sicuteri *et al.* [196] investigated patients with cluster headache and found that the intravenous administration of somatostatin produced a symptomatic relief, similar to that seen after ergotamine. This effect was suggested to be due to an inhibition of the release on the vasodilator substance P. On account of these data Caleri *et al.* [197] carried out a study on superficial hand veins of such patients and found that somatostatin produced a marked venoconstrictor effect. Further studies are required to test whether this constriction is due to inhibition of a substance P effect or a direct venoconstrictor action of the drug.

Bedarida *et al.* [198, 199] reported, that although in hypercholesterolaemic subjects endothelium-dependent relaxation of arteries is impaired, this is not the case on superficial hand veins, where the dilator effects of bradykinin and nitroglycerine were even somewhat higher than in normocholesterolaemic subjects. This shows that in hypercholesterolaemic patients endothelium-dependent venodilatation is not impaired.

Raynaud's disease

In order to investigate mechanisms potentially contributing to altered vascular responsiveness in patients with Raynaud's disease Bedarida *et al.* [200] compared the dose-response relationship of the constrictor effect of noradrenaline and the venodilator effect of bradykinin on superficial hand veins of affected patients and healthy subjects. The results showed that the constrictor action of noradrenaline was not enhanced in the patients, whereas the dilator action of bradykinin was reduced, thus suggesting that endothelin-dependent venodilatation is impaired in Raynaud's disease. The authors proposed that therapeutic approaches augmenting nitric oxide release from endothelial cells might be useful in this condition.

The studies carried out on superficial hand veins of patients with various diseases indicate the usefulness of such investigations for the further analysis of the pathophysiology and the pathogenesis of many diseases.

References

See part 2 *Br J clin Pharmac* 1994; **38**: 289–305.