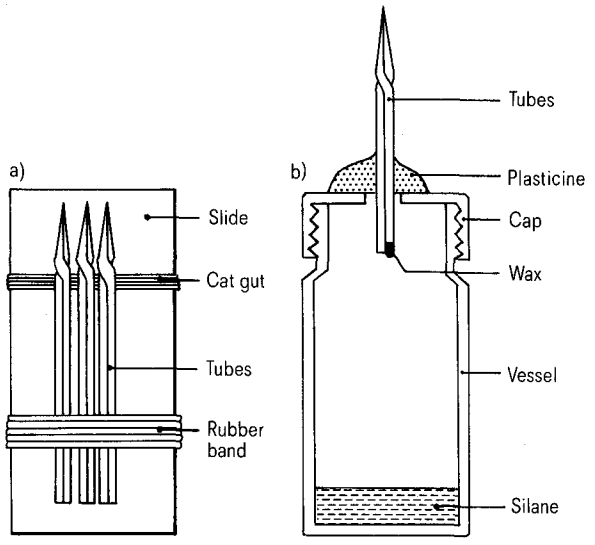
tubes are stored in beakers with silica gel on the bottom to keep the atmosphere dry.
The electrodes produced in this way should have a resistance of 20–30 M Ω on the reference side. If a K^+-exchanger of the type Corning 477317 is used they will then have a resistance of 2000–5000 M Ω on the ion-selective side.
Using amplifiers of 10^{12} M Ω input resistance, there will be enough resistance to ensure a correct recording on the ion-selective channel. The slope of the ion-selective stem is usually about 50 mV/decade using the K^+-exchanger from Corning. The ideal value of 58 mV is only reached sometimes.
Such electrodes were used very successfully to measure the intracellular K^+-activity of Malpighian tubules of *Drosophila hydei*[13].



a Object slide for transfer handling; *b* scintillation vessel for silanization.

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An apparatus for the calibration of electromagnetic flowprobes on small veins in situ

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Summary. Electromagnetic flowprobe calibration must be done under controlled conditions similar to those encountered experimentally. This in situ calibration apparatus is simple in design, inexpensive, and provides pressure and flow conditions analogous to those found in small veins in vivo.
Key words. Vein, gracilis, dog; dog gracilis vein; blood flowmetry, electromagnetic; calibration in situ.

For cuff-type, non-cannulating electromagnetic blood flowprobes, probe fit (electrode contact), vascular constriction, hematocrit, method of zero baseline determination (occlusive vs non-occlusive zero), flow profile, intraluminal pressure, and vessel conductivity and wall thickness (i.e., vessel type: artery or vein) are all important determinants of probe sensitivity that must be controlled during probe use[2,3]. As such, any calibration system should closely mimic experimental conditions regarding all these factors. Considering this, it has previously been suggested that an in situ apparatus is the best practical way of simulating the in vivo probe environment, and calibration of larger probes on large vessels has been accomplished in this way[4]. However, no previously described in situ method was suitable for calibrating our small flowprobes (1.0–2.0 mm internal diameter) on small veins (1.0–3.0 mm external diameter) with low luminal pressures and low flow rates. We have previously demonstrated that, given an appropriate calibration method, probes of this size are accurate for measuring blood flow rates from 0–30 ml/min[3]. Here, we describe a calibration apparatus that provides constant flows at low luminal pressures in small veins in situ.

Materials and methods. All calibrations were done on in situ gracilis veins in dogs anesthetized with sodium pentobarbital (30 mg/kg). After dissection to clear a suitable length for flowprobe placement, the gracilis vein on one side was cannulated proximally and distally to the probe with polyethylene intravenous catheters (16 gauge or larger). The remainder of the apparatus was then assembled (fig. 1). A constant pressure reservoir from a liquid chromatography column was positioned at the proper height to provide a hydrostatic pressure of 10–20 mm Hg (1.33–2.67 kPa) during flow in the vein. This was monitered using a pressure transducer as shown. Tygon tubing with the largest internal diameter possible (5 mm) was used to allow high flow at low pressure. All glassware and tubing were siliconized to inhibit blood clotting and to improve flow. After an i.v. bolus injection of 4000 units of sodium heparin, 200 ml of blood was withdrawn from the dog at a separate site and used to fill the apparatus as shown. Hematocrit was determined by the standard centrifuge method. Stepwise changes in the flow rate were obtained by adjusting the distal roller clamp. A 1-min timed collection into a graduated cylinder quantified the actual blood flow. Constancy of flow over the

timed interval was checked by collecting timed fractions and comparing the volumes.

Gould Statham SP75 series, cuff-type flowprobes were connected to a Gould Statham SP2202 electromagnetic flowmeter for calibration[5]. Flowprobes were used in a standard fashion: the probe was of the proper size to provide 0–35% vascular constriction, a null reading of less than 10% full scale was obtained before each measurement, and a mechanical, occlusive zero was obtained before and after each reading. Inconstant flow, drifting baseline, or excessive null (indicating poor probe fit) were criteria for rejecting an observation. At least 8 paired observations of the flowmeter reading and true flow rate as measured by the timed collection were recorded for each flow run. Flow rates were increased in a stepwise fashion from 0 to greater than 30 ml/min for each run. At least 2 runs were performed under each set of conditions (same probe, vascular constriction, and hematocrit). After plotting meter reading vs actual flow rate, the least squares regression lines obtained for corresponding runs were tested for equivalence by analysis of covariance. Identical lines (slope and intercept equal, $p > 0.05$) for similar conditions were pooled to produce a calibration line for that probe under those conditions. Using the predicted value of the meter reading from the calibration line (E(meter)), error for each line was defined as the average of the absolute values of ((E(meter)-meter)/meter) × 100 (percent).

Results and discussion. Canine gracilis veins were chosen because other work in our laboratory required measurement of flow from an isolated gracilis muscle. No difficulties were encountered with the isolation of the vein or its cannulation. The apparatus, which was assembled from spare parts at negligible cost, was capable of providing constant flows from 0 to 30 ml/min over a 1-min period. This was verified by the fractional collections. Obstructing the outflow to decrease the flow rate was associated with minimal changes in intraluminal pressure, as venous pressure never varied more than 8 mm Hg (1.07 kPa) over the entire flow range. No changes in vessel diameter, measured using an operating microscope, were evident over this range of pressures. Although previous authors have stated that luminal pressure is not an important calibration determinant for pressures from 20 to 200 mm Hg (2.67 to 26.7 kPa) in arteries[6], there have been no previous reports of calibration at low pressures in small veins. This apparatus allowed both a low pressure (10–20 mm Hg or 1.33–2.67 kPa) and a flow range suitable for our purposes, and provided a probe environment similar to that found in vivo. The constant pressure reservoir is the essential feature; attempts to produce these conditions (especially the low pressure) using pumps or simple gravity feed systems were unsuccessful.

Eight pooled calibration lines for the 1.5 mm and 2.0 mm flowprobes are shown in the table. Results for probes with other diameters were analogous. Calibration plots were linear

Pooled flowprobe calibration lines

Probe size (mm)	No. runs (No. points)	Vascular constriction* (%)	Hematocrit (%)	Linear regression defined Slope	Intercept	r	Error (%)
1.5	2 (20)	0	37	0.83	0.12	0.99	4.7
1.5	2 (20)	15	37	0.81	− 0.08	0.99	2.7
1.5	2 (19)	25	37	0.84	− 0.24	0.99	2.4
1.5	2 (17)	35	38	0.85	− 0.45	0.99	2.1
2.0	2 (20)	15	38	1.02	0.15	0.99	2.7
2.0	2 (22)	20	38	1.02	− 0.89	0.99	2.6
2.0	2 (19)	26	38	0.98	− 0.18	0.98	2.8
2.0	4 (43)	33	37	1.01	− 0.25	0.99	3.7

* Note: Vascular constriction is expressed as the percentage ratio of the difference between the external vascular diameter and the probe lumen diameter, to the external vascular diameter.

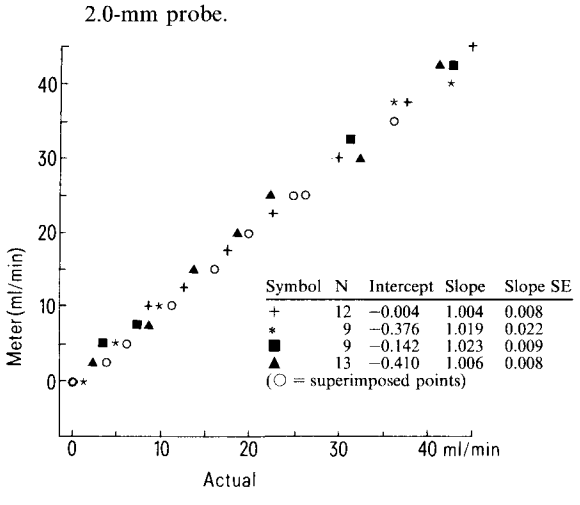

Figure 1. Calibration apparatus showing constant pressure reservoir, venous pressure recording, gracilis muscle (G), flowprobe on gracilis vein, distal outflow occlusion, and graduated cylinder for timed collection.

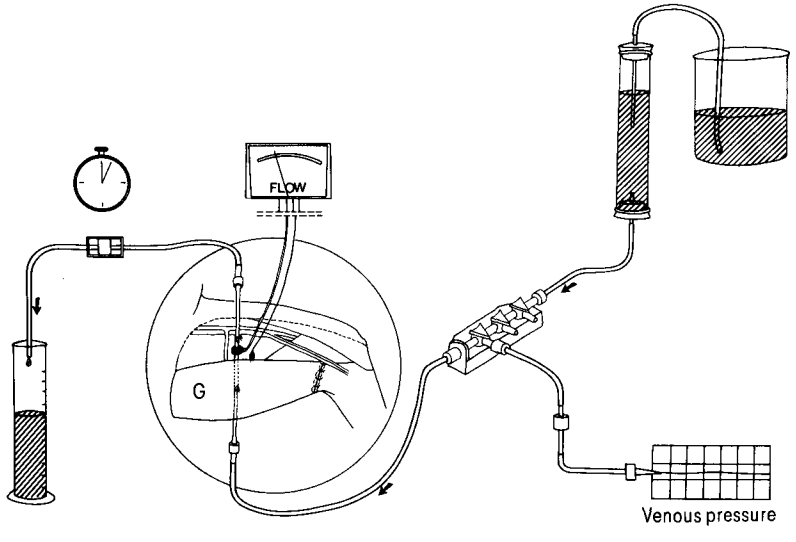

Figure 2. Flowmeter output vs actual flow rate as determined by timed blood collection for 4 calibration runs done under similar conditions (2.0-mm probe, 33% vascular constriction, hematocrit 35–40%). For each run, the slope, intercept, and standard error of the slope were computed and compared to those from runs done under similar conditions. If identical, run calibration lines were pooled to form a pooled calibration line for those conditions. The 4 runs shown here were statistically equivalent and were pooled to form the pooled calibration line for corresponding conditions shown in the table.

through zero and all correlation coefficients exceeded 0.97. Error was less than ± 5.0%. The lines were reproducible; calibration lines from individual runs done under similar conditions on different days were identical. An example of the measurements and variation of results is shown in figure 2. These results show that even under the marginal conditions of low luminal pressure and low vascular constriction (i.e. when the concern is poor electrode contact), reliable, repeatable calibrations can be achieved if care is exercised.

This in situ apparatus was designed to be applied to an isolated canine gracilis vein at the end of an acute experiment using the gracilis to study skeletal muscle ischemia. Although the animal can survive since only a small aliquot of blood is required to fill the system, the vein is destroyed because of a need to cannulate both ends. Therefore, this system is not suited to chronic work or repeated calibrations in the same location. Another potential problem is the sedimentation of red cells in the system, producing inhomogeneity of hematocrit at the probe. However, flow through the small reservoir is great enough to ensure adequate mixing, and this difficulty was not encountered.

In conclusion, the apparatus described employs a low volume, constant pressure reservoir to provide a range of flow rates in an in situ gracilis vein at low luminal pressures. This has allowed repeatable, reliable calibration of our electromagnetic flow equipment for use on gracilis veins in vivo. The apparatus is simple in design, inexpensive, and can be used on virtually any small vessel in situ.

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Enzyme immunometric assay for the determination of pregnancy associated plasma protein A (PAPP-A) with the antigen as solid phase (conjoint IEMA)

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12 December 1983

Summary. Purified pregnancy associated plasma protein A (PAPP-A) can be effectively bound to polystyrene microtitre plates. This immobilized antigen competes with the added serum PAPP-A of unknown concentration for the limited amount of peroxidase-labeled monospecific anti-PAPP-A antibody incubated simultaneously. The sensitivity is 0.1 WHO unit/ml and non-specific binding is 1.0%.
Key words. Serum, pregnancy; placental proteins; enzyme immunoassay.

Pregnancy associated plasma protein A (PAPP-A) is one of the new generation of placental proteins. This large glycoprotein (M_r 730,000) was first described by Lin et al.[2] and has been purified[3]. Its concentration in the maternal circulation increases as pregnancy advances[4]. In order to elucidate the function of this protein, various assays have been developed, beginning with a modified Laurell immunoelectrophoresis[5]. The low sensitivity of this method restricted its use to measurements in late pregnancy. Later radioimmunoassays (using labeled PAPP-A)[6-8] and immunoenzymometric assays (ELISA, using labeled antibody)[9,10] have been developed to increase the sensitivity. The above ELISA's are two-site (sandwich) immunoassays where immunoglobulin is bound to the solid phase. Here the efficient adsorption of the antigen itself (PAPP-A) to polystyrene plates and the determination of PAPP-A by competition of the latter with the solid phase for a limited amount of peroxidase-labelled antibody is described (Conjoint immunoenzymometric assay, CIEMA).

Materials and methods. PAPP-A was purified from a pool of late pregnancy (32–36 weeks) serum as described elsewhere[3], but the ConA-Sepharose and negative affinity chromatography steps were replaced by chromatography on heparin-Sepharose (Pharmacia, Uppsala, Sweden) as reported by Davey et al.[11]. Eluted PAPP-A was pooled and stored at −20 or −70 °C. The activity of this fraction was measured by immunoelectrophoresis[5] and its protein content determined[12]. Polystyrene microtitre plates (96 wells, Kontron Ltd, St. Albans, England) were coated with this PAPP-A fraction at various dilutions (see results) in sodium carbonate buffer, 0.05 M, pH 9.2. Incubation was 24 h at room temperature. Blocking of remaining sites and the subsequent washes were performed as described[10]. The assay itself was run by incubating the test serum or standard (0.1 ml) together with the diluted enzyme-antibody conjugate (see results) in sodium phosphate, 0.01 M, pH 7.0; NaCl, 0.14 M; Tween-80, 0.05% v/v; normal donkey serum, 10% v/v (0.1 ml) in a PAPP-A-coated well. The absence of cross-reaction with various other proteins has been demonstrated for this conjugate[10]. It is important that the standard or test serum is added to the well containing immobilized PAPP-A before the addition of the conjugate, or that the 2 solutions are added to the well immediately after mixing in a separate tube. This is in order not to favor the soluble over the insoluble PAPP-A to be encountererd first by the antibody. The incubation was 4 h at 37 °C. The wells were then washed with 4 × 0.3 ml of 0.9% NaCl containing Tween-80 (0.1% v/v). Then the amount of peroxidase bound in each well was determined with orthophenylene-diamine as chromogen at pH 5.0 as described earlier in detail[10]. The absorption was measured in an automatic microplate reader (Kontron SLT-210, 486.1 nm). Nonspecific binding was determined in wells not coated with PAPP-A, and zero values were obtained by using 0.9% NaCl or a pool of male serum as test sample in coated wells. All measurements throughout were performed in triplicate.