The catheter was rinsed daily with heparinized saline and the wounds kept clean with cotton swabs soaked in betadine. Usually in the same surgical operation some other, arterial, venous and urinary bladder catheters were also implanted and exteriorized in the same site as previously described[2,3].

The surgical procedures did not last more than 10 min. Intra- and postoperative mortality was very low when only the splenic catheter was placed. When this method was used in a group of 39 normal rats, 2 had to be discarded because the tip of the catheter appeared in the spleen's surface, 2 died at days 3 and 5 after surgery and an other revealed a splenic hematoma in the postmorten examination. From the 34 rats studied only 1 showed signs of catheter obstruction as verified in the postmorten examination. More difficulties were found when this technique was applied on animals made cirrhotic by a method previously described[4,5]. From a group of 26 cirrhotic rats, 6 were discarded because of intraoperative problems and 3 died in the first 4 days after surgery. Splenic bleeding, hypotension and infection were the most frequent complications. No catheters were found to be obstructed during the 7-day period after surgery.

Experiments were performed from 4 h to 7 days after the surgical preparation. We prefer to use large cages in which the rat can walk freely rather than restraining cages, because more stable arterial pressure and pulse rates have been found. Experiments of more than 6 h duration can be performed. To carry out the portal infusions, the external end of the catheter is connected to a syringe infusion pump (Unita I, Braun Melsungen, Germany) and a pressure transducer (Statham) and recorder (Poligraph, Grass Inst Co. Quincy, Mass., USA) using a 3-way stopcock. Basal intrasplenic pressure was measured by injecting 0.1 ml of heparinized saline in the catheter as a bolus. This procedure causes an abrupt rise in the pressure which slowly decreases and stabilizes at basal intrasplenic pressure. Intrasplenic pressures recorded for different experiments averaged 13.3 ± 0.6 cm H_2O for 26 control Wistar male rats and 29 ± 3.7 cm H_2O for 18 cirrhotic rats of the same strain, sex and weight. These values are very similar to the ones previously reported by our laboratory using direct splenic puncture[3] and do not increase with the time of cannulation.

The possibility of using high infusion rates without altering the splenic pressure has also been studied. Actual pressures in the tip of the catheter have been obtained by subtracting from the pressures recorded with the catheter in place, the pressures obtained at the same infusion rates with the tip of the catheter open at air. Isotonic saline infusion at rates as high as 9 ml/h can be performed without detectable changes in intrasplenic pressure. The infusion of isotonic saline into the spleen for 6 h at a rate of 15 ml/h did not cause any increment in organ weight (0.25 ± 0.06% of b.wt in 8 animals, compared with 0.24 ± 0.04% in 8 not infused animals). Also, 3 h after infusion of isotonic saline containing 0.1 μCi of $^{22}NaCl$, less than 0.05% of the activity injected remained in the spleen, an amount similar to the activity measured in the spleen of rats which had received the ^{22}Na infusion i.v. This fact demonstrates that no significant amounts of the substance injected are retained by the spleen.

Some pieces of spleen have been histologically studied after 7 days of cannulation, showing a slight fibrous reaction around the catheter and in the surface in contact with the adhesive.

Aziz et al.[6] have reported a method for cannulation of the vena porta and vena cava in conscious rats using a 4 mm long steel cannula attached to a polyethylene catheter. This method has been successfully used by the same authors[7,8], but it requires considerably more time and greater handling of the viscera that the procedure here described. Furthermore the possibility of vein damage by the steel cannula in more chronic studies can be important.

We think the present method allows repeated intraportal infusions, pressure measurement and angiographic studies of the portal area in conscious or anesthetized rats without a requirement for extensive surgery or alteration of the normal pattern of vascular flow in the splanchnic area.

1 The author thanks Providencia Cruz for excellent secretarial assistance. Present address: Veterans Administration Center, Medical Research Division, San Juan, Puerto Rico.

2 M. Gellai and H. Valtin, Kidney Int. *15*, 419 (1979).
3 J.M. López-Novoa, M.A. Rengel and L. Hernando, Am. J. Physiol. *238*, F353 (1980).
4 E.K. McLean, E.M. McLean and P.M. Sutton, Br. J. exp. Path. *50*, 502 (1969).
5 J.M. López-Novoa, M.A. Rengel and L. Hernando, Am. J. Physiol. *232*, F315 (1977).
6 O. Aziz, F.J. Haberich and P.E. Nowacki, Naturwissenschaften *51*, 557 (1964).
7 F.J. Haberich, O. Aziz and P.E. Nowacki, Pflügers Arch. *279*, R30 (1964).
8 J.H. Permutt, O. Aziz and F.J. Haberich, Pflügers Arch. *357*, 1 (1975).

A simple filtration device to study the interaction of RNA-polymerase with DNA

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Summary. An accessory for a commercial 10-place filtration apparatus is described, which allows the easy recovery of both filter-bound material and flow-through in the study of DNA-protein interactions by assaying binding of protein to cellulose nitrate filters.

The specific interaction of DNA with some proteins can be monitored by making use of the binding of proteins to cellulose nitrate filters. In this case, if the protein has a high affinity in binding to DNA, the DNA will also be retained by the filter. Using DNA fragments produced by restriction endonucleases these fragments can be characterized by their differing ability to bind a certain protein and the sites where the protein binds specifically can be determined. This type of study is especially useful when promotor sites of RNA polymerase are studied[2-6]. As these assays are normally carried out, only the retained DNA fragment is recovered. We found, however, that the relation of bound

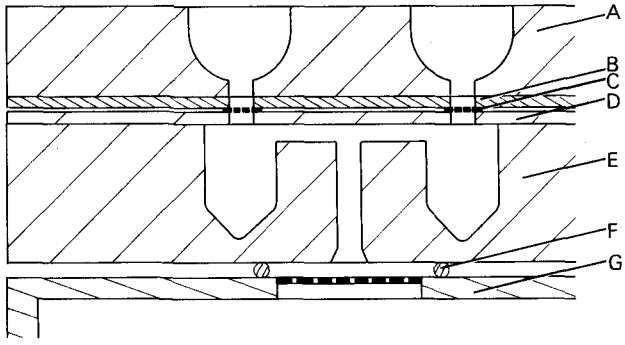
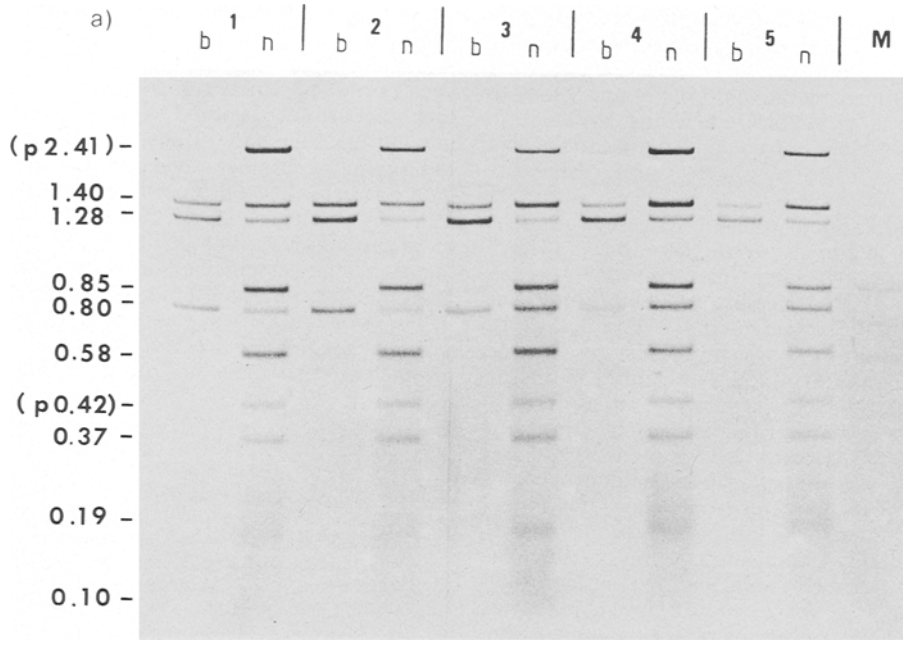


Fig. 1. Schematic diagram of the filtration device. A, Upper perspex plate with moulds (12 mm in diameter at the top, 3 mm at the bottom) to retain the assay solution; B, rubber plate; C, cellulose nitrate filters (6 mm in diameter); D, removable perspex plate as support for C with holes 3 mm in diameter; E, lower perspex plate with cavities to hold up to 1.5 ml of flow-through and outlet to vacuum pump; F, O-ring; G, part of commercial filtration apparatus DNA-10 (New Brunswick, Inc.).



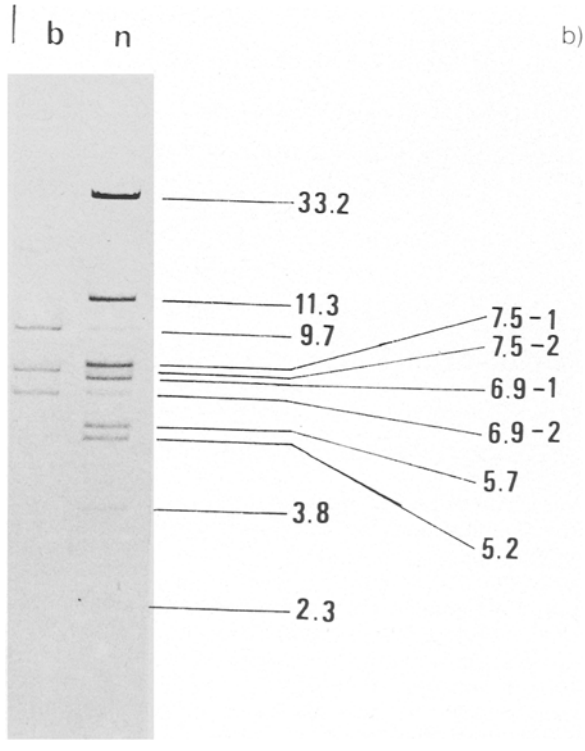


Fig. 2. Demonstration of RNA-polymerase binding sites. *a* The stability of DNA/RNA-polymerase complexes at high ionic strength. The spinach chloroplast DNA (pSoc S15) fragment Sal I-F (5.7×10^6 dalton) was digested with the enzymes Sal I and EcoRI. The DNA fragments were incubated with RNA polymerase (ratio 1:12 in 20 mM Tris/HCl, pH 8.0, 60 mM KCl, 10 mM Mg Cl_2, 0.1 mM EDTA and 5% glycerol at 37 °C for 10 min), then adjusted to 200 mM KCl and further incubated at 15 °C as follows: 1. assay minus KCl; 2. + KCl, 0 min; 3. + KCl, 5 min; 4. + KCl, 10 min; 5. + KCl, 20 min. After filtration, filter bound fragments (b) and fragments in the flow-through (n) were separated on a 1% agarose gel. M, marker DNA is ΦX 174/Hae III. The numbers are fragment sizes in 10^6 dalton. The arrow points to the KCl-sensitive binding site. *b* The assay monitors DNA/RNA polymerase complexes which are known as promotors. Phage λ DNA digested with Bam HI and Hind III was separated in bound (b) and nonbound (n) fragments (DNA:RNA polymerase = 1:20) and the DNA fragments were then separated on a 0.9% agarose gel. The numbers close to the individual bands give the DNA fragment size in % of the total length (32.3×10^6 dalton)[11,12].

to unbound DNA fragments is of importance for comparison and internal control. In order to save the flow-through we designed a simple attachment for a commercially available filtration apparatus which allowed the recovery of DNA fragments which passed the filter. DNA from phage λ was given to us by Dr H. Bujard (University of Heidelberg). Chloroplast DNA from spinach, and also spinach chloroplast DNA fragments in pBR 322 (from Dr M. Hartley, Coventry) were isolated according to standard procedures[7]. *E. coli* RNA polymerase (holoenzyme) and restriction endonucleases were purchased from Boehringer, Mannheim. Restriction endonuclease digestion and electrophoresis were performed as described[7]. The filtration apparatus as outlined was built by a local workshop, a first version was made without difficulty in the laboratory workshop. The device was designed to replace the top plate of a 10-fold filtration apparatus (DNA-10, New Brunswick) and could hold a total of 20 cellulose nitrate filters.

The assays contained between 0.04 and 0.1 pmole of DNA. The amount of DNA was measured in principle as described by Reis[8] using the densitometric comparison of the DNA to be estimated with a standard dilution. The DNA was digested in 5–10 µl total volume with various restriction endonucleases and after dilution 0.2–4 pmole RNA polymerase holoenzyme was added. After 10 min at 37 °C poly rI (20–150 µg/ml final concentration) or heparin (100–500 µg/ml final concentration) in prewarmed buffer solution was added and the incubation was continued for another 10 min.

The assay solution was pipetted into the cavity of the filtration device (fig. 1, A). It was then filtered slowly through the cellulose nitrate filter (fig. 1, C) which was held in place by a rubber plate (fig. 1, B). The flow-through, which was collected in the cavities of the lower perspex plate (fig. 1, E), could easily be obtained since the top plate (fig. 1, D) was removable. The whole apparatus was evacuated through the bottom part (fig. 1, G) of a commercially available filtration apparatus (New Brunswick, filtration apparatus DNA-10).

The DNA in the solution which passed the filter was precipitated in the presence of 0.2 M K-acetate and 0.01 M $MgCl_2$ by 2.5 vol. of ethanol at −80 °C for 60 min. DNA retained by the filters was eluted at low temperatures by shaking the filters in 2×200 µl 0.01 M Tris/HCl pH 8.0, 1 mM EDTA, 0.2% SDS[9] and the DNA was precipitated. DNA fragments were electrophoretically separated in 0.5–1.8% agarose or 4–8% polyacrylamide gels[7,9].

The figure 2a gives an example. A cloned DNA fragment of spinach chloroplast DNA (pSoc S15) was digested using the restriction endonucleases EcoRI and Sal I and the DNA fragments were incubated with RNA polymerase at 37 °C in a ratio of 1:12. In the 5 individual assays the KCl concentration was adjusted to 200 mM and the assays were then incubated at 15 °C for 0–20 min to demonstrate the varying stability of the DNA-RNA polymerase complexes at low temperature and high salt concentration. After filtration the DNA fragments were separated on an agarose gel. The fragments labelled p 2.41 and p 0.42 respectively, are derived from the cloning vector pBR 322 and show the molecular weight of these subfragments in 10^6 dalton. It is obvious that no strong RNA polymerase binding site comparable to the chloroplast DNA sites exists on pBR 322. The promotor for the plasmid DNA would be expected[10] on the subfragment p 2.41 which is in fact slightly bound. This weak promotor becomes evident only at much higher polymerase/ DNA ratios. The region of the binding site on the (1.28×10^6) dalton DNA fragment from the chloroplast DNA contains some very AT-rich segments which by their arrangement most probably account for the strong polymerase binding (to be published).

Similar experiments, using the phage λ, have shown that binding occurs to DNA fragments on which promotors have been located by others[9,11,12]. The figure 2b shows the DNA fragments produced by a Bam HI/Eco RI double digestion. The strongly bound fragment '9.7' contains the λ promotors P_L, P_{rm}, P_R and P_O, the bound fragment '6.9-2' contains promotor P_R, and '7.5-2' contains the strongest promotors of the λ b_2 region[9,11,12].

The function of the several *E. coli* RNA polymerase binding sites on the chloroplast DNA insert pSoc S15 is not yet known. We were however able to show that the binding site on the (1.28×10^6 dalton) Sal I/Eco RI subfragment (fig. 2) is located within 120 base pairs upstream of a 32,000 dalton protein gene on the chloroplast DNA (to be published elsewhere). The filtration device described permitted easy and highly reproducible monitoring of such DNA-protein interactions.

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2 D.C. Hinkle and M. Chamberlin, J. molec. Biol. *70* 157 (1972).
3 B.B. Jones, H. Chan, S. Rothstein, R. Wells and W. Reznikoff, Proc. natl Acad. Sci. USA *74*, 4914 (1977).
4 P.H. Seeburg and H. Schaller, J. molec. Biol. *92*, 261 (1975).
5 A. von Gabain and H. Bujard, Molec. gen. Genet. *157*, 301 (1977).
6 W.E. Taylor and R.R. Burgess, Gene *6*, 331 (1979).
7 H.J. Bohnert K.H.J. Gordon and E.J. Crouse, Molec. gen. Genet. *179*, 539 (1980).
8 R.J.S. Reis, Analyt. Biochem. *90*, 8 821 (1978).
9 M. Zech, Diplomarbeit, Universität Düsseldorf, 1980.
10 R.W. West and R.L. Rodriguez, Gene *9*, 175 (1980).
11 H.J. Vollenweider and W. Szybalski, J. molec. Biol. *123*, 485 (1978).
12 B. Allet, R.J. Roberts, R.F. Gesteland and R. Solem, Nature *249*, 217 (1974).

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