Group of diseases	No. of cases	Folic acid Mean ± SEM (ng/ml) CSF	Serum	Vitamin B_{12} Mean ± SEM (pg/ml) CSF	Serum
Controls	19	> 18.5	5.4 ± 1.3	50.8 ± 10.0	562.2 ± 73.8
Meningitis	19	> 18.5	4.1 ± 2.2	98.1 ± 30.2	401.2 ± 73.8
Demyelinating diseases	7	> 18.5	7.5 ± 1.5	48.6 ± 16.0	1021.0 ± 238.0*
Tumors	10	11.2 ± 2.2**	8.3 ± 2.4	42.0 ± 15.2	730.0 ± 150.3

Mean values ± SEM of CSF and serum folic acid and vitamin B_{12} in different studied groups. **$p < 0.001$; *$p < 0.05$ (exogenous administration of vitamin B_{12}); $p > 0.05 - p < 0.1$. In all remaining mean values.

folic acid values were within normal limits. The CSF and serum vitamin B_{12} levels in all groups were found within normal limits, (mean value ± SEM 515.5 ± 169.2 ng/ml), except for a slight increase in CSF in the group with meningitis and a borderline higher value in the serum in the demyelinating diseases group.

Discussion. The values obtained in the control group indicate that the CSF folic acid concentration under normal conditions is much higher than that of the serum (> 18.5 ng/ml vs 5.4 ng/ml), as has been reported[4,5]. This is probably due to a passage from serum to CSF predominately by a diffusion mechanism[6]. Thus, all the obtained values above 18.5 ng/ml in the group with meningitis and demyelinating diseases must be considered as normal.

On the contrary, in the group with cerebral tumors the CSF folic acid mean value was found to be significantly lower than that of the controls (CSF folic acid 11.2 ± 2.2 ng/ml, $p < 0.001$). Thus the main point which deserves comment is the low CSF folic acid level which was observed in the group with cerebral tumors. All the patients of this group had metastatic cerebral tumors, which had originated from the lung (6 cases), from the thyroid gland (2 cases) and from kidneys (2 cases) as confirmed by clinical and laboratory data. The low CSF folic acid levels in the group with cerebral tumors may be attributed to the ability of tumor cells to metabolize folates rapidly[7], due to the increased synthesis of protein and nucleic acids for which folates are essential. The increased folate consumption by the neural tumor cells in such a limited compartment as the CNS may lead to a more evident change in the folic acid level, than it does in other organs. The specificity of the method used here is supported by the fact that other tested substances such as methotrexate, N_5-formyltetrahydrofolic acid and pteroic acid not interfere with the binding assay of labeled M.T.H.F.A.[2]. Other substances used, such as chloramphenicol, penicillin G, tetracyclin, phenytoin, ethanol and vitamin B_{12} did not show cross-reactivity either.

The increased CSF vitamin B_{12} levels observed in some cases of the meningitis group may be due to increased permeability through the damaged blood-brain barrier caused by the infection. The upper normal limits of serum vitamin B_{12} values noted in the group with demyelinating diseases must be considered to be a result of exogenous high dose administration of this agent in the patients of this group.

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Modification of a piston-type perfusion pump for delivery of low flow rates [1]

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Summary. A new remote pumping head and valve unit has been constructed to allow the delivery of low flow rates (with 0.05–0.3 ml stroke volume) from a Harvard Instruments blood pump.

Reciprocating, or piston type perfusion pumps are most useful for simulating the action of the heart during vascular perfusion. Pumps which produce a fixed phase of systole: diastole of 1:2 are commercially available. The Harvard Blood Pump (Model 1405, Harvard Apparatus Co., Inc., Mass., USA[4]) is such a pump. However, these perfusion pumps do not accurately deliver constant volumes at low flow rates, when stroke volume is small. We therefore have constructed a replacement piston system with newly designed valves, to allow the delivery of low minute volumes, with stroke volumes in the range of 0.05–0.3 ml per stroke.

The complete perfusion apparatus is shown in figure 1. The reciprocating mechanism of the piston type pump is attached to the plunger of a small syringe (fig. 2). The small syringe (e.g. 1 ml) allows production of small stroke volumes. A piece of thick-walled polyethylene tubing connects the lumen of the syringe to the remote pumping head (fig. 3, A). The remote pumping head enables the pump to be located away from the preparation and avoids the hazard of salines in electrical connections. The one-way valves which we have designed and constructed connect to the input and output of the remote head so that only one-way flow is allowed (fig. 3, B).

The existing reciprocating mechanism of the pump (in our case the plunger rod of the Harvard blood pump) was connected to the plunger of a disposable 1-ml syringe (Tuberculin; B & D Co., Mississauga, Canada) by a U-shaped steel connector (fig. 2, A). The barrel of the syringe was cut off at the 1.0 ml mark and clamped into a machined perspex block (fig. 2, B). This block, with the syringe barrel, then was mounted onto the pump chassis

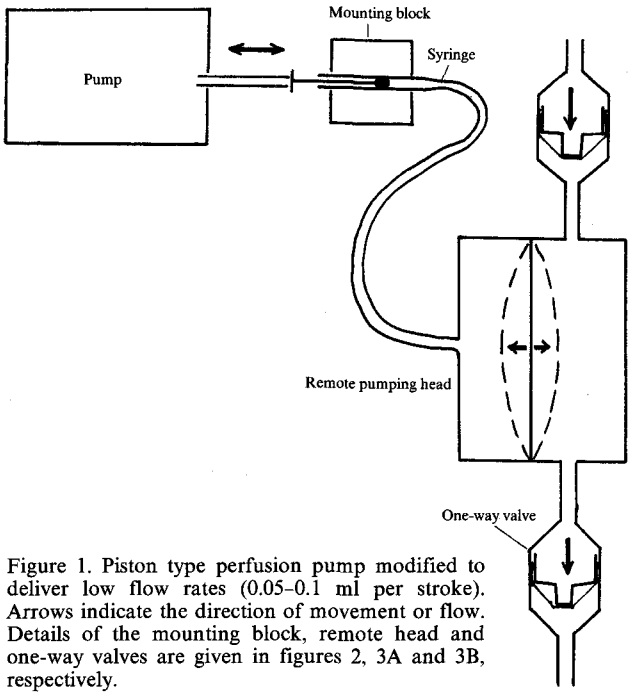

Figure 1. Piston type perfusion pump modified to deliver low flow rates (0.05–0.1 ml per stroke). Arrows indicate the direction of movement or flow. Details of the mounting block, remote head and one-way valves are given in figures 2, 3A and 3B, respectively.

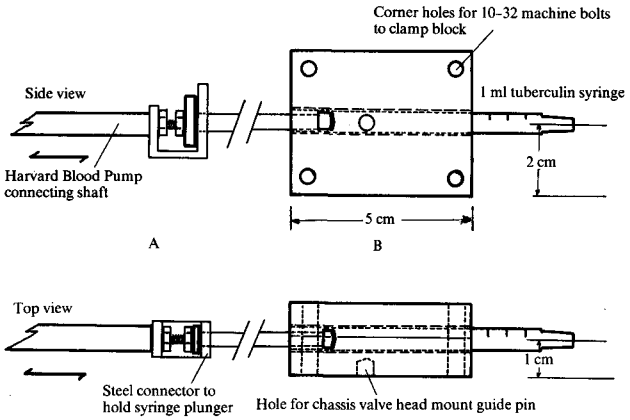

Figure 2. Modified block and syringe barrel assembly.

with machine bolts. The syringe barrel was connected to a length of thick-walled polyethylene tubing (ID 1.2 mm). Figure 3A is a diagram of this remote pumping head which is similar in concept to that available from Harvard Apparatus Co.

The remote pumping head is simply a chamber divided in half by a flexible latex rubber dam (No. 7 Dental Dam. B & D). From figure 1 we can see that as the syringe plunger moves back and forth, the fluid contained in the polyethylene tubing will cause rhythmic convexity and concavity of the rubber partition. This provides the suction (diastole) and pumping (systole) phases of the pumping cycle. These forces are transmitted to the perfusion fluid on the opposite side of the partition. As the plunger withdraws, the partition concaves, drawing perfusion fluid down through the upper one-way vale into the chamber, while the lower one-way valve is closed. When the plunger moves forward, the convexion of the rubber partition forces the perfusion fluid from the chamber out of the lower one-way valve, the upper valve now being closed. Thus the phase relationship of the reciprocating pump is transmitted to the perfusion fluid.

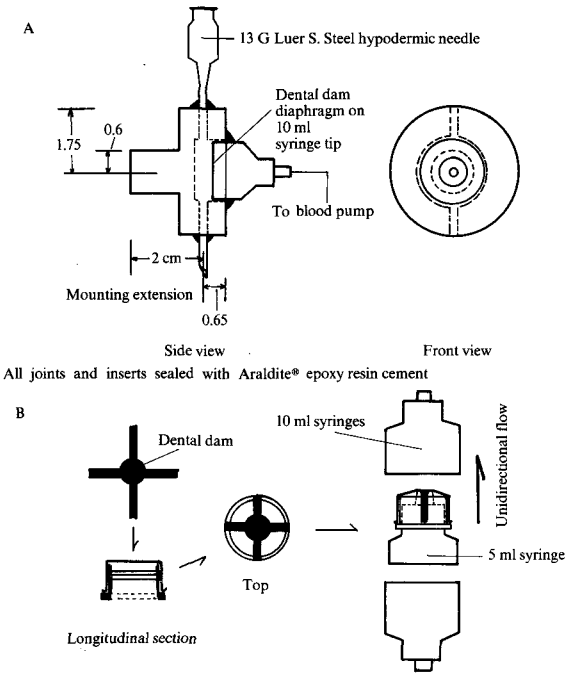

Figure 3. *A* Re-designed remote pumping head (machined perspex). *B* Checking mechanism and unidirectional valves attatched to inflow and outflow of pumping head.

Our newly designed valves, which attatch to input and output Luer needles on the redesigned remote pumping head, are shown in exploded view in figure 3B. The checking mechanism of the unidirectional valves was provided by a piece of latex rubber dental dam stretched lightly over a collar cut from a 1.5-ml Eppendorf centrifuge tube (Brinkman Co.). The collar and dam (valve assembly) was press-fitted over the tip of a 5-ml disposable Luer-Lok syringe (B & D Co.), and then this assembly was mounted inside a housing constructed from the tips of two 10-ml disposable Luer-Lok syringes (B & D Co.). The positive valve action provided by the stretched dam actively prevents flow when low pressures are exerted by the pumping mechanism. That is there is no delay in shut off of flow. Other valve types (e.g. ball bearing in socket) require a finite time to check backflow, and in doing so allow a small amount of fluid to pass the valve. Where low flow rates are required, this becomes unacceptable.

When assembled and attached to a reciprocating perfusion pump, our new remote pumping head and valve unit allowed only unidirectional flow, regardless of orientation. Flow rates were also independent of outflow back pressure at stroke volumes as small as 0.05 ml. There was no discernable backflow during the suction (diastole) phase of the pumping cycle. All other features of the reciprocating perfusion pump used were unaffected by these modifications. Valves were inexpensive and easily constructed, and could therefore be discarded after each experiment.

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