Oestrus induction and synchronization in unisexually grouped female mice

Group and treatment	Number of females returning to oestrus within 7 days Days 1	2	3*	4	5	6	7	Percent of females returning to oestrus on day 3
I Continuous exposure to males for 7 days	1	6	14	2	2	1	1	46.6
II Intermittent (30 min) exposure to males, thrice daily, for 7 days	4	6	11	3	4	0	0	36.6
III Intermittent (10 min) exposure to males, thrice daily, for 7 days	4	4	14	0	2	0	1	46.6
IV Housed in a room containing males for 30 min, thrice daily, for 7 days	3	3	3	5	4	3	4	10.0
V Controls: housed in a male-free room	3	2	5	3	6	5	0	16.6

*Significance of differences: I vs V, $p < 0.001$; II vs V, $p < 0.001$; III vs V, $p < 0.001$; IV vs V, NS.

habitat may influence the oestrous cycle in mice. Implantation failure (the Bruce effect) in newly inseminated mice can be achieved by topical application of microquantities of male urine[8] or by multiple short-term exposures to males[9]. It appears that brief exposures of female mice to male odour is sufficient to trigger the chain of neuroendocrine events which culminate either in ovulation or implantation failure depending upon the physiological state of the female. These findings suggest that primer pheromonal control on oestrous cycle and implantation in wild populations of rodents cannot be ruled out.

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Radioisotopic determination of cerebrospinal fluid (CSF) folic acid and vitamin B_{12} in neurological disorders

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Summary. In a total of 55 samples of cerebrospinal fluid (CSF) and an equal number of serum samples obtained from 45 patients with neurological disorders and 10 controls, folic acid and vitamin B_{12} were measured. A radioisotopic assay method was used. A significant decrease of CSF folic acid was noted in the group with cerebral tumors.

The determination of cerebrospinal fluid (CSF) levels of folic acid and vitamin B_{12} in neurological disorders has been thoroughly studied. The purpose of this paper is to report our results on the concentrations of folic acid and vitamin B_{12} in the CSF of patients suffering from meningitis of different types, cerebral tumors and demyelinating diseases.

Materials and methods. In a total of 55 CSF samples obtained from the same number of patients after lumbar puncture performed for diagnostic purposes, folic acid and vitamin B_{12} were measured. 19 out of the 55 samples were from patients with meningitis, 10 with a cerebral tumor, 7 with demyelinating disease and 19 were from normal controls. Serum folic acid and vitamin B_{12} were also determined. All samples were stored at $-20\,°C$ in small aliquots until analysis. Folic acid in CSF and serum was estimated by a radioisotopic assay[2]. Tritiated methyl-tetrahydrofolic acid as a label, pig plasma as a specific binding agent in a saturation analysis and dextran coated charcoal for separating the free from the bound fractions of folic acid were used. All samples (after separation) were counted in a 'Packard' liquid scintillation counter using 'Insta-Gel' as a scintillation fluid. The counting rate of the samples containing hemolysates was corrected for quenching due to color, by the method of internal standardization. The results are expressed in ng/ml of serum and CSF.

Vitamin B_{12} in CSF and serum was measured by a radioisotopic method based on saturation analysis[3]. Radioactive ^{57}Co-vitamin B_{12} of high specific activity obtained from Amersham was used as label, human serum as a source of vitamin B_{12} binders, and a Sephadex G-25 medium column for separation of the free and the bound portions. The samples were then counted in a 'Nuclear Chicago' well type scintillation counter. The results are expressed in pg/ml of serum and CSF. Estimation of both vitamins was performed in duplicate in each case. The statistical analysis was performed using Student's t-test.

Results. As can be seen in the table, the CSF folic acid levels in the groups with meningitis and demyelinating diseases were above 18.5 ng/ml, as was also observed in the control group. In the group with cerebral tumor the mean value $\pm$SEM was 11.2 ± 2.2 ng/ml with a statistically significant difference ($p < 0.001$) in comparison to the control group. In all the above mentioned groups, serum

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