Cytological Examination of Cerebrospinal Fluid in Eight Patients with Neuro-Behçet's Disease

SHOZO NAKAMURA, SADAO TAKASE and KATSUYA ITAHARA

*Division of Clinical Neurology, Institute of Brain Diseases,
Tohoku University School of Medicine, Sendai 980*

NAKAMURA, S., TAKASE, S. and ITAHARA, K. *Cytological Examination of Cerebrospinal Fluid in Eight Patients with Neuro-Behçet's Disease*. Tohoku J. exp. Med., 1980, 132 (4), 421-430 — In eight patients with Neuro-Behçet's disease cerebrospinal fluid (CSF) cells were studied in relation to the clinical symptoms, clinical course and treatment. The results obtained were as follows: (1) The mean value of CSF cell counts was $126 \pm 177/3 \text{ mm}^3$ in 88 specimens from the patients, and $2.9 \pm 2.4/3 \text{ mm}^3$ in 30 specimens from healthy control subjects, the former being significantly higher than the latter ($p < 0.01$). (2) In the patients' CSF, the average percentages of mononuclear and polymorphonuclear cells were $67 \pm 23\%$ and $32 \pm 23\%$, respectively. (3) In CSF cytogram from 37 specimens from the patients, the average percentage of small lymphocytes was $59 \pm 19\%$, which was significantly lower ($p < 0.01$) than control ($71 \pm 8\%$). The average percentage of activated lymphocytes, activated monocytes and neutrophils which were not observed in control were $0.5 \pm 1.2\%$, $1.9 \pm 2.2\%$ and $17 \pm 16\%$, respectively. (4) The ratio of nucleic acids (RNA/DNA) measured by microspectrophotometry (Zeiss UMSP-1) in CSF lymphocytes stained with methylgreen and pyronin was 0.58 ± 0.12 in 17 specimens from the patients and $0.55 \pm 0.07\%$ in 10 specimens from the control; there was no significant difference between them. These results suggested that the pleocytosis in Neuro-Behçet's disease may represent perivascular inflammatory process in the central nervous system. — Neuro-Behçet's disease; CSF cells

So-called Neuro-Behçet's disease with neurological symptoms as its major signs is said to have increased in incidence along with a sharp rise in the number of cases of Behçet's disease in recent years (Shimizu 1972). According to an epidemiological survey conducted in Japan in 1972, Neuro-Behçet's disease accounts for 257 (13.7%) of 1,876 cases of Behçet's disease (Shimizu 1974).

Neuro-Behçet's disease is an intractable disorder which affects mainly young men and brings death within several years after onset. Among a variety of abnormal findings concerning blood and cerebrospinal fluid (CSF) in this disease which have been pointed out, it has been reported that pleocytosis in CSF, one of the CSF abnormalities, also exists in many cases (Wolf et al. 1965; Shimizu 1972).

Reports on pleocytosis in CSF in this disease, however, have hitherto dealt mainly with cell counts and ratio of polymorphonuclear cells to mononuclears.

Received for publication, December 11, 1979.

This study was partly supported by Grant for the Ministry of Health and Welfare Multiple Sclerosis Research Committee of Japan.

Recently, we have examined the CSF cell counts, cytogram and ratio of nucleic acids in eight patients with Neuro-Behçet's disease and studied them in reference to the clinical symptoms, clinical course and treatment.

The present paper reports the results obtained along with some comment.

MATERIALS AND METHODS

The subjects were eight patients whose CSF cytogram could be examined on a time-course basis among 14 patients who were seen at the Division of Clinical Neurology, Institute of Brain Diseases, Tohoku University School of Medicine and diagnosed as Neuro-Behçet's disease between April, 1969 and August, 1978.

These patients were all males aged 22 to 39 years (Table 1). Their initial neurological symptoms consisted of gait disturbance in 3 cases, memory disturbance in 2 cases, and speech disturbance, cranial nerve palsy or sensory disturbance in 1 case each. Neurological symptoms which appeared during the clinical course were multifarious. As for the type of illness (possible site of lesion which brought forth the major neurological symptoms), there were 3 cases of the brain stem type, 2 cases of encephalomyelitic type, 1 case of meningitic type and 2 cases of myelitic type.

Thirty normal controls were also examined for CSF cell counts and cytogram.

About 5 ml of CSF was withdrawn by lumbar puncture and subjected to general examination. CSF cells were counted in a Fuchs-Rosenthal chamber after treatment with Samon's solution and the ratio of mononuclear cells to polymorphonuclear cells was estimated.

Immediately after withdrawal of CSF, CSF smear was prepared with a Tomy cell collection apparatus (Model SC-2) and subjected to May-Grünwald Giemsa staining.

CSF cells were classified into (1) small lymphocytes, (2) activated lymphocytes, (3) plasma cells, (4) monocytes, (5) activated monocytes, (5) neutrophils, eosinophils, basophils and (7) others (Itahara et al. 1976); and, 100 cells were counted under a microscope for classification.

Following methylgreen-pyronin staining (Kurnick 1955) of CSF cell smears, measurement was made of DNA at 630 nm and RNA at 550 nm with a micro-spectrophotometer

TABLE 1. *Clinical features of eight cases*

Cases	Sex & age	Age of onset	Initial symptom	Initial neurological symptom
1. S.M.	M 23	22	Uveitis	Gait disturbance
2. K.T.	M 39	36	Epilepsy	Memory disturbance
3. K.T.	M 32	28	Oral aphtha	Memory disturbance
4. T.A.	M 32	25	Uveitis	Disturbance of ocular movement
5. N.I.	M 22	19	Uveitis	Speech disturbance
6. T.H.	M 30	24	Oral aphtha	Gait disturbance
7. A.T.	M 38	35	Oral aphtha	Sensory disturbance
8. E.O.	M 21	20	Oral aphtha	Gait disturbance

(Zeiss UMSP-1) and the RNA/DNA ratio was calculated. Twenty lymphocytes per preparation were counted, and the cell diameters were also measured.

RESULTS

A total of 88 lumbar punctures were performed in eight cases (Table 2).

CSF cell counts. The mean value for the 30 controls was $2.9 \pm 2.4/3 \text{ mm}^3$ and the mean value for the eight cases of Neuro-Behçet's disease ($n=88$) was $126 \pm 177/3 \text{ mm}^3$, which was significantly larger than control ($p<0.01$). One case of the meningitic type (Case 1) showed marked pleocytosis (a maximum value of $1,312/3 \text{ mm}^3$), whereas three cases of the brain stem type (Cases 3, 4, 8) had maximum values of $540/3 \text{ mm}^3$ and two myelitic type cases (Cases 6, 7) had values less than $360/3 \text{ mm}^3$.

The average percentages of mononuclear and polymorphonuclear cells were $67 \pm 23\%$ and $32 \pm 23\%$, respectively.

CSF cytogram. In 21 control cases, the average percentages of small lymphocytes and monocytes were $71 \pm 8\%$ and $28 \pm 8\%$, respectively; no activated lymphocytes, activated monocytes, neutrophils nor plasma cells were observed. In Neuro-Behçet's disease ($n=37$), the average percentage of small lymphocytes was $59 \pm 19\%$, which was significantly lower ($p<0.01$) than that of the controls. Activated lymphocytes and activated monocytes appeared in a small number. The average percentage of neutrophils showed a slight increase to $17 \pm 16\%$.

Nucleic acid ratio (RNA/DNA) in CSF lymphocytes. The ratio in 10 control cases was 0.55 ± 0.07 . As seen in Table 3, there was a significant increase to 2.82 ± 0.06 ($p<0.01$) in the acute phase of viral meningitis and to 1.10 ± 0.38 ($p<0.01$) in the exacerbated phase of multiple sclerosis. In Neuro-Behçet's disease ($n=17$),

of Neuro-Behçet's disease

Main neurological symptoms & signs	Type of nervous system involvement	Clinical course
Headache, stiff neck, diplopia, urinary disturb.	Meningitic type	Deteriorated
Spastic gait, diplopia, memory disturb., urinary disturb.	Encephalomyelitic type	Deteriorated
Euphoria, spastic gait, hiccup, right hemiparesis	Brain stem type	Died (autopsy)
Left oculomotor palsy, right hemiparesis, euphoria	Brain stem type	Slowly progressive
Spastic gait, depressive state, visual impairment, character change	Encephalomyelitic type	Progressive
Spastic gait, constipation, urinary disturb., depressive state	Myelitic type	Deteriorated
Paraparesis, urinary disturb.	Myelitic type	Slowly progressive
Memory disturb., euphoria, spastic gait, cerebellar ataxia	Brain stem type	Slowly progressive

TABLE 2. *Summary of CSF cell counts and cytogram in eight cases of Neuro-Behçet's disease*

	Case								Total
	1. S.M.	2. K.T.	3. K.T.	4. T.A.	5. N.I.	6. T.H.	7. A.T.	8. E.O.	
Number of lumbar punctures	7	9	17	22	8	11	5	9	88 (Mean $\pm$ S.D.)
Cell counts (/3 mm ³)	72-1,312	15-184	14-263	6-540	41-150	30-146	21-360	29-352	126 $\pm$ 177
MNC (%)	18-86	60-100	6-86	18-100	61-71	52-95	19-70	50-100	67 $\pm$ 23
PMN (%)	14-82	0-40	14-96	0-82	29-39	5-48	30-81	0-50	32 $\pm$ 23
Cytogram (%)				(n=10)	(n=5)	(n=9)	(n=5)	(n=8)	(n=37)
Small lymphocytes				46-77	44-65	14-71	8-79	23-89	59 $\pm$ 19
Activated lymph.				0-2	0-2	0-4	0-2	0-2	0.5 $\pm$ 1.0
Plasma cells	Not examined	Not examined	Not examined	0	0	0	0	0	0
Monocytes				9-32	2-29	14-40	15-38	9-30	23 $\pm$ 12
Activated mono.				0-10	3-7	0-3	2-5	0-1	1.9 $\pm$ 2.2
Neutrophils				4-29	6-28	15-43	3-57	0-61	17 $\pm$ 16

MNC, mononuclear cells; PMN, polymorphonuclear cells.

TABLE 3. *Nucleic acids of lymphocytes in CSF*

Diseases	RNA/DNA (10 μ m)
Control (n=10)	0.55 $\pm$ 0.07
Neuro-Behçet (n=17)	0.58 $\pm$ 0.12
Viral meningitis acute phase (n=4)	2.82 $\pm$ 0.06*
Multiple sclerosis exacerbation (n=3)	1.10 $\pm$ 0.38*
	(1.23 $\pm$ 0.20)†
Remission (n=8)	0.52 $\pm$ 0.18

* Significant with $p<0.01$. † Cell diameter $>17 \mu$ m.

it was 0.58 ± 0.12 , not significantly different from the control.

Presentation of a case (Fig. 1). A male, 30 years of age (Case 6).

Chief complaint was gait disturbance and dysuria from around age 28. He began to suffer from recurring oral aphtha and constipation. Then gait disturbance developed gradually and about a year later genital ulcer appeared. In February 1976, he was seen at the Division of Clinical Neurology, Institute of Brain Diseases.

Physically, neither the chest nor the abdomen was remarkable, but an aphthous ulcer was found in the buccal mucosa. Neurologically, consciousness was clear and no abnormality in the cranial nerves was found, but the tendon reflex of the extremities was hyperactive and Babinski's reflex was elicited in the lower limbs. Additionally, he had urinary disturbance and was diagnosed as neurogenic bladder at the Department of Urology. On the basis of the clinical course described above, this case was diagnosed as Neuro-Behçet's disease.

Fig. 1. Clinical course and CSF cytogram in a patient with Neuro-Behçet's disease (Case 6, T.H., 30 years old, myelitic type).

Fig. 2. CSF cells smear (May-Grünwald Giemsa stain $\times 200$). Large quantities of neutrophils are present, but no activated lymphocytes nor plasma cells are found.

After admission, the symptoms improved temporarily following oral administration of prednisolone, but the urinary disturbance and gait disturbance became aggravated again in May. At that time, CSF cells consisted of 52% of mononuclear

cells and 48% of polymorphonuclear cells. The cytogram revealed neutrophils in 43% and small lymphocytes in 14%.

Thereafter, the symptoms aggravated and improved alternately and in August 1977 the CSF cell count increased again to $114/3 \text{ mm}^3$ (Fig. 2). An indwelling catheter was inserted into the bladder because of inability to void, and the patient became unable to walk by himself because of spastic paraparesis.

With oral administration of prednisolone, however, the symptoms improved and the patient became able to void, although the passage of urine was quite frequent.

Lumbar puncture performed about two months later revealed a marked decrease in the cell counts to $19/3 \text{ mm}^3$.

DISCUSSION

Behçet's disease is considered a chronic inflammatory disease with aphthous ulcer in the oral mucosa and external genitals, uveitis and dermal symptoms. But its etiology still remains obscure.

Of Behçet's disease, Neuro-Behçet's disease was so named by Cavara and D'Ermo (1954). Its clinical symptoms are multifarious and involve the entire central nervous system, though most neurological symptoms arise from the disturbance of the brain stem (Shimizu and Yamamoto 1969).

An increase in immunoglobulin IgG and IgA was pointed out as abnormality in CSF in Neuro-Behçet's disease (Takase 1974). It was also reported that mild pleocytosis in CSF is found in about 80 percent or more of the patients (Shimizu 1972).

From a study of 26 cases of Neuro-Behçet's disease, in which pleocytosis ($18-3,600/\text{mm}^3$) was found in all 20 cases from which CSF findings were obtained, Shimizu and Yamamoto (1969) maintained that pleocytosis exists subclinically even when neurological symptoms are not present. They pointed out, however, that the degree of pleocytosis is not necessarily in parallel with the intensity of inflammatory symptoms of the meninx, although changes in CSF cell counts serve as an index of disease activity.

Of the eight cases in the present study, the meningitic type showed marked pleocytosis in the aggravated phase, but with oral administration of steroid preparations the CSF cell count decreased sharply and neurological symptoms also improved.

In the two myelitic type cases, three brain stem type cases and two encephalomyelitic type cases, the relationship between the clinical disability and cell count was not clear, there being cases (Cases 4, 6, 7) in which clinical symptoms were improved and CSF cell counts were decreased by oral administration of steroid preparations and cases (Cases 2, 5, 8) in which clinical symptoms improved, but no change in CSF cell counts was observed. Further, oral administration of immuno-suppressants in one case (Case 3) showed no change in symptoms or CSF findings (Nakamura and Takase 1975).

In a review of 68 cases of Neuro-Behçet's disease, including his own and cases from the literature, Inaba et al. (1977) stated that the ratio of patients improved by administration of steroid preparations was 36 percent and reported that many effective cases were found among those which were of the acute type and which lacked brain stem symptoms.

In determining the effectiveness of steroid preparations on Neuro-Behçet's disease in the future, it appears important to use CSF cell counts as an index of activity.

In recent years there has been a trend to examine the CSF cytogram, likewise the differential count of peripheral white blood cells, in investigations of the pathology of diseases of the central nervous system (Tourtellotte 1968; Dyken 1975).

The cytogram of the normal CSF is composed mostly of lymphocytes and monocytes. According to reports of various authors, however, there is a considerable difference in values — lymphocytes ranging between 61 and 92% and monocytes between 12.5 and 37% (Oehmichen 1976). Such differences are probably due to differences in the method of collecting cells, indicating a need for controlling the collection method among various laboratories. Reported percentages include $1 \pm 1\%$ (Dyken 1975) and $0.5 \pm 0.9\%$ (Tourtellotte 1970) for neutrophils, 0.1% (Ito et al. 1969) for eosinophils and $0.3 \pm 0.5\%$ (Tourtellotte 1970) for plasma cells.

Neutrophils are taken as indicating a pathologic state if they appear in pleocytosis (Oehmichen 1976). Plasma cells are never found in normal CSF (Péter 1967) and their appearance is considered pathologic.

As to CSF cytogram in Neuro-Behçet's disease, mononuclear cells accounted for about 67% and polymorphonuclear cells for 32% in our eight cases. However, Wolf et al. (1965) studied 53 out of 64 cases in the literature and reported that the percentage was the same for polymorphonuclear cells and lymphocytes. Shimizu (1972) obtained similar results.

As far as we have been able to determine, however, there has been no report on CSF cytogram in Neuro-Behçet's disease in Japan.

Kölmel (1976) maintained that neutrophils account for about 10% of the CSF cytogram in Neuro-Behçet's disease and that there are fewer plasma cells and stimulating lymphocytes compared with viral meningitis. He states that this increase in neutrophils is similar to that found in tuberculous meningitis and is never found in multiple sclerosis.

In our examination of eight Neuro-Behçet's cases ($n=37$), the incidence of neutrophils was as high as $17 \pm 16\%$. It is known that cells appearing in aqueous humor in the early stage of ophthalmic symptoms of Behçet's disease involves vasculitis with infiltration of neutrophils as the chief symptom (Shimada et al. 1971) in its early stage according to histopathological findings of mucosal ulcer and erythema (Nishiyama 1971).

Miyoshi et al. (1974) studied the function of neutrophils in blood and reported that NBT reducing activity of neutrophils, random motility and migration by casein chemotaxis were all increased in the active phase of Behçet's disease.

According to findings of CSF neutrophilia during increased vascular permeability (Delank 1972), the white cell counts increased, lymphocytes and monocytes decreased and the relative number of neutrophils increased (Yu et al. 1974) in peripheral blood 6 hr after oral administration of prednisolone.

In cases in which CSF cell count decreased following oral administration of prednisolone among our Neuro-Behçet's cases, the cytogram showed a decrease in neutrophils and a relative increase in lymphocytes.

As for the difference in action of this adrenocorticosteroid preparation in the peripheral blood and CSF cells, it is deduced that the former results from an immunosuppressant action and that the latter is due to an anti-inflammatory action which stabilizes the endothelial cell membrane and thereby prevents permeability (Fishman 1976).

As described above, examining the function of neutrophils in Behçet's disease is important in determining its pathology, and apparently there is need to study the relationship between the function of CSF neutrophils and steroid preparations in the future.

Generally, small lymphocytes, when subjected to antigenic stimulation, are differentiated into stimulated lymphocytes to participate in various immune reaction (Gowans 1962). Similarly, CSF cells have an important function in humoral and cellular immunity. Therefore, the appearance of stimulated lymphocytes or plasma cells in CSF cavity suggests the existence of immune reaction.

Olischer (1972) maintained that pyronin staining of CSF mononuclear cells in neurological diseases is effective in demonstrating the presence of stimulated lymphocytes in the course of immune or inflammatory reactions. Schlote and Roos (1974) reported on the significance of CSF large pyroninophilic cells in multiple sclerosis and assumed that an immune reaction might be involved in its pathology.

Generally, pyroninophilic cells are said to contain plasma cells derived from B-cells, precursor cells and larged lymphoid cells derived from T-cells (Oort and Turk 1965). Morphologically, these cells cannot be distinguished from one another except plasma cells.

Pyroninophilic cells originated from T-cells are said to be derived from small lymphocytes of the pool of immune eligible cells and to be involved in the secondary immune reaction (Ford et al. 1966; Gowans 1966).

In addition to the findings of pleocytosis and neutrophilia mentioned earlier, the fact that almost no pyroninophilic cells were observed among the CSF cells from our cases of Neuro-Behçet's disease suggests repeated inflammatory reaction centering around blood vessels in the central nervous system and indicates the existence of a disturbance of the immune response.

References

- 1) Cavara, V. & D'Ermio, F. (1954) A case of Neuro-Behçet's syndrome. *XVII Concilium Ophthal. Acta, Canada, USA.*, **3**, 1489-1495.
- 2) Delank, H.W. (1972) Klinische Liquordiagnostik. *Nervenarzt*, **43**, 57-68.

- 3) Dyken, P.R. (1975) Cerebrospinal fluid cytology. Practical clinical usefulness. *Neurology (Minneapolis)*, **25**, 210-217.
- 4) Fishman, R.A. (1976) Steroids in neurological disorder. In: *Recent Advances in Neurology*, University of California School of Medicine, San Francisco, Extended Progress in Medical Education, pp. 28-35.
- 5) Ford, C.E., Mickelm, H.S., Evans, E.P., Gray, J.G. & Ogden, D.A. (1966) The inflow of bone marrow cells to the thymus. Studies with past-body irradiated mice injected with chromosome-marked bone marrow and subjected to antigenic stimulation. *Ann. N.Y. Acad. Sci.*, **129**, 283-296.
- 6) Gowans, J.L. (1962) The fate of parental strain small lymphocytes in F₁ hybrid rats. *Ann. N.Y. Acad. Sci.*, **99**, 432-455.
- 7) Gowans, J.L. (1966) Life-span, recirculation, and transformation of lymphocytes. *Int. Rev. exp. Path.*, **5**, 1-22.
- 8) Inaba, G., Aoyama, J. & Shimizu, T. (1977) Treatment of Neuro-Behçet's syndrome (Abstract in Japanese). *Clinical Neurology (Tokyo)*, **17**, 965.
- 9) Itahara, K., Nakamura, S., Takase, S., Iijima, K., Endo, M. & Ohara, Y. (1976) The cerebrospinal fluid cellular differential in multiple sclerosis and other neurological diseases. *Annual Report of the Ministry of Health and Welfare Multiple Sclerosis Research Committee, Japan*, Tokyo, pp. 83-90. (in Japanese with English abstract)
- 10) Ito, U., Hiratsuka, M., Kamisasa, A. & Komatsu, K. (1969) A new collecting device for cells in cerebrospinal fluid: Centrifuging sedimentation chamber method. *Naika (Tokyo)*, **24**, 99-104. (Japanese)
- 11) Kölmel, M.W. (1976) *Atlas of Cerebrospinal Fluid Cells*. Springer-Verlag, Berlin, pp. 54-55.
- 12) Kurnick, N.B. (1955) Histological staining with methylgreen-pyronin. *Stain Technol.*, **27**, 233-242.
- 13) Miyoshi, K., Shirakami, R., Miyamoto, H., Harada, H., Masuda, K. & Yamano, T. (1974) Neutrophils function and chemotactic factor of serum in Behçet's disease. *Annual Report of the Ministry of Health and Welfare Behçet's Disease Research Committee, Japan*, Tokyo, pp. 241-248. (Japanese)
- 14) Nakamura, S. & Takase, S. (1975) Two siblings with Behçet's syndrome. *Shinkeinaika (Tokyo)*, **2**, 169-175. (in Japanese with English abstract)
- 15) Nishiyama, S. (1971) Histopathogenesis of Behçet's syndrome. *Saishinigaku (Osaka)*, **26**, 445-450. (Japanese)
- 16) Oehmichen, M. (1976) *Cerebrospinal Fluid Cytology. An Introduction and Atlas*. George Thieme, Stuttgart, pp. 85-87.
- 17) Olischer, R.M. (1972) Reaktionen mononuclearer Zellen im Liquor cerebrospinalis. *Wien. Z. Nervenheilk.*, **30**, 261-275.
- 18) Oort, J. & Turk, J.L. (1965) A histological and autoradiographic study of lymph nodes during the development of contact sensitivity in the guinea pig. *Brit. J. exp. Path.*, **46**, 147-154.
- 19) Péter, A. (1967) The plasma cells of the cerebrospinal fluid. *J. neurol. Sci.*, **4**, 227-239.
- 20) Schlote, W. & Roos, W. (1974) Gibt es ein charakterisches Liquor-Zellbild bei multipler Sklerose? Beitrag zur Bedeutung der grossen pyroninophilen Rundzellen in Liquor cerebrospinalis. *Nervenarzt*, **45**, 576-587.
- 21) Shimada, K., Yaoita, H. & Shikano, S. (1971) Chemotactic activity for polymorphonuclear leucocytes of the aqueous humor in patients with Behçet's disease. *Jap. Ophthalmol. (Tokyo)*, **75**, 2100-2105. (Japanese)
- 22) Shimizu, T. (1972) Epidemiological and clinicopathological studies on Neuro-Behçet's syndrome. *Advanc. neurol. Sci. (Tokyo)*, **16**, 167-178. (Japanese)
- 23) Shimizu, T. (1974) Behçet's disease. — Epidemiological and clinical statics. *Nihonrinsho (Osaka)*, **32**, 2093-2102. (Japanese)
- 24) Shimizu, T. & Yamamoto, K. (1969) Neuro-Behçet's syndrome. *Nihonrinsho (Osaka)*, **26**, 18-30. (Japanese)
- 25) Takase, S. (1974) Quantitative analysis of immunoglobulins in cerebrospinal fluid and

- relationship between alterations of CSF fractions and neurological diseases. *Physico-chem. Biol. (Tokyo)*, **18**, 79–89. (Japanese)
- 26) Tourtellotte, W.W. (1968) Cerebrospinal fluid and its reactions in diseases. In: *Pathology of the Nervous System*. Vol. 10, edited by J. Minckler, Mc Grow-Hill, New York-Toronto-Sydney-London, pp. 434–456.
- 27) Tourtellotte, W.W. (1970) Cerebrospinal fluid in multiple sclerosis. In: *Handbook of Clinical Neurology*. Vol. 9, edited by P.J. Vinken & G.W. Bruyn, North-Holland Publishing Co., Amsterdam, pp. 324–382.
- 28) Wolf, S.M., Schotland, D.L. & Phillips, L.L. (1965) Involvement of nervous system in Behçet's syndrome. *Arch. Neurol.*, **12**, 315–325.
- 29) Yu, D.T.Y., Clements, P.J., Paulus, H.E., Peter, J.B., Levy, J. & Barnett, E.V. (1974) Human lymphocyte subpopulations. Effect of corticosteroids. *J. clin. Invest.*, **53**, 565–571.
-