Differential cellular increase in cerebrospinal fluid after encephalography in mentally retarded patients¹

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SYNOPSIS The behaviour of the CSF cells during gas encephalography (GEG) with O₂, N₂O, and halothane is poorly known in cerebral developmental disorders. One hundred and fifty CSF samples taken during pneumoencephalography from 75 mentally retarded patients were examined cytologically by the millipore technique with Papanicolaou staining. The results were processed automatically. An approximately 25-fold increase in CSF cells ($P < 0.001$), mainly meningeal, reticulohistiocytic, and monocytic types, was found to occur. The cortical gas filling rate had a positive correlation ($P < 0.001$) with the increase of number of CSF cells. There were no significant differences between the cellular changes in different cerebral disorders. Thus, though the irritant effect of GEG gives a rich cell yield, diagnostic atypical cells in developmental disorders of the central nervous system probably rarely exfoliate into the lumbar CSF.

The normal cell count in the cerebrospinal fluid (CSF) varies from 300 to 6,200 with a mean of 1,450 cells per ml (Sörnäs, 1972). We know that this number of cells increases during gas encephalography (GEG) (Tschugunoff, 1929; Schwab and von Storch, 1937; Bickerstaff, 1950) but no detailed qualitative or statistical study of this increase has ever been made (Marrack *et al.*, 1961; Sayk, 1966). We therefore took CSF samples for cytological examination when GEGs were being done in patients with mental retardation.

METHODS

The GEGs were performed in the Rinnekoti Institution on 35 males and 40 females aged between six and 49 years, in an attempt to identify the causes of their mental retardation. These patients formed part of a larger study (Iivanainen, 1974). The mental retardation was mild in six cases, moderate in 13, severe in 11, and profound in 45 cases. Forty-one patients had epilepsy and 40 had cerebral palsy, while six were psychotic. Abnormal GEGs were found in 61 of the patients. Enlargement of ventricles was the most

TABLE 1
MAIN FINDINGS AT GAS ENCEPHALOGRAPHY

Findings	No. of cases
Enlarged ventricles (symmetrical, asymmetrical, or unilateral)	31
Brain malformation	9
Cerebellar atrophy or dysplasia	9
Temporal or other local deformity	8
Local expansion	3
Cortical atrophy or dysplasia	1
Nothing definitely abnormal	12
Insufficient gas filling	2
Total	75

common finding (Table 1). Most of the mental retardation was of prenatal origin (Table 2). Details of the combined anaesthesia and fractionated GEG technique have been described previously (Iivanainen *et al.*, 1970; Iivanainen and Kostiaainen, 1971).

Two samples of CSF were taken at each of the 75 pneumoencephalographic examinations. The first CSF sample (3 ml) was drawn immediately after lumbar puncture, before injection of contrast gas (a mixture of oxygen, nitrous oxide, and halothane). The second sample (5 ml) was taken between 15 and

¹ National Welfare Association for the Mentally Deficient in Finland (Kehitysvammaliitto r.y.) provided financial support.

TABLE 2

AETIOLOGICAL CLASSIFICATION OF 75 MENTALLY RETARDED PATIENTS ACCORDING TO WHO (1967)

Aetiological category	No. of cases
Infections and intoxications	12
Trauma or physical agents	8
Disorders of metabolism	6
Gross brain disease	3
Unknown prenatal influence	19
Chromosomal aberrations	2
Prematurity	3
Multiple causes	5
Other and unspecified causes	17
Total	75

40 minutes later (average 25 minutes), immediately before withdrawal of the lumbar puncture needle and before the patient was moved from a sitting to a lying position. On average 30 ml CSF were drawn for other laboratory studies. A total of approximately 57 ml gas was introduced per study.

The samples were fixed immediately with an equal volume of 96% ethyl alcohol. From each sample a millipore specimen was made with a millipore filter of 3 μ m pore size using a minimal suction. The cells were stained by the Papanicolaou technique. A Permount mounting medium and a cover slip were put onto the filter. The average cell count per 100 microscope fields was determined from three different places and the area of the filter was measured. From these results the relative number of cells per ml was calculated. A differential count of 100 consecutive cells was also made following the cytological criteria of Sayk (1966).

TERMINOLOGY The terminology for CSF cells is confusing (Schultz and Knibbe, 1952a, b; Hutton, 1958; Bischoff, 1960; Marks and Marrack, 1960; Wieczorek, 1964; Sayk, 1966; Pariante and Scala, 1967; Kolář and Zeman, 1968), which makes comparison of results difficult. This confusion particularly concerns phagocytic and immature cells. Terms such as macrophage, histiocyte, monocytoïd cell, G cell, phagocytierende mononucleäre Zelle, meningeal histiocytoïd cell (Bischoff, 1960; Marks and Marrack, 1960; Sayk, 1966; Pariante and Scala, 1967; Kolář and Zeman, 1968; Sörnäs, 1971; Oehmichen and Schütze, 1973) have been used to describe the former. Synonyms such as reticulohistiocytoïd, reticulomonocytoïd, lymphoid, plasmacytoïd, *lymphoide Zelle*, lymphocytoïd cell (Bischoff, 1960; Naylor, 1964; Sayk, 1966; Kolář and Zeman,

1968; Drewinko *et al.*, 1973; Oehmichen and Schütze, 1973) have been used for the immature types.

To us, monocytoïc cells include both monocytes and macrophages or mature mononuclear phagocytic cells. If any phagocytosed material was found in the cytoplasm, further differentiation into erythrophages and lipophages was made. The immature mononuclear and round cells in the connective tissue and perivascular areas of the leptomeninges were termed reticulohistiocytoïc cells. We tried to type each of these into the lymphoid, plasmacytoïd, or monocytoïd series according to Sayk (1966). Endothelial cells of the leptomeninges and fibroblasts were regarded as meningeal cells.

STATISTICAL ANALYSIS The data were processed automatically at the Computing Centre, University of Helsinki. The influence of the following variables on the cytological appearance of the CSF was investigated: sex, age, level of intelligence, epilepsy, malformations, cerebral palsy, cause of mental retardation, anaesthesia, GEG findings such as ventricular dilatation, its grade and character, size of the third ventricle, displacement of midline structures, state of the septum pellucidum, other supratentorial and infratentorial central and cortical findings, degree of gas filling in the ventricles and over the cortex, interval between samples I and II, volume of contrast medium before the second sample, and the CSF protein concentration. Student's *t* test was used as the test of significance. A correlation matrix was used only in cases of continuous variables.

TABLE 3

CEREBROSPINAL FLUID CELL COUNT (CELLS PER ML) DURING GAS ENCEPHALOGRAPHY

Cell type	CSF fluid cell count (cells/ml)				Significance* of pleocytosis
	First sample		Second sample		
	Mean	Deviation	Mean	Deviation	
Lymphocytes	399	264	6,675	5,312	P < 0.001
Neutrophils	7	12	57	150	P < 0.05
Meningeal cells	27	43	7,787	5,142	P < 0.001
Monocytic cells	271	275	5,138	4,628	P < 0.001
lipophages	3	8	96	222	P < 0.01
Reticulohistiocytic cells	130	140	1,985	2,434	P < 0.001
lymphoid	68	88	1,005	1,238	P < 0.001
plasmacytoid	15	27	121	257	P < 0.01
monocytoid	47	52	860	1,518	P < 0.001
Total cell count	885	650	22,189	14,238	P < 0.001

* Tested by Student's *t* test.

FIG. 1. *Effect of gas encephalography on cytology of CSF. Surface areas indicate quantity of cells. Different sectors correspond to percentage fractions of cells in differential counts. I: cellularity before encephalography, II: after irritation during encephalography. Note striking rise of meningeal cells. (Preliminary report: Iivanainen and Taskinen (1973).)*

RESULTS

The 25-fold increase in the total cell number (Fig. 1, Table 3) during GEG was statistically significant ($P < 0.001$). The increase could first be detected within 15 minutes, which was the shortest period between the first and second samples. The increase was greatest in meningeal cells ($P < 0.001$) (Fig. 1). The increase in lymphocytes and monocytic and reticulohistiocytic cells was also statistically highly significant ($P < 0.001$) (Table 3), although in fact their percentage proportions decreased (Fig. 1). In a case of astrocytoma, the monocytic cells contained some undefined phagocytosed pigment. A large cell which we interpreted as a ganglion cell was encountered in the second sample of another case (Fig. 2). Erythrophages were found only in one first and in one second sample. Large sheets of meningeal cells with some fibroblasts were almost always seen in the second sample (Fig. 3). Some single ependymal cells occurred in five of the first samples, though in none of the second. No glial cells were detected. Reticulohistiocytic cells (see examples in Fig. 4) were found in all samples except two second ones. Of the different variables the degree of cortical gas filling correlated most closely and most significantly with the increase in total cell number. A marked degree of gas

filling over the cortex was found in six cases in which the total cell number increased significantly more than with mild cortical gas filling (mean

FIG. 2. *A large cell interpreted as a ganglion cell. Millipore technique, Papanicolaou staining, $\times 3,300$.*

FIG. 3. An exfoliated sheet of meningeal cells. Millipore technique, Papanicolaou staining, $\times 650$.

increase in total cell count in some cases, though this number was too small for reliable statistical analysis.

The correlation matrix showed a positive statistically significant correlation between the total cell count and the following types: lymphocytes, meningeal cells, monocytic cells, and the total number of reticulohistiocytic cells ($P < 0.001$) as far as lymphoid ($P < 0.001$) and monocytoid ($P < 0.01$) subtypes of reticulohistiocytic cells. Of the changes in the individual cell types, statistically significant positive correlation occurred between each of the cell types of probable tissue origin—that is, reticulohistiocytic and monocytic cells ($P < 0.001$). No other statistically significant correlations were found.

DISCUSSION

There are several different methods for processing the CSF specimen for cytology (McCormick and Coleman, 1962; Bots *et al.*, 1964; Eneström, 1966; Sayk, 1966; Tourtellotte, 1966; Pariante and Scala, 1967; Sörnäs, 1967; Kolář and Zeman, 1968; Sörnäs *et al.*, 1972; Drewinko *et al.*, 1973). Modifications of Sayk's (1966) sedimentation chamber and May-Grünwald Giemsa staining probably are the most common. The cytocentrifuge is also good (Drewinko *et al.*, 1973; Iivanainen and Taskinen, 1973). Gas encephalography was performed at some distance from the cytological laboratory and the

71.3 versus 23.4; $P < 0.01$) and in the cases with moderate cortical gas filling (mean 71.3 versus 37.8; $P < 0.05$). The width of the posterior cranial fossa also correlated positively with the

FIG. 4. Reticulohistiocytic cells: (a) a lymphocytoid type, (b) a monocytoid type, and (c) a plasmocytoid type. Millipore technique, Papanicolaou staining, $\times 3,300$.

CSF samples could not be treated within one hour, so we selected the technique of alcohol fixation and millipore filtration. In Papanicolaou staining the cellular details cannot be separated so well as in May-Grünwald-Giemsa staining. On the other hand, Papanicolaou staining is the only one which can be used with the millipore filter. The other methods stain the filter too darkly thus disturbing the recognition of the cells.

Some artefacts, particularly shrinkage of the cells and destruction of the cytoplasm, are known to be associated with the millipore technique. However, these changes do not affect the cell count of the specimen. In the network of the millipore filter the cells retain the three-dimensional form so the cellular details are impossible to focus on one film plane when photographing.

In the treatment of CSF cells it is impossible to develop an ideal method which could be applied simultaneously for different purposes. On the basis of our experience, we believe that different methods (at least the sedimentation chamber or cytocentrifuge and the millipore filtration) are needed simultaneously for the most effective cytological examination of the CSF (Iivanainen and Taskinen, 1973). The sedimentation chamber with different modifications and cytocentrifuge combined with May-Grünwald-Giemsa staining is suitable for qualitative analysis of the CSF cells. In the millipore technique the cells are settled vertically down on the filter surface. There is no loss of the cellular material to the horizontal direction, which occurs in the sedimentation chambers. The millipore technique with Papanicolaou staining is therefore used to advantage for quantitative purposes and for detection of neoplastic cells.

As lymphocytes, plasma cells, and monocytic cells are developed in the leptomeninx, particularly in the connective tissue of the arachnoid and pia maters and in the adventitia of the vessels, the leptomeninx is therefore functioning as a part of the reticuloendothelial system (Schultz and Knibbe, 1952a).

A significant increase in CSF cells (approximately 25-fold) during GEG was demonstrated in the present study. The great increase in the meningeal cells (Fig. 1) may be explained partly by the change in the subarachnoid pressure due to the contrast gas. The change in pressure is

naturally first focused on the meningeal cells covering the subarachnoid space and thus causes them to exfoliate. It is easy to see that extensive cortical gas filling could stretch and rupture the tissues in the subarachnoid space, which would account for the large sheets of meningeal cells we often found (Fig. 3). We think wide variations in the numbers of cells shed is due chiefly to (1) technical effects of the GEG procedure itself such as the varying degree of cortical gas filling, and (2) the varying numbers of cells originally in the leptomeningeal tissues, which must be related to any underlying disease, such as infection.

The large proportion of reticulohistiocytic cells in our samples is partly due to our classification, where all the cell types—for example, medium-sized lymphocytes—which could not with certainty be regarded as mature cells, were classified as reticulohistiocytic cells. We tried further to differentiate the cells as accurately as possible into subgroups, to see whether particular cell types were associated with particular variables but no clear correlations were found.

The typing of a large cell in the CSF of one patient was difficult (Fig. 2). In the differential diagnosis the following possibilities were considered: atypical meningeal cell, atypical ependymal cell, atypical choroid plexus cell, atypical glial cell, neoplastic cell, and ganglion cell. We interpret it as a ganglion cell, which are known to be encountered on the leptomeninges (Schultz and Knibbe, 1952a).

The absence of glial cells from both CSF samples suggests that the pia mater and ependyma either protect the brain against the irritation produced by the GEG or prevent glial cells from entering the CSF.

The fact that the increase of some cell types such as plasma cells was not significant can be explained in part by their small proportions in the differential count. The scarcity of ependymal cells suggests that cell exfoliation from the ventricular surface is very slight. The absence of correlation between the degree of intraventricular gas filling and CSF cell increase suggests that even good intraventricular gas filling does not cause marked mechanical changes in the cerebral ventricular space. Thus exfoliation does not occur with intraventricular gas filling to the same extent as with subarachnoid gas filling. The observation

that neither ependymal cells nor plexus cells are increased significantly during GEG supports this.

The small increase in the number of red cells as a result of GEG suggests that the mechanical effect of the contrast gas on the walls of the capillaries was slight. The significant positive correlation of the different tissue cells with each other (reticulohistiocytic cells, monocytoïds) indicates that the irritant effect of gas filling affects all leptomeningeal tissue equally. The correlation between the tissue and blood cells could not be determined more accurately because the origin of some cells remains somewhat uncertain. However, some changes in capillary permeability are known to occur—for instance, in the changes in the electrophoretic fractions of the CSF proteins during GEG (decrease of albumin, increase of α_2 - and β -globulins) (Iivanainen and Kostiaïnen, 1971).

Significant increase of cells in the CSF during GEG occurred rapidly within 15 minutes. This phenomenon could be used to get good yield of CSF cells for the diagnosis of suspected tumours of the CNS (Iivanainen and Taskinen, 1973). We know that CSF cell increase due to GEG lasts about one week (Bickerstaff, 1951). The volume of CSF removed does not essentially affect the differential cell count of the second sample. The percentage of meningeal cells was practically the same in all the second specimens.

When we looked at the underlying neurological disorder we found that the greatest increase in CSF cells during GEG was in those who had had infections or in conditions whose causes were unknown. A much smaller, though still significant, cellular increase was found in conditions classified as due to unknown pre-natal influences by WHO (1967). Roskamp's (1961) increase in CSF cells during GEG was rather small (about 13%) and depended on the underlying infectious disease. Our results are not really comparable with his when we remember that in our patients the disease was not acute. He used smaller volumes of contrast gas than we did, and he did not mention the degree of gas filling over the cortex. Perhaps any small differences in CSF cellularity in different diseases during GEG are hidden when the increase in cells is as marked as in our study. On the other hand, neurological conditions with unknown

aetiology may actually be due to brain damage from infections or from active degenerative diseases or from trauma so we want to emphasize particularly the effect of destructive non-infectious processes on the cellularity of the leptomeninges and thus on the increase of CSF cells during GEG (Schultz and Knibbe, 1952b). Roskamp (1961) did not deal with this aspect. The possible correlation of increase in CSF cells with the width of the posterior fossa, a sign of cerebellar atrophy, supports this view. On the other hand, in cases with a wide posterior fossa gas filling over the cerebral cortex was generally good. The cerebellum is known to be vulnerable to exogenous substances (Dreyer, 1966; Dobbing *et al.*, 1973). The differential cell count, however, did not reveal anything diagnostic of known disorders. So we conclude that the changes in the CSF cells were distributed without statistical significance between the different types of mental retardation. It is doubtful, however, whether more sensitive methods would have been able to reveal anything significant.

Generally, CSF cytology is most applicable to proliferative conditions of the central nervous system which cause leptomeningeal cell exfoliation and it is less rewarding in chronic degenerative disorders. In malformations and generally in dysplastic conditions exfoliation of cells seems to be minimal. The irritant effect of GEG gave a rich yield of cells but the possible cellular atypia which would indicate a developmental disorder of the central nervous system was either absent or so poor that it was not seen in the millipore technique even in the rich cellular material produced by the GEG irritation (Marrack *et al.*, 1961; Iivanainen and Taskinen, 1973).

REFERENCES

- Bickerstaff, E. R. (1950). Changes in the cerebrospinal fluid during pneumo-encephalography. *Lancet*, **2**, 683–685.
- Bickerstaff, E. R. (1951). Changes in the cerebrospinal fluid after pneumo-encephalography. *Lancet*, **1**, 1209–1210.
- Bischoff, A. (1960). Der derzeitige Stand der Liquor-Cyto-diagnostik. *Schweizerische Medizinische Wochenschrift*, **90**, 479–487.
- Bots, G. T. A. M., Went, L. N., and Schaberg, A. (1964). Results of a sedimentation technique for cytology of cerebrospinal fluid. *Acta Cytologica (Balt.)*, **8**, 234–241.
- Dobbing, J., Levitsky, D., Richardson, S., and Tizard, J. (1973). Undernutrition and the developing brain. *Symposium in the Third International Congress of the International Association for the Scientific Study of Mental Deficiency*, The Hague, 1973. Newsletter IASSMD, **1**, No. 1, 3.

- Drewinko, B., Sullivan, M. P., and Martin, T. (1973). Use of the cytocentrifuge in the diagnosis of meningeal leukemia. *Cancer*, **31**, 1331–1336.
- Dreyer, R. (1966). Kleinhirndauerschädigung infolge Diphenylhydantoinintoxikation. *Fortschritte der Neurologie, Psychiatrie und ihrer Grenzgebiete*, **34**, 224–235.
- Eneström, S. (1966). Die Saugkammermethode. In *Symposium über die Zerebrospinalflüssigkeit*, Rostock, 1964. Edited by J. Sayk. Fischer: Jena.
- Hutton, W. E. (1958). A survey of the application of the 'molecular' membrane filter to the study of cerebrospinal fluid cytology. *American Journal of Clinical Pathology*, **30**, 407–410.
- Ivanainen, M. (1974). A study on the origins of mental retardation. *Clinics in Developmental Medicine*, No. 51. Heinemann Medical: London.
- Ivanainen, M., Collan, R., and Donner, M. (1970). Adverse effects of pneumoencephalography performed under general anaesthesia in children. *Annals of Clinical Research*, **2**, 71–78.
- Ivanainen, M., and Kostianen, E. (1971). Changes in the electrophoretic pattern of the lumbar cerebrospinal fluid during fractional gas encephalography. *Acta Neurologica Scandinavica*, **47**, 91–105.
- Ivanainen, M., and Taskinen, E. (1973). Cytological examination of the cerebrospinal fluid. *Annals of Clinical Research*, **5**, 80–86.
- Kolář, O., and Zeman, W. (1968). Spinal fluid cytomorphology. *Archives of Neurology (Chic.)*, **18**, 44–51.
- McCormick, W. F., and Coleman, S. A. (1962). A membrane filter technic for cytology of spinal fluid. *American Journal of Clinical Pathology*, **38**, 191–197.
- Marks, V., and Marrack, D. (1960). A technique for the staining of cells from the C.S.F. *Confinia Neurologica*, **20**, 310–315.
- Marrack, D., Marks, V., and Couch, R. S. C. (1961). Changes in the lumbar cerebro-spinal fluid during air-encephalography. *British Journal of Radiology*, **34**, 635–639.
- Naylor, B. (1964). The cytologic diagnosis of cerebrospinal fluid. *Acta Cytologica (Balt.)*, **8**, 141–149.
- Oehmichen, M., and Schütze, G. (1973). Erythrophagen in der Liquorzell Diagnostik der Subarachnoidalblutung. *Nervenarzt*, **44**, 407–416.
- Pariente, F., and Scala, A. (1967). Tecniche moderne per lo studio citologico e citochimico del liquido cefalorachidiano. *Ospedale Psichiatrico*, **35**, 287–304.
- Roskamp, H. (1961). Zellzahlveränderungen bei fraktionierter Liquorentnahme während der Pneumoencephalographie. *Archiv für Psychiatrie und Nervenkrankheiten*, **201**, 426–437.
- Sayk, J. (1966). Cytologie der cerebrospinalflüssigkeit. *Wiener Zeitschrift für Nervenheilkunde und deren Grenzgebiete*, Suppl. 1, 86–102.
- Schultz, A., and Knibbe, H. J. (1952a). Neue Erkenntnisse über die normale und pathologische Histologie der weichen Hirnhäute durch die Untersuchung in 'Häutchenpräparaten'. Teil 1. Normale Anatomie und funktionelle Reaktionen. *Frankfurter Zeitschrift für Pathologie*, **63**, 455–471.
- Schultz, A., and Knibbe, H. J. (1952b). Neue Erkenntnisse über die normale und pathologische Histologie der weichen Hirnhäute durch die Untersuchung in 'Häutchenpräparaten'. Teil 2. Pathologische Anatomie. *Frankfurter Zeitschrift für Pathologie*, **63**, 472–492.
- Schwab, R. S., and von Storch, T. J. C. (1937). Alterations of the cerebrospinal fluid subsequent to pneumoencephalography. *New England Journal of Medicine*, **217**, 21–24.
- Sörnäs, R. (1967). A new method for the cytological examination of the cerebrospinal fluid. *Journal of Neurology, Neurosurgery, and Psychiatry*, **30**, 568–577.
- Sörnäs, R. (1971). Transformation of mononuclear cells in cerebrospinal fluid. *Acta Cytologica (Balt.)*, **15**, 545–552.
- Sörnäs, R. (1972). The cytology of the normal cerebrospinal fluid. *Acta Neurologica Scandinavica*, **48**, 313–320.
- Sörnäs, R., Östlund, H., and Müller, R. (1972). Cerebrospinal fluid cytology after stroke. *Archives of Neurology (Chic.)*, **26**, 489–501.
- Tourtellotte, W. W. (1966). A selected review of reactions of the cerebrospinal fluid to disease. In *Neurological Diagnostic Techniques*, pp. 25–50. Edited by W. S. Fields. Thomas: Springfield, Ill.
- Tschugunoff, S. A. (1929). Zur Frage über die Veränderungen der cerebrospinalen Flüssigkeit nach der Encephalographie. *Zeitschrift für die Gesamte Neurologie und Psychiatrie*, **122**, 452–456.
- Wieczorek, V. (1964). Liquorveränderungen bei Blutungen in den Subarachnoidalraum mit besonderer Berücksichtigung des Liquorzellbildes. *Deutsche Zeitschrift für Nervenheilkunde*, **186**, 87–100.
- World Health Organization (1967 and 1969). *Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death*. 8th revision, 2 volumes, W.H.O.: Geneva.