Spontaneous Mitoses in Direct Preparations from Peripheral Blood of Schizophrenic Patients

Cells in mitosis in the peripheral blood can be detected in several hematological disorders such as leukemia[1], hemoblastoses[2], erythroleukemia[3], Cooley's anemia[4], and are clearly correlated with the presence in these diseases of circulating immature – malignant or not – myeloid or erythroid elements.

The circulating atypical lymphocytes present in the active phase of infectious mononucleosis are also spontaneously capable of undergoing mitosis: dividing forms have occasionally been seen in routine blood smears[5–7], and PEARMAIN and LYCETTE[8] have demonstrated the presence of mitoses in direct preparations obtained by a 4–9 h incubation of peripheral blood. On the basis of these observations, PEARMAIN and LYCETTE[8] have suggested that infectious mononucleosis lymphocytes are similar to PHA cells.

Several authors[9,10] have observed in the peripheral blood of schizophrenic patients atypical lymphocytes, like those present in infectious mononucleosis, and recently a striking resemblance between these elements and PHA cells has been noted[11], but, as far as we know, hitherto there are no reports of increased frequency of mitoses in the circulating blood of these patients. We supposed that atypical lymphocytes of schizophrenia, as true PHA cells, can spontaneously undergo mitosis; the following experimental evidence appears to support this supposition.

Materials and method. A series of direct chromosome preparations from the peripheral blood of 13 treated chronic schizophrenic patients, from long-term inmates in the Ospedale Psichiatrico of Ferrara, and 6 untreated schizophrenic patients in the acute phase of the disease, at their first hospitalization in the neurological department of the Arcispedale S. Anna of Ferrara were examined. The criteria for acceptance of the patients into the study were: (1) undoubted diagnosis of schizophrenia; (2) significant abnormalities of peripheral blood lymphocytes; (3) no clinical, radiological and serological signs of additional diseases. The patients were aged between 19 and 54 and peripheral blood leucocyte counts between 4400 and 9400/mmc. Data were compared, in a blind study, with those obtained from 5 mentally retarded subjects from the Ospedale Psichiatrico of Ferrara and 8 healthy donors.

This method was followed: soon after drawing, 20 ml of heparinized venous blood were mixed with 0.1 ml of a $1^0/_{00}$ colchicine solution (Colcemid – CIBA), and incubated at 37 °C for 2 h. During this time some sedimentation of erythrocytes occurred, but thereafter, to obtain a more complete separation of the erythrocytes, the blood was centrifuged at 500 rpm for 10 min. All the supernatant plasma with the uppermost layer of W.B.C. was collected and mixed with 3 volumes of 1% Na citrate. The cells were then resuspended in 45% acetic acid, and from this squash preparations stained with Giemsa were made. All the final suspension of fixed cells was used for preparing air-dried slides and all slides were scanned under low power. All metaphases with clearly distinguishable chromosomes were scored.

Results and conclusions. The clinical and hematological details of the subjects studied and the number of observed metaphases in singular cases are summarized in the Table. Here it may be seen that in direct preparations from peripheral blood, a significant increase of metaphases was detected in 17 of 19 schizophrenic patients in comparison with control subjects. The absence of immature myeloid and/or erythroblastic cells in the peripheral blood

Clinical and hematological details and number of observed metaphases in schizophrenic patients and normal controls

Patient number, sex and age	Leukocytes/mmc	Lymphocytes/mmc (% of atypical lymphocytes)	No. of mitoses	Diagnosis
1, ♀, 24	4500	1015 (53%)	13	Acute untreated schizophrenia
2, ♂, 33	7100	1562 (36%)	14	
3, ♂, 27	4800	1296 (19%)	12	
4, ♀, 22	5800	1856 (31%)	14	
5, ♂, 35	6000	1860 (32%)	15	
6, ♀, 19	6700	2342 (28%)	14	
7, ♀, 45	7400	2516 (23%)	11	Chronic treated schizophrenia
8, ♂, 23	8200	2296 (18%)	7	
9, ♀, 28	5500	1815 (17%)	7	
10, ♂, 41	6300	2142 (9%)	2	
11, ♂, 33	5900	2065 (34%)	9	
12, ♂, 27	9400	3102 (15%)	11	
13, ♂, 37	7000	2030 (23%)	12	
14, ♀, 35	6700	1541 (19%)	1	
15, ♀, 28	5200	1508 (28%)	7	
16, ♂, 54	5600	1624 (29%)	7	
17, ♂, 43	4400	1452 (18%)	8	
18, ♂, 54	6900	2415 (49%)	21	
19, ♂, 40	7300	1971 (21%)	7	

Control subjects

Patient number, sex and age	Leukocytes/mmc	Lymphocytes/mmc (% of atypical lymphocytes)	No. of mitoses	Diagnosis
1, ♀, 19	7400	2550 (6%)	3	Treated mental retardation
2, ♂, 51	5100	1173 (4%)	0	
3, ♂, 30	4400	1118 (5%)	0	
4, ♂, 22	5800	1566 (7%)	1	
5, ♂, 29	6200	1922 (1%)	0	
6, ♀, 32	4900	1078 (1%)	1	Healthy donor
7, ♀, 54	7100	1704 (0%)	0	
8, ♀, 34	6300	1764 (1%)	0	
9, ♂, 27	6500	1495 (0%)	0	
10, ♂, 48	7100	2059 (3%)	1	
11, ♂, 28	5500	1055 (0%)	0	
12, ♂, 21	5900	1586 (0%)	0	
13, ♂, 26	6800	2108 (2%)	0	

[1] M. A. KINLOUGH and H. N. ROBSON, Br. med. J. *2*, 1052 (1961).
[2] A. BASERGA, L. BOSI, G. L. CASTOLDI, E. PUNTURIERI and N. RICCI, Haematologica *47* (Suppl.), 83 (1962).
[3] N. RICCI, Prog. Med. *21*, 309 (1965).
[4] L. BOSI, G. L. CASTOLDI, N. RICCI, E. PUNTURIERI and A. M. TUNIOLI, Policlinico, Sez. Prat. *69*, 1209 (1962).
[5] E. E. SCHWARTZ, Ergebn. inn. Med. Kinderheilk. *43*, 1 (1932).
[6] H. BOWCOCK, Am. J. med. Sci. *198*, 384 (1939).
[7] R. L. CARTER, Blood *26*, 579 (1965).
[8] G. E. PEARMAIN and R. R. LYCETTE, Lancet *2*, 1072 (1963).
[9] H. V. KAMP, J. Neuropsych. *4*, 1 (1962).
[10] W. J. FESSEL and M. HIRATA-HIBI, Arch. Ges. Psychol. *9*, 601 (1963).
[11] F. BELLONI and E. STROSSELLI, Acta Neurol. *135*, 1 (1967).

of patients studied indicates that dividing elements belong to lymphoid cell line and are, as in infectious mononucleosis, the atypical circulating lymphocytes. The number of detected metaphases seems not to be related to age and sex of the subjects studied, and a precise linkage with the treatment could not be demonstrated.

The significance of the presence in schizophrenia of circulating cells spontaneously capable of undergoing mitosis is still questioned, but our findings allow us to suppose that the abnormal peripheral lymphocytes present in this disease are blast-transformed cells.

The presence of 'atypical lymphocytes', like those detected in schizophrenia and in infectious mononucleosis, in several other disorders[12,13], suggests that mitoses in short-term peripheral blood cultures can be found in other pathological conditions in which circulating 'reactive lymphocytes' are present[14].

Riassunto. Vengono riportati i risultati di una ricerca che dimostra come preparazioni cromosomiche dirette praticate su sangue periferico di schizofrenici contengano mitosi in quantità sensibilmente superiori a quelle evidenziabili con lo stesso metodo in soggetti di controllo.

P. MALACARNE and B. DALLAPICCOLA

Clinica Medica dell'Università, 44100 Ferrara (Italy), 26 September 1968.

[12] R. KLIMA, Schweiz. med. Wschr. *91*, 1165 (1961).
[13] T. A. WOOD and E. P. FRENKEL, Am. J. Med. *42*, 923 (1967).
[14] This investigation was supported by C.N.R. (Consiglio Nazionale delle Ricerche; Contract No. 115-1135-04690).

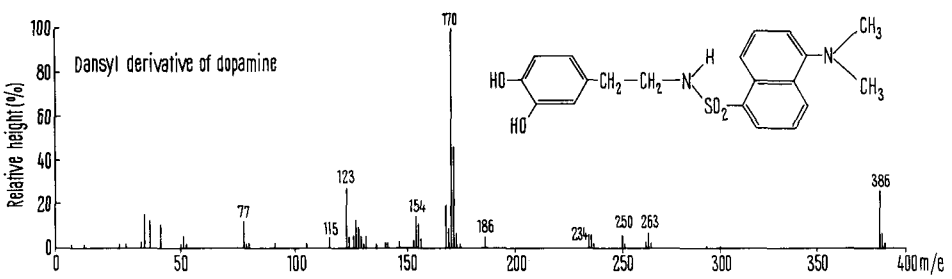
On the Biogenic Amines in the Carotid Body: Identification of Dopamine by Mass Spectrometry

In connection with investigations on the fine structure of the carotid body, it was of interest to identify the biogenic amines, which should be localized in electron-opaque cored vesicles of the glomus cells. These osmiophilic granules show under various experimental conditions[1-4] differences in numbers, size, and density. Unfortunately, controversial results exist both about the kinds and the amounts of the biogenic amines[5-7]. This might be due to the methods applied, such as histochemical techniques, fluorescence microscopy, and pharmacological tests. It is difficult to exclude errors under these experimental conditions. Therefore, an attempt was made in initial experiments to identify biogenic amines in the carotid body by a combination of chromatographic and spectrometric methods[8,9].

Materials and methods. The common carotid bifurcations from 30 healthy horses were removed within 3 min after death. The horses had been killed by a shot in the head. The bifurcations were frozen in dry ice/ethanol. The fine preparation of the carotid bodies from the connective tissues was done under an operation microscope. During the chemical investigation, a microscopical survey of small pieces of the exstirpated tissues was made to test whether only carotid body cells had been removed.

For the chromatographic separation, the biogenic amines of the carotid bodies were treated with 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl chloride) in the presence of an excess of sodium hydrogen carbonate[10]. Thus, each carotid body was immersed in a solution of 25 mg of dansyl chloride in acetone/water = 2/1 (v/v), and homogenized in the solution. After 2 days, the reaction medium was filtered, the acetone removed in vacuo, and the dansyl derivatives extracted with ethyl acetate. After separation by thin-layer chromatography on silica gel G MERCK (solvent systems: ethyl acetate/cyclohexane = 3/2; benzene/triethylamine = 5/1), the dansyl derivative of dopamine was identified as one of the main components (Figure). The fragmentation mechanism was reported recently[9]. The question whether the biogenic amines are localized in some or all electron-opaque cored vesicles, could not be solved by these experiments. It was for instance, shown recently by CHIOCCHIO et al.[6] by electron microscopy, using a special

[1] J. D. LEVER, P. R. LEWIS and J. D. BOYD, J. Anat. *93*, 478 (1959).
[2] D. DUNCAN and R. YATES, Anat. Rec. *157*, 667 (1967).
[3] S. BLÜMCKE, J. RODE and H. R. NIEDORF, Z. Zellforsch. *80*, 52 (1967).
[4] H. KNOCHE and S. DECKER, Z. mikrosk.-anat. Forsch., in press.
[5] A. E. BECKER, J. DRUKKER and A. E. F. H. MEIJER, Histochem. *11*, 195 (1967).
[6] S. R. CHIOCCHIO, A. M. BISCARDI and J. H. TRAMEZZANI, Science *158*, 790 (1967); and cited references of earlier papers.
[7] D. P. DEARNALEY, M. FILLENZ and R. J. WOODS, Proc. R. Soc., Ser. B *170*, 195 (1968).
[8] H. MÖLLMANN, J. REISCH, H. ALFES and N. JANTOS, Z. analyt. Chem. *243*, 29 (1968).
[9] J. REISCH, H. ALFES, N. JANTOS and H. MÖLLMANN, Acta pharm. suecica *5*, 393 (1968).
[10] N. SEILER and M. WIECHMANN, J. Chromat. *28*, 351 (1967).



Mass spectrum of the dansyl derivative of dopamine, isolated from the carotid body of the horse (Hitachi-Perkin Elmer RMU-6D mass spectrometer, direct inlet, 70 eV, 365 °C).