Horizontal Cells in the Zona Incerta of the Developing Human Telencephalon

Its is usually presumed that most, if not all, cells in the developing human telencephalic wall during fetal life originate in the germinal layer, by mitosis, and proceed to travel towards the external surface of the growing brain. Thus, with one process attached, or pointing to, the germinal layer and the other toward the cortical anlage, the long axis of these cells is vertical to the telencephalic wall. The moving cells thus cross successively the germinal layer, the mantle layer, the pars interna and pars externa of the zona incerta and reach the cortical anlage (Figure 1).

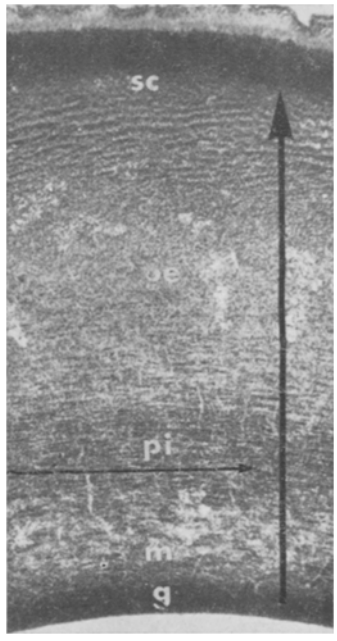

Fig. 1. This is a photograph of the telencephalic wall in a human fetus, age 14 weeks. The thick arrow stretching from the germinal layer (g) to the cortical enlage (sc) is the presumed direction of cells originating in the germinal layer. The thin arrow in the pars interna (pi) indicates the pattern of the long axis of the horizontal cells. The mantle layer is indicated by the letter (m) and the pars externa of the zona incerta is indicated by the letters (pe). Hematoxylin-eosin. ×10.

In a study of Golgi preparation of cerebral tissue from human fetuses ranging in crown rump length from 75 to 130 mm (12 to 16 weeks estimated ovulation age), cells were observed in the zona incerta whose long axis is horizontal to the surface of the brain. These cells are presumed to be neurons because of their size, the length and extent of their ramifications. During the 2nd, 3rd and 4th months there is a gradual creation of a vascular system within the neural parenchyma of the telencephalic wall[1]. Budding capillaries originate in the meninges, cross the cortical anlage, reach the zona incerta where they form branches parallel to the cortical surface, referred to as parallel or horizontal vessels. From these horizontal vessels vertical branches originate, cross the mantle and reach the subependymal area. It is presumed at this time that the horizontal cells in the zona incerta described here move in the direction of the blood vessels, as is usually the case in most developing tissues. The purpose and eventual destiny of these cells are unknown.

Résumé. Durant le 4me mois de la vie foetale humaine, il existe dans la zone incerta du télencéphale, des cellules dont l'axe est parallèle à la surface du cerveau, c'est-à-dire perpendiculaire à la majorité des autres cellules nerveuses. Cette position serait en rapport avec la position, parallèle aussi, des vaisseaux dans cette région du cerveau. Le rôle et le destin de ces cellules sont inconnus.

S. DUCKETT

Department of Neurology and Pathology, Jefferson Medical College of the Thomas Jefferson University Philadelphia (Pennsylvania 19107, USA), 14 February 1973.

[1] S. DUCKETT, Acta Anat. *80*, 107 (1971).

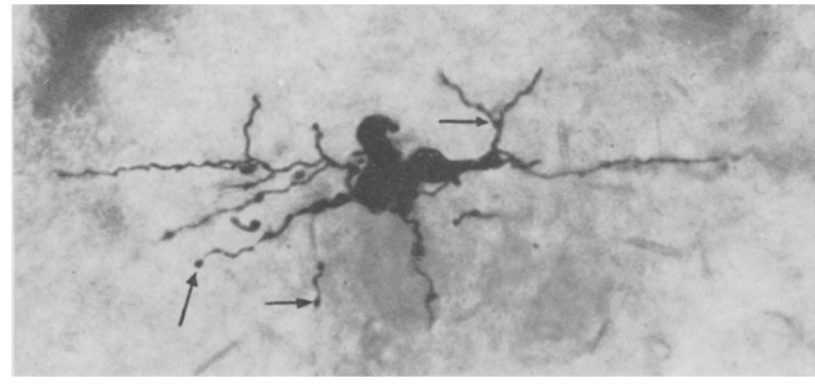

Fig. 2: A horizontal cell as seen in a Golgi preparation. The arrows point to some boutonx-en-passage. ×300.

The Effects of Antineoplastic Agents on the Respiratory Activity of Murine Lymphocytic Leukemia L1210[1]

The effects of several established antineoplastic agents on the oxygen consumption of murine lymphocytic leukemia L1210 in vitro have been studied and compared to the antitumor activity of each compound in an attempt to determine if oxygraph analysis can be utilized to effectively screen chemical compounds for antitumor potential.

Materials and methods. Murine lymphocytic leukemia

strain L1210 was obtained through the Battelle Memorial Institute, Columbus, Ohio, and standard leukemic cell suspensions (7.5×10^6 cells/ml) were prepared in 4 ml aliquots for subsequent oxygraph analysis as reported by GOODMAN et al.[2]. Antineoplastic agents used in this study were the alkylating agents mechlorethamine (Merck, Sharp and Dohme) and chlorambucil (Burroughs Wellcome), the antimetabolite methotrexate (Lilly), the antibiotic actinomycin D (National Cancer Institute) and the corticosteroid prednisone (McKesson). Each drug required solubilization prior to administration to cell suspensions. Chlorambucil, 6-mercaptopurine, vinblastine, actinomycin D and prednisone were dissolved in dimethyl sulfoxide while mechlorethamine and methotrexate were solubilized in phosphate-buffered sucrose-lactate media since they proved to be insoluble in DMSO.

Effects of these agents on the respiratory activity of L1210 cells were evaluated at dose levels based on the LD_{10} values for each drug since this is considered to be the highest therapeutically effective dose that can be administered to an animal with one injection without causing extensive toxicity to the host. Dosage levels administered to each sample were determined by converting LD_{10} values for each drug to the in vitro system utilizing a ratio between the fluid volumes of the experimental animal and the in vitro system as a conversion factor. Since prednisone proved to be non-toxic to BDF_1 mice, a dosage level was selected for this drug which exceeded those calculated for the other agents. The trypan blue exclusion test[3] was employed to estimate cell survival following 1 h of exposure to the antitumor agents.

Results. Results have been presented in the Table and summarize the effects of selected antineoplastic agents on the oxygen uptake of leukemic cell suspensions. No significant depression in the respiratory activity of L1210 cells occurs following 1 h of exposure to a converted LD_{10} dosage of mechlorethamine, chlorambucil, methotrexate, 6-mercaptopurine, actinomycin D, vinblastine or prednisone. A marked inhibition in the oxygen consumption rates of leukemic cells is produced after exposure to 10 times the converted LD_{10} dosage of mechlorethamine (52%), chlorambucil (32%) and prednisone (27%) while methotrexate, 6-mercaptopurine, actinomycin D and vinblastine fail to appreciably alter cell respiration at this dose level.

Viability of leukemic cell suspensions following exposure to each antitumor agent utilized in this study has been determined using the trypan blue exclusion test (Table). Exposure of L1210 cells to the converted LD_{10} dose level of mechlorethamine, chlorambucil, methotrexate, 6-mercaptopurine, actinomycin D, vinblastine and prednisone produces minimal loss of cell viability after 1 h of incubation in vitro. Administration of 10 times the converted LD_{10} dosages of mechlorethamine and chlorambucil demonstrates that these compounds exert a considerable cytotoxic effect on L1210 cells, however, the percentage of non-viability is significantly less than the percentage depression in respiratory activity produced by these agents. Methotrexate, 6-mercaptopurine, vinblastine and prednisone fail to appreciably alter the viability of leukemic cell suspensions at this dose level when compared to controls.

Discussion. Mechlorethamine and chlorambucil have been shown to impair the oxygen consumption of leukemic cell suspensions at 10 times the converted LD_{10} dosage and a loss of cell viability has been observed to accompany this depression in cell respiration. In contrast, prednisone which also has been demonstrated to inhibit the oxygen consumption at 10 times the converted LD_{10} dosage does not significantly decrease the number of viable cells. This indicates that although prednisone does not produce extensive cytotoxicity, it is capable of altering the metabolism of the tumor system.

Methotrexate, 6-mercaptopurine, vinblastine and actinomycin D are known to exert a direct biochemical effect on the synthesis of nucleic acids[4–7]. Therefore, the action of these agents would not necessarily be expected to measurably influence oxygen utilization following short term exposure unless a rapid cytotoxic effect is produced. Viability studies demonstrate these agents do not exert a lethal effect on L1210 cells after 1 h of incubation.

[1] Supported by Grant No. AM-HE-12084-15 from the National Institutes of Health, Bethesda, Maryland, and Grant No. 7132 from The Ohio State University General Research Support Fund.

[2] M. T. GOODMAN, R. M. FOLK and G. A. ACKERMAN, J. natn. Cancer Inst., in press (1973).

[3] J. R. PENNANT, Transplantation *2*, 685 (1964).

[4] U. C. WERKHEISER, Cancer Res. *23*, 1277 (1963).

[5] R. W. BROCKMAN, Cancer Res. *23*, 1191 (1963).

[6] J. F. RICHARDS, Cancer Chemother. Rep. *52*, 463 (1968).

[7] J. HURWITZ and J. J. FURTH, Proc. natn. Acad. Sci. USA *48*, 1222 (1962).

Effects of antineoplastic agents on the respiratory activity and viability of lymphocytic leukemia L1210 cells

Drug	Dose	Mean O_2 consumption rates		P	Depression (%)	Non-viability (%)
Mechlorethamine	0.008	0.89 ± 0.18	0.87 ± 0.14	N.S.	2	3
	0.08	0.75 ± 0.21	0.36 ± 0.12	<0.001	52	20
Chlorambucil	0.09	0.86 ± 0.14	0.85 ± 0.11	N.S.	1	1
	0.90	0.90 ± 0.20	0.61 ± 0.20	<0.02	32	18
Methotrexate	0.05	0.86 ± 0.18	0.87 ± 0.17	N.S.	1	4
	0.50	0.81 ± 0.13	0.82 ± 0.15	N.S.	1	4
6-mercaptopurine	0.35	0.84 ± 0.19	0.83 ± 0.13	N.S.	1	4
	3.50	0.86 ± 0.17	0.84 ± 0.15	N.S.	2	4
Actinomycin D	0.0006	0.85 ± 0.16	0.85 ± 0.19	N.S.	0	3
	0.006	0.82 ± 0.14	0.80 ± 0.16	N.S.	2	4
Vinblastine	0.0006	0.84 ± 0.14	0.83 ± 0.12	N.S.	1	2
	0.006	0.80 ± 0.16	0.79 ± 0.15	N.S.	1	3
Prednisone	0.46	0.87 ± 0.12	0.88 ± 0.11	N.S.	1	3
	4.60	0.82 ± 0.21	0.60 ± 0.18	<0.02	27	7

Dose levels administered are expressed as mg/30×10^6 cells. Mean oxygen consumption rates represent 4–10 observations and are included in the S.D. P-values are based on a comparison between the mean oxygen consumption rates of control and experimental samples.

Data obtained in this study indicate that mechlorethamine, chlorambucil, methotrexate, 6-mercaptopurine, vinblastine, actinomycin D and prednisone do not inhibit oxygen utilization of leukemic cells at the converted LD_{10} dosage. Correspondingly, these agents do not produce a significant increase in the survival time of tumor-bearing mice following a single i.p. injection of an LD_{10} dosage (< 20% increase in survival over untreated controls). Cells exposed to 10 times the converted LD_{10} dosage of mechlorethamine and chlorambucil are shown to depress oxygen consumption, however, in vivo dosages which correspond to this in vitro dose level fail to demonstrate antitumor activity and have proven highly toxic to the host. In contrast prednisone, which does not exhibit antitumor activity against L1210, does inhibit oxygen uptake at 10 times the converted LD_{10} dose level. It appears, therefore, that while a positive correlation exists between these 2 parameters of drug action at the converted LD_{10} dose level, no such relationship exists at other dosages. This suggests that drug effects on oxygen consumption does not necessarily provide a completely reliable or sensitive indicator of antitumor potential.

Résumé. On a évalué des effets de plusieurs agents antinéoplastiques sur la consommation d'oxygène des lymphocytes leucémiques L1210 de la souris et on les a mis en corrélation avec l'activité antitumorale de chaque composition. Cette étude indique qu'il n'y a pas de rapport apparent entre ces deux paramètres de l'action des drogues.

M. T. GOODMAN and G. A. ACKERMAN[8]

The Ohio State University, Department of Anatomy, 333 West 10th Avenue, Columbus (Ohio 43210, USA),
3 April 1973.

[8] Acknowledgments: The authors wish to express their appreciation to Dr. R. M. FOLK of Battelle's Columbus Laboratories for his consultation and advice and to MELANIE CROSSIN for her technical assistance.

Short-Term Effects of Colcemid on the Rapid Axonal Transport of Proteins in the Optic Pathway of Chick Embryos

An axonal transport of proteins occurs in the optic pathway of chick embryos[1,2] and of hatched chicks[3,4]. Its rate increases progressively throughout embryonic and post-embryonic development[5]. In analogy with evidence showing that in non-neuronal cells microtubules are involved in the intracellular movements of organelles like chromosomes and melanine granules, it has been proposed that axonal transport also depends on microtubules[6-9]. The observation that colchicine, a drug which binds to microtubular protein[10], blocks axonal transport[11-14] supports this view.

In this investigation the effects of Colcemid, a colchicine derivative, was tested upon the retino-tectal transport of proteins in chick embryos at two stages of development.

Method. Colcemid (Ciba, Basel) dissolved in saline was injected into the right eyeball of chick embryos at 13 and 18 days of incubation. 5 h after drug injection, ^{3}H-proline (10 Ci/mmole, New England Nuclear) or ^{3}H-fucose (5 Ci/mmole, New England Nuclear) was injected into the right eye. The dose of radioactivity for both precursors was 3 and 5 μCi respectively for 13 and 18 day embryos. All the embryos were decapitated 6 h after precursor injection when a wave of rapidly transported protein has reached the contralateral tectum[5]. The right retina and the paired optic tecta were removed and homogenized in ice-cold 5% trichloroacetic acid (W/v). After washing as

Table I. Effect of Colcemid on ^{3}H-proline and ^{3}H-fucose incorporation into proteins of chick embryo retina

Interval between drug and precursor injection (h)	Drug dose (μg)	Disintegration/min × 10^{-3}	Incorporation (% of control)	n
13 day				
^{3}H-proline				
–	0	2,569 ± 694	100	4
5	0.3	1,461 ± 150	56*	6
5	1.0	1,051 ± 262	40*	6
5	3.0	527	20	2
^{3}H-fucose				
–	0	1,002 ± 157	100	3
5	3.0	603 ± 215	60*	4
18 day				
^{3}H-proline				
–	0	3,315 ± 416	100	5
5	0.5	2,905 ± 530	87	6
5	5.0	2,390 ± 240	72*	3
5	10.0	2,052 ± 505	61*	5
24	0.5	3,076	92	2
24	10.0	2,866	86	2
^{3}H-fucose				
–	0	2,712 ± 986	100	3
5	5.0	1,853 ± 313	68*	6

The drug was dissolved in saline and injected into the right eye of embryos. The injected radioactivity was 3 μCi and 5 μCi of either proline or fucose at 13 and 18 days respectively. All the embryos were sacrificed 6 h after precursor injection. Data represent the whole protein-bound radioactivity recovered in right retinas (mean ± S.E.M.) Statical significance of the difference between control and treated retinas is indicated by an asterisk.

[1] P. C. MARCHISIO and J. SJÖSTRAND, Brain Res. *62*, 204 (1971).
[2] S. C. BONDY and C. J. MADSEN, J. Neurobiol. *2*, 279 (1971).
[3] P. C. MARCHISIO and F. GREMO, Abstr. of 3rd int. Meet. Neurochem. Budapest 1971, p. 153.
[4] S. C. BONDY, Expl. Brain Res. *13*, 127 (1971).
[5] P. C. MARCHISIO, J. SJÖSTRAND, M. AGLIETTA and J. O. KARLSSON, Brain Res., in press (1973).
[6] F. O. SCHMITT, Proc. natn. Acad. Sci. *60*, 1092 (1968).
[7] P. F. DAVISON and F. C. HUNEEUS, J. molec. Biol. *52*, 429 (1970).
[8] R. J. LASEK, Int. Rev. Neurobiol. *13*, 289 (1970).
[9] S. OCHS, Science *176*, 252 (1972).
[10] G. C. BORISY and E. W. TAYLOR, J. Cell. Biol. *34*, 525 (1967).
[11] A. DAHLSTRÖM, Eur. J. Pharmac. *5*, 111 (1968).
[12] G. W. KREUTZBERG, Proc. natn. Acad. Sci. *62*, 722 (1969).
[13] M. CUENOD, J. BOESCH, C. SANDRI, P. MARKO and J. P. SUSZ, Abstr. of 3rd int. Meet. Neurochem., Budapest 1971, p. 155.
[14] J. O. KARLSSON, H. A. HANSSON and J. SJÖSTRAND, Z. Zellforsch. mikrosk. Anat. *115*, 265 (1971).