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.

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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.

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Table II. Effect of Colcemid on the rapid phase of axonal transport of ^{3}H-proline and ^{3}H-fucose labelled proteins in the embryonic optic pathway

	Drug	(μg)	Disintegration/min				
13 day			Left	Right	L/R	*P*	*n*
^{3}H-proline		0	34,870 ± 3.875	3,767 ± 427	9.71 ± 1.73	0.001	4
	Colcemid	0.3	8,911 ± 1.531	3,386 ± 474	2.63 ± 1.16	0.01	6
	Colcemid	1.0	8,796 ± 1.102	6,025 ± 1.412	1.45 ± 0.56	n.s.	6
	Colcemid	3.0	7,971 ± 1.506	7,995 ± 319	0.99 ± 0.08	n.s.	3
^{3}H-fucose		0	25,599 ± 1.466	8,695 ± 825	2.69 ± 1.39	0.01	3
	Colcemid	3.0	7,316 ± 690	7,750 ± 855	0.95 ± 0.08	n.s.	4
18 day							
H-proline		0	63,743 ± 3.600	5,644 ± 292	12.45 ± 3.07	0.001	5
	Colcemid	0.5	36,111 ± 4.777	3,724 ± 690	9.69 ± 3.45	0.001	6
	Colcemid	5.0	11,714 ± 1.087	5,135 ± 493	2.27 ± 0.20	0.05	3
	Colcemid	10.0	11,917 ± 2.200	3,928 ± 581	3.40 ± 0.81	0.01	5
	Colcemid	0.5 *	41,862	4,222	9.90	—	2
	Colcemid	10.0 *	22,253	3,765	5.93	—	2
^{3}H-fucose		0	128,869 ± 23.845	15,974 ± 1.128	7.98 ± 1.05	0.001	3
	Colcemid	5.0	48,422 ± 13.066	11,542 ± 2.834	4.39 ± 1.15	0.01	6

The drug was injected into the right eye of embryos 5 h before precursor injection. In 2 groups (marked with asterisk) the precursor was given 24 h after drug administration. The injected radioactivity was 3 μCi and 5 μCi of either proline or fucose respectively at 13 and 18 days. All the embryos were sacrificed 6 h after precursor injection when a maximum of rapidly transported protein may be detected in the contralateral tectum[5]. Data represents the total protein-bound radioactivity recovered in tecta (mean ± S.E.M.). The statistical significance of the left vs. right difference was calculated on the basis of Student's *t*-test.

previously described[1], the whole acid-insoluble radioactivity was determined by liquid scintillation spectrometry with a Packard Tri-Carb model 3320.

Results. Table I shows the effects of Colcemid on proline and fucose incorporation into retinal proteins. The administration of the drug 5 h before precursor injection caused a significant reduction in retinal protein synthesis. When 24 h elapsed between drug and precursor injection in the 18-day embryo, no significant effect was detected. The apparent drug inhibition on the overall retinal protein synthesis was more marked in younger embryos.

Table II shows the effects of Colcemid on the rapid retinotectal transport of proteins. The drug caused a complete block of the rapid axonal transport when injected at a dose of 1 μg or higher. At the 18 day stage intraocular Colcemid reduced but never abolished the asymmetry in tectal protein-bound radioactivity, even after 24 h. The somatofugal transport of ^{3}H-fucose labelled glycoproteins was also inhibited by Colcemid.

Discussion. Colcemid decreases amino acid incorporation into retinal proteins shortly after its administration into the eye of chick embryos. The depression of protein synthesis caused by Colcemid is peculiar to the embryonic retina since it does not occur in hatched chicks (unpublished data); a transient block of glial cell proliferation may be involved and may explain why the effect is more marked in 13-day embryos when cell division is more active than at later stages.

The block caused by Colcemid on the retino-tectal migration of proteins and glycoproteins was complete in 13-day embryos but could not be obtained in 18-day embryos, even by administering larger doses of the drug. At the latter stage, conversely, colchicine and vinblastine at much lower doses gave a total block of transport (GREMO and MARCHISIO, in preparation). The failure of Colcemid to block axonal transport completely in older embryos could depend on the incomplete diffusion of the drug to the whole retina and (or) on a rapid inactivation of the drug by retinal ganglion cells. The latter possibility is supported by the reversibility of Colcemid effect on protein synthesis after only one day.

The effect of Colcemid on axonal transport is more marked than the effect on protein synthesis in the whole retina: the ratio between the net amount of protein-bound radioactivity transported to the contralateral tectum and the amount of radioactivity actually incorporated by the corresponding retina decreases as a function of Colcemid dose.

The finding that Colcemid blocks axonal transport during embryonic development indicates that microtubule integrity is required for axonal transport also in the embryo. The results of the present paper emphasize the importance of microtubules in neurogenesis and suggest that the rapid synthesis of microtubular protein in the immature nervous system[15] and its high concentration in embryonic brain[16] may reflect the high demand for transport involved microtubules during development.

Riassunto. La somministrazione endoculare di piccole dosi di Colcemid in embrioni di pollo al 13° e al 18° giorno di incubazione diminuisce la sintesi proteica nella retina. Pur in misura diversa, i due farmaci bloccano il trasporto assonico di proteine dalle cellule gangliari retiniche al tetto ottico. Anche nell'embrione quindi il trasporto assonico dipende dalla integrità dei microtubuli neuronali.

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