tyrosine hydroxylase, it is reasonable to expect that exposure of crude extracts containing tyrosine hydroxylase, activated by optimal K^+-depolarization to phosphorylating conditions, should produce no further or at least only a small increase in the activity of the enzyme. However, as shown in Table 2, the addition of Mg^{2+}, ATP and cAMP to tyrosine hydroxylase isolated from striatal slices incubated in 100 mM K^+ resulted in a further significant increase in enzyme activity. In fact, the absolute increase in tyrosine hydroxylase activity produced by exposure of the enzyme to phosphorylating conditions in this last experimental situation was slightly higher when compared to the absolute increase in enzyme activity resulting after adding Mg^{2+}, ATP and cAMP to enzyme obtained from slices incubated in normal KRP media (Table 2, third column). Suprisingly, exposure to phosphorylating conditions also significantly increased the activity of tyrosine hydroxylase in homogenates obtained from striatal slices incubated in the presence of optimal concentrations of dBcAMP (10 mM).

In summary, the observations reported here, albeit necessarily indirect and with the limitation of being obtained with crude enzyme preparations, do not support the conclusion that K^+ depolarization leads to an activation of tyrosine hydroxylase primarily via a cAMP-dependent phosphorylation process. Our findings offer no alternative explanation for the activation of tyrosine hydroxylase caused by neuronal depolarization. However, reports from this and other laboratories have emphasized the role that Ca^{2+} might play in this activation. Calcium addition has been shown to activate soluble tyrosine hydroxylase in a manner similar to that caused by neuronal depolarization [14,22,23] and the K^+-depolarization-induced activation of tyrosine hydroxylase appears to exhibit a calcium requirement [24]. Unexpectedly, our findings also suggest that dBcAMP activates tyrosine hydroxylase in striatal slices primarily by mechanisms other than cAMP-dependent phosphorylation. Dibutyryl cAMP could produce this activation through mobilization of intraneuronal Ca^{2+} compartments. Further experimentation with more purified enzyme preparations of activated tyrosine hydroxylase is needed in order to provide more definite answers to the above questions.

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Biologic properties of three anthracyclines as a function of lipophilicity

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Adriamycin and daunorubicin are well-known anthracycline antibiotics which inhibit growth of animal tumors [1] and are useful in the clinic [2]. Carminomycin [3] is a structural analog of daunorubicin, in which the —OCH_3 substituent on the C-4 position (ring 1) is replaced by —OH.

Accumulation of the anthracyclines is believed to involve a "leak and pump" system [4, 5]; enhanced drug exodus is one mode of drug resistance [5–7]. Once inside the cell, the

anthracyclines bind to DNA and inhibit subsequent biosynthesis of DNA and RNA [8–10]; a free radical process may be involved in these biologic effects [11] and in another mode of drug action involving lipid peroxidation [12, 13]. This report describes the relation between some physical and biologic properties of three anthracyclines.

Adriamycin and daunorubicin were supplied by the Division of Cancer Treatment, NIH and cardinomycin by Bristol Laboratories, Syracuse, N.Y. Methods for the maintenance of L1210 cells in culture, together with procedures for the measurement of drug-induced inhibition of DNA synthesis, have been described previously [14].

Suspensions of 7×10^6 cells/ml in 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES)-buffered growth medium or in phosphate-buffered saline [20 mM sodium phosphate (pH 7.0) + 140 mM NaCl containing 1 mg/ml of glucose] were incubated with drug concentrations of 0–30 μg/ml for 0–30 min. The cells were collected by centrifugation, washed with fresh incubation medium, and the anthracycline concentration was measured fluorometrically [15]. The effect of drug on the incorporation of labeled thymidine into DNA was measured by suspension of drug-treated cells in HEPES-buffered growth medium over 5-min intervals [14].

Chromatography of anthracyclines and of chloroform–methanol (1:1) extracts of drug-treated cells was carried out on thin-layer sheets of silica using chloroform–methanol–acetic acid (76:20:4). In this solvent, the aglycones ran with the solvent front, and the individual drugs were clearly separable.

Partitioning of drugs was measured by dissolving 50 μg of each in 500 μl of octanol-saturated 20 mM sodium phosphate buffer (pH 7.2), which was then mixed with 500 μl of buffer-saturated octanol. The phases were then separated by centrifugation, and a 100 μl portion of each phase was mixed with 900 μl of ethanolic HCl for determination of drug level by fluorometry [15].

A summary of results obtained is shown in Fig. 1 and Table 1, and is described below. Transport of the drugs was initially examined in serum-free medium to discriminate against effects related to binding of drugs to plasma proteins, and data are reported in the figure and table. When growth medium containing 10% serum was employed, the extent of uptake of all three drugs was reduced to 85 per cent of the numbers shown. In this report, drug concentrations are given in terms of μg: a 1 μg/ml anthracycline solution is approximately 1.4 μM.

Carminomycin was more rapidly accumulated by L1210 cells than was daunorubicin or adriamycin (Fig. 1) at either 10° or 37°. Uptake of the latter agent was almost temperature-insensitive in this system; the uptake of daunorubicin and of carminomycin was markedly temperature-sensitive. As a result, uptake of adriamycin was faster at 10°, but slower at 37° than was daunorubicin accumulation.

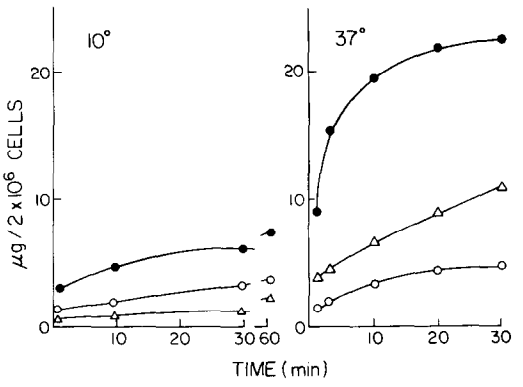

Fig. 1. Uptake of anthracyclines as a function of temperature: Key: (o) adriamycin; (△) daunorubicin; and (●) carminomycin. Extracellular drug concentration = 30 μg/ml.

The extracellular drug concentration required for 50 per cent inhibition of DNA synthesis varied among the three agents, and was correlated with lipophilicity (Table 1). But while the ID_{50} levels of the anthracyclines were different, the intracellular drug level associated with a 50 per cent inhibition of DNA synthesis was almost identical for the three drugs. Lipophilicity was estimated both from octanol:water partition results and from chromatographic behavior of drugs in a relatively polar system where the more polar agents migrated faster. There was no evidence of drug metabolism when cell extracts were chromatographed.

Comparisons between transport vs biologic effects of adriamycin and daunorubicin have been made before [4, 6, 9, 16–20]. the latter agent is generally the better accumulated, perhaps accounting for its more potent capacity for inhibition of DNA synthesis when both drugs are employed at the same extracellular concentration. Daunorubicin is also the more lipophilic of the two (21).

In the present study, carminomycin was found to be more lipophilic than either daunorubicin or adriamycin. Carminomycin was also more rapidly and extensively accumulated *in vitro* by L1210 cells than were the other agents. Avery and Cruze [21] have reported carminomycin to be substantially more potent than adriamycin against L1210 *in vitro*, as would be expected from the results described here.

It is surprising that a marked alteration in fluorescence emission spectrum and in lipophilicity is associated with replacement of the C-4 methoxyl of daunorubicin with a hydroxyl group, expecially since the latter are generally considered to be less lipophilic than the former. Capacity of the phenolic H of carminomycin at C-4 to participate in hydrogen-bonding interactions may account for these results. In a recent report, Formelli *et al.* [22] described studies on 4-

Table 1. Properties of anthracyclines*

Compound	ID_{50}† (μg/ml)	Intracellular drug level‡ (μg/2 × 10^6 cells)	Partition ratio§	R_f value (t.l.c.)	Emmision maximum‖ (nm)
Adriamycin	15 ± 0.9	1.9 ± 0.3	0.1 ± 0.01	0.6 ± 0.05	585
Daunorubicin	7.5 ± 0.8	1.7 ± 0.2	0.22 ± 0.03	0.7 ± 0.05	585
Carminomycin	3.0 ± 0.4	1.5 ± 0.2	1.44 ± 0.15	0.9 ± 0.08	545

* Data represent means ± S.D. for five determinations.

† Extracellular drug level which, during 30-min incubations at 37°, inhibits incorporation of a subsequent 5-min pulse of labeled thymidine into DNA by 50 per cent.

‡ Intracellular drug level resulting from the incubation described above.

§ Ratio of drug concentration in the octanol:aqueous phase.

‖ Fluorescence emission maximum upon excitaion at 467 nm in ethanolic-HCl solution.

demethoxydaurorubicin; absence of the C-4 methoxyl substituent was associated with an increase in potency over daunorubicin. Therefore, it appears that the nature of the C-4 substituent on the chromophore substantially affects anthracycline pharmacology. The bearing of the enhanced lipophilic character of carminomycin on the nature and severity of adverse reactions to the drug remains to be established.

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GABA depletion and GABA-transaminase activity increase after intraventricular 6-hydroxydopamine

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Several studies have shown that intraventricular or intracerebral injection of 6-hydroxydopamine (6-OHDA) produce a rather selective, dose-dependent degeneration of catecholaminergic neurons [1–3] associated with a long-lasting depletion of brain catecholamines [4–6]. Similar results have been obtained in mammalian and avian species for adrenergic neurons in the periphery [7-11]. On the other hand, brain concentrations of serotonin [3, 4, 12], GABA [4, 13], acetylcholine, glycine, glutamic acid and other free amino acids [13] were not changed. In addition, low doses of 6-OHDA given intraventricularly (i.v.) do not seem to affect gross behaviour in rats [14], whereas after higher doses severe convulsions have been shown to occur [6, 13].

The aim of the present work was to study in chicks the effects of a single large intraventricular dose of 6-OHDA on GABA content, GAD and GABA-T activities in diencephalon and brain-stem.

Rhode Island Red chicks, 1 week old were used. For implanting cannulae the chickens were anaesthetized with halothane (Fluothane ICI) in oxygen delivered through a Vapor halothane vaporizer, according to Marley and Stephenson [15]. Chicks were tested at least 24 hr after implantation of cannulae when recovery was complete. The cannula position was verified by the outflow of clear cerebro-spinal fluid at the implantation time. Eight chicks received a single intraventricular dose (100 μg as free base) of 6-OHDA dissolved in 2.5 μl of distilled H_2O containing ascorbic acid (1 mg/ml); the pH was adjusted to 5.0 with hydrochloric acid.

The solution was made up just before use. Control animals ($n = 6$) received an equal volume of the vehicle solution. After decapitation the diencephalon and brain-stem were quickly dissected out within 30 sec and frozen in liquid nitrogen.

GABA was assayed by a C. Erba aminoacid analyzer with AMINEX A-5 (0.9 × 13 cm) ion exchange resin as previously described [16]

GAD-activity was assayed using the conditions of Beaven *et al.* [17], slightly modified. A 15 μl sample of the tissues homogenate (1:10) was mixed with 35 μl of reagent inside a 1.5 ml polypropylene Eppendorf vial. The vial was placed inside a 20 ml screw-cap liquid scintillation counting vial. A drop (20 μl) of 30% 2-phenylethylamine in methanol was adsorbed on a square (1 cm^2) of filter paper (Whatman 3MM), placed at the bottom of the counting vial away from the Eppendorf vial. The reagent consists of (i) L-[U-^{14}C]glutamic acid, 20 nCi; (ii) unlabeled L-glutamic acid to bring the concentration of aminoacid to 5×10^{-4}M; (iii) 1×10^{-5}M PLP; and (iv) 0.1 M sodium phosphate buffer, pH 6.8. Reaction blanks were prepared by substituting buffer for the sample. The counting vials were tightly capped and incubated at 37°C for 30 min; for deproteinization the vials were then placed on ice and uncapped one at a time and 20 μl of 2 N perchloric acid was added to the Eppendorf vials. The counting vials were quickly recapped and reincubated for