for 7 days, however, was found to bring about a decline in the copper content to 1.40 mg%. On continued feeding for 15 days, the normal value was obtained. The other organs, such as brain, heart, kidney, lung and muscle, did not indicate any significant difference in the copper content of normal and psoralen-fed rats for the period of 3, 7 and 15 days.

The decrease in the copper content of liver is in close agreement with the observation of MOFTY et al.[4] who reported a marked rise in blood copper and a drop in liver after the administration of 8-methoxypsoralen. It would thus appear that, in view of the requirement of copper at other sites, psoralen administration depletes copper from liver giving rise to its increase in the peripheral blood circulation. Having accepted that psoralen induces copper to migrate from liver to peripheral blood, its increase in spleen could probably be explained on the basis of spleen being a blood filter. Copper is probably bound to such proteins in the circulatory blood as are easily permitted by the spleen to pass through.

The initial increase of copper in the skin in the first 3 days of psoralen supplementation could possibly be due to the uptake of copper from the peripheral blood stream. In albino skin, this increase in copper did not bring about any material advantage and was therefore once again excreted into the blood and the values were found to return to normal. The decrease after 7 days of psoralen feeding may possibly point out some sort of excretory mechanism which may have been developed in this tissue, after accumulated copper was not required. The inertness in other organs such as brain, heart, kidney, lung and muscle would perhaps indicate the possible insignificance of these organs in the action of psoralen in pigment production.

It would thus appear from these studies that one of the mechanisms by which psoralen may exert its action in the production of melanin may be that it somehow (exact mechanism for this process is still unknown) releases the stored copper from the liver and through peripheral blood circulation, makes it available to the depleted vitiliginous areas. However, this hypothesis will have to await further confirmation by the use of radioactive copper and finally clinical studies in human beings.

Zusammenfassung. Psoralenfütterung von Ratten bewirkt eine Vermehrung des Kupfergehaltes in der Milz und eine Verminderung in der Leber. In der Haut sind die Werte nicht einheitlich.

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22 July 1968.

Comparative Fractionation of DNA from Ascites Tumour and Normal Cells on Hydroxyapatite Column

Applicability of hydroxyapatite column to the fractionation of nucleic acids has been demonstrated by several workers[1–5]. So far, however, there has been no attempt to investigate possible differences in the mammalian DNA from normal and tumour cells using this column. In this laboratory DNA samples from normal and malignant mice tissues were examined by chromatography using hydroxyapatite column. The following is the report of these observations.

Materials and methods. Dalton's lymphoma in the form of an ascites tumour from DBA mice obtained from Dr. G. KLEIN which originally arose as a thymus tumour in DBA 212 mouse at the National Cancer Institute (USA) was selected for the present experiments. It was maintained as ascites tumour in DBA (-MTI) mice in our laboratory. High polymer DNA was prepared according to the method of BERNS and THOMAS[6], where they have been able to obtain DNA of molecular weight as high as 4.0×10^8. Residual RNA was removed by digesting the preparation with RNAase at 37 °C and with subsequent dialysis. Ratios of optical density at $\lambda_{260}/\lambda_{280}$ and $\lambda_{260}/\lambda_{230}$ were of the order of 2.0:2.2 when the material was dissolved in physiological saline. The protein test carried out according to the method of LOWRY et al.[7], was negative. DNA prepared as above from the thymus of the normal DBA (-MTI) mice, was used as control.

Hydroxyapatite column was prepared according to the method of MIYAZAWA and THOMAS[5]. Fractionation of DNA was carried out on 1×3 cm of this column by continuous elution with linear molarity gradient of phosphate buffer (Na_2HPO_4, NaH_2PO_4, pH 6.8).

Results and discussion. Fractionated DNA from both normal as well as tumour cells appeared in a single peak eluting at 0.26 *M* PO_4 when the concentration of PO_4 was raised from 0.001–1.0 *M*. However, these 2 varieties of DNA samples showed a marked difference in the proportion of DNA which eluted at the above peak and that which still adhered to the column at 1.0 *M* PO_4 concentration and could be recovered from it by suspending the column material in a 1.0 *M* PO_4 buffer in a test-tube shaking the suspension for 3–4 min and by its subsequent centrifugation. More than 90% of the thymus DNA which served as the control eluted at 0.26 *M* PO_4 and about 5% was separated from the column with 1.0 *M* PO_4 in the manner stated above. On the other hand, 54–62% of the tumour DNA was obtained at 0.26 *M* PO_4 and about 40% was recovered from the column with 1.0 *M* PO_4. The typical elution patterns are shown in the Figure. Thus DNA from tumour source was always found poorer in DNA eluting at 0.26 *M* PO_4 and between 5- to 10-fold richer in the 'sticking' kind (separated with 1.0 *M* PO_4) when compared to the corresponding fractions from the normal tissue. This feature was observed repeatedly in number of independent experiments.

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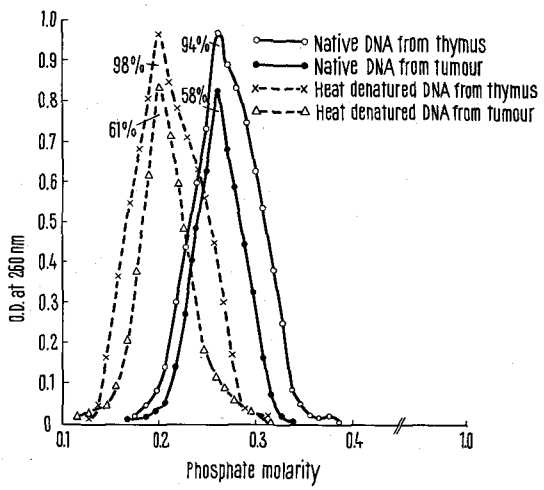
On heat denaturation (100 °C, 15 min) of the DNA in tumour as well as control samples, the peaks at 0.26 *M* PO_4 shifted close to 0.2 *M* PO_4 while no significant change in the behaviour in the 1.0 *M* region was observed in both these cases.



The chromatographic elution patterns of DNA on hydroxyapatite column using phosphate buffer gradient.

The fractions eluting at 0.26 *M* PO_4 from both the present sources of DNA conform to the observations of earlier workers with native DNA from chicken erythrocyte, calf thymus, *Escherichia coli*[4], as well as T2 and λ bacteriophage[5]. The observed shift to lower PO_4 molarity of the eluent for these fractions on heat denaturation of the DNA samples also supports such a correlation and could be due to conversion of double stranded DNA into single stranded variety. On the other hand, the observation of the more adherring type of DNA in the present investigations which seems to form a significant fraction in the samples from tumour cells only, does not find easy correlation with known data. The stability of this fraction to heat treatment is also noteworthy. It would be of interest to know if this fraction showed any characteristic differences in its biological behaviour when compared with the more common native DNA fraction eluting in the PO_4 concentration range 0.2–0.3 *M*.

Zusammenfassung. Zwischen DNS normaler und neoplastischer Zellen besteht ein wesentlicher biochemischer Unterschied.

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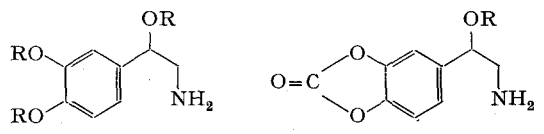
Labile Lipophilic Derivatives of Norepinephrine Capable of Crossing the Blood-Brain Barrier

One method of modifying the activity of pharmacological agents is through synthesis of labile derivatives which undergo either enzymatic or non-enzymatic hydrolysis in vivo to regenerate the parent drug[1–5]. Differences in metabolism, excretion, active or passive transport and tissue localization of these derivatives should influence greatly the onset, duration and type of activity characteristic of the parent drug. The catechol amines, (nor) epinephrine and dopamine may be modified either at the basic nitrogen or at the phenolic groups. Labile lipophilic derivatives of catechol amines would be expected to cross the 'blood-brain barrier', enter the central nervous system (CNS) and, by hydrolysis, generate the parent neurohumoral transmitters in situ. In addition, such labile derivatives of norepinephrine-^{3}H and other neurohumoral transmitters might provide a method of labeling central storage sites with radioactive amine for the study of amine uptake, storage, release and metabolism. Such CNS studies normally make use of radioactive metabolic precursors which readily cross the 'blood-brain barrier', for example: tyrosine[6,7], dopa[6,7], or 3,4-dihydroxyphenylserine[8], or involve the direct, intracisternal injection of the amine[9].

Our synthetic approach to 'latentiation' of norepinephrine was guided by the observation that phenolic derivatives of catecholamines, such as the 0,0-dibenzoate, 0,0-diacetate or 0,0-*bis*(ethylcarbonate), caused release of norepinephrine-^{3}H from cardiac tissue in 2 h[10], a clear indication that rapid hydrolysis of these esters to the active catechols must have occurred in vivo.



I: R = Ac
II: R = $(CH_3)_3Si$

Two lipophilic derivatives of norepinephrine were prepared. One, 3,4,β-triacetyl-L-norepinephrine (I) was prepared by acetylation of *N*-carbobenzyloxy-L-norepine-

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