Resumen. En 12 ratas normales y en 12 hipofisectomizadas 150 días antes se determinaron las concentraciones de ácidos urónicos, glicoproteínas, ácido hialurónico, heparitín sulfato, condroitín-4-sulfato, condroitín-6-sulfato, dermatán sulfato y heparina en piel y cartílago traqueal. Estos estudios demostraron dos hechos: (1) un efecto distinto de la hipofisectomía sobre piel y tráquea en relación con el metabolismo de las glicoproteínas y el ácido hialurónico, cuyas concentraciones aumentaron en la primera y descendieron en la segunda; (2) una disociación entre el comportamiento del ácido hialurónico y los glicosaminoglicanos sulfatados en piel, aumentando la concentración del primero y disminuyendo las correspondientes a los segundos en forma variable según la fracción considerada.

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Effect of Feeding Psoralen on the Copper Content of Different Organs in Albino Rats

Furocoumarins have been known to induce pigmentation in vitiliginous skin when administered along with longwave UV- or solar-irradiation[1-3]. The precise mechanism of this induction is at present largely unknown. MOFTY et al.[4] have reported that feeding of 8-methoxypsoralen to albino rats caused a marked rise in blood copper and a significant drop in its content in liver. However, 8-isoamyleneoxypsoralen was found to be ineffective. Hence it was considered worthwhile to investigate the effect of feeding psoralen for different periods and observing its effect on total copper of the different organs in albino rats. The result of such studies are reported in the present communication.

Male albino rats (100 g) were divided into 4 groups of 8 rats each. The composition of the feeding mixture was 250 mg gum tragaconth, 750 mg glucose, 125 mg psoralen, 2.5 ml ethyl alcohol and water to make 25 ml. In the normal feeding mixture psoralen was omitted. Each rat was fed 0.5 ml of the solution daily. After the requisite period of feeding the rats were killed, organs immediately removed, weighed and digested to a transparent colourless liquid. The digested samples of various organs containing concentrated sulphuric acid were polarographed using Lange's manual polarograph with a multiflex galvanometer for recording current. In all the samples, the halfwave potential, measured against Hume and Harris saturated calomel electrode at 37 °C, was exactly at 0 V, a potential reported for copper ions in this medium. The concentration of copper was calculated by measuring the diffusion current at -0.200 V. The effect of different concentrations of sulphuric acid, different ionic strength obtained by the additions of potassium chloride and potassium sulphate was also investigated and found to be negligible.

Before actually doing the copper content of different organs of normal and psoralen-fed albino rats, some recovery experiments were done by adding different amounts of copper to all the organs under study in order to know the error in the estimation. The error was found to vary from $\pm$ 2 to $\pm$ 5% in most of the organs and that is well within the limit of polarographic analysis[5].

The Table represents the total copper in different organs of psoralen-fed rats for 3, 7 and 15 days. The copper content of spleen indicated a rise of around 46.2% and liver on the other hand exhibited a decrease of about 42.87% after 3 days of psoralen administration. These changes were more or less maintained in both the organs even after 15 days of feeding. Skin was found to give a variable response at different periods. Initial feeding for 3 days indicated a rise of about 43.52%. Further feeding

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Total copper in different organs of normal and psoralen-fed albino rats

Groups	Copper content of various organs (in mg%)							
	Liver	Skin	Heart	Brain	Kidney	Lung	Spleen	Muscle
Normal	8.00 $\pm$ 3.27	3.35 $\pm$ 2.77	4.95 $\pm$ 1.32	1.49 $\pm$ 0.67	3.61 $\pm$ 2.41	5.25 $\pm$ 3.70	9.16 $\pm$ 4.64	2.07 $\pm$ 1.59
Psoralen-fed								
3 days	4.50 $\pm$ 3.27	4.81 $\pm$ 2.27	5.22 $\pm$ 4.26	1.58 $\pm$ 0.90	3.14 $\pm$ 1.16	5.27 $\pm$ 2.87	13.40 $\pm$ 2.58	2.29 $\pm$ 1.07
7 days	4.75 $\pm$ 1.45	1.40 $\pm$ 1.29	5.47 $\pm$ 3.38	1.45 $\pm$ 0.56	3.28 $\pm$ 2.47	4.77 $\pm$ 3.57	12.24 $\pm$ 8.05	2.02 $\pm$ 1.32
15 days	4.46 $\pm$ 1.67	3.25 $\pm$ 1.84	4.75 $\pm$ 2.46	1.98 $\pm$ 0.73	3.48 $\pm$ 0.75	5.43 $\pm$ 2.71	13.99 $\pm$ 5.10	2.79 $\pm$ 1.32

Values given are mean of 8 animals.

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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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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