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The Effect of the Toxohormone on the Liver Porphyrin and the Non-heme Iron Level in the Blood Serum of a Syrian Hamster

Wpływ toksohormonu na poziom porfiryn wątrobowych
oraz żelaza niehemowego w surowicy krwi u chomika syryjskiego

Влияние таксогормона на уровень печеночных порфиринов и безгемового железа
в сыворотке крови у сирийского хомяка

INTRODUCTION

In 1948 Nakahara and Fukuoka (1) isolated a substance causing a depression of the liver catalase from a neoplastic tissue and called it a toxohormone.

The biological effect of the toxohormone does not limit itself only to decreasing the liver catalase level, but at the same time causes an increase of the level of free porphyrin in the liver, the lowering of non-heme iron in the blood serum and a decrease of ferritin in the liver.

Ono et al (3) examined the effect of the toxohormone, isolated from the tumor *Rhadomina sarcoma*, on the level of iron in the blood serum. They obtained a 50% decrease with a dosage of 150 μg per rat.

Nixon and Zinman (2) extracted the toxohormone from ten different tumors. The most active preparation was obtained from gastric *adeno-carcinoma*, which, after being purified on Amberlite XE-64 and administered in a dose of 100 μg per rat, decreased the iron level by 50%.

Ono et al (5) examined the effect of the toxohormone of the protoporphyrin level in the liver and the coproporphyrin level in urine. They ascertained that the level of free porphyrin in the liver of rats with *Rhodamina sarcoma* increased by 87.5% on average and by the administration of the toxohormone by injection by 40%.

The level of coproporphyrin in the urine also significantly increased.

In our previous paper (7) we ascertained a decrease in the liver catalase activity of a Syrian hamster with an implanted melanoma tumor. The activity level of the enzyme in the liver was inversely proportional to the tumor mass.

The aim of the present paper is a comparison of the effect of the toxohormone discharged by the malignant melonoma tumor *in vivo* and that isolated from melonoma tumors on the liver protoporphyrin and coproporphyrin level and on the non-haem iron level in the blood serum.

MATERIALS AND METHODS

The material for the experiments was the malignant melanoma tumor which was passaged on the Syrian hamster (*Mesocricetus auratus* W a t e h r).

The porphyrin was determined by the method worked out for erythrocytes by S c h w a r t z and W i k o f f (6), adapted by us for the examined material. The liver after being washed in 0.9% of NaCl solution was homogenized in a mixture of ethylacetate and acetic glacial acid. After being centrifuged, protoporphyrin and coproporphyrin were extracted with 3 N HCl from the supernatant. Next the solution was neutralized

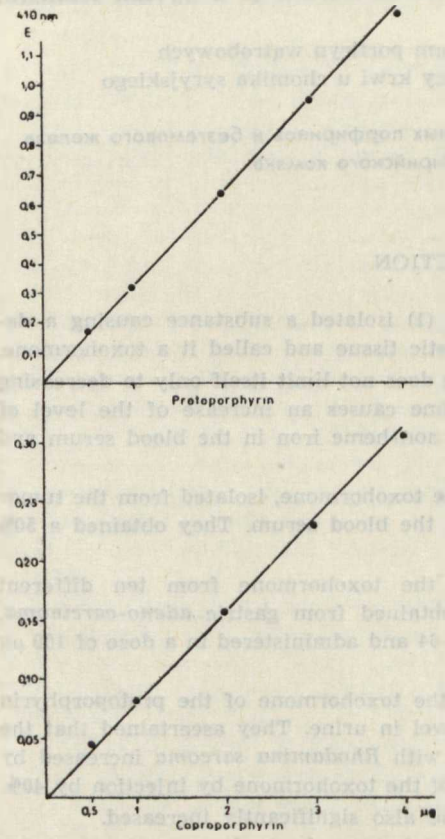


Fig. 1. The absorption spectrum of protoporphyrin

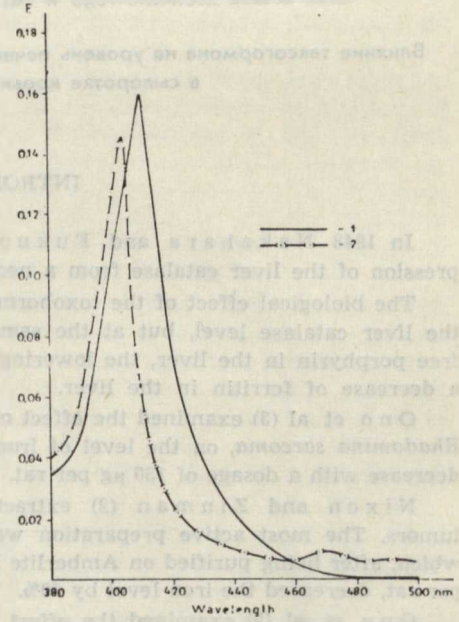


Fig. 2. The calibration curves of protoporphyrin and coproporphyrin preparations; 1 — the standard preparation of protoporphyrin, 2 — the extracted protoporphyrin from liver

with sodium acetate and after taking up the porphyrin in ethylacetate, coproporphyrin was extracted with 0.1 N HCl and protoporphyrin with 3 N HCl. After measuring the absorption at 400—410 nm wave-length, the quantity of protoporphyrin and coproporphyrin in μg was read from the standard curve (Fig. 1).

The absorption curve of protoporphyrin extracted from the liver of hamsters injected by cancerous tissue or a toxohormone preparation was unsignificantly moved to the left and had an unsignificant peak at 460—480 nm in comparison with a control porphyrin preparation (Fig. 2).

The determination of the iron level in the blood serum was carried out according to a somewhat modified W o o d r u f f's method (8). In this method the ability of iron (Fe^{+2}) to create a coloured complexed

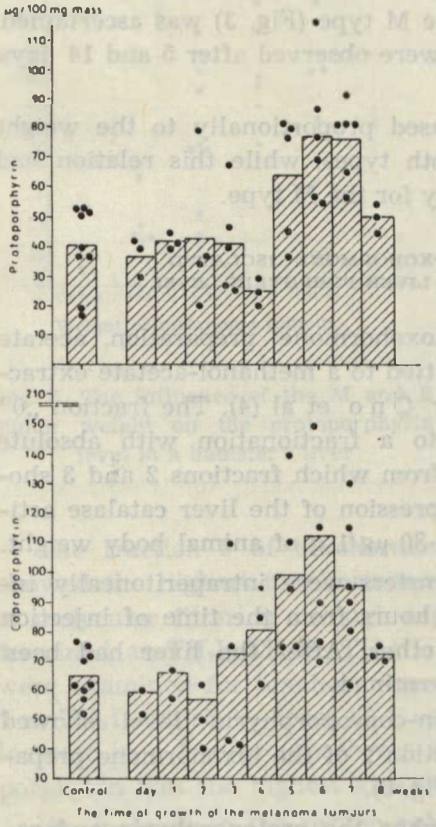


Fig. 3. The influence of the time of tumor M growth on the protoporphyrin and coproporphyrin levels in hamster's liver

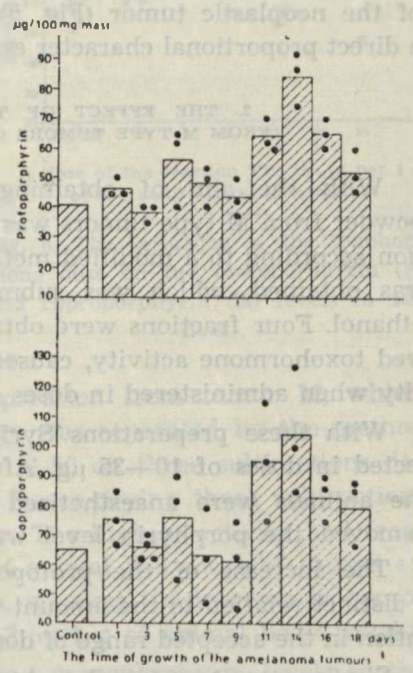


Fig. 4. The influence of the time of tumor B growth on the protoporphyrin and coproporphyrin levels in hamster's liver

compound with 2.2-dipyridine was measured. The absorption of this compound was measured at 510 nm.

1. THE EFFECT OF THE TOXOHORMONE DISCHARGED FROM TUMORS IN VIVO ON THE LIVER PORPHYRIN LEVEL

The implantation of the malignant melanoma tumor of the melanotic type (M) and the amelanotic (B) consisted in administering 120 mg of neoplastic tissue homogenized in 0.9% of NaCl solution injected subcutaneously.

The first determination of porphyrin for both of these types was carried out after 24 hours from the moment of injection, next it was carried out for the M tumor in weekly intervals, while for the B type, because of the different rates of growth of both types, every 2 days.

The increase in the porphyrin level and the attainment of the maximum after 6 weeks in the case of the M type (Fig. 3) was ascertained. In animals with tumor B two maxima were observed after 5 and 14 days, respectively (Fig. 4).

The liver porphyrin content increased proportionally to the weight of the neoplastic tumor (Fig. 5) in both types, while this relation had a direct proportional character especially for the M type.

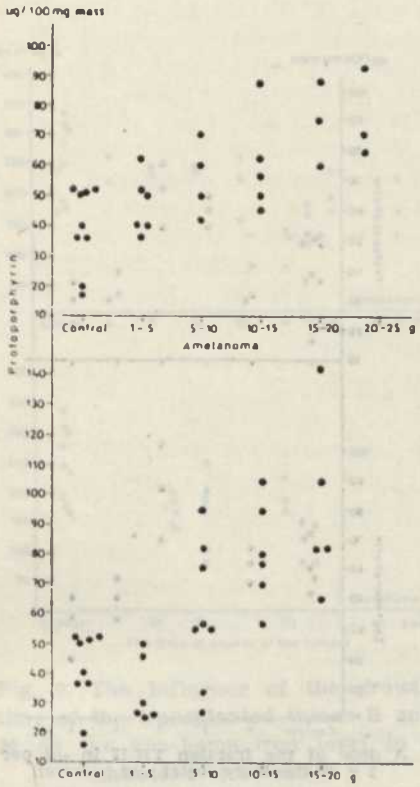
2. THE EFFECT OF THE TOXOHORMONE ISOLATED FROM M TYPE TUMORS ON THE LIVER PORPHYRIN LEVEL

With the aim of obtaining the toxohormone preparation, acetate powder from M type tumors was submitted to a methanol-acetate extraction according to a modified method by O n o et al (4). The fraction „0" was obtained, which was submitted to a fractionation with absolute ethanol. Four fractions were obtained from which fractions 2 and 3 showed toxohormone activity, caused a depression of the liver catalase activity when administered in doses of 20—30 μg/1 g of animal body weight.

With these preparations Syrian hamsters were intraperitoneally injected in doses of 10—35 μg. After 24 hours from the time of injection the animals were anaesthetized with ether. After the liver had been removed the porphyrin level was determined.

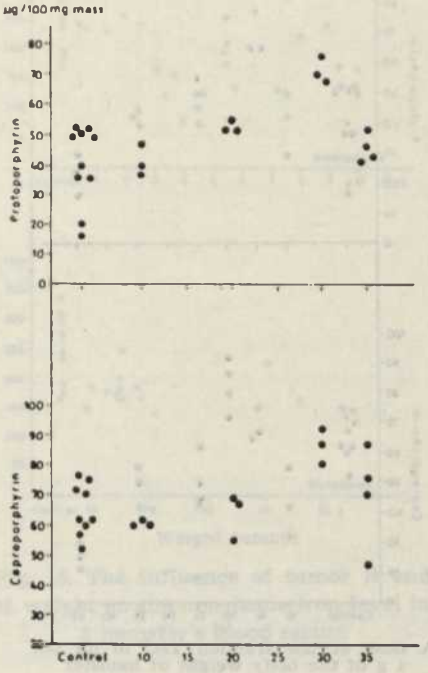
The increase of the protoporphyrin-coproporphyrin level showed a distinct relation to the amount of fraction 2 of the toxohormone preparation in the accepted range of dosage (Fig. 6).

Similar results were also obtained when the protoporphyrin and coproporphyrin levels were determined in animals which had been administered fraction 3 of the toxohormone in doses of 10—45 μg per g of animal body weight (Fig. 7).



Weight of melanoma tumour

Fig. 5. The influence of the M and B tumor weight on the protoporphyrin level in a hamster's liver

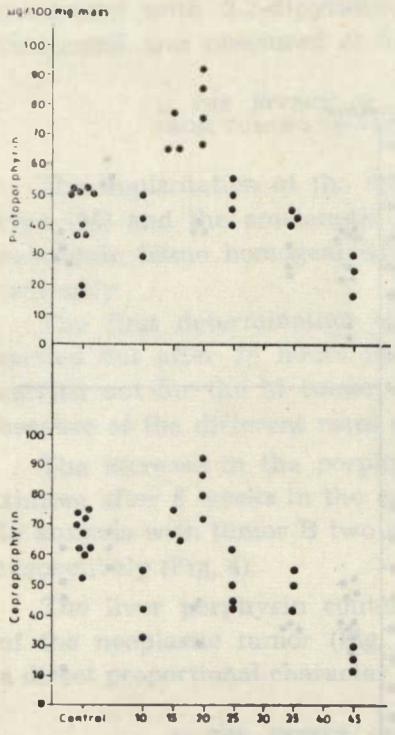


A dose of the fraction TH_2 in µg per 1 g of the body weight of hamster

Fig. 6. The influence of the toxohormone dose on the protoporphyrin (P) and coproporphyrin (K) levels in the liver

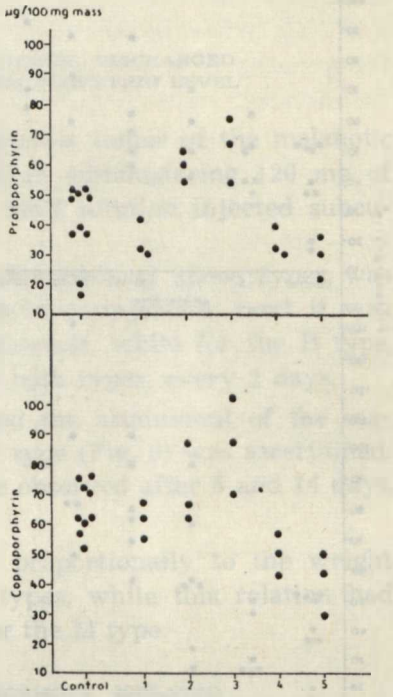
The fraction 3 of toxohormone preparation from tumor M, which showed the highest toxohormone activity was separated by the method of molecular filtering through sephadex G-50 on three subfractions determined as TH_3I, TH_3II, TH_3III. After freeze-drying these subfractions were examined for toxohormone activity. The highest activity had fraction TH_3II which in doses of 2 µg/1 g of animal body weight caused an increase in a free protoporhyrin by 48%, whereas the increase in coproporphyrin was the highest at a dose of 3 µg/1 g of animal body weight.

An increase in the protoporphyrin and coproporphyrin levels in the liver was observed with increasing doses of preparation TH_3II until the maximum was reached, similarly as in the experiment with fractions 2 and 3. However after reaching the maximum a fall in the protopor-



A dose of the fraction TH_3 to µg per 1 g of the body weight of hamster

Fig. 7. The influence of the toxohormone dose of the fraction 3 (TH_3) on protoporphyrin (P) and coproporphyrin (K) levels



A dose of the fraction TH_3II in µg per 1 g of the body weight of hamster

Fig. 8. The influence of the dose of toxohormone preparation TH_3II on coproporphyrin and protoporphyrin levels in a hamster's liver

phyrin level occurred in spite of the increased dose of toxohormone (Fig. 8).

3. THE EFFECT OF DISCHARGED TOXOHORMONE IN VIVO ON THE IRON LEVEL IN THE BLOOD SERUM

With the aim of determining the level of non-haem iron in the blood serum, blood was taken by a puncture into the heart of animals with implanted tumor. A distinct fall in the iron in the blood serum of the hamsters was found while the lowest level of Fe was observed for tumor B on the 18th day from the moment of tumor implantation and for the M type on the 53rd day (Fig. 9).

A decrease in the non-heme iron level in the blood serum was proportional to the weight of the neoplastic tumor for both types, but this relation had a direct proportional character especially for the B type (Fig. 10).

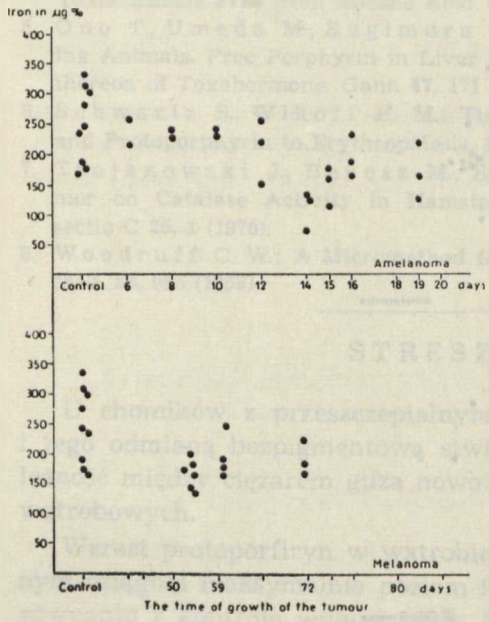


Fig. 9. The influence of the growth time of the transplanted tumor B and M on the non-heme iron level in a hamster's blood serum

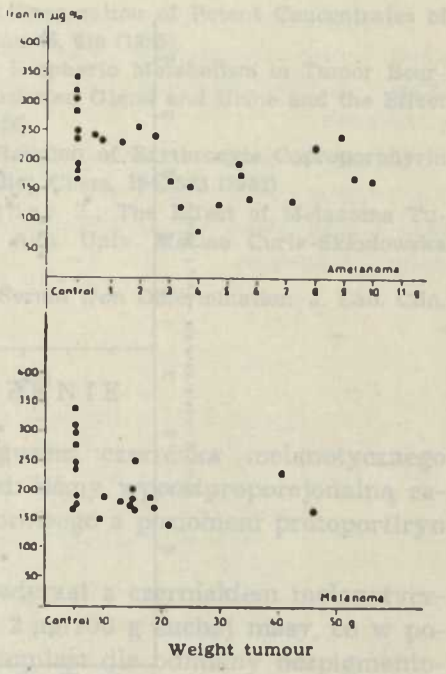


Fig. 10. The influence of tumor B and M weight on the non-heme iron level in a hamster's blood serum

Furthermore a correlation between the activity of the liver catalase and the level of Fe in the animals with tumors B and M (Fig. 11) was noticed.

CONCLUSIONS

Summing up the presented results it can be ascertained that:

1. The increase in the free porphyrin level in the liver is proportional to the neoplastic tumor mass.

2. In a hamster with an implanted M tumor the maximum increase in free porphyrin occurred 6 weeks after the moment of implantation and was 89.8% for protoporphyrin and 72.6% for coproporphyrin. In the following weeks a gradual decrease was observed. In animals with an implanted amelanotic tumor the maximum increase in protoporphyrin occurred after 14 days of tumor growth and was 108.3% for protoporphyrin and 59.7% for coproporphyrin.

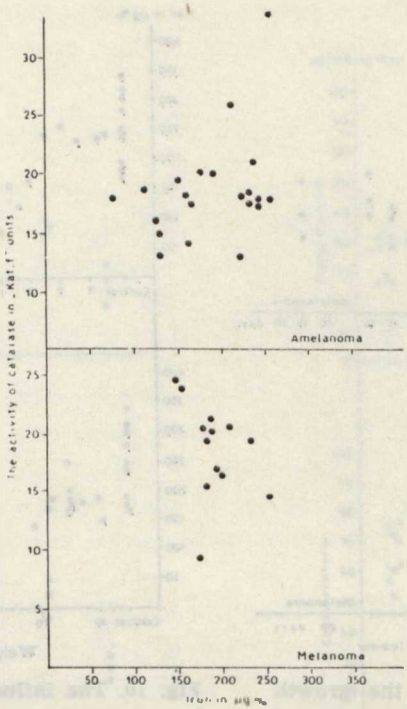


Fig. 11. The comparison of the liver catalase activity and non-hem iron in serum blood in hamsters with transplanted Melanoma B and M tumors

3. The administration of the toxohormone preparations TH_3 and TH_3II, isolated from malignant M tumors, by way of injection causes an increase of the level of free porphyrin when given in doses which are increased of the level of free porphyrin when given increasing doses in until the maximum is reached, and next a decrease. Moreover, it was observed that the level of protoporphyrin was the highest after 24 hours following intraperitoneal injection of the hormone.

4. The level of iron in the blood serum of a hamster with an implanted malignant melanoma tumor of both types distinctly decreased with the development of tumour and growth of its mass. At the same time a fall in the liver catalase activity occurred.

REFERENCES

1. Nakahara W., Fukuoka F.: Toxohormone a Characteristic Toxic Substance Produced by Cancer Tissue. Gann **40**, 45 (1949).
2. Nixon J. C., Zinman B.: Toxohormone in Bacteria-Free Tumors. Canadian Journal of Biochemistry **44**, 1069 (1966).
3. Ono T., Okashi M., Yago N.: The Effect of Toxohormone on Iron Metabolism. Gann **51**, 213 (1960).

4. Ono T., Sugimura T., Umeda M.: Preparation of Potent Concentrates of Toxohormone Free from Nucleic Acid. Gann **46**, 619 (1955).
5. Ono T., Umeda M., Sugimura T.: Porphyrin Metabolism in Tumor Bearing Animals. Free Porphyrin in Liver Harderian Gland and Urine and the Effect thereon of Toxohormone. Gann **47**, 171 (1956).
6. Schwartz S., Wikoff H. M.: The Relation of Erythrocyte Coproporphyrin and Protoporphyrin to Erythropoiesis. J. Biol. Chem. **194**, 563 (1952).
7. Trojanowski J., Benesz M., Hejnar Z.: The Effect of Melanoma Tumor on Catalase Activity in Hamsters. Ann. Univ. Mariae Curie-Skłodowska sectio C **25**, 1 (1970).
8. Woodruff C. W.: A Micromethod for Serum Iron Determination. J. Lab. Clin. Med. **53**, 955 (1959).

STRESZCZENIE

U chomików z przeszczepialnym guzem czerniaka melanotycznego i jego odmianą bezpigmentową stwierdziliśmy wprostproporcjonalną zależność między ciężarem guza nowotworowego a poziomem protoporfiryn wątrobowych.

Wzrost protoporfiryn w wątrobie zwierząt z czerniakiem melanotycznym osiągnął maksymalnie poziom 98, 2 μg/100 g suchej masy, co w porównaniu z kontrolą wynosi 142%. Natomiast dla odmiany bezpigmentowej — 75 μg/100 g suchej masy, tj. 84% w stosunku do kontroli.

Poziom koproporfiryn wątrobowych wzrastał dla obu odmian o ok. 50%.

Frakcja toksohormonu izolowana z guzów czerniaka melanotycznego i oczyszczona chromatograficznie, wprowadzona drogą iniekcji, wywoływała wzrost poziomu protoporfiryn dla frakcji TH_3II 61,5% w dawce 3 μg/1 g ciężaru zwierzęcia.

Równocześnie ze zmianami poziomu porfiryn w czasie rozwoju choroby nowotworowej zaobserwowaliśmy spadek poziomu żelaza niehemowego w surowicy krwi chomików oraz spadek aktywności katalazy wątrobowej.

РЕЗЮМЕ

У хомяков с трансплантированной опухолью меланомы и ее беспигментным видом установили прямо пропорциональную зависимость между весом опухоли и уровнем печеночных протопорфиринов.

Рост протопорфиринов в печени животных с меланомой достиг максимум уровня 98,2 μg/100 г сухой массы, что по сравнению с контролем дает 142%; для беспигментного вида — 75 μg/100 г сухой массы, т. е. 84% по отношению к контролю.

Уровень печеночных копропорфиринов для обоих видов увеличился почти на 50%.

Фракция таксогорма изолированная от опухолей меланомы и очищена хроматографически и введенная путем инъекции, вызывала рост уровня протопорфиринов для фракции TH_3 II 61,5% в дозе 3 μg/1 г веса животного.

Одновременно с изменениями уровня порфиринов во время развития опухолевой болезни заметили понижение уровня безгемового железа в сыворотке крови хомяков, а также понижение активности печеночной каталазы.