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THE PATHOGENESIS OF PORPHYRINURIA IN HEMOCHROMATOSIS AND IN LEAD INTOXICATION

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The appearance of porphyrines in the urine is one of the most reliable initial symptoms of lead intoxication. In the course of the intoxication not only one kind of porphyrine is formed, because the cause of the disturbance in the porphyrine metabolism is not uniform, either. In the urine we mostly find koproporphyrine III. ; in the serum and in the erythrocytes the quantity of the protoporphyrine is increased. This latter belongs to the III. isomeric group, too. Increase of the I. isomeric group koproporphyrine can be observed in the bile, in the duodenal juice and in the stool, but it may appear in the urine as well in abnormally large quantities, together with the already mentioned kopro III.

The most important role in the formation of porphyrines was attributed to the hemoglobin ; from the investigations of *H. Fischer*, however, we know, that according to the dualistical theory no koproporphyrine I can be formed from the hemoglobin, that belongs to the isomeric group III. Another cause is to be sought for to explain its increase.

Lead and all heavy metals have the bone marrow as their point of attack. In the adult organism hemoglobin is synthetised in the bone marrow, inasmuch as the iron and pyrrolring compounds necessary to its formation are stored in the cells of the reticulo-endothelial system (RES) ; the chromogen takes up the iron in the erythroblasts. No porphyrine, as intermediaer product, could be demonstrated in normal bone marrow by means of methods at our disposal.

In order to determine, how koproporphyrine III. is formed in the bone marrow in the course of lead intoxication, the following experiments have been perfomed. We utilized white rats as experimental animals ; they were intoxicated with 1 per cent solution of lead acetate. The animals were given 1 ml. pro 100 g body weight of this solution through a stomach tube every other day. The urine of normal animals does not contain porphyrine in demonstrable quantities. Porphyrine in the urine is demonstrated in 24 hour quantities. The urine is acidified slightly with acetic acid than it is shaken out with ether. The ether is then washed out three times with water (important!) After this the dissolved porphyrine is shaken out into a known quantity (0,5—1,0 ccm) of 0,25 per cent hydrochloric acid and is examined in Wood's light.

With the aid of standard comparative solutions even the quantity of porphyrine can be determined. This solution of hydrochloric acid contains the mixture of kopro I. and III. These two fractions can be separated each other the following way: from the ether extract is poured on aluminium oxyde chromatographic microtubes and after letting it drop through, the colored ring containing porphyrine at the top is eluated with a mixture containing 9 parts of ether and 1 part of glacial acetic acid. The koproporphyrine III. migrates rapidly downward, while the kopro I. remains suspended. Determination and elution are performed in Wood's light.

Of the 25 rats observed in several series 3 died too early. The other 22 rats tolerated intoxication relatively well; if their conditions turned to the worse, the number, of treatments was reduced. Only slight losses of weight occured during the experimental period of 3 to 4 weeks. The losses in hemoglobin and erythrocytes did not amount to more than 15 to 20 per cent. After the period of 3 to 4 weeks, if treatment was continued with the original intensity, the animals usually lost a great deal of weight, became anemic and died within a few days.

Urinanalysis revealed that in 50 per cent of the animals porphyrine could be demonstrated in the urine by the end of the first week; during the second week it could be demonstrated in all rats already. In the first week the entire quantity of porphyrine corresponded to that of kopro III.

In a few animals killed at the end of the first, week we found that porphyrine could be demonstrated in the gastrointestinal tract of the animals only and that fluorescence was only slightly more intesive than in normal animals. In the bone marrow at that time we could demonstrate porphyrine neither with macroscopic fluorescence, nor with chemical methods; it was only the number of fluorescytes that was obviously higher, than that in the control animals. By implanting bone marrow into a tissue culture (rat plasma + chicken embryo extract), we could not demonstrate porphyrine formation. Technique: The culture material was ground in a Widal tube with ether and acidified with acetic acid. The protein precipitate was solved in acetic ether and extracted with 0,2 ml of 25 per cent hydrochloric acid. The drop of fluid that collected at the bottom of the tube, was examined in Wood's light.

During the second week marked red fluorescence could be demonstrated in the gastrointestinal tract, in the kidneys, as well as in the chiseled-up bone marrow. In the bone marrow smear preparate numerous fluorescytes are seen. With chemical methods we could extract porphyrine from the bone marrow; the hydrochloric acid, however, showed minimal fluorescence only, the quality of the porphyrine could thus not be determined. On implanting parts of bone marrow into a tissue culture, intensive emigration of cells could be observed after the lapse of 48 hours already. At the same time formation of considerable quantities of porphyrine could be demonstrated. The implanted material that was dark at the time of implantation, glared up in a vivid red light under the fluorescence microscope, in the emigration zone it was only occasionally that we could see one or two glistening red cells. The bone marrow of control animals produced either minimal quantities of porphyrine or none at all.

During the third week porphyrin was found in the urines of all animals. It was remarkable that in the small chromatographic column not the entire quantity of porphyrine was eluated with the 10 per cent glacial acetic acid ether; A ring representing a minimal quantity always remaned in the top layer. This ring could be solved out with concentrated acidum aceticum glaciale only. This fact suggests that in addition to kopro III. there appeared also a minimal quantity of kopro I. in the urine.

After killing the animals that had lost a great deal ot their original weight, we made the following observations. The gastrointestinal tract, the kidneys, but first of all the bone marrow, on naked eye examination in Wood's light showed a vivid red fluorescence. In smears numerous fluorescytes could be seen. Studying tissue cultures immediately following implantation, the bone marrow particle was found to show intensive red fluorescence. 48 hours later the intensity of the fluorescence was found to have become only very slightly more intensive, or, in the majority of instances, underwent no change at all. In the emigration zones porphyrine was demonstrable in individual cells only.

On basis of data published in literature (Vannotti) and of the above investigations as well, the appearence of porphyrine can be explained in the following way. As the lead attacks the bone marrow, it is probable, that the normal cooperation between bone marrow parenchyma and reticulum becomes disturbed or ceases completely and therefore the incorporation of iron into the pyrrol ring containing molecule is rendered impossible. The synthesis of hemoglobin becomes disturbed, too, and is stopped at a lower developmental niveau (*Watson*), as a consequence of which ironfree hemoglobin, i. e. porphyrine belonging to the isomeric group III. is formed in pathological quantities. On grounds of this theory the appearance of koproporhyrine in the urine of the patient or of the experimental animal, suffering from lead intoxication, may be considered as to be indicative of *a special lesion of the bone marrow*. After a certain period of time — which was approximately 3 weeks in the experiments described above — the bone marrow becomes exhausted and unable to produce more porphyrine, even under the conditions of a tissue culture.

This lesion of the bone marrow may be one of the causes of the anemia, that presents itself quite early in the course of lead intoxication of man ; this anemia could be observed in the experimental subacut intoxications to be only of a lesser degree. Of course, other pathogenesis of this anemia is also imaginable. It is most probable, that it is not only in the bone marrow, but also in other organs — for instance in the liver — that the RES suffers a lesion. In these instances cooperation between the cells of the liver parenchyma and the cells of Kupffer is also disturbed, the cells of Kupffer are unable to transfer iron slowly and gradually to the cells of the parenchyma and through them to the circulation. In consequence of this, the iron is fixed in the liver and there is no sufficient quantity of iron at the disposal of the bone marrow for the formation of hemoglobin. This may be therefore another cause of the anemia — apart from the drift toward porphyrine formation — and hemolysis brought about by metals should be considered in addition. The latter may account for the increase of protoporhyrine in the erythrocytes.

In hemochromatosis we see an extreme degree of the liver manifestation of the here described pathological condition. Since, according to data in literature, this disease also leads to porphyrinuria, and since no experimental evidence can account for the appearance near the end of lead intoxication of koproporphyrine I., more detailed study of this disease appeared to be worth while.

Opportunity to do so presented itself in connection with the case of a 35 years old man (for details see : Orvosi Hetilap, No 6., 1950, article written by *Dávid* and *Sümegi*), in which hemochromatosis was suspected clinically and this diagnosis could be confirmed and set up with the aid of biopsy material, taken from the skin. The patient was an alcohol addict ; he noted some six months previously that his skin became brown, at first only in areas exposed to sunlight, later all over his body. On admittance abdominal tenderness, especially in the liver region was found, he complained of sensations of meteorism in this region ; his liver and spleen were found to be enlarged and a minimal ascites could be demonstrated. Blood count was normal,

the ESR slightly increased, urobiliuria, positive liver function tests, increased serum bilirubin, direct diazo reaction were found. Histological examination of the skin revealed increased melanin content. The dye was found to be located exclusively to the basal layer of the skin. In addition to melanin, hemosiderin could be demonstrated in the basal membrane of sweat glands, in the gland cells proper, in the wals of smaller vessels, peri- vascularly, in a few wider bundles of connective tissue, as well as in the form of smaller aggre- gates in the upper layer of the chorium. The skin particle also gave very marked macroscopical prussian blue reaction. Copper-containing pigment could be demonstrated neither with alkaline fuchsin, nor with Mallory's copper test.

The function of the endocrine glands was more closely studied in this patient and the tests revealed marked hypofunction of them (diabetic blood sugar curve, low basic metabolism values, hypogonadism). In general, the hypofunction of glands is attributed to the deposition of iron pigment.

Urinanalysis brought the following results. The first investigations had been carried out at a time when the patient was in a relatively good condition. At that time only such quantities of porphyrine could be demonstrated in his urine, as corresponds to the upper limit of normal values. A few weeks later, when he began to become anemic (3 million red cells, Hb : 72 per cent) urin-analysis revealed excretion of 200 gamma of porphyrine. This porphyrine proved to be mainly koproporphyrine I. ; it was surprising, however, that small, but demontsrable quantities of kopro III. were also excreted.

Excretion of koproporphyrine, belonging to this latter izomeria, in the large group of toxic porphyrias has been known to occur in connection with heavy metal intoxications only. *Dobriner* et al., as well as *Bodansky* have been the only authors, who reported the presence in the urine of this dye in liver cirrhosis (1 case each). *Vigliani* and *Libowitzky* have found it also in one case of liver cirrhosis, but when the patient's history was carefully studied, it was found, that the patient had completed a Hg cure immediately prior to the examination. Experimentally excretion of kopro III. can be brought about not only with lead and mercury, but also with other heavy metals (Bi, Cu, Zn etc.) *(Putnoky-Sümegi)*.

How can we explain the observations we made with the patient's urine

Hemochromatosis is essentially a thesaurismosis of the mineral salts, characterised by a serious disturbance in the iron metabolism. This disturbance is probably a constitutional abnormity, precipitated in the middle aged by some other lesion, e. g. by excessive consumption of alcohol, more serious liver lesion, heavy metal intoxication. In consequence of the disturbed metabolism, iron is deposited in the usually already cirrhotic liver, in the pancreas (glycosuria, or at least diabetic blood sugar curve), in the skin (melanodermia), in smaller quantities in the spleen, kidneys, lacrimal and salivary glands and in the endocrine glands, containig RES elements.

As we have seen, when the process deteriorated, the patient excreted a mixture of koproporphyrine I. and III. We can explain the appearance of koproporphyrine III. in a way similar to the lead intoxication, inasmuch as the iron, stored in the liver is lost for the organism, the bone marrov becomes poor in iron and in a way discussed above »iron-free hemoglobin«, i. e. koropor-

phyrine III. is formed. In accordance with this, the appearance of koproporphyrine III. in this disease is a sign indicative of such *a special lesion of the bone marrow, as usually is brought about by intoxications with heavy metals in the first place.*

This disturbance of the hemoglobin synthesis and the consecutive koproporphyrine III. excretion and anemia occured in a very advanced stage of the disease, when, presumably due to the iron blockade, the bone marrow not only had insufficient quantities of iron at its disposal, but also the possibility for storing pyrrolring-compounds necessary for building up chromogen was reduced or ceased to exist.

It was this theory we wanted to verify with the aid of the following experiment. The white rats were blockaded with intravenous injections of china ink; on each of four consecutive days we gave 0,5 ml of this material. Following this treatment lead intoxication was initiated in a manner described above. For controls we used animals that were poisoned with lead only

At the very end of the first experimental week, just as it was seen in the first series, in the urines of the majority of the control animals porphyrine appeared; at the same, time, no porphyrine could be demonstrated in the urines of the blockaded rats. Examination of the bone marrow revealed that no prophyrine production was demonstrable macroscopically, chemically or in tissue cultures in either the blockaded, or in the control animals.

During the second week some controls were killed, some died; in the urine of each of these animals porphyrine was found. This proved to be kopro III. At the same time we could demonstrate minimal amounts of porphyrine in the urines of only four of the 16 blockaded animals. The bone marrows of control animals showed in every case vivid red fluorescence and in tissue cultures production of large amounts of porphyrine could be demonstrated, just as it was the case in previous experiments. No fluorescence was exhibited by the bone marrows of the blockaded rats. In their tissue cultures the bone marrows of seven of the 10 examined animals produced no porphyrine at all, three produced amounts that were less than those of non-blockaded animals.

Blockaded animals, as compared to those poisoned with lead only, tolerated intoxication much worse and remained alive for a period of 15 days, at the most. At the same time of the 12 controls 7 were still alive and these rats died only on the 20th—28th day resp. following the intoxication. It was also interesting to note, that anemia developed considerably earlier in blockaded animals, than in the control ones. Animals poisoned with lead only loose 15 to 20 per cent of their erythrocytes and hemoglobin up till the 3d to 5th day prior to their death; at the same time, an average loss of 40 per cent can be demonstrated in the blockaded animals.

From the literature we must mention *Vannotti's* data. He conducted experiments on basis of a somewhat similar theory, in which he examined porphyrine excretion in the urine only and demonstrated the inhibition thereof in blockaded animals poisoned with lead.

These investigations appear to confirm our theory. While in lead intoxication the produced masses of porphyrine give rise to intensive fluorescence in the bone marrow of the animal and porphyrin production can be demonstrated in tissue cultures as well, there is, if transport of pyrrolring-compounds, necessary for the formation of chromogen is disturbed by the blockade, diminished porphyrine production in the bone marrow, or none at all. At the same time the disturbance in the synthesis of hemoglobin is increased to such an extent, as may give rise to a more or less serious anemic condition.

On grounds of these experiments it may be explained why some authors (*Günther*) found no porphyrinuria in their patients suffering from hemochro-

matosis, while others (***Eppinger***) demonstrated serious, again others (***Lageder Vannotti, Dobriner, Bodansky***) only slighter excretion of that dye. These investigations explain in addition, why some authors observed kopro I. excretion, while others — just as we did — found a mixture of koproporhyrines. It can be namely understood from these experiments, that the degree and quality of porphyrinuria, together with the degree of anemia, are in correlation with the condition of the bone marrow and may disappear sub finem vitae.

As we have seen, in lead poisoning it is the excretion of kopro III. that dominates, while in hemochromatosis that of kopro I. According to the dualistical theory of *H. Fischer* kopro I. cannot be derived from the hemoglobin structure, as can be kopro III., since the hemoglobin structure belongs to the pyrrolring-compounds of the III. izomeria and from these no kopro I. can be formed without complete dissolution and re-synthesis of these rings. Several investigations prove, that kopro I. is produced by direct synthesis in the liver. In tissue cultures the liver of the chicken embryo produced, in the presence of bilirubin surplus, a technically undeterminable kind of porphyrine in our earlier experiments. As we have seen, in hemochromatosis, beside the functional lesion of the RES and the disturbance of the iron transport — it is the lesion of the liver parenchyma that dominates the condition. As a consequence of this, the kopro, I. normally synthetised there, cannot be excreted with the bile and omitting this route, it is absorbed into the blood and is excreted with the urine. Its appearance is therefore indicative of the degree of the hepatic lesion. Due to difficulties in absorption, porphyrine is deposited in the cells of the liver, too, and can be demonstrated therein at autopsy of hemochromatosis cases by means of adequate fluorescence instruments. From all this we may draw the conclusion, that in the composition of porphyrine mixtures, excreted with the urine in any kind of pathological condition, the liver also plays a decisive part.

In lead poisoning it is the lesion of the bone marrow that dominates : therefore, as we have seen, we find mainly koproporphyrine III. in the urine.

In hemochromatosis, the most seriously affected organ is, without doubt, the liver : in the urines therefore, we find kopro I., that was not excreted with the bile. Due to the great regenerability of the liver and to the course by pushes the cirrhosis may take, there may be periods, in which the urine is completely free of porphyrine ; *then, after the generalization of the process had manifested itself, together with kopro I., kopro III. may also appear in the urine.*

In both diseases, the appearance of porphyrines, belonging to the two different isomeric groups, is indicative of the general lesion of the RES and may be interpreted as a sign indicating the generalization of the process.

Summary

According to the reported animal experiments, as well as to morphological and chemical evidence obtained in them and to observations made on tissue cultures, the site of koproporphyrine III. production in lead poisoning is the bone marrow. Its appearance is indicative of a special lesion suffered by the bone marrow. This lesion may be, together with the disturbed iron metabolism and metal-hemolysis, in part responsible for the anemia developing in the course of lead poisoning. Koproporphyrine belonging to the isomeric group I., demonstrable in serious cases, is synthetized in the liver and it is the consequence of the hepatic lesion, that it appears in the urine. In cases of hemochromatosis the excretion of koproporphyrine I. was dominant, while the presence at the same time of minimal amounts of koproporphyrine III. was indicative of a sub finem vitae lesion of the bone marrow.

In both diseases the appearance of porphyrines, belonging to the two different isomeric groups, is indicative of the general lesion of the RES and may be interpreted as a sign indicating the generalization of the process.

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ПАТОГЕНЕЗ ПОРФИРИНУРИИ, НАБЛЮДАЕМОЙ ПРИ ГЕМОХРОМАТОЗЕ И ПРИ ОТРАВЛЕНИИ СВИНЦОМ

И. Шюмеги

Резюме

Опыты, проведенные на животных, морфологическое и химическое исследование этих животных, а также наблюдения, проведенные на тканевых культурах говорят за то, что при отравлении свинцом, местом образования копропорфирина III является костный мозг и это проявление этого вещества является признаком повреждения костного мозга. Кроме нарушения обмена железа и гемолиза, вызванного металлсв, это повреждение костного мозга является одной из причин ведущих при отравлении свинцом к анемии. Копропорфирин принадлежащий к первой группе изомеров проявляется в тяжелых случаях. Он синтетизируется в печени и проявляется в большом количестве в моче вследствие повреждения печени. При гемохроматсзе доминирует выделение копропорфирина I. проявляющееся в то же время ничтожное количество копропорфирина III. Указывает на наличие предсмертного повреждения костного мозга.

При обоих заболеданиях проявление порфирина принадлежащего к одной или другой группе изомеров, является признаком генерализации процесса и общего повреждения ретикуло-эндотелиальной системы.