A SUGGESTED CONTROL GENE MECHANISM FOR THE EXCESSIVE PRODUCTION OF TYPES I AND III PORPHYRINS IN CONGENITAL ERYTHROPOIETIC PORPHYRIA*  

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Communicated June 19, 1964

The curious phenotype of erythropoietic porphyria, both human and bovine, is directly related to the large amounts of type I porphyrins produced by the developing red cells of the bone marrow. The excessive uroporphyrin (URO-) I is responsible for the cutaneous manifestations, as well as the red urine, teeth, and bones. The spleen enlarges and is at least partly responsible for the increased destruction of circulating red cells so often observed. This stimulates erythropoiesis and heightened formation of porphyrins, as described elsewhere.1-3

It has been proposed4-7 that the genetic error is a deficiency of uroporphyrinogen (UPG) isomerase. This enzyme directs cyclization to UPG III, of the polypyrryl methane first formed from porphobilinogen (PBG) by PBG deaminase. UPG III is preferentially converted by a decarboxylase to coproporphyrinogen (CPG) III and this by virtue of a coproporphyrinogenase (CPGase), to the corresponding protoporphyrin (PROTO-) and heme. In the absence or relative paucity of isomerase the polypyrrol methane from PBG cyclizes to UPG I, and is decarboxylated in varying proportion to CPG I. With possible rare and minor exceptions,8 the specificity of CPGase for type III is maintained, and CPG-I is not converted to a protoporphyrin. Thus, type I porphyrinogens or their respective porphyrins are excreted or accumulated. This has been discussed in recent reviews.4-7

If the inborn error were an isomerase deficiency, a markedly reduced production of type III porphyrin and hemoglobin might be anticipated. It is true that anemia is a frequent accompaniment of the disease and may be severe,2 3 9 but there is no evidence of deficient heme or hemoglobin formation; in fact, this is considerably increased, at times compensating fully for the increased blood destruction.2 Due to the conversion of type III CPG to PROTO-, thence to heme and bile pigment, the principal evidence of increased type III porphyrin production is found in the excessive fecal urobilinogen excretion, a regular manifestation of the disease when it is sought.4 8 10 The excess is due in part to increased destruction of circulating red cells, especially in the spleen, but also to a significant, often marked, increase in formation of "early labeled" bile pigment11-12 unrelated to destruction of the more mature circulating red cells. This fraction may be formed by destruction of young cells, or heme derived from them, even before their entrance into the circulation. A small proportion may be of extraerythropoietic source.

The theory that the basic genetic abnormality is a deficiency of isomerase stimulated re-examination of data derived from our earlier studies in the human and bovine disease5 3 9 and led to some additional observations.

Materials and Methods.—The three human cases studied during the past 15 years have been described in detail in earlier papers.1 3 9 In these, methods were cited for the fecal urobilinogen, urinary and fecal porphyrins, and erythrocyte-free porphyrins. These methods were used in
obtaining data which will be considered presently. In addition, a modification of Erikson's paper chromatographic technique for the (free) coproporphyrin (COPRO-) isomers as well as the method of Cornford and Benson for the uroporphyrin (URO-) isomers (methyl esters) have been used. Hemoglobin PROTO- and free erythrocyte PROTO- were reduced catalytically to mesoporphyrin. The zinc and sodium salts of the latter porphyrin were subjected individually to ascending chromatography on Whatman no. 1 paper with solvent systems of 2,6 lutidine:5% aq. zinc acetate (4:1) or 0.8% NaOH (6:1). The zinc method permits somewhat sharper separation, detecting type I isomer at the 3% level. It is advantageous to use both, as the isomers move in opposite fashion in the two systems. The sodium salts run in the same relative order as the ammonium salts which have also been used.

Normal and porphyric Holstein-Friesian cattle have provided blood and bone marrow.

In a series of experiments, hemolysates of human or bovine red cells, prepared essentially according to the later method of Dreezel and Falk, were incubated with varying amounts of PBG for 2-8 hr, whereupon the unchanged PBG and the porphyrins formed from it were determined. In some experiments the red cells were first separated by repeated centrifugation, the lighter fraction being reticuloocyte- and uroporphyrin-rich, as contrasted with the cells of greater density.

Bone marrow was obtained from bovine porphyrics before and after repeated bleeding, amounts of 1500-4000 cc being removed in 2-4 days, depending on the size of the animal. The percentage of fluorescing normoblasts was counted on dry smears mounted in glycero-alcohol (3:1) in a modified Reichert fluorescence microscope allowing superposition of fluorescence and phase contrast image.

Results.—Table 1 includes data for fecal urobilinogen, urinary and fecal URO- and COPRO-. As noted by others the proportion of type III porphyrin

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Hemo-globin, gm %</th>
<th>Retic. %</th>
<th>UBG, mg/d</th>
<th>Urine and feces URO- + COPRO-, mg/d</th>
<th>URO, % III</th>
<th>COPRO- after decarboxylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. H.</td>
<td>♂</td>
<td>4</td>
<td>5.0</td>
<td>7.8</td>
<td>422 (25-40)*</td>
<td>195.0 (&lt;0.5)*</td>
<td>11.0</td>
<td>5.5</td>
</tr>
<tr>
<td>M. H.</td>
<td>♂</td>
<td>5</td>
<td>10.5</td>
<td>5.4</td>
<td>461 (25-40)*</td>
<td>82.4 (&lt;0.5)*</td>
<td>15.0</td>
<td>5.1</td>
</tr>
<tr>
<td>M. M.</td>
<td>♂</td>
<td>25</td>
<td>9.8</td>
<td>13.1</td>
<td>731 (100-200)*</td>
<td>177.0 (&lt;1.6)*</td>
<td>21.0*</td>
<td>5.5</td>
</tr>
</tbody>
</table>

* Approximate normal range.
† Cornford-Benson method. Accuracy with this method is stated to be considerably reduced below 17%, and hence the values for coproporphyrin obtained after decarboxylation are regarded as more accurate.
‡ Av. of two runs ± 4 weeks apart.

is relatively small, but the actual amount is much greater than in the normal.

The porphyrin-forming activity of both human and bovine porphyric hemolysates is regularly much greater than that of hemolysates of normal red cells, when incubated with PBG. In accord with this there is more efficient utilization of PBG. Data from such an experiment are given in Table 2. As indicated, this animal had been bled to provide greater stimulus to erythropoiesis, prior to the experiment. It is seen that the lighter, reticuloocyte and uroporphyrin-rich cells produced considerably more PROTO-9 than either the heavier or the normal control cells, the last two types forming about equal amounts of total porphyrin. The differing proportions of URO- isomers formed by the three hemolysates are evident. An increased formation of PROTO- by the porphyric blood might be anticipated as it regularly contains increased numbers of reticuloocytes; nevertheless, this also reveals a highly efficient isomerase activity. Similar data have been obtained with other porphyric hemolysates, human as well as bovine, the latter subjects both with and without preliminary bleeding. As yet, we have not obtained comparable data from otherwise normal subjects in which erythropoiesis has been stimulated by bleeding. In Table 3 the much greater utilization of PBG and increased formation of PROTO-9 by the porphyric
hemolysate is noted. The relatively large proportion of type I uroporphyrin formed by the heterozygote's hemolysate may be due at least in part to inadequate cooling of the blood prior to incubation although the ratio of PBG to red cells and hence to isomerase may also have been significant. Since no attempt was made to determine newly formed heme, any additional PROTO-9 used in heme formation would not have been detected.

Bleeding of the porphric animals was regularly followed by an increase in the percentage of fluorescing normoblasts in the bone marrow from a range of 35–50 per cent to one of 80–93 per cent. In the controls the initial percentage and increase was negligible, from a range of 1.5–2.5 to one of 2.0–2.5.

**Discussion.**—Fischer and Kirrmann first provided direct evidence that the hemoglobin PROTO-in erythropoietic porphyria is type 9 (series III). This finding has recently been confirmed by more detailed studies of both human and bovine material. The increased amount of free PROTO- of the circulating red cells in this disease does not support the concept of an isomerase deficiency. This PROTO- has also been identified repeatedly as type 9, series III.

Heilmeyer and co-workers have recently noted outspoken increases of PROTO- when human porphric hemolysates were incubated with ALA. They observed that this finding did not support the concept of an isomerase deficiency.

The data emphasize the greater activity of deaminase which must be taken into account in considering the basic genetic abnormality. The overproduction of both type I and III porphyrins suggests that the underlying error is a control gene mutation with lack of repression of enzyme synthesis, in accord with the Jacob-Monod concept. While there is a considerable list of control gene mutations in various human diseases, nearly all have been characterized by a deficiency rather than an increase of protein synthesis. The increased propphyrin production in erythropoietic porphyria is more consistent with a constitutive regulator mutation which is expected to be recessive. Lack of repression on this basis might be due to failure of the regulator gene to provide repressor or an
“aporepressor” sensitive to its normal effector.30 Several models may be considered, one with a single operon including the genes for ALA synthetase through ferrochelatase; a second in which two operons control, respectively, the synthesis of ALA and PBG, and the segment from PBG to heme; a third in which at least the genes of the latter segment are unlinked, under noncoordinate control. Each of these presents theoretical advantages and with certain assumptions is consistent with the (unbalanced) excessive porphyrin production. A single operon would accord with evidence thus far derived from bacterial enzyme systems in which clustering of determinant genes with a single operator is usual;30 nevertheless, exceptions have been noted and may well be more frequent in higher forms. Assuming a single operon of the above linkage, a lack of repression would effect increased formation of ALA synthetase and might thus explain the excessive porphyrin production. Excessive induction of ALA synthetase in liver cells may be caused by a variety of “porphyrogenic” chemicals;4 this is followed by rapid conversion of ALA, in part, to PBG and porphyrins. ALA synthetase, in contrast to PBG deaminase and UPG isomerase, is scarcely demonstrable in normal cells.6 As Granick4 has suggested, it seems not unlikely that the genetic abnormality in the liver in acute intermittent porphyria is a lack of repression and consequent induction of ALA synthetase, although this has not been demonstrated. The characteristics of this dominant form of porphyria differ greatly from those of the recessive erythropoietic porphyria, the urine containing large amounts of ALA and PBG, which are not observed in the latter disease.3,4 Although the two diseases represent entirely distinct genetic errors, a primary overproduction of ALA might nevertheless be common to both, in which event the widely differing phenotypes might conceivably depend on differences in liver cells and normoblasts in terms of rates of use or release of ALA and PBG. In other words, much of the excessive ALA and PBG would escape from liver cells but would remain in normoblasts for a sufficient period to be converted to porphyrin. This would require that the physiological concentration of isomerase be limited in relation to that of deaminase, but sufficient to convert most of the PBG normally formed, to UPG III. Cornford,32 in fact, has recently reported such a limitation of isomerase in normal red cells. Thus, if lack of repression of a single operon is postulated for erythropoietic porphyria, the normal amount of isom-

![Table 3: Incubation of Bovine Porphyrin Hemolysates with PBG](image)

<table>
<thead>
<tr>
<th>Prelim. data</th>
<th>Retio., %</th>
<th>Packed cells, ml</th>
<th>Final vol., ml</th>
<th>Eryth. Porph. Conc., µg</th>
<th>Mg PBG added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygote; cow #2120</td>
<td>12.1</td>
<td>10.0</td>
<td>60.0</td>
<td>35.5</td>
<td>7.0</td>
</tr>
<tr>
<td>Heterozygote; cow #2121</td>
<td>0</td>
<td>10.0</td>
<td>60.0</td>
<td>6.2</td>
<td>0.4</td>
</tr>
<tr>
<td>PBG only Incubation 37° 8 hr</td>
<td>0</td>
<td>0</td>
<td>60.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Results**

<table>
<thead>
<tr>
<th></th>
<th>PROTO-</th>
<th>COPRO-</th>
<th>URO-</th>
<th>Mg PBG remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>#2120</td>
<td>365†</td>
<td>257‡</td>
<td>1008*</td>
<td>0.25</td>
</tr>
<tr>
<td>#2121</td>
<td>42‡</td>
<td>19</td>
<td>280§</td>
<td>1.2</td>
</tr>
<tr>
<td>PBG only</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*95-100% I.
†95% I.
‡85-90% I.

30 After conversion to mesoporphyrin and paper chromatography, only meso-9 (Series III) was demonstrable.

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merase might be able to compete successfully for some of the excess PBG, to form UPG III, the remainder providing the large increases in amount of type I porphyrins. There is some indication of a difference in relative permeability of normoblasts versus liver cells, to ALA, \textit{in vivo}.\textsuperscript{33-35} It is possible that the rate of conversion of PBG to uroporphyrinogen is much more rapid in normoblasts than liver cells, thus preventing accumulation and leakage of PBG from the former.

A model having two operons, the first for ALA-PBG, the second for PBG-Heme requires some consideration, not only for erythropoietic porphyria but for hepatic acute porphyria as well as the newly described \textit{protoporphyria}.\textsuperscript{36, 37} With a regulator constitutive mutation of the first operon the result would be similar to that just discussed except that the amounts of deaminase and isomerase formed in the second, repressed operon, would be expected to be much smaller in relation to the excessive PBG and hence less appropriate for erythropoietic porphyria, though consistent with acute porphyria.

With such a mutation in a second operon for the segment PBG-Heme, a proportional increase of ALA formation related to the first operon would be required. With lack of repression of the second operon it might be assumed that utilization of PBG from the first would be more efficient, thus employing a greater proportion of the total ALA formed, at the expense of the fraction ordinarily available for alternative pathways.\textsuperscript{34} Some form of positive feedback to formation of ALA synthetase may also be considered, especially in view of the observation that the rate of hemoglobin synthesis may increase in individual normoblasts, especially those which are precursors of so-called "stress" red cells.\textsuperscript{39} It is recognized that there is also evidence of a negative feedback control of ALA synthesis.\textsuperscript{6, 40}

The imbalance favoring deaminase might relate to proximity of the structural gene for deaminase to the derepressed operator. In a general way this relates to the problems of coordinated transcription and operon polarity.\textsuperscript{29-31} Recent studies\textsuperscript{41-43} indicate that the rate of synthesis of one enzyme whose cistron is operator-distal may be considerably less than that of another whose cistron is operator proximal. Thus S\textsubscript{1} (deaminase) proximal to the operator might receive greater influence than S\textsubscript{2} (isomerase), at least under the conditions of a regulator constitutive mutation.

The third possibility involves unlinked genes with a single regulator and repressor for individual operator and structural genes.\textsuperscript{30, 31} Derepression due to a regulator gene mutation would be fully consistent with the deaminase-isomerase imbalance on the basis of a differential activity of homologous operator genes.\textsuperscript{31} The regulator gene for arginine synthesis by \textit{E. coli} induces acetyl ornithine \(\delta\)-transaminase while other unlinked genes in the pathway are being repressed.\textsuperscript{44} In terms of the overproduction in the various forms of porphyria, there might be one or two sets of regulator genes and repressors, comparable in location to those in the single or double operon models discussed in the foregoing. With unlinked structural genes at least for the segment PBG-Heme, noncoordinate repression might be the basis of the deaminase-isomerase imbalance in erythropoietic porphyria.

Constitutive operator (\(O^c\)) mutations would appear more reasonable for the dominant forms of porphyria. Advantages are apparent in respect to acute porphyria, whether the structural genes for ALA and PBG are linked or unlinked. Such mutations in bacteria have been found to be \textit{partially constitutive},\textsuperscript{29} perhaps
in accord with the more variable course of the dominant forms of porphyria, as contrasted with the recessive erythropoietic type. The latent or intermittent character of acute porphyria as well as the adverse effects of various chemicals such as barbiturates\textsuperscript{4} and estrogens,\textsuperscript{43} and of variations in diet,\textsuperscript{46} are perhaps more consistent with an $O$ mutation controlling ALA synthetase induction, as these factors might inhibit an allosteric repressor.\textsuperscript{47} This could also explain the spontaneous improvement which may occur in protoporphyria\textsuperscript{48, 49} and which has been noted but rarely, and then in any marked degree only after splenectomy, in the recessive erythropoietic porphyria. A partially constitutive mutation might also account for the lesser increases of protoporphyrin and negligible increase of type I porphyrins in protoporphyria, as the amount of isomerase might be adequate for conversion of most of the PBG to UPG III, thence to PROTO-. Globin formation may be rate-limiting in so far as the final heme synthesis is concerned.\textsuperscript{50}

Heilmeyer and associates have recently mentioned repressor deficiency as a possible basic abnormality in erythropoietic porphyria. Although the above suggestions are based on the repressor-operator hypothesis,\textsuperscript{28, 29} other possibilities are not excluded, among which the "modulation"\textsuperscript{42} or "operatorless" model\textsuperscript{42} may be mentioned. According to this concept there is direct control of messenger RNA transcription, with modulation up or down for varying cistrons in a given pathway. "Up modulations," requisite for porphyria in terms of increased production, are believed indistinguishable from $O$ mutations,\textsuperscript{42} and hence would be expected to be dominant and thus unlikely for erythropoietic porphyria.

The localization of the genetic disturbance in the same biosynthetic pathway, to the liver cells in hepatic porphyria and to the developing red cells in erythropoietic porphyria, poses highly interesting questions in relation to tissue differentiation and genetic control systems. The possibility exists that differing ALA synthetases are formed by separate control systems in liver cells and erythroblasts, in analogy to the differing phosphorylases in heart, liver, and muscle and the genetic abnormality in McArdle's disease restricted to muscle phosphorylase.\textsuperscript{31} The presence of more than one ALA synthetase in \textit{Rh. spheroides} could not be demonstrated.\textsuperscript{40} In erythropoietic protoporphyria the liver as well as the bone marrow may be responsible for the excessive protoporphyrin production,\textsuperscript{52, 53} but the data suggesting this can also be interpreted in support of an exclusive erythropoietic formation.\textsuperscript{54}

The close relationship of the excessive type I porphyrin formation to hemoglobin synthesis in erythropoietic porphyria deserves some emphasis in the present context. Bieding is followed by increased erythropoiesis, a prompt and marked increase of fluorescing normoblasts in the bone marrow and of free porphyrins in the circulating red cells. It was initially believed\textsuperscript{1, 2, 25} that there were two lines of normoblasts in erythropoietic porphyria, one representing the genetic error forming free porphyrin in excess, hence fluorescing, the other nonfluorescing and believed to be normal. The present finding that the fluorescing normoblasts in the bone marrow rapidly increase to a range of 80–95 per cent, when porphyrine animals are bled, indicates that the normoblasts are unimodal in respect to the genetic error. Those which fluoresce may simply represent cells in which hemoglobin synthesis is more active,\textsuperscript{39} and the others those in which it is less active or has stopped.

Rimington\textsuperscript{46} observed that a porphyrin hemolysate, on incubation with PBG, yielded equal amounts of types I and III uroporphyrins, while in the normal control
only type III appeared. Under appropriate conditions our incubation experiments indicate that the lighter cells form a greater amount of URO-I than the heavier, more mature cells, at the same time producing larger amounts of PROTO-I.

Evidence has also been obtained that at least a considerable proportion of the younger, uroporphyrin-rich cells are short-lived,\textsuperscript{46} similar in this respect to "stress" red cells.\textsuperscript{38} The greater imbalance between the soluble enzymes, deaminase and isomerase, in younger cells would appear to be more consistent with the single operon model. It remains to be determined whether this imbalance is characteristic of (porphric) "stress" red cells and especially the corresponding reticulocytes.

Summary.—Erythropoietic porphyria is characterized by an excessive production of type III as well as type I porphyrins. The erythrocyte-free PROTO-9 is increased, especially in the bovine disease, and hemolysates of porphyrin red cells, both human and bovine, form more PROTO- than normal hemolysates. The amount of type III formed per day generally exceeds that of type I, but much of the type III is represented as bile pigment derived from PROTO- while the type I is not converted to the corresponding protoporphyrin and appears as URO- or COPRO-I. The amount of type I formed normally is very small, and hence the excess in this disease is relatively great. This is regarded as a greater than normal imbalance in the activity of PBG deaminase and UPG isomerase, favoring the deaminase. On the basis of this unbalanced overproduction a lack of repression related to a constitutive regulator mutation, in accord with the concept of Jacob and Monod, is believed to provide a reasonable explanation of the genetic error. Hypothetical models are considered, involving certain assumptions consistent with present knowledge of the classical erythropoietic as well as certain other forms of porphyria. These should be regarded simply as approaches to an understanding of the remarkable overproduction of porphyrin which characterizes this disease. At the present time there are serious obstacles to experimental testing with special reference to problems of permeability and cell culture, as well as the application of bacterial genetic techniques to the human or bovine species.

We gratefully acknowledge the advice and stimulus provided by Prof. John Spizizen, during the preparation of this manuscript, and also valuable discussions with Profs. Elving Anderson, Heinrich Kluver, and S. Schwartz. We are indebted to Drs. LaRue Johnson, William Moore, and Wallace Wass of the University of Minnesota Veterinary Clinic, for their cooperation in procuring samples of bovine blood and bone marrow.

\textsuperscript{*} Supported by RG AM 04435 and A 2096, USPHS.


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