DIETARY PROTEIN AND PROTOPORPHYRIN FORMATION IN THE RAT*

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The wide-spread occurrence of the porphyrins as constituents of many important respiratory catalysts, such as hemoglobin, the cytochromes, and catalase, attaches fundamental importance to the problem of the origin and metabolism of these substances in the organism. Progress in this field has been retarded by a lack of satisfactory methods of quantitative analysis. However, in 1943, Grinstein and Watson (1) published a colorimetric method for the determination in erythrocytes of protoporphyrin, an alleged intermediate in the formation of heme. The introduction of this method appeared to offer an opportunity for a quantitative study of protoporphyrin in other body tissues and in the excreta.

Earlier work by other investigators has demonstrated that protoporphyrin is the predominant porphyrin appearing in the feces of the rat and that only a trace is eliminated in the urine (2, 3), even after the parenteral administration of protoporphyrin (3). Little is present in other body fluids or tissues with the exception of the Harderian glands (4), the intestinal contents (4), erythrocytes, especially reticulocytes (1), and in the white matter of the central nervous system (5). The fecal protoporphyrin appears to be that formed in excess of the requirement for hemoglobin synthesis and excreted in the feces perhaps by way of the Harderian glands in the rat, since the removal of these structures results in a marked decrease in fecal protoporphyrin to practically nil (4). Some protoporphyrin may be synthesized by the bacteria of the intestinal flora, although the amount from this source appears to be extremely small (4, 6) unless there is hemorrhage into the intestine (7).

In view of the above evidence, the assumption that protoporphyrin synthesis in the rat could be ascertained from fecal protoporphyrin excretion appears justified. The present study was designed, therefore, to determine whether dietary protein serves as a precursor of protoporphyrin

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in the rat, as is indicated by the studies of Schoenheimer and his coworkers (8), by means of certain amino acids containing isotopic nitrogen, and, if so, the proportion of the protein intake allotted to porphyrin synthesis in vivo. Fecal protoporphyrin excretion was followed quantitatively in groups of rats fed adequate or low protein diets.

EXPERIMENTAL

Male rats of the Connecticut Agricultural Experiment Station strain weighing 40 to 50 gm. at weaning were used. They were divided litterwise into two groups and were housed in individual wide mesh screen bottom cages. One group (twelve rats) received an "adequate protein" diet having the following percentage composition: casein 22.5; sucrose 10; white corn dextrin 36.5; hydrogenated cottonseed oil 27; Wesson's salt mixture 4. The other group (twelve rats) was given a low protein diet having the same composition with the following exceptions: casein 3.5; white corn dextrin 55.5. All animals were given the following vitamin supplements: 200 mg. of ryzamin-B ² and 200 mg. of liver extract ³ daily; 3 drops of halibut liver oil with viosterol twice weekly.

The animals of both groups were weighed and the food consumption was carefully measured weekly. Each day the feces were collected quantitatively on absorbent paper. A 16 week period of observation was used and duplicate protoporphyrin determinations were made biweekly on the feces of each animal.

The following procedure was used for protoporphyrin analysis: The daily feces collection was weighed to 0.01 gm. and was placed immediately in 95 per cent ethyl alcohol and stored in a refrigerator. Pooled weekly samples for each rat were then homogenized in 95 per cent ethyl alcohol in a Waring blender, dried at room temperature, and ground to a fine powder which passed through a 60 mesh sieve. This powder was then dried in vacuo at 60°, and duplicate 0.500 gm. aliquots were taken for protoporphyrin determination by a slight modification of the Grinstein and Watson (1) method. The final colored solution was read in a photoelectric colorimeter with a 410 μm filter. In addition, readings were made with a special 554 μm filter, a wave-length at which protoporphyrin has a characteristic absorption maximum. ⁴ The values obtained at both wave-lengths agreed closely. A calibration curve was constructed with crys-

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¹ No. 453, Casein Manufacturing Company, New York.
² No. 2, Burroughs Wellcome and Company, Tuckahoe, New York. Appreciation is expressed to Mr. D. M. Cypher for supplying this material.
³ Liver extract "B," from The Wilson Laboratories, Chicago. Appreciation is expressed to Dr. David Klein for furnishing a generous supply of this extract.
⁴ Dr. R. H. McCoy, University of Pittsburgh; personal communication.
talline protoporphyrin dimethyl ester. Satisfactory recoveries (average 96.9 per cent) of 30 to 45 γ samples of protoporphyrin added to dried feces were obtained.

Analyses of the two diets (10 gm. samples) employed and of daily portions of the vitamin supplements used demonstrated that the protoporphyrin content of each was only a questionable trace, and hence of no practical significance.

Hemoglobin determinations were made by a photoelectric acid hematin method at regular intervals.

### TABLE I

<table>
<thead>
<tr>
<th>Protein diet</th>
<th>Wks.</th>
<th>Dried feces</th>
<th>Per rat</th>
<th>Body weight</th>
<th>Protein ingested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>γ per 0.5 gm.</td>
<td>γ per day</td>
<td>γ per 100 gm.</td>
<td>γ per gm.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>55 (31–98)</td>
<td>146 (67–224)</td>
<td>56 (24–91)</td>
<td>55 (23–90)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>68 (50–102)</td>
<td>182 (136–282)</td>
<td>53 (38–75)</td>
<td>76 (50–129)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>73 (33–113)</td>
<td>202 (107–295)</td>
<td>52 (32–81)</td>
<td>78 (38–115)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>89 (52–104)</td>
<td>232 (165–344)</td>
<td>54 (39–68)</td>
<td>91 (63–118)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>97 (62–122)</td>
<td>241 (179–367)</td>
<td>52 (38–70)</td>
<td>103 (62–149)</td>
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<tr>
<td></td>
<td>16</td>
<td>86 (52–107)</td>
<td>212 (122–305)</td>
<td>43 (29–55)</td>
<td>92 (59–110)</td>
</tr>
<tr>
<td>Low</td>
<td>2</td>
<td>29 (25–35)</td>
<td>19 (16–22)</td>
<td>41 (35–44)</td>
<td>127 (93–164)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>31 (16–40)</td>
<td>17 (7–22)</td>
<td>38 (18–48)</td>
<td>143 (86–217)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>37 (31–46)</td>
<td>15 (8–19)</td>
<td>30 (19–38)</td>
<td>153 (72–193)</td>
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<tr>
<td></td>
<td>8</td>
<td>28 (11–55)</td>
<td>13 (5–24)</td>
<td>31 (12–58)</td>
<td>121 (43–232)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>23 (2–46)</td>
<td>13 (1–37)</td>
<td>28 (3–67)</td>
<td>123 (11–256)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>16 (13–19)</td>
<td>8 (6–11)</td>
<td>17 (12–22)</td>
<td>93 (76–120)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>13 (6–19)</td>
<td>7 (2–12)</td>
<td>14 (7–21)</td>
<td>71 (31–111)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>12 (11–14)</td>
<td>6 (6–6)</td>
<td>13 (13–13)</td>
<td>94 (92–95)</td>
</tr>
</tbody>
</table>

* The values given are group averages with the minimum and maximum for individual rats in parentheses.

### Results

The averaged results for the two groups of rats, together with the range of individual values for fecal protoporphyrin excretion at biweekly intervals, are given in Table I. It is evident that the controls fed the adequate protein diet excreted considerably more protoporphyrin per 0.5 gm. sample, per day, and per 100 gm. of body weight per day than did the

* Sincere appreciation is expressed to Dr. C. J. Watson, University of Minnesota School of Medicine, to Dr. W. J. Turner, Veterans Administration, and Captain John Mason, Army Medical School, for generous samples of protoporphyrin.
rats fed the low protein ration. The difference was evident within 4 weeks after the experiment began and became more pronounced as the study progressed. The rather constant protoporphyrin excretion per 100 gm. of body weight per day observed in the control animals is of interest, as likewise is the steady decrease in this value seen in the low protein rats. This decrease paralleled the development of the chronic anemia usually observed in protein-deficient rats (9), the average value at the end of the 16 week period being 10.7 gm. of hemoglobin per 100 cc. of blood, as compared with 16.8 gm. per 100 cc. for the controls.

The values obtained for fecal protoporphyrin excretion when calculated on the basis of micrograms per gm. of dietary protein consumed show a rather striking contrast between the two groups of rats. The averaged results show clearly that in the first part of the experiment the low protein rats showed a much greater utilization of dietary protein for protoporphyrin formation than did the adequate protein controls. This difference became less pronounced as the experiment progressed, however, perhaps for two reasons. First, there was a general failure in hemopoiesis in the low protein rats, as evidenced by the development of an anemia. Second there was a decreased rate of growth in the control animals, and hence perhaps more protein became available to the organism for porphyrin formation. The data thus suggest that protoporphyrin synthesis, like hemoglobin synthesis (10, 11), has a "high priority call" on available protein in the organism and that porphyrin formation takes precedence over the formation of general body tissue protein when the intake of protein is limited.

DISCUSSION

The foregoing results indicate that if fecal protoporphyrin excretion is a measure of porphyrin synthesis in the organism, as appears to be demonstrated by the existing evidence, dietary protein serves as the ultimate precursor of the porphyrins in vivo. This conclusion is in accord with the observations of Schoenheimer and his coworkers (8) previously mentioned.

The question of the identity of the amino acid or acids which serve as precursors for the porphyrin nucleus is one of fundamental and practical importance. Years ago Fischer and others (see (12)) suggested that such substances as proline or tryptophane might serve this purpose. In this connection it is pertinent to note that Shemin and Rittenberg (13) have now demonstrated, using the isotopic nitrogen technique, that glycine is involved in hemin formation, thus in protoporphyrin formation in man. Under the same experimental conditions, no direct utilization of either proline, glutamic acid, leucine, or ammonium citrate for porphyrin synthesis could be demonstrated. It is also of interest that acetic acid may be
used in the *in vivo* synthesis of porphyrin, as indicated by the use of isotopic carbon as a tracer (14). Evidently an adequate supply of this important chemical nucleus is thus assured to the organism by synthesis from simple plentiful units. Such a general concept of the importance attached to porphyrin synthesis in the organism is further supported by the data obtained in the present study, demonstrating that protoporphyrin formation, like hemoglobin formation, ranks high in the preferential allotment of protein for anabolic purposes *in vivo*.

**SUMMARY**

The effect of a diet low in protein (3.5 per cent casein) on fecal protoporphyrin excretion in the rat was followed for a 16 week period.

Porphyrin excretion of the low protein animals was consistently much less than that of control rats when calculated either as micrograms of protoporphyrin per 0.50 gm. of sample, per rat per day, or per 100 gm. of body weight per day, but was significantly greater when expressed as micrograms per gm. of dietary protein ingested.

Protoporphyrin excretion of the low protein rats decreased progressively during the period of observation, while the usual chronic anemia developed.

If fecal protoporphyrin excretion is an index of porphyrin synthesis *in vivo*, as the available evidence indicates, these data demonstrate that dietary protein serves as a precursor of the porphyrin nucleus in the rat and that porphyrin formation, like hemoglobin formation, has a high "priority rating" for available protein in the organism.

**BIBLIOGRAPHY**

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