Effect of Ingestion of Hemoproteins on Fecal Excretion of Hemes and Porphyrins
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Stools from asymptomatic volunteers on diets containing red meat, whole blood, or high fiber were analyzed for the content of hemes and dicarboxylic (heme-derived) porphyrins by the "HemoQuant" assay, the "Hemocult" test, and "high-performance" liquid chromatography (HPLC). In 49 subjects, ingestion of red meat increased HemoQuant-determined combined fecal heme plus dicarboxylic porphyrins by an average 375%: the contribution of heme-derived porphyrins to total fecal porphyrins increased from 37% to 78%. Of subjects on a red-meat diet, 27% passed stools with a porphyrin content suggestive of a porphyria, compared with only 4% on a red-meat-free diet. These increases were due largely to protoporphyrin and its derivatives pempithophore and deuteroporphyrin, all of which were present in feces as iron-free porphyrins and iron-ligated (heme) forms. Ingestion of blood had an effect similar to that of red meat, but ingestion of fiber had no effect. These effects of dietary and endogenous hemoproteins must be considered when such methods are used to test feces for occult blood or to test for excess fecal porphyrins as an indicator of a porphyria.

Additional Keyphrases: variation, source of • protoporphyrin • diet-related effects • fluorometry • chromatography, reversed-phase • porphyrin digestion

Detection in feces of the iron-porphyrin blood pigment, protoporphyrin, and its iron-free component, protoporphyrin, along with the further dicarboxylic derivatives indicated in Figure 1, is of clinical importance as an indicator of gastrointestinal pathology or disordered metabolism of the porphyrins. Heme6 in the intestinal lumen may be derived from bleeding caused by a broad range of intestinal disorders, including cancer (1–4). Nonpathological sources of luminal heme and heme-related porphyrins include physiological shedding of cells and (or) nonpathological microbleeding (5). Only 5% to 15% of this heme is absorbed (6); most of the heme or its derivatives in the lumen is excreted in feces. Ingested heme would be expected to follow the same fate, but opinions on this are contradictory.

Evidence based on studies with the guaiac (7) and fluorimetric "HemoQuant" (8) (patent now owned by SmithKline Bio-Science Laboratories, Philadelphia, PA) tests indicates that dietary heme or its derivatives will appear in feces. Yet van den Berg et al. (9), using a fluorimetric assay for heme derived from the HemoQuant assay, have claimed that meat ingestion does not affect fecal heme excretion.

Furthermore, Beukeveld et al. (10) have stated that dietary heme is "mostly converted to bile pigments, not giving rise to creation of porphyrins." These workers suggest that fecal porphyrins related to heme are almost solely derived from de novo synthesis by bacteria. An alternative is that these porphyrins are derived from heme in the gut by bacterial or biochemical action; i.e., they are "intestinally derived."

If measurement of fecal excretion of heme and dicarboxylic porphyrins is to be used as a means of detecting or measuring gastrointestinal blood loss, then these opposing views on the fate of ingested heme and the origin of fecal heme and dicarboxylic porphyrins must be resolved.

Fluorimetry provides a relatively simple means for measuring fecal porphyrins; two fluorimetric approaches are available: the HemoQuant assay (11) and "high-performance" liquid chromatography (HPLC) with fluorimetric detection (12, 13). HemoQuant, the principal nonchromatographic method for quantifying the hemes and dicarboxylic porphyrins naturally present in feces (4, 8, 11, 14, 15), quantifies the fluorescence of these porphyrins when exposed to ultraviolet light. For measuring the nonfluorescing hemes, HemoQuant incorporates a reduction step with oxalic acid to remove the iron responsible for quenching the fluorescence. Another fluorimetric assay for heme, derived from the HemoQuant assay (9), is so recent that there is little experience with its practical application.

HemoQuant cannot differentiate between individual dicarboxylic porphyrins. The separation and quantification of the various porphyrins present in feces is best achieved by HPLC combined with fluorescence detection (12, 13). In its standard form, HPLC does not detect any of the hemes because of the quenching of fluorescence by iron. The sensitivity and precision of HPLC and HemoQuant contrast with those of the simple, inexpensive leukodye tests (e.g., guaiac-based Hemocult II; SmithKline Diagnostics

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2 We use "heme" or "hemes" to refer to any iron-containing dicarboxylic porphyrin, including protoporphyrin (the prosthetic group of hemoglobin and myoglobin), pempithophore, and deuteroporphyrin. Heme-derived porphyrins (HDPs) refers to any porphyrin remaining after removal of iron from a heme, whether this removal be in vivo (i.e., intestinally derived) or in vitro.

Received April 10, 1989; accepted August 14, 1989.

2290 CLINICAL CHEMISTRY, Vol. 35, No. 12, 1989
Inc., San Jose, CA). Guaiac tests are qualitative (16), and they detect only the pseudoperoxidase activity of intact heme and not the derived porphyrins (5). The ability of intestinal factors, recently shown to be luminal bacteria, to remove iron from heme was observed many years ago (9, 11, 17). Heme that has undergone such modification cannot be detected by the leukoyde tests.

In this study we have clarified the effect of challenge diets containing red meat or blood on the fecal excretion of heme and heme-derived porphyrins. These were detected or measured by three methods: qualitative guaiac tests ("Hemoccult II" and "HemoccultSENSA" [SmithKline Diagnostics Inc.]), HemoQuant, and HPLC. We also determined whether the vinyl side chains of heme could be modified before the in vivo removal of iron.

**Materials and Methods**

**Diet-Study Subjects**

Forty-nine asymptomatic volunteers, ages 16–30 years, were studied. None had a history of gastrointestinal bleeding, including hemorrhoidal bleeding, or any other gastrointestinal disease that could cause bleeding. None was taking non-steroidal anti-inflammatory drugs or was menstruating during the study period.

**Study Design**

Each subject was studied on days 4 to 6 of three different dietary periods. The diets were: (a) an exclusion diet based on recommendations for guaiac-based occult blood tests (7) in which red meat and high-peroxidase plant foods were excluded; (b) a meat challenge diet of 250 g or more of rare red meat (beef) per day, while peroxidase-containing plant foods were excluded; and (c) a vegetable challenge diet of three cups of raw high-peroxidase plant foods per day (e.g., cantaloupe, radish, turnip, broccoli, and horseradish), with no red meat (18).

**Samples**

After the initial three-day period on a diet, the subjects used a plastic scoop to collect 5–10 g of feces from three consecutive stool specimens, which was then sealed in a screw-capped container. The subjects also prepared two Hemoccult slides (two windows per slide) from each stool. Samples were refrigerated until collection of the subjects' third specimen, then delivered to the laboratory without delay. At the laboratory, samples were stored at −20 °C for later assay. The delay between collection of the final sample and freezing was usually 24 to 48 h. Hemoccult slides were developed without hydration (2) within three days of delivery to the laboratory.

**Blood-Ingestion Study**

Four presumably healthy subjects on a red-meat-prohibited diet made total stool collections during the 24 h before and three 24-h periods after ingestion of either 2, 5, or 10 mL of their own blood (in 200 mL of cold tomato juice). Stools were stored at −20 °C as soon as they were passed. The experimental protocol was approved by the Board of Medical Research and the Ethics Committee, The Royal Melbourne Hospital.

**HemoQuant Assay**

The method we used followed that of Schwartz et al. (11). Aliquots of approximately 10 mg wet weight of feces were weighed to the nearest 0.1 mg. Iron was removed from the hemes by heating the sample with oxalic acid at 100 °C for 60 min to yield dicarboxylic porphyrins, which are fluorescent in ultraviolet light. We used solvent extraction to isolate porphyrins with two carboxylic acid groups. These heme-derived porphyrins (HDPs) include protoporphyrin, mesoporphyrin, pempetoporphyrin, and deuteroporphyrin (see Figure 1), whether generated in vivo or in vitro. Other possible interfering substances in feces include coproporphyrin and uroporphyrin, which have four and eight carboxylic acid side-chains, respectively (see Figure 1), and chlorophyll, which has none and a significantly modified ring structure; these all remain behind in the extraction steps (11). Dicarboxylic porphyrins were measured in extracted material by fluorimetry at specific excitation (402 nm) and emission (653 nm) wavelengths, and their fluorescence intensity was compared with a series of hemoglobin (Hb) standards run as an internal control. We used Hitachi F-4000 fluorescence spectrophotometer with monochromators and wavelength slits set as described (11). The results were expressed as milligrams Hb-equivalent per gram of feces (wet weight).

When we wanted to measure only the dicarboxylic porphyrins already present [i.e., "intestinal derived," also referred to as the "intestinal converted fraction" (15)] in feces and not the hemes as well, the assay was modified as described (11) so that fecal samples were treated with citric acid rather than hot oxalic acid. This treatment does not remove iron from the heme and so the fluorescence remains quenched.

For quality control, 50 unknown fecal samples were assayed both by ourselves and by SmithKline Bio-Science Laboratories. Regression of our results against theirs gave a slope of 0.976, a y-intercept of 0.16, and a correlation coefficient of 0.980.

**HPLC**

**Sample preparation (19).** We weighed small (20–50 mg) samples of dried feces and put them in 10-mL propylene tubes as described (19), then added 1 mL of concentrated HCl and vortex-mixed thoroughly until the feces were completely dissolved. Within 5 min of adding the HCl, we extracted the samples with 3 mL of diethyl ether to remove interfering colored compounds. Adding ether to each tube, we mixed the contents gently in uncapped tubes to allow the gas formed to escape, then capped the tubes and thoroughly vortex-mixed the samples. We then added 3 mL of distilled water to each tube and vortex-mixed again. Any porphyrins present pass into the acid aqueous phase, whereas interfering pigments such as those of carotenoid and chlorophyll origin remain in the ether phase. After separating the two phases by centrifugation, we scanned the aqueous phase spectrophotometrically (370–440 nm) to determine the total porphyrin concentration, calculated from the height of the Soret peak by assuming an average molar absorptivity of 3.03 × 10⁶ mol cm⁻¹ L⁻¹ (20). The percentage dry weight was determined from another aliquot of the same fecal sample, and we expressed total fecal porphyrin in micromoles per kilogram of dry weight. The acid aqueous extract was applied directly to the HPLC column after filtration through a 5-μm (pore size) filter. If too concentrated, the samples were diluted with a 2.7 mol/L solution of HCl.

This method of sample preparation does not render the hemes fluorescent and so they remain undetected. In some cases, we first treated samples as in the HemoQuant assay,
using hot oxalic acid to convert the hemes to dicarboxylic porphyrins. These samples were applied to the HPLC column with no further treatment other than filtration.

**Chromatography.** Reversed-phase gradient HPLC on an SAS-Hypersil C1 250-mm column (5-µm spherical silica chemically bonded with trimethylsilyl groups; Shandon Southern, Runcorn, Cheshire, U.K.) was used to separate the fecal porphyrins, as described by Lim and Peters (12). In brief, we used fluorimetric detection at 310–410 nm excitation and 585–650 nm emission wavelengths and a linear gradient between an aqueous ammonium acetate buffer (1 mol of ammonium acetate and 100 mL of acetonitrile per liter, adjusted to pH 5.16 with glacial acetic acid) and an organic phase consisting of a 100 mL/L solution of acetonitrile in methanol. The linear gradient began with 100% of the acetate buffer and changed to 100% of the methanolic acetonitrile over 30 min, the flow rate being 1 mL/min. The porphyrins most soluble in the aqueous phase were eluted from the column first (uroporphyrin), and the increasing concentration of methanol eluted the other porphyrins, with the dicarboxylic porphyrins coming off last. We used a 50 µL standard mixture of porphyrins (MK-1A marker kit; Porphyrin Products, Logan, UT), each at 10 nmol/L in HCl (2.7 mol/L), to identify the retention times of the porphyrins. The percentage of each porphyrin in the sample as calculated from the area under each peak. Absolute amounts were calculated by referring to the total porphyrin concentration of the sample.

Using this method, we determined total porphyrin (uroporphyrin, coproporphyrin, and dicarboxylic porphyrins) concentration in feces from 475 subjects screened for porphyrin but subsequently found to be nonporphyrin. The distribution was skewed and was not normalized by log-transformation; 95.1% had total porphyrin concentrations <200 µmol/kg dry weight of feces. This agrees closely with previous reports (19, 21) in which samples with total porphyrin >200 µmol/kg were considered above the normal range.

**Results**

**Effect of Diet on Fecal Heme and HDPs Measured by HemoQuant**

As shown in Figure 2, meat ingestion had a substantial effect on the fecal excretion of heme and HDPs. HemoQuant-determined fecal heme plus intestinally derived dicarboxylic porphyrins was 0.65 ± 0.42 mg (mean ± SD) Hb equivalent per gram of feces in the 49 subjects which they were on the red-meat-prohibited diet. During red-meat challenge this increased to 2.44 ± 2.15 mg/g. The mean HemoQuant value for the three samples collected by each subject on the meat challenge diet was higher than exclusion diet values in 45 of the 49 subjects (P <0.0001, paired t-test). The upper limit of normal was defined as the mean (0.65) on the exclusion diet plus two standard deviations (0.84), i.e., 1.5 mg Hb-equivalent per gram of feces. On the meat diet, 40 of 49 subjects (82%) passed at least one stool in which the concentration exceeded this upper limit. Furthermore, 34 subjects (69%) passed at least one stool with >2 mg/g, and 24 subjects (49%) exceeded 3 mg/g (Table 1). The vegetable challenge diet, which is high in plant peroxidases, did not significantly affect HemoQuant values as compared with the exclusion diet. The coefficient of variation on each diet indicated greatest intersubject variation during meat consumption: 62% for the exclusion diet, 60% for the vegetable challenge diet, and 58% for the meat challenge diet.

Fig. 2. Effect of diet on fecal concentration of heme plus dicarboxylic porphyrins, as determined by the HemoQuant assay

Each point represents the mean of three samples from an individual subject. Group means and SD are shown by the large symbols and vertical bars. Diets: FOBT, the exclusion diet; MEAT, the meat challenge diet; VEGE, the vegetable challenge diet.

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* Three fecal samples were tested in each subject on each diet.
<sup>b</sup> Units are milligrams of Hb-equivalent per gram of feces.

Measurement by HemoQuant of the “intestinal converted fraction” showed that the hemes comprised 39% ± 11% (mean ± SD) of combined total heme and dicarboxylic porphyrins on the meat-free diet, which was similar to the value of 44% ± 12% during meat consumption.

**Effect of Diet on Hemoccult Results**

Testing for intact heme by Hemoccult II gave four positive results. Results are compared with HemoQuant in Table 1. Results of the HemoQuant assay, expressed as
milligrams Hb-equivalent per gram of feces, in the guaiac-positive samples were: exclusion diet, 0.51 mg/g (51% as heme); meat diet, 7.6 mg/g (69% as heme), 4.5 mg/g (45% of heme), and 10.7 mg/g (52% as heme). The more sensitive HemocultSENSA test gave positive results with the same four stools and with three additional stools from two subjects on the meat diet. The HemoQuant results for these stools were 0.73 mg/g (45% as heme), 3.8 mg/g (45% as heme), and 1.7 mg/g (51% as heme). Thus, with the exception of two instances, Hemocult test results were positive for stools with increased HemoQuant values.

Although Hemocult results were generally positive for stools with high HemoQuant values, there were 79 other stools that exceeded 1.5 mg/g but for which neither Hemocult test was positive.

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Effect of Diet on Specific Fecal Porphyrins

Meat ingestion increased dicarboxylic porphyrins. It can be seen from the typical HPLC profiles shown in Figure 3 that, in addition to increased deuteroporphyrin, an isomer of pemptoporphyrin, and protoporphyrin, there were increases in at least two other dicarboxylic porphyrins. Mesoporphyrin was seen only in occasional samples. The peak seen between deuteroporphyrin and the main pemptoporphyrin isomer is probably the other isomer of pemptoporphyrin. Figure 4 shows that, on a meat-free diet, intestinally derived dicarboxylic porphyrins accounted for 37% (SD 25%) of total fecal porphyrins (including copro- and uroporphyrins). This increased to 78% (SD 16%) on the meat diet. The absolute concentrations of coproporphyrin and uroporphyrin were unaffected, whereas the dicarboxylic porphyrins increased by 6.6-fold. On the meat diet, 27% of samples measured had a total fecal porphyrin concentration >200 μmol/kg dry weight. Only 3% of samples obtained from subjects on a meat-free diet were above this value.

The effect of meat ingestion on individual dicarboxylic porphyrins is shown in Figure 5. The relative proportions of porphyrins derived in vivo from heme were not significantly altered during meat ingestion (analysis of variance). However, absolute amounts increased substantially: deuteroporphyrin by 11-fold, pemptoporphyrin isomers by 11-fold, and protoporphyrin by sixfold.

The standard method of sample preparation for HPLC results in detection of heme-related porphyrins only and not the hemes.

Effect of Blood Ingestion

Ingestion of blood also results in an increase in total heme and dicarboxylic porphyrins, in proportion to the amount ingested, as measured by HemoQuant. As shown in Table 2, ingestion of as little as 2 mL of blood increased fecal porphyrins. In one subject who ingested 10 mL, fecal porphyrins remained slightly increased at 72 h. Ingestion of 2-5 mL of blood gave values comparable to those reached during meat consumption.
Total fecal porphyrin measurements determined by scanning spectrophotometry corresponded to the changes in dicarboxylic porphyrins observed by HPLC. HPLC analysis of fecal porphyrins formed in vivo after ingestion of 10 mL of blood is shown in Figure 6 for subject A, referred to in Table 2. Total fecal porphyrins were 114 μmol/kg dry weight at time zero, increasing to 390 μmol/kg at day 1 and 359 μmol/kg at day 2. As with meat ingestion, the increase was exclusively due to an increase in the amount of dicarboxylic porphyrins and not to uro- or coproporphyrin. With increasing time spent by heme in the gut, the proportion of dicarboxylic porphyrins attributable to deuteroporphyrin was increased, from 50.8% on day 1 to 66.7% on day 2. The proportion due to protoporphyrin decreased from 6.3% to 1.5%.

Figure 6 also shows results of HPLC analysis of fecal samples after pretreatment with hot reducing oxalic acid, as in the HemoQuant assay, compared with simple pretreatment with citric acid. Treatment with citric or hydrochloric acid was equivalent (data not shown). The oxalic acid treatment led to an increase not only in protoporphyrin but also in pemptoporphy and deuteroporphyrin. This latter treatment in itself is not capable of converting the protoporphyrin of heme (either as hemoglobin or myoglobin) to pemptoporphy or deuteroporphyrin (Figure 7). The two unidentifed peaks accompanying protoporphyrin in the HPLC analysis of oxalic acid-treated blood were also observed by Schwartz and Ellerison (8); the nature of these is uncertain, but probably they represent hydroxylation of one or another vinyl group. Hydroxylation of both vinyl groups would lead to formation of hematoporphyrin, which would not be identified in the tracings.

**Discussion**

**Sources of Fecal Porphyrins**

Under conditions of a meat-prohibited diet, fecal excretion of hemes and HDPs averaged 0.65 mg Hb-equivalent per gram of feces, which is approximately equivalent to 0.65 mL of blood in a 150-g stool. These compounds are likely to come from multiple sources, including microbleeding, shedding of epithelium, biliary excretion, and perhaps, as suggested by Beukoveld et al. (10), de novo synthesis by bacteria. Ingestion of protoheme as myoglobin or hemoglobin resulted in increased excretion of a series of hemes—including protoheme, pempho, and deuteroheme—and of dicarboxylic porphyrins. During meat ingestion, average fecal hemes plus HDPs was 2.44 mg Hb-equivalent per gram of feces. Thus, exogenously derived fecal porphyrins can be quantitatively greater than those of endogenous origin. Under unrestricted dietary conditions, de novo synthesis by bacteria (10) can provide only a minor contribution to fecal hemes and dicarboxylic porphyrins; this is contrary to some previous assertions (9, 10). If one were to use fecal excretion of hemes and HDPs as an indicator of gut pathology or disordered porphyrin metabolism, the possibilities that the porphyrins were of dietary origin or due to pathological blood loss would need consideration.

**Fecal Porphyrins and Diagnosis of the Porphyras**

HPLC (12, 13) is especially useful for establishing the
diagnosis of the porphyrias, the normal anabolic sequence that leads to controlled formation of protoporphyrin and protoheme being disordered. Thus, excess porphyrin may be excreted in bile to eventually appear in feces (22). Porphophyrin reaching the intestine by this route would be subject to bacterial or biochemical action, which modifies the two vinyl side-chains to produce other dicarboxylic porphyrins, including pempetoporphyrin and deuteroporphyrin (11, 17). This conversion is not associated with any major changes in fluorescent characteristics (17). Beukeveld et al. (10) showed that the bacteria that cause this are anaerobic and that measurement of fecal protoporphyrin but not the other dicarboxylic porphyrins derived from protoporphyrin might cause the diagnosis of a porphyria to be missed.

Luminal protoporphyrin may also have a dietary origin, in addition to its biliary origin. It was recognized many years ago that bacteria of then-unspecified type could remove iron from porphine with resultant production of various porphyrins (11, 17, 23). Except for the work of Schwartz and associates (8, 11, 14), this observation has been largely ignored. Our findings show that meat ingestion of >250 g per day did not affect fecal uro- or coproporphyrin concentrations but did cause up to 11-fold increases in certain dicarboxylic porphyrins, including protoporphyrin. Importantly, 27% of samples obtained during meat ingestion exceeded the upper limit of the normal range of fecal porphyrins defined for our center. Similarly, ingestion of a modest amount of blood (up to 10 mL) was followed by a marked increase in fecal dicarboxylic porphyrins, which exceeded increases seen in most subjects during meat ingestion.

The possibility that fecal dicarboxylic porphyrins can be derived from dietary heme or blood loss should not be overlooked when one is testing for the porphyrias. To minimize intersubject scatter of results, red-meat consumption should be proscribed in the 72 h before collection of the sample for analysis; indeed, the normal range needs to be redefined for these conditions. If fecal heme-related porphyrins were increased under these conditions of dietary restriction, then occult gastrointestinal bleeding must be excluded as the source. This can be done by testing feces for heme. A simple guaiac test would be useful if positive but, unfortunately, the guaiac tests lack sensitivity for the hemes, as seen during meat ingestion in this study, and a negative test would not exclude blood loss with certainty. A more reliable but more complex approach would be to perform a HemoQuant assay including calculation of the intestinal converted fraction to determine if fecal hemes were increased.

Catabolism of Heme in Feces

After ingestion of heme, not only pempetoporphyrin, protoporphyrin, and deuteroporphyrin were increased but also a number of other porphyrins with a longer retention time than coproporphyrin (Figures 2 and 4). They accounted for about 20% of total dicarboxylic porphyrins on both meat-free and meat-containing diets and increased in absolute amounts during meat ingestion (as opposed to coproporphyrins). These additional "heme-derived" porphyrins are probably dicarboxylic porphyrins also, as judged from their retention times, and would include mesoporphyrin (10). Hematoporphyrin has not been identified in significant amounts in feces (10, 12). HPLC analysis showed that these additional porphyrins are detected by the HemoQuant assay, because they are extracted by solvents along with known dicarboxylic porphyrins (unpublished observations). It is possible that they might also be derived from protoporphyrin excreted in bile, and their contribution should not be ignored when fecal porphyrins are being measured in the diagnosis of the porphyrias.

After ingestion of blood, there was a time-dependent change in the proportional contribution made by each of the three major dicarboxylic porphyrins. The longer the time after ingestion, the lower the protoporphyrin and the higher the deuteroporphyrin content. These changes are consistent with sequential removal of the two vinyl side-chains of protoporphyrin by bacteria—protoporphyrin to pempetoporphyrin to deuteroporphyrin—the process being more complete with increased exposure.

When feces were pretreated with hot oxalic acid before HPLC, there was a substantial increase in all HDPs and not only protoporphyrin (Figure 6). Because oxalic acid does not remove vinyl groups from protoporphyrin under these conditions (Figure 7), the vinyl side-chains can be modified independently of the presence of iron. In other words, fecal "heme" consists of not only protoheme as ingested but also as deuteroheme and pempetoheme.

HemoQuant and the Detection of Fecal Heme

The HemoQuant test has its principal application in the detection and (or) quantification of gastrointestinal bleeding, because of its simplicity compared with HPLC and its ability to readily quantify hemes and HDPs, the latter of which are not detected by guaiac tests. In this study, we found that fecal concentrations of heme plus intestinally derived dicarboxylic porphyrins by HemoQuant were increased in 90% of subjects during meat consumption. Many (81%) achieved values exceeding the "normal" range estab-
lished for the red-meat-free diets (<1.5 mg Hb-equivalent per gram of feces), and the intersubject scatter of results broadened. Ingestion of blood also increased fecal heme plus HDPs, as would be expected. There is no reason to assume that any factor other than myoglobin-heme or hemoglobin-heme was responsible for these increases. Unrecognized variation in the plant-food content of the diet would not be responsible, because ingestion of porphyrin-containing plant foods had no effect whatsoever, either on fecal heme excretion or intersubject variation.

It has been claimed with little evidence that meat ingestion does not elevate fecal hemes (9), but our results indicate that optimal conditions for detection and (or) quantification of gastrointestinal bleeding by quantifying hemes and HDPs include strict avoidance of red meat. It is possible to make allowance for heme ingestion by adjusting upward the upper limit of normal, but the resulting wide scatter of results is likely to reduce the precision of this approach.

The HemoQuant assay may give a false-positive result for blood in patients with certain forms of porphyria, owing to increased excretion of heme-related porphyrins (11). However, plant porphyrinases, which do cause false-positive results with the guaiac tests, do not affect HemoQuant. Comparison of the Hemoccult and HemoQuant tests showed that the latter was more affected by red-meat ingestion; but this was simply due to the inability of the Hemoccult tests to measure HDPs. The few false-positive Hemoccult results obtained during meat ingestion occurred largely in samples with an increased heme content; i.e., they were not falsely positive for heme. Note that many other samples with similar HemoQuant-determined total heme and HDP values did not give positive guaiac results. It seems probable that most of the heme in these samples had been converted to dicarboxylic porphyrins during transit through the gut or possibly that feces contained a substance capable of interfering with the guaiac test (15). As anticipated, HemoccultSENSA was more sensitive for heme than Hemoccult II in that it gave more positive results during red-meat challenge.

In conclusion, fecal porphyrin and heme content is altered by ingestion of red meat or simulation of gastrointestinal bleeding. When one is determining fecal porphyrins by fluorimetry, either by HemoQuant or HPLC, heme-rich red meats should be strictly proscribed for sufficiently long times (ideally 72 h) before testing to allow clearance of unabsorbed previously ingested heme and heme-derived porphyrins. Failure to control for meat ingestion in the past has probably accounted for the wide variation observed in fecal porphyrin content between subjects (10). When fecal porphyrins are measured in the investigation of patients for a porphyria, especially the difficult case, Beukeveld et al. (10) have suggested that subjects be tested while receiving antibiotics to suppress anaerobic bacteria. We would recommend strict avoidance of red meat in the diet and follow-up investigation of subjects with abnormally increased dicarboxylic porphyrins for the presence of exces-

This study was supported in part by grants from the Anti-Cancer Council of Victoria and SmithKline Diagnostics Inc., San Jose, CA. We thank Masha Alexeyeff (research nurse) and Jenny Mather (medical student) for recruiting subjects and supervising the diets.

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