A METHOD FOR THE ISOLATION OF BILIRUBIN FROM FECES*

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(Received for publication, November 24, 1952)

The recent observation that certain antibiotics depress or even temporarily eliminate the bacterial reduction of bilirubin in the colon suggested that the feces under such circumstances might be an excellent source of bilirubin. The possibility was also at hand that bilirubin labeled with N\textsuperscript{15} or C\textsuperscript{13} might thus be made available in significant amounts, assuming a prior administration of isotopic glycine or acetate.

Crystalline bilirubin is customarily prepared from bile or cattle gallstones and numerous procedures for its isolation from these sources have been described (1). Fischer and Libowitsky (2) obtained it, together with stercobilin, from the feces of an individual with hemolytic anemia. In this instance, it appeared that either the transit time of the intestinal contents was too short or the bacterial flora too inefficient to reduce the bilirubin completely. As will be noted in the following, this method has not proved satisfactory nor has any previous description been found of a satisfactory method for obtaining crystalline bilirubin from feces.

Suppression of urobilinogen formation and excretion of bilirubin in the feces can readily be induced by the use of aureomycin (3), terramycin (4), and neomycin. After oral administration of these drugs for several days (in daily doses of 2, 4, 6 gm. respectively), the fecal urobilinogen drops to very low values, and the feces now give a strongly positive Fouchet reaction for bilirubin. To isolate it in crystalline form, the method of Fischer and Libowitsky (2) was first tried. Essentially, this procedure consists in acidification with tartaric acid and prolonged extraction of the feces with a mixture of ether and alcohol. After drying the fecal residue, the bilirubin is extracted with chloroform. In our hands, this extraction has proved inefficient, and from normal individuals receiving terramycin only traces of bilirubin were obtained. The following method was therefore developed.

* This work was done in part under a contract from the Office of the Surgeon General, United States Army, and under sponsorship of the Commission on Liver Disease of the Armed Forces Epidemiological Board.
† Aided by a fellowship from The National Foundation for Infantile Paralysis, Inc.

1 Unpublished data with R. Wise.
Method

Feces of individuals receiving one of the above antibiotics, and after marked reduction of urobilinogen values, are collected for convenient periods (1 to 4 days). Although not essential, the feces are more easily handled if their bulk is first reduced by several extractions with an equal mixture of ether and alcohol. These extractions are carried out in a mortar. The supernatant solution is decanted through a sintered glass filter and discarded. If it is also desired to isolate any preformed urobilin which may be present, these initial extractions are made with alcohol which is then treated further according to a method described separately (5).

After being extracted and packed dry on the filter, the feces are transferred to a Waring blender, where they are mixed with a 2.5 per cent sodium hydroxide solution, also containing 2 per cent ascorbic acid. This solution removes practically all of the pigment from the feces, including the bilirubin. The inclusion of ascorbic acid is essential to prevent oxidation of bilirubin to biliverdin, which otherwise proceeds rapidly in an alkaline medium. After blending, the dark brown homogenate is diluted with an equal volume of a 3:1 mixture of alcohol and ether, which causes the fecal residue to become flocculent. The extract is then filtered through a Büchner funnel. This filtration proceeds much more rapidly if the paper is first layered with infusorial earth. After filtration, the residue is again extracted with the sodium hydroxide and ascorbic acid solution to remove any remaining bilirubin. On only a few occasions have more than two extractions been necessary.

The combined extracts are saturated with ether and then weakly acidified with a 4 per cent solution of tartaric acid. On acidification a large precipitate forms which includes the greatest part of the bilirubin. This precipitate is allowed to settle overnight at 4\(^\circ\). The next day the supernatant fluid is decanted and the solid material centrifuged and washed once with a 1:1 mixture of alcohol and ether, to which several mg. of hydroquinone are added to maintain reduction of the bilirubin. After washing twice more with ether alone, the orange-yellow precipitate obtained is allowed to dry. After drying it is pulverized and extracted for several hours with chloroform in a Soxhlet extractor. The chloroform solution is then shaken with a small amount of animal charcoal, filtered, and washed twice with water. After concentration to a small volume, the bilirubin readily crystallizes in the form of micro prisms. It is recrystallized out of chloroform (Fig. 1).

The final product shows all of the reactions characteristic of bilirubin; i.e., amalgam reduction to mesobilirubinogen, oxidation to biliverdin, the Gmelin color play, Fouchet reaction, and diazo reaction. It is a bright

\* Hyflo Super-Cel; Johns Manville.
Fig. 1. Crystals of bilirubin isolated from human feces. × 40

Fig. 2. Spectral distribution curve of bilirubin isolated from feces compared with Armour bilirubin in chloroform solution, 0.83 mg. per 100 ml. in each. Complete identity of the curves is seen.

orange powder which is identical in appearance with crystalline bilirubin from hog bile. Armoured Company. The diazo reaction, spectral distribution, and absorption intensity indicate that the material obtained by this method is of equal

Armour and Company.
purity. Both show equal intensity at absorption in chloroform from 320 to 490 mp\(\mu\), with the same maximum at 450 mp\(\mu\) (Fig. 2). The diazo reaction is identical with that of commercial bilirubin\(^3\) recrystallized from chloroform (Fig. 3).

The yields have been relatively satisfactory. 40 mg. of bilirubin per day have been obtained from a normal individual and 419 mg. per day from a patient with congenital hemolytic anemia, in both instances during the administration of terramycin. The method has been particularly use-

![Graph](http://www.jbc.org/)

**Fig. 3.** Comparison of diazo reaction of Armour bilirubin and bilirubin isolated from feces. Appropriate dilutions were made so that 1 ml. of a chloroform solution of bilirubin was mixed with 6 ml. of methyl alcohol and 1 ml. of diazo reagent. After standing 15 minutes, readings were made on an Evelyn colorimeter with a No. 540 filter.

ful in \(\text{N}^{15}\) studies in which it has been desirable to obtain not only frequent samples but a supply of \(\text{N}^{15}\) bilirubin. The latter, for example, has been reduced to mesobilirubinogen, which has then been used to prove that fecal bacteria readily convert mesobilirubinogen to stercobilinogen. This study will be described in a separate communication.

**SUMMARY**

1. A method has been described for the isolation of crystalline bilirubin from human feces. This depends on (a) administration of suitable antibiotics to depress the reduction of bilirubin by the fecal flora and thus permit its excretion in the feces in significant amounts, (b) suitable pre-
liminary treatment of the feces with ether and alcohol, and (c) the use of ascorbic acid during an alkaline extraction to prevent oxidation to biliverdin.

2. The bilirubin thus obtained is identical with the purest material isolated from hog bile.

We gratefully acknowledge the technical assistance of Miss Ruth Cardinal and Miss Mary Ann Farisy.

BIBLIOGRAPHY
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