tained. 2. Mesobilirubinogen added to bile and acholic feces was converted at least in part to d-urobilin. In one instance the addition of acholic feces to urobilin-free bile was instrumental in the development of d-urobilin.

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IV. Formation of (Laevorotatory) Stercobilin from Mesobilirubinogen in Human Feces.*

C. J. WATSON, VICTOR SBOROV AND SAMUEL SCHWARTZ.

From the Division of Internal Medicine, University of Minnesota Hospital, Minneapolis, Minn.

In Paper I of the present series it was shown that stercobilin could not be obtained by the amalgam reduction of native bilirubin in various bile samples. This led only to mesobilirubinogen and, on oxidation, urobilin IX,a. Nor was stercobilin obtained by incubating mesobilirubinogen with acholic feces, or with bile, as described in Paper III. In these experiments, the hitherto undescribed d-urobilin was formed.

In the present study the following experiments were carried out in order to determine whether mesobilirubinogen is converted to stercobilinogen in the intestinal tract, or when incubated *in vitro* with normal feces.

1. 210 mg of crystalline mesobilirubinogen were dissolved in 50 cc of dilute Na_2CO_3 (0.2%) to which was added 0.33 g bile salts (Bilien, Abbott). The solution was diluted further to 100 cc with physiological saline. This was administered over a period of one-half hour through a Miller-Abbott tube, into the duodenum of a patient having a T-tube in the common bile duct, and with a continuous suction fistula.[1] The urobilinogen excretion in the feces was determined for two-day periods before and after the administration of mesobilirubinogen as shown in Table I. The feces for the various periods indicated by the brackets on the left in Table I were subjected to the chromatographic isolation method as described in Paper II of this series, with the one exception that the primary extraction and adsorption was carried out with 95% ethyl alcohol rather than with $CHCl_3$. The relative advantages of various isola-

* Aided by a grant from Mr. A. C. Randall, Minneapolis.

1 Layne, J. A., and Bergh, G. S., *Surgery*, 1941, **10**, 563.

TABLE I.

Date	Feces urobilinogen in mg per day	Crystals obtained	Degrees rotation in 15 cc $CHCl_3$	Comment
3/6 -3/8	11.6	—	(−)0.11°	Continuous suction on 3/2-3/18
3/10-3/12	7.0			
3/12-3/14	19.8	+	(−)1.02°	210 mg mesobilirubinogen on 3/13
3/14-3/16	47.3			
3/16-3/18	11.4	Isolation not attempted		
3/18-3/21	55.8	+	(−)0.97°	Bile suction discontinued

tion procedures for urobilin and stercobilin will be discussed in a separate communication. It is seen in Table I that stercobilin increased markedly in the feces after the administration of mesobilirubinogen. A comparison of the total urobilinogen and optical activity values for this period with those of 3/18 to 3/21 reveal striking agreement. In the latter period the only source of urobilinogen was the native bilirubin of the patient's bile.

2. Feeding of mesobilirubinogen. Subject A.R.,‡ ♂, 73. 775 mg of crystalline mesobilirubinogen were fed, in capsules, over a period of 12 hours. The feces urobilinogen was determined before, during, and after the time of administration. The various samples were then subjected to alcohol extraction and chromatographic separation as already referred to. The data are shown in Table II.

A significant increase in stercobilin is observed in Table II during the period following administration of the mesobilirubinogen.

3. Incubation of mesobilirubinogen with feces. The methods employed in these experiments were entirely similar to those described above and in the preceding papers of this series. In Table III are shown the data from four experiments in which mesobilirubinogen was added either to acholic or normal feces. The fourth of these experiments was already described in the preceding paper and the

TABLE II.

Date	Feces urobilinogen in mg per day	Mg stercobilin*		
		Crystals	Mother liquor	Total
6/19-6/23	197.5	11.5	38	49.5
6/23-6/27	243.8	19	21	40
6/27	775 mg mesobilirubinogen *per os.*			
6/27-6/29	235	49	44	93
6/29-7/1	308			
7/1 -7/5	165	29	5	34

*In terms of optical activity. Calculated from $[\alpha]_{20°}^{589} = -3300°$ ($CHCl_3$).

‡ This patient had a mild refractory anemia of undetermined origin.

TABLE III.

	Grams feces	Total mg urobilinogen in feces	Material added	Incubation days	Method of purification	Stercobilin in mg* Crystals in $CHCl_3$	Mother liquor	Total mg
1.								
a.	20	33.5	—	3	Chromatographic	<1	1.8	2+
b.	20	33.5	58 mg mesobilirubinogen	3	"	5	9.5	14.5
2.								
a.	20	0	64 mg "	3	"	0†	0	0
b.	20	0	69 mg "	0	"	0†	0	0
3.								
a.	10	0	100 mg "	5	7% HCl-$CHCl_3$	0†	0	0
b.	10	0	100 mg " + 5 cc bile‡	5	7% " "	0.8	not determined	>1
4.								
a.	10	1.4	—	6	7% " "	0	0	0
b.	10	1.4	46 mg mesobilirubinogen	6	7% " "	0†	0	0

*Calculated on basis of optical activity $[\alpha]_{20°}^{589} = (-)\ 3300°$ ($CHCl_3$).

†Crystals of urobilin IX,a, isolated (optically inactive).
‡Weakly positive Ehrlich reaction in the native bile.

significant data are included again for purposes of direct comparison.

The question arose as to whether urine alone was capable of converting mesobilirubinogen to stercobilinogen. Mesobilirubinogen was therefore added to 3 separate urobilin and urobilinogen-free samples, as shown in Table IV. After incubation for 3 days, the samples were acidified with sufficient HCl to make a concentration of approximately 5% and they were then extracted repeatedly with $CHCl_3$. The combined $CHCl_3$ was filtered and subjected to chromatographic analysis as described in Paper II. As noted in Table IV, urobilin IX,a was identified on the basis of optical inactivity and there was no evidence of stercobilin formation.

Discussion. The results of the present study based upon oral administration of mesobilirubinogen and upon *in vitro* incubation of mesobilirubinogen with feces, indicate that this substance is converted in part to stercobilin. A survey of the above data would indicate at first glance that the degree of conversion was not large. Other factors, however, must be taken into account. For one thing, meso-

TABLE IV.
Addition of Mesobilirubinogen to Normal Urine.

	Amt urine in cc	Amt mesobilirubinogen in mg	Type of urobilin obtained
1.	100	64	Urobilin IX,a*
2.	100	64 (+ ¼ g acholic feces)	" IX,a*
3.	500	58	" IX,a*

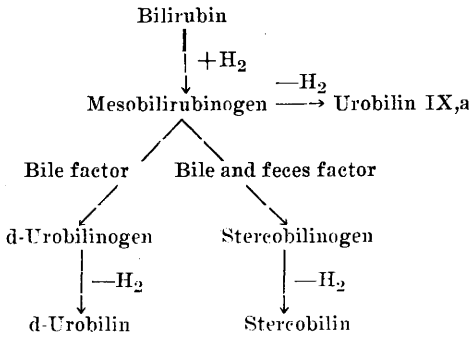
*Optically inactive in $CHCl_3$.

bilirubinogen is very easily oxidized so that a considerable portion of the added material in the above *in vitro* experiments may well have been changed before it could be converted to stercobilin. In the *in vivo* studies the factor of absorption cannot be assayed. In both instances considerable amounts of mesobilirubinogen appeared in the urine during the 24 hours after its administration. This was identified by the subsequent identification of urobilin IX,a from each. Another factor to be taken into account is the yield of stercobilin which one may expect especially when working with very small amounts of material. It will be noted that less than 1 mg of crystals were obtained in the control feces of Experiment 1, Table III, although 20 g of feces contained 33 mg of urobilinogen. Under the best of conditions and working with large amounts of feces, the yield of stercobilin is usually from 5-15 and rarely exceeds 20% of the initial urobilinogen. In this connection it is of interest that Fischer and Libowitzky[2] added 100 mg of mesobilirubinogen to a daily amount of feces and were able to recover but a very few crystals. This was not incubated and the possibility that some conversion to stercobilin had occurred was not mentioned. We have observed repeatedly that incubation of feces causes a sharp decrease in yield, so that the question arises in experiments of the above type as to whether much more stercobilinogen than was present at the time of extraction had been successively formed and oxidized during incubation. Most important, however, is the fact that significant increases of stercobilin were obtained, whose only source could have been the added mesobilirubinogen.

It is significant that in one of the present experiments (No. 3 in Table III), stercobilin formation was noted when mesobilirubinogen was incubated with urobilin-free bile and acholic feces. This is in contrast with the formation of d-urobilin in an entirely similar experiment (3c) in Paper III. Since this was carried out with bile and feces from different individuals, it can only be assumed that unknown and variable factors in bile or feces determine the direction of conversion.

The present results when correlated with those of the preceding papers indicate clearly that mesobilirubinogen is the primary chromogen or urobilinogen, and stercobilinogen and d-urobilinogen are to be regarded as secondary products, the former requiring both bile and fecal factors and the latter only a bile factor for its elaboration. This concept is perhaps best shown in the following diagram:

[2] Fischer, H., and Libowitzky, H., *Z. physiol. Chem.*, 1939, **258**, 255.



The primary reduction to mesobilirubinogen is probably effected by a number of bacterial reducing systems, while the secondary transitions to d-urobilinogen and stercobilinogen evidently depend on more complex factors, as indicated. The possibility cannot be excluded that bilirubin may be reduced directly to d-urobilinogen or stercobilinogen without mesobilirubinogen as an intermediate stage, also, that given the proper conditions, d-urobilinogen may be converted to stercobilinogen. Still another possibility is that stercobilin or d-urobilin may be formed directly from mesobilirubinogen without intermediary differing chromogens.

Summary and Conclusions. 1. The oral or intraduodenal administration of mesobilirubinogen resulted in an increased excretion of stercobilin in the feces. 2. The incubation of mesobilirubinogen with normal feces, or with acholic feces plus bile resulted in formation of stercobilin. Incubation with acholic feces alone resulted in no change; urobilin IX,a was subsequently isolated. 3. Mesobilirubinogen was not converted to stercobilin in the 3 urine samples studied. It is believed that mesobilirubinogen is the primary chromogen or urobilinogen formed by bacterial reduction of bilirubin. The further elaboration of d-urobilin requires bile, while that of stercobilin requires bile and feces.

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Experimental Studies in Metaplastic Ossification.

SHELDON A. JACOBSON. (Introduced by Valy Menkin.)

From the Laboratory of Pathology, Hospital for Joint Diseases, and the Richard Morton Koster Research Laboratory.

Huggins[1-4] has discovered that transplanted epithelium of bladder, ureter, or kidney pelvis induces ossification of the connective tissue