STUDIES OF THE DIPYRRYLMETHENE ("FUSCIN") PIGMENTS.
I. THE ANABOLIC SIGNIFICANCE OF THE FECAL
MESOBILIFUSCIN *

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In the study of bile pigments relatively little attention has been given to the "fusin" category. Siedel and co-workers (1-3) have contributed much of the information available about these compounds. The term bilifusin was first used by Städeler (4) to describe a dark brown pigment observed during isolation of bilirubin from cattle gallstones. Siedel has shown that this is a dipyrromethene resulting from halving of the (tetrapyrromethene) bilirubin molecule, each half retaining one of the vinyl ($\text{CH}=\text{CH}_2$) groups characterizing bilirubin. According to Siedel and Möller (1), mesobilifusin, as found in the feces or obtained by oxidation of mesobilirubinogen, is the analogous compound in which the vinyl groups have been reduced to ethyl groups. Theoretically, mesobilifusin could be formed by schism of bilirubin and subsequent reduction of the resulting bilifusin, or by primary reduction of bilirubin to its meso-derivatives and subsequent schism. This assumes only a catabolic origin and it is also quite conceivable that dipyrromethene pigments of mesobilifusin type might be elaborated during the anabolic sequences of heme metabolism.

Siedel, Stich and Eisenreich (3) have emphasized that mesobilifusin occurs normally in the feces partly as a dark brown pigment and partly as a colorless chromogen which is readily converted to the pigment by heat and acidification. Mesobilifusin does not have a distinct absorption band but exhibits diffuse absorption below 500 $\text{m}\mu$. It has not been crystallized. It is readily precipitated as a zinc complex from alcoholic solution. Similar properties characterize the amorphous dark brown pigment accompanying stercobilin in the feces and first designated as copro-nigrin (5, 6).

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Although the color of normal feces is probably dependent in considerable measure on the presence of the intensely colored "fusin" pigments, their significance in hemoglobin metabolism has remained a matter of speculation. Since mesobilifusin can be obtained *in vitro* by oxidation of bilirubinoid compounds, the corresponding fecal pigment has been assumed to be a product of hemoglobin breakdown. Technical difficulties have discouraged attempts to clarify this question. Siedel and Möller (1) described a method of purification of mesobilifusin from feces, but it is clear that this included varying amounts derived by (artificial) schism of bilirubinoids during the procedure. This is considered again in the following.

The present study was undertaken to determine the significance of mesobilifusin in respect to hemoglobin metabolism by means of N^{15} labeled glycine.

MATERIAL AND METHODS

Four individuals were studied using N^{15} glycine according to well established methods (7, 8). The first, C. B., a 55 year old male, was studied eight months after a left radical nephrectomy and splenectomy for hyper-nephroma. No known hematologic disease existed. The second case, J. P., was a 65 year old male with congenital hemolytic anemia. Mention of this patient was previously included in studies describing the isolation of crystalline N^{15} bilirubin from feces and demonstrating conversion of N^{15} labeled mesobilirubinogen to stercobilin by fecal bacteria (9, 10). Case 3, L. H., a 54 year old female, was a patient with sporotrichosis and reticuloendotheliosis. Despite an eventual fatal outcome the patient's condition was relatively good at the time of the present study; there was no evidence of hemolytic anemia. The fourth subject, S. G., a 29 year old female, was a normal volunteer. Thus three of the four subjects studied had normal or relatively normal hemoglobin metabolism. The life span of the erythrocytes approximated 120 days on the basis of the hemoglobin protoporphyrin N^{15} data.

Glycine was prepared by the method of Schoenheimer and Ratner (11) from potassium phthalimide containing 33 atom per cent excess N^{15} for the first three cases and 62 atom per cent excess for the fourth (normal) subject. All four were given 20 Gm. protein diets for several days before and during the period of glycine administration. The labeled glycine was fed in total quantities of 15 Gm. to the normal subject and 53 Gm. to the three others. It was given over a three day period with equal doses at hourly intervals except for triple doses at midnight and 3 a. m. according to the schedule of Shemin and Rittenberg (7). Blood specimens were drawn at one to four day intervals for the first 16 days and at irregular intervals thereafter. Feces were collected continuously for the first several weeks and were divided into either two or four day periods. After five weeks of study the collections were sporadic in Cases 1 and 3. The exact periods of collection for Cases 1, 2 and 4 are shown in Figures 1, 2 and 3, and for Case 3 in Table I.

The hemoglobin protoporphyrin and fecal stercobilin were crystallized according to methods previously described (12, 13). The alcohol- Al_2O_3 -water modification (13) was used for the isolation of stercobilin. Case 2 (J. P., Figure 2) had been given Terramycin® for seven days prior to administration of glycine; hence

TABLE I
Comparison of N^{15} concentration in fecal stercobilin and mesobilifuscin in subjects with normal hemoglobin metabolism, at the time of the early and late stercobilin N^{15} peaks

Case	Day after glycine N^{15}	Atom per cent excess in		
		Hemo-globin protoporphyrin	Fecal stercobilin or urobilin	Fecal dipyrromethene or mesobilifuscin
1	2	0.007	0.382	0.309
3	1-4	0.049	0.651	0.666
3	5-8		0.239	0.182
4	3-4	0.028	0.374	0.693
4	5-6	0.242	0.573	0.391
1	133-134	0.111	0.202	0.024
3	128-129	0.072	0.200	0.057
4	117-120	0.248	0.987	0.126

d-urobilin and bilirubin rather than stercobilin were isolated, by methods previously described (9, 13). The method used for obtaining mesobilifuscin in the first three cases provided a relatively crude material as compared with the modification of Siedel and Möller's method (1) employed in Subject 4. The dark brown or

FIG. 1. N^{15} CONCENTRATION IN CIRCULATING HEMOGLOBIN PROTOPORPHYRIN, FECAL STERCIBILIN AND FECAL MESOBILIFUSCIN IN SUBJECT NO. 1 (NORMAL ERYTHROCYTE LIFE SPAN)

Determinations of stercobilin and mesobilifuscin were performed on 24 hour collections of feces during the first 16 days and on 48 hour feces collections thereafter.

almost black precipitate appearing at several stages during the isolation of stercobilin or bilirubin by earlier methods (5, 9, 14) was precipitated by zinc from an alcoholic (zinc acetate) solution in the same manner as previously described for copronigrin. As will be noted in the following, the nitrogen content of the samples thus obtained was considerably lower than the theory for mesobilifuscin or any pure dipyrromethene. In all of these the nitrogen determinations were made on the zinc precipitated material; hence the low values are due at least in part to zinc which may have been present in excess. Certain of the more important samples, in point of time, were analyzed for zinc¹ by the method of Vallée and associates (15, 16). These and certain other samples were also recycled according to Siedel and Möller's method after which ordinary and isotopic nitrogen percentages were again determined.²

The method used in the fourth or normal subject (S. G.) was a modification of Siedel and Möller's pro-

¹ The zinc determinations were carried out by Miss Margaret Giebenhain in the Research Laboratory of the Department of Obstetrics and Gynecology, through the courtesy of Dr. J. L. McKelvey.

² The ordinary nitrogen determinations were made either in the Microanalytical Laboratory, Division of

cedure for isolation of mesobilifuscin(ogen) from feces. Most of the fecal samples from Subject 4 were first subjected to the ferrous hydroxide method for stercobilin isolation as previously described (13). With two of the samples (Days 33 to 36 and 41 to 44) the alternative alcohol— Al_2O_3 —water method (13) was used. In either event the aqueous filtrate or eluate was extracted repeatedly at pH 4.0 with petroleum ether (30 to 60° B. P.) in order to remove most or all of the urobilinogen group. This was converted in the usual way (13) to the urobilin group and crystallized for N^{15} analysis. The remaining aqueous solution, after petroleum ether extraction, was heated to 80° C. on a boiling water bath and allowed to stand overnight. This results in conversion of colorless chromogen to pigment. The next day it was extracted three times with butyl alcohol containing 2 per cent acetic acid; approximately 75 ml. of butyl alcohol was used for each extraction. The aqueous fraction was then discarded. The butyl alcohol fraction was washed three times with water and filtered through a

Organic Chemistry, University of Minnesota, or in the Clark Microanalytical Laboratory, Urbana, Illinois. The atom per cent excess N^{15} was determined in the mass spectrometer through the courtesy of Professor A. O. Nier, Department of Physics, University of Minnesota.

FIG. 2. N^{15} CONCENTRATION IN CIRCULATING HEMOGLOBIN PROTOPORPHYRIN, FECAL BILIRUBIN, FECAL d-UROBILIN AND FECAL MESOBILIFUSCIN IN A PATIENT WITH CONGENITAL HEMOLYTIC ANEMIA (SUBJECT NO. 2)

Determinations were carried out on 24 hour collections of feces during the first 21 days and on 48 hour collections thereafter. The patient had received Terramycin® shortly before the period of study.

butyl alcohol moistened filter paper. It was then concentrated under reduced pressure to less than 5 ml. after which approximately 30 ml. of methyl alcohol and 10 ml. of a saturated alcoholic zinc acetate solution were added. This was allowed to stand overnight or until the zinc complex of the dipyrromethene group precipitated. The precipitate was separated by filtration and washed with methyl alcohol. It was esterified with a 20:1 mixture of methyl alcohol:sulfuric acid. Four to 24 hours were allowed for esterification. The total ester was then extracted into chloroform. This was washed once with water, once with 3 per cent ammonium hydroxide and once with 7 per cent sodium chloride. The chloroform was then concentrated to a small volume and was passed through a column of Al_2O_3 , first saturated with ethyl ether. The column was first eluted with a 1:10 mixture of chloroform:ethyl ether, then with a 1:3 mixture of chloroform:ethyl ether, then with chloroform alone. Finally the top brown band containing the mesobilifuscin ester was eluted with hot glacial acetic acid. This was mixed with several volumes of chloroform and washed repeatedly with water to remove much of the acetic acid. The chloroform was then concentrated to a small volume and the mesobilifuscin ester precipitated by addition of 10 volumes of petroleum ether. For purposes of compari-

son with the N data in the first three cases, a fecal sample from Subject 4 (Period 31) was subjected to the method used in obtaining the zinc precipitates as analyzed in Cases 1, 2 and 3. The material was subjected to ordinary N and N^{15} analysis, then esterified and chromatographed according to Siedel and Möller's method, after which the N and N^{15} determinations were repeated.

Rittenberg's procedure (17) was used for collection of nitrogen from the samples.

Since mesobilifuscin has not been crystallized, a comparative study was made of the yields of well defined oxidation products of the fecal pigment and mesobilifuscin prepared by Siedel and Möller's method (1). Strenuous oxidation either with the 20 per cent sulfuric acid-chromic oxide method (18, 19) or the nitric acid method (1) was carried out on four samples of the mesobilifuscin methyl ester isolated from Subject 4 (Periods 10 to 11, 14 through 20, 32 to 33, and 35) and two samples of the zinc complexed mesobilifuscin isolated from Cases 1 and 2. The methyl ethyl maleimide was crystallized by sublimation and the hematinic acid recrystallized from ether petroleum ether. The yields of methyl ethyl maleimide and hematinic acid from each sample were compared with those obtained, after like treatment, of mesobilifuscin methyl ester prepared from mesobilirubinogen (1).

FIG. 3. N^{15} CONCENTRATION IN CIRCULATING HEMOGLOBIN PROTOPORPHYRIN, FECAL STERCOBILIN AND FECAL MESOBILIFUSCIN IN A NORMAL INDIVIDUAL (SUBJECT NO. 4)

Determinations of stercobilin and mesobilifuscin were performed on 48 hour collections of feces during the first 12 days and on 96 hour collections thereafter.

TABLE II
Percentage of N, atom per cent excess N^{15} and Zn before and after recycling

Case	Day after glycine N^{15}	Initial zinc precipitate					Methyl ester obtained after chromatography according to Siedel and Möller (1)		
		N %	Atom % excess N^{15}	Zn %	Ash %	Zn % in ash	N %	Atom % excess N^{15}	Zn % (ash)
		Theoretical	6.45*	30*			8.8		0
1	2		2.7	28.3	24.6	48.0	6.43		0
1	5		2.44				6.78	0.130	0
1	133		0.024					0.020	
1	146		0.030					0.040	
2	1		1.29	87.1	22.1	79.0	2.22	0.638	0
2	4		2.4				4.6	0.647	
2	10-13		3.97	27.2	22.6	73.7	5.99	0.466	0
2	49		2.5	41.1	16.2	98.4	6.95	0.268	0
3	5-8		1.36				4.99	0.190	
3	126-129		0.057					0.056	
4	8-9						8.96	0.347	
4	107-110		3.42				8.81	0.133	
4	115-118		0.105				8.94	0.126	

* Calculated as the di-zinc complex of mesobilifuscin.

RESULTS

The curves for N^{15} concentration in the circulating hemoglobin protoporphyrin, fecal dipyrromethenes and the bilirubinoid pigments for Cases 1 and 2 and Subject 4 are shown in Figures 1, 2 and 3, respectively. Table I permits comparison of the N^{15} percentages in Subjects 1, 3 and 4 (having normal erythrocyte life spans) at the early and late peaks of the stercobilin N^{15} ; the former shortly after the administration of N^{15} glycine, the latter at the time of destruction of mature circulating red cells as denoted by the decline of the protoporphyrin N^{15} and the marked secondary rise of N^{15} in fecal stercobilin or urobilin.

The data in these three individuals with normal hemoglobin metabolism are generally similar and may be considered together. The red blood cell survival, determined on the basis of the N^{15} concentration in the circulating hemoglobin protoporphyrin, approximated the normal survival curve described by Shemin and Rittenberg (7). The stercobilin N^{15} data and curves are those to be anticipated in the presence of normal hemoglobin metabolism. The early peak, occurring between four and 12 days, has been described in a normal individual by London, West, Shemin, and Rittenberg (8). It may be derived either from anabolic sources or from destruction of young erythrocytes in the bone marrow or spleen. The second stercobilin N^{15} increase beginning

at about 90 days with the peak at 120 to 130 days corresponds with the period of destruction of mature circulating erythrocytes. It is evident that the N^{15} data for mesobilifuscin and stercobilin differ markedly. Only one mesobilifuscin N^{15} peak is observed occurring between two and eight days after the glycine administration. In the normal subject, Figure 3, this peak definitely precedes that of stercobilin and the N^{15} concentration in the mesobilifuscin is already declining while the concentration in stercobilin is still rising. There is some suggestion of this in Case 3 and the precedence is well defined in Case 2 (Figure 2), the patient with hemolytic anemia (see below). By way of contrast, in the subjects with normal hemoglobin metabolism, the stercobilin N^{15} concentration shows a striking increase at the time of red cell destruction but no mesobilifuscin peak is seen. A slight increase in the mesobilifuscin N^{15} is noted in Figure 3. This may well represent artifactual formation from bilirubinoids either in the colon or during the chemical procedure, though the possibility that a small proportion owes its formation to bilirubinoid schism in the body cannot be excluded (see below). The small increase noted in Figure 3 occurs gradually and later than in the corresponding stercobilin curve. Furthermore, it is apparent that the greater part of the mesobilifuscin rise occurs while the N^{15} concentration of stercobilin is decreasing. There was no significant increase in mesobilifuscin N^{15}

at the time of the second stercobilin peak in Cases 1 and 3 (Table I and Figure 1).

It is also noteworthy that in the subjects with normal red cell life span (Nos. 1, 3 and 4) the N^{15} in mesobilifuscin more nearly approaches the base line during the heme (protoporphyrin) N^{15} plateau than does the N^{15} of the stercobilin. If the persistent small elevation of the latter is construed as due to a minor, random destruction of hemoglobin during this period, the lower level of N^{15} in the dipyrromethene would be in accord with an anabolic origin.

In Case 2, that of congenital hemolytic anemia (Figure 2), a marked shortening of the red cell survival time is demonstrated by the N^{15} curve for hemoglobin protoporphyrin. In the presence of active hemolysis only one peak is noted in the N^{15} curve for bilirubin and for *d*-urobilin. The mesobilifuscin N^{15} curve reveals an early peak clearly preceding that of bilirubin and *d*-urobilin. The mesobilifuscin N^{15} at first falls more rapidly but levels off between 15 and 30 days, then exhibits a definite secondary rise at the same time that the curves for hemoglobin protoporphyrin, fecal bilirubin and *d*-urobilin are steeply falling.

In the foregoing description of method it was pointed out that the procedure used in the first three cases yielded a crude product of lower total nitrogen percentage than that obtained in the fourth and most detailed study (S. G.). The ordinary nitrogen percentages, with the atom per cent excess N^{15} on the same samples, are given in Table II. The low ordinary nitrogen percentages

in the material from the first three cases obviously restrict any conclusions based on these data. The theoretical concentration for a mono-zinc complex is 7.6 per cent N and 18 per cent Zn, for a di-zinc complex 6.45 per cent N and 30 per cent Zn and for the (zinc free) methyl ester 8.8 per cent N. The results of the nitrogen and zinc determinations are given in Table II. The zinc content approximated that of a di-zinc complex in two samples, while in the others zinc was present in excess of the theoretical for such a complex. It is apparent that many of the precipitates analyzed contained excessive zinc due to coprecipitation or as complexes of impurities. The nitrogen analyses both before and after chromatographic purification by Siedel and Möller's method (1) are shown in Table II. It is seen that in the majority of samples analyzed there was essentially no change in atom per cent excess N^{15} before and after application of Siedel and Möller's method. In Case 2 the atom per cent excess N^{15} decreased moderately in one sample and increased slightly in another. The nitrogen content increased consistently after this additional purification recycling, although the theoretical nitrogen percentage was reached in but one sample (No. 4). These observations indicate that most of the nitrogen in the crude samples was in the dipyrromethene molecule, the low nitrogen percentage being mainly due to the presence of non-nitrogenous impurities. It is not clear why the nitrogen percentage failed to reach the theoretical after purification of these samples. In the only instance where this did occur (No. 4)

TABLE III
Oxidation of mesobilifuscin (Mbf) from feces as compared with that from mesobilirubinogen (Mbg)

	Yields (mg.) and melting point			
	Methyl ethyl maleimide	M. P.	Hematinic acid	M. P.
1. $H_2SO_4 - Cr_2O_3$ method (18, 19)				
a. 25 mg. from Mbg	5.4	62-65	2.4	110-113
b. 50 mg. from Period 35; Subject No. 4	10.0	63-65	4.0	113-114
c. 50 mg. from Zn complex; Mbf Subject No. 1	3.0	60-65	1.2	98-106
d. 51 mg. from Periods 32, 33; Subject No. 4	7.6	62-65	3.9	112-115
e. 87 mg. from Periods 10, 11; Subject No. 4	12.0	62-65	3.8	113-114
2. Nitric acid method (1)				
a. Siedel and Möller's data (1) 200 mg. Mbf methyl ester from Mbg	14.0	67		
b. 200 mg. Mbf Periods 14-20; Subject No. 4	20.0	63-67	2.1	100-108
c. 200 mg. Zn Mbf; Subject No. 2	6.0	63-68	0.5	110-112

the material was freshly prepared and the purification by Siedel and Möller's method was carried out at once, while the earlier samples had been kept for long periods in the crude zinc precipitate; thus the possibility of a slow complex formation with a non-nitrogenous material must be entertained.

The amounts of methyl ethyl maleimide and hematinic acid obtained after oxidation of mesobilifuscin by two different methods are given in Table III. Use of the sulfuric acid-chromic oxide method resulted in better yields of methyl ethyl maleimide (38 per cent of theoretical) and hematinic acid (22 per cent of theoretical) from the standard (mesobilifuscin prepared from mesobilirubinogen) than did the nitric acid method. The yields obtained from the mesobilifuscin methyl ester isolated from Subject 4 compare favorably with those from the standard by either method, indicating at least a high degree of similarity. In the case of the crude pigments, prepared as the zinc complexes in Cases 1 and 2, the yields were considerably lower than from either the standard or the more highly purified material from Subject 4. Nevertheless the isolation and identification of these oxidation products from the crude pigments, considered in relation to their N percentage and other characteristics, provides strong evidence that their nitrogen was mainly present in a dipyrromethene compound. The experiment in the normal subject, No. 4, is regarded as the most decisive, particularly because of the regular use of Siedel and Möller's method with each sample, shortly after the period of collection. The data in Experiments 1 through 3 are less decisive because of the initial method of preparation, nevertheless the results are in general agreement with those of Experiment 4.

DISCUSSION

It has been widely assumed that the naturally occurring fecal dipyrromethenes or mesobilifuscins are products of hemoglobin catabolism and are formed by oxidative schism of the bilirubinoids. Siedel, Stich and Eisenreich (3) believed that quantitative measurement of fecal mesobilifuscin might reconcile known discrepancies between the rate of hemoglobin breakdown and fecal urobilinogen excretion. A considerable amount of data collected in various types of ane-

mia reveals that discrepancies of this type are not accounted for by proportionate increases of fecal mesobilifuscin or dipyrromethene. These observations are to be described in an ensuing paper of this series. Also, if mesobilifuscin were mainly catabolic, like the urobilinogen group, one would anticipate that after feeding N^{15} glycine there would be a second mesobilifuscin N^{15} peak corresponding to the second stercobilin peak at the time of destruction of mature circulating erythrocytes. It is evident from examination of the present N^{15} curves for mesobilifuscin that a second peak does not occur. Similarly, in the presence of active hemolysis the mesobilifuscin N^{15} falls much earlier than that in the hemoglobin protoporphyrin and bilirubinoid compounds. A secondary rise as the latter continues to fall may be due to reutilization of N^{15} made available from the globin of destroyed hemoglobin. This would accord with the concept of a main anabolic origin of the fecal dipyrromethenes, also with that of the amino acid pool (20). Although mesobilifuscin is readily prepared from bilirubinoid compounds and may be formed from them in small amount under appropriate conditions, the present results indicate that the relation of the naturally occurring dipyrromethene compounds to hemoglobin catabolism, is insignificant. In this connection, however, the special significance of Bingold's "pentdyopent" (21) must be recognized. This appears to represent a small moiety of dipyrromethene formed by the kidney, by schism of heme or bilirubinoids. There is no evidence that it occurs in the feces.

The mesobilifuscin N^{15} curves exhibit an early peak, as seen in Figures 1, 2 and 3. As already noted, this clearly precedes the early stercobilin peak in Subjects 2 and 4 (Figures 2 and 3) and this is suggested in Case 3 (Table I), though here the data are not decisive. In Figures 2 and 3 it is seen that the N^{15} concentration in mesobilifuscin falls earlier and more rapidly than in the corresponding stercobilin N^{15} curve. The early concentration of N^{15} in mesobilifuscin together with the lack of any marked N^{15} concentration at the time of mature erythrocyte destruction indicates that the dipyrromethene pigments are mainly anabolic in origin. This concept is also supported by certain clinical observations, to be published separately, which indicate that mesobilifuscin excretion is better correlated with erythropoietic ac-

tivity in the bone marrow than with hemoglobin destruction.

The slight gradual rise in the mesobilifuscin N^{15} curve at 100 to 130 days in the normal subject (Figure 3) merits consideration. The curve is a continuous gradual upward slope with the greatest increase actually occurring when the N^{15} concentration in stercobilin is declining. It is unfortunate that the N^{15} determinations were discontinued at 130 days when the mesobilifuscin N^{15} value had not yet commenced to fall. This was due to delay of many weeks in the isolation and N^{15} analyses. Nevertheless, it is clear that the stercobilin N^{15} peaked at about 120 days and had fallen considerably when the experiment was broken off. Thus, even had there been further increase of mesobilifuscin N^{15} it could not well have been ascribed to hemoglobin destruction. The small sustained increase associated with and following the stercobilin N^{15} peak may be explained on the basis of reutilization of N^{15} made available from the globin of destroyed hemoglobin, as discussed in the foregoing. It is also possible that some part of this minor increase is due to (artifactual) conversion of bilirubinoid to dipyrromethene. It should be emphasized that an earlier peak in the mesobilifuscin curve was not overlooked, for each consecutive four day collection of feces was analyzed throughout this period.

It should be re-emphasized that the fecal pigment(s) isolated in the first three cases was relatively crude with a nitrogen percentage considerably lower than the theoretical concentration for a dipyrromethene compound. Thus, conclusions based on these data alone would be hazardous. However, when Siedel and Möller's procedure (1) was used from the outset, as in Case 4, the total nitrogen content agreed regularly with the theoretical and the yields from oxidative degradation compared favorably with those from standard preparations. Since the configuration of the mesobilifuscin curve is similar in all four experiments, the validity of the data in the first three is considered to be enhanced, especially in view of the fact, already discussed, that strenuous oxidation provided methyl ethyl maleimide and hematinic acid in amounts indicating that the nitrogen was mainly contained in dipyrromethenes.

The means by which mesobilifuscin might be

derived from anabolic sources have not been determined. In the broader sense one must consider derivation from a pigment pool or "complex" such as suggested by Whipple (22) in 1922. This would require that a certain amount of bilirubinoid or other pyrrole pigment or precursor be manufactured in excess of that used in heme synthesis and that the excess be converted to mesobilifuscin or similar dipyrromethenes. A more specific concept in this regard involves the theory of a tripyrromethene intermediate on the pathway from porphobilinogen to porphyrin. The tripyrromethene theory was first suggested by Turner (23), more recently by Bogorad and Granick (24) and Shemin, Russell and Abramsky (25). The latter investigators, in particular, have emphasized this possible pathway, pointing out that differing dipyrromethanes could thus be provided to account for the formation of the porphyrin isomers. By the same token, an overproduction and excretion of excess dipyrromethanes, on this basis, is easily envisioned. This would also be true in respect to the dipyrromethane intermediaries postulated for the biosynthesis of uroporphyrin III in the differing concepts of Treibs and Ott (26) and of Bullock, Johnson, Markham and Shaw (27). The dipyrromethenes which might be formed, either by oxidation of excessive dipyrromethanes or by schism of a hypothetical tripyrromethane, might be expected to have acetic and propionic B substituents rather than methyl, ethyl and propionic as found in pure mesobilifuscin, according to Siedel, von Pölnitz and Eisenreich (2). It is quite conceivable, however, that acetic and propionic groups are decarboxylated in part to methyl and ethyl groups, in which event the differences in elementary analysis might well be undetected, especially as the fecal mesobilifuscin has not thus far been crystallized. Siedel and co-workers (2, 3) believe that it is excreted in the form of polymers of varying chain length which may account for the variations in solubility of different fractions and the failure to crystallize.

SUMMARY AND CONCLUSIONS

1. The relation of fecal dipyrromethene compounds or mesobilifuscin to hemoglobin metabolism has been investigated in four human subjects with the aid of N^{15} labeled glycine. A modification of Siedel and Möller's method (1) of

partial purification of mesobilifuscin was employed. This included preliminary removal of urobilinogen to prevent addition by oxidative schism to the preformed dipyrromethene moiety.

2. The lack of significant concentration of N^{15} in mesobilifuscin at the time of destruction of mature circulating erythrocytes when the stercobilin N^{15} was greatly elevated is presented as evidence that mesobilifuscin is mainly anabolic in origin rather than a product of hemoglobin breakdown.

3. The early incorporation of N^{15} in mesobilifuscin with a peak which often precedes the early stercobilin N^{15} peak also indicates that mesobilifuscin is derived principally from anabolic sequences. The lower N^{15} values for mesobilifuscin during the plateau of the protoporphyrin N^{15} in the circulating erythrocytes are in accord with this concept.

4. The possible mechanisms of mesobilifuscin derivation are considered.

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