FAT AND NITROGEN METABOLISM IN A CASE OF CON-
GENITAL ABSENCE OF THE BILE-DUCTS WITH A
STUDY OF FERMENTS OF THE PAN-
CREATIC SECRETION AND
THE FECES

HENRY KOPLIK, M.D., AND BURRILL B. CROHN, M.D.

NEW YORK

In this study of fat metabolism, with a consideration of the nitrogen balance in a case of congenital absence of the bile-ducts, we were especially concerned from a clinical standpoint, not only with the fat and nitrogen disposition in the body, but with the activity of the pancreatic ferments. In this, one of us (Koplik) was fortunate in having the active co-operation of a colleague skilled in studies of such a nature.

From a clinical standpoint it is of importance to ascertain whether the ducts are stenosed or absent, or practically occluded. There are cases of stenosis of the bile-ducts in which considerable bile passes into the intestine; such a case we have seen lately. In this case also there was the anomaly of a very large colon (Hirschsprung). In such a case metabolism studies would be of uncertain value on account of the abnormal state of the large, or even the small, intestine.

In the case which forms the subject of this present study the abnormal condition, as far as could be defined clinically, was that of a pure absence of the bile-ducts without any anomaly of the stomach or intestine being evident. Such cases are not numerous in the literature. The finding of a definite pancreatic secretion, with the study of its fermentative powers, is of great interest, occurring, as it does, in so young an infant.

METABOLISM BED

A word of explanation may not be out of place as to the metabolism bed. This bed was devised by one of us (Koplik) to confine the movements of the infant as little as possible. Of course all ideas of any metabolism bed must be traced back to the original bed of Heubner. From this all the others must derive their construction. The bed used in this case is an inclined Bradford frame devised in such a way as to maintain the pelvis of the baby in a fixed position. The frame itself is suspended from the head and foot of the bed like a hammock. The arms and lower extremities are absolutely free, the abdomen is not pressed on, the baby is safe and happy and does not seem to be the least incommoded. To prevent the baby from falling off the frame the chest is dressed in a loose corset-waist with bands at the sides so that the baby cannot fall to one or the other side, but at the same time has some latitude of motion. The baby also is not chilled, for by means of radiation from warm water bags beneath the frame an equable temperature is maintained. The exposed buttock and other parts are not chilled. The babies may actually gain in weight, at least do not lose, and seem perfectly happy. We have used this bed quite often and are well pleased,

inasmuch as we feel that the ultimate results are vitiated as little as possible by the enforced rest.

CASE REPORT

M. E., aged 10 weeks, was studied from Jan. 5 to Feb. 4, 1912. The diagnosis was congenital absence of the bile ducts.

Family History.—Negative for tuberculosis. The mother was married two years; this was the first child; no miscarriages.

Past History.—Breast-fed since birth; feedings q. 2 h. 15 to 30 minutes. Mother had little milk. Labor not especially difficult. Child was in a hospital for two weeks.

Present History.—The mother first noticed that the child was jaundiced when she was a week old. She did not know whether the child was yellow during the first week. Since the first week the child's stools were whitish. For over seven weeks the urine was dark. The child was becoming more jaundiced. The mother had noticed spots on the back only during the previous week.

Physical Examination.—General condition good; well nourished.

Skin: Intense greenish jaundice of skin and of mucous membranes. On back about middle of lumbar region there were three large, separate hematomata, the areas being infiltrated and just under the skin. Just under the free border of ribs on left side below axilla, another hematoma. Hematoma on right arm and right buttock.

Eyes: Icterus of conjunctiva, otherwise negative.

Mouth: Tongue clean, frenum short, prevents active movement of tongue.

Pharynx, ears and mastoids, glands, spine, chest, lungs and heart were negative.

Liver: Edge felt about four fingers (9 cm.) below free costal border in mammary line; moderately firm; gall-bladder not palpable.

Spleen: Edge felt one finger below free border.

Abdomen: Negative except for small umbilical hernia.

Extremities: Hematomata as noted. No Kernig or Babinski.

January 8.—*Stool:* Silvery white, of pasty consistency. The child was placed on the metabolism bed January 8.

January 9: Coagulation time of blood 5½ minutes. W. B. C., 7,600; Polynuclears, 48 per cent.; S. L., 44 per cent.; L. L., 8 per cent.; Hgb., 55 per cent. Blood contained bile. Urine normal except for heavy bile staining.

January 13: General condition fair; jaundice seemed somewhat more intense. Child for four days was on metabolism bed with apparently no discomfort. Stools clay colored. Urine contained considerable bile; weight practically at a standstill.

January 17, 9 a. m.: Soft rubber catheter No. 15 French passed gently into stomach, and slowly pushed to length of 25 cm. from the gum line. Felt apparently to enter duodenum. Aspirated with negative pressure for one hour. About 10 c.c. clear mucoid fluid obtained, very mucoid and stringy, neutral in reaction. The child was quiet throughout, though apparently hungry.

January 18: General condition fair; jaundice at a standstill; greenish yellow. Temperature normal. Weight seemed about stationary. The ecchymotic spots had practically disappeared.

January 26: Condition fair; temperature normal; jaundice seemed, if anything, somewhat lighter. Only remnants of ecchymoses were present on body; no new ecchymoses anywhere. General physical condition otherwise unchanged. Liver in mid-clavicular line was 7.5 cm. below free border; gaining in weight.

February 4: Condition about the same. Jaundice seemed stationary; temperature normal; stationary in weight. No new ecchymoses. Heart and lungs normal. Liver edge felt 6 cm. below free border in mid-clavicular line. Spleen felt at free border of ribs. Stools still show no evidence of bile.

February 6: Discharged with weight 8 pounds 5 ounces, but in very good condition.

February 9: It was learned that soon after leaving the hospital the child began to fail rapidly. Death took place three days later. No autopsy was obtainable.

TABLE OF WEIGHT OF THE BABY

	Pounds	Ounces
January 6, after admission	8	11
January 8, put in metabolism bed, 10 a. m.	8	11½
January 11, just before removal from bed, 12 m.	8	11
January 12, 6 p. m., taken out of metabolism bed.		
January 14	8	8
January 18	8	9
January 22	8	8
January 25	8	10
January 29	8	8
February 1	8	6
February 3	8	5

Temperature and pulse normal throughout the period of observation.

LABORATORY FINDINGS

1. Methods: The following chemical and metabolism studies were effected by placing the child in a metabolism frame for a period of four days. During this time the intake of the child was most carefully noted, an exact duplicate diet being reserved for chemical analysis. The excreta were collected, urine and stool separately, as far as possible (stool being strained off through a sieve), the whole being iced immediately and so maintained for analysis. The twenty-four-hour specimen of urine was daily examined chemically and for ferments. The daily stool was similarly analyzed for the presence of ferments; the chemical data on the stool was ascertained from the accumulated total period collection.

Nitrogen estimations were made by the Kjeldahl process; total fat analysis of the stool with estimations of the split fat, by means of the Folin-Wentworth method.

The total solids of the milk was reckoned by drying and difference; fat reckoned by extraction of the dried residue with warm ether. The methods of ferment analyses will be described later.

2. Metabolism Studies: A computation of the intake of the child during the four-day period is represented in Table 1.

TABLE 1.—ANALYSIS OF FOOD INTAKE IN AUTHOR'S CASE

Date	Milk Amount,	Total Solids,	Total N,	Fat, Gm.	Remarks
....	c.c.	Per Cent.	Gm.	...	
January 9 ...	900	5.52	2.091	13.95	Diet, Jan. 9-10: Whole milk,
January 10 ...	825	5.2	1.478	10.31	3viii; barley gruel, 3xvii; milk-sugar 3v.
January 11 ...	1,110	6.8	1.771	23.20	Diet, Jan. 11-12: Whole milk,
January 12 ...	1,260	6.4	1.842	50.78	3xv; barley gruel, 3xx; milk sugar 3 vi.
Total amount..	4,095		7.182	98.24	
Daily average..	1,024	5.98	1.7955	24.56	

The output of the child is estimated as urinary excretion (Table 2) and as fecal excretion (Table 3).

TABLE 2.—URINARY EXCRETION

	Amount, c.c.	Nitrogen, Gm.
January 9	275	0.67
January 10	400	1.0192
January 11	400	0.9520
January 12	500	1.2040
Total amount	1,575	3.8452
Daily average	393.8	0.9613

Large amount of bilirubin present.

TABLE 3.—STOOL EXCRETION (DRIED=61 Gm.)

	Gm.
Nitrogen—	
Total for period	0.991
Daily average	11.89
Fat, per cent—	
Folin-Wentworth	77.95
Split fat (soaps and fatty acids)	22.6
Neutral fat	77.4
Fat, total amount	47.55
Daily average	11.89

Chemical tests of the stool were repeatedly negative for bile.

From a comparison of Table 1 with Tables 2 and 3, we are able to compute the absorption and the utilization of the food products (Table 4).

TABLE 4.—ABSORPTION AND RETENTION (DAILY AVERAGE)

	Nitrogen, Gm.	Fat, Gm.
Intake	1.7955	24.56
Output—		
Urine	0.9613	
Stool	0.2480	11.89
Absorption (from intestine)	1.5475 (86.2%)	12.67 (48.4%)
Retention (per cent. of intake) ..	0.5862 (32.6%)	

Weight of child during study was stationary.

It will thus be seen in this child, 10 weeks of age, that the nitrogen absorption from the intestinal tract and its retention in the body were but slightly, if at all, interfered with. A very marked disturbance in the absorption of fat from the intestine is noted. In contradistinction to the normal fat absorption of 88 to 96 per cent. (Rubner and Heubner,¹ Freund² and L. F. Meyer³), there is seen in this case an absorption of only 48.4 per cent. Not only is the absorption poor, but the splitting of the fat and saponification seem also to be interfered with. Thus Hecht⁴ and Keller⁵ offer from observations on normal and slightly pathological children, fat-splitting in the stool ranging from 88 to 90 per cent.

1. Rubner and Heubner: *Ztschr. f. Biol.*, No. 38, p. 315.
2. Freund: *Biochem. Ztschr.*, 1909, No. 16.
3. Meyer, L. F.: *Biochem. Ztschr.*, 1908, No. 12, p. 422.
4. Hecht: *Jahrb. f. Kinderh.*, 1905, No. 62, p. 612.
5. Keller: *Monatschr. f. Kinderh.*, 1903, i, 234.

We may compare the findings in our case with the results of analyses offered by Niemann⁶ in an instance of congenital absence of the bile-ducts with advanced biliary cirrhosis in a child of 9 months. Niemann notes a nitrogen absorption of 80 to 93 per cent. and a fat absorption of 28 to 39 per cent. During one period of his investigations, when the child was on a very low diet, more fat was found excreted in the stool than was ingested in the diet. Further analyses are not noted in his case.

3. Pancreatic Studies: It appeared of interest to us to further investigate this disease with a view of ascertaining the condition of the pancreatic secretion, its presence or absence from the intestinal tract, and if possible, the enzymatic potency of the elaborated ferments. This was undertaken by means of ferment analyses of the duodenal juice obtained by the Hess⁷ method, and, second, by study of the ferments present in the stool and urine. The technic employed by Hess was followed closely, the duodenal catheter being passed two hours after the first feeding in the morning. During the course of one hour we obtained by aspiration 8 c.c. of slightly milky, cloudy material, of neutral reaction, containing much stringy mucus. This material was differentiated from the gastric contents by the strong acidity and increased milkiness of the latter.

The duodenal material was diluted with twice as much distilled water before testing. The stool was extracted in twenty-five times its volume of distilled water made slightly alkaline with sodium hydrate. Both materials were tested for pancreatic ferments in the following manner:

For amylase, by adding to 1 c.c. of the stool extract, urine or duodenal juice varying amounts of 1 per cent. starch solution and allowing this to incubate at 40 C.; the duodenal contents or urine was incubated for one hour, the stool contents for twenty-four hours (under toluol.). The persistence of starch was tested for by adding liberal excess of iodine solution.

For lipase, to 1 c.c. of material was added 1 c.c. of ethyl butyrate and 0.5 c.c. toluol; the whole made up to 25 c.c. volume, brought to exact neutral point (one drop of 1 per cent. alcoholic phenolphthalein solution as indicator) and incubated for twenty-four hours. The amount of acidity produced was titrated with N/10 NH_4OH ; a boiled specimen served as a blank control.

Trypsin, Gross-Fuld method.

The following results were obtained:

Duodenal Contents:

Amylase—1 c.c. digests 9 c.c. of 1 per cent. starch solution in one hour.

Lipase—1 c.c. frees 0.1 c.c. of N/10 acid in twenty-four hours.

Trypsin—10 c.c. of 0.1 per cent. casein is digested by duodenal juice in dilution of 1:1,000 in twenty-four hours.

6. Niemann: *Ztschr. f. Kinderh.*, 1912, No. 4, p. 152.

7. Hess: *AM. JOUR. DIS. CHILD.*, 1912, iii, 133; 1912, iii, 304.

We are not possessed of figures for the values of pancreatic ferments in duodenal contents of normal children. However, judging from the results obtained by one of us (Crohn) in normal adults (to be published) we are enabled roughly to deduce in this case (1) that the pancreatic ferments are present and therefore the pancreatic ducts are patent; (2) that the amylase and trypsin are strongly present; (3) that the lipase is very weak. This latter fact is the important fact, and is probably best explained by the absence of the normally present bile salts which act to increase the strength of the lipolytic ferment from ten to twenty times.

The examination of the urine for amylase, and of the stool for the presence of the three ferments, revealed the following:

TABLE 5.—RESULTS OF EXAMINATION OF URINE AND STOOLS FOR FERMENTS

		Stool		
		Amylase	Lipase,	Protease
			c.c.	
Jan. 9		Neg.	0.1	
Jan. 10		100 T. D. E.	50 D. E.	1:500 (casein)
Jan. 11		Neg.		Neg. (Mett; fibrin flakes)
Jan. 12		70 T. D. E.		1:62 (casein)

In order to appreciate the meaning of these findings several observations were made on infants a few weeks of age and in normal health. The results are given in Table 6.

TABLE 6.—FINDINGS IN NORMAL INFANTS

URINE: AMYLASE (TWENTY-FOUR-HOUR SPECIMENS)

A—1 c.c. urine digests	0.5	c.c. 1 per cent starch in 1 hour (200 c.c. total excretion) =	100 T. D. E.
B—1 c.c. urine digests	0	c.c. 1 per cent. starch in 1 hour =	0
C—1 c.c. urine digests	0	c.c. 1 per cent. starch in 1 hour =	0

STOOL: AMYLASE (EXTRACT = 2:50 DILUTION)

A—1 c.c. extract digests	0.25	c.c. 1 per cent starch in 24 hours =	12.5 D. E.
B—1 c.c. extract digests	0	c.c. 1 per cent. starch in 24 hours =	0
C—1 c.c. extract digests	10	c.c. 1 per cent. starch in 24 hours =	250 D. E.
D—1 c.c. extract digests	1.5	c.c. 1 per cent. starch in 24 hours =	37½ D. E.
E—1 c.c. extract digests	0.5	c.c. 1 per cent. starch in 24 hours =	12 D. E.

STOOL: CASEIN TEST (GROSS-FULD METHOD. DILUTION 2:50)

A—0.3	c.c. stool extract digests	10	c.c. 1 per cent. casein in 24 hours =	1:825
B—0.01	c.c. stool extract digests	10	c.c. 1 per cent. casein in 24 hours =	1:25,000
C—0.7	c.c. stool extract digests	10	c.c. 1 per cent. casein in 24 hours =	1:357
D—0.3	c.c. stool extract digests	10	c.c. 1 per cent. casein in 24 hours =	1:825

STOOL: LIPASE

A—0	c.c. acid produced from 1 c.c. ethyl butyrate in 24 hours.
B—0	c.c. acid produced from 1 c.c. ethyl butyrate in 24 hours.
C—1.9	c.c. acid produced from 1 c.c. ethyl butyrate in 24 hours.
D—0.1	c.c. acid produced from 1 c.c. ethyl butyrate in 24 hours.
E—7.1	c.c. acid produced from 1 c.c. ethyl butyrate in 24 hours.

In examining such findings in normal infants we are struck by the variability of the results in spite of an unvarying technic in carrying out the tests. Notwithstanding this, by comparison of the findings in the

pathologic case with those in normal controls we may note (a) that on two occasions strong amylase was present in the urine; (b) that a weak amylolytic ferment was present in the stools; (c) the proteolytic ferments of the stool, tested against casein, shows no diminution in the strength of this enzyme as normally present; (d) the lipolytic ferment, on the one occasion present, was very weak.

To do more than draw the most general conclusions from this scant data would lead to error. The question of the specificity of the ferments of the stool, and their relation to the diagnosis of pancreatic disease, must be reserved for a future discussion, as also the value of the casein test in the stool as an index of pancreatic-trypsin activity. However, in general, we may say that the pancreatic enzymes are present, and of normal strength, except the lipase of the stool, which, like the lipase of the duodenal secretion, is weak.

GENERAL CONCLUSIONS

From the study of this case of congenital absence of the bile-ducts, the conclusions are as follows:

1. Nitrogen metabolism is normal.
2. Fat metabolism is greatly disturbed, characterized by poor absorption and deficient splitting of neutral fats.
3. The pancreatic ducts are patent and the pancreatic secretion is of normal strength.
4. The lipolytic ferment of the pancreas, while present, performs its function weakly on account of the absence of the bile salts, which normally accelerate its enzymatic activity.

The authors wish to express their obligations to Dr. Samuel Bookman for helpful suggestions and material assistance during the course of this study.