METABOLIC STUDIES IN PATIENTS WITH GASTRO-INTESTINAL CANCER. IV. FAT METABOLISM, A METHOD OF STUDY[1]

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The results of recent studies have added significantly to the understanding of the intermediary metabolism of fat, particularly as concerns the role of the liver in the utilization of fat by the body (1 to 3). On the other hand, the available information concerning the absorption and digestion of fat in the gastro-intestinal tract leaves much to be desired, primarily because of a lack of satisfactory technical methods.

In the course of an investigation of the metabolism of essential dietary factors by patients with gastro-intestinal cancer (4, 5), the ability of these patients to digest and absorb fats was determined. For this purpose, it was necessary to devise a simple and satisfactory quantitative method. A description of this method and the results of its application to patients with various disorders form the subject of the present communication.

CLINICAL MATERIAL

The clinical material studied consisted of the following:

Two male individuals were admitted to the hospital, one for the surgical treatment of a plantar wart, and the other for a saphenous vein ligation. Both were entirely free of gastro-intestinal complaints and had taken normal diets for several years.

One male patient with gastric cancer was admitted to the hospital for an exploratory laparotomy. At the time of operation, the cancer could not be resected. Both before and after the operative procedure, the patient received an adequate diet which he ate without difficulty. Post-operatively, he remained afebrile and essentially free of all symptoms referable to his disease. There was no significant weight change during the period of observation.

One male patient had had a complete gastrectomy for cancer of the stomach and was not subjected to study until 20 months after the operative procedure. During that time he had lost 35 pounds, but was apparently free from any recurrent or metastatic disease. His diet was considered to be adequate and was ingested daily in several small, frequent feedings. His only complaints were postprandial distention and loose, bulky stools.

The one patient with atrophic gastritis was a woman in whom the diagnosis was established by repeated gastroscopic examinations. She had symptoms referable to her disease, but did not suffer from nausea, vomiting, or diarrhea. She had taken a grossly deficient diet for a considerable period.

Two male individuals with hepatic cirrhosis were admitted to the hospital and included in this study. One was a chronic alcoholic who had marked hepatomegaly, moderate splenomegaly, and frequent episodes of icterus, but no ascites, edema, anemia, or diarrhea. The other had hepatomegaly and splenomegaly, with macrocytosis but no anemia. No edema, ascites, jaundice, or diarrhea had been present at any time. The diets of both patients were moderately deficient in animal proteins and vitamin B complex. Both had considerable evidence of hepatic insufficiency as demonstrated by several liver function tests.

METHODS

1. *Clinical*

a. *Diet and fat load.* From the time of admission, all but 1 of the subjects studied received daily a diet, calculated from standard tables to contain approximately 1.5 grams of protein, 1.5 grams of fat, and 3.0 grams of carbohydrate per kilogram of body weight. The exception, A. Z., was given a diet which by analysis was found to contain 1.1 grams of protein, 0.7 gram of fat, and 4.9 grams of carbohydrate per kilogram of body weight.[4] The patients were allowed a preliminary period of from 3 to 5 days on these diets before any studies were begun, in order that they might become accustomed to the rigid routines employed in their feedings and in the collection of their excreta.

When each subject had taken the basal diet satisfactorily for from 6 to 9 days, a supplement of fat was given in order to obtain a measure of the individual's ability to absorb fat, at both a normal and high level of fat ingestion. This supplement consisted of 2 grams of fat per kilogram of body weight (the "fat load"). Twenty per cent of the fat in this supplement was provided by butter,

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[4] The low fat diet in this one instance was employed because it had constituted the basal diet fed to a group of patients who had undergone gastrectomy. The results of that study will be reported in a subsequent communication.

and eighty per cent by heavy cream. The fat load was taken at 7 a.m. of the test day.

The basal diet was eaten in 3 equal meals ordinarily at 7 a.m., 12 noon, and 6 p.m. No food was taken at any other time. However, on the day when the fat load was administered, the meals were taken at 1 p.m., 4 p.m., and 7 p.m.

b. *Collection of feces.* When the subject had taken the basal diet for from 3 to 5 days, a saline enema of 500 ml. was given at 7 a.m. and the returns discarded. From that hour, all stools excreted were collected, for from 3 to 4 days, in a large metal container and kept in a moist ice chest. At the end of each period, another 500 ml. saline enema was given and the returns were added to the stool collection.

From 1 to 4 such collections were obtained from each patient, and the fat content of these collected stools was considered to represent the fecal fat output of the individual under the basal conditions of the study.

At the end of each basal period, each subject was given the supplementary fat meal, and his stools were collected for from 2 to 3 days in 24-hour lots. At the beginning and end of each 24-hour period, the 500 ml. saline enema was administered and the returns added to the proper specimen. Finally, another 2 or 3-day stool collection was obtained in the manner described for the basal period.

2. *Chemical*

Determination of stool fat. The Van Slyke combustion method for the determination of lipid in blood (6) was adapted for liquid in stool specimens in the following manner: A homogeneous suspension of the feces in water was made by means of a mechanical stirrer. An aliquot of this aqueous stool suspension, chosen to contain from 0.05 to 0.15 mgm. of lipid carbon, was used. The fat was extracted from this aliquot by 9 volumes of a mixture of equal parts of absolute alcohol and ethyl ether. The extract thus obtained was then dried on a steam bath, cooled to room temperature, and the carbon content of the residue measured.

To ascertain the validity of the results obtained by this adapted technique, some of them were checked by the method of Bloor (7).

RESULTS

The results of the investigation are presented in 2 parts: 1. The results of studies to test the validity of the method used, and 2. The results obtained when this method was applied to a study of the absorption of fat by the subjects employed.

1. *Results of studies to test the validity of the method used*

a. *Homogeneity of stool suspensions.* Stool collections were diluted with tap water to make a volume of from 500 to 1500 ml., and the whole stirred mechanically for about 1 hour. To ascertain whether or not this technique provided homogeneous suspensions, immediately after stirring, stearin determinations were made on samples which were removed by a large bore pipette from different levels of suspension. Differences of from 1 to 4.5 per cent were found between the stearin content of the various aliquots (Table I). Thus,

TABLE I

Experiments to ascertain the homogeneity of the stool suspension

Stool of patient	Sample number	Stearin content
		grams per cent
F. P.	1	1.23
	2	1.25
S. L.	1	2.20
	2	2.32
	3	2.20
E. F.	1	1.42
	2	1.43

it would appear that an adequate mixture of the stool specimens was obtained by the technique used.

b. *Comparison of values obtained by the technique used with those obtained by the gravimetric method of Bloor.* It is recognized that the method of Van Slyke (6) measured only the combustible lipid carbon which is extracted by a mixture of equal parts of alcohol and ether from an aqueous suspension. It was desirable, therefore, to ascertain whether or not the stearin values calculated from those of lipid carbon were in agreement with the values for total fat as determined by another (gravimetric) technique. The technique chosen for this comparison was that of Bloor (7).

In Bloor's method (7), the lipid is extracted from 1 volume of aqueous suspension by 9 volumes of a mixture of 1 part ether and 3 parts alcohol. This extraction mixture of Bloor is not the same as that used in the present investigation. Accordingly, it was necessary also to determine whether or not different values were obtained by the use of different extraction mixtures.

Ten ml. samples of each of 2 different suspensions of feces were diluted to 100 ml. with Bloor's solution (alcohol 3 volumes: ether 1 volume) and the fat content of the extract deter-

mined. By the gravimetric method of Bloor, the 10 ml. samples were found to contain 0.145 gram, and 0.117 gram of fat; by the gasometric method of Van Slyke, the fat (stearin) content of the extracts were 0.165 gram and 0.1165 gram, respectively, or 11 and 0 per cent greater than those obtained by the former procedure (Table II).

TABLE II

Comparison of the fat analyses in stool suspension and in evaporated milk by the adapted gasometric technique of Van Slyke et al., *and that of Bloor*

Material	Fat content by method of		Fat content by method of	
	Van Slyke	Bloor	Van Slyke	Bloor
	extracted with alcohol-ether 1 : 1		extracted with alcohol-ether 3 : 1	
Stool suspension number 1, 10 ml. samples.	0.17	0.17	0.165	0.145
Stool suspension number 2, 10 ml. samples.	0.124	0.122	0.116	0.117
Evaporated milk number 1, 10 ml. samples.	1.03	0.97	0.93	0.95
Evaporated milk number 2, 10 ml. samples.	1.04	0.99	0.99	0.93

When the 10 ml. samples of the above 2 stool suspensions were extracted with the mixture used by Van Slyke (alcohol 1 volume: ether 1 volume), the fat contents of the extracts determined by the method of Bloor were 0.170 gram and 0.122 gram, values 17 and 4 per cent greater than those obtained gravimetrically by the use of the Bloor solution. Furthermore, the values obtained by both methods when the Van Slyke solution was used were in better agreement than were those which were obtained by extraction with the Bloor solution. By the gasometric technique and use of the Van Slyke solution, the fat (stearin) [5] contents of the samples studied were 0.170 and 0.124 gram.

Determinations of the same nature also were made of the fat content in two 10 ml. samples of evaporated milk. By the gravimetric and gasometric techniques, the Bloor solution extract contained 0.95, 0.93 and 0.93, 0.99 gram respectively. The values obtained when the Van Slyke solution was employed were, for the gravimetric technique, 0.97 and 0.99 gram, and for the gasometric method, 1.03 and 1.04 grams (Table II).

Thus, it would appear that the values of fat in stool suspensions and in milk, determined by the gasometric technique of Van Slyke, are in good agreement with those obtained by the gravimetric method of Bloor. This agreement is better still when the Van Slyke solution is used in both methods for extraction of the lipid.

2. *Measurement of the absorption of fat from the gastro-intestinal tract of the individuals studied*

a. *Normal individuals.* Two normal male subjects (F. P. and P. R.) were fed the standard diet which contained 1.5 grams of fat per kilo, or a total of 135 and 120 grams per day. During the basal period, neither individual excreted more than 6.3 grams of stearin (fat) per day (Table III). The daily stearin outputs of F. P. for 3 days ranged from 5.0 to 6.3 grams and averaged 5.5 grams, and those of P. R. for 4 days ranged from 3.1 to 4.0 grams, and averaged 3.5 grams. These average excretions of stearin represented, for F. P. and P. R., 4 and 3 per cent respectively of their daily fat consumption.

When the fat supplement was administered to the 2 normal subjects, a sharp increase in the fecal fat output resulted. In the first instance (F. P.), the stearin excretion during the 48-hour period after the ingestion of the fat load rose from the average base level of 5.5 grams per day to 12 grams per day. This increase of 6.5 grams per day represented a loss of 7.6 per cent of the administered fat supplement. Differently expressed, 92 per cent of the fat load had been absorbed. Forty-eight hours after the ingestion of the fat meal, the stearin output of this individual returned to the base level.

In the second instance (P. R.), a significantly increased output of fecal fat was noted only during the 24-hour period which followed the fat load test. In that interval, the subject excreted 12.2 grams of stearin in contrast to his average basal excretion of 3.5 grams per day. This increased fat output represented a loss of 6 per cent of the

[5] Mgm. stearin = mgm. lipid carbon × 1.15.

TABLE III

The excretion of fat (stearin) in the stools of the individuals studied

Subject	Disease	Excretion of fat while on basal diet		Days	Average fat excreted. Fat ingested	Average amount of fat excreted per day above the basal output after the ingestion of the fat supplement	Days	Extra fat excreted after fat supplement
		Range	Average					
		grams			*per cent*	*grams*		*per cent*
F. P.	Normal	5.0 to 6.3	5.5	3	4.0	6.5	2	7.6
P. R.	Normal	3.1 to 4.0	3.5	4	3.0	8.7	1	6.0
A. A.	Gastric cancer	4.5 to 10.4	6.5	16	7.0	8.5	1	7.0
A. Z.	Gastrectomy for cancer	26.5 to 32.1	29.4	6	73.0	120	1	90.3
M. Z.	Atrophic gastritis	9.6 to 19.5	14.5	6	14.0	29	2	22.0
E. H.	Cirrhosis of liver; chronic alcoholism	7.0 to 9.5	7.6	6	8.1	8.8	1	7.5
E. M.	Cirrhosis of liver	4.9 to 5.0	4.95	3	5.0	2.4	3	3.0

supplement, and indicated that 94 per cent had been absorbed.

In summary, 2 normal adult males, who ingested a constant diet, absorbed 96 and 97 per cent of the fat content of that diet. Furthermore, the fat load of the amount employed does not decrease significantly their ability to absorb fat from their gastro-intestinal tracts.

b. *Patients with gastric disease.* In this group are included 3 individuals: One bearing gastric cancer (A. A.), 1 who 20 months before the present study had undergone a complete gastrectomy for the removal of a gastric cancer (A. Z.), and 1 with atrophic gastritis (M. Z.).

During a basal period of 16 days, the patient with gastric cancer excreted from 4.5 to 10.4 grams of stearin per day, or from 5 to 11 per cent of the dietary fat ingested. The administration of the fat supplement was followed during the next 24 hours by a fecal stearin output of 15.5 grams, or 7 per cent of that load. The fecal excretion of stearin returned to within basal levels (6.7 to 6.8 grams per day) within 48 hours after the fat meal. The findings indicate, therefore, that this patient who bore a gastric cancer apparently absorbed from 89 to 95 per cent of his dietary fat, values only a little lower than those absorbed by the normal individuals. The addition to his diet of a large fat supplement did not decrease this efficiency of absorption.

In sharp contrast to the findings thus far presented were those noted in the patient who had undergone a total gastrectomy. During his basal period of 6 days, this patient excreted from 26.5 to 32.1 grams of stearin, or from 66 to 80 per cent of that ingested.[6] The one-day addition to his diet of 130 grams of fat as a supplement resulted in an increase of the fecal stearin output during the next 24 hours of 120 grams. This would indicate an absorption of only 10 per cent of the fat load. The return to his basal stearin excretion occurred within 48 hours after the ingestion of the fat supplement.

These observations indicate that not only did the gastrectomized patient have a considerable steatorrhea while taking a comparatively low fat diet, but the addition of the fat load to this diet significantly increased the fat loss.

A detailed investigation into the nature and cause of steatorrhea in the gastrectomized patient is beyond the province of the present study but is presented in a subsequent communication. However, the possibility that a relationship might exist between the absence of a gastric mucosa and the occurrence of steatorrhea was examined in a patient who had marked and generalized atrophic gastritis (M. Z.).

During the 6 day basal period, this patient (M. Z.) excreted from 10 to 20 per cent of the fat ingested. Although these stearin outputs were not as great as were those of the gastrec-

[6] It is to be recalled that the diet of this subject included only 0.7 gram of fat per kilo.

tomized patient (66 to 80 per cent), still they were considerably above the normal values (3 to 4 per cent). Furthermore, the administration of the fat supplement to this patient with atrophic gastritis was promptly followed by an increased stearin excretion of from 28 to 30 grams per day for 48 hours. The increase represented an output of 22 per cent of the fat meal, and was significantly more than those of the normal individuals who excreted 6 and 7.6 per cent of their fat supplements.

Thus, it would appear that the patient who lacked a stomach, and the patient whose gastric mucosa was markedly atrophic, suffered from an impaired ability to absorb fat from their gastro-intestinal tracts. This impairment was accentuated by the ingestion of a fat load. Although no conclusions can be drawn from such studies on isolated patients, it is possible that some relation may exist between the presence of normal gastric mucosa and the ability to absorb fat from the intestinal tract.

c. *Patients with hepatic diseases.* The fact has been demonstrated that patients with hepatic cirrhosis no longer can properly metabolize intravenously administered fat (3). However, no data are available to indicate whether or not these patients likewise might suffer from any impaired ability to absorb fat from their gastro-intestinal tracts. Should such an impairment exist, then the benefit which patients with hepatic disease would derive from ingested fat must be very limited. To provide, in part, some answer to these questions of fat absorption, 2 patients with hepatic cirrhosis were studied. One of these was a chronic alcoholic and the diets of both had been grossly deficient in animal protein but not in fat.

While on the basal diet for from 3 to 6 days, the stearin excreted by these 2 individuals ranged from 4.9 to 9.5 grams per day. These outputs accounted for from 2 to 8.1 per cent of the fat ingested, or indicated an absorption of from 92 to 98 per cent of the fat from their gastro-intestinal tracts.

The addition of the fat supplement to their diets increased the fecal fat excretions to from 2.4 to 8.8 grams per day. These values represented an absorption of from 93 to 97 per cent of the supplements. Thus no significant defect appeared to exist in the absorption of fat from the gastro-intestinal tract of the patients with hepatic cirrhosis included in this study.

DISCUSSION

The results of the present investigation demonstrate the efficiency of fat absorption from the gastro-intestinal tract of normal individuals, of patients with hepatic cirrhosis, and of a patient with gastric cancer. The percentage values obtained for the absorption of ingested fat probably are slightly higher than the true value, for no correction has been made for the small amounts of fecal lipid which is known to be of endogenous origin. Ordinarily, this endogenous fat varies from 0.2 to 3.0 grams per day (8), which would indicate that only from 1 to 5 grams of the fecal lipid excreted by this group of subjects who ingested 1.5 grams of fat per kilogram of body weight, was of dietary origin.

It is worthy of note that the addition of large amounts of fat to the diet of normal subjects, of the individual with gastric cancer, and of the patients with hepatic cirrhosis, failed to produce a striking rise in the output of fecal fat. Previous studies have demonstrated a considerable degree of physiologic hepatic dysfunction both in patients with gastric cancer (9) and in those with portal cirrhosis (10). It is, therefore, interesting to observe that hepatic dysfunction apparently plays no constant, significant role in the absorption of fat from the gastro-intestinal tract.

Finally, increased intestinal motility must be considered a causative factor for the steatorrhea. To exclude this possibility, studies were carried out by means of barium and fluoroscopic roentgenography and the marking of diet periods with carmine and charcoal. These studies indicated no significant departure from the normal. Thus, it would appear that increased intestinal motility was not a causative factor for the steatorrhea.

However, the possibility must be considered that the proper absorption of lipids is influenced by the presence of a normal, functioning gastric mucosa. Both the patient with severe atrophic gastritis, and the individual who lacked a stomach entirely, suffered from a decreased efficiency of fat absorption. The nature of this defect, and the

measures necessary for its effective treatment, will be presented in a subsequent report.

CONCLUSIONS

1. The gasometric method of Van Slyke *et al.* was adapted to measure the absorption of fat from the gastro-intestinal tract of a group of subjects. This included 2 normal individuals, 1 patient bearing gastric carcinoma, 1 patient who had undergone total gastrectomy, 1 patient with generalized atrophic gastritis, and 2 patients with hepatic cirrhosis.

2. An abnormal absorption of fat was demonstrated only in the gastrectomized patient and in the patient with atrophic gastritis.

3. The question is raised of a relationship between the absence of an intact gastric mucosa and the normal absorption of fat from the gastro-intestinal tract.

BIBLIOGRAPHY

1. Sinclair, R. G., Physiology of phospholipids. Physiol. Rev., 1934, 14, 351.
2. Ralli, E. P., Paley, K., and Rubin, S. H., Liver lipids and their distribution in disease. Analysis of sixty human livers. J. Clin. Invest., 1941, 20, 413.
3. Nachlas, A., Duff, G. L., Tidwell, H. C., and Holt, L. E., Jr., Liver function as tested by lipemic curve after intravenous fat administration. J. Clin. Invest., 1936, 15, 143.
4. Rekers, P. E., Pack, G. T., and Rhoads, C. P., Metabolic studies in patients with cancer of the gastro-intestinal tract. V. Pancreatic insufficiency in a patient treated surgically for carcinoma of the ampulla of Vater. (In press.)
5. Rekers, P. E., Pack, G. T., and Rhoads, C. P., Metabolic studies in patients with cancer of the gastro-intestinal tract. VI. Disorders in digestion and absorption in patients who have undergone total gastrectomy. (In press.)
6. Van Slyke, D. D., Page, I. H., and Kirk, E., A manometric micromethod for determination of carbon in organic compounds. J. Biol. Chem., 1933, 102, 635.
7. Bloor, W. E., A method for the determination of fat in small amounts of blood. J. Biol. Chem., 1914, 17, 377.
8. Muller, F., Untersuchungen über Icterus. Ztschr. f. klin. Med., 1887, 12, 45.
9. Abels, J. C., Rekers, P. E., Binkley, G. E., Pack, G. T., and Rhoads, C. P., Metabolic studies in patients with cancer of the gastro-intestinal tract. II. Hepatic dysfunction. Ann. Int. Med., 1942, 16, 221.
10. Cates, H. B., Relation of liver function to cirrhosis of the liver and to alcoholism: Comparison of results of liver function tests with degree of organic change in cirrhosis of liver and with results of such tests in persons with alcoholism without cirrhosis of liver. Arch. Int. Med., 1941, 67, 383.