used in this study. It would appear, therefore, that loss of ovarian function in B_6 deficiency may be partly due to impaired ovarian sensitivity. In protein-deficient rats, where no such impairment exists, reduction of circulating gonadotrophins is a more likely explanation. Lack of circulating gonadotrophins has previously been suggested as an explanation for the gonadal atrophy resulting from inanition(8,9,10).

The reason for the greater effectiveness of HCG and PMS in protein-deficient rats than in normal immature rats is not clear. These hormones may be more rapidly inactivated or excreted in normal immature rats, or the ovaries of sexually immature animals may be refractory to low levels of these gonadotrophins. Both HCG and PMS exert gonadotrophic action at lower doses in normal than in hypophysectomized rats(6). It is commonly assumed that these hormones stimulate the pituitary in the normal rat, the FSH released acting synergically with the ICSH-like component of the injected hormone. Follicular growth following injection of HCG and PMS into protein-deficient rats might then result from a similar mechanism. The pituitaries of protein-deficient rats have already been shown by bioassay to contain FSH (14), and this may be released under the stimulus of these hormones.

Summary. Adult female rats were given a protein-free diet for 5 weeks and their response to graded amounts of gonadotrophic hormones determined. Protein-deficient rats responded to FSH with follicular growth at the same dosage levels as adult hypophysectomized rats of similar age and body weight. ICSH, as judged by stimulation of interstitial tissue, was slightly more effective in protein-deficient than in hypophysectomized rats. HCG and PMS induced follicular growth, corpus luteum formation and increased uterine weights in protein-deficient rats at one-half the dosages necessary in normal immature rats.

1. Guilbert, H. R., Goss, H., *J. Nutrition,* 1932, v5, 251.

2. Nelson, M. M., Evans, H. M., *ibid.,* 1953, v51, 71.

3. Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 65.

4. Nelson, M. M., Evans, H. M., *Endocrinol.,* 1954, v55, 543.

5. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., Hart, E. B., *J. Biol. Chem.,* 1941, v138, 460.

6. Fraenkel-Conrat, H., Li, C. H., Simpson, M. E., Evans, H. M., *Endocrinol.,* 1940, v27, 793.

7. Evans, H. M., Simpson, M. E., Tolksdorf, S., Jensen, H., *ibid.,* 1939, v25, 529.

8. Mulinos, M. G., Pomerantz, L., Smelser, J., Kurzrok, R., PROC. SOC. EXP. BIOL. & MED., 1939, v40, 79.

9. Werner, S. C., *ibid.,* 1939, v41, 101.

10. Maddock, W. O., Heller, C. G., *ibid.,* 1947, v66, 595.

11. Rinaldini, L. M., *J. Endocrinol.,* 1949, v6, 54.

12. Courrier, R., Raynaud, R., *Comptes rend. Soc. Biol.,* 1932, v109, 881.

13. Wooten, E., Nelson, M. M., Simpson, M. E., Evans, H. M., *Endocrinol.,* in press.

14. Srebnik, H. H., Nelson, M. M., *Anat. Rec.,* 1957, v127, 372.

Received June 17, 1958. P.S.E.B.M., 1958, v99.

Effect of Pre-Feeding of Fat on I^{131} Triolein Absorption in Subtotal Gastrectomy Patients. (24247)

MALCOLM P. TYOR AND JULIAN M. RUFFIN
(Introduced by E. A. Stead)
Departments of Medicine, Duke University Medical Center, Veterans Administration Hospital, Durham

Oral administration of I^{131} labeled triolein to patients with subtotal gastrectomy (Billroth II) frequently yields abnormal results consisting of reduced levels of blood radioac-

* Work supported in part by U. S. Public Health Grant A1987.

tivity and increased concentrations of radioactive material in the stool(1). Blood and fecal values of radioactivity obtained from the same patients given I^{131} labeled oleic acid have been normal in most instances, indicating that the defect exhibited is primarily one of digestion of triglyceride rather than absorption of fatty acid(1). Following *intraduodenal injection* of I^{131} labeled lipids (triolein and oleic acid), similar results for blood and fecal radioactivity have been found in normal subjects(2). Recently, this induced defect in normal subjects has been partially or completely corrected by oral or intraduodenal administration of untagged lipid emulsion given prior to intraduodenal infusion of tagged triolein (unpublished). Since the results following oral I^{131} lipid administration to patients with subtotal gastrectomy were similar to those observed in normal subjects following duodenal intubation, an untagged lipid meal was given to patients with subtotal gastrectomy prior to administration of tagged triolein.

Methods. Twelve male patients, age 30 to 72 years, who had had a subtotal gastrectomy (Billroth II) 2 to 9 years previously were studied. In no instance was there clinical or laboratory evidence to suggest an associated disorder of pancreas or small intestine. Each patient was studied twice. The first test was conducted in the fasting state; 58 to 80 ml of a lipid emulsion, which contained I^{131} labeled triolein being administered orally. The second study period began 96 to 168 hours after the first and was similar in every respect, except that 50 ml of non-radioactive lipid emulsion (peanut oil and water) was given orally 30 minutes prior to second test dose of tagged lipid. The methods of preparation and administration of the I^{131} labeled triolein emulsion as well as collection and counting of blood and fecal samples have been previously described(3). Blood and fecal radioactivity were expressed as percentage of administered dose/liter of whole blood and 48 to 72 hour collection period, respectively (3). Blood samples were obtained at hourly intervals for 6 hours. Fecal samples were collected for 24 to 72 hours and were weighed. Liquid stools were judged to be possibly contaminated with urine and were discarded. Blood radioactivity data were analyzed by comparing differences between means of paired values obtained at each sampling time during the 2 study periods.

Results. (Table I). When patients were pre-fed with lipid, concentrations of blood radioactivity measured 3 to 6 hours after labeled lipid drink were significantly greater, $P = <0.01$, than previous values obtained after labeled triolein was given without lipid pre-feeding. Blood values obtained at 1 and 2 hours were similar for both study periods, as was the time of maximum blood radioactivity, $P = >0.20$.

Fecal radioactivity was increased in 10 patients during initial study period, when compared with previously established normal values (upper limit = 5.0% of dose/48 hr collection period)(3). One patient had normal fecal radioactivity (HC), and in another the value was borderline (JP). Fecal collections obtained during second study period were incomplete in 4 patients (AP, CL, JP and JD). Significant reduction of fecal radioactivity was observed in the remaining 8 patients, during the second study period (mean difference = $10.3 \pm 2.3\%$ of dose/comparable collection period, $p = <0.01$). Five of the 7 patients who showed initial abnormalities had normal fecal samples when lipid was fed 30 minutes prior to tagged triolein.

Discussion. It is apparent from the fecal radioactivity results that 10 of the 12 patients studied exhibited impaired digestion or absorption of a "standard" dose of I^{131} labeled triolein. This abnormality was either partially or completely corrected by oral ingestion of fat 30 minutes before administration of a "standard" dose of I^{131} labeled triolein. These data support the concept that the defect in such patients is primarily if not completely digestive in origin(1,4,5). Indeed, the reported data suggest that the major digestive disturbance is one of suboptimal mixing of the labeled triglyceride bolus with bile and pancreatic secretions, since optimal hormonal stimulus with a subsequent physiologic response of gall bladder and pancreas is suggested by the normal fecal values obtained with lipid pre-feeding. Although an overall

TABLE I. Effect of Pre-feeding of Fat on Blood and Fecal Radioactivity in Subtotal Gastrectomy Patients.

Patient	Study*	Blood values (% of dose/l) 1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	Fecal values (% of dose)
RT	1	.71	.87	.95	1.13	1.21	1.62	16.6/48†
	2	1.11	2.14	2.66	2.61	2.36	1.95	6.0/48
AP	1	.70	1.00	1.14	1.21	1.24	1.20	36.0/48
	2	.52	1.28	2.25	3.05	3.55	2.82	.4/24
WH	1	.27	.65	1.63	2.24	2.67	2.58	10.0/48
	2	.14	.82	1.80	2.67	3.45	3.29	1.0/72
RD	1		3.13		2.90	2.51	2.26	15.6/48
	2		1.74		4.43	3.69	3.31	3.5/72
CL	1	.62	.89	1.48	2.00	1.75	1.20	27.0/24
	2	.78	1.73	2.30	3.28	3.30	2.72	.2/24
JP	1	.84	1.62	1.65	1.71	1.83	1.74	5.0/72
	2	.77	1.22	1.98	2.14	2.24	2.16	‡
ER	1	.17	.26	.26	.29	.42	.54	23.3/72
	2	.33	1.12	1.50	2.40	2.46	2.08	2.9/72
GM	1	.95	1.50	1.67	1.55	2.02	1.48	14.8/48
	2	.96	1.97	2.85	2.51	2.24	2.10	1.5/72
CD	1	.74	1.28	1.70	1.65	1.67	1.59	14.7/72
	2	.85	2.17	3.63	2.98	3.02	3.00	.4/72
JD	1	.43	.58	1.58	1.60	1.74	1.56	21.4/72
	2	.94			2.35	2.34	1.60	‡
HC	1	1.33	1.27	1.91	1.23	1.63	1.93	2.8/72
	2	1.23	2.74	3.13	2.72	2.61	2.27	.3/72
WD	1	1.52	2.50	2.65	2.51	2.51	2.30	8.3/48
	2	2.32	2.37	2.56	2.36	2.20	2.23	6.8/48

* Study 1: I^{131} labeled triolein. Study 2: I^{131} labeled triolein preceded 30 min. by peanut oil.
† Collection period (hr).
‡ Incomplete; contaminated with urine.

decrease in intestinal transport time is not present in most patients with subtotal gastrectomy(6), the bolus may enter the lower small intestine within 15 minutes after ingestion(7). In doing so, it rapidly traverses the intestinal level where maximum lipid absorption may normally occur(8). From these observations and the reported data, one may postulate a mechanism to explain the correction of abnormal fecal radioactivity when lipid was fed 30 minutes prior to administration of tagged triolein to patients with subtotal gastrectomy (Billroth II): 1. Pre-fed lipid stimulates hormonal secretion with resultant physiologic flow of bile and pancreatic juices. 2. Bile and pancreatic juices are available for mixing with the tagged lipid bolus at an intestinal site where lipids are maximally absorbed(5). Since the intestinal hormones are characterized by a short period of latency and little persistence of action(9), the timing of the "priming" dose may be variable in individual patients, which might explain the fact that fecal radioactivity was only partially corrected in 2 patients.

Summary. The I^{131} tagged triolein test was performed in 12 patients with subtotal gastrectomy (Billroth II) in the fasting state and 30 minutes after oral ingestion of a fat meal. In the fasting state, increased fecal radioactivity was observed in 10 patients. When lipid was pre-fed, there was a significant decrease in radioactive content of stool and increase in blood radioactivity. The data support the concept that the defect exhibited by these patients is primarily digestive and suggest that the major disturbance is one of inadequate mixing of labeled triglyceride with the upper intestinal contents necessary for proper lipid digestion.

1. Shingleton, W. W., Isley, J. K., Floyd, R. D., Sanders, A. P., Baylin, G. J., Postlethwaite, R. W., Ruffin, J. M., *Surgery,* 1957, v42, 12.

2. Tyor, M. P., Janssen, B., Jr., Ruffin, J. M., *Clin. Res.,* 1958, v6, 144.

3. Chears, W. C., Jr., McCraw, B. H., Tyor, M. P., Ruffin, J. M., *South. Med. J.*, 1958, v51, 433.
4. Volwiler, W., *Am. J. Med.*, 1957, v23, 250.
5. Lundh, G., *Acta Chir. Scand.*, 1958, Suppl. 231, 1.
6. Kirsh, I. E., *Gastroenterology*, 1956, v31, 251.
7. Polak, M., Pontes, J. F., *ibid.*, 1956, v30, 489.
8. Borgstrom, B., Dahlqvist, G., Lundh, G., Sjovall, J., *J. Clin. Invest.*, 1957, v36, 1521.
9. Grossman, M. I., *Physiol. Rev.*, 1950, v30, 33.

Received June 19, 1958. P.S.E.B.M., 1958, v99.

Glucose as Adjunct to 3.3-Dimethyl-1-Phenyltriazene in its Action upon Sarcoma 180. (24248)

CHARLES D. STEVENS, J. A. MUHLENPOH, MALCOLM E. TURNER, KLAUS STEMMER, I. RUCHMAN AND CHARLES J. FELDHAKE

Kettering Laboratory in Dept. of Preventive Medicine and Industrial Health, College of Medicine, University of Cincinnati, and Scientific Labs., Wm. S. Merrell Co., Cincinnati, O.

The characteristic acidity of cells of malignant tumors may make them more susceptible than normal cells to damaging, acid-sensitive chemicals(1,2). One such chemical is 3,3-dimethyl-1-phenyltriazene (PT). It inhibits Sarcoma 180 and other tumors(1,3), perhaps by releasing diazonium ion in a reaction catalyzed by acid(1). To learn whether the increased acidity of tumors might alter the action of PT on Sarcoma 180 in mice it was proposed to inject glucose prior to each day's injection of PT. The administration of glucose is known to increase acidity of tumors (4). This report presents results of such an experiment, together with ancillary observations.

Materials and methods. PT was prepared by the method of Elks and Hey(5). It was characterized by boiling range and absorption spectra in 3 solvents. It distilled at 100-101°C (uncorr.) and 5 to 5.5 mm Hg pressure. Common logarithms of molar extinction coefficients at 225, 285 and 308 mμ were, for PT in 95% ethanol, 3.93, 4.08 and 4.06, respectively. LeFevre and Liddicot(6) reported 3.97, 4.14 and 4.11 as the corresponding values. In iso-octane the logarithms were 3.99, 4.12 and 4.06 at the same wave lengths. In water they were 3.91, 4.10 and 4.10, and a maximum value of 4.12 was obtained at 302 mμ. No comparable data are available. Ultraviolet measurements were made with 1 cm cells and a Beckman DK spectrophotometer. The effect of PT upon tumor growth was measured in female albino mice weighing 17 to 20 g. Each was given by trocar a subcutaneous implant of Sarcoma 180 weighing 7.5 mg average. Injections of PT were begun one day after implantation and continued once daily for 5 days (7 days in preliminary assay). PT was dissolved in peanut oil (1 g/100 ml) and given intraperitoneally. Some of these mice were also injected subcutaneously with glucose in a solution containing 20 g/100 ml in 0.85% sodium chloride. They were given 0.8 ml/mouse/day (8 g glucose/kg) always one hour before PT. On 8th day after implantation of tumor, the mice were again weighed and the external diameters of their tumors were measured. The tumors were then excised, weighed, fixed in formalin, imbedded in paraffin and stained with hematoxylin-eosin for microscopic examination.

Results. The results of a preliminary assay and of the main experiment are presented in Table I. A difference in degree of effect of PT between preliminary assay and main experiment is unexplained. A similar variability is to be found in the data of Clarke *et al.* (1).

To analyze data from the main experiment, that with glucose, measurements of tumor weight and body weight, summarized in Table I, were transformed into a new metric of response by taking the common logarithm of the ratio of each tumor weight to the difference between initial and final body weight after adding 10 g to the difference to make