

Title: Neomycin Enteropathy and Malabsorption.

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### Objectives:

Neomycin, an aminoglycoside antibiotic, causes a reversible malabsorption syndrome in man. Faloon and colleagues have demonstrated increased fecal excretion of fat, nitrogen, and sodium and potassium and decreased serum cholesterol as well as malabsorption of B-carotene, vitamin B<sub>12</sub>, d-xylose, iron and <sup>131</sup>I-labelled trioleate, due to oral neomycin administration. These workers have reported changes in the jejunal mucosa consisting of clubbing of the villi and edema and round cell infiltration of the lamina propria due to the drug. Hvidt and Kjeldsen reported malabsorption of both fat and d-xylose on 3 gm. per day of neomycin, and Samuel and Steiner showed the cholesterol lowering effect to occur with as little as 0.5 gm of neomycin daily.

The pathogenesis of the syndrome is not known. Gluten restriction or steroid administration may or may not effect neomycin-induced steatorrhea. Recently attention has been focused on alterations in bile salt metabolism due to neomycin which may effect mucosal function or intraluminal hydrolysis of fat. The histologic changes reported by Jacobson et al and thought to result in "physical blockage" of absorption are disputed by Rubin, and may not correlate with changes in function.

The present study was undertaken to evaluate the acute effects of neomycin and to study the evolution of functional and structural changes.

### Material and Methods:

Thirty normal Thai adults, 27 females and 3 males gave informed consent for the studies to be described. The mean age was 25.3 (16-47) years. All were hospitalized and studied before, during and after drug administration. Neomycin was given orally as the sulfate, 0.5 gm tablets equivalent to 0.35 gm neomycin base.

Effects of neomycin were assessed with the following:

1. Twenty-five gram xylose tolerance test, with measurement of 5 hour urinary excretion and 2 hour serum xylose by the method of Roe and Rice.

2. Sucrose tolerance test with measurement of blood total reducing substance by a modification of the method of Hoffman adapted to the autoanalyzer before and 30, 45, and 90 minutes following oral administration of 1.5 g/KBW sucrose.

3. Fecal fat content, determined by the method of Van de Kamer et al in 3-5 day collections marked with carmine-red dye. A daily supplement of 75 grams of fat in the form of butter was given during collection periods.

In 5 subjects Vit B<sub>12</sub> absorption was tested by the Shilling technique using Co<sup>57</sup> labelled vitamin\* with exogenous intrinsic factor.

In 6 cases intestinal water absorption was assessed by administration of an oral water load of 20 cc of water/KBW. All urine was collected and volume and osmolarity (Fiske Osmometer) were measured at 60, 80, 95, 110, 125, and 140 minutes. Maximum water diuresis was maintained by oral water replacement equal to the urine volume at the end of each collection period. Osmolar clearance (Cosm) and free water clearance (C<sub>H2O</sub>) were calculated. The collection period during which maximum diuresis was obtained was noted and the end of that collection period recorded as the time to reach maximum C<sub>H2O</sub>.

Biopsy specimens were obtained with the Crosby-Kugler capsule from the region of the ligament of Treitz, oriented on filter paper, and divided. One section of tissue was fixed in 10% buffered neutral formalin for histologic examination. The second portion of the specimen was oriented villus-side up on a block of kidney tissue and quick frozen with liquid nitrogen or dry ice. Sections were cut at -5° to -15°C and examined for the activities of adenosine triphosphatase, alkaline phosphatase, glucose-6-phosphate dehydrogenase, non-specific esterase, and succinic dehydrogenase. In most cases sections were processed within 4 hours of biopsy. In a few cases tissue was kept for 24 hours in a freezer at -70°C before staining. Sections from normal Thai and mouse intestine served as positive controls. Addition of inhibitor substances or substrate withdrawal established negative controls. Sections were post-fixed in formalin and stained with Oil-Red-O.

In 4 cases, jejunal tissue was homogenized in 1 cc of saline and cultured on blood agar pour plates for total aerobes and anaerobes. Classification of organisms was made on the basis of colonial characteristics and microscopic morphology of gram stained smears. Growth of C. perfringens insured that strict anaerobiosis was maintained.

### Results:

Xylose Absorption: Five hour urinary xylose excretion decreased in all subjects given neomycin (Table I) from the mean control value of  $6.26 \pm 1.60^*$  gm to  $2.01 \pm 0.75$  gm ( $p < .01$ ). Recovery was rapid and by 1 week following cessation of drug administration the mean xylose excretion was  $5.64 \pm 1.76$  gm.

The 2 hour serum xylose concentration paralleled urinary excretion. The mean control value of  $39.60 \pm 7.0$  mg per 100 ml decreased to  $17.44 \pm 3.83$  mg per 100 ml during the drug period. The 2 hour serum level was  $34.70 \pm 5.92$  mg per 100 ml within 1 week of stopping the drug.

Xylose tolerance tests were begun within 6 hours of a single 2 gram dose of neomycin in 6 subjects. An additional 5 out-patients were similarly tested. Xylose excretion decreased in 10 of the 11 subjects and the 2 hour serum level was depressed in 9 of the 10 subjects in whom this measurement was obtained (Table II). These changes were highly significant ( $p = < .01$ ) for both urine and serum determinations.

Xylose absorption was tested on alternate days in 5 subjects given increasing doses of neomycin (Fig 1,2). In 4 of the 5, xylose was malabsorbed when first tested after 24 hours on drug. The remaining subject (CH) malabsorbed xylose on study day 4 after receiving neomycin for 3 days. This subject did not

\* Racobalmin, Abbott Laboratories

\* All data expressed as mean  $\pm$  1 S.D.

manifest other stigmata of neomycin malabsorption, i.e. steatorrhea or sucrose malabsorption. Changes in the 2 hour serum xylose concentration (Fig 2) were a less sensitive indicator of neomycin induced malabsorption. There was little apparent effect from increasing the dose of neomycin once xylose malabsorption was present.

Sucrose absorption: The mean maximal rise in blood glucose following an oral sucrose load in 10 subjects was  $59 \pm 12.4$  mg per 100 ml (Fig 3). During neomycin administration this decreased to  $24.5 \pm 10$  mg per 100 ml ( $p = <.01$ ). In 9 of the 10 subjects the maximal rise in blood glucose during drug administration was less than 20 mg per 100 ml. When neomycin was discontinued the mean maximal rise was  $69.5 \pm 17.1$  mg per 100 ml. Recovery was rapid and values returned to control within 1-2 days of discontinuing neomycin.

In 7 patients a sucrose tolerance test was performed within 6 hours of a single 2 gm dose of neomycin (Table III). Three of the 7 showed a "flat" response, and the blood sugar rise was blunted in the remaining 4.

Fecal Fat Excretion: Twelve of 15 subjects given neomycin excreted increased fat in the stool, including one subject taking only 1 gm per day of neomycin (Fig 4). In the entire group mean fecal fat content during the control period was  $1.8 \pm 1.2$  gm per day. This increased to  $5.0 \pm 3.0$  gm per day during neomycin administration. The post neomycin value was  $1.8 \pm 1.1$  gm per day.

Vitamin B<sub>12</sub> Absorption: In 5 subjects studied during control and neomycin periods, 3 showed impaired vitamin B<sub>12</sub> absorption during drug administration, including 1 subject given only 1 gm per day of drug. In the remaining 2 subjects no significant change occurred.

Water Load Tests: Water load data are summarized in Table IV. Although the maximum C<sub>H2O</sub> decreased during neomycin administration in every case, there was no detectable delay in the time to maximum diuresis.

Bacteriologic Culture of Jejunal Tissue: Small numbers of streptococci or staphylococci were found which diminished somewhat after 7 days of drug administration. No new organisms were found during or after drug except in one post neomycin biopsy (SW) from which a gram-negative, anaerobic fusiform rod was recovered.

Histologic Study: Pretreatment Biopsies: The surface pattern under the dissecting microscope was regular, with tall leaf shaped villi the predominant form present. There were occasional tongue shaped villi. Finger-like forms were rarely seen. Sections showed nonspecific changes in varying degrees as previously described in normal Thais.

Neomycin Treatment Biopsies: 6 hour Biopsies: There was no change in appearance under the dissecting microscope. In sections, there was some shortening and broadening of the villi. The vascular loops were usually inconspicuous. Polymorphonuclear leucocytes and lymphocytes in small numbers were present in the epithelial layer. The columnar epithelial cells appeared shrunken. Many nuclear fragments were present in the epithelial cells. These changes were more marked near the villus tip. (The changes are similar to those shown in the report on Paramomycin effects on gut structure and function).

24 hour Biopsies: There were no uniform changes in the dissecting microscope appearance. A few specimens were coated with thick opaque mucus. The changes in the epithelial cells were similar to those seen at 6 hours. Mitotic figures appeared more abundant than in the 6 hour material.

72 hour Biopsies: Most of the specimens were covered with heavy mucus containing particulate debris. In comparison to the previous specimens the epithelial cells were more normal in appearance. Most villi contained prominent vascular loops. Mitoses were frequent. Goblet cells were irregularly distributed.

Increased numbers of plasma cells were present in the lamina propria. Many macrophages containing nuclear debris and bacteria-like objects were present near the villus tips. The latter particles, variable in shape, ranged in size from 2.5 microns to the lower limit of resolution and were visible in biopsy material fixed in neutral 10% formalin, Zenker's (AFIP) and Bouin's fluids, although less sharply defined in the latter fixative. They were intensely basophilic with alum hematoxylin, PAS negative, stained red with Brown-Brenn and MacCallum-Goodpasture stains, and were metachromatic with Giemsa and azure-eosin stains. The Feulgen reaction was positive with only a portion of this material.

Post-Drug Biopsies: Biopsies secured from 5 to 14 days after cessation of neomycin could be distinguished from control only by the presence of mononuclear phagocytes containing particulate debris. These progressively diminished in number and were usually not detectable by the end of the second week following the drug.

Enzyme Histochemistry: The pattern of enzymatic staining in the control specimens was normal. Neomycin administration resulted in patchy abnormalities in staining for ATP'ase, alkaline phosphatase, and succinic dehydrogenase (to be reported in detail elsewhere). In 2 subjects studied at 6, 24, and 72 hours on neomycin, 4 gm per day, there were more profound abnormalities in the 6 and 24 hour specimens than in the 72 hour specimen.

In 4 patients with neomycin-induced steatorrhea, fat droplets were present within epithelial cells as well as in the upper portion of the lamina propria 12 hours after a high-fat meal.

#### COMMENTS

The present studies of neomycin-induced malabsorption differ in two respects from previous reports. Firstly, the acute effects of the drug were evaluated, and secondly the subjects were Thai people whose jejunal mucosa is histologically different than normal Americans. The fact that neomycin administration can lead to histologic, histochemical and functional changes within 6 hours of a therapeutic dose is previously undescribed.

The question naturally arises whether or not this extreme sensitivity of the Thai small bowel is a consequence of pre-existing damage represented by non-specific jejunal abnormalities. Function studies in Thais, however, have failed to reveal significant abnormalities. When Americans are studied after only 24 hours on a small dose of neomycin the d-xylose test, including both urinary excretion and the 2 hour serum level is significantly depressed from control (unpublished data). Rogers et al administered high doses of neomycin to Americans and described epithelial cell changes including flattening, vacuolization, and loss of nuclear polarity, as well as macrophages in the lamina propria containing "ingested cellular fragments" after 1-2 weeks of drug. It is not certain if the latter observation is similar to our findings in Thais. It appears, however, that the response to the drug in Americans both in terms of function and structure may be qualitatively, if not quantitatively, similar to Thais.

The pathogenesis of neomycin-induced malabsorption is not known. Jacobson et al suggested that inflammatory changes in the jejunum were responsible for "physical blockage of absorption". These histologic changes have been the subject of controversy and are disputed by some authorities. The features described by Jacobson et al as typical for neomycin enteropathy resemble the usual Thai bowel in whom there is no accompanying malabsorption. When neomycin is given to Thai subjects the most striking early abnormality is in the absorptive epithelial cell where nuclear and cytoplasmic changes occur within 6 hours of the first dose of drug. Accompanying this are histochemical abnormalities and malabsorption of xylose and sucrose. It is clear that the absorptive cell itself is being affected by neomycin, and one need not implicate changes in villus shape or in the degree of round cell infiltration in the lamina propria to account for functional abnormalities. While some histologic and histochemical improvement in the appearance of the epithelium

appears with continued administration of drug, other evidence of continued injury is present, e.g. mucus discharge, increased numbers of mitoses in the crypts, and abnormal function. It may be that neomycin causes generalized poisoning of the epithelial cells acutely and a more specific biochemical lesion with chronic administration.

The nature of this lesion is open to speculation. The mechanism of action of neomycin in bacterial systems is to interfere with ribosomal reading of m-RNA information in protein synthesis. The result of this is synthesis of inefficient or inactive protein. Each absorptive function studied by us probably relies upon an intermediary protein for hydrolysis and/or absorption. Sucrose for example, is not absorbed unless enzymatically split into its component monosaccharides which are then actively transported across the intestinal wall. Xylose transport is mediated by a carrier molecule, and some evidence suggests this is a protein. Fat, once within the cell as triglyceride, required attachment to protein to form chylomicrons in order to escape the cell. Evidence that these proteins in man are affected by neomycin includes disaccharidase assay, histochemical evidence of depressed enzyme activity, and demonstration of fat within the epithelial cells after a 12 hour fast suggesting an "exit block" similar to the abnormality caused by paromomycin administration in rats. Furthermore, we have found that the structurally related antibiotic paromomycin causes xylose and sucrose malabsorption and similar histologic changes as neomycin while tetracycline does not (unpublished data). This suggests that non-absorbable aminoglycoside antibiotics as a class possess the ability to significantly affect mammalian intestinal epithelial cells. The data are consistent with an effect on protein synthesis, however direct proof is lacking.

The exact nature of the "bacteria-like" structures within the macrophages during neomycin administration awaits electron microscopic study. Their appearance and staining characteristics by light microscopy are consistent with bacteria. Although we were unable to culture significant numbers of organisms in intestinal tissue, interpretation is difficult. It is possible that bacteria were dead or in altered form, such as protoplast or L-form and therefore difficult to grow. This has been suggested in Whipple's Disease where bacteria in and around macrophages have been identified morphologically but culture studies have been inconclusive. It is also possible that these structures are merely cellular debris. Whatever their true identity, absorptive abnormalities correlate temporally with epithelial cell damage rather than the appearance and disappearance of these structures.

#### Summary:

Thirty normal Thai subjects were given oral neomycin and intestinal function and structure studied sequentially. Within 6 hours of a single dose of neomycin, xylose and sucrose were malabsorbed. Intestinal epithelial cells appeared injured and histochemical abnormalities were found. Fat malabsorption occurred and an apparent epithelial cell "exit block" was produced. "Bacteria-like" bodies were noted to accumulate within macrophages in the lamina propria. All changes reverted to normal after the drug was stopped. It is suggested that neomycin may directly affect protein synthesis in the human intestinal epithelial cell, and that this may be the underlying pathogenesis of neomycin-induced malabsorption.

Table I  
Effect of Chronic Neomycin Administration  
On the 25 Gram D.Xylose Tolerance Test

Pt.	Dose of Neomycin (gm per day)	Urine Xylose, gm per 5 hrs			2 Hour Serum Xylose, mg per 100 ml		
		Control	Neomycin	Recovery	Control	Neomycin	Recovery
SC	1	8.5	2.4	8.0	—	—	—
WI	1	9.6	2.7	7.3	—	—	—
JA	2	5.4,4.1	1.2	3.7	56.1, 26.5	20.0	45.1
PB	2	5.2	3.8	7.3	—	—	—
SO	3.5	4.3, 6.4	0.9,0.5,2.3	3.5,3.2	22.8, 32.5	8.0,8.0,14.3	32.2, 37.7
BO	4	6.8, 4.9	1.7	5.8	60,2, 41.8	13.4	38.6
CC	4	6.6	2.0,1.4,2.3	4.7,6.2	31	9.6,23.9,14.3	18.4, 47.8
LA	4	9.6	2.5	9.8	47.8	19.5	27.4
MA	4	7.7	3.3	6.2	35.1	14.3	30.6
UR	4.0	4.7,4.3, 4.4,6.0	2.0,2.4	3.7	35.7, 31.2 36.4, 41.0	15.9, 22.9	27.1
AM	4.5	6.3,5.7	1.5,2.3	4.8,4.9 8.6	42.1, 43.6	19,26.1	41.9,45.7,51.8
CH	4.5	7.2,6.9	3.4,4.0	7.0,6.4,6.3	32.4, 40.9	19.8,26.3	34.7,37.2,28.3
SU	4.5	6.4,6.5	2.5,2.6	6.1,5.5,6.0	39.7, 34.5	15.2,18.6	42.4,31.5,31.1
SW	4.5	6.0,5.1	1.8,1.3	4.5,4.6,4.0	46.7, 45.3	23.8,22.7	45.1,45.7,40.2
CL	6	4.0,3.5	1.1	3.9,3.8	40.8, 26.1	14.4	35.4,30.2
LJ	6	4.6,4.6	2.8,1.1	4.2,4.7	39.6, 28.7	16.6,14.4	34.5,34.8
SR	8	4.4,4.3	1.3	2.0,3.9,5.7	56.7, 45.9	16.2	29.7,34.8 45.7
Mean ± 1 SD		6.26 ± 1.60	2.10 ± 0.75	5.64 ± 1.76	39.60 ± 7.0	17.44 ± 3.83	35.7 ± 5.92

Table II  
 Acute Effect of a Single 2 Gram Dose Neomycin on the Xylose  
 Tolerance Test

Patient	Urine, gm per 5 hours		2 Hour Serum, mg per 100 ml	
	Control	Neomycin	Control	Neomycin
KA	6.6	4.6	49.6	21.4
KI	5.8, 5.0	3.9	50.5, 53.6	24.5
PA	4.6, 4.9	3.9	43.5, 42.2	43.4
SA	3.1	2.4	36.0	20.9
SR	5.7	0.9	52.7	37.2
WA*	6.2	4.2	—	—
21	5.2	3.1	54.1	28.4
22	6.4	4.1	45.8	28.4
23	5.2	5.0	39.6	25.9
24	6.0	4.7	56.3	40.9
25	5.0	0.3	47.9	25.1
Mean $\pm$ 1 S.D.	5.42 $\pm$ 0.94	3.37 $\pm$ 1.5	47.70 $\pm$ 6.35	29.61 $\pm$ 7.74

\* Intraduodenal administration of 25 gm d-xylose before and 2 hours after intraduodenal administration of 1 gm neomycin.

Table III

Acute Effect of a Single Dose of 2 Grams of Neomycin on the Sucrose Tolerance Test

Patients	Maximum Rise Blood Glucose, mg. per 100 ml			
	Control	Hours Post Neomycin		
		6	18	30
BO	93,64	23	76	91
JA	41,33	6	34	6
KA	65	51	—	80
KI	53	37	—	35
NI	116	90*	40	—
PA	80	13	—	55
SA	149	17*	59 <sup>+</sup>	—

\* 2 hours post neomycin

+ 10 hours post neomycin

Table IV

## Oral Water Load

Pt.	Control				Neomycin				Recovery			
	U <sub>osm</sub> mOsm/L	Cosm ml/min	Max. CH <sub>2</sub> O ml/min	Time to Max. Diuresis minutes	U <sub>osm</sub> mOsm/L	Cosm ml/min	Max. CH <sub>2</sub> O ml/min	Time to Max. Diuresis minutes	U <sub>osm</sub> mOsm/L	Cosm ml/min	Max. CH <sub>2</sub> O ml/min	Time to Max. Diuresis minutes
SY	50	2.4	11.3	80	54	2.1	8.0	80	59	2.5	9.5	80
MY	60	2.2	7.9	60	64	2.1	6.4	80	59	2.6	9.8	80
PS	70	2.2	6.8	105	74	2.2	6.2	80	65	1.8	6.0	80
NT	51	1.5	7.2	80	50	1.5	6.3	80	63	1.6	5.6	80
SA	66	2.3	6.8	80	81	1.6	3.9	80	85	2.1	5.1	80
TB	48	1.7	8.4	80	54	1.6	6.8	95	62	2.3	8.1	80

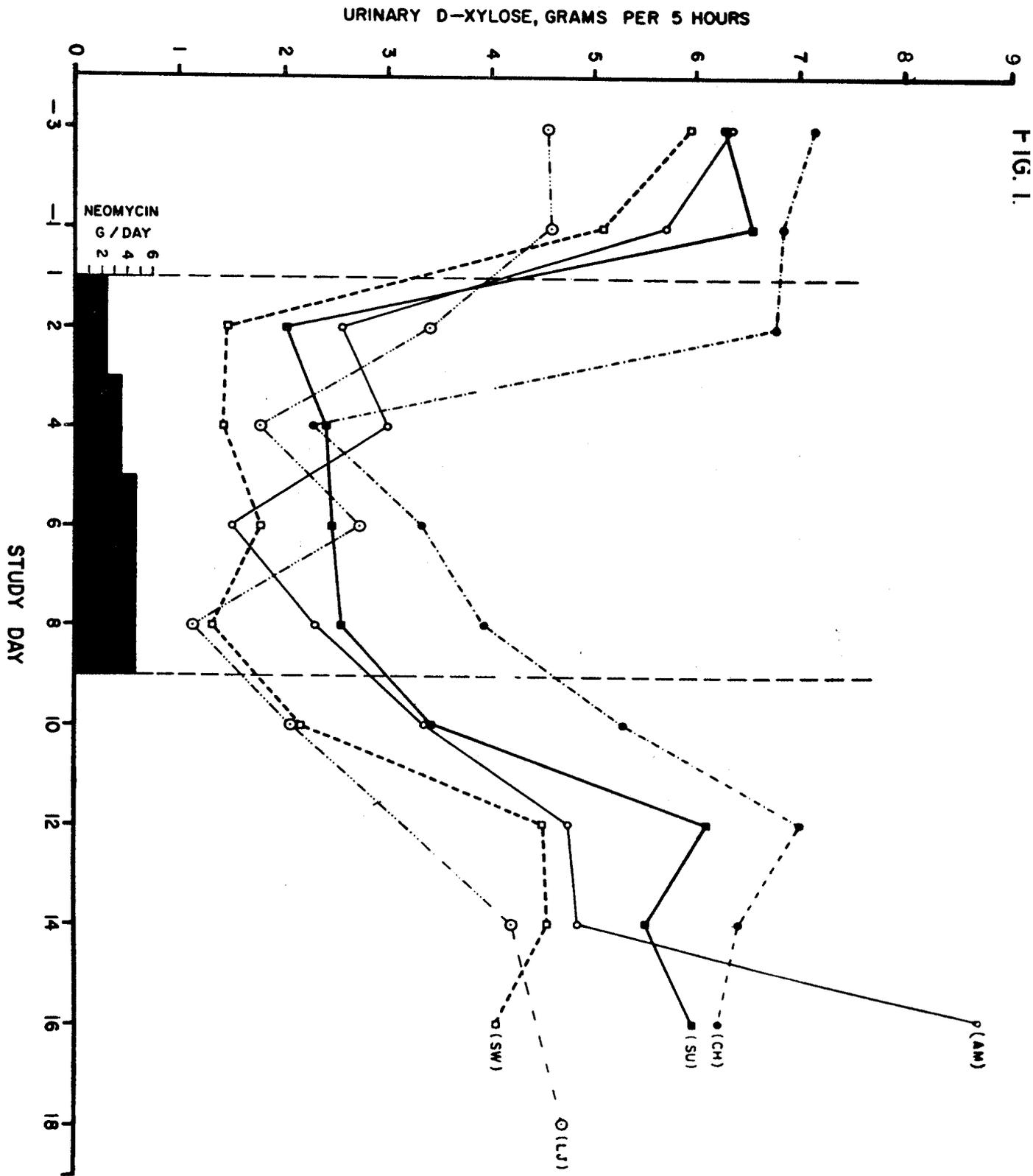


FIG. 1.

FIG. 2.

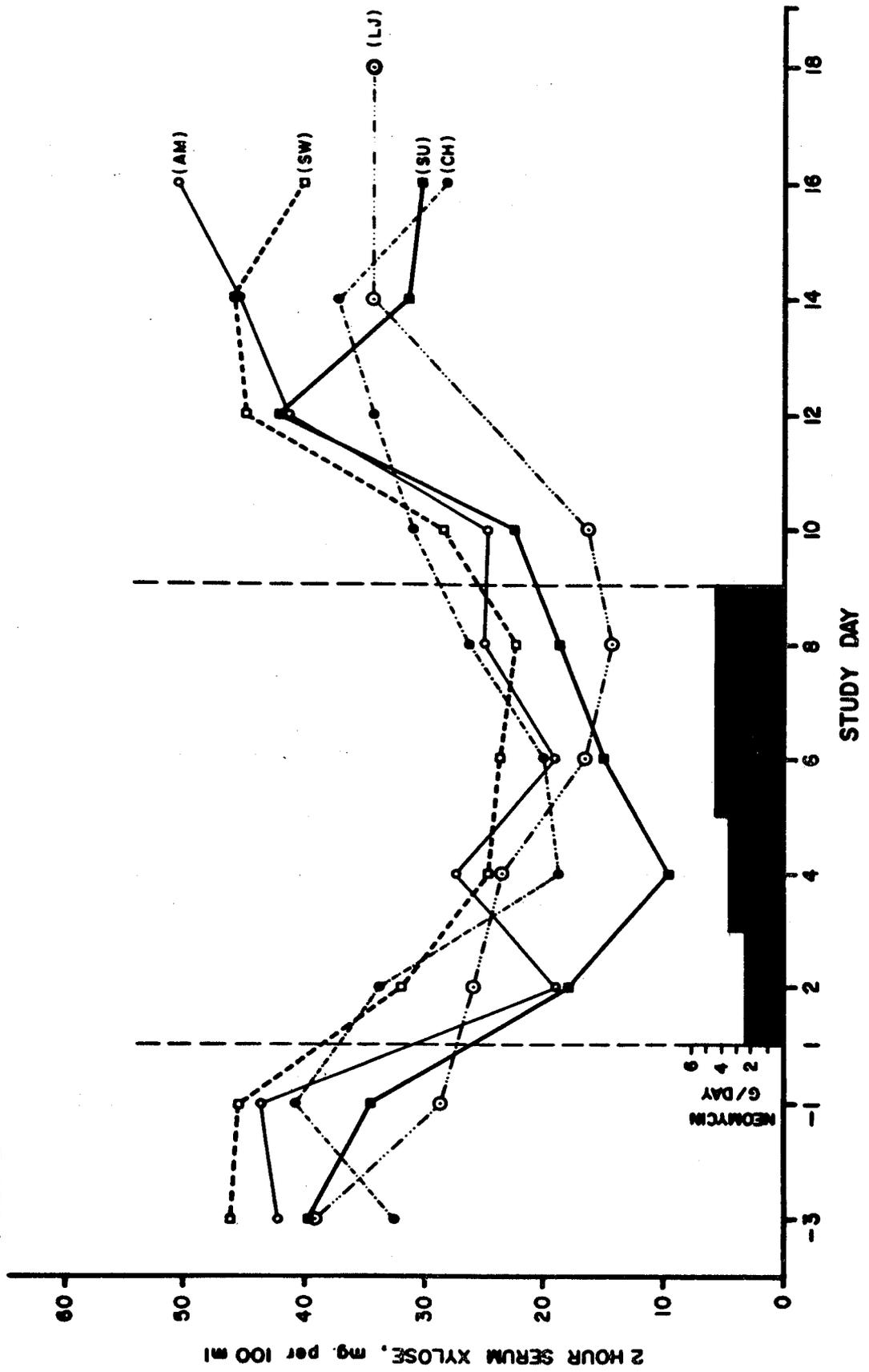


FIG. 3. EFFECT OF NEOMYCIN ON SUCROSE ABSORPTION

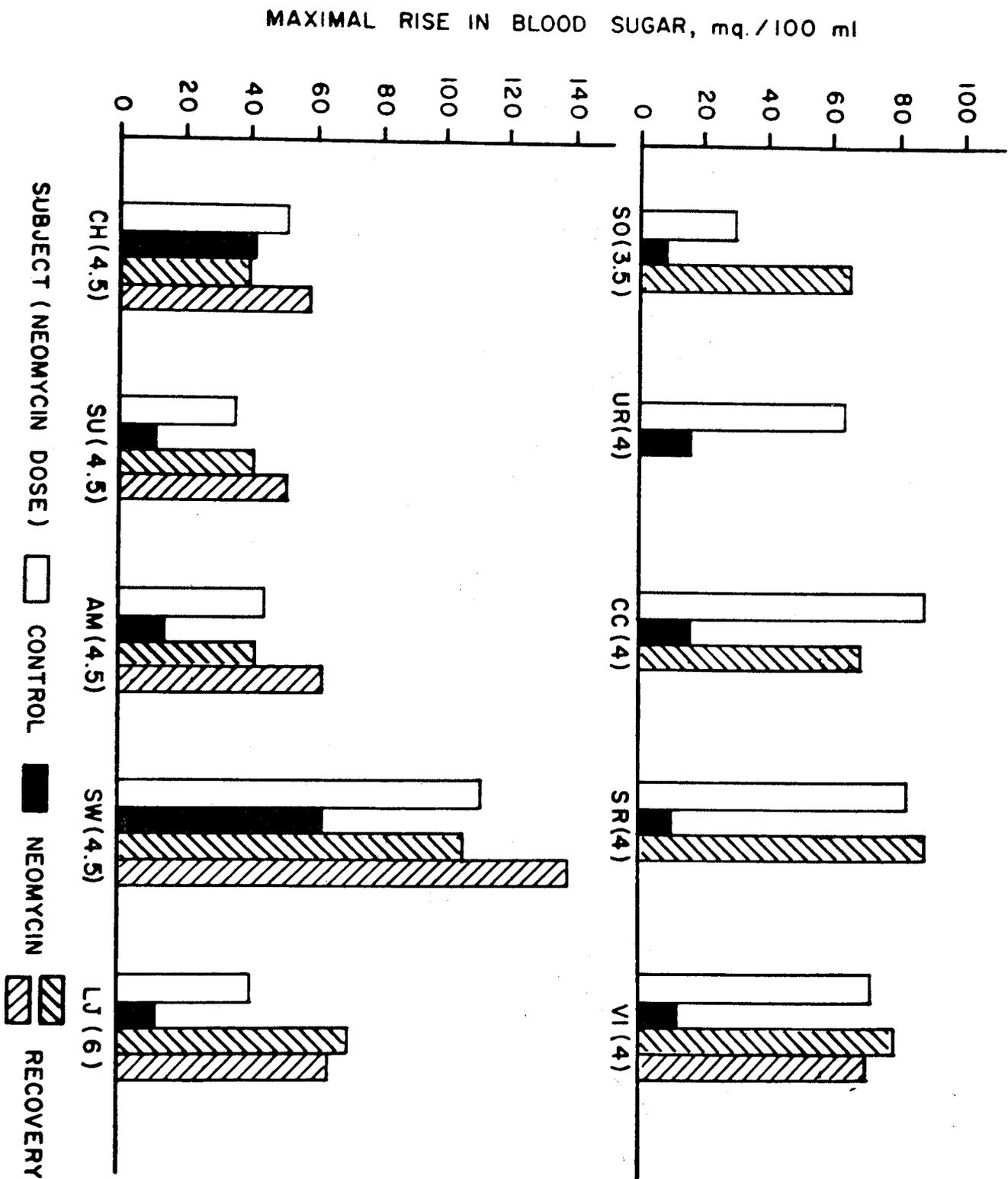


FIG.4. EFFECT OF NEOMYCIN ON FECAL FAT EXCRETION

