The Effect of Neomycin on Cholesterol Metabolism

J. G. HAMILTON, J. E. MULDREY, B. H. McCRACKEN, GRACE A. GOLDSMITH and O. N. MILLER,
Laboratory of Nutrition and Metabolism, Department of Medicine and the Department of Biochemistry,
Tulane University School of Medicine, New Orleans, Louisiana

Abstract

Oral neomycin lowers serum cholesterol in man, whereas intramuscular doses are ineffective. Probably it exerts its effect in the gastrointestinal tract. It has been suggested that neomycin specifically involves alteration of the intestinal flora. An increased excretion of bile acids and the failure of the conversion of cholic acid to deoxycholic acid has been demonstrated, which supports this hypothesis. It has also been suggested that the decrease in serum cholesterol is related to a malabsorption syndrome. Although large doses of neomycin do cause a malabsorption syndrome there is no agreement as to the occurrence or severity of a malabsorption syndrome related to therapeutic doses of neomycin.

The Effect of Neomycin on Serum Cholesterol in Man. Since the original observation by Samuel and Steiner (1) that oral neomycin therapy lowers serum cholesterol levels, surprisingly few reports have appeared of the use of neomycin for this purpose. The evidence for this depression of serum cholesterol by neomycin is convincing and stands undisputed although little is understood of the mechanism whereby this effect is produced. Samuel and Waithe (2) and Steiner et al. (3) have studied some 50 patients and obtained statistically significant decreases of serum cholesterol in every patient. Although their experiments were well designed, a question might be raised regarding the adequacy of dietary control of outpatients and patients on ordinary hospital diets. Confirmation of their observations was obtained, however, under conditions of careful dietary control on metabolic wards, by Goldsmith et al. (4) and by Powell et al. (5). All four groups of workers observed a depression of serum cholesterol levels of 10–30% of control levels with oral doses of 0.5–2.0 g neomycin sulfate/day.

Jacobson et al. (6) reported a similar effect on serum cholesterol with daily doses of 12 grams of neomycin in 8 patients; in the same study they reported two patients in which there was no response of serum cholesterol. Their report provides the single exception in which neomycin did not lower serum cholesterol in man.

The Effect of Neomycin on Intestinal Flora. Neomycin is often used as preoperative treatment for operations involving the large intestines and is loosely referred to as "gut sterilization." It is obvious that not all bacteria are affected to the same extent. Steiner et al. (3) found that Escherichia coli were most affected with a marked reduction to absence of these organisms and a marked reduction of other enteric organisms except for the enterococci and Clostridium welchii which tend to become more abundant. Powell et al. (5) also found a complete absence of coliform bacteria, but in a later report (Leveille et al., 7) found several patients in which coliform organisms were found in the stools during periods in which their serum cholesterol had been reduced with neomycin.

A study of the fecal excretion of bile acids and sterols has generally confirmed these observations (4, 8). Taurine and glycine conjugates of bile acids are split. This has been shown by Danielsson et al. (9) to be due to Clostridia. Usually cholic acid (8) is the predominant bile acid found in the feces after oral neomycin but occasionally large amounts of 7-ketodeoxycholic are found. The conversion of cholic acid to 7-ketodeoxycholic acid is readily accomplished by coliform organisms. A single exception was found in which the bacteria that convert cholesterol to coprosterol were not inhibited (8). All samples thus far tested have shown a complete inhibition of the conversion of cholic acid to deoxycholic acid. Although these bacteria are ubiquitous in their occurrence, efforts to cultivate them *in vitro* have been singularly unsuccessful (8,10,11). If indeed effects on intestinal bacteria are involved in the mechanism by which neomycin lowers serum cholesterol, the organism (not necessarily the bacteria that converts cholic acid to deoxycholic) is probably an unidentified organism. Efforts to elucidate the mechanism of action of neomycin on serum cholesterol by cultivation of known bacteria from feces may indeed be "sterile."

The Effect of Neomycin on Cholesterol Metabolism in Experimental Animals. Neomycin has been reported to elevate serum cholesterol in cholesterol-fed rats (12) and rabbits (13) over that of cholesterol-fed controls. These elevations in serum cholesterol have been postulated by both groups of workers to involve changes in the intestinal bacterial flora.

So little is known about absorption from the large intestines and the role that bacteria play in absorption that discussion of mechanism becomes highly conjectural for lack of factual information. Broitman et al. (12) suggest that a reduction in the number of bacteria which control the formation of nonabsorbable coprosterols from cholesterol and thus allows reabsorption of cholesterol might be involved in the elevation of serum cholesterol by neomycin in rats. As attractive as this hypothesis sounds, there is no information on the absorbability of cholesterol from the large intestines. The nonabsorbability of coprosterol must refer to the large and small intestines combined whereas information on the absorbability of cholesterol refers to the small intestine. A corollary to the cholesterol-coprosterol absorption theory when applied to man is that coprosterol is better absorbed than cholesterol.

Some information is available on bile acid absorption from the large intestines since both lithocholic acid and deoxycholic have been shown to be formed by intestinal bacteria (9). These bile acids both appear in significant amounts in bile. The absorption of deoxycholic acid in the large intestines has been estimated by Linstedt and Samuelsson (14). The absorbability or nonabsorbability of cholic acid and conjugated bile acids from the large intestines is unknown although the slower excretion of bile acids in the germ-free rat (15) suggests that conjugated bile acids may be rather efficiently absorbed from the large intestines.

Some Observations on Methodology. There has been much discussion of methods for determination of serum cholesterol. In our hands the method has proved to be less important than care in carrying out the determination and good experimental design. A

single determination of cholesterol, regardless of accuracy or precision, has little meaning. Our general procedure is to have at least five different samples in any experimental period and to precede and follow the experimental period with control periods and to maintain dietary control on a metabolic ward. The cholesterol values presented by Jacobson et al. (6) on patients receiving 12 g neomycin/day, as they so well point out, are of questionable value. The Sobel and Mayer method used by Jacobson is an excellent method and when carefully done, yields cholesterol values of great accuracy and precision. They measured the serum cholesterol only once in the control period and once in the neomycin period. Much more valuable results could have been obtained with a simple cholesterol method and a well-designed experiment.

The determination of fecal bile acids has proved to be a difficult and frustrating problem. Adequate spectrophotometric methods exist for the determination of cholic, deoxycholic and chenodeoxycholic acids and, when applied to bile, yield good results. These methods when applied to fecal bile acids yield uninterpretable results. Often significant amounts of cholic acid are reported in feces, when, in fact, the amount is usually quite insignificant. Methods based on careful extraction and chromatographic procedures which separate fatty acids from hydroxy and keto-carboxylic acids and chemical determination by some reaction for carboxyl groups again are only some compromise with the ideal since there are no simple methods for separating hydroxy fatty acids from bile acids. Methods involving the use of C^{14}-cholesterol and determination of radioactivity in the sterol free fraction yield valuable quantitative but no qualitative information. Recent advances in bile acid methodology offer great promise for the future. Noteworthy are the ion exchange procedures of Kuron and Tennent (16) and Gordon et al. (17); the glass paper and TLC procedures of Eneroth (18) and Hamilton (8); and gas chromtographic methods of Sjovall (19) and Horning (20).

Of inestimable value is the work of the last 14 years of Bergstrom, Sjovall, Linstedt, Samuelsson, Danielsson, Norman et al. on bacterial transformation of bile acids and the most recent identification of the major intestinal bacterial metabolites of cholic and chenodeoxycholic by these workers (9).

On the Neomycin Malabsorption Syndrome. Neomycin in large doses (12 g/day, orally) has been implicated by Faloon et al. (21,22) as the etiologic agent in "malabsorption syndrome" resembling idiopathic steatorrhea. In an extensive study of this "experimental malabsorption syndrome produced by neomycin" Jacobson et al. (6) investigated certain parameters of absorption, including carotene, glucose, vitamin B_{12}, d-xylose and iron, as effected by 12-g daily doses of neomycin. In their study of 33 patients, they followed one or two of these absorptive indices in some six or eight patients, and measured serum cholesterol in nine patients, in six of whom they also performed Schilling tests for B_{12} absorption. Their findings showed decreased absorption of carotene in six patients, iron in four out of six patients, vitamin B_{12} in four out of six subjects, *d*-xylose in six out of eight subjects, glucose in four out of six. The general pattern of this work was with few exceptions the application of a single "absorption parameter" to a single patient. These authors emphasized the similarity of the absorption defects seen in nontropical sprue and in the neomycin syndrome: Steatorrhea, azotorrhea, impaired absorption and increased excretion of calcium, potassium, sodium, iron, vitamin B_{12}, carotene, glucose, *d*-xylose and lowered serum cholesterol. Hypermotility, per se, was thought to be ruled out as a direct cause of these malabsorption syndromes because carotene absorption was not decreased in $MgSO_4$ catharsis. The observation of these authors ("patients receiving regular hospital diets, frequently tend to exhibit some degree of fall in serum cholesterol with time") is an indication of incomplete dietary control of these patients. Jacobson and Faloon (23) also studied malabsorptive effects of oral neomycin in daily doses of four to six g in ten patients. Reduced carotene absorption was observed in six of eight subjects, reduced xylose absorption in three of four subjects and a rise of fecal fat from 3.7–7.3 g/day in one patient. Jacobson et al. (24) reported neomycin-induced morphologic alterations in the jejunal mucosa which resembled those seen in idiopathic steatorrhea. This observation was interpreted by Jacobson and by Leveille et al. (7) as supporting the concept of impairment of mucosal permeability to foodstuffs due to an inflammatory effect of neomycin on the mucosa. Leveille et al. (7) studied six subjects for 84 days on a metabolic ward, measuring body weight, plasma lipids, fecal fat, bile acids and digitonin precipitable sterols, and coliform organisms in stool during the control and neomycin periods. They administered neomycin at daily dosages of 0.2 and 2.0 g orally and at 0.2 g/day parenterally during separate experimental periods. Only the oral administration of neomycin produced a significant effect on plasma cholesterol and fecal fat, and this only at the dosage of 2.0 g daily. The plasma cholesterol level fall averaged 25% below the preceding control period and rose by 18% during the recovery period of eight days. The fecal fat excretion averaged 2.5 g/day during the control periods and rose to 5.5 g/day during the period of 2.0 g of oral neomycin, returning to about the control level during the last three days of the eight-day recovery period. They reported an increase in bile acids and in digitonin precipitable sterols during the same period, but it should be noted that the highest bile acid excretion which they observed occurred during the period of parenteral neomycin (0.2 g daily) and during the recovery period. It is significant that the weight of the subjects remained constant throughout the study.

In the opinion of the present authors, it appears that a malabsorption syndrome has been shown to be induced by the high dosages of neomycin administered by Faloon et al., [12 g/day (22) and by Jacobson et al.,4–6 g/day (23)] but has not been shown to result from the 2.0-g daily doses used by Leveille et al. The latter authors reported a doubling of fecal fat (from 2.5–5.5 g/day), but presented no other data indicative of malabsorption. Samuel and Waithe (2) measured absorption via I^{131}-triolein in patients receiving 2 g neomycin/day orally. They observed normal absorption in two patients and delayed but complete absorption in four others. Steiner et al. (3) in a study of 20 patients on oral neomycin at 0.5–2 gm daily, reported: "In the present study, significant diarrhea did not occur. Several loose stools daily for short periods were recorded in three patients. It was not necessary to discontinue the medication in any individual. Steatorrhea was not encountered. The weights of our patients were not altered significantly during this study."

The present authors were so unimpressed with the clinical manifestations of malabsorption in their patents receiving 2 g neomycin/day that no use was

made of the usual parameters of malabsorption. Although the mechanism by which neomycin lowers serum cholesterol is still subject to some difference in interpretation of the data from various laboratories, the evidence favors a reciprocal relationship between the amount of bile acids excreted and levels of serum cholesterol. This could be a part of a mild "malabsorption syndrome" although the choice of words in this case may be unfortunate. The unfavorable consequences of a doubling of fat excretion may have to be balanced against the consequences of a doubling of bile acid excretion and its concomitant decrease of serum cholesterol.

The generalized impression that neomycin sterilizes the gut should be corrected. There is a sharp decrease in some organisms and an increase in the numbers of other organisms which are apparently normal organisms since there have been no reports of side effects due to pathogenic bacteria.

The primary side effect is an initial diarrhea which usually subsides within two weeks. Seldom is the diarrhea severe enough to cause withdrawal of the drug. The mild "malabsorption syndrome" produced by 2 g neomycin/day appears to be medically inconsequential, whereas the decrease in serum cholesterol appears to be significant.

REFERENCES

1. Samuel, P., and A. Steiner, Proc. Soc. Exptl. Biol. Med. *100*, 193 (1959).
2. Samuel, P., and W. I. Waithe, Circulation *24*, 578 (1961).
3. Steiner, A., E. Howard and S. Akgul, Circulation *24*, 729 (1961).
4. Goldsmith, G. A., J. G. Hamilton and O. N. Miller, Trans Assoc. Am. Physicians *72*, 207 (1959).
5. Powell, R. C., W. T. Nunes, R. S. Harding and J. B. Vacca, Am. J. Clin. Nutri. *11*, 156 (1962).
6. Jacobson, E. D., R. B. Chodas and W. W. Faloon, Am. J. Med. *28*, 524 (1960).
7. Leveille, G. A., R. C. Powell, H. E. Sauberlich and W. T. Nunes, Am. J. Clin. Nutri. *12*, 421 (1963).
8. Hamilton, J. G., Arch. Biochem. Biophys. *101*, 7 (1963).
9. Danielsson, H., P. Eneroth, K. Hellstrom, S. Linstedt and J. Sjovall, J. Biol. Chem. *238*, 2299 (1963).
10. Gustafsson, B. E., A. Norman and J. Sjovall, J. Arch. Biochem. Biophys. *91*, 93 (1960).
11. Portman, O. W., S. Shah, A. Antonis and B. Jorgensen, Proc. Soc. Exptl. Biol. Med. *109*, 959 (1962).
12. Broitman, S. A., D. G. Kinnear, L. S. Gottlieb, A. L. Begman, J. J. Vitale and N. Zamcheck, J. Lab. Clin Med. *55*, 55 (1960).
13. Fisher, E. R., Proc. Soc. Exptl. Biol. Med. *103*, 857 (1960).
14. Lindstedt, S., and B. Samuelsson, J. Biol. Chem. *234*, 2036 (1959).
15. Danielsson, H., and B. Gustafsson, Arch. Biochem. Biophys. *83*, 482 (1959).
16. Kuron, G. W., and D. M. Tennent, Fed. Proc. *20*, 268 (1961).
17. Gordon, B. A., A. Kuksis and J. M. R. Beveridge, Fed. Proc. *20*, 248 (1961).
18. Eneroth, P., J. Lipid Res. *4*, 11 (1963).
19. Sjovall, J., Acta Chem. Scand. *16*, 1761 (1962).
20. Horning, E. C., JAOCS *41*, 707–716 (1964).
21. Faloon, W. W., and C. J. Fisher, J. Clin. Res. Proc. *5*, 215 (1957).
22. Faloon, W. W., C. J. Fisher and K. C. Duggen, J. Clin. Invest. *37*, 893 (1958)
23. Jacobson, E. D., and W. W. Faloon, J.A.M.A. *175*, 187 (1961).
24. Jacobson, E. D., J. T. Prior and W. W. Faloon, J. Lab. Clin. Med. *56*, 245 (1960).

[Received April 15, 1964—Accepted July 8, 1964]

Thin-Layer Chromatography of Lipids[1]

HELMUT K. MANGOLD,[2] University of Minnesota, The Hormel Institute, Austin, Minnesota

Abstract

Technique

Thin-Layer Chromatography as an Analytical Tool

Qualitative Analysis

Quantitative Analysis

Thin-Layer Chromatography as a Preparative Tool

Applications

Analysis of Lipids in Microorganisms, Plants and Laboratory Animals

Fats, Oils, Waxes and Their Hydrolysis Products

Phospholipids, Sulfolipids and Glycolipids

Steroids, Including Bile Acids

Terpenes, Including Carotenoids, Balsams and Resins

Fat-Soluble Vitamins and Biologically Active Quinones

Topographic Lipid Analysis of Human Tissues

Lipid Patterns in Healthy Humans

Lipid Patterns in Pathological Cases

Conclusion

Introduction

IN THE LAST FEW YEARS, TLC has become widely accepted and is now considered an indispensable tool in many laboratories. Monographs on this technique have been written or edited by Bobbitt (35), Marini-Bettolo (273), Randerath (341), Stahl (394) and Truter (430). Several chapters on TLC have been published in a book edited by Morris and James (296). In another volume, an article providing a survey of equipment and material used in TLC has just been printed (261). A publication of von Arx and Neher (7) is also of interest in this connection.

Two reviews (267,323) on applications of TLC in lipid analysis were published almost simultaneously in 1961. Progress in the development of TLC was discussed more recently in articles by Hofmann (165) and by Lines (255). These two publications appeared in a volume which also contains several other contributions pertaining to the subject to be discussed here.

Chapters reviewing the use of TLC and other chromatographic techniques for the analysis and isolation of lipids have been prepared by Morris (292) and by Schlenk (366). Applications of chromatographic techniques to a more specific problem, the study of the biosynthesis of fatty acids, have been discussed by Pascaud (320).

Reference is made here to the treatise on lipid analysis that has been written by Entenman (102), to the first volume of a new serial publication on the same subject which has been edited by Boekenoogen (36) and to Neher's (301) well-known monograph on steroid analysis. The second edition of the latter work has just been released.

[1] This review is an extension of an article included in AOCS Lectures of the 12th Annual Short Course on *Newer Lipid Analyses*, Chicago, 1961. It is based on talks given at the International Course on *Chromatographic Methods in Lipid Research* conducted at Milano, Italy, 1962 and at the Symposium: *Drugs in Lipid Metabolism*, AOCS Meeting, Minneapolis, 1963.

[2] The author's work supported by the U.S. Public Health Service, National Institutes of Health, Grant GM-5817, GM-11364 and AM-6674, and by The Hormel Foundation.