(2) a decrease in the rate at which the epidermal cells keratinize. Since there is no significant increase in the mitotic activity of the basal cells[10], it seems more probable that the epidermal cells of mice deficient in fatty acid are retarded in their rate of keratinization by their involvement.

I thank Dr. Richard A. Ellis of Brown University and Dr. William Montagna of the Oregon Regional Primate Research Center for their advice, and Dr. Elizabeth Leduc of Brown University for providing the animals. This investigation was supported in part by a fellowship from the Government of the United Arab Republic and in part by U.S. Public Health Service traineeship 5-*TI-GM*-582-04 and research grant *GM*-08380-04 from the Division of General Medical Sciences.

Ahmed N. Nasr*

Biology Department,
Brown University,
Providence,
Rhode Island.

* Present address: Department of Pathology, Boston University Medical Center, Boston, 18, Mass.

[1] Haldi, J., Giddings, G., and Wynn, W., *Amer. J. Physiol.*, **135**, 392 (1942).
[2] Wynn, W., and Haldi, J., *Amer. J. Physiol.*, **142**, 508 (1944).
[3] Kooyman, D. J., *Arch. Dermat. and Syph.*, **29**, 342 (1934).
[4] Smedley-MacLean, I., and Hume, E. M., *Biochem. J.*, **35**, 990 (1941).
[5] Williamson, R., *Biochem. J.*, **35**, 1003 (1941).
[6] Baker, J. R., *Quart. J. Micr. Sci.*, **87**, 441 (1946).
[7] Baker, J. R., *Quart. J. Micr. Sci.*, **85**, 1 (1944).
[8] Cain, A. J., *Biol. Rev.*, **25**, 73 (1950).
[9] Rothman, S., *Physiology and Biochemistry of the Skin* (Univ. Chicago Press, 1954).
[10] Nasr, A. N., and Shostak, S. (in preparation).

Influence of Basic Antibiotics on Serum- and Liver-cholesterol Concentrations in Chicks

It has been reported[1] that orally administered neomycin lowers the serum-cholesterol level in man, and these results were confirmed in other studies[2–4]. Neamine and kanamycin have only a moderate effect, whereas streptomycin and dihydrostreptomycin are inactive[3,4].

As neomycin is very poorly absorbed from the gut, its action is probably restricted to the intestinal lumen. The modifications of the intestinal flora, induced by the antibiotic, are generally considered to be responsible for the cholesterol-lowering effect of this drug. Nevertheless, the observation that streptomycin, a substance with a closely related antibiotic spectrum, has no influence on serum-cholesterol concentration casts some doubt on this explanation.

An incidental observation that neomycin precipitates solutions of bile acids led us to suppose that the activity of this product might be due to its ability to form salts which interfere with the absorption of lipids from the intestine.

To examine this hypothesis, we have prepared and tested two types of neomycin derivatives which are devoid of antibiotic activity: *N*-methylated neomycin (Vanderhaeghe and Claes, unpublished work), which is basic, and *N*-acetylated neomycin[5], which is a neutral substance.

Five groups of 15 neonatal broiler chicks were fed a basic casein-sucrose diet containing 6 per cent corn-oil as the sole source of fat[6]. To this basic diet were added 0·2 per cent cholesterol and 0·15 per cent of the different substances under investigation, as indicated in Table 1. The experimental diets were fed for 2 weeks. After fasting overnight, the birds were bled by cardiac puncture and the serum- and liver-cholesterol concentrations were determined by the method of Abell *et al.*[7]. Fæcal fat excretion was determined daily according to Van de Kamer *et al.*[8].

As shown in Table 1, feeding 0·15 per cent neomycin or 0·15 per cent methylated neomycin reduced serum-cholesterol concentrations by 26 per cent and 17 per cent respectively. The *N*-acetylated neomycin was ineffective, and streptomycin rather tended to increase cholesterol levels. Table 1 also shows that the administration of neomycin or methylated neomycin resulted in an increased fæcal fat excretion; streptomycin and *N*-acetylated neomycin had no such effect.

Table 1. Effect of Neomycin, Streptomycin, and Two Neomycin Derivatives on Serum- and Liver-cholesterol and on Fæcal Fat Excretion in Chicks

	—	Neomycin 0·15 %	Strepto-mycin 0·15 %	Methyl-neomycin 0·15 %	*N*-acetyl. neomycin 0·15 %
Average serum-cholesterol, (mg/100 ml.)	267	198	284	222	270
Average total liver-cholesterol, (mg/100 g liver)	762	502	771	589	765
Fæcal fat, (mg/g dried fæces)	57	104	49	92	52
Body weight (g)	143	127	148	140	141

Our results indicate that the cholesterol-lowering effect of neomycin is due not to its antimicrobial activity but to the presence of basic groups in the molecule. This hypothesis could also explain the results obtained in human experiments by Samuel and Waithe[4]. Streptomycin, which does not lower serum-cholesterol, has only three basic groups. Kanamycin and neamine, with four amino groups, present a weak activity. Neomycin, an antibiotic with six amino groups, is the most active of the four drugs.

Further experiments in this laboratory have shown that both neomycin and methylated neomycin, but neither streptomycin nor acetylated neomycin, can break emulsions stabilized by lecithin (to be published). A similar effect on the emulsification of lipids in the intestine could explain the increase in fæcal fat output observed in the present work.

During these studies it was also found that neomycin and methylated neomycin when added to human or to chick bile cause precipitation of bile acids and phospholipids, and a correlation was observed between the bile-acid-precipitating activity of different compounds and their effect on the serum-cholesterol level in chicks. Moreover, feeding chicks with 0·25–0·5 per cent of methylated neomycin caused a five-fold to ten-fold increase in the excretion of fæcal bile acids, and addition of neomycin or methylated neomycin to a diet containing lithocholic acid protected chicks from the hepatotoxic effects of lithocholic acid[9], thus indicating interference with the absorption of bile acids from the intestine.

These experiments support the hypothesis that basic substances such as neomycin and methylated neomycin interfere with the intestinal absorption of fats, cholesterol, and bile acids by disturbing the emulsification of lipids in the intestine or by complexing bile acids. The cholesterol-lowering effect could then be explained by an impaired intestinal absorption of cholesterol and an increased degradation of cholesterol to bile acids in the liver.

P. De Somer
H. Vanderhaeghe
H. Eyssen

Rega Institute for Medical Research,
University of Louvain, Belgium.

[1] Samuel, P., and Steiner, A., *Proc. Soc. Exp. Biol. and Med.*, **100**, 193 (1959).
[2] Samuel, P., *Proc. Soc. Exp. Biol. and Med.*, **102**, 194 (1959).
[3] Steiner, A., Howard, E., and Akgun, S., *Circulation*, **24**, 729 (1961).
[4] Samuel, P., and Waithe, W. I., *Circulation*, **24**, 578 (1961).
[5] Rinehart, K. L., Argoudelis, A. D., Goss, W. A., Sohler, A., and Schaffner, C. P., *J. Amer. Chem. Soc.*, **82**, 3938 (1960).
[6] Eyssen, H., and De Somer, P., *J. Exp. Med.*, **117**, 127 (1963).
[7] Abell, L. L., Levy, B. B., Brooke, B. B., and Kendall, F. E., *J. Biol. Chem.*, **195**, 357 (1952).
[8] Van de Kamer, J. H., Ten Bokkel Huininck, H., and Weyers, H. A., *J. Biol. Chem.*, **177**, 231 (1949).
[9] Eyssen, H., and De Somer, P., *Poultry Sci.*, **42**, 1020 (1963).

©1964 Nature Publishing Group