DRUGS AND ATHEROSCLEROSIS[1]

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In the last two decades, concerted attempts have been made to clarify the role of various factors causing atherosclerosis in man. This search has involved what is perhaps the broadest interdisciplinary approach ever utilized in the history of medical investigation. Internists, pathologists, biochemists, clinical nutritionists, epidemiologists, and representatives of many other disciplines have participated. As basic information on the nature of the disease has accumulated, numerous drugs have been studied in an attempt either to ameliorate or alter the course of the atherosclerotic process.

In the present review, emphasis will be given only to those drugs that have been used extensively in human subjects. Consideration will be given principally to preparations concerning which sufficient biochemical data are available as a foundation for clinical trial. No attempt will be made to discuss pharmacologic agents still in an early stage of development. Studies of drug action on intermediary lipid metabolism at the cellular or subcellular levels have been adequately covered by recent reviews [Paoletti (1)].

The atherosclerotic process appears to develop over a period of years, and necessarily exhibits a lack of immediate response to drugs. Consequently, relatively short-term drug studies (months or even several years) may not provide acceptable information regarding their effectiveness. The problem is further complicated by a lack of certainty that a metabolite affected by a drug, such as cholesterol, plays a fundamental role in the development of atherosclerosis.

Definitions and Assumptions

The term "atherosclerosis" has a variety of meanings, depending on the point of view of the student of the problem. For example, internists conducting clinical drug trials must necessarily diagnose the disease on the basis of specific clinical signs and symptoms that indicate atherosclerosis or atherosclerotic heart disease. The statistical probability of obtaining a correct clinical diagnosis depends on the kind of clinical criteria used and on the rigor with which they are applied. For example, if a patient with diagnosed atherosclerosis dies of "myocardial infarction," the clinician will state the cause of death as atherosclerotic heart dis-

[1] The survey of the literature pertaining to this review was concluded in July 1965.

ease. However, if no autopsy is done, the diagnosis remains an assumption. Thus, a large element of vagueness and uncertainty enters into clinical drug trials, particularly when a disorder as diffuse and generalized as atherosclerosis is involved. For the clinician, atherosclerosis encompasses a wide scale of clinical entities which may include myocardial infarction, angina pectoris, cerebrovascular disease, peripheral vascular disease, various forms of cardiac failure, arrhythmias, and sometimes changes that can be seen only on the electrocardiogram [Biorck (2)].

In contrast, for the pathologist atherosclerosis is "a variable combination of changes of the intima of arteries (as distinguished from arterioles) consisting of focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue and calcium deposits, and associated with medial changes" [WHO, Tech. Rept. Ser. (3)].

It is now well recognized that a number of "risk factors" may be involved in the development of atherosclerotic heart disease. These factors include hypertension (diastolic blood pressure of 95 mm or above), hypercholesterolemia (serum cholesterol 260 mg/100 ml or above), marked obesity, heavy cigarette smoking, diabetes mellitus, and a family history of vascular disease [Kannel et al. (4); Stamler et al. (5, 6); Doyle et al. (7)].

Recently there has been a growing suspicion that carbohydrates in the diet, possibly by virtue of an influence on the serum triglycerides and phospholipids, may play a role in the development of atherosclerotic heart disease [Albrink (8); Kuo & Bassett (9); Ostrander et al. (10)]. However, the etiologic significance of serum triglycerides and phospholipids (to say nothing of the various serum lipoprotein fractions) awaits clarification. In fact, the serum total cholesterol level appears to remain the best single predictor of risk in coronary heart disease [Kannel (11)], prior to the development of myocardial infarction. The survival rate of patients following myocardial infarction, however, was not influenced by the plasma concentrations of cholesterol [Little et al. (12)]. For this reason, the present review will give preference to drugs that are currently in use for the correction of hypercholesterolemia (and hyperlipidemia) in patients without evidence of advanced atherosclerosis.

Thyroid Hormone and its Analogues

That thyroid hormone exerts a strong influence on lipid metabolism is well accepted. An excellent review on this subject [Myant (13)] covers the literature up to 1961 and discusses in detail both the *in vivo* and *in vitro* effects of thyroid. Recent clinical studies [Moses et al. (14)] have demonstrated that desiccated thyroid administered to euthyroid volunteers lowers serum α- and β-lipoprotein cholesterol and α-lipoprotein triglycerides. However, serum total triglycerides and nonesterified fatty acid

concentrations were not affected by daily ingestion of 0.2 to 1.6 g of desiccated thyroid. These seemingly beneficial effects of the administered thyroid were offset by variable degrees of tachycardia, an increase in the systolic blood pressure, minor but persistent changes of electrocardiogram, and occasional hyperkalemia [Danowski et al. (15)]. It is obvious that doses of thyroid needed to accomplish a reduction of serum lipids also may produce undesirable side effects. In fact, thyroid-induced hypermetabolism may aggravate coronary insufficiency or lead to circulatory failure in patients whose cardiac status is already marginal because of atherosclerotic coronary heart disease.

Several structural modifications of the thyroid hormone have been produced and evaluated [Best & Duncan (16); Furman & Howard (17)] in an effort to separate the serum lipid lowering effect from the calorigenic effect. The most extensive clinical trials were carried out on the dextrorotatory isomer (D-T4) of L-thyroxine (L-T4) [Schneeberg et al. (18)]. When appropriate quantities of D-T4 were given in double blind studies it did not alter basal metabolic rate, pulse rate, and body weight [Owen, Neely & Owens (19)]. A daily dosage of 4 to 8 mg was well tolerated by patients with angina pectoris and heart failure [Hansen (20)]. Current evidence suggests [Winters & Soloff (21)] that a 20 to 36 per cent reduction in serum cholesterol can be expected in hypercholesterolemic patients receiving D-T4 for several weeks. (In the case of serum triglycerides, the response is often pronounced but highly variable.) No tendency to "escape" from treatment was apparent after three years' use. Xanthomatous skin and tendon lesions diminished in size or even disappeared altogether in some cases [Owen, Owens & Neely (22); Nichols (23)].

The primary effect of D-T4 on cholesterol metabolism appears to be similar to that of natural thyroid hormones [Owen (24)], i.e., an acceleration of catabolism and an excretion of cholesterol and its products through the gastrointestinal tract.

Nicotinic Acid

In 1956, Altschul observed that administration of large doses of nicotinic acid protected cholesterol-fed rabbits from the development of hypercholesterolemia and lipid deposition in the aorta. Similarly, he was the first to report [Altschul, Hoffer & Stephen (25); Altschul & Hoffer (26, 27)] that hypercholesterolemic patients respond with a decrease in serum cholesterol when this vitamin is given in sufficient quantity. It is noteworthy, however, that the doses of niacin necessary to induce such effects in man are 15 to 30 times the recommended daily allowance for this vitamin. These results were rapidly confirmed and extended, in patients [Parsons et al. (28); Miller, Hamilton & Goldsmith (29); Kottke,

Bollman & Juergens (30)] and in a variety of experimental animals [Merril & Lemley-Stone (31); Gaylor, Hardy & Baumann (32)]. One species found to be unresponsive to niacin was the rat [Friedman & Byers (33)].

In addition to the serum cholesterol lowering property of niacin, clinical studies have indicated that a slight to moderate depression of serum phospholipids [Gurian & Adlersberg (34)], triglycerides [Miller, Hamilton & Goldsmith (35)], and β-lipoproteins [Galbraith, Perry & Beamish (36)] may be associated with niacin administration. Niacin also decreases the mobilization of free fatty acids into the blood during exercise without, however, affecting the rate of their removal by other tissues [Carlson et al. (37)].

Most of these effects of niacin were obtained as a result of daily oral administration of 3 to 6 g of this vitamin. It soon became evident, however, that such quantities of niacin were not without certain clinical and laboratory side effects in patients. The most commonly observed clinical effects of niacin therapy were transitory peripheral vasodilation, occasional tingling sensations, and itching lasting for several minutes following ingestion of the drug. These effects, however, usually did not occur after a week or two. The laboratory abnormalities were suggestive of hepatic dysfunction, with impairment of sulfobromophthalein excretion in some patients [Parsons (38)]. Furthermore, these changes were often accompanied by elevations of serum uric acid and alkaline phosphatase and transaminase levels [Christensen et al. (39)]. Deterioration of glucose tolerance during niacin treatment also has been observed. No other major clinical or pathological abnormalities resulting from the oral administration of niacin have been reported.

Several other compounds, structurally somewhat similar to nicotinic acid, have been tested for serum cholesterol decreasing properties in man. Of these, only two related compounds, ethyl nicotinate [Miller, Hamilton & Goldsmith (40)] and 3-pyridineacetic acid [Antonini & Masi (41)] appeared to have cholesterol lowering effects. However, on a weight basis they are appreciably less potent than niacin.

The site of action of nicotinic acid in cholesterol metabolism is not well defined at present. It is generally accepted that niacin inhibits cholesterol biosynthesis at some point. There is good evidence from radioactive carbon studies *in vivo* and *in vitro* that incorporation of acetate [Parsons (42); Gamble & Wright (43)] is inhibited by the presence of niacin. The incorporation of C^{14} mevalonic acid into cholesterol remains unimpaired. The locus of the inhibitory action of the molecular configuration of niacin has been postulated by Miller & Hamilton (44). From evaluation of 22 structurally similar compounds in their test system, they suggest that the relative position of the carboxyl group to the nitrogen atom on the pyri-

dine ring is the stereochemical molecular configuration important for inhibitory action.

Ethyl Chlorophenoxyisobutyrate (CPIB)

Thorp & Waring (45) reported in 1962 that CPIB administration to rats resulted in reduction of serum and liver cholesterol concentrations. This observation generated widespread interest and, indeed, there was such an upsurge of research on the effect of CPIB on lipid metabolism that a symposium on the subject was held only one year later in Buxton, England. The 46 papers presented there were later published in one volume (46). It was reported that when CPIB was given to hypercholesterolemic patients in doses of 2 g per day there was a tendency for serum cholesterol concentrations to fall to normal. This effect on serum cholesterol was documented in some 1300 cases. The majority of subjects were patients with atherosclerotic heart disease who were treated for an average period of six months. In eight cases, however, hypercholesterolemic patients had been treated with the drug for periods up to two years. Also reported was a reduction of serum triglycerides which in most cases nearly paralleled the serum cholesterol changes. Similarly, a depression of plasma lipoproteins was found, with an especially profound effect on the S_f 20-400 class. This latter finding is consistent with the effect of CPIB on plasma triglycerides, since the triglycerides would be expected to reflect a decrease in low density lipoproteins. The serum phospholipids and free fatty acids apparently show little or no response to the drug.

The side effects of CPIB may provide a clue to its action on blood lipids, particularly since it has been suggested that one of its main effects may be on lipoprotein lipase. It is well known that heparin, in addition to its effect on the blood clotting mechanism, also acts as an activator of lipoprotein lipase (clearing factor). Apparently, CPIB also has both of these characteristics; namely an action on the blood clotting mechanism as well as on lipoprotein lipase. Patients already stabilized on certain doses of anticoagulant medication (warfarin) had to be given lower doses of the anticoagulant when administration of CPIB was instituted because of development of a bleeding tendency. Moreover, when CPIB and androsterone are administered simultaneously, prolongation of the prothrombin time, a decrease of platelet adhesiveness, and increased fibrinolytic activity ensue. These effects on coagulation may appear somewhat surprising in view of the fact that androgens usually have an effect on fibrinolysis only when given systemically in large doses [Fearnley & Chakrabarti (47)]. When CPIB is given alone it has no influence on known components of the fibrinolytic system [Sweet, Rifkind & McNicol (48)]. The apparently synergistic action of CPIB and androsterone on the blood clotting mechanism awaits clarification.

The ability of CPIB to lower postprandial plasma optical density as well as triglycerides and lipoproteins [Strisower & Strisower (49)] suggests an action of this drug on lipoprotein lipase. The mechanism is believed to involve either induction or increase of lipoprotein lipase or blockade of an inhibitor of that enzyme (46).

The clinical side effects of CPIB are mostly gastrointestinal, these include nausea, vomiting, and diarrhea in a small proportion of cases. No other major clinicopathological disturbances have been reported but it must be remembered that the drug is still at an early stage of development. Any assessment of the therapeutic value of this promising agent must await the result of comprehensive long-term clinical trials.

Cholestyramine

Cholestyramine, a quaternary ammonium anion exchange resin sequesters bile acids in the intestinal lumen, exchanging them for chloride. The high molecular weight of cholestyramine ($> 1{,}000{,}000$) apparently prevents its absorption from the intestine. These two critical factors, namely efficient binding of bile acids and lack of intestinal absorption of the cholestyramine-bile acid complex, provide a desirable combination for increasing the rate of fecal excretion of bile acids.

In order to appreciate the novel approach utilized to lower serum and tissue cholesterol with cholestyramine resin, it is appropriate to review briefly the metabolic fate of the cholesterol molecule.

Numerous isotope tracer studies indicate that cholesterol in the body either is manufactured from acetyl coenzyme A (endogenous) or is derived as the intact molecule from food sources of animal origin (exogenous cholesterol). It is assumed (but not yet definitely determined) that endogenous and exogenous cholesterol share the same metabolic fate in every respect. At present, it is possible to visualize the cholesterol in the body as being partitioned into a number of "pools." This concept is a useful one as long as one remembers that each "pool" is in some sort of equilibrium with the other pools. Cholesterol is continually being exchanged [Danielsson (50)] among these at different, yet characteristically constant, rates. For example, one may consider plasma, erythrocyte, liver, and arterial walls as separate cholesterol pools. In certain pathologic states, one or more pools may increase disproportionately while others remain normal. Examples are found in patients with familial hypercholesterolemia in whom the incidence of early coronary atherosclerosis and cutaneous xanthomatous tumors indicate expansion of plasma, arterial and subcutaneous cholesterol pools. However, erythrocyte, brain and, perhaps, liver pools appear to remain normal in such individuals. Since cholesterol enters and leaves the more labile pools fairly readily, one can envisage a situation in which an agent may deplete one pool and thereby indirectly

affect others. Thus, an increased removal of cholesterol from the liver may promote an increased rate of transfer of cholesterol from other tissues to the liver. That such a phenomenon occurs is suggested by well documented biochemical [Tennent et al. (51)] and clinical [Van Itallie & Hashim (52)] studies with cholestyramine.

It is known that a part of liver cholesterol is continually being converted to bile acids that are excreted into the intestinal lumen via the biliary tract. Bile acids in their conjugated form participate in various digestive-absorptive processes and are then in large part reabsorbed through the portal system and reutilized [Bergstrom (53)]. A small fraction is excreted from the body with the feces.

Blockade of the reabsorption of bile acids from the intestine appears to have at least two effects on cholesterol metabolism. One is to increase the rate of hepatic conversion of cholesterol to bile acids to compensate for the bile acids that are no longer being recovered from the intestine. A second effect is an increase in rate of cholesterol biosynthesis by the intestinal wall, particularly terminal ileum, owing to interruption of a feedback mechanism in which the bile acids available for reabsorption are controlling factors in enteric cholesterol manufacture. Furthermore, other effects can be predicted; for example, removal in sufficient quantity of bile acids from the intestinal lumen may interfere with absorption of exogenous cholesterol.

Knowledge of the feedback role of the bile acids in hepatic cholesterol metabolism makes it obvious that agents affecting bile acid reabsorption have a potential value in the treatment of hypercholesterolemia in man.

Use of cholestyramine in the treatment of hypercholesterolemia has been reported by a number of workers. Hashim & Van Itallie (54) studied nine patients with "primary" hypercholesterolemia for periods of one month to four years. Cholestyramine was taken in daily quantities of 13.3 g in four equally divided doses. A 20 to 50 per cent lowering of total serum cholesterol was observed during treatment. In two subjects, simultaneous analysis of fecal bile acid excretion showed a marked (three- to elevenfold) increase without a corresponding change in neutral sterol output. Further evidence, indicating depletion of certain cholesterol pools within the body, was provided by the gradual disappearance of large xanthomatous skin lesions in the patients [Keczkes, Goldberg & Fergusson (55)]. In a similar study [Gherondache & Pincus (56)] in elderly males, the reduction in serum total cholesterol was accompanied by a fall in β-lipoproteins. It is noteworthy that patients with familial hypercholesterolemia [Horan, Di Luzio & Etteldorf (57)], who usually resist most attempts to reduce their serum cholesterol, responded well to cholestyramine administration.

The metabolic side effects of the drug are apparently minimal or negli-

gible. It has been suggested, however, that cholestyramine may have had a causative role in biliary calcification in three cases of biliary cirrhosis. Yet, subsequent studies (54) showed neither X-ray evidence of liver calcification nor changes in serum calcium levels when the drug was given to hypercholesterolemic patients for periods up to four years. No abnormal values of liver function tests, including alkaline phosphatase, SGOT, prothrombin time, serum albumin-globulin concentrations, and serum bilirubin were seen. Chloride ions which were exchanged for bile acids by the resin in the intestinal lumen did not chronically alter serum chloride levels. The clinical side effects of the drug, as could be predicted, were limited to the gastrointestinal tract. Sensations of gastric fullness, nausea, gaseous distention, constipation, and occasionally diarrhea were reported by the patients. Improvement of these symptoms usually occurred by the end of the second week of treatment.

LITERATURE CITED

1. Paoletti, R., Ed., *Lipid Pharmacology* (Academic Press, New York, 1964)
2. Biorck, G., *J. Atherosclerosis Res.*, **5,** 261–66 (1965)
3. *World Health Organ., Tech. Rept. Ser.*, No. 143 (1958)
4. Kannel, W. B., Dawber, T. R., Kagan, A., Revotskie, N., and Stokes, J., III, *Ann. Internal Med.*, **55,** 33–50 (1961)
5. Stamler, J., Berkson, D. M., Young, Q. D., Lindberg, H. A., Hall, Y., Miller, W., and Stamler, R., *J. Am. Dietet. Assoc.*, **40,** 407–16 (1962)
6. Stamler, J., Berkson, D. M., Young, Q. D., Lindberg, H. A., Hall, Y., Mojonnier, L., and Andelman, S. L., *Med. Clin. N. Am.*, **47,** 3–31 (1963)
7. Doyle, J. T., Dawber, T. R., Kannel, W. B., Heslin, A. S., and Kahn, H. A., *New Engl. J. Med.*, **266,** 796–801 (1962)
8. Albrink, M. J., *Ann. Internal Med.*, **62,** 1330–33 (1965)
9. Kuo, P. T., and Bassett, D. R., *Ann. Internal Med.*, **62,** 1199–1212 (1965)
10. Ostrander, L. D., Francis, T., Jr., Hayner, N. S., Kjelsberg, M. D., and Epstein, F. H., *Ann. Internal Med.*, **62,** 1188–98 (1965)
11. Kannel, W. B., Dawber, T. R., Friedman, G. D., Glennon, W. E., and McNamara, P. M., *Ann. Internal Med.*, **61,** 888–99 (1964)
12. Little, J. A., Shanoff, H. M., Roe, R. D., Csima, A., and Yano, R., *Circulation*, **31,** 854–62 (1965)
13. Myant, N. B., *Lipid Pharmacology*, 299–323 (Paoletti, R., Ed., Academic Press, New York, 1964)
14. Moses, C., Sunder, J. M., Vester, J. W., and Danowski, T. S., *Metab., Clin. Exptl.*, **13,** 717–28 (1964)
15. Danowski, T. S., Sarver, M. E., D'Ambrosia, R. D., and Moses C., *Metab., Clin. Exptl.*, **13,** 702–16 (1964)
16. Best, M. M., and Duncan, C. H., *Circulation*, **24,** 58–67 (1961)
17. Furman, R. H., and Howard, R. P., *Metab., Clin. Exptl.*, **11,** 76–93 (1962)
18. Schneeberg, N. G., Herman, E., Menduke, H., and Altschuler, N. K., *Ann. Internal Med.*, **56,** 265–75 (1962)
19. Owen, W. R., Neely, W. B., and Owens, J. C., *J. Am. Med. Assoc.*, **178,** 1036–38 (1961)
20. Hansen, P. F., *J. Atherosclerosis Res.*, **3,** 584–90 (1963)
21. Winter, W. L., and Soloff, L. A., *Am. J. Med. Sci.*, **243,** 458–68 (1962)
22. Owen, W. R., Owens, J. C., and Neely, W. B., *Angiology*, **13,** 75–78 (1962)
23. Nichols, F. L., *J. Am. Med. Assoc.*, **181,** 1074–76 (1962)
24. Owen, W. R., *Med. Clin. N. Am.*, **48,** 347–53 (1964)
25. Altschul, R., Hoffer, A., and Stephen, J. D., *Arch. Biochem. Biophys.*, **54,** 558–59 (1955)
26. Altschul, R., and Hoffer, A., *Z. Kreislaufforsch.*, **44,** 129–34 (1955)
27. Altschul, R., and Hoffer, A., *Arch. Biochem. Biophys.*, **73,** 420–24 (1958)
28. Parsons, W. B., Jr., Achor, R. W. P., Berge, K. G., McKenzie, B. F., and Barker, N. W., *Proc. Staff Meetings Mayo Clinic*, **31,** 377–90 (1956)
29. Miller, O. N., Hamilton, J. G., and Goldsmith, G. A., *Circulation*, **18,** 489–90 (1958)
30. Kottke, B. A., Bollman, J. L., and Juergens, J. L., *Circulations Res.*, **11,** 108–14 (1962)
31. Merril, J. M., and Lemley-Stone, J., *Circulation Res.*, **5,** 617–19 (1957)
32. Gaylor, J. L., Hardy, R. W. F., and Baumann, C. A., *J. Nutr.*, **70,** 293–301 (1960)
33. Friedman, M., and Byers, S. O., *J. Clin. Invest.*, **38,** 1328–33 (1959)
34. Gurian, H., and Adlersberg, D., *Am. J. Med. Sci.*, **237,** 12–22 (1959)
35. Miller, O. N., Hamilton, J. G., and Goldsmith, G. A., *Am. J. Clin. Nutr.*, **8,** 480–90 (1960)
36. Galbraith, P. A., Perry, W. F., and Beamish, R. E., *Lancet*, **I,** 222–23 (1959)
37. Carlson, L. A., Havel, R. J., Ekelund, L. G., and Holmgren, A., *Metab., Clin. Exptl.*, **12,** 837–45 (1963)
38. Parsons, W. B., Jr., *Arch. Internal Med.*, **107,** 653–67 (1961)
39. Christensen, N. A., Achor, R. W. P., Berge, K. G., and Mason, H. L., *Diseases Chest*, **46,** 411–16 (1964)
40. Miller, O. N., Hamilton, J. G., and

Goldsmith, G. A., *Am. J. Clin. Nutr.*, **10**, 285–96 (1962)
41. Antonini, F. M., and Masi, R., *Reforma Med.*, **74**, 262–66 (1960)
42. Parsons, W. B., Jr., *Circulation*, **24**, 1099–1100 (1961)
43. Gamble, W., and Wright, L. D., *Proc. Soc. Exptl. Biol. Med.*, **107**, 160–62 (1961)
44. Miller, O. N., and Hamilton, J. G., *Lipid Pharmacology*, 275–98 (Paoletti, R., Ed., Academic Press, New York, 1964)
45. Thorp, J. M., and Waring, W. S., *Nature*, **194**, 948–49 (1962)
46. Symposium on atromid, *J. Atherosclerosis Res.*, **3**, 347–75 (1963)
47. Fearnley, G. R., and Chakrabarti, R., *Lancet*,, **II**, 128–32 (1962)
48. Sweet, B., Rifkind, B. M., and McNicol, G. P., *J. Atherosclerosis Res.*, **5**, 347–50 (1965)
49. Strisower, E. H., and Strisower, B., *J. Clin. Endocrinol. Metab.*, **24**, 139–44 (1964)
50. Danielsson, H., *Advan. Lipid Res.*, **1**, 335–85 (1963)
51. Tennent, D. H. Siegel, H., Zanetti, M. E., Kuron, G. W., Ott, W. H., and Wolf, F. J., *J. Lipid Res.*, **1**, 469–73 (1960)
52. Van Itallie, T. B., and Hashim, S. A., *Med. Clin. N. Am.*, **47**, 629–48 (1963)
53. Bergstrom, S., *Federation Proc.*, **20**, No. 1, Part III, 121–26 (1961)
54. Hashim, S. A., and Van Itallie, T. B., *J. Am. Med. Assoc.*, **192**, 289–93 (1965)
55. Keczkes, K., Goldberg, D. M., and Fergusson, A. G., *Arch. Internal Med.*, **114**, 321–28 (1964)
56. Gherondache, C. N., and Pincus, G., *Metab. Clin. Exptl.*, **13**, 1462–68 (1964)
57. Horan, J. M., Di Luzio, N. R., and Etteldorf, N. J., *J. Pediat.*, **64**, 201–9 (1964)

CONTENTS