

# Reports on Individual Drugs

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## Carotene supplementation for vitamin A deficiency

It has been recognized for the past 10 years that vitamin A deficiency is a substantial factor in much childhood morbidity and mortality, as well as being the cause of blinding xerophthalmia. Although the deficit is readily and rapidly corrected by high doses of preformed vitamin A, it is probable that some 40 million children still remain at risk. Indeed, the prevalence of clinical deficiency may be widely underestimated since most attention has been directed to children of preschool age, whereas deficiency also occurs among older children (1, 2).

Regression of clinical signs and early cytological evidence of xerophthalmia can be expected in children aged one year or more after a single oral dose of vitamin A given as 200 000 IU retinol palmitate (3-7). Preventive strategies are commonly based on the administration of this dose at 3 or 6-month intervals, whereas the same dose administered on two consecutive days and again after an interval of 4 weeks is widely used to treat xerophthalmia (8). There is general agreement, none the less, that the best preventive strategy is assurance of an adequate dietary intake of vitamin A. Not only does dietary sufficiency assure maintenance of adequate stores of vitamin A at all times, it also avoids any possibility of toxicity resulting from intermittent massive dosage which is claimed occasionally to result in signs of raised intracranial tension, including bulging of the fontanelle, restlessness, fever and vomiting (9).

The difficulty is that in areas where deficiency is most prevalent, foods rich in vitamin A — meat, dairy products and fish — are rare, while fruits and vegetables rich in carotenoids — the main source of vitamin A in most developing countries — are often not offered in sufficient quantities to children (10-12). In some of these areas, it seems, minor dietary adjustments would be sufficient to correct endemic vitamin A deficiency. In one study, Brazilian children who received a supplement of buriti palm fruit sufficient to provide 40% of their daily betacarotene requirement (13) were free of clinical and subclinical signs of vitamin A deficiency within 20 days (14).

Further replacement studies have recently been undertaken in Senegal, where vitamin A deficiency is endemic despite the availability for 5 months of the year of mangoes rich in vitamin A (15). These studies have shown that a massive dose of 1 200 000 IU of the provitamin, betacarotene, which is less efficiently absorbed from the intestine (16), is as effective as 200 000 IU vitamin A in reversing abnormal eye cytology in children (17, 18). However, whereas the same dose of vitamin A would be expected to produce a marked clinical response in virtually nearly all vitamin-deficient children in Asia, only about half the Senegalese children within each treatment group responded favourably (18). This, it is suggested, may reflect the co-existence of protein malnutrition and, in particular, deficiency of retinol-binding protein and transthyretin which are synthesized in the liver and are implicated in the transport of hepatic stores of vitamin A (19, 20).

Although, as yet, there has been no direct assessment of the impact of betacarotene replacement on survival of vitamin A deficient children, the indications are that it offers an effective alternative to vitamin A supplementation. Its ready availability in fruits and vegetables in some areas in which vitamin A deficiency is endemic, its apparent lack of toxicity (9, 21), and a claim that it enhances the immune response (22), identify it as a valuable and sustainable dietary means of maintaining adequate vitamin A stores.

### References

1. Carlier, C., Etchepare, M., Ceccon, J., Amédée-Maneme, O. Assessment of the vitamin A status of pre-school and school age Senegalese children during a cross-sectional study. *International Journal of Vitamin and Nutritional Research*, **62**: 209-215 (1992).
2. Carlier, C., Etchepare, M., Ceccon, J., Amédée-Maneme, O. Annual assessment of the vitamin A and nutritional status of children during two cross-sectional surveys. *International Journal of Vitamin and Nutritional Research*, **62**: 216-220 (1992).
3. Wittpenn, J., Scheffer, C., Tseng, G., Sommer, A. Detection of early xerophthalmia by impression cytology. *Archives of Ophthalmology*, **104**: 237-239 (1986).

4. Natadisastra, G., Wittpenn, J., West, K. et al. Impression cytology for detection of vitamin A deficiency. *Archives of Ophthalmology*, **105**: 1224-1228 (1987).
5. Natadisastra, G., Wittpenn, J., Muhilal, I. et al. Impression cytology: a practical index of vitamin A status. *American Journal of Clinical Nutrition*, **48**: 695-701 (1988).
6. Reddy, V., Rao, V., Reddy, A., Reddy, A.M. Conjunctival impression cytology for assessment of vitamin A status. *American Journal of Clinical Nutrition*, **50**: 814-817 (1989).
7. Semba, R., Wirasasmita, S., Natadisastra, G. et al. Response of Bitot's spots in preschool children to vitamin A treatment. *American Journal of Ophthalmology*, **110**: 416-420 (1990).
8. World Health Organization. *Control of vitamin A deficiency and xerophthalmia. Report of a joint WHO/UNICEF/USAID/Helen Keller International/IVAGC meeting*. WHO Technical Report Series, No. 672 (1982).
9. Hathcock, J., Hattan, D., Jenkins, M. et al. Evaluation of vitamin A toxicity. *American Journal of Clinical Nutrition*, **52**: 183-202 (1990).
10. Le François, P., Chevassus, A., Benefice, E. et al. Etat vitaminique A de la population dans trois pays de l'Afrique de l'Ouest. *Bulletin de l'Organisation de Coopération et de Coordination des Grandes Endémies*, **73**: 50-61 (1981).
11. Mele, L., West, K., Kusciono, J. et al. Nutritional and household risk factors for xerophthalmia in Aceh, Indonesia: a case-control study. *American Journal of Clinical Nutrition*, **53**: 1460-1465 (1991).
12. Nathanail, L., Powers, H. Vitamin A status of young Gambian children: biochemical evaluation and conjunctival impression cytology. *Annals of Tropical Paediatrics*, **12**: 67-73 (1992).
13. Olson, J. Recommended dietary intakes of vitamin A in humans. *American Journal of Clinical Nutrition*, **45**: 704-716 (1987).
14. Mariath, J., Lima, M., Santos, L. Vitamin A activity of buntii (*Mauritia vinifera marj*) and its effectiveness in the treatment and prevention of xerophthalmia. *American Journal of Clinical Nutrition*, **49**: 849-853 (1989).
15. Rankins, J., Hopkinson, S., Diop, M. Palatability and nutritional significance of solar dried mangoes for Senegal. *Ecology, Food and Nutrition*, **23**: 131-140 (1989).
16. Brubacher, G., Weiser, H. The vitamin A activity of beta carotene. *International Journal of Vitamin and Nutritional Research*, **55**: 5-15 (1985).
17. Carlier, C., Etchepare, M., Ceccon, J. et al. Efficacy of massive oral doses of retinyl palmitate and mangoes consumption to correct an existing vitamin A deficiency in Senegalese children. *British Journal of Nutrition*, **68**: 529-540 (1992).
18. Carlier, C., Etchepare, M., Périquet, B., Amédée-Maneme, O. A randomised controlled trial to test equivalence between retinyl palmitate and beta carotene for vitamin A deficiency. *British Medical Journal*, **307**: 1106-1110 (1993).
19. Smith, F., Goodman, D., Zaklama, M. et al. Serum vitamin A, retinol binding protein and prealbumin concentrations in protein-calorie malnutrition. I: a functional defect in hepatic retinol release. *American Journal of Clinical Nutrition*, **26**: 973-981 (1973).
20. Glover, J., Muhilal, I. Nutritional factors affecting the biosynthesis of retinol binding protein in the liver and its release into the plasma. *International Journal of Vitamin and Nutritional Research*, **46**: 239-243 (1976).
21. Goodman, D. Overview of current knowledge of metabolism of vitamin A and carotenoids. *Journal of the National Cancer Institute*, **73**: 1375-1379 (1984).
22. Bendich, A. Beta-carotene and the immune response. *Proceedings of the Nutritional Society*, **50**: 263-274 (1991).

## New perspectives on ACE inhibitors: diabetic nephropathy...

About one-third of patients with insulin-dependent diabetes mellitus ultimately develop nephropathy characterized by proteinuria and a decreasing glomerular filtration rate (1). Treatment of associated hypertension, particularly with beta-adrenoreceptor blocking agents, has been shown to reduce the rate of decline of renal function (2-6). However, most patients develop end-stage renal failure within 10 years and, in North America, these account for 1 in every 3 patients newly accepted for dialysis (7).

More recently, interest has centred upon angiotensin-converting enzyme (ACE) inhibitors. Early uncontrolled studies of captopril as an antihypertensive in diabetic patients with end-stage renal failure suggested that it slowed the decline of glomerular filtration rate in a manner dissociated from its systemic antihypertensive effect (8). Some 5 years ago this finding was confirmed in an animal model: enalapril was shown to slow progression of albuminuria and glomerulosclerosis in rats with

streptozocin-induced diabetes at a dose that had a marginal antihypertensive effect (9). The effect was attributed to a reduction in efferent glomerular arteriolar tone, which is mediated by angiotensin II, and a consequent fall in glomerular filtration pressure. Subsequently, it has been claimed that administration of ACE inhibitors (but not of other antihypertensive drugs) to diabetic rats over extended periods, results in some restoration of the functional and structural integrity of the glomerulus (10).

The general applicability of these findings to diabetic patients has been supported by results generated in various controlled studies (8, 11–13), and an analysis of some 100 published studies to show that, at comparable blood pressure levels, ACE inhibitors reduce urinary protein excretion to a greater extent than other antihypertensive agents (14). No evidence was obtained from the meta-analysis, however, to suggest that ACE inhibitors delay end-stage renal failure, as signalled by time of transfer to dialysis, renal transplant or death.

However, a recent placebo controlled study undertaken in the United States that examines the effect of captopril, 25 mg three times daily, has provided more encouraging results (15). Some 400 patients with diabetic nephropathy who were already receiving other antihypertensives, as necessary, to bring and maintain their blood pressures within a predetermined range, were randomly allocated to receive captopril or placebo and subsequently followed for periods ranging from 1.5 to nearly 5 years. Overall, the cumulative risk of a doubling of the serum creatinine concentration was reduced by 50% among patients receiving captopril, as was the combined risk of death, dialysis or transplantation. Within the captopril-treated group, the effect was most marked among patients with relatively severe degrees of renal impairment.

In contrast to results obtained in previous studies (3, 4, 11, 12), this trial provided no indication that use of other types of antihypertensive agent to control blood pressure in such patients also substantially reduces the rate of progression of diabetic nephropathy. The patients who did not receive captopril lost renal function at a rate higher than was expected from earlier reports.

In this trial, as has been noted in previous studies (16, 17), administration of captopril was associated with a marked decrease in proteinuria. The authors acknowledge that this may result simply from a

decrease in glomerular transcapillary pressure (9). There is now evidence that local tissue renin-angiotensin systems — determined by local renin gene expression (18) — are implicated in a broad range of cardiovascular regulatory mechanisms, including vascular growth, atheroma and cardiac hypertrophy (19). Given these findings, it could well be that ACE inhibitors reduce proteinuria by blocking one or more processes predisposing to glomerular scarring, including the trophic effect of angiotensin II on glomeruli (20–22), and the accumulation of mesangial matrix (23).

It is also possible that reduction in proteinuria itself tends to reduce glomerulosclerosis and preserve renal function (24). Both captopril and enalapril have been claimed, on the basis of small studies, to slow the development of nephropathy in normotensive insulin-dependent diabetic patients with microalbuminuria (25–27). This has very recently been confirmed in an important study undertaken in 12 hospital-based centres in Europe involving 92 patients with insulin-dependent diabetes and persistent microalbuminuria but without hypertension (28). The patients were randomly allocated to receive either captopril 50 mg twice daily or placebo and all were subsequently followed for a period of 2 years. During this period, albumin excretion rates rose among patients taking placebo from a geometric mean of 52 to 76 micrograms/min, while among patients taking captopril, they fell from 52 to 41 micrograms/min ( $P < 0.01$ ). Similarly, mean blood pressure remained unchanged in the placebo group, but fell significantly, by 3 to 7 mm Hg, in the captopril group. In all, 12 patients receiving placebo and 4 receiving captopril progressed to clinical proteinuria ( $P = 0.03$  by log-rank test), defined as an albumin excretion rate persistently greater than 200 micrograms/min and at least a 30% increase from baseline.

Although the effect on blood pressure was small in magnitude in this study and did not correlate with changes in albumin excretion rates throughout the group as a whole, blood pressure rose independently of treatment category in the patients who developed clinical proteinuria. It is thus impossible to dismiss change in blood pressure as an important factor in determining progression of nephropathy. None the less, evidence is accumulating that captopril may have a direct effect on the permeability of renal glomeruli. ACE inhibitors have been shown to reduce selectively the fractional clearance of high-molecular-weight dextrans (29) and this effect has been claimed to be independent of changes in systemic blood pressure (30).

There is no doubt, having regard to all the available evidence, that ACE inhibitors now hold a secure place in the management of established diabetic nephropathy (see also page 30). Whether advantage is gained by starting treatment earlier remains uncertain but distinctly possible. Longer observation periods are needed to determine whether slowing the progression of albuminuria while nephropathy remains subclinical also retards the onset of decline in glomerular filtration rates (27, 28). It is on patients with subclinical evidence of nephropathy that the focus of interest will now impinge.

#### References

1. Andersen, A., Christiansen, J., Andersen, J. et al. Diabetic nephropathy in type 1 (insulin dependent) diabetes: an epidemiological study. *Diabetologia*, **25**: 496-501 (1983).
2. Christlieb, A., Warram, J., Krolewski, A. et al. Hypertension: the major risk factor in juvenile-onset insulin-dependent diabetes. *Diabetes*, **30** (suppl 2): 90-96 (1981).
3. Mogensen, C. Long-term antihypertensive treatment inhibiting progression of diabetic nephropathy. *British Medical Journal*, **285**: 685-688 (1982).
4. Parving, H., Andersen, A., Smidt, U., Svendsen, P. Early aggressive antihypertensive treatment reduces rate of decline in kidney function in diabetic neuropathy. *Lancet*, **1**: 1175-1179 (1983).
5. Parving, H., Andersen, A., Hommel, E., Smidt, U. Effects of long-term antihypertensive treatment on kidney function in diabetic nephropathy. *Hypertension*, **7** (suppl 2): 114-117 (1985).
6. Parving, H., Hommel, E. Prognosis in diabetic retinopathy. *British Medical Journal*, **299**: 230-233 (1989).
7. Remuzzi, G., Ruggenenti, P. Slowing the progression of diabetic nephropathy. *New England Journal of Medicine*, **329**: 1496-1497 (1993).
8. Björck, S., Nyberg, G., Mulec, H. et al. Beneficial effects of angiotensin-converting enzyme inhibition on renal function in patients with diabetic nephropathy. *British Medical Journal*, **293**: 471-474 (1986).
9. Zatz, R., Dunn, B., Meyer, T. et al. Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *Journal of Clinical Investigation*, **77**: 1925-1930 (1986).
10. Andersen, S., Rennke, H., Garcia, D., Brenner, B. et al. Short and long-term effects of antihypertensive therapy in the diabetic rat. *Kidney International*, **36**: 526-536 (1989).
11. Hommel, E., Parving, H., Mathiesen, E. et al. Effect of captopril on kidney function in insulin dependent diabetic patients with nephropathy. *British Medical Journal*, **293**: 467-470 (1986).
12. Parving, H., Hommel, E., Smidt, U. Protection of kidney function and decrease in albuminuria by captopril in insulin dependent diabetics with nephropathy. *British Medical Journal*, **297**: 1086-1091 (1988).
13. Björck, S., Mulec, H., Johnsen, S. et al. Renal protective effect of enalapril in diabetic nephropathy. *British Medical Journal*, **304**: 339-343 (1992).
14. Kasiske, B., Kalil, R., Ma, J. et al. Effect of anti-hypertensive therapy on the kidney in patients with diabetes: a meta-regression analysis. *Annals of Internal Medicine*, **118**: 129-138 (1993).
15. Lewis, E., Hunsicker, L., Bain, R., Rohde, R. for the Collaborative Study Group. The effect of angiotensin-converting enzyme inhibition on diabetic nephropathy. *New England Journal of Medicine*, **329**: 1456-1462 (1993).
16. Taguma, Y., Kitamoto, Y., Futaki, G. et al. Effect of captopril on heavy proteinuria in azotemic diabetics. *New England Journal of Medicine*, **313**: 1617-1620 (1985).
17. Praga, M., Hernandez, E., Montoyo, C. et al. Long-term beneficial effects of angiotensin-converting enzyme inhibition in patients with nephrotic proteinuria. *American Journal of Kidney Disease*, **20**: 240-248 (1992).
18. Samani, N., Swales, J., Brammer, W. Expression of the renin gene in extrarenal tissues of the rat. *Biochemistry Journal*, **253**: 907-910 (1988).
19. Schelling, P., Fischer, H., Ganteen, D. Angiotensin and cell growth: a link to cardiovascular hypertrophy. *Journal of Hypertension*, **9**: 3-15 (1991).
20. Berk, B., Vekshtein, V., Gordon, H., Tsuda, T. Angiotensin II-stimulated protein synthesis in cultured vascular smooth muscle cells. *Hypertension*, **13**: 305-314 (1989).
21. Fogo, A., Ichikawa, I. Evidence for the central role of glomerular growth promoters in the development of sclerosis. *Seminars in Nephrology*, **9**: 329-342 (1989).
22. Fogo, A., Yoshida, Y., Yared, A., Ichikawa, I. Importance of angiogenic action of angiotensin II in the glomerular growth of maturing kidneys. *Kidney International*, **38**: 1068-1074 (1990).
23. Remuzzi, A., Puntorieri, S., Battaglia, C. et al. Angiotensin-converting enzyme inhibition ameliorates glomerular filtration of macromolecules and water and lessens glomerular injury in the rat. *Journal of Clinical Investigation*, **85**: 541-549 (1990).
24. Remuzzi, Bertani, T. Is glomerulosclerosis a consequence of altered glomerular permeability to macromolecules? *Kidney International*, **38**: 384-394 (1990).

25. Marre, M., Chatellier, G., Leblanc, H. et al. Prevention of diabetic nephropathy with enalapril in normotensive diabetics with microalbuminuria. *British Medical Journal*, **297**: 1092-1095 (1988).

26. Parving, H., Hommel, E., Damkjaer Nielsen, M., Giese, J. Effect of captopril on blood pressure and kidney function in normotensive insulin-dependent diabetics with nephropathy. *British Medical Journal*, **299**: 533-536 (1989).

27. Methiesen, E., Hommel, E., Giese, J., Parving, H. Efficacy of captopril in postponing nephropathy in normotensive insulin-dependent patients with microalbuminuria. *British Medical Journal*, **303**: 81-87 (1991).

28. Viberti, G., Mogensen, C., Groop, L., Pauls, J. for the European Microalbuminuria Captopril Study Group. Effect of captopril on progression to clinical proteinuria in patients with insulin-dependent diabetes mellitus and microalbuminuria. *Journal of the American Medical Association*, **271**: 275-279 (1994).

29. Morelli, E., Loon, N., Meyer, T. et al. Effects of angiotensin-converting enzyme inhibition in diabetic glomerulopathy. *Diabetes*, **39**: 76-82 (1990).

30. Pinto, J., Walker, J., Tumer, C. et al. Renal response to lowering of arterial pressure by angiotensin-converting enzyme inhibition or diuretic therapy in insulin-dependent diabetic patients with nephropathy. *Kidney International*, **37**: 516 (1990).

### ... and cardiac infarction

Survivors of cardiac infarction are at increased risk of death from subsequent cardiovascular events (1), and this risk rises with the severity of left ventricular dysfunction (2-4). Dysfunction correlates with various factors, including the age of the patient, the extent of coronary artery disease, propensity to arrhythmia and, most markedly, the degree of post-myocardial left ventricular enlargement (5, 6). In animal models, long-term administration of captopril following infarction has been shown both to attenuate this ventricular enlargement (7) and to prolong survival (8), and this protective effect has since been demonstrated in post-infarction patients, initially in relation to ventricular enlargement (9, 10) and, more recently, in relation to congestive cardiac failure and mortality (11).

Within 3 to 16 days after myocardial infarction, over 2000 patients with a left ventricular ejection fraction of 40% or less, but without symptoms of myocardial ischaemia, were randomly assigned, within a

double-blind study, to receive either captopril or placebo (11). Patients with signs of congestive cardiac failure were also excluded because of the established value of angiotensin converting enzyme (ACE) inhibitors in these patients (12-14). Other drugs, including thrombolytic agents, acetylsalicylic acid and beta-adrenoreceptor blocking agents were administered as appropriate. The dosage of captopril was increased progressively from 12.5 mg to a maximum of 50 mg three times daily, and follow-up was maintained for an average of 36 months after randomization. During this period, 25% of patients receiving placebo and 20% of those receiving captopril died. The reduction in risk of death from all cardiovascular causes was 37% (95% confidence interval, 20-50%;  $P < 0.001$ ). Somewhat smaller, yet still significant reductions in risk were recorded for congestive heart failure requiring admission to hospital, and for recurrent myocardial infarction. These differences were independent of the effects of other interventions, suggesting that treatment with captopril results in improvement supplementary to that provided by other therapy (15-17).

Evidence obtained from this study indicates that the beneficial effect of captopril could well reflect both attenuation of ventricular enlargement (18) and the inhibition of the renin-angiotensin system (19). Plasma renin activity was increased independently of ventricular size and function in a quarter of the patients studied, which suggests that ACE inhibitors may be of value in a broader population of post-infarction patients.

Indeed, the clinical possibilities extend yet further: deletion polymorphism in the ACE gene has been shown to be associated with an increased risk of coronary occlusion (20, 21), and individuals homozygous for this deletion are at considerably increased risk. This polymorphism is associated with raised circulating concentrations of ACE (22). This, in turn, is associated with an increased risk of myocardial infarction, at least among patients with hypertension (23). ACE inhibitors, it seems, may eventually find a place in the prevention as well as the treatment of cardiac infarction.

### References

1. Kannel, W., Sorlie, P., McNamara, P. Prognosis after initial myocardial infarction: the Framingham Study. *American Journal of Cardiology*, **44**: 53-59 (1979).
2. The Multicenter Postinfarction Research Group. Risk stratification and survival after myocardial infarction. *New England Journal of Medicine*, **309**: 331-336 (1983).

3. Stadius, M., Davis, K., Maynard, C. et al. Risk stratification for 1-year survival based on characteristics identified in the early hours of acute myocardial infarction: the Western Washington Intracoronary Streptokinase Trial. *Circulation*, **74**: 703-711 (1986).
4. White, H., Norris, R., Brown, M. et al. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation*, **76**: 44-51 (1987).
5. Jeremy, R., Allman, K., Bautovitch, G., Harris, P. Patterns of left ventricular dilation during the six months after myocardial infarction. *Journal of the American College of Cardiologists*, **13**: 304-310 (1989).
6. Gaudron, P., Eilles, C., Ertl, G., Kochsiek, K. Early remodelling of the left ventricle in patients with myocardial infarction. *European Heart Journal*, **11** (suppl B): 139-146 (1990).
7. Pfeffer, J., Pfeffer, M., Braunwald, E. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. *Circulation Research*, **57**: 84-95 (1985).
8. Pfeffer, M., Pfeffer, J., Steinberg, C., Finn, P. Survival after an experimental myocardial infarction: beneficial effects of long-term therapy with captopril. *Circulation*, **72**: 406-412 (1985).
9. Pfeffer, M., Lamas, G., Vaughan, D. et al. Effect of captopril on progressive ventricular dilatation after anterior myocardial infarction. *New England Journal of Medicine*, **319**: 80-86 (1988).
10. Sharpe, N., Murphy, J., Smith, H., Hannan, S. Treatment of patients with symptomatic left ventricular heart dysfunction after myocardial infarction. *Lancet*, **1**: 255-259 (1988).
11. Pfeffer, M., Braunwald, E., Moyé, L. et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. *New England Journal of Medicine*, **327**: 669-677 (1992).
12. Cohn, J., Archibald, D., Ziesche, S. et al. Effect of vasodilator therapy on mortality and chronic congestive heart failure: results of a Veterans Administration Cooperative study. *New England Journal of Medicine*, **314**: 1547-1552 (1986).
13. The CONSENSUS Trial Study Group. Effects of captopril on mortality in severe congestive heart failure: results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). *New England Journal of Medicine*, **316**: 1429-1435 (1987).
14. The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *New England Journal of Medicine*, **325**: 293-302 (1991).
15. Yusuf, S., Wittes, J., Friedman, L. Overview of results of randomized clinical trials in heart disease. 1. Treatments following myocardial infarction. *Journal of the American Medical Association*, **260**: 2088-2093 (1990).
16. Moss, A., Benhorin, J. Prognosis and management after a first myocardial infarction. *New England Journal of Medicine*, **322**: 743-753 (1990).
17. Gunnar, R., Passamani, E., Bourdillon, P. et al. Guidelines for the early management of patients with acute myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Assessment of Diagnostic and Therapeutic Cardiovascular Procedures. *Journal of the American College of Cardiology*, **16**: 249-292 (1990).
18. St John Sutton, M., Pfeffer, M., Plappert, T. et al. Survival and ventricular enlargement (SAVE) quantitative 2D echo substudy: effects of ACE inhibition therapy on ventricular enlargement. *Journal of the American College of Cardiology*, **19**: 205A (1992).
19. Rouleau, J., Moyé, L., de Champlain, J. et al. Activation of neurohumoral systems following acute myocardial infarction. *American Journal of Cardiology*, **68** (Suppl): 80D-86D (1991).
20. Cambien, F., Poirier, O., Lecerf, L. et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature*, **359**: 641-644 (1992).
21. Tiret, L., Kee, F., Poirier, O. et al. Deletion polymorphism in angiotensin-converting enzyme gene associated with parental history of myocardial infarction. *Lancet*, **341**: 991-992 (1993).
22. Rigat, B., Hubert, C., Alhenc-Gelas, F. et al. An insertion/deletion polymorphism in the angiotensin-1 converting enzyme gene accounting for half the variance of serum enzyme levels. *Journal of Clinical Investigation*, **86**: 1343-1346 (1990).
23. Alderman, M., Madhavan, S., Ooi, W. et al. Association of the renin-sodium profile with the risk of myocardial infarction in patients with hypertension. *New England Journal of Medicine*, **324**: 1098-1104 (1991).

## **Malaria vaccination: encouraging evidence of immunogenicity in African children**

One year ago, the medical scientific establishment was largely taken by surprise on learning that a chemically-synthesized subunit malaria vaccine developed in Colombia and targeted empirically against blood-stage antigens of *Plasmodium*

*falciparum* had been found to be partially protective against clinical episodes of the disease in both adults and children in an area of low malaria transmission on the Colombian coast (1).

It became critically important to extend clinical studies of the vaccine to areas of Africa where transmission of *P. falciparum* is intense, where the indigenous community has a higher degree of naturally-acquired immunity against the disease, and yet where most malaria-related deaths occur. A large-scale study of the clinical efficacy of the vaccine in such an environment is now underway in Tanzania (2). The definitive trial has been preceded by three small double-blind, randomized, placebo-controlled studies designed to assess the safety and immunogenicity of the preparation, firstly in non-immune expatriates living in Tanzania, secondly in semi-immune adults from the indigenous population, and thirdly in semi-immune children aged from 1 to 5 years (3). In each study the effects of three doses of the vaccine (administered at 0, 4, and 26 weeks and delivered as peptide adsorbed on aluminium hydroxide adjuvant) were investigated. Children received half the adult dose of 2 mg peptide, and placebo injections contained the vaccine adjuvant to which tetanus toxoid was added in the first dose only.

The results of the studies were notable in that each of 12 non-immune adult volunteers seroconverted after receiving two doses of the vaccine. This response was more encouraging than that previously obtained in Colombia, where conversion rates of 33-76% were recorded (1, 4), and in US soldiers, of whom only 67% developed an antibody response (5). The response, which was several orders higher than that of 2 subjects who received the placebo, and which was further boosted by the third dose, could not reasonably be attributed to a non-specific immune activation induced by the adjuvant, as has previously been suggested (6).

A limited assessment of acceptability in both adults and children provided no grounds for concern. No signs of hypersensitivity or anaphylaxis, no other generalized systemic reactions, and no severe local reactions were recorded. No recipient developed autoantibodies, while biochemical and haematological perturbations were slight and comparable among recipients of the vaccine and placebo. These results were particularly reassuring in that previous intense exposure to *P. falciparum* does not appear to enhance susceptibility of allergic reactions to SPf66.

Two-thirds of 50 young semi-immune children were shown to have anti-SPf66 titres of at least 1:100 before vaccination. After 3 doses of vaccine, the antibody titres increased substantially and consistently (mean increase: 60-fold; 95% confidence interval: 37-100). It is important, however, not to regard evidence of immunogenicity as necessarily indicative of clinical efficacy, since the trials in Colombia failed to establish a correlation between anti-SPf66 antibody titres and clinical protection (1, 7). None the less, the results must be regarded as providing a highly encouraging basis for undertaking a phase III field trial, which is already in progress, to estimate the level of protective efficacy of SPf66 against clinical malaria among young African children in an area of high endemicity.

#### References

1. Valero, M., Amador, L., Galindo, C. et al. Vaccination with SPf66, a chemically synthesised vaccine, against *Plasmodium falciparum* malaria in Columbia. *Lancet*, **341**: 705-710 (1993).
2. Alonso, P., Tanner, M., Smith, T. et al. A trial of SPf66, a synthetic malaria vaccine, in Kilombero (Tanzania). Rationale and design. *Vaccine*, **12**: 181 (1994).
3. Teuscher, T., Armstrong Schellenberg, J., Bastos de Azevedo, I. et al. SPf66, a chemically synthesized subunit malaria vaccine, is safe and immunogenic in Tanzanians exposed to intense malaria transmission. *Vaccine*, **12**: 328-336 (1994).
4. Amador, R., Moreno, A., Murillo, L. et al. Safety and immunogenicity of the synthetic malaria vaccine SPf66 in a large field trial. *Journal of Infectious Diseases*, **166**: 139-144 (1992).
5. Gordon, D., Sadoff, J., Heppner, D. et al. Safety and immunogenicity of alum-adjuvanted SPf66 produced in the United States under CGMP standards. *American Journal of Tropical Medicine and Hygiene*, **49** (suppl 3): 319 (1993).
6. Millet, P., Campbell, G., Sulzer, A. et al. Immunogenicity of the *Plasmodium falciparum* asexual blood stage synthetic peptide vaccine SPf66. *American Journal of Tropical Medicine and Hygiene*, **48**: 424-431 (1993).
7. Salcedo, M., Barretto, L., Rojas, M. et al. Studies on the humoral immune response to a synthetic vaccine against *Plasmodium falciparum* malaria. *Clinical and Experimental Immunology*, **84**: 122-128 (1991).

#### ***Pneumocystis* prophylaxis: what is achieved?**

Before effective prophylaxis was widely available, *Pneumocystis carinii* pneumonia was the most

frequent manifestation of the acquired immunodeficiency syndrome (AIDS) among patients in developed countries. Within the United States, it was estimated to occur in 3 of every 4 patients with HIV infection (1, 2). Its frequency declined considerably from the late 1980s onwards as a result of increasing preventive use of aerosolized pentamidine, trimethoprim-sulfamethoxazole and dapsone (3, 4).

Much evidence is available that regimens based upon each of these drugs are highly effective in preventing and treating *P. carinii* pneumonia (5-8). However, none of these drugs has demonstrable influence, either on other AIDS-related illnesses — with the lone exception of toxoplasmosis (7-9) — or on the progression of the underlying HIV infection. It is consequently important to measure their effect not only in suppressing pneumocystis pneumonia, but in changing the course of the illness. Do patients benefitting from prophylaxis live longer? And, if so, what is the quality of their life? To what other AIDS-related illnesses do they become particularly vulnerable? And might these be more effectively treated or prevented?

Answers to some of these questions have been provided by prospectively following the course of AIDS in 844 HIV-positive homosexual men presenting in centres across the United States (10, 11). In all, 255 of these men reported using zidovudine, and 138 reported receiving prophylaxis against *P. carinii* pneumonia before the diagnosis of AIDS. Only one man reported receiving *P. carinii* prophylaxis, but not zidovudine.

Among the 138 subjects who received primary pneumocystis prophylaxis the mean CD4+ cell count had dropped to 48 (95% confidence interval 40-57) within 6 months of the first clinical evidence of AIDS. Among the remaining subjects AIDS supervened when the preceding CD4+ cell count was substantially higher (geometric mean: 118/mm<sup>3</sup>; 95% confidence interval 107-130). These results, it is estimated, indicate that patients receiving prophylactic therapy benefit from an additional 6 to 12 months of life free from AIDS-related illness.

*P. carinii* pneumonia occurred in some 15% of men who had received prophylactic treatment, and it was the first AIDS-related illness reported in almost half the 706 men who did not receive specific protection against the disease. Among the latter, many did not receive secondary prophylaxis. These have all subsequently died and many did not report any other type of AIDS-related illness during life.

Among patients who received prophylaxis against infection by *P. carinii*, four diseases — *Mycobacterium avium* complex disease, wasting syndrome, cytomegalo-virus disease, and oesophageal candidiasis — were significantly more common as initial manifestations of AIDS than *P. carinii* pneumonia. Collectively, however, these accounted for less than half of all initial AIDS-related illnesses in these patients. No illness was responsible for more than 20% of diagnoses of AIDS, and each of 6 illnesses occurred in at least 18% of the subjects at some stage in their disease.

The variety and dissimilarity of these diseases mitigates against the likelihood of developing additional prophylactic regimens that are broadly beneficial to the community of patients described in this study. Even therapeutic management of other supervening diseases apparently offers little prospect of substantial extension of life: the geometric mean of the last CD4+ cell count recorded before death among 50 patients who received primary *P. carinii* prophylaxis was only 15/mm<sup>3</sup>. At this degree of immunodeficiency, therapeutic intervention can do little to relieve the risk of imminent death.

As the authors of this study emphasize, this cohort is not representative of patients elsewhere and, least of all, where *M. tuberculosis* is highly endemic. In such settings, other strategies are evidently needed, and analogous prospective surveillance needs to be undertaken to provide firm data on the influence of prophylactic and therapeutic interventions on the clinical course of HIV infection.

#### References

1. Pneumocystis pneumonia — Los Angeles. *Morbidity and Mortality Weekly Report*, **30**: 250-252 (1981).
2. Hay, J., Osmond, D., Jacobson, M. Projecting the medical costs of AIDS and ARC in the United States. *Journal of the Acquired Immune Deficiency Syndrome*, **1**: 466-485 (1988).
3. Graham, N., Zeger, S., Park, L. et al. Effect of zidovudine and *Pneumocystis carinii* pneumonia prophylaxis on progression of HIV-1 infection to AIDS: the Multicenter AIDS Cohort Study. *Lancet*, **338**: 265-269 (1991).
4. Muñoz, A., Schragger, L., Bacellar, H. et al. Trends in the incidence of outcomes defining the acquired immunodeficiency syndrome (AIDS) in the Multicenter AIDS Cohort Study: 1985-1991. *American Journal of Epidemiology*, **137**: 423-438 (1993).

5. Leoung, G., Feigal, D., Montgomery, A. et al. Aerosolized pentamidine for prophylaxis against *Pneumocystis carinii* pneumonia: the San Francisco Community Prophylaxis Trial. *New England Journal of Medicine*, **323**: 769-775 (1990).
6. Hirschel, B., Lazzarin, A., Chopard, P. et al. A controlled study of inhaled pentamidine for primary prevention of *Pneumocystis carinii* pneumonia. *New England Journal of Medicine*, **324**: 1079-1083 (1991).
7. Schneider, M., Hoepelman, A., Eeftinck Schattenkerk, J. et al. A controlled trial of aerosolized pentamidine or trimethoprim-sulfamethoxazole as primary prophylaxis against *Pneumocystis carinii* pneumonia in patients with human immunodeficiency virus infection. *New England Journal of Medicine*, **327**: 1836-1841 (1992).
8. Girard, P., Landman, R., Gaudebout, C. et al. Dapsone-pyrimethamine compared with aerosolized pentamidine as primary prophylaxis against *Pneumocystis carinii* pneumonia and toxoplasmosis in HIV infection. *New England Journal of Medicine*, **328**: 1514-1520 (1993).
9. Carr, A., Tindall, B., Brew, B., et al. Low dose trimethoprim-sulfamethoxazole prophylaxis for toxoplasmic encephalitis in patients with AIDS. *Annals of Internal Medicine*, **117**: 106-111 (1993).
10. Kaslow, R., Ostrow, D., Detels, R. et al. The Multi-center AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. *American Journal of Epidemiology*, **126**: 310-318 (1987).
11. Hoover, D., Saah, A., Bacellar, H., for the Multicenter AIDS Cohort Study. Clinical manifestations of AIDS in the era of pneumocystis prophylaxis. *New England Journal of Medicine*, **329**: 1922-1926 (1994).

## Spermicides: a viable protection against STD?

The possibility of developing intravaginal preparations possessing both spermicidal and microbicidal properties has attracted attention for more than 20 years. With the emergence of human immunodeficiency virus (HIV) infection, it is a possibility that has been accorded greater impetus (1). Nonoxynol-9 and other non-ionic surfactant spermicides have long been recognized to have significant *in vitro* microbicidal action against *Treponema pallidum*, *Neisseria gonorrhoeae*, *Candida albicans*, and *Trichomonas vaginalis* (2). Nonoxynol-9 has since been claimed, additionally, to be rapidly active at low doses *in vitro* against *Chlamydia trachomatis* (3), herpes simplex viruses (4), and even against HIV (5). Preliminary clinical studies have suggested that this activity is preserved to a demonstrable

degree *in vivo*, at least in relation to the gonococcus, chlamydia, and candida (6-9).

In the most extensive of these studies, a placebo-controlled randomized comparison among women prostitutes in Bangkok has indicated that regular use of nonoxynol before coitus can reduce rates of gonococcal and chlamydial cervicitis by some 40% (9). The degree of protection was probably underestimated since, for ethical reasons, all women enrolled in the study were also supplied with condoms. It has been suggested on the basis of this and other studies that spermicides as well as condoms should be supplied routinely to women at high risk (10).

The method may have its disadvantages, however. High cumulative doses of nonoxynol-9 can cause irritation and inflammatory lesions of the vaginal epithelium. They may also promote overgrowth of pathogenic organisms by a microbicidal effect on protective *Lactobacilli* present in the normal vaginal flora (11, 12). Cumulation was averted in the Bangkok study because the women washed and doused between partners. In contrast, pessaries containing 150 mg nonoxynol-9 inserted 4 times at hourly intervals on 14 consecutive days has been reported to produce epithelial disruption of the cervix and vagina in six of 14 women volunteers (13). Such lesions may well increase the risk of HIV infection both by facilitating entry of the retrovirus and by attracting increased numbers of T-lymphocytes, including the targeted CD4+ cells, into the mucosa (11, 14).

Non-ionic surfactants also share the disadvantage that they do not penetrate cervical mucus (15). This may explain some apparent contraceptive failures associated with these products, and it suggests that they provide little or no protection against HIV contained in ejaculate deposited directly into the cervical os.

Other antiseptic compounds have been developed that hold advantage in these respects. Chlorhexidine, which is positively charged, is claimed to have a spermicidal effect, to be miscible with cervical mucus, and to be well tolerated in the vagina (15). Confirmation of these properties would seem to establish it as a prime candidate for further investigation as a multipurpose spermicide.

### References

1. Editorial. Multipurpose spermicides. *Lancet*, **340**: 211-213 (1992).

2. Singh, B., Cutler, J., Ujidian, H. Studies on the development of a vaginal preparation providing both prophylaxis against venereal disease, other genital infections and contraception. *British Journal of Venereal Disease*, **48**: 52-62 (1972).
3. Benes, S., McCormack, W. Inhibition of growth of *Chlamydia trachomatis* by nonoxynol-9 *in vitro*. *Antimicrobial Agents and Chemotherapy*, **27**: 724 (1985).
4. Asculai, S., Weis, M., Rancourt, M., Kupferberg, A. Inactivation of herpes simplex virus by nonionic surfactants. *Antimicrobial Agents and Chemotherapy*, **13**: 686 (1978).
5. Hicks, D., Martin, L., Getchell, P. Inactivation of HTLV-III/LAV-infected cultures in normal human lymphocytes by nonoxynol-9 *in vitro*. *Lancet*, **2**: 1422-1423 (1985).
6. Cutler, J., Singh, B., Carpenter, U. et al. Vaginal contraceptives as prophylaxis against gonorrhoea and other sexually transmitted diseases. *Advances in Planned Parenthood*, **12**: 45 (1977).
7. Rendon, A., Covarubias, J., McCamey, K. et al. A controlled comparative study of phenylmercuric acetate, nonoxynol-9 and placebo vaginal suppositories as prophylactic agents against gonorrhoea. *Current Therapeutic Research*, **27**: 780-783 (1980).
8. Rosenberg, M., Rojanapithayakorn, W., Feldblum, P., Higgins, J. Effect of the contraceptive sponge on chlamydial infection, gonorrhoea, and candidiasis: a comparative trial. *Journal of the American Medical Association*, **257**: 2308-2312 (1987).
9. Niruthisard, S., Roddy, R., Chuitivongse, S. Use of nonoxynol-9 and reduction in the rate of gonococcal and chlamydial infections. *Lancet*, **339**: 1371-1375 (1991).
10. Alexander, N., Gabelnick, H., Spieler, J. eds. *Heterosexual transmission of AIDS: Second Contraceptive Research and Development (CONRAD) Program*. Proceedings of an International Workshop, February 1989, pp. 69-79. Wiley-Liss, New York, 1990.
11. Kirkman, R., Chantler, E. Contraception and the prevention of STD. *British Medical Bulletin*, **49**: 171-181 (1993).
12. Klebanoff, S., Hillier, S., Eschenbach, D., Waltersdorff, A. Control of the microbial flora of the vagina by H<sub>2</sub>O<sub>2</sub>-generating lactobacilli. *Journal of Infectious Diseases*, **164**: 94-100 (1991).
13. Niruthisard, S., Roddy, R., Chuitivongse, S. The effects of frequent nonoxynol-9 on the vaginal and cervical mucosa. *Sexually Transmitted Diseases*, **18**: 176-179 (1991).
14. Women, contraception and HIV. *Drug and Therapeutics Bulletin*, **31**: 97-98 (1993).
15. Chantler, E. Vaginal spermicides: some current concerns. *British Journal of Family Planning*, **17**: 118-119 (1992).

## Tamoxifen: a role in the prevention of breast cancer?

From data generated in the United States, it is estimated that 2 of every 5 new cancers diagnosed in women is hormonally influenced (1). The use of combined oral contraceptives offers women considerable protection against two such cancers — ovarian and endometrial (2, 3) — but these preparations are not protective against breast cancer. Indeed they may slightly increase the risk of diagnosis of the disease among pre-menopausal women (4, 5) and particularly among those who relied upon them before the birth of their first child, and they may be associated with a substantially greater risk when employed in hormone replacement therapy in post-menopausal women (6).

These epidemiological findings accord with experimental evidence that cell proliferation within the lobular unit of the terminal duct, from which most breast cancers arise, is relatively low during the follicular (estrogen) phase of the cycle and accelerates markedly during the luteal (estrogen plus progesterone) phase (5). They also accord with the finding that specific estrogen-receptor proteins, that mediate cell division by acting as transcription modulators, are found in duct cells in some 70% of breast tumours, and that the presence of these receptors is an important determinant of the response of tumours to endocrine therapy (7, 8).

The possibility consequently exists that long-term administration of an anti-estrogenic compound may exert a clinically important protective effect. In fact, tamoxifen, a synthetic compound with anti-estrogenic effects — but partial estrogenic activity in some tissues (9, 10) — has already been widely used in the treatment of breast cancer for more than 20 years (see also pp. 22-23). An early overview of its use within 28 trials as an adjuvant to surgical resection of early-stage breast cancer, has indicated an overall advantage in survival among treated patients in excess of 15% within two years of surgery, when compared with placebo or no adjuvant treatment (11). The advantage was most apparent among post-menopausal women with estrogen receptor-positive tumours, but in some trials advantage has also been claimed among women with no demonstrable estrogen receptors.

A subsequent overview has not only confirmed these initial findings, it has demonstrated that immediate adjuvant treatment with tamoxifen decreases the incidence of second primary breast cancers by some 40% (12). Given this demonstration of an apparent suppressive action, and evidence that less than 2% of treated women withdraw from therapy, interest has been aroused in using tamoxifen in the primary prevention of breast cancer among healthy women at increased risk of the disease (13, 14). However, the need for maintaining treatment for periods of 5 years or more (15–17), and the need to examine the effects of tamoxifen on other estrogen-responsive physiological processes has demanded a highly cautious approach to the enrolment of subjects in these trials (18, 19).

The initial concern was that, by exerting an anti-estrogen effect, tamoxifen would promote development of atherosclerosis and osteoporosis. This was a strong supposition since hot flushes and other menopausal symptoms are reported by up to 40% of patients treated with tamoxifen (7, 20–22). Instead, its influence on lipid metabolism in postmenopausal women is that of an estrogen agonist: this is beneficial in that low-density lipoprotein cholesterol is substantially lowered and sex-hormone binding globulin is raised (23–27). As yet, however, only one trial has generated data indicating that tamoxifen, like estrogen replacement therapy, may reduce mortality due to myocardial infarction (28). Similarly, tamoxifen appears to exert an estrogenic effect by reducing postmenopausal osteoporosis, at least in the trabecular bone of the lumbar spine (29–30). However, little is known about the effect of tamoxifen on bone metabolism in younger women. Since the possibility of an anti-estrogenic effect cannot be excluded (32), relevant data need to be collected with some urgency.

Other estrogenic effects of tamoxifen clearly operate to the disadvantage of the patient. Thromboembolic disease has been reported to occur in 1–2% of women treated for prolonged periods (33), and haematological disturbances may underlie a number of cases of severe retinopathy that have been reported (34–36). At recommended dosages tamoxifen induces nuclear cellular hyperplasia (37) and benign proliferative lesions within the endometrium (38), while *in vitro* it has been shown to stimulate some endometrial carcinoma cell lines (39), and to inhibit some others (40, 41). A recent case-control study undertaken in the Netherlands, which involved analysis of information on

some 100 patients who were diagnosed to have endometrial cancer at least three months after a diagnosis of primary breast cancer, has shown a significant trend of increasing risk of endometrial cancer with duration of tamoxifen use ( $P=0.049$ ) and also with cumulative use ( $P=0.046$ ) (42). Women who had used tamoxifen for more than 2 years had a 2.3 (95% confidence interval: 0.9–5.9) greater risk than non-users of endometrial cancer. Only in one prospective trial has an increased risk of endometrial cancer been recorded among women receiving tamoxifen (43), but at least three reports suggest that when such cancers do occur, they tend to be of high-grade malignancy (44–46).

There is no direct evidence that tamoxifen is associated with other cancers. However, because the hormonal effects of tamoxifen may well differ in pre- and postmenopausal women (47), it may not be valid to extrapolate data obtained in older women to younger age groups. In premenopausal women tamoxifen significantly increases secretion of estradiol and progesterone. Because tamoxifen is structurally similar to clomifene (48), this effect may reflect maturation of multiple ovarian follicles. If so, it is possible that excessive stimulation of ovulation may in the long term increase the risk of ovarian cancer (49, 50).

Concern about hepatocellular carcinoma has also recently arisen (51). Like some other estrogenic compounds, tamoxifen induces tumours in the rat liver (52–54). Hepatocellular carcinoma occurs rarely in women, and this risk has become accepted in the use of oral contraceptives. None the less, at least two such tumours have been reported in women receiving tamoxifen (19), and it has been emphasized that a related anti-estrogen, toremifene, does not appear to induce liver tumours in rodents (54).

None of these concerns weighs heavily in the balance when tamoxifen is used in older women who already have breast cancer. On the basis of some 4.5 million women-years of experience already obtained, the general consensus is that routine use of tamoxifen in the treatment of the established disease would significantly extend many lives (55, 56). It is evident that any exploration of the use of tamoxifen in the primary prevention of breast cancer will be problematic. None the less, three large multicentre studies involving many thousands of subjects have already been organized to this end in Italy, the United Kingdom and the United States, respectively (18,

19). The objective is not simply to determine whether tamoxifen can prevent breast cancer, but to assess its effect on cardiovascular disease, on the incidence of bone fractures, and on the nature and incidence of any associated adverse effects in young women at high risk of breast cancer.

The justification for this intervention, which remains controversial (54, 57), is twofold. Firstly, many women are at highly increased risk of breast cancer as a result of family history, reproductive factors, or presence of benign breast disease (14, 58). For instance, a 40-year-old woman who has undergone multiple breast biopsies for benign disease and who has two first-degree relatives with a history of breast cancer faces a 1:5 risk of developing the disease before she is 50. At present she is entirely reliant upon early diagnosis to decrease her chances of dying from the condition. Secondly, the available data indicate that tamoxifen is as effective in women under 50 as in older women in protecting against recurrence of disease (59).

Meticulous sequential monitoring within these trials for unwanted effects, and particularly for premonitory signs of endometrial carcinoma, is clearly mandatory. Indeed, there is already speculation that some investigators are modifying their policies for recruitment of subjects into the trials as a result of substantiated concerns (60). Of comparable consequence, however, is a recent claim that commonly-encountered acquired resistance to tamoxifen results from reduced concentration of the drug within tumour cells (61). As yet it remains uncertain whether this results from impaired uptake, increased metabolism or active extrusion. To place preventive therapy on a sound footing, there must be reasonable certainty that prolonged pretreatment with tamoxifen does not ultimately impair its protective potential.

#### References

- Henderson, B., Ross, R., Pike, M. Toward the primary prevention of cancer. *Science*, **254**: 1131-1138 (1991).
- WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Epithelial ovarian cancer and combined oral contraceptives. *International Journal of Epidemiology*, **18**: 538-545 (1989).
- Grimes, D. The safety of oral contraceptives: epidemiological insights from the first thirty years. *American Journal of Obstetrics and Gynecology*, **166**: 1950-1954 (1992).
- WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Breast cancer and combined oral contraceptives: results from a multinational study. *British Journal of Cancer*, **61**: 110-119 (1990).
- Henderson, B., Ross, R., Pike, M. Hormonal chemoprevention of cancer in women. *Science*, **259**: 633-638 (1993).
- Persson, I., Yuen, J., Bergkvist, L. et al. Combined oestrogen-progestogen replacement and breast cancer risk. *Lancet*, **340**: 1044 (1992).
- Johnston, S., Dowsett, M., Smith, I. et al. Towards a molecular basis for tamoxifen resistance in breast cancer. *Annals of Oncology*, **3**: 503-511 (1992).
- Robertson, J., Bates, K., Pearson, D. et al. Comparison of two oestrogen receptor assays in the prediction of the clinical course of patients with advanced breast cancer. *British Journal of Cancer*, **65**: 727-730 (1992).
- Buckley, M., Goa, K. Tamoxifen: a reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic use. *Drugs*, **37**: 451-490 (1989).
- Jordan V., Murphy, C. Endocrine pharmacology of antiestrogens as antitumor agents. *Endocrine Reviews*, **11**: 578-610 (1990).
- Early Breast Cancer Trialists Collaborative group. Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. An overview of 61 randomized trials among 28,896 women. *New England Journal of Medicine*, **319**: 1681-1692 (1988).
- Early Breast Cancer Trialists Collaborative group. Systemic treatment of early breast cancer by hormonal, cytotoxic and immune therapy: 133 randomized trials involving 33 000 recoveries and 24 000 deaths among 75 000 women. *Lancet*, **339**: 1-15 (1992).
- Powels, T., Hardy, J., Ashley, S. et al. A pilot study to evaluate the acute toxicity and feasibility of tamoxifen for the prevention of breast cancer. *British Journal of Cancer*, **60**: 126-131 (1989).
- Nayfield, S., Karp, J., Ford, L. et al. Potential role of tamoxifen in the prevention of breast cancer. *Journal of the National Cancer Institute*, **83**: 1450-1459 (1991).
- Fisher, B., Brown, A., Wolmark, N. et al. Prolonging tamoxifen therapy for primary breast cancer: findings from the National Surgical Adjuvant Breast and Bowel Project clinical trial. *Annals of Internal Medicine*, **106**: 649-654 (1987).
- Breast Cancer Trials Committee, Scottish Cancer Trials Office (MRC). Adjuvant tamoxifen in the management of operable breast cancer: the Scottish Trial. *Lancet*, **2**: 171-175 (1987).
- Falkson, H., Gray, R., Wolberg, W., et al. Adjuvant trial of twelve cycles of CMFPT followed by observation of continuous tamoxifen versus four cycles of CMFPT in postmenopausal women with breast cancer: an Eastern Cooperative Oncology Group phase III study. *Journal of Clinical Oncology*, **8**: 599-607 (1990). [Erratum, *ibid.* **8**: 1603 (1990)].

18. Davidson, N. Tamoxifen: panacea or Pandora's box? *New England Journal of Medicine*, **326**: 885-886 (1992).
19. Jordan, V. How safe is tamoxifen? Only large randomised controlled trials can decide. *British Medical Journal*, **307**: 1371-1372 (1993).
20. Love, R. Tamoxifen therapy in primary breast cancer: biology, efficacy and side effects. *Journal of Clinical Oncology*, **7**: 803-815 (1989).
21. Rostom, A., Gershuny, A. Adjuvant treatment in breast cancer. *Lancet*, **339**: 424 (1992).
22. Kaplan, H. Adjuvant therapy in breast cancer. *Lancet*, **339**: 424-425 (1992).
23. Rossner, S., Wallgren, A. Serum lipoproteins and proteins after breast cancer surgery and effects of tamoxifen. *Atherosclerosis*, **52**: 339-346 (1984).
24. Bagdade, J., Wolter, J., Subbiah, P. et al. Effects of tamoxifen treatment on plasma lipids and lipoprotein lipid composition. *Journal of Clinical Endocrinology and Metabolism*, **70**: 1132-1135 (1990).
25. Bruning, P., Bonfrer, J., Hart, A. et al. Tamoxifen, serum lipoproteins and cardiovascular risk. *British Journal of Cancer*, **58**: 497-499 (1988).
26. Love, R., Newcomb, P., Weibe, D. et al. Effects of tamoxifen therapy on lipid and lipoprotein levels in postmenopausal patients with node-negative breast cancer. *Journal of the National Cancer Institute*, **82**: 1327-1332 (1990).
27. Dewar, J., Horobin, J., Preece, P. et al. Long-term effects of tamoxifen on blood lipid values in breast cancer. *British Medical Journal*, **305**: 225-226 (1992).
28. McDonald, C., Stewart, H. Fatal myocardial infarction in the Scottish adjuvant tamoxifen trial. *British Medical Journal*, **303**: 435-437 (1991).
29. Love, R., Mazess, R., Barden, H. et al. Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. *New England Journal of Medicine*, **326**: 852-856 (1992).
30. Turken, S., Siris, E., Seldin, D. et al. Effects of tamoxifen on spinal bone density in women with breast cancer. *Journal of the National Cancer Institute*, **81**: 1086-1088 (1989).
31. Ryan, W., Wolter, J., Bagdade, J. Apparent beneficial effects of tamoxifen on bone mineral content in patients with breast cancer: preliminary study. *Osteoporosis International*, **2**: 39-41 (1991).
32. Ursin, G., Spicer, D., Pike, M. Tamoxifen and prevention. *Lancet*, **341**: 693 (1993).
33. Saphner, T., Tormey, D., Gray, R. Venous and arterial thrombosis in patients who received adjuvant therapy for breast cancer. *Journal of Clinical Oncology*, **9**: 286-294 (1991).
34. Griffiths, M. Tamoxifen retinopathy at low dosage. *American Journal of Ophthalmology*, **104**: 185-186 (1987).
35. Gerner, E. Ocular toxicity of tamoxifen. *American Journal of Ophthalmology*, **21**: 420-423 (1989).
36. Bentley, C., Davies, G., Aclimandos, W. Tamoxifen retinopathy: a rare but serious complication. *British Medical Journal*, **304**: 495-496 (1992).
37. Rayter, Z., Shepherd, J., Gazet, J-C. et al. Tamoxifen and endometrial lesions. *Lancet*, **342**: 1124 (1993).
38. Neven, P., De Muylder, X., Van Belle, Y. et al. Hysteroscopic follow-up during tamoxifen treatment. *European Journal of Obstetrics, Gynaecology and Reproductive Biology*, **35**: 235-238 (1990).
39. Anzai, Y., Holinka, C., Kuramoto, H., Gurdipe, E. Stimulatory effect of 4-hydroxytamoxifen on proliferation of human endometrial adenocarcinoma cells (Ishikawa line). *Cancer Research*, **49**: 2362-2365 (1989).
40. Greenman, S., Roberts, J., England, B. et al. *In vitro* growth regulation of endometrial carcinoma cells by tamoxifen and medroxyprogesterone acetate. *Gynecological Oncology*, **30**: 239-250 (1988).
41. Van Leeuwen, J., Benraadt, J., Coebergh, J. et al. Risk of endometrial cancer after tamoxifen treatment of breast cancer. *Lancet*, **343**: 448-452 (1994).
42. Van Leeuwen, J., Benraadt, J., Coebergh, J. et al. Risk of endometrial cancer after tamoxifen treatment of breast cancer. *Lancet*, **343**: 448-452 (1994).
43. Fomander, T., Rutqvist, L., Cedermark, B. et al. Adjuvant tamoxifen in early breast cancer: occurrence of new primary cancers. *Lancet*, **1**: 117-120 (1989).
44. Anderson, M., Storm, H., Mouridsen, H. Carcinogenic effects of adjuvant tamoxifen treatment and radiotherapy for early breast cancer. *Acta Oncologica*, **31**: 259-263 (1992).
45. Magriples, U., Naftolin, F., Schwartz, P. et al. High grade endometrial carcinoma in tamoxifen breast cancer patients. *Journal of Clinical Oncology*, **11**: 485-490 (1993).
46. McAuliffe, F., Foley, M. Tamoxifen and endometrial lesions. *Lancet*, **342**: 1124 (1993).
47. Spicer, D., Pike, M., Henderson, B. Ovarian cancer and long-term tamoxifen in premenopausal women. *Lancet*, **337**: 1414 (1991).
48. Messinis, I., Nillius, S. Comparison between tamoxifen and clomifene for induction of ovulation. *Acta Obstetrica Gynecologica Scandinavica*, **61**: 377-379 (1982).
49. Groom, G., Griffiths, K. Effect of the anti-estrogen tamoxifen on plasma levels of luteinizing hormone, follicle-stimulating hormone, prolactin, estradiol and progesterone in normal pre-menopausal women. *Journal of Endocrinology*, **70**: 421-428 (1976).

50. Sherman, B., Chapler, F., Crickard, K., Wycoff, D. Endocrine consequences of antiestrogen therapy with tamoxifen in postmenopausal women. *Journal of Clinical Investigation*, **64**: 398-404 (1979).
51. Williams, G., Iatropoulos, M., Hard, G. Long-term prophylactic use of tamoxifen: Is it safe? *European Journal of Cancer Prevention*, **1**: 386-387 (1992).
52. Greaves, P., Goonetilleke, Nunn, G. et al. Two year carcinogenicity study of tamoxifen in Alderley Park Wistar-derived rats. *Cancer Research*, **53**: 919-924 (1993).
53. Williams, G., Iatropoulos, M., Djordjevic, M., Kaltenberg, O. The triphenylethylene drug tamoxifen is a strong liver carcinogen in the rat. *Carcinogenesis*, **14**: 315-317 (1993).
54. Hard, G., Williams, G., Iatropoulos, M. Tamoxifen and liver cancer. *Lancet*, **342**: 444-445 (1993).
55. Jordan, V. Can all postmenopausal women with a diagnosis of breast cancer receive benefit from tamoxifen? *Reviews in Endocrine Related Cancer*, **43**: 23-31 (1993).
56. Plowman, P., Allum, W. Adjuvant treatment in breast cancer. *Lancet*, **339**: 423 (1992).
57. Bluming, A. Tamoxifen and prevention, *Lancet*, **341**: 693-694 (1993).
58. Gail, M., Brinton, L., Byar, D. et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *Journal of the National Cancer Institute*, **81**: 1879-1886 (1989).
59. Powles, T., Smith, T. Adjuvant treatment in breast cancer. *Lancet*, **339**: 423 (1992).
60. Seachrist, L. Restating the risks of tamoxifen. *Science*, **263**: 910-911 (1994).
61. Johnston, S., Haynes, B., Smith, I. et al. Acquired tamoxifen resistance in human breast cancer and reduced intra-tumoral drug concentration. *Lancet*, **342**: 1521-1522 (1993).

### Vitamin K and cancer: reassuring evidence

Vitamin K, a fat-soluble naphthoquinone, is an essential component of the blood clotting mechanism. Through a process of carboxylation, it converts prothrombin into active forms that bind calcium and proteins. During the first week of life, however, a physiologically-determined deficit in vitamin K commonly occurs as a result of poor placental transfer, an absence of vitamin K<sub>1</sub>-

producing bacteria in the gut, and immature hepatic metabolism. In as many as 50% of neonates, plasma concentrations of vitamin K fall transiently below levels required to sustain effective blood coagulation (1). Surveys undertaken in both developed and developing countries indicate that, among infants who do not receive supplementary vitamin K, as many as 0.2–2% develop signs of haemorrhagic disease during the first week of life (2) and that, over the next six months, a small number subsequently die or sustain permanent brain damage as a result of bleeding into the central nervous system (3–5). Perversely, since human milk contains only trace amounts of vitamin K, the incidence of spontaneous bleeding is higher among breast-fed babies than among those fed upon vitamin-supplemented infant formulas (6, 7).

Routine prophylactic administration of vitamin K to all children at birth has been recommended by professional associations and health authorities in North America and Western Europe for more than 30 years (8). The preparation used is an intramuscular formulation of phytomenadione or vitamin K<sub>1</sub>, a water-soluble analogue of vitamin K in a vehicle of polyethoxylated castor oil, propylene glycol, and phenol. In the USA, this is delivered in a single intramuscular injection containing 0.5 to 1.0 mg vitamin K (9). In Europe, the same formulation is sometimes administered orally, notwithstanding evidence that this route of administration is less effective in preventing late onset haemorrhagic disease (5).

In 1990, the possibility was raised that post-natal administration of vitamin K might exert a carcinogenic action. The association was established from records relating to 33 children born in the UK in April 1970 who were included within a national cohort and who subsequently developed cancer before the age of 10 years (10). This result was not anticipated, and obviously required confirmation. Many events in early life have been reported to be associated with the development of cancer of childhood (11); this study had not been designed to explore this specific association; and the hypothesis had been established on data derived from a small number of children.

In 1992, the association was again examined by the same group. Using a retrospective case-control design, 195 children who had developed cancers — 50% of which were leukaemias and lymphomas — were compared with a larger number of children without cancer born within the same period of time in the same hospitals in South-West England (12).

Again, a positive association was established: intramuscular — but not oral — administration of vitamin K was linked with a doubling of the risk of childhood cancer (odds ratio 1.97, 95% confidence interval 1.3 to 3.0).

This association is strong in statistical terms and several experimental observations have been cited to suggest that the finding is biologically plausible. The transient excessive plasma concentrations of vitamin K resulting from intramuscular administration — up to 5000 times greater than those of normal breast-fed infants (13) — have been claimed to increase sister chromatid exchanges in human placental lymphocytes *in vitro* (14) although this has not been evident in cells obtained from a small number of treated infants (15). Moreover, vitamin K has been claimed to promote the carcinogenic action of benzopyrenes (16, 17), and the incidence of spontaneously generated tumours has been claimed to be reduced among animals artificially deficient in vitamin K (18).

None the less, the general relevance of these findings has always been held in question. An increase in risk of this magnitude should have become apparent in national cancer statistics, particularly in the USA where vitamin K is largely administered intramuscularly. However, there is no evidence that increasing post natal administration of vitamin K over the past 30 years has been accompanied in this country by an increasing trend in the incidence of childhood leukaemia (19). Nor have the British findings been supported by subsequent studies undertaken in Sweden and the USA (20-21).

The first of these was a record linkage study undertaken to identify cases of cancer developing in children born within maternity hospitals in Sweden between 1973 and 1989 (20). In all, more than 1 million infants were born in hospitals where vitamin K was given routinely by intramuscular injection, while some 270 000 were born where it was given orally. No difference in the risk of childhood cancer was detected between the two groups (odds ratio 1.01; 95% confidence interval 0.88 to 1.17). The second study utilized data obtained prospectively in a broader study of pregnancy, delivery and childhood conducted in a cohort of over 50 000 children born at 12 centres in the USA between 1959 and 1966 (21). A nested case-control study involved 48 children who had developed cancer within 8 years of birth. Of these children, 68% had received vitamin K intramuscularly at birth, as compared with 71% of matched controls (odds ratio 0.84; 95% confidence interval 0.41 to 1.71).

Both these studies are more secure in their design than the definitive British study, in which the route of administration of vitamin K was sometimes inferred exclusively from beliefs about policy. Both, also, are more representative of national trends. However, the reassurance that they provide is qualified (22). The small number of cases in the US study resulted in wide confidence limits that do not exclude the existence of a clinically important association, whereas the incidence of cancer among the controls in the Swedish study (2.5 cases per 1000 by 15 years) is some 20% higher than expected from pre-existing data (23). Statistical imprecision leaves open the possibility of an association between vitamin K and cancer, but the magnitude of the risk, if it exists, is likely to be considerably less than was first proposed. Meanwhile, "the risk of haemorrhagic disease is certain; that of cancer is not" (24).

#### References

1. Aballi, A., De Lamerens, S. Coagulation changes in the neonatal period and in early infancy. *Pediatric Clinics of North America*, **9**: 785-817 (1962).
2. Andrew, M. The hemostatic system in the infant. In Nathan, D., Oski, F. eds. *Haematology of infancy and childhood*, pp 115-153, Vol. 1, 4th edn., W. B. Saunders, Philadelphia, 1993.
3. Hathaway, W. New insights on vitamin K. *Hematology and Oncology Clinics of North America*, **1**: 367-379 (1987).
4. McNinch, A., Tripp, J. Haemorrhagic disease of the newborn in the British Isles: two-year prospective study. *British Medical Journal*, **303**: 1105-1108 (1991).
5. von Kries, R. Vitamin K prophylaxis — a useful public health measure. *Paediatric and Perinatal Epidemiology*, **6**: 7-13 (1992).
6. Greer, F., Marshall, S., Cherry, J., Suttie, J. Vitamin K status of lactating mothers, human milk, and breast-feeding infants. *Pediatrics*, **88**: 751-756 (1991).
7. Hilgartner, M. Vitamin K and the newborn. *New England Journal of Medicine*, **329**: 957-958 (1993).
8. Committee on Nutrition, American Academy of Pediatrics. Vitamin K compounds and the water-soluble analogues: use in therapy and prophylaxis in pediatrics. *Pediatrics*, **28**: 501-507 (1961).
9. American Academy of Pediatrics Vitamin K ad hoc Task Force. Controversies concerning vitamin K and the newborn. *Pediatrics*, **91**: 1001-1003 (1993).
10. Golding, J., Paterson, M., Kinlen, L. Factors associated with childhood cancer in a national cohort study. *British Journal of Cancer*, **62**: 304-308 (1990).

11. Gilman, E., Kinnear-Wilson, L., Kneale, G., Waterhouse, J. Childhood cancers and their association with pregnancy, drugs and illnesses. *Paediatrics and Perinatal Epidemiology*, **3**: 66-94 (1989).
12. Golding, J., Greenwood, R., Birmingham, K., Mott, M. Childhood cancer, intramuscular vitamin K, and pethidine given during labour. *British Medical Journal*, **305**: 341-346 (1992).
13. McNinch, A., Upton, C., Samuels, M. et al. Plasma concentrations after oral and intramuscular vitamin K<sub>1</sub> in neonates. *Archives of Diseases of Children*, **60**: 814-818 (1985).
14. Israels, L., Friesen, E., Jansen, A., Israels, E. Vitamin K<sub>1</sub> increases sister chromatid exchange *in vitro* in human leukocytes and *in vivo* in fetal sheep cells: a possible role for 'vitamin K deficiency' in the fetus. *Pediatric Research*, **22**: 405-408 (1987).
15. Comelissen, M., Smeets, D., Merckx, G. et al. Analysis of chromosome aberrations and sister chromatid exchanges in peripheral blood lymphocytes of newborns after vitamin K prophylaxis at birth. *Pediatric Research*, **30**: 550-553 (1991).
16. Israels, L., Walls, G., Ollmann, D. et al. Vitamin K as a regulator of benzo(a)pyrene metabolism, mutagenesis and carcinogenesis. *Journal of Clinical Investigation*, **71**: 1130-1140 (1983).
17. Dogra, S., Israels, L. Vitamin K<sub>1</sub> amplification of benzo(a)pyrene metabolism in chick embryos. *International Journal of Biochemistry*, **19**: 471-473 (1987).
18. Hilgard, P. Experimental vitamin K deficiency and spontaneous metastases. *British Journal of Cancer*, **35**: 891-892 (1977).
19. Miller, R. Vitamin K and childhood cancer. *British Medical Journal*, **305**: 1016 (1992).
20. Ekelund, H., Finnström, O., Gunnarskog, J., Källén, B., Larsson, Y. Administration of vitamin K to newborn infants and childhood cancer. *British Medical Journal*, **307**: 89-91 (1992).
21. Klebanoff, M., Read, J., Mills, J., Shiono, P. The risk of childhood cancer after neonatal exposure to vitamin K. *New England Journal of Medicine*, **329**: 905-908 (1993).
22. Passmore, S., Draper, G., Stiller, C. Vitamin K and childhood cancer. *British Medical Journal*, **307**: 1140 (1993).
23. Parkin, D., Stiller, C., Draper, G. et al. eds. *International incidence of childhood cancer* (IARC Scientific Publication No. 87). International Agency for Research on Cancer, Lyons, 1988.
24. Hull, D. Vitamin K and childhood cancer. *British Medical Journal*, **305**: 326-327 (1992).

## L-tryptophan: a postscript on eosinophilia-myalgia syndrome

In October 1989, three cases of acute severe myalgia and marked eosinophilia were notified to the state health authorities in New Mexico, USA (1, 2). Each of the patients had been taking oral preparations of the amino acid, L-tryptophan. A national surveillance system was immediately established and within three weeks 287 cases of this newly recognized disorder had been reported (3). Some 9 months later, more than 1500 cases had been reported and in 27 instances the disease was fatal (4).

Sporadic cases of myalgia associated with eosinophilia have been reported previously (5-7). It has been claimed (4) that the clinical features relate most closely to the epidemic of toxic-oil syndrome reported from Spain in 1981 (8-10). The cause of this epidemic, which affected some 20 000 persons and resulted in more than 300 deaths, was associated with the use of adulterated rape seed oil but the precise cause has never been established (11). Both epidemics were characterized clinically in the acute phase by severe myalgia, intense eosinophilia, and diverse signs of multisystem involvement, including fever, rash, and pneumonitis. Both diseases also shared a potential to induce chronic sequelae such as neuromuscular abnormalities, pulmonary hypertension and scleroderma-like changes (3, 9).

In all, 97% of patients notified to the national surveillance system as having the eosinophilia-myalgia syndrome also reported taking oral tryptophan preparations (4). This was to be expected since the presumed causal association had been widely publicized at an early stage of the epidemic. Subsequently, the association has been confirmed in several independent case-control studies (3, 4, 12-14). However, these studies do not explain why a sudden clustering of serious adverse events was associated with products that had been available for many years and that contained, as the sole active ingredient, a constituent of dietary protein.

An obvious possibility is that batches of one or more products contained a toxic contaminant. This was decisively confirmed by a survey undertaken by the state health authorities in Oregon, USA (15): 45 of 46 patients notified as having the syndrome had taken a product made by one manufacturer, and this possibility was not excluded in the remaining patient. The search for a causal con-

taminant initially centred upon the di-tryptophan N- $\alpha$ -aminal of acetaldehyde (16). However, more extensive analysis of this compound and of other contaminants has provided only weak support for an association between risk and any one contaminant (17).

Since many trace contaminants have been found in batches of L-tryptophan associated with cases of the disease (18), it has been suggested that other contaminants — or interactions among several contaminants — may account for the pathological changes. Certainly, the etiology of the condition is likely to be complex, and host factors may well be involved since the incidence of the disease among individuals who used products manufactured from the one suspect source probably did not exceed one in 100 (15).

Given the lack of evidence that L-tryptophan products produced by other manufacturers are associated with risk, the decision taken in the United Kingdom on the advice of the Committee on Safety of Medicines (and reported on p. 35) to re-introduce oral preparations under carefully monitored conditions for depressed patients unresponsive to other therapy, seems well founded. There is reason, none the less, to continue with the attempt to unravel the cause of eosinophilia-myalgia syndrome since, in doing so, knowledge may be increased about the pathogenesis of scleroderma and other connective tissue disease (16).

#### References

- Centers for Disease Control. Eosinophilia-myalgia syndrome — New Mexico. *Morbidity and Mortality Weekly Report*, **38**: 765-767 (1989).
- Flannery, M., Wallach, P., Espinosa, L. et al. A case of the eosinophilia-myalgia syndrome associated with the use of an L-tryptophan product. *Annals of Internal Medicine*, **112**: 300-301 (1990).
- Centers for Disease Control, Eosinophilia-myalgia syndrome and L-tryptophan-containing products — New Mexico, Minnesota, Oregon, and New York. *Morbidity and Mortality Weekly Report*, **38**: 785-788 (1989).
- Swygert, L., Maes, E., Sewell, L. et al. Eosinophilia-myalgia syndrome: results of national surveillance. *Journal of the American Medical Association*, **264**: 1698-1703 (1990).
- Yonker, R., Panush, R. Idiopathic eosinophilic myositis with preexisting fibromyalgia. *Journal of Rheumatology*, **12**: 165-167 (1985).
- Symmans, W., Beresford, C., Bruton, D. et al. Cyclic eosinophilic myositis and hyperimmunoglobulin-E. *Annals of Internal Medicine*, **104**: 26-32 (1986).
- Lakhanpal, S., Duffy, J., Engel, A. Eosinophilia associated with perimyositis and pneumonitis. *Mayo Clinic Proceedings*, **63**: 37-41 (1988).
- Toxic Epidemic Syndrome Study Group. Toxic epidemic syndrome, Spain, 1981. *Lancet*, **2**: 697-702 (1982).
- Kilbourne, E., Rigau-Perez, J., Heath, C. et al. Clinical epidemiology of toxic-oil syndrome. *New England Journal of Medicine*, **309**: 1408-1414 (1983).
- Grandjean, P., Tarkowski, S. eds. *Toxic oil syndrome: mass food poisoning in Spain. Report of a World Health Organization meeting, Madrid 1983*. WHO Regional Office for Europe, Copenhagen, Denmark, 1984.
- Kilbourne, E., Bemert, J., Posada, M. et al. Chemical correlates of pathogenicity of oils related to the toxic oil syndrome epidemic in Spain. *American Journal of Epidemiology*, **127**: 1210-1227 (1988).
- Eidson, M., Philen, R., Sewell, C. et al. L-tryptophan and eosinophilia-myalgia syndrome in New Mexico. *Lancet*, **335**: 645-648 (1990).
- Hertzman, P., Blevins, W., Mayer, J. et al. Association of the eosinophilia-myalgia syndrome with the ingestion of tryptophan. *New England Journal of Medicine*, **322**: 869-873 (1990).
- Silver, R., Heyes, M., Maize, J. et al. Scleroderma, fasciitis, and eosinophilia associated with the ingestion of tryptophan. *New England Journal of Medicine*, **322**: 874-881 (1990).
- Slutsker, L., Hoesly, F., Miller, L. et al. Eosinophilia-myalgia syndrome associated with exposure to tryptophan from a single manufacturer. *Journal of the American Medical Association*, **264**: 213-217 (1990).
- Mayeno, A., Lin, F., Foote, C. et al. Characterization of "peak E", a novel amino acid associated with eosinophilia-myalgia syndrome. *Science*, **250**: 1707-1708 (1990).
- Philen, R., Hill, R., Flanders, W. et al. Tryptophan contaminants associated with eosinophilia-myalgia syndrome. *American Journal of Epidemiology*, **138**: 154-159 (1993).
- Hill, R., Caudill, S., Philen, R. Contaminants in L-tryptophan associated with eosinophilia-myalgia syndrome. *Archives of Environmental Contamination and Toxicology*, **25**: 134-142 (1993).
- Stenberg, E., Van Woert, M., Young, S. et al. Development of a scleroderma-like illness during therapy with L-5-hydroxytryptophan and carbidopa. *New England Journal of Medicine*, **303**: 782-787 (1980).