Tumours and Coley's toxins

SIR — Starnes[1] discusses the treatment of tumours with Coley's toxins, and makes the interesting point that 90% of Coley's successes occurred in patients with tumours of mesodermal origin, perhaps attributable to a greater probability that such tumours are immunogenic. Then, as there is some evidence that tumour necrosis factor (TNF) is also more effective against sarcomas, Starnes assumes a strong parallel between TNF therapy, endotoxin therapy and the use of Coley's toxins. But any therapy relying on the immune response is more likely to be effective if the tumour is immunogenic, so this argument does not indicate that Coley's toxins worked via TNF; indeed, there are reasons for suggesting that the mechanism was more complex.

Although TNF can be directly toxic for tumour cells, this is a rare mode of action *in vivo*, where the antitumour effects seem to operate via the tumour vasculature[2]. Thus the functional state of the endothelium, or perhaps a minor degree of pre-existing inflammation in some tumours, results in a TNF-sensitive state analogous to that induced in the "prepared sites" described by Shwartzman[3]. Shwartzman injected bacterial suspensions into the skin of rabbits, and 24 h later injected a similar endotoxin-rich suspension intravenously. The result, after a further 20–24 h, was haemorrhagic necrosis confined to the previously injected skin site.

This response was almost certainly mediated by systemically released TNF reaching the "prepared site", as similar necrosis can be evoked in such sites by the direct injection of TNF[4,5]. When a bolus of the cytokine reaches such a site there is rapid destruction of the vasculature, and subsequently of the dependent tissue. Infusion of large doses of TNF into perfused limbs together with interferon-γ and melphalan has reproduced this effect in human malignant melanomata and sarcomata[6]. In animals, this phenomenon, like the Shwartzman reaction in sites 'prepared' with lipopolysaccharide (LPS) or endotoxin, or other bacterial extracts, is rapid. The necrosis is seen about 24 h after TNF (or TNF-releasing trigger) is given. Moreover, after sublethal doses of LPS or TNF in several experimental systems, TNF-mediated tissue damage cannot be elicited again for at least 24 h (refs 5, 7, 8). The mechanism of this refractory state may be tachyphylaxis (though there is disagreement as to whether this affects antitumour activity[9,10]), temporary exhaustion of the TNF-releasing potential of macrophages, or the triggering of counteracting physiological pathways such as cortisol secretion and free TNF receptors.

Does this fit the descriptions of Coley's observations[11]? First, Coley's effects were usually seen after 2–3 weeks, not after 24 h, and in some cases no tumour regression was seen until the toxin administration had continued for several months (for example, the case described on page 84 of ref. 11). Second, the toxins were usually administered daily or on alternate days, so for these reasons a 'Shwartzman-like' mechanism is improbable. Finally, before *Serratia marcescens* (which contains LPS and other toxic entities) was added to the formulation, results were seen by causing a skin infection with group A streptococci (erysipelas), or following injection of the group A *Streptococcus* alone. Is there something special about this organism in this context? In the pre-antibiotic era, when infection of ulcerating tumours was common, was remission also observed following infection with LPS-containing Gram-negative bacteria (which must have been common), or only if the organism was a group A *Streptococcus*?

I agree strongly with Starnes' speculation that there is more to be learnt about Coley's fascinating observations, and I suggest that we should focus attention on the group A *Streptococcus* itself, and on novel effects of chronic release of many cytokines, rather than on short-term effects of single large doses of TNF.

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1. Starnes, C.O. *Nature* **357**, 11–12 (1992)
2. Havell, E. A., Fiers, W. & North, R. J. *J. exp. Med.* **167**, 1067–1085 (1988).
3. Shwartzman, G. *Phenomenon of Local Tissue Reactivity and its Immunological Pathological and Clinical Significance*, 1–461 (Hoeber, New York, 1937).
4. Rothstein, J. & Schreiber, H. *Proc. natn. Acad. Sci. U.S.A.* **85**, 607–611 (1988).
5. Al-Attiyah, R., Rosen, H. & Rook, G. A. W. *Clin. exp. Immun.* (in the press).
6. Lienard, D., Ewalenko, P., Delmotte, J. J., Renard, N. & Lejeune, F. J. *J. clin. Oncol.* **10**, 52–60 (1992).
7. Freudenberg, M. A. & Galanos, C. *Infect. Immun* **56**, 1352–1357 (1988).
8. Sheppard, B. C., Fraker, D. & Norton, J. A. *Surgery* **106**, 156–161 (1989).
9. Takahashi, N., Brouckaert, P. & Fiers, W. *Cancer Res.* **51**, 2366–2372 (1991).
10. Fraker, D. L., Sheppard, B. C. & Norton, J. A. *Cancer Res.* **50**, 2261–2267 (1990).
11. Nauts, H. C., Fowler, G. A. & Bokatko, F. H. *Acta med. scand. Suppl.* **274–277**, 1–93 (1953).

Protein crystallization cont . . .

SIR — The discussion by Pitts[1,2] and Abad-Zapatero[3] on the crystallization of proteins by centrifugation deserves some further comments, if only to clarify some misunderstandings. The crystallization by centrifugation reported by Wyckoff and Corey[4] in 1936 was not of tobacco mosaic virus (TMV) coat protein but was a pellet of the ribonucleoprotein viral rods with X-ray diffraction properties similar to those of the liquid crystals or tactoids of TMV previously observed by Stanley[5]. TMV solutions/suspensions at concentrations above 50 mg per ml under low salt conditions will separate at 1*g* into two phases; the lower, concentrated phase exhibits some properties of liquid crystals (for a description of the behaviour of concentrated solutions of TMV see refs 6, 7). Centrifugation only enhances this separation, but true three-dimensional crystals of TMV rods are not obtained. On the other hand, crystals of TMV coat proteins suitable for X-ray diffraction analysis were obtained by conventional means from ammonium sulphate solutions much later[8].

The salient point about Pitts' procedure[1] is that ultrafiltration by centrifugation led to a condition of supersaturation for his protein under the particular solvent conditions. This is, in principle, no different from the results obtained with *Penicillium vitale* catalase in a solution containing 2-methyl-2,4-pentanediol[9]. The effectiveness of a protein precipitant is, generally, a linear function of the concentration of the precipitant and the logarithm of the solubility of the protein in a relationship that resembles the Setschenow equation for salting-out[10]. In the case of *P. vitale* catalase[9], the initial concentration of the protein was very low and because of its relatively low molecular mass, a long centrifugation at high *g* forces would be required to establish a sufficiently high concentration of protein for supersaturation under the particular solvent condition. One must recognize the individuality of proteins; their maximum solubilities under various solvent conditions are part of this individuality. Thus it is unlikely that, in either case, crystals of these proteins have been obtained by centrifugation *per se* but rather that centrifugation produced a critical protein concentration favouring crystallization.

The important point of the observa-

1. Pitts, J. E. *Nature* **355**, 117 (1992).
2. Pitts, J. E. *Nature* **356**, 392 (1992).
3. Abad-Zapatero, C. *Nature* **356**, 392 (1992).
4. Wyckoff, R. W. G. & Corey, R. B. *Science* **84**, 513 (1936).
5. Stanley, W. M. *Science* **81**, 644 (1935).
6. Gregory, J. & Holmes, K. C. *J. molec. Biol.* **13**, 796–801 (1965).
7. Lauffer, M. A. *Trends biochem. Sci.* **9**, 369–371 (1984).
8. Finch, J. T. *et al. Nature* **212**, 349–350 (1966).
9. Vainshtain, B. K. *et al. J. molec. Biol.* **188**, 49–55 (1986).
10. Cohn, E. J. *Physiol. Rev.* **5**, 349–437 (1925).