and lymphocytes, submucosal oedema and vascular congestion. Ulceration and crypt abscess were not features of this model.

Experiments were then performed with the object of enhancing this inflammation. These included cyclophosphamide pretreatment (250 mg/kg, i.p.) given once 3 days prior to sensitization, prolonging the challenge period to 10 days and incorporating Freunds complete adjuvant into the sensitizing application of DNCB. All failed to potentiate the inflammatory response, which being maximal 24 h after the final challenge was no longer apparent after 72 h, either grossly or histologically.

Using the method of Askenase, *et al.*, (1978) we also investigated the effects of intrarectal sensitization using 2.5% DNCB in Orabase administered for 4 days followed by intrarectal challenge. We were unable to confirm their finding of colonic ulceration and indeed, were unable to produce a systemic sensitization by this method, as shown by a negative skin test with DNCB.

We are currently evaluating further modifications of this model in an attempt to increase chronicity of the response.

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A simple method for the measurement of phospholipase activity

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The mobilization of arachidonic acid (AA) from fat cell ghosts is inhibited by anti-inflammatory steroids suggesting that these drugs might inhibit the action of phospholipase A_2 (Lewis, Piper & Vigo, 1979). In order to investigate this we have developed a novel method for measuring the action of phospholipase A_2.

Multilayer liposomes of dipalmitoyl lecithin (DPL) were prepared in tris buffer (0.1 M, pH 7.2 containing 1 mM $CaCl_2$). A suspension of DPL (1 mg/ml) was prepared in tris buffer, heated above the transition temperature (Tc) of 41°C and whirlimixed. Throughout all experiments the liposomes were kept at the Tc since the optimum hydrolysis by phospholipase A_2 occurs at this temperature (Op den Kamp, de Gier & Van Deenen, 1974).

Liposomes were hydrolysed by incubation with various concentrations (2.5, 5.0, 7.0 iu) of phospholipase A_2 from pig pancreas or Naja Naja venom for 40–70 minutes. Reactions were terminated with 1 ml methanol, 15 mM EDTA.

The actions of drugs were studied by adding various concentrations to the buffer in which the liposomes were formed. The optical density of 1 ml aliquots of liposomes (1 mg DPL/1 ml) was measured in a Pye Unicam SP 1800 spectrophotometer using wavelength 340 nm. When different concentrations of phospholipase A_2 were added to the liposomes, hydrolysis of the phospholipids occurred and the optical density of the liposome suspension fell. In order to establish that the change in optical density was a reflection of hydrolysis of phospholipid, phospholipids and lysophospholipids were separated by t.l.c. in chloroform:methanol:water (65:25:4) and estimated by phosphorus assay. The degree to decay in light scattering corresponded to the degree of hydrolysis measured chemically and both were directly related to enzyme concentration and time of incubation. In the presence of mepacrine (0.1–1 mg/ml), which is known to inhibit phospholipase A_2 (Vargaftig & Dao Hai, 1972; Flower & Blackwell, 1976), hydrolysis was inhibited in a dose-related manner up to 100%.

The method provides a simple technique for studying the effects of drugs on the rate of hydrolysis of lipid membranes by purified phospholipases.

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A comparison of linear and nonlinear parameter estimates in drug receptor quantitation

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Many quantitative estimates of drug receptor number (B_{max}) and affinity (K_d, dissociation constant) in radioreceptor experiments are derived from linear and nonlinear transformations of the general hyperbolic binding curve, analogous to kinetic constants derived from the transformation of a Michaelis-Menton plot of initial velocity of reaction, against concentration of substrate (Dowd & Riggs, 1965):-

$$\text{Amount Bound} = \frac{B_{max} \cdot K_a \cdot F_c}{(1 + K_a \cdot F_c)}$$

(B_{max} = total number of binding sites, K_a = association constant, F_c = free concentration)

In the absence of experimental error, transformations of the hyperbolic curve based on the law of mass action, such as the Scatchard, double reciprocal and direct linear plots provide good estimates of the binding parameters, B_{max} and K_d (Scatchard, 1949; Cornish-Bowden & Eisenthal, 1974; Madsen & Robertson, 1974). However, when data is subjected to random error of the nature and magnitude encountered in radioligand binding studies, discrepancies are apparent between parameter estimates obtained using different transformations. Theoretically, the most accurate, simple and unbiased way to fit experimental data to such a model is by iterative, computer assisted, nonlinear regression providing a direct least squares estimate of the parameters (Batchelor, 1977).

Parameter estimates of B_{max} and K_d were set at 200×10^{-15} mols and 3.0 nM respectively, and a theoretical saturation binding curve constructed. This was subjected to random noise of 0–10% and B_{max} and K_a calculated by Gauss-Newton iterations, providing a least squares fit, utilising an IBM360 computer and University of California BMDP3R nonlinear regression programme. The results obtained were compared with those from conventional transformations of the curve (Table 1).

Table 1

Data treatment	B_{max} $\times 10^{-15}$ *mols*	95% *Confidence limits*	K_d *nM*	95% *Confidence limits*
Nonlinear regression	199	186–211	2.83	2.52–3.23
Double reciprocal	173	150–203	1.49	1.49–2.16
Scatchard plot	198	184–210	2.93	2.53–3.30
Direct linear plot	196	170–226	3.1	0.4–4.6

D.S.M. is a Medical Research Council student.

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