of phenolphthalein was determined by procedures described by Storey (1964) and Dutton (1966) respectively, using the 2000 *g* supernatant of 10% (w/v) rat liver homogenates in 0.25 M sucrose. Microsomal protein was determined on the fraction of the liver homogenates sedimenting between 10,000 and 100,000 *g*.

There were significant ($P < 0.05$) decreases in liver to body weight ratios, mg total protein g^{-1} liver, and mg microsomal protein g^{-1} liver in the 30-month-old control rats compared with 8-month-old control animals. The rates of conjugation of both substrates were also significantly ($P < 0.05$) lowered to a small extent in the older rats: rates (n mole substrate conjugated mg^{-1} microsomal protein min^{-1}; means ± s.e. of 5 rats) for 8- and 30-month-old animals respectively, were 4.6 ± 0.1 and 3.5 ± 0.1 for 4-nitrophenol (at a substrate concentration of 2 mM) and 11.9 ± 0.2 and 10.8 ± 0.1 for phenolphthalein (at a substrate concentration of 0.2 mM). The Km values for both substrates were unaltered by old age with respect to glucuronidation.

Nephrectomy had no significant effect on the rate of conjugation of 4-nitrophenol by liver from both 8- and 30-month-old rats. However, the rate of glucuronidation of phenolphthalein was significantly ($P < 0.05$) reduced to a small extent 1 day after total and 14 and 35 days after sub-total nephrectomy in the rats of both age groups, e.g. at a substrate concentration of 0.2 mM, the rate of conjugation (n mole phenolphthalein conjugated mg^{-1} microsomal protein min^{-1}; means ± s.e. of 5 rats) was 12.0 ± 0.5 (controls) and 8.9 ± 0.4 (nephrectomized) in 8-month-old rats, 14 days after sham-operation or nephrectomy. The Km value for phenolphthalein in this system was unaltered by nephrectomy.

If decreases of a similar magnitude occur in the hepatic glucuronidation pathway in old age and renal failure in man, it is unlikely that they would be of great clinical importance.

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References

CROOKS, J., O'MALLEY, K. & STEVENSON, I.H. (1976). Pharmacokinetics in the elderly. *Clin. Pharmacokin.*, **1**, 280–296.

DUTTON, G.J. (1966). The biosynthesis of glucuronides. In: *Glucuronic acid: free and combined*, ed. Dutton, G.J., pp 185–299. Academic Press, New York.

MCCANCE, R.A. & MORRISON, A.B. (1956). The effects of equal and limited rations of water, and of 1, 2 and 3 per cent solutions of sodium chloride on partially nephrectomized and normal rats. *Quart. J. exp. Physiol.*, **41**, 365–386.

REIDENBERG, M.M. (1975). Drug metabolism in uraemia. *Clin. Nephrol.*, **4**, 83–85.

STOREY, I.D.E. (1964). The inhibition of the uridine diphosphate-transglucuronylase activity of mouse liver homogenates by thiol agents. *Biochem. J.*, **95**, 201–208.

Studies on a cell-mediated immune response in the guinea-pig colon

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The guinea-pig colon has been shown to participate in delayed-type hypersensitivity reactions (Rosenberg & Fischer, 1964), using the contact allergen, 2,4-dinitro-1-chlorobenzene, (DNCB) for cutaneous sensitization and intrarectal challenge. This model has subsequently been proposed for the investigation of the pathogenesis of inflammatory bowel disease by several workers (Bicks & Rosenberg, 1964; Askenase, Boone & Binder, 1978).

We have examined the effects of varying the sensitization and challenge regimens using DNCB, in an attempt to establish such a model with a view to making it a potential screening system for studying the action of anticolitic drugs and other therapeutic agents.

Male, Dunkin Hartley guinea-pigs (170–200 g) were used in all experiments. Several groups were sensitized on the shaved skin of the neck to 50 µl of 2.5% DNCB in ethanol for three consecutive days. After an interval of seven days the animals were challenged intrarectally using 0.25% DNCB in Orabase (E.R. Squibb Ltd.) for five successive days. Confirmation of systemic sensitization was achieved the day prior to intrarectal challenge by skin testing on the ear and measuring the increased skin thickness after 24 hours. Fifty µl of 0.25% DNCB in ethanol produced a 36% ± 9% increase in skin thickness. Macroscopically, the most intense inflammatory response in the colon was observed 24 h after the final challenge and showed erythema and fine punctate lesions. Histologically the lesion was characterized by increased cellular infiltration, including macrophages, plasma cells

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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References

ASKENASE, P.W., BOONE, W.T. & BINDER, H.J. (1978). Colonic basophil hypersensitivity. *J. Immunol.*, **120**, 198–201.

BICKS, R.O. & ROSENBERG, E.W. (1964). A chronic delayed hypersensitivity reaction in the guinea-pig colon. *Gastroenterology*. **46**, 543–549.

ROSENBERG, E.W. & FISCHER, R.W. (1964). DNCB allergy in the guinea-pig colon. *Archs. Derm.*, **89**, 99–112.

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.

References

FLOWER, R.J. & BLACKWELL, G.J. (1976). The importance of phospholipase-A_2 in prostaglandin biosynthesis. *Biochem. Pharmac.*, **25**, 285–291.

LEWIS, G.P., PIPER, P.J. & VIGO, C. (1979). The distribution and mobilisation of arachidonic acid in fat cell ghosts and its modification by glucocorticoids. *Br. J. Pharmac.*, (in press).