A survey of 160 adult epileptic patients in a long-stay residence has demonstrated a 22·5% incidence of mild hypocalcaemia, which was related to the daily dose and number of major anticonvulsants received and particularly to use of pheneturide and primidone (Richens & Rowe, unpublished). The tendency for a raised liver alkaline phosphatase isoenzyme to be related to hypocalcaemia suggested that these drugs may be interfering with calcium metabolism by an action on the liver, perhaps by disturbing vitamin D metabolism. This hypothesis has been tested in rats. Two of four equal groups of male albino rats (seven in each group) were given phenobarbitone (1 mg/ml) in their drinking water for 10 days, while the remaining two groups received distilled water. One phenobarbitone-treated group and one control group were given 40,000 units of calciferol in arachis oil daily by mouth for 8 days, and then killed. Their livers, kidneys and hearts were removed and weighed separately. Serum calcium, phosphorus and alkaline phosphatase, and the calcium content of the ashed kidneys were estimated. Satisfactory liver enzyme induction was demonstrated by a liver weight 44% higher in the phenobarbitone-treated ($P<0·001$). Table 1 summarizes the results.

A comparison of the differences of the means revealed that phenobarbitone (*a*) reduced the weight-loss caused by calciferol ($P<0·001$), (*b*) reduced renal calcinosis ($P<0·01$), (*c*) reduced the rise in serum calcium ($P<0·01$) and (*d*) prevented the fall in serum phosphorus ($P<0·005$). In addition, phenobarbitone appeared to lessen the increase in heart weight ($P<0·01$) and kidney weight ($P<0·05$), but these were probably relative increases due to the loss of body weight. The reduction in alkaline phosphatase produced by calciferol was not significant but phenobarbitone potentiated this effect ($P<0·01$). The mechanism of this action is uncertain. The results of these experiments support the hypothesis that phenobarbitone interferes with calciferol metabolism possibly by enhancing its liver degradation.

One of us (A. R.) was supported by a Wellcome Research Fellowship.

Pharmacologically active substances in malignant ascites fluid

JENNIFER A. C. SYKES (introduced by K. HELLMANN), *Cancer Chemotherapy Department, Imperial Cancer Research Fund, London WC2A 3PX*

There is a low degree of peritoneal inflammation of uncertain origin in mice bearing various types of ascitic tumours. Prostaglandins of the E series are present in carrageenin-induced inflammatory exudate (Willis, 1969) and in medullary carcinoma of the thyroid and its venous effluent (Williams, Karim & Sandler, 1968). Fluid from malignant ascites tumours has now been investigated for the occurrence of prostaglandins and other pharmacologically active substances.

Mice (C3H/He) were inoculated intraperitoneally with 0·2 ml ascitic fluid (BP8/P_1). Seven days later about 0·7–5 ml of peritoneal fluid from each of fifteen to twenty inoculated mice was aspirated, combined and collected in polythene centrifuge tubes. Total fluid volume was measured and cell counts were made. The fluid was centrifuged at 3,000 *g* for 10 min and the supernatant was decanted off and stored at −20° C until required.

The crude supernatant had smooth muscle stimulating activity when assayed on the rat stomach strip (Vane, 1957), chick rectum (Mann & West, 1950) and the rat

ascending colon (Regoli & Vane, 1964). These tissues were superfused (Gaddum, 1953) at 5 ml/min with Krebs solution at 38° C. Ethyl alcohol extracts of ascites fluid contained prostaglandin-like activity when tested on the above tissues in the presence of various antagonists (Gilmore, Vane & Wyllie, 1968). Activity was also extracted from acidified fluid into ethyl acetate. After evaporation to dryness, the extract was redisssolved in Krebs solution and assayed. The presence of an E-type prostaglandin was confirmed: this assayed as $13{\cdot}6 \pm 2{\cdot}26$ (mean $\pm$S.E.M.) ng prostaglandin E_2 per ml ascites fluid. Thin-layer chromatography of the ethyl acetate extract using the AII solvent system (Gréen & Samuelsson, 1964) showed zones corresponding to prostaglandins E_1 and E_2. Prostaglandin-like activity was eluted from the plate and bioassayed; the zones gave similar activity to prostaglandins E_1 and E_2 with some traces of $F_{2\alpha}$.

To detect other pharmacologically active substances, ethyl alcohol extracts were submitted to paper chromatography. These results and those from various control experiments will be shown.

REFERENCES

GADDUM, J. H. (1953). The technique of superfusion. *Br. J. Pharmac. Chemother.*, **8**, 321–326.
GILMORE, N., VANE, J. R. & WYLLIE, J. H. (1968). Prostaglandins released by the spleen. *Nature, Lond.*, **218**, 1135–1140.
GRÉEN, K. & SAMUELSSON, B. (1964). Thin-layer chromatography of the prostaglandins. *J. Lipid Res.*, **5**, 117–120.
MANN, MONICA & WEST, G. B. (1950). The nature of hepatic and splenic sympathin. *Br. J. Pharmac. Chemother.*, **5**, 173–177.
REGOLI, D. & VANE, J. R. (1964). A sensitive method for the assay of angiotensin. *Br. J. Pharmac. Chemother.*, **23**, 351–359.
VANE, J. R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.
WILLIAMS, E. D., KARIM, S. M. M. & SANDLER, M. (1968). Prostaglandin secretion by medullary carcinoma of the thyroid. *Lancet*, **1**, 22–23.
WILLIS, A. L. (1969). Release of histamine, kinin and prostaglandins during carrageenin-induced inflammation in the rat. In *Prostaglandins, Peptides and Amines*, ed. Mantegazza P. and Horton, E. W. London: Academic Press.

A method for the determination of residual quantities of halothane in blood

D. DENISON DAVIES and J. A. MATHIAS, *Research Department of Anaesthetics, Royal College of Surgeons of England, London W.C.2*

Blood levels of halothane, chloroform and trichloroethylene can be determined by a technique using a gas-liquid chromatographic column and an electron capture detector.

The anaesthetic agent is extracted from the blood with the solvent *n*-heptane. A small quantity of the extract (2·5 μl) is injected into a heated injection port and swept on to the columns by the carrier gas (nitrogen) and thereafter on to a detector. To determine the amount of the agent in the injected sample, the heights of the peaks produced are compared with those of known amounts of the agent dissolved in *n*-heptane. Three standards are necessary to define the calibration curve which is not truly linear.

The electron capture detector is extremely sensitive to most halogenated anaesthetics, halothane in particular. Blood halothane levels of the order of 1 μg/100 ml may be measured.

The degree of accuracy is high. When ten separate analyses of an identical blood sample containing halothane were compared, a mean concentration of 6·024 mg with