The data show that omission of calcium and/or phosphorus has no effect on food intake. In contrast, weight gain is significantly reduced if the diet does not contain phosphorus. This is probably due to the importance of phosphorus in many metabolic processes besides those concerned with bone.

Fresh and dry weight of callus was reduced in all deficient diets. The reduction was most pronounced in those rats which received no phosphorus. This indicates either a quantitative reduction or a retardation in the rate of development. The absolute amounts of calcium and phosphorus in the callus as well as the mineralization product $\left(\frac{Ca \times P}{100}\right)$ follow the same pattern, thus implying a reduced mineralization caused by the dietary deficiency.

The hydroxyproline content, representative of the callus matrix, increases particularly in phosphorus deficient animals. The absolute values and the ratio of the mineralization product to hydroxyproline content demonstrates that matrix formation of the callus is not affected by the diet deficiencies investigated, whereas its mineralization is markedly reduced, particularly if phosphorus is omitted.

REFERENCES

DAY, H. G. & MCCOLLUM, E. V. (1939). Mineral metabolism, growth, and symptomatology of rats on a diet extremely deficient in phosphorus. *J. biol. Chem.*, **130**, 269–283.

FOURMAN, P. & ROYER, P. (1968). *Calcium Metabolism and the Bone*, 2nd ed. Oxford and Edinburgh: Blackwell.

MCLEAN, F. C. & URIST, M. R. (1968). *Bone*, 3rd ed. Chicago and London: University of Chicago Press.

The behaviour of diethylstilboestrol, a lipid-soluble drug, in simulated intestinal content

J. A. BARROWMAN and A. D'MELLO*, *Departments of Physiology and of Pharmacology and Therapeutics, The London Hospital Medical College, London, E.*1

During the digestion of a meal containing fat, intestinal contents have been shown to consist of an oil phase dispersed in an aqueous system which contains bile salts in micellar solution (Hofmann & Borgström, 1964). The oil phase consists mainly of undigested triglyceride and some diglyceride, while monoglyceride and fatty acids are found partly in the oil phase and partly as solutes in the bile salt micelles. These polar products of lipolysis and other lipids such as sterols and phospholipids are distributed between the two phases (Borgström, 1967; Arnesjö, Nilsson, Barrowman & Borgström, 1969). In such a system lipid-soluble drugs, such as the synthetic oestrogens, might be expected to partition between the two phases in a manner related to their polarity. The solubility of hexoestrol in mixed micellar solutions of bile salts and lipids has been studied by Bates, Gibaldi & Kanig (1966). In order to study the process of absorption of diethylstilboestrol, an initial study has been made of the solubility of this drug in bile salt solutions and its distribution between oil and aqueous-micellar phases in simulated intestinal content.

In a buffer solution (pH 6·3) the solubility of the drug was enhanced by the addition of sodium taurodeoxycholate in concentrations above the critical micellar concentration. Inclusion of mono-olein in the bile salt micelles increased the solubility of octadecane, a non-polar solute, but did not increase the solubility of diethylstilboestrol.

Emulsions of triolein (20 mM) at pH 6·3 in sodium taurodeoxycholate were prepared at 20° C; the system also contained 0·5 mM labelled diethylstilboestrol. The aqueous phase was separated by centrifugation and the partition of the drug between oil and aqueous-micellar phase (M/O) calculated (Freeman, 1969). An increase in the concentration of bile salt over a range 3–20 mM resulted in a proportional increase in the partition ratio (M/O) of diethylstilboestrol.

Diethylstilboestrol, therefore, is soluble in micellar solutions of sodium taurodeoxycholate and in mixed intestinal content will be distributed partly in any oil present and partly in aqueous micellar solution. The drug does not behave like non-polar solutes such as the sterols, which show enhanced solubility in mixed monoglyceride-bile salt micelles.

J.B. acknowledges financial support for the work from the Wellcome Trust.

REFERENCES

ARNESJÖ, B., NILSSON, Å., BARROWMAN, J. & BORGSTRÖM, B. (1969). Intestinal digestion and absorption of cholesterol and lecithin in the human. *Scand. J. Gastroenterol.*, **4**, 653–665.

BATES, T. R., GIBALDI, M. & KANIG, J. L. (1966). Solubilizing properties of bile salt solutions II. Effect of inorganic electrolyte, lipids and a mixed bile salt system on solubilization of gluthimide, griseofulvin, and hexoestrol. *J. Pharm. Sci.*, **55**, 901–906.

BORGSTRÖM, B. (1967). Partition of lipids between emulsified oil and micellar phases of glyceride-bile salt dispersions. *J. lipid Res.*, **8**, 598–608.

FREEMAN, C. P. (1969). Properties of fatty acids in dispersions of emulsified lipid and bile salt and the significance of these properties in fat absorption in the pig and the sheep. *Br. J. Nutr.*, **23**, 249–263.

HOFMANN, A. F. & BORGSTRÖM, B. (1964). The intraluminal phase of fat digestion in man: The lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption. *J. clin. Invest.*, **43**, 247–257.

Effects of circulating oestrogen on function of the cholinergic dilator nerves supplying the guinea-pig uterine artery

C. BELL (introduced by MARTHE VOGT), *Department of Physiology, Institute of Animal Physiology, Babraham, Cambridge*

Recent published evidence has been advanced to indicate that the extrinsic uterine arterial supply of the guinea-pig is innervated by cholinergic vasodilator nerves. These nerves are functional only during the last half of pregnancy, and it has been suggested that their existence is concerned with the production of uterine hyperaemia of pregnancy (Bell, 1968, 1969). In the present study, release of acetylcholine (ACh) from the vasodilator nerves in response to nervous stimulation was examined in tissues taken from guinea-pigs in different hormonal states. The arteries were cleared of surrounding tissues and mounted at *in vivo* length in a 2·5 ml bath of Krebs solution (37° C) containing physostigmine (2×10^{-5} M). Trains of 3,000 pulses at 5 Hz and 25 Hz were delivered with platinum electrodes at either end of the bath. The bathing fluid was collected 10 min after commencement of a stimulation period, diluted 10 : 4 with distilled water and assayed on the dorsal muscle of the leech.

Arteries from dioestrous virgin animals released $0{\cdot}48 \pm 0{\cdot}11$ (mean $\pm$ standard error, six arteries) and $0{\cdot}5 \pm 0{\cdot}11$ ng (ten arteries) ACh/artery per train at 5 Hz and 25 Hz respectively. During oestrus these rose to $2{\cdot}6 \pm 0{\cdot}36$ (eight arteries) and $4{\cdot}0 \pm 0{\cdot}64$ ng (eight arteries) ACh/artery per train. Values obtained in tissues from animals in mid to late pregnancy were still high in comparison to those obtained in dioestrus, but lower than those in oestrus ($1{\cdot}1 \pm 0{\cdot}14$ (twelve arteries) and $1{\cdot}8 \pm 0{\cdot}31$ ng (twelve arteries) ACh/artery per train). This disparity might be attributable to lessened diffusion of ACh out of the hypertrophied pregnant tissues.