a possible physiological role for the adrenergic nerves might be a modulation of uterine sensitivity to oxytocin as well as to other hormones.

USPHS-HE-10187; Deutsche Forschungsgemeinschaft, R.U. 134–1.

REFERENCES

ABRAHAMS, V. C., LANGWORTHY, E. P. & THEOBALD, G. W. (1964). Potentials evoked in the hypothalamus and cerebral cortex by electrical stimulation of the uterus. *Nature, Lond.*, **203**, 654–656.

FOLKOW, B. (1952). Impulse frequency in sympathetic vasomotor fibres correlated to the release and elimination of transmitter. *Acta physiol. scand.*, **25**, 49–75.

Consequences of calcium and/or phosphorus deficient diets on various parameters of callus formation and on growth rate in young rats

W. DOEPFNER (introduced by B. BERDE), *Department of Pharmacology, Biological and Medical Research Division, Sandoz Ltd., Basle, Switzerland*

The role of calcium in the process of bone mineralization, as well as the optimum dietary requirements of this element, have been investigated extensively (Fourman & Royer, 1968; McLean & Urist, 1968). Phosphorus, which is assumed to be in excess in the diet, has attracted somewhat less interest in this respect, presumably because of very different requirements of calcium during life and the established importance of maintaining calcium homoeostasis, factors which inevitably affect bone turnover. Although the effects of phosphorus deficient diets on mineral metabolism, growth and general symptomatology of rats have been analysed (Day & McCollum, 1939), no data concerning fracture healing (callus formation) are apparently available in the literature.

Male, 5 week old rats were used. They were anaesthetized with ether and the tibia and fibula of both hind legs were fractured. Animals were randomly allocated to the different diet groups and kept in single cages. The diets (Altromin) and distilled water were supplied *ad lib.* On day 11 the animals were killed, the callus removed, and various parameters obtained by standard methods (Table 1).

TABLE 1. *Effect of calcium and phosphorus deficient diets on callus formation and growth rate in young rats*

Diet deficiency	None (Control)	Calcium and phosphorus	Calcium	Phosphorus
Number of animals	38	24	24	24
Food intake g/day	13·3±1·8 (100)	13·0±1·0 (98)	12·7±1·5 (96)	12·5±1·7 (94)
Body weight gain g/day	3·81±1·11 (100)	3·29±0·70* (86)	3·28±0·74*(86)	1·76±1·05*(46)
Callus fresh weight mg/rat	646 ±161 (100)	480±113* (74)	576 ±119 (89)	480±99* (74)
Callus dry weight mg/rat	198±41 (100)	138±27* (70)	167±25* (84)	131±24* (66)
Calcium (Callus) mg/100 mg	16·9±1·6 (100)	13·9±2·0* (82)	15·0±2·6* (89)	12·9±1·7* (76)
Phosphorus (Callus) mg/100 mg	8·6±1·0 (100)	7·2±1·0* (83)	7·8±1·3* (91)	6·6±0·8* (76)
Ratio: calcium/phosphorus	1·98±0·1	1·96±0·08	1·9±0·06*	1·98±0·08
$\frac{\text{Calcium} \times \text{Phosphorus}}{100}$	2·97±0·60 (100)	2·04±0·57* (69)	2·43±0·76* (82)	1·78±0·45* (60)
Hydroxyproline mg/100 mg	2·88±0·39 (100)	3·04±0·32 (106)	3·06±0·35 (106)	3·41±0·47* (118)
$\frac{\text{Calcium} \times \text{Phosphorus}}{100 \times \text{Hydroxyproline}}$	0·54±0·15 (100)	0·34±0·12* (64)	0·42±0·14* (78)	0·27±0·06* (50)

Mean values±S.D. * $P<0·05$. Figures in parentheses are percentages of control value.

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.