of pituitary prolactin cells in male and pregnant rats. The APG of pregnant rats is primarily composed of mammotrophs. In male rats clomiphene also abolishes the effect of sulpiride on the incorporation of tritiated thymidine in the APG. In this respect the results are similar to those obtained with pregnant animals (table 3).

Discussion. The results of these experiments confirm our previous work on the relationship between prolactin content and DNA synthesis in the APG. In APGs with a high intracellular prolactin content (as in pregnant rats) DNA replication is markedly depressed, but when prolactin is released with sulpiride, DNA synthesis increases. This last effect is abolished by clomiphene, which is an oestrogen receptor blocking agent. This suggest that the intracellular prolactin content in the APG plays a role in the regulation of DNA synthesis through a mechanism mediated by oestrogens. It appears that prolactin depletion from the APG enhances the oestrogenic action on prolaction cells resulting in a stimulation of cell proliferation. On the other hand, clomiphene may alter the release of hypothalamic hormones and of pituitary gonadotropins and as a result of this alteration may block the stimulation of DNA synthesis produced by sulpiride.

A similar effect is observed in male rats. There is strong evidence that androgens are aromatized to oestrogens in the APG[10], and that this may be the mechanism by which the androgenic hormones affect prolactin cells. When several androgenic steroids were administered to male rats, serum prolactin increased only with those which could be aromatized to oestrogens[11]. Therefore we propose that, in male and female rats, prolactin cell proliferation could be regulated by a similar mechanism.

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2 Abbreviations: APG, anterior pituitary gland; TCA, trichloroacetic acid.

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Substance P in medullary carcinoma of the thyroid

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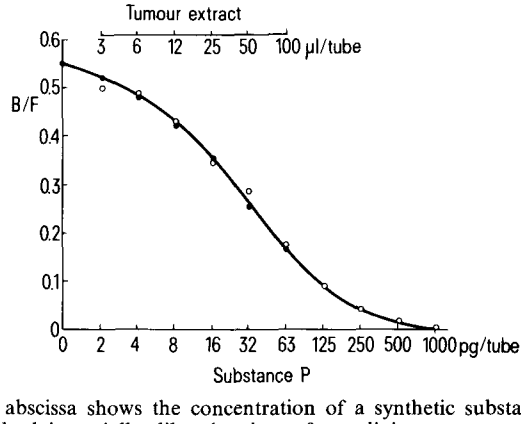
Summary. High levels of substance P-like immunoreactivity were demonstrated by radioimmunoassay in the plasma and tumour of a patient with a medullary carcinoma of the thyroid.

Medullary carcinoma of the thyroid (MCT) is an endocrine tumour capable of producing calcitonin, somatostatin, ACTH, histaminase, prostaglandins, and serotonin (5-HT)[1-3]. Light and electron-microscopic studies show a close resemblance between MCT and carcinoid tumours and several reports have described the carcinoid syndrome in patients with MCT.

We have reported previously on the production of substance P (SP) by carcinoid tumours[4,5]. We now report the finding of immunoreactive SP in the plasma and tumour of a patient with MCT.

Materials and methods. Tumour tissue was removed at autopsy from a 65-year-old man with a mediastinal mass and the carcinoid syndrome. The tumour was found in the left thyroid lobe extending into the anterior mediastinum and metastasizing into thoracic lymphnodes and liver. Histology of the tumour and metastases showed a typical MCT with abundant amyloid and argentophilia. The tumour and adjacent normal thyroid tissue were extracted according to the method of Chang and Leeman[6], the extract was evaporated and the residues dissolved in assay buffer, pH 7.6, and assayed for calcitonin and SP[8,9]. Blood was withdrawn into 10-ml plastic tubes containing 150 I.U. of heparin, spun, and the plasma was assayed for calcitonin and SP. The antiserum used in the SP radioimmunoassay was characterized previously and shown to be directed against the biologically active end of the SP molecule, i.e. its C-terminus[10]. The sensitivity of the assay is 2 pg, i.e. 1.5 fmoles[9]. The urinary 5-hydroxyindole-acetic acid was 15 mg/24 h.

Results and discussion. The tumour content of calcitonin was 1.25 µg/g wet tissue. The plasma level of calcitonin was 50 ng/ml. The tumour content of SP was 48 ng/g wet tissue. Serial dilution of the tumour extract showed a close parallelism with the SP standard (MRC, London) and is shown in the figure. The normal thyroid gland contained less than



The abscissa shows the concentration of a synthetic substance P standard in serially diluted points of a radioimmunoassay curve (open circles). The ordinate shows the antibody binding as the bound over free ratio corrected for 'damage' of the tracer. The closed circles superimposed on the standard curve represent values of antibody displacement for the tumour extract. The volume of the extract reconstituted in the assay buffer is shown on the upper scale.

2 ng/g wet tissue. The plasma level of SP was 180 pg/ml. The normal level of SP is 41±7 (SEM) pg/ml[4].

The pathophysiology of the carcinoid syndrome in MCT is unknown and the elevation of 5-hydroxyindole-acetic acid in urine, if present, is usually slight[11]. Prostaglandins and kallikrein have also been implicated in the genesis of the carcinoid syndrome but the evidence is inconsistent[11]. SP accompanies 5-HT in enterochromaffin cells[12]. In human volunteers, ng-quantities of SP infused parenterally induced carcinoid-like flush, hypotension, and increased intestinal motility[13]. The biological effects of SP suggest the possibility that SP may contribute to the pathophysiology of the carcinoid syndrome in MCT or carcinoid tumours. The finding of SP in MCT and in carcinoid tumours support the concept that MCT and carcinoid tumours are related[14].

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Effect of phosphate omission on glucose-induced insulin release in vitro

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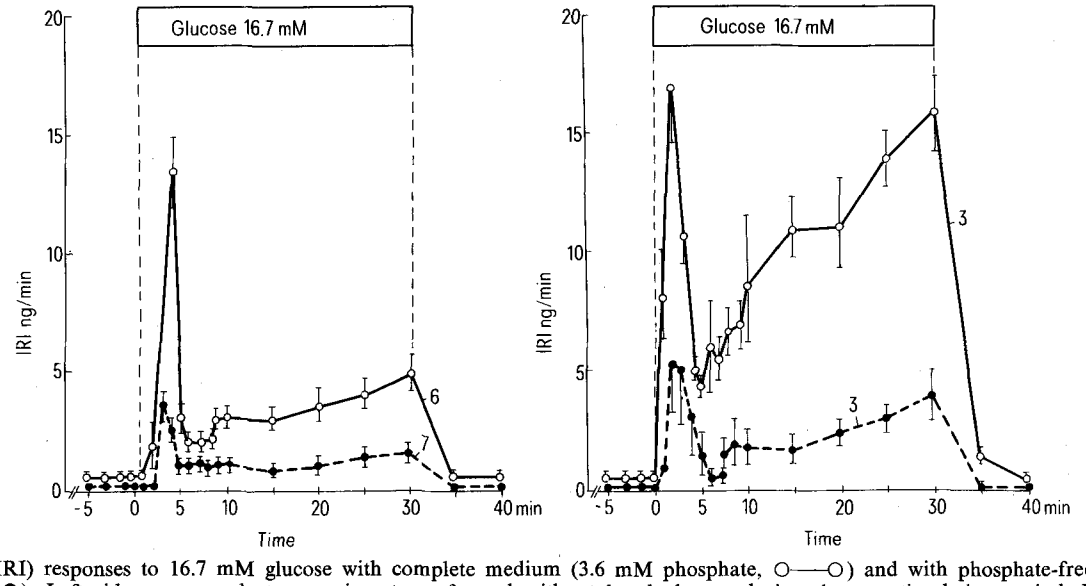
Summary. In the isolated perfused rat pancreas, omission of extracellular phosphate ($H_2PO_4^-$) significantly reduces the insulin secretion in response to 16.7 mM glucose.

Increasing evidence suggests that phosphate anions could play an important role in the mechanism of insulin release from the stimulated beta cell[1-9]. This study aimed at investigating the effect of extracellular phosphate ($H_2PO_4^-$) on the insulin response to 16.7 mM glucose.

Materials and methods. Overnight fasted male wistar rats (200-250 g) were utilized. Techniques for isolation and perfusion of the rat pancreas have been described[10]. The perfusate contained: NaCl 120 mM; KCl 1.4 mM; $MgSO_4$ 0.7 mM; $NaHCO_3$ 25 mM; $CaCl_2$ 1.0 mM; with or without 3.6 mM KH_2PO_4 for each experimental condition. In the absence of KH_2PO_4, an equivalent amount of KCl was added to avoid variations in the potassium concentration. The perfusate was supplemented with 0.5% (w/v) bovine



Insulin (IRI) responses to 16.7 mM glucose with complete medium (3.6 mM phosphate, O——O) and with phosphate-free medium (●– – –●). Left side corresponds to experiments performed without basal glucose during the pre-stimulation period. Right side corresponds to experiments performed in the presence of 2.7 mM basal glucose. Number of experiments are indicated with arrows. Results are expressed as mean ± SEM.