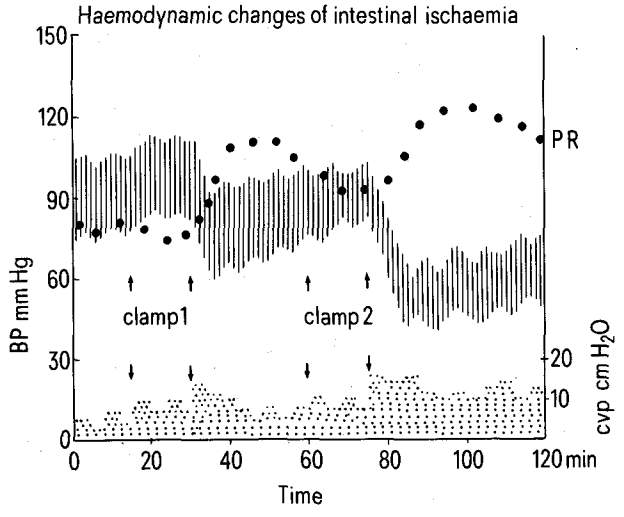

Fig. 2. The haemodynamic changes produced by 2 episodes of intestinal ischaemia in a single pig. The marked hypotension and tachycardia after ischaemia are particularly severe after the 2nd episode. This correlates well with the magnitude of portal VIP release. BP = blood pressure; PR = pulse rate; CVP = central venous pressure.

intervals from the femoral artery and portal vein during a basal period of 15 min then throughout the experiment, except after each ischaemic episode when samples were taken every 2 min. The samples for VIP estimation were taken into lithium heparinised tubes containing 10% volume of aprotinin (trasylol 10,000 KIU/ml) at 4 °C and centrifuged immediately. Plasma VIP levels were measured using a specific radioimmunoassay technique developed to a sensitivity of 1.5 pmol/l with a 95% confidence limit.

Results. The basal systemic plasma VIP level was 8±3 pmol/l (SEM) and the portal 12±5. There was no significant change in either level during the 1st 15-min period of clamping. However after the 1st ischaemic period the plasma VIP rose in the portal blood from 12±6. The arterial levels of VIP however did not change significantly (figure 1). Concomitant with the release of the vascular clamp a rapid fall in the blood pressure occurred of 110/70 ±25/15 to 90/60±12/8 mm Hg within 30 sec. Simultaneously the pulse rate rose sharply from 86±9 to 125±18/min (figure 2). During the rest period these changes slowly reversed and by 20 min were hardly apparent. The elevated portal plasma VIP level followed a similar course returning to its basal values at approximately 20 min after relaease of the 1st clamp. After the 2nd ischaemic episode a similar but far greater release of VIP was evident in the portal circulation. Portal plasma VIP levels rising from 18±6 pmol/l to 90±12 pmol/l. The arterial VIP levels rose from 8±3 to 14±6 pmol/l but this was not significant. In addition the haemodynamic changes were far more severe.

Discussion. VIP is widely distributed in high concentrations throughout the length of the gastrointestinal tract hence its massive and rapid release during intestinal ischaemia may have important pathophysiological connotations. Although very high VIP levels occur in the watery diarrhoea syndrome the elevation is achieved slowly and the effects of a sudden increment in level have not been previously documented. The sudden massive release of a potent vasodilator with a cardiac chronotropic action would contribute to the production of hypotension and tachycardia. Alterations in the autonomic nervous response of shock might reflect the postulated role of VIP as a neurotransmitter agent.

The fact that systemic VIP levels remain low throughout the ischaemia and post ischaemic periods suggests that the liver is degrading VIP rapidly or that the peptide is in some way altered so as to be undetectable by radioimmunoassay. Such an altered form of VIP might still be a highly potent vasoactive substance. The mechanisms producing shock in intestinal ischaemia are complex and ill understood. It does however seem possible that excessive and inappropriate release of a highly vasoactive compound such as VIP into the portal circulation might play an important part in modulating the gross and often fatal haemodynamic changes observed.

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An immunofluorescent method for identification of isolated thyrotropic cells

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Summary. A sensitive and specific immunofluorescent method for identification of thyrotropic cells was developed. The TSH-producing cells were found to be heterogenic in their morphology and intensity of staining.

A sensitive method for the localization of proteins of low molecular weight is an indirect technique which utilizes fluorescin-labelled antirabbit globulin[1]. With this method, a protein localized in a tissue reacts with a specific antiserum and the protein antiserum complex is then detected by the use of fluorescin labelled antiglobulin.

Emmant et al.[2] localized prolactin in the rat pituitary, using fluorescent antibody. Bain and Ervin[3] demonstrated the immunofluorescent localization of the LH cells of the human adenohypophysis. Localization of gonadotrophic, somatotrophic and corticotrophic hormones in the pituitary gland of the *Neotophyroides occidentalis* has been demonstrated by Zuber-Vögeli et al.[4]. Greenspan and Hargadine[5] show the intracellular localization of the pituitary thyrotrophic hormone in various tissues.

This paper presents the results of an immunofluorescent

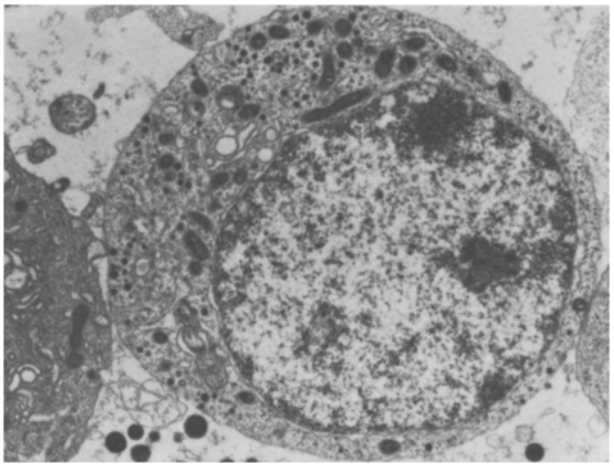

Fig. 1. Electron micrograph of a thyrotrophic cell. The granules range between 62 and 125 nm. × 12,200.

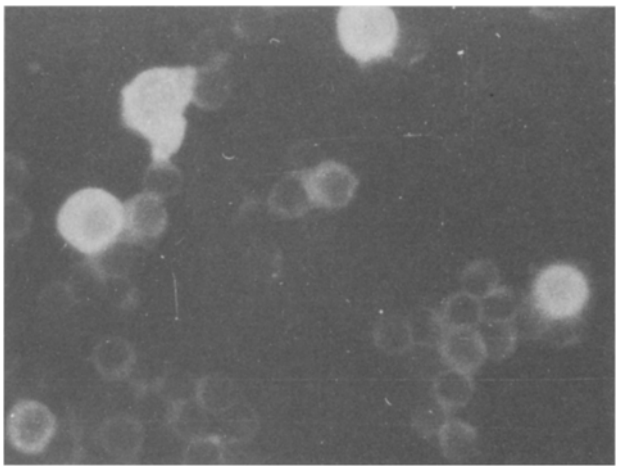

Fig. 2. Fluorescent positive thyrotrophic cells from the rat pituitary gland reacting specifically with anti-TSH. × 400.

method for identification of isolated thyrotrophic cells from the rat pituitary gland. This method provides a possibility to identify specifically thyrotrophic cells in a cell suspension after isolation of these cells[6].

Materials and methods. The experimental animals were 30–45-day-old male rats of the Hebrew University Sabra strain, and weighed 60–70 g each. The animals were killed by decapitation, their pituitaries were removed quickly and the anterior lobe was transferred into Earle's medium pH 7.2, without Mg^{+2} or Ca^{+2}, and cut into small fragments.

All glassware was pretreated with siliclad (Clay Adams). The tissue fragments were dispersed in Earle's medium, without Mg^{+2} and Ca^{+2}, containing 0.1% trypsin (Difco 1:250) and 0.1% bovine serum albumin (BSA, Armour Pharmaceutical Company, fraction V). The trypsin solution was buffered with 1.4% $NaHCO_3$ to pH 7.2–7.4. Dispersion was performed in a 25-ml Erlenmeyer flask and the solution was kept at 37 °C with constant stirring. The cells were expelled through a Pasteur pipette 20–30 times every 30 min and the entire dissociation took about 1–1.5 h.

After trypsinization, the cells were concentrated by centrifugation, washed twice and resuspended in Hank's solution containing 1% fetal calf serum. Cell counts were made using a hemacytometer. The number of cells per pituitary varied within a range of $1–1.5 \times 10^6$ cells.

For histological studies, smears of the cell suspension were prepared and fixed for 4 min in a formaldehyde solution. The slides were air-dried and stained with Herlant's tetrachrome[7]. Sections of intact pituitary tissue, prepared in the standard manner, were stained concurrently to ensure that optimal differentiation had been achieved[8].

The gamma globulin fraction of goat anti-rabbit gamma globulin serum (GARGG) was labelled with fluorescein isothiocynate (California Corporation for Biochemical Research, Los Angeles) according to the method of McKinney et al.[9]. Non-specific staining was removed by absorbing the fluorescein conjugated GARGG (fl. GARGG) twice with dried rat-liver powder (100 mg/ml gamma globulin). The indirect 'sandwich' fluorescent technique was used throughout the study. Smears of the cell preparations were prepared on the same day the test was performed. They were then air-dried at room temperature of 22 °C and fixed for 20 min with cold acetone (−20 °C). Before staining, the slides were washed twice in saline for 20 min. Non-conjugated rabbit anti-rat TSH serum was applied to the slides and they were incubated for 1 h at +4 °C in a moist chamber, and then washed twice in saline for 20 min. In the second stage, the slides were incubated with fl. GARGG for 1 h at +4 °C, then washed twice in saline for 20 min and mounted with buffered glycerol (pH 7.2). Specificity controls included the use of a) normal rabbit serum instead of rabbit anti-TSH (rat pituitary program of the National Institute of Arthritis Metabolism and Digestive Diseases NIAMDD RP-1 dilution 1:10), followed by fl. GARGG, and b) fl. GARGG alone. Results were evaluated in a Zeiss fluorescent microscope with an Osram high pressure mercury lamp HBO 200. Excitation filters BG12 and BG38 and barrier filter 44 with dark illumination were used.

Results and discussion. The isolated cells were examined by histological staining and by electron microscopy (figure 1). These examinations showed that using 0.1% trypsin with 0.1% BSA, prevented most of the cells from being damaged. It was obvious that not all the thyrotrophic cells would be in the same physiological stage during treatment with the antibodies, i.e. the majority of cells were secreting different amounts of TSH. We assumed that the strength of the staining would express the hormonal level of the cell. When various cell preparations were tested, fluorescence positive cells were detected at a frequency of 3%. Control preparations treated with normal rabbit serum followed by fl. GARGG were always negative. The cells reacting positively were found to be heterogenic in their morphology and their degree of fluorescence staining (figure 2). Most of them were large irregular cells rich in cytoplasm, while the remainder were smaller and more regular. We believe that we have succeeded in developing a specific and sensitive immuno-fluorescent method of identification of thyrotrophic cells from a pituitary cell suspension.

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