selenite by erythrocytes, but we could not confirm this under our conditions. Thus, the present findings indicate that stimulation of metHb reduction by selenite depends upon the GSH level in the cells.

The figure shows the species differences in metHb reduction in erythrocytes from various species in the absence and presence of selenite. In the absence of selenite, the rate of metHb reduction was lowest in the erythrocytes of dogs and highest in those of rabbits, the rate in erythrocytes of humans, rats and guinea-pigs being intermediate. Selenite (especially at high concentration), stimulated the reduction most in rabbit erythrocytes and least in dog erythrocytes. The rate of metHb reduction in these erythrocytes in the presence of selenite decreased in the following order: rabbit > human, rat, guinea-pig > dog. Lower concentrations of selenite resulted in similar rates of metHb reduction in erythrocytes of the various species, except guinea-pigs whose rate was considerably lower than others.

Discussion. Reduction of metHb in erythrocytes is mainly enzymatic (i.e. due to metHb reductase)[11], and thus the reduction observed in the absence of selenite is probably due to metHb reductase. The species differences in the rates of reduction of metHb observed in the absence of selenite were similar to those reported previously[12]. As shown in the table, GSH was necessary for stimulation of metHb reduction by selenite. We determined the GSH levels in erythrocytes of various animals to be as follows: rabbit 3.01±0.07 mM, guinea-pig 2.83±0.15 mM, rat 2.16±0.11 mM, human 2.25±0.22 mM and dog 1.72±0.22 mM (means±SE of 5 determinations). The stimulation of metHb reduction by a high concentration of selenite was found to be high in rabbit erythrocytes, in which the GSH level is high, and lower in dog erythrocytes in which the GSH level is low. However, this correlation was not observed with lower concentrations of selenite, suggesting that a certain molar ratio of selenite to GSH is important for selenite-catalyzed reduction of metHb by GSH[2]. We also found that selenite did not influence NADH-metHb reductase activity in vitro, measured by the method of Hegesh et al.[13] (data not shown). Therefore, we conclude that the species difference in the stimulation of metHb reduction by selenite is related to a species difference in the level of GSH in erythrocytes.

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Plasma vasoactive intestinal polypeptide (VIP) levels and intestinal ischaemia

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Summary. Vasoactive intestinal polypeptide (VIP) is released into the portal circulation in large quantities by ischaemic bowel. In view of its known high concentration in the gut and potent vasoactive properties it may well be implicated in the pathogenesis of the serious haemodynamic changes produced by gut ischaemia.

Vasoactive intestinal polypeptide is a pharmacologically active pepetide with a broad biological activity[1] which has been isolated from both the gut and the brain[2]. It has as yet no proven physiological role and no concensus exists as to its release mechanisms though both calcium provocation[3] and vagal stimulation[4] have been demonstrated to produce a rise in plasma levels. The causative role in the pathogenesis of the watery diarrhoea syndrome has recently been directly established[5] but its relationship to any other pathological process has yet to be determined. Many of the clinical changes that follow ischaemia of the gut such as tachycardia, profound hypotension and watery diarrhoea could well be explained by the release of VIP.

Materials and methods. 6 English white pigs (20–25 kg) were anaesthetised with nitrous oxide and oxygen. The internal jugular vein, portal vein and femoral artery were cannulated for plasma sampling and monitoring of the haemodynamic status. Ischaemia was produced by clamping the superior mesenteric vascular pedicle for 2 periods of 15 min each with an intervening rest period of 30 min. Haemodynamic changes were continually recorded by an intra arterial pressure manometer, a central venous catheter and an electrocardiograph. Blood samples were taken at 5 min

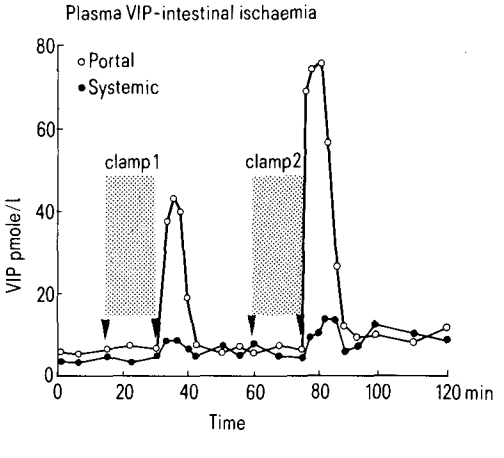

Fig. 1. The portal and plasma VIP levels during intestinal ischaemia in a single pig. The portal VIP levels are significantly higher than the systemic levels. The 2 15-min ischaemic periods are indicated by the shaded areas.

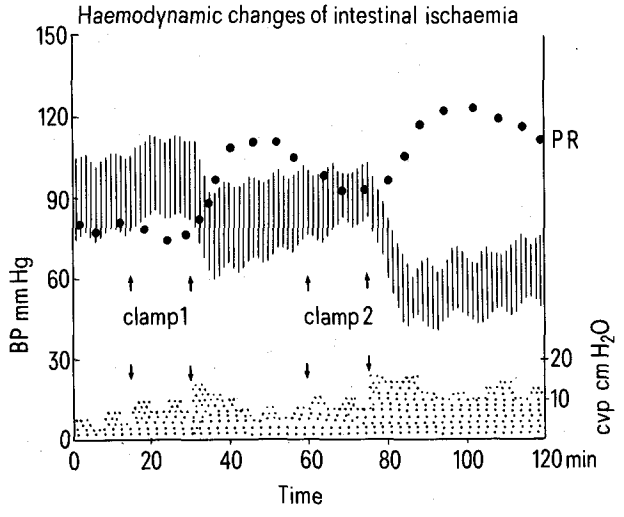

Fig. 2. The haemodynamic changes produced by 2 episodes of intestinal ischaemia in a single pig. The marked hypotension and tachycardia after ischaemia are particularly severe after the 2nd episode. This correlates well with the magnitude of portal VIP release. BP = blood pressure; PR = pulse rate; CVP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