

Fig. 4. For comparison with Figure 3. Block from duodenal mucosa after 30 min perfusion with acid. 7 S cells are visible and some exhibit very little fluorescence. $\times 220$.

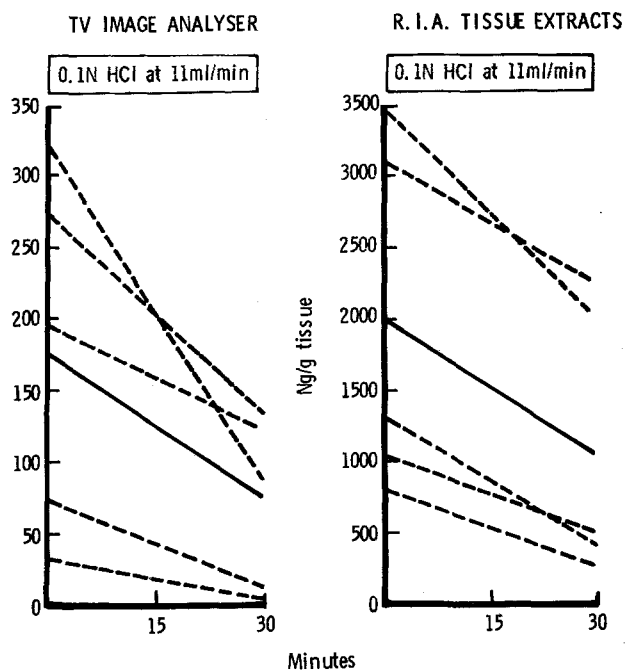


Fig. 5. Comparison of results obtained in the test series.

which correlates well with the fall in tissue secretin as estimated by radioimmunoassay. There was also a rise in blood secretin levels with a peak at 15 min and a pancreatic bicarbonate response which correlated in time well with the peak plasma secretin levels.

Total immunofluorescent cell count is not a sensible way of quantifying hormone secretion because, of course, one can record the same number of cells whether full or partially discharged. We propose, therefore, that quantification of variation in endocrine cell hormone content, under physiological or pathological conditions, should be carried out by procedures of the type described here, that is to say, by quantitative immunocytochemistry.

We conclude that secretin can be released in response to acid in the duodenum and that it probably is so released when the acidity falls below a threshold level, shown by MAYER, WAY and GROSSMAN¹¹ to be pH 4.5. Furthermore, acid appears not to stimulate discharge in the case of other endocrine cells in the upper intestine, in so far as these were covered by the antisera we employed.

Very little work has been done on cellular aspects of hormone secretion following an appropriate stimulus. Studies of the kind we report here should provide greater understanding of gut hormone physiology and also of the nature of disorders where increased hormone secretion or abnormal storage consequent on failure of release is suspected.

Zusammenfassung. Die Reaktion auf das Einträufeln einer 0,1 N HCl (11 ml/min) in das Duodenum des Schweines wurde parallel mit dem Radioimmuntest zur Bestimmung des Sekretgehaltes der Mucosa und mit quantitativer immunhistochemischer Analyse der duodenalen Sekretionzellen untersucht. Nach einer 30minütigen Einwirkung der Säure betrug der durchschnittliche Abfall des zellulären Sekretgehaltes in 5 Schweinen 52%. Der entsprechende Wert, der mit der Radioimmuntestmethode ermittelt wurde, betrug 72%.

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A Comparison of the Composition of Epididymal Plasma from the Cauda Epididymidis of the Rat, Hamster and Guinea-Pig

The composition of epididymal plasma obtained from the intact cauda epididymidis of the anaesthetized rabbit and rat has recently been described^{1,2} The present paper reports on the chemical composition of epididymal plasma from the intact cauda epididymidis of the hamster and the guinea-pig and a comparison is made with previous² and present findings in the rat. Earlier observations in the hamster³ were on fluid collected by puncturing the epididymal tubules in post-mortem material.

Methods. 7 adult male golden hamsters (110 to 140 g) and 7 adult male guinea-pigs (700 to 800 g) were anaesthetized with sodium pentobarbitone (Nembutal, Abbott

¹ R. JONES and T. D. GLOVER, *J. Reprod. Fert.* 34, 395 (1973).

² D. J. BACK, J. C. SHENTON and T. D. GLOVER, *J. Reprod. Fert.* 40, 211 (1974).

³ R. JONES, Ph. D. thesis, University of Liverpool (1973).

The composition of epididymal plasma from the cauda epididymidis of the rat, hamster and guinea-pig

Characteristic or constituent	Rat (Mean \pm SE)	Hamster (Mean \pm SE)	Guinea-pig (Mean \pm SE)
Spermatocrit (%)	52.80 \pm 2.05 (16)	48.45 \pm 0.58 (7)	49.61 \pm 0.51 (7)
Sodium (mequiv./l)	23.94 \pm 2.11 ^c (10)	24.94 \pm 1.18 (6)	15.95 \pm 1.11 (6)
Potassium (mequiv./l)	50.82 \pm 1.47 ^c (12)	33.41 \pm 0.66 (6)	28.98 \pm 2.44 (6)
Glycerolphosphorylcholine (mg/100 ml)	612.0 \pm 16.1 ^c (10)	560.0 \pm 23.0 (7)	934.0 \pm 13.0 (7)
Acid phosphatase (IU) ^a	430.0 \pm 7.90 ^c (12)	1,100 \pm 50.0 (6)	58,540 \pm 5,060 (7)
Alkaline phosphatase (IU) ^a	47.39 \pm 4.50 ^c (10)	107,000 \pm 6,500 (6)	240.0 \pm 30.0 (7)
Glutamic-oxaloacetic transaminase (IU) ^b	2695 \pm 100 (10)	570.0 \pm 42.0 (6)	260.0 \pm 32.0 (7)
Lactate dehydrogenase (IU) ^b	7741 \pm 309 (10)	5,340 \pm 440 (6)	13,400 \pm 1,270 (7)
Total protein (g/100 ml)	3.80 \pm 0.04 ^c (5)	3.41 \pm 0.12 (5)	3.67 \pm 0.23 (5)

Figures in brackets refer to the number of animals. ^a(IU, International Units (μ mol substrate hydrolyzed/min/l at 37°C)). ^bIU, International Units (μ mol substrate hydrolyzed/min/l at 25°C). ^cValues taken from BACK, SHENTON and GLOVER².

Laboratories; 30 mg/kg i.p.). 10 adult male rats were anaesthetized with urethane (14% w/v in water; 10 ml/kg, i.p.). The contents of the caudae epididymides were collected by the procedure previously described by BACK et al.² Using this technique, between 30 and 50 μ l of uncontaminated fluid were obtained from the rat and guinea-pig and 50 to 80 μ l of uncontaminated fluid from the hamster.

The epididymal contents were transferred to microhaematocrit centrifuge tubes and centrifuged at 12,000 *g* for 25 min (guinea-pig) or 45 min (rat and hamster) in a microhaematocrit centrifuge (Hawksley Ltd.). The spermatocrit was measured and the epididymal plasma separated and stored in polythene 'BEEM' capsules (TAAB Laboratories) at -20°C. Plasma was analyzed for sodium and potassium ions, glycerolphosphorylcholine (GPC), acid phosphatase, alkaline phosphatase, and total protein as previously described by BACK et al.² Glutamic-oxaloacetic transaminase (GOT) and lactate dehydrogenase (LDH) were determined spectrophotometrically at 340 nm (Boehringer test kits).

Results and discussion. The chemical composition of the luminal plasma from the 3 rodents is shown in the Table. The ratio of sodium to potassium was greater than 1:1 in each species, being 1:2.1 in the rat, 1:1.3 in the hamster and 1:1.8 in the guinea-pig. It is clear that a characteristic of rodent plasma, at least in the species so far examined, is the relatively high concentration of potassium ions compared to the boar⁴, the bull^{5,6}, the rabbit¹ and the ram^{3,7} in which the sodium to potassium ratios are approximately 1:1. The significance of this finding is not clear.

GPC has previously been shown to be present in the contents of the vas deferens of guinea-pigs⁸. The findings of the present study are that plasma from the guinea-pig has a greater concentration of this specific secretory product of the epididymis than either the rat or the hamster.

The most striking differences between the 3 species is in the relative concentrations of phosphatases in epididymal plasma. Histochemical studies in the hamster⁹, have shown that the luminal contents of the epididymis have a marked positive reaction for alkaline phosphatase, but that the enzyme activity is not in the cytoplasmic droplet¹⁰. The present finding confirms that in the hamster alkaline phosphatase is present in vast amounts in the epididymal plasma. In contrast, the rat and guinea-pig have a low level of the enzyme. A different situation

exists for acid phosphatase, the guinea-pig being rich in the enzyme with correspondingly less in the hamster and the rat. Histochemical studies on epididymides of the rat^{10,11} and the hamster¹⁰ have also indicated acid phosphatase to be present in the epithelial cells lining the duct.

LDH and GOT are known to be released from spermatozoa in the rabbit¹ and the rat¹² after prolonged high speed centrifugation or cold shock. It may well be that in comparison with the rabbit¹, the higher levels of these enzymes found in rodents is at least in part the result of leakage from spermatozoa due to the increased time of centrifugation which is necessary to obtain a constant spermatocrit. It is striking that guinea pig plasma contains the highest concentration of LDH and the lowest concentration of GOT. The significance of this is not yet clear.

Résumé. On a étudié la composition du plasma de l'épididyme chez le rat, le hamster et le cobaye. Les relations entre les composants sont discutées.

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⁴ B. CRABO, *Acta vet. scand.* 6, Suppl. 5 (1965).

⁵ B. CRABO and B. GUSTAFSSON, *J. Reprod. Fert.* 7, 337 (1964).

⁶ R. G. WALES, J. C. WALLACE and I. G. WHITE, *J. Reprod. Fert.* 12, 139 (1966).

⁷ T. W. SCOTT, R. G. WALES, J. C. WALLACE and I. G. WHITE, *J. Reprod. Fert.* 6, 49 (1963).

⁸ R. M. C. DAWSON and I. W. ROWLANDS, *Q. Jl. exp. Physiol.* 44, 26 (1959).

⁹ K. A. MONIEM, Ph. D. thesis, University of Liverpool (1972).

¹⁰ K. A. MONIEM and T. D. GLOVER, *J. Anat.* 111, 437 (1972).

¹¹ J. MARTAN, *Biol. Reprod., Suppl.* 1, 134 (1969).

¹² D. J. BACK and J. C. SHENTON, unpublished observations.

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