cellular activity in the bone marrow and the lymph nodes[13,14] and enhances humoral immunity[15], suggesting an adjuvant-like behaviour. Previous data account also for the differences observed in the action of BCG or LPS mR595 in the 2 dietary groups.

The influence of the FDD on the immune system is known to be multileveled and sex-related[2]. From the present data, the following conclusions can be drawn: a) Lipopolysaccharides are a diet-independent class of adjuvants, in contrast to BCG; b) FDD3 alone provides only intermediate immunostimulation. When adjuvants are used in conjunction with the diet, proper agent and administration timing are required to achieve full stimulation; c) given the right conditions, the action of BCG may be equivalent to the combined effects of LPS mR595 and FDD3. Our results suggest that using BCG or lipopolysaccharides in conjunction with formula-defined diets may be analogous to dealing with interacting adjuvants affecting different elements of the immune system.

Action of formula-defined diet 3 on the immune system response to SRBC in the female rat: Effect of sequential or concomittant BCG and LPS mR595

Treatment	Anti-SRBC[a] antibody levels in serum[b]	
	Animals fed laboratory chow	Animals fed diet 3
SRBC	3.5 ± 0.5[c] 11[d] (8-16)[e]	6.5 ± 0.4 90 (69-119)
SRBC + BCG^{f}_{seq}	8.2 ± 1.2 294 (128-657)	6.5 ± 0.2 91 (78-105)
SRBC + LPS $mR595^{g}_{seq}$	5.9 ± 0.5 59 (43-80)	8.4 ± 0.3 337 (274-416)
SRBC + BCG_{con}	6.2 ± 0.6 72 (48-109)	8.2 ± 0.5 288 (207-402)
SRBC + LPS $mR595_{con}$	4.5 ± 1.0 23 (12-44)	6.5 ± 0.56 91 (61-133)

[a]Sheep erythrocytes (1 ml, 10 cells, injected i.p. [b]Determined by passive hemagglutination, 7 days after SRBC challenge. [c]Mean number of wells (x) ± SE (6 animals per group, repeated once). [d]Titer as obtained by 2^x. [e]Titer range as obtained from SE of x. [f]Bacillus Calmette-Guérin (Institut Armand Frappier, Laval des Rapides, Québec, Canada) 1 mg/animal, injected i.p. [g]Lipopolysaccharides of *Salmonella minnesota* mR595 (provided by Dr O. Lüderitz, Max-Planck-Institut für Immunobiologie, Freiburg, FRG), 40 µg/animal, injected i.p.

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Release of gastrointestinal hormones following an oral water load

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Summary. The ingestion of 2 different water loads (7.5 and 15 ml/kg) by healthy subjects stimulated the release of plasma motilin, gastrin, pancreatic polypeptide and VIP. Atropine was found to block the release of PP but not the other hormones.

The mechanisms involved in the postprandial release of intestinal hormones are ill understood. The nutriment component of food is clearly important but the role of other stimuli such as water ingestion have not been previously investigated. This study provides evidence that it results in the release of several intestinal hormones.

Subjects and methods. All subjects were healthy and were studied after an overnight fast in 2 groups. a) 7.5 ml of distilled water (temperature 20 °C)/kg b.wt was given orally to 6 healthy volunteers (aged 25-35 years, weighing 70-85 kg). b) 15 ml water/kg b.wt was given to a 2nd group of 6 healthy subjects (aged 26-34 years, weighing 75-85 kg). c) The last experiment was repeated 2 weeks later on 5 of the group (b) subjects who received 600 µg atropine i.v. 2 min prior to ingestion of the water load (15 ml/kg). The mean ingestion time of the water for both groups was 2 min.

Hormone radioimmunoassays were performed with conventional methodology using antisera raised to human gastrin[2] and pancreatic polypeptide (pp)[3] to porcine gastric inhibitory polypeptide (GIP)[4], vasoactive intestinal polypeptide (VIP)[5], glucagon[6] and motilin[7] and to bovine insulin[8]. The assays could detect the following changes in plasma hormone concentration with 95% confidence: gastrin 2 pmoles/l, PP 2 pmoles/l, GIP 8 pmoles/l, enteroglucagon 10 pmoles/l, pancreatic glucagon 2 pmoles/l, VIP

1.5 pmoles/l, motilin 3 pmoles/l and insulin 6 pmoles/l. No cross-reaction was detectable between any of the peptides assayed with the exception of enteroglucagon which was derived by subtraction of the true pancreatic glucagon from total glucagon-like immunoreactivity[9].

Plasma hormone concentrations are expressed graphically as mean ± SEM and in the text as median and range. Statistical analysis was carried out by the Wilcoxon sum of ranks test.

Results. Figure 1 shows the response of plasma motilin after the water load and the effect of atropine. Following ingestion of the 7.5 ml/kg of water (bottom figure 1), motilin levels rose from a median zero value of 68 pmoles/l (range 15–83 pmoles/l) to peak value at 20 min of 107 pmoles/l (40–220) ($p < 0.05$). Levels remained significantly elevated for 40 min before returning to basal values at 50 min. Following 15 ml/kg of water, plasma motilin levels rose from a median zero value of 40 pmoles/l (22–97) to a peak level at 30 min of 103 pmoles/l (43–184) ($p < 0.05$). Levels remained significantly elevated up until the end of the experiment, the 60 min motilin value being 87 pmoles/l (46–198) ($p < 0.05$). Following atropine, ingestion of 15 ml/kg water (top figure 1) caused plasma motilin to rise from a zero value of 39 pmoles/l (20–112) to a peak level at 50 min of 73 pmoles/l (36–240) ($p < 0.05$).

Figure 2 shows the response of plasma gastrin. After 7.5 ml/kg of water (bottom figure 2) plasma gastrin levels rose from a zero value of 1.7 pmoles/l (0.5–3) to a peak level at 5 min of 9 pmoles/l (2.5–23) ($p < 0.05$) and returned to basal values at 20 min. Following the 15 ml/kg water (middle figure 2) plasma gastrin rose from a zero value of 1.2 pmoles/l (1–5) to a peak of 10 pmoles/l (5.5–36) at 5 min ($p < 0.05$) and returned to basal values at 40 min. Figure 2 (top) shows the plasma gastrin levels following 15 ml/kg water in the atropinised subjects. Levels rose from a zero value of 2.5 pmoles/l (1–3) to a peak of 12 pmoles/l (3–16) at 5 min ($p < 0.05$) but plasma gastrin remained significantly above basal until the end of the experiment.

Plasma pancreatic polypeptide (PP) following the different water loads and atropine is shown in figure 3. The 7.5 ml/kg water load (bottom figure 3) caused PP levels to rise from a zero level of 16 pmoles/l (4–56) to a peak at 20 min of 63 pmoles/l (24–78) ($p < 0.05$). Levels then fell returning to a value not significantly different from basal at 40 min. Following ingestion of the 15 ml/kg water load, plasma PP levels rose from a zero value of 23 pmoles/l (6–30) to an early peak value at 10 min of 47 pmoles/l (28–136) ($p < 0.05$) and fell slowly to basal by 50 min. In the atropinised subjects PP levels fell from a zero value of 16 pmoles/l (12–31) to a median nadir value at 30 min of 9 pmoles/l (4–18) ($p < 0.05$). Levels remained significantly below basal until the end of the experiment at 60 min.

Following ingestion of the low volume water load plasma VIP levels rose reaching a small but significant peak at 30 min of 4 pmoles/l (1–8). The large volume of water produced a rise of 6 pmoles/l (2–11) at 30 min. An identical rise was also observed at 30 min in the atropinised subjects. Ingestion of the water load or administration of atropine

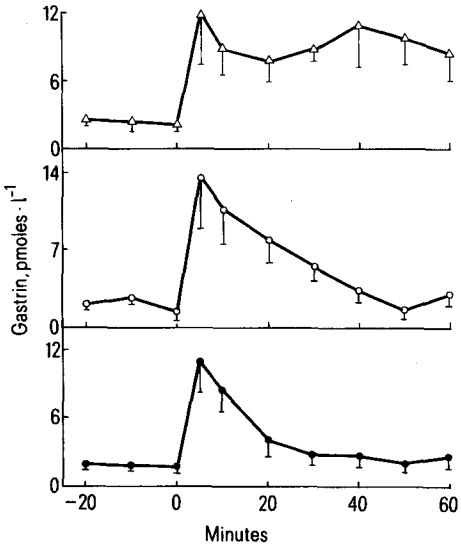

Fig. 2. Plasma gastrin concentrations (mean ± SEM) following ingestion of 7.5 ml/kg of water (bottom), 15 ml/kg (middle) and 15 ml/kg following i.v. atropine (top).

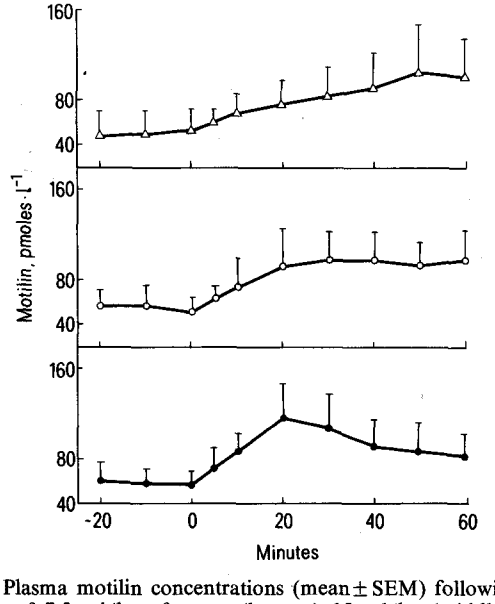

Fig. 1. Plasma motilin concentrations (mean ± SEM) following ingestion of 7.5 ml/kg of water (bottom) 15 ml/kg (middle) and 15 ml/kg following i.v. atropine (top) by healthy subjects.

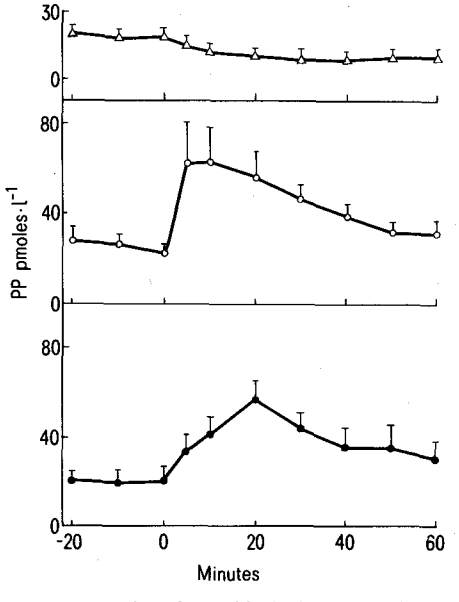

Fig. 3. Plasma pancreatic polypeptide (PP) (mean ± SEM) following ingestion of 7.5 ml/kg of water (bottom), 15 ml/kg (middle) and 15 ml/kg following i.v. atropine (top).

did not result in any significant change in the plasma concentration of GIP, pancreatic glucagon, enteroglucagon or insulin.

Discussion. We have shown that a substantial release of various gastrointestinal hormones occurs after ingestion of water by normal subjects. The mechanisms involved in the release of these hormones, however, appear to differ. The release of PP was blocked in the atropinised subjects suggesting an important influence of the cholinergic innervation. This observation is in accord with that of Schwartz et al.[10] where the PP rise after gastric distension was also blocked by atropine. Adrian et al.[11] have shown that the PP release following insulin hypoglycaemia, caerulein and Boot's secretin can also be abolished by atropinisation. Thus both the present study and previous work emphasise the importance of cholinergic tone in control of PP release.

Plasma motilin appears to have a different release mechanism. Levels rose following ingestion of the water load and this rise was not significantly affected by atropine. It is interesting to note that the peak rise of motilin occurred at least 20 min after ingestion of water when presumably a significant amount of water had left the stomach. It is thus possible that direct stimulation of the duodenum was responsible for the motilin release. Motilin in man is indeed found mainly in the duodenum and jejunum with only minute amounts being present in the stomach.

Vasoactive intestinal polypeptide (VIP) is found in fine nerves in the plexuses of the bowel wall and it is thought to function as a peptidergic neuromodulator or neurotransmitter[12]. There are only a few stimulants causing systemic release of VIP but small rises are seen after intraduodenal instillation of hyperosmolar solutions[13] and acid[14]. Ingestion of water can now be added to this list. It is known that considerable VIP destruction may occur in the liver[15], thus when the systemic plasma dilution factor is taken into account, it is possible that the VIP levels in the local venous drainage are much higher than those found in the systemic plasma. Like motilin, the rise of VIP occurred late (30') and was not blocked by atropine.

Gastric distension in the dog is a powerful stimulant of gastrin release[16] but its influence in man is less well established. Richardson et al.[17] recently reported that 500 ml or 750 ml isotonic saline meals stimulated gastric acid secretion but had no effect on gastrin release. This appears to be in contrast to the gastrin release found in the present study. The discrepancy, however, may be due to the different methodologies used. Richardson et al. infused the saline intragastrically in their subjects and maintained the antral pH constant at 5.0 throughout their experiment. In the present study the gastric pH was not monitored. A rise in gastric pH may thus have been an important factor. This study has provided additional evidence that the PP release is under vagal tone. In addition, it has been shown that water ingestion releases PP, VIP, motilin and gastrin, although except for the first mentioned, the mechanisms involved are presently quite unknown.

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Seasonal variations in vasopressin secretion in rats

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Summary. Plasma vasopressin concentrations and vasopressin content in neurohypophysis in rats show seasonal variations; namely, high in summer and low in winter.

MacFarlane and Robinson[1] first documented seasonal changes in plasma vasopressin (VP) concentration in humans and sheep, higher in summer and fall and lower in winter and spring. When the heat stimulus was apllied in warm seasons, plasma VP concentration increased, but it did not change in cold seasons. Therefore, season-related sensitivity to stimuli was suggested. Later, Morimoto et al.[2] also described the same seasonal variations in man. We are presenting data accumulated during 1976–1978 providing evidence of seasonal variations in VP secretion in rats.

Materials and methods. Adult male Sprague-Dawley rats were kept at constant room temperature (22.5 ± 1.0 °C) with a 12:12 dark-light cycle at least 2 weeks before the experiment. Rat Purina Chow diet and tap water were given ad libitum. A minimum of 6 rats was used in each experiment for each season (table). The rats were decapitated, trunk blood collected into heparinized dishes, and plasma kept frozen for VP extraction. Neurohypophysis (NH) including the intermediate lobe, and the hypothalamus (HT) tissue block bound by chiasma opticum anteriorly, corpora mamillaria posteriorly and approximately 2 mm lateral to the midline on each side at the base of the brain were quickly removed and dried in acetone before VP extraction. Plasma VP was extracted with cold acetone and