design B. Sets of 'experimental' data (500) were generated from the perfect data by using series of normally-distributed pseudo-random numbers. 2 sorts of random error were incorporated:
AN: normally-distributed eror of constant absolute magnitude, v having an SD of 0.10 (= 10% of V).
RN: normally distributed error of constant relative magnitude, v having a coefficient of variation of 10%.
The Michaelis-Menten equation was fitted to the data by 2 numerical methods:
WLS: weighted non-linear least-squares[2], the weighting factor being unity for data sets AN, and the reciprocal of the square of the predicted velocity for RN.
RLP: the revised version of the direct linear plot, in which the axes are scaled in K_m/V and $1/V^3$.
Results and discussion. The results (table) are for K_m rather than V because the former is the more difficult to estimate reliably; those for V were similar. It is evident that the 2-concentration design (A) was a little less precise than the conventional one (B) when the error was of constant absolute magnitude (AN), presumably because of the high coefficient of variation of the lower velocity (60%). On the other hand it was considerably the more precise when the error was of constant relative magnitude (RN). Since in general errors in v increase with the magnitude of v[4], one may conclude that design A will usually give the more precise estimates of K_m and V. Certainly this is our experience when assaying erythrocyte acetylcholinesterase[5]. We have also found the 2-concentrations design helpful when deciding whether an inhibitor acts in a linear competitive, uncompetitive or mixed fashion (unpublished work). Finally, the table shows that, as one would expect, the method of least-squares (WLS) is more efficient than the distribution-free alternative (RLP).
The major limitations of the 2-concentration design are that it gives no information as to whether or not the Michaelis-Menten equation fits the data, and that it requires a provisional estimate of K_m. However, these are relatively unimportant when, for example, kinetic variants of an enzyme are being sought, because one is looking for differences in kinetic behaviour and will have a standard value of K_m. The design also has a number of peripheral advantages; such as: 1. There is no need to weight the data when least-squares is used, unless standard errors of K_m and V are required. In contrast, incorrect weighting of the data from the conventional design is likely to give biased estimates of K_m and V as well as of their standard errors. 2. Running replicate assays at only 2 concentrations of substrate is convenient in practice, and gives a day-by-day check on the reproducibility of one's methods.

Values of K_m from the 2 experimental designs

Experimental design	Error type	
	AN	RN
Weighted least-squares (WLS)		
A	1.056 ± 0.451 (0.950)	1.004 ± 0.098 (1.000)
B	1.106 ± 0.442 (1.010)	1.025 ± 0.159 (1.021)
Revised linear plot (RLP)		
A	1.105 ± 0.620 (1.036)	1.007 ± 0.105 (1.006)
B	1.093 ± 0.523 (0.991)	1.036 ± 0.178 (1.024)

Values of K_m are mean ± SD (median in parentheses) of 500 simulated experiments. Design A: 6 replicate initial velocities at each of 2 concentrations of substrate; design B: 1 velocity at each of 12 concentrations of substrate.

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Evidence against cyclic GMP acting as a direct modulator of active sodium absorption in rat cecum[1,2]

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Summary. Cyclic GMP concentrations were measured in rat cecum mucosa in vivo when the net absorption of sodium, chloride and fluid was stimulated by methylprednisolone (MP). Whereas Na-K-ATPase specific activity was increased by MP, suggesting enhanced active sodium transport, cyclic GMP levels remained unaffected.

Cyclic guanosine 3′,5′-monophosphate (cGMP) has been considered to be related to sodium absorption in the mammalian intestine[4-6] and toad urinary bladder[7], although the postulated connection is poorly understood. Under some conditions, elevated cGMP concentrations in the epithelial cell are associated with increased active sodium absorption[4] whereas in others, the correlation is inverse[5,7]. Recently, it has been reported in a preliminary communication that the activity of guanylate cyclase (GC) in rat ileum was increased by methylprednisolone[8] (MP) treatment which enhances sodium absorption and the specific activity of the Na^+-K^+-activated adenosine triphosphatase (Na-K-ATPase) in the small and large intestine[9]. It may, therefore, be anticipated that cGMP levels also increase under MP. The present experiments were designed to test this assumption in the cecum mucosa of the rat. Although we confirm that the glucocorticoid stimulated sodium transport and Na-K-ATPase specific activity, cGMP concentrations were not significantly affected.
Materials and methods. Male Wistar rats (180–220 g) were kept on a standard diet with free access to water. MP (Urbason®, crystalline suspension, Hoechst), 30 mg/kg b.wt

Table 1. Effect of methylprednisolone on fluid and electrolyte net transport and on the transmural electrical potential difference (PD) in rat cecum in vivo

	Controls	Methylprednisolone	p-value
Fluid absorption ($\mu l/cm^2 \cdot h$)	24.5 ±2.1 (6)	61.0 ±4.4 (4)	<0.001
Sodium absorption ($\mu Eq/cm^2 \cdot h$)	4.4 ±0.3 (6)	10.1 ±0.6 (4)	<0.001
Chloride absorption ($\mu Eq/cm^2 \cdot h$)	9.4 ±0.4 (6)	13.1 ±0.6 (4)	<0.001
Potassium secretion ($\mu Eq/cm^2 \cdot h$)	0.87±0.15 (6)	1.27±0.08 (4)	<0.05
PD (at 2 h) (mV)	−14.9 ±0.7 (9)	−27.9 ±3.7 (8)	<0.005

Data are means ± SEM with the number of observations in parenthesis.

Table 2. Effect of methylprednisolone on Na-K-ATPase and Mg-ATPase specific activity and on cyclic nucleotide levels in rat cecum mucosa

	Controls	Methylprednisolone	p-value
Na-K-ATPase (μmoles P_i/h · mg protein)	1.74 ±0.11 (8)	3.67 ±0.32 (8)	<0.001
Mg-ATPase (μmoles P_i/h · mg protein)	6.71 ±0.49 (8)	6.83 ±0.44 (8)	NS
cGMP (pmoles/mg protein)	0.642±0.090 (11)	0.626±0.064 (11)	NS
cAMP (pmoles/mg protein)	9.69 ±1.81 (11)	10.33 ±1.89 (11)	NS

Data are means ± SEM with number of observations in parenthesis.
NS = not significant.

per day, was injected i.m. while control rats received an equivalent amount of 0.9% NaCl. Following the last injection at 72 h, rats were anesthetized with sodium thiobarbital (80 mg/kg i.p.). The cecum was ligated at the ileocecal and cecocolonic junctions, washed free from contents and, after insertion of a saturated KCl-agar a defined (2–3 ml) volume of 37 °C isotonic saline was introduced into the lumen. The transmural electrical potential difference (PD) was monitored using the deskinned tail tip as reference. After 2 h, the luminal fluid was analyzed for volume, sodium, potassium and chloride contents. Net transport was calculated from initial and final volumes and concentrations, and expressed in reference to the mucosal macrosurface. At the end of the experiments, the mucosa was gently scraped off to determine the specific activity of the Na-K- and Mg-ATPase in the whole homogenate. Details and validation of the various procedures have been described[10–12]. Cyclic nucleotides were measured in a separate set of experimental and control rats. At the end of an absorption period, the scraped mucosa was immediately transferred into 1 N perchloric acid, and cGMP and cAMP were extracted and assayed using methods also established in this laboratory[5]. Cyclic nucleotides were separated by column chromatography with recoveries of 75% for cGMP and 84% for cAMP. Succinylated cGMP was determined by radioimmunoassay[13], cAMP by a protein binding assay[14], and protein according to Lowry et al.[15] using bovine serum albumin as a standard.

Results and discussion. In the rat cecum, the net absorption of sodium chloride and water was stimulated by MP, as was the net secretion of potassium and the PD (table 1). MP enhanced the specific activity of the Na-K-ATPase whereas the specific activity of the Mg-ATPase remained unchanged (table 2, upper part). These data are consistent with previous findings in the jejunum, ileum and colon of the rat[9]. Table 2, lower part, presents the cell concentrations of cGMP and cAMP. The cAMP level was not affected by MP. This is not surprising since cAMP probably is not involved in Na-K-ATPase mediated sodium absorption[16,17]. Although it has been reported[8] that GC specific activity increased in the ileum under analogous conditions, cGMP levels in the cecum were not significantly altered in the present experiments (table 2). Because increased formation of cGMP may be followed by increased degradation without a major change in the cGMP level, GC activity can be augmented while the cGMP concentration is normal, as demonstrated in this communication.

The present data do not clarify the relation between intestinal sodium transport and cGMP. Enhancement of active sodium absorption by different stimuli, e.g., treatment with epinephrine in vitro[4], dietary polyethylene glycol in vivo[5] or MP can be associated with elevated, depressed or, as in the present case, unchanged levels of cGMP in the cell. In addition, exogenous cGMP or 8-bromo cGMP failed to affect sodium transport in the rabbit ileum or toad urinary bladder in vitro[4,7]. High doses of 8-bromo cGMP, however, do elicite fluid secretion in the rabbit and mouse intestine in vivo[18]. In response to heat-stable *E. coli* enterotoxin in the rabbit ileum, the cGMP cell concentration increases[18,19] via activation of guanylate cyclase[19], fluid is secreated into the lumen, and net chloride absorption is abolished[19], while a reduction in net sodium absorption has only been reported in a preliminary form[6]. Taken together, these observations suggest that there is no direct interrelation between the overall level of cGMP in the cell and active sodium absorption. It is nonetheless possible that cGMP may modulate active sodium absorption under special conditions, e.g., as a negative feedback signal to increased cytoplasmic calcium as previously proposed[5]. This concept is supported by recent experimental evidence suggesting that an increased intracellular calcium concentration diminishes sodium absorption[20] and stimulates chloride secretion[20,22] in the intestine. Increased intracellular calcium concentrations also decrease the short-circuit current and elevate cell cGMP in the urinary bladder[7]. Furthermore, the possibility exists that cGMP distribution and action may be compartmentalized within the cell[22]. Thus, future studies on the formation and distribution of cGMP during changes in ion transport, and its relation to intracellular calcium, may lead to a better understanding of cGMP actions in intestinal sodium and fluid transport.

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Changes in the free amino acids of the haemolymph of diapause and non-diapause pupae of the cotton bollworm, *Heliothis armigera* Hbn. (Lepidoptera: Noctuidae)

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Summary. The concentration of free amino acids in the haemolymph of non-diapausing and diapausing pupae of *Heliothis armigera* was investigated. 20 amino acids were detected in the haemolymph of the studied stages. Asparagine, glutamine, cystine, ornithine, histidine and valine were the predominant free amino acids at all stages. The diapause resulted in increased levels of most of the amino acids.

Insects are known to contain high levels of free amino acids in their haemolymph and tissues[1]. The significance of the high titre of free amino acids in insect haemolymph is still not fully understood. Different studies have shown that the titres of some of these amino acids change during metamorphosis[2,3]. The haemolymph of diapausing pupae of several Lepidoptera was found to contain abnormally high concentrations of several free amino acids as compared to non-diapausing species[4]. Jeffery et al.[5] also reported high amino acids in diapause larvae of *Pectinophora gossypiella.*

This paper presents quantitative data on the changes in the levels of free amino acids of the haemolymph of non-diapausing and diapausing pupae of the cotton bollworm, *Heliothis armigera.*

Materials and methods. Heliothis armigera was reared on green beans *(Phaseolus vulgaris)* in the laboratory according to Abdallah and Salama[6]. Haemolymph was collected from 2-day-old non-diapausing pupae, 15-week-old diapausing pupae and 18-week-old pharate adults by piercing the integument in the region of the 1st abdominal segment and microcapillary tubes were used to collect the haemolymph.

For the preparation of amino acid extracts, 0.5–1 ml of the haemolymph was deproteinized and freed from fats according to the method of Pant and Agrawal[7]. Free amino acids in the haemolymph extracts were separated and determined quantitatively by 2-dimensional paper chromatography according to the method detailed in a preceding paper by Boctor and Salem[8]. For each sample 6 chromatographic separations were carried out, and the average and the experimental error were calculated (table).

Results and discussion. The concentrations of 18 amino acids and 2 amides in the haemolymph of the non-diapausing and diapausing pupae of *Heliothis armigera* are listed in the table. There are some quantitative but no qualitative changes in the composition of the free amino acid pool of the non-diapausing and diapausing *Heliothis* pupae. Asparagine, glutamine, cystine, ornithine, histidine and valine are the predominant free amino acids at all stages, asparagine is the most abundant amino acid. These data agree with those obtained with *Spodoptera littoralis* which showed that asparagine is characteristic of the studied pupae[9]. Glutamine, asparagine, histidine, ornithine and cystine were also found in high concentrations in *Spodoptera* pupae. In the present investigation diapause in *Heliothis* is associated with great increase of most of the amino acids which results in about 30% increase of the free amino acid pool of the diapausing pupae as compared with the non-diapausing

Free amino acids of the haemolymph of diapausing and non-diapausing pupae of *H. armigera*

Amino acids	Non-diapausing pupae (2-day-old)	Diapausing pupae (15-week-old)	Pharate adults (18-week-old)
Glycine	155.4± 5.5	188.2± 9.6	220.5± 15.9
Alanine	60.0± 3.2	122.8± 8.4	138.4± 8.4
Serine	135.9± 7.4	225.8± 14.7	220.4± 15.0
Threonine	176.3± 12.3	282.0± 12.1	259.3± 17.7
Valine	327.8± 16.5	491.1± 31.4	553.9± 22.4
Leucine	222.4± 8.9	324.4± 12.7	343.1± 17.5
Aspartic acid	29.0± 1.8	43.0± 2.8	75.1± 3.4
Asparagine	1185.3± 36.2	1192.8± 46.5	1281.6± 95.8
Glutamic acid	67.4± 3.0	45.8± 3.8	35.5± 2.6
Glutamine	362.2± 19.5	1064.5± 80.9	631.0± 46.9
Proline	126.7± 4.5	175.2± 10.3	173.7± 12.7
Lysine	281.3± 14.7	222.6± 15.0	287.2± 14.5
Arginine	72.0± 2.8	59.7± 4.7	53.6± 3.6
Histidine	267.9± 12.5	568.4± 35.2	288.5± 12.5
Tyrosine	63.9± 4.6	89.7± 3.6	73.6± 5.7
Citrulline	54.4± 3.3	75.3± 5.3	54.2± 2.4
Ornithine	339.9± 16.9	891.8± 70.5	346.4± 17.5
Methionine	109.5± 8.1	129.5± 3.8	165.3± 9.6
Cystine	934.6± 70.9	1047.0± 41.8	628.4± 30.5
Phenylalanine	112.4± 7.3	145.8± 9.2	157.6± 11.7
Totals	5084.3	7385.4	5987.3

The values are given as μmoles amino acids/100 ml of haemolymph.