μmole respectively) were added to the solution used to hemolyse the cells. After the incubation, the radioactivity of cells and medium was determined in the supernatant after precipitation of membranes and proteins with trichloroacetic acid, using a Packard Tri-Carb liquid scintillation spectrometer, Model 3324. The leaking of the cells was estimated by measuring hemoglobin in the cells and the medium[16].

Results and discussion. Figure 1 shows the ATP driven calcium transport out of the sealed ghosts. As found by others[15], the calcium concentration inside the cells is higher than in the hemolysing solution added before resealing. This effect is probably due both to the shrinking of the ghosts upon reversal of hemolysis and some binding of calcium[17]. Cl_2MDP showed no effects, whether it was present inside or outside the cells, PPi did not produce any effect either (not shown). It is unlikely that PPi was hydrolysed during the experiment, since calcium is a strong inhibitor of inorganic pyrophosphate[18,19]. Actually we found that not more than 10% of the PPi was hydrolysed, when given at 1 mM concentration in the absence of ATP. Addition of ATP will not bind enough calcium to abolish the inhibitory effect of the latter.

Results are somewhat different for EHDP (figure 2). When EHDP was introduced into the cells, calcium concentrations found inside the cell at time 0 was higher than in the control experiment. The opposite was the case, when EHDP was added outside into the incubation medium. To analyse a possible effect on the rate of transport, assuming a first order reaction, the calcium concentrations within the cells were plotted on a logarithmic scale versus time. The following rate constants were found ($\pm$ 1 SEM): 0.0622 $\pm$ 0.0023 min^{-1} for control; 0.0522 $\pm$ 0.0035 min^{-1} for EHDP inside and 0.0693 $\pm$ 0.0032 min^{-1} for EHDP outside. The latter 2 values are significantly different ($p < 0.025$), suggesting that EHDP has a slight effect on the calcium transport out of the cell. It is possible that EHDP may bind to the cell membrane, where it may act differently on the transport whether it is outside or inside. The small difference in the rate constants could explain the difference of the Ca^{2+} content of the cells at time 0, when EHDP was present, since the calcium transport is probably not completely stopped during the time from sealing of the cells up to the beginning of the experiment, even though the cells were kept in ice. The difference in the calcium content could also be explained in that EHDP may influence the shrinking of the ghosts or possibly the binding of Ca^{2+} to the membrane. Furthermore one could argue that EHDP binds Ca^{2+} and that the transport is changed because the free Ca^{2+} concentration is decreased. This, however, is very unlikely since PPi and Cl_2MDP have a similar affinity for Ca^{2+} as does EHDP[20].

When ghosts containing labelled PPi or labelled diphosphonates were incubated, a loss of these compounds was found which was only slightly faster than hemoglobin in the case of PPi and EHDP and was the same as hemoglobin in the case of Cl_2MDP (figure 3). Thus is appears that PPi and the diphosphonates cannot easily permeate the erythrocyte membrane.

In conclusion, it seems that PPi and the 2 diphosphonates EHDP and Cl_2MDP have no striking effect on the calcium transport of red blood cells and that these compounds do not easily permeate the erythrocyte membrane.

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Influence of protein free aqueous extract of parathyroid powder on serum vitamin A level in rats

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Summary. Administration of the protein-free extract of bovine parathyroid powder to rats resulted in a significant increase of the serum vitamin A level.

The oral administration of bovine parathyroid powder, as well as its protein-free aqueous extract, showed widerange vitamin A-like effects[2]. This observation prompted us to test whether or not the protein-free and calcium-inactive (consequently parathormone-free) extract of the powder has any influence on serum vitamin A level in rats.

Preparation of protein-free parathyroid extract. Freeze-dried and micronized bovine parathyroid glands (Biofac A/S, Copenhagen) were defatted according to Aurbach[3]. Eventual presence of thyroid hormones in the powder was excluded by the organically bound iodine assay of US Pharmacopoeia[4]. 10 g of the defatted powder was successively stirred for 1 h with 3 × 100 ml of distilled water at 30 °C. The combined supernatant separated by centrifugation was freeze-dried. The residue was dissolved in 20 ml of water, then 8 ml of 60% (w/v) aqueous solution of trichloroacetic acid was added dropwise, with stirring, and left to stand for 2 h at 5 °C. The aqueous supernatant separated by centrifugation from precipitated proteins was extracted with 20 × 120 ml of ether. Traces of ether were removed by evaporation at reduced pressure at 25 °C, then the aqueous solution was freeze-dried to yield 1.2 g of dry protein free extract.

Effect of protein-parathyroid extract. 9 groups of male Wistar rats (LATI, Gödöllö, 200–220 g b.wt) were treated with different daily doses of the protein-free extract, the first one serving as control group. Each group consisted of 20 animals. Each animal was kept in a separate cage and received 20–25 g of feed daily. The standard feed contained 6 IU of vitamin A per g. The protein-free extract was administered on 8 consecutive days in 2 ml drinking water. On the 9th day, the rats were killed by decapitation. The vitamin A concentration in the serum

Effect of the protein free parathyroid extract on concentration of vitamin A in the sera of rats

Group	Daily dose (μg)	Serum vitamin A concentration (μg/100 ml)
1	–	10.10 ± 0.18
2	30	14.33 ± 0.17*
3	50	15.80 ± 0.09*
4	100	14.90 ± 0.18*
5	300	14.10 ± 0.17*
6	600	13.30 ± 0.09*
7	1500	11.60 ± 0.25*
8	3000	10.90 ± 0.17
9	6000	9.60 ± 0.11

Values are the mean ± SE of 20. * $p < 0.001$. The third group indicates the maximum level of the dose-response curve.

of each animal was determined spectrophotometrically, according to the method of Neeld and Pearson[5].

Data of the table show that administration of the protein-free extract of parathyroid gland powder to rats results in a significant increase of the serum vitamin A concentration. This suggests that the influence observed on the serum vitamin A level is connected with the presence of a new bioactive substance in the parathyroid powder. Attempts to isolate this substance are in progress.

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A new histamine metabolite, quantitatively important in chicken[1]

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Summary. After i.v. injection of ^{14}C-histamine to chicken, we identified one of the histamine metabolites as N-acetyl-methylhistamine in the urine. This new metabolite accounted for about 20% of the urinary or 14% of the administered radioactivity.

Based on analyses of faeces from chickens fed large amounts of histamine, Shifrine et al.[3] concluded that acetylation seems to be the principal pathway for histamine detoxication in this species. Since acetylation is of minor quantitative importance for the inactivation of histamine in other species, it was deemed of interest to examine whether acetylation is of major importance also in the inactivation of parenterally administered histamine in chickens.

Materials and methods. In preliminary experiments, isotope dilution technique as well as paper chromatographic methods were used unsuccessfully in an attempt to establish the catabolic pattern of histamine. However, when the ion-exchange chromatographic method of Bergmark and Granerus[4] was substituted for the above mentioned methods, it was possible to identify more than 90% of the urinary radioactivity after i.v. administration of ^{14}C-histamine. 5–10 μCi of ring-2-^{14}C-histamine with

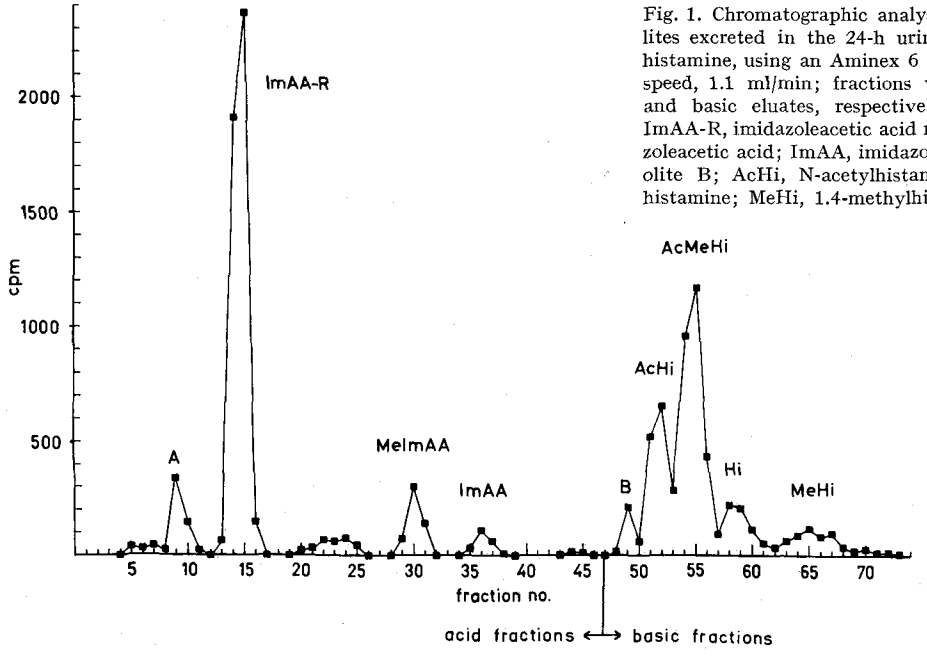

Fig. 1. Chromatographic analysis of radioactive histamine metabolites excreted in the 24-h urine after i.v. injection of ring-2-^{14}C-histamine, using an Aminex 6 resin column (0.9×60 cm). Eluation speed, 1.1 ml/min; fractions volumes, 2.2 ml and 1.1 ml for acid and basic eluates, respectively. A, unidentified metabolite A; ImAA-R, imidazoleacetic acid riboside; MeImAA, 1.4-methylimidazoleacetic acid; ImAA, imidazoleacetic acid; B, unidentified metabolite B; AcHi, N-acetylhistamine; AcMeHi, N-acetyl-1.4-methyl histamine; MeHi, 1.4-methylhistamine.