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

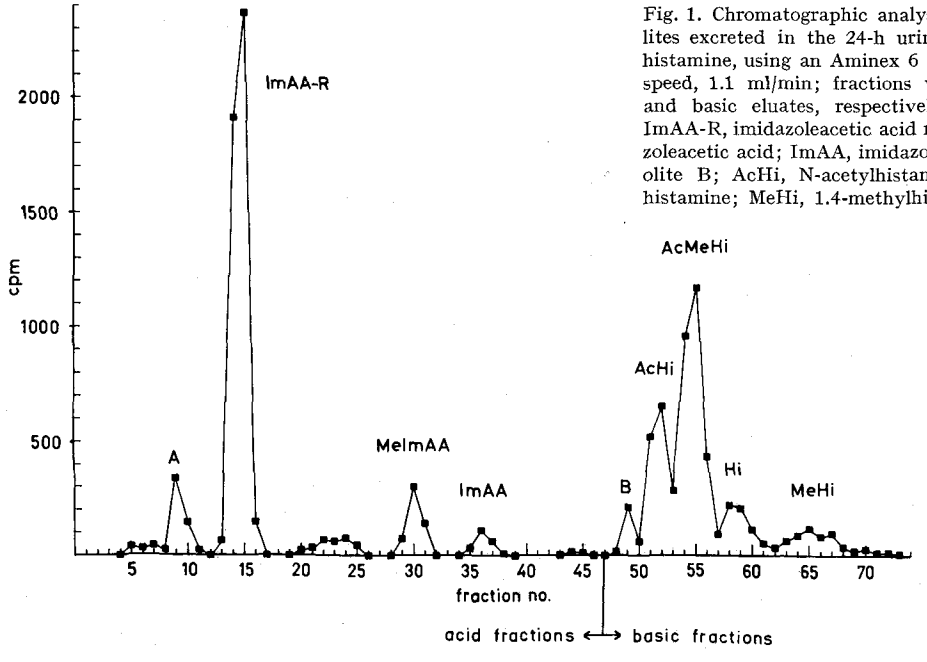

Fig. 1. Chromatographic analysis of radioactive histamine metabolites excreted in the 24-h urine after i.v. injection of ring-2-^{14}C-histamine, using an Aminex 6 resin column (0.9×60 cm). Eluation speed, 1.1 ml/min; fractions volumes, 2.2 ml and 1.1 ml for acid and basic eluates, respectively. A, unidentified 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.

a specific activity of 59 mCi/mmoles (The Radiochemical Centre, Amersham, England) were injected into the brachial vein of 'White Leghorn' chickens of either sex, weighing 2.0–3.6 kg. Urine and faeces were collected together on a shallow through placed under the birds' cages. To prevent microbiological growth in the excreta, pH was kept below 2 by adding 0.1 N HCl. In 3 hens, faeces was collected by way of a colon fistula. A plastic bag for sampling faeces was sutured to the fistula. Urine was sampled as described above.

To enable identification of the radioactive substances, reference substances[5] were added to the urine sample prior to chromatography.

Results and discussion. More than 50% of the administered radioactivity could be accounted for in the urine the first 6 h after administration of ^{14}C-histamine, while the recovery for 24 h was 70.1 ± 5.9% (SD) (n = 4). Only a small percentage of the radioactivity was excreted with the faeces. The urinary excretion of free histamine in the 3 hens fitted with a colon fistula was determined on a guinea-pig ileum after ion-exchange chromatography. The values were found to be 24, 113 and 145 μg histamine base/24 h. These values are corrected for experimental losses.

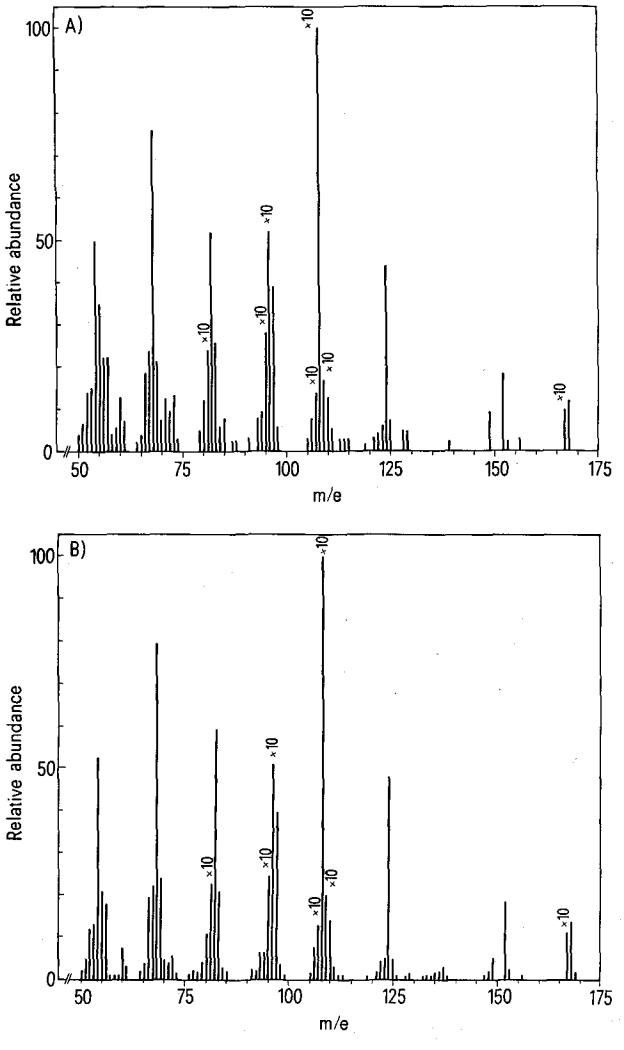

Fig. 2. Mass spectrum of: A) a histamine metabolite isolated from chicken urine, B) synthesized N-acetylmethyl-histamine. LKB 9000 mass spectrometer and direct inlet was used. Electron energy 70 eV, temperature 50 °C.

Urinary excretion of ^{14}C-histamine metabolites in 24-h specimens of urine and faeces of chicken after i.v. injection of ring-2-^{14}C-histamine, expressed as percent of excreted radioactivity

^{14}C-metabolite	A Urine and faeces, 3 pooled samples from 3 chickens	B Untreated urine from 2 chickens		C Same urine as B, after acid hydrolysis	
Acid fractions:					
Unidentified metabolite A	3.8– 8.1	4.3	6.1	1.8	2.4
Imidazole-acetic acid riboside	23.3–29.6	42.3	34.4	6.3	6.2
1.4-methylimi-dazole-acetic acid	3.5–5.8	4.5	4.0	4.9	4.5
Imidazole-acetic acid	4.2– 7.2	1.7	2.2	41.8	36.3
Basic fractions:					
Unidentified metabolite B	2.3– 2.9	2.2	2.3	1.9	3.3
N-acetyl-histamine	10.1–13.9	13.8	16.5	2.5	3.4
N-acetyl-methyl histamine + histaminol	20.4–24.1	20.4	19.6		1.6
Histamine	6.0–13.3	6.1	6.1	15.8	18.8
1.4-methyl-histamine	5.2– 7.2	2.5	4.5	19.3	22.7
Sum	– –	97.8	95.7	94.5	99.2

The elution pattern of the ^{14}C-histamine-metabolites from the column showed peaks corresponding to the kown histamine metabolites. In addition, 3 unknown peaks were observed. The largest one was found between the N-acetylhistamine and the histamine peaks (figure 1), in the histaminol fraction. The unknown substance also overlapped N-acetylhistamine to such an extent that quantitation of these metabolites was often very difficult.

Acid hydrolysis of the urine resulted in a nearly complete disappearence of the unidentified metabolite, indicating that the radioactivity of the unidentified peak was mostly originating from another substance than histaminol, since the last-mentioned compound is not hydrolysable under the present conditions. However, hydrolysis led to an increase in the radioactivity, not only in the fractions corresponding to imidazoleacetic acid and histamine, as would be expected due to deconjugation of imidazoleacetic acid-riboside and acetylhistamine respectively, but also in the 1.4-methylhistamine fraction. Since none of the known histaminemetabolites forms 1.4-methylhistamine upon hydrolysis, it was assumed that the increment in the radioactivity of 1.4-methylhistamine upon hydrolysis came from the unidentified metabolite. This was later confirmed by hydrolysis of partly purified fractions of

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the new metabolite. This observation, as well as chromatographical data, indicated that the new metabolite was acetylated methylhistamine or another hydrolysable conjugate of methylhistamine.

The radioactivity remaining in the fraction of the new metabolite after hydrolysis may be due to incomplete hydrolysis. However, the possibility also exists that at least part of the activity, but more than 2–3%, may stem from histaminol. By the methods used, quantitation of histaminol was impossible. To identify the unknown metabolite, it was necessary to separate it from N-acetylhistamine. The main fractions of the new metabolite were rechromatographed on an Aminex column until the metabolite seemed to move as a single compound.

The new metabolite is easily extractable from a basic solution with n-butanol and can be reextracted as histamine, from the organic solution with HCl. Butanol extraction followed by reextraction with HCl was combined with repeated column chromatography for isolation and purification. By starting with a 24-h urine, enough substance for direct mass-spectrographic analysis was obtained. The mass-spectra (figure 2a) indicate that the substance is N-acetyl-1.4-methylhistamine.

Reference N-acetylmethylhistamine was synthesized by methylation of N-acetylhistamine with dimethylsulfate and purified by means of TLC and butanol-HCl extractions. The purified compound could be visualized neither with ninhydrin nor by diazotation. The mass-spectrum for synthetic N-acetylmethylhistamine HCl (figure 2b) is nearly identical to that of the new metabolite isolated from the hens' urine. From this evidence, we conclude that N-acetyl-1.4-methyl-histamine is a metabolite of histamine in chickens.

About 90% of the radioactivity could be accounted for by identified histamine-metabolites, the remaining 10% may represent other metabolites such as fragments of the histamine-ring[6]. However, one cannot exclude the possibility that chromatographical effects may result in underestimation of some of the metabolites. The table demonstrates the metabolic pattern of exogenous histamine in chickens. The metabolic pattern of histamine in chicken differs largely from that found in other species. This difference is first and foremost a reflection of the new metabolite, N-acetylmethylhistamine, which accounted for about 20% of the excreted radioactivity, but also of the great quantitative importance of N-acetylhistamine. The identity of N-acetylhistamine was confirmed with mass-spectrography.

The present experiments support the conclusion of Shifrine et al.[3] that acetylation is of great importance in the detoxication of histamine in chicken. This might apply to fowls generally since in vitro experiments with liver slices and cell free extracts of pigeon liver are potent to conjugate histamine[7].

The fact that Bergmark and Granerus[4] found only very low radioactivity in the fractions where N-acetylmethylhistamine should occur suggests that this metabolite, quantitatively important in chickens, is negligible, if present at all, in man.

Whether acetylation or methylation of histamine is the first step in the formation of N-acetylmethylhistamine, and in which tissues these reactions take place, is under examination. Since neither N-acetylhistamine nor 1.4-methylhistamine possess biological activity, it seems likely that this is true also for N-acetyl-1.4-methylhistamine.

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Reduction of adenylylsulfate and 3′-phosphoadenylylsulfate in phototrophic bacteria

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Summary. Extracts of 14 species of phototrophic bacteria, partly grown with different sulfur compounds, were tested for their ability to form volatile sulfur compounds from adenylylsulfate (APS) and 3′-phosphoadenylylsulfate (PAPS). The Rhodospirillum species showed marked activities with both APS and PAPS while the Rhodopseudomonas species seem to prefer PAPS. The Chromatiaceae exhibited the strongest activities with APS, whereas Chlorobium limicola had equally high activity with PAPS.

During dissimilatory sulfur metabolism in Chlorobiaceae and Chromatiaceae, sulfite is oxidized by APS[2] reductase[3]. The relatively high levels of APS reductase found in Chromatium vinosum and Thiocapsa roseopersicina after photoheterotrophic growth with sulfate, as the sole sulfur source, led to the assumption that APS reductase might be involved also in assimilatory sulfur metabolism in these bacteria[4], i.e. in the reduction of APS to sulfite. On the other hand, none of the Rhodospirillaceae contain APS reductase[4], and the formation of PAPS[2] from sulfate and ATP has been reported to occur in chromatophores of Rhodospirillum rubrum[5].

The aim of this study was to test a number of representative species of the phototrophic bacteria as to whether they are able to form volatile sulfur compounds from PAPS or APS (or both).

Material and methods. The Rhodospirillaceae were grown on conventional media[6,7] with sulfate, or special media[8,9] with reduced sulfur compounds. The Chlorobium strain and the Chromatiaceae were cultivated in Pfennig's medium[10] with sulfide, C. vinosum also photoheterotrophically. Incubation occurred in 500 ml screw-capped bottles at 25–30 °C and about 2000 lux.

Frozen cells were thawed and suspended in a buffer containing 0.1 m Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 0.1 M KCl,

1 We thank J. F. Imhoff for skilful assistance and the Deutsche Forschungsgemeinschaft for financial support.
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