ice-like lattice surrounding these molecules need not be invoked to explain the acid shifts in pK_a. Moreover, if such lattices are responsible for lowering of the pK_a of the DNS conjugate of bovine serum albumin, it seems reasonable to expect similar behavior in the case of other protein conjugates—a conclusion unsupported by the present study.

TABLE I

pK_a VALUES FOR DNS CONJUGATES OF RNAASE AND LYSOZYME

Preparation	*Solvent*	pK_a
RNAase	0.10 M KCl	3.20
RNAase (calculated) *	0.10 M KCl	3.05
Reduced RNAase	0.10 M KCl	3.55
RNAase	8 M urea	3.75
Lysozyme	water	2.50
Lysozyme	0.10 M KCl	3.55
Lysozyme	8 M urea	3.05
Lysozyme	8 M urea, 0.10 M KCl	3.90

* Calculated from data of TANFORD AND HAUENSTEIN[11].

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Isolation of 1-methylimidazole-4-acetic acid, A metabolic product of histamine, from human urine

The major urinary excretory product of histamine in man is 1-methylimidazole-4-acetic acid; it has been identified by isotope-dilution techniques in urine of humans injected with radioactive histamine[1–3]. No reliable non-isotopic analytical method

for this substance has been published. This paper reports the first isolation and identification of the endogenous methylimidazoleacetic acid of human urine.

A 72-h specimen of urine (3.4 l), from a male patient suffering from an extensive inflammatory condition of the skin*, was adjusted to pH 10.7 with conc. ammonium hydroxide and insoluble material removed by centrifugation. 175 μg of 1-methyl-[2-^{14}C]imidazole-4-acetic acid (total activity 164000 counts/min) was added as an indicator. The urine was extracted 3 times with butanol and the latter discarded. The aqueous phase was freeze-dried, extracted with methanol and insoluble material discarded. The soluble portion was passed through an Amberlite IR-120 column on the hydrogen cycle and the 2 N ammonium hydroxide eluate collected and evaporated to dryness. This fraction was dissolved in water, passed through an Amberlite IRA-400 column on the hydroxide cycle and the effluent discarded. The 2 N HCl eluate was collected and evaporated to dryness. Passage through an Amberlite IR-120 column, as described above, was repeated to remove remaining acidic compounds. Lyophilization of the eluate yielded 1.9 g "amphoteric" materials. This fraction was dissolved in methanol and after passage through columns of silicic acid, celiulose and silicic acid again, 15 mg solids, containing 74000 counts/min, were obtained. This crude methylimidazoleacetic acid was converted to the styphnate and recrystallized. 10 mg of the styphnate, m.p. 195–197°, containing 46500 counts/min, were obtained. The isolated material was shown to be identical with the synthetic compound styphnate by mixed melting point and by comparison of infrared spectra. From the recovered radioactivity, it was calculated that the total urine sample contained 12.5 mg base, or 3.7 mg/l.

An attempt to isolate methylimidazoleacetic acid from urine of one normal male (E.A.H.) was unsuccessful.

The present work was part of a proposed study to evaluate, in man, the hypothesis that newly synthesized histamine is the mediator of the microcirculatory change during the slowly developing phase of inflammation[4,5]. However, because of the long periods of time required for the isolation procedures, only one normal and one pathological urine were examined; hence these experiments cannot be construed as evidence on the role of histamine in inflammation in man. The results do show, by unequivocal means, the existence of endogenous methylimidazoleacetic acid in the urine of one individual, and suggest to future investigators a rough guide to the concentrations which might be expected.

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* This urine sample was obtained through the kind cooperation of Professor C. T. NELSON and Mrs. J. EINBINDER, Department of Dermatology, College of Physicians and Surgeons, Columbia University. The patient had an intense petechial and exfoliative drug eruption (*dermatitis medicamentosa*); at the time of collection of the urine, the patient had not been given any of the offending drugs for several weeks.