The nucleotides of eluate peak fractions (1–7, Table I) represented less than 1 % (as measured by absorbancy at 260 mμ, pH 2) of the total nucleotides extracted. Adenine and uracil nucleotides of peaks 8–10 (Table I) constituted about 5 % of the total pool.

Ethanol–acid extracts of *Mycobacterium smegmatis* (500 mg dry weight) were evaporated under vacuum to dryness, and the resultant crystals hydrolysed with perchloric acid (0.5 ml) to give a solution of purines and pyrimidines. Using the paper chromatographic technique of BENDICH[7], adenine and guanine were estimated in these hydrolysates. These values were compared with totals obtained by summing the adenine and guanine contents of the eluate fractions.

Four-day-old *Mycobacterium smegmatis* was found to contain 11.25 ± 0.75 μmoles adenine/g dry weight of organism extracted, by paper chromatography; and 10.45 ± 0.15 μmoles adenine/g dry weight of organism extracted, by column chromatography. Corresponding results for guanine were 1.1 ± 0.1 and 1.15 ± 0.35 μmoles/g dry weight extracted, by paper chromatography and column chromatography respectively.

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Chromatography of *im*-benzyl-L-histidine

The use of derivatives of *im*-benzyl-L-histidine[1] in the chemical synthesis of peptides containing histidine is well known[2]. As *im*-benzyl-L-histidine is strongly bound to the resins normally used in amino acid analyzers, the determination of this amino acid derivative in hydrolysates of synthetic peptides can pose problems. The use of a

separate column for the determination of *im*-benzyl-L-histidine has been reported by MARSHALL AND MERRIFIELD[3].

We have resolved mixtures of *im*-benzyl -L-histidine and amino acids on a single column of an amino acid analyzer. A Technicon Amino Acid Analyzer was used with a 75 × 0.6 cm column of Chromobeads Type C-2 (Lot B-110) in the sodium form (Technicon Corp., Ardsley, N.Y.). The column was operated at 60° and eluting buffers were pumped through the columns at 0.8 ml/min. Ninhydrin color was measured at 570 mμ. The nine-chamber Autograd device[4] was used with the buffer gradients shown in Table I.

TABLE I

COMPOSITION OF BUFFER GRADIENTS

The stock solutions of citrate buffer, pH 2.75, pH 2.875, pH 3.10, pH 3.80, and pH 6.10, were prepared as directed by the Technicon Corp[4]. The column was washed with 0.2 *N* sodium hydroxide followed by equilibration with pH 3.10 buffer after every run. At 5 h running time the Autograd supplying gradient A is replaced with a reservoir of pH 6.10 buffer.

Autograd chamber no.	*Gradient A*		*Gradient B*	
	ml sodium citrate buffer	*pH*	*ml sodium citrate buffer*	*pH*
1	38	2.75 (+ 2 ml methanol)	60	3.80
2	12	2.75 (+ 28 ml pH 2.875)	60	6.10
3	40	2.875	60	6.10
4	40	3.80	60	6.10
5	40	3.80	60	6.10
6	40	6.10	60	6.10
7	40	6.10	60	6.10
8	40	6.10	60	6.10
9	40	6.10	60	6.10

Gradient A allows the resolution of an Amino Acid Calibration Mixture-Type 1 (Beckman Instruments, Palo Alto, Calif.) and *im*-benzyl-L-histidine. In this system histidine elutes at 282 min and *im*-benzyl-L-histidine at 498 min. The ninhydrin color value for *im*-benzyl-L-histidine was 0.93 that of norleucine. Gradient B allows a more rapid determination of histidine (118 min) and *im*-benzyl-L-histidine (383 min) accompanied by a loss in resolution of acidic and neutral amino acids.

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