associated with only one of the peptides produced by tryptic digestion of the inactivated enzyme protein.

These results suggest that the microbial aminopeptidase may be composed of two similar, if not identical, chains, each containing 1 super-reactive and essential tyrosine residue/protein unit of mol.wt. 30 000. A similar result was obtained with aminopeptidase from pig-kidney particles (Wachsmuth, 1967), which was found to contain 10 essential and super-reactive tyrosine residues/mol. (mol.wt. 280 000) or only 1 tyrosine residue/sub-unit. Additional experiments, involving in each case the isolation and characterization of the radioactive iodinated tyrosine peptide, are clearly necessary before the sub-unit structure of the aminopeptidases is further established.

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Polarographic Determination of Dipicolinic Acid in the Presence of Bacterial Spores and Vegetative Cells

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DPA* is a major and characteristic component of bacterial spores (Powell, 1953). The determination of DPA released from spores is important in studies of heat-resistance (Church & Halvorson, 1959) and germination (Levinson & Hyatt, 1966). Methods available for estimation of DPA involve separation of the spores from the assay system and are time-consuming (Janssen, Lund & Anderson, 1958).

DPA gives a cathodic polarographic wave in acid solution (Volke & Volkova, 1954). A polarographic procedure has therefore been devised that affords a rapid and sensitive method of determining DPA in the presence of nutrient broth, vegetative cells and spores.

Experimental procedure. A Davis differential cathode-ray polarograph (Southern Analytical A1660) was used with mercury-pool anodes. All determinations were made at 25° with 5 ml. samples

* Abbreviation: DPA, dipicolinic acid.

and each solution was flushed with N₂ (5 min.) before polarography. Peak current readings were corrected to maximum instrument sensitivity.

Method 1. Direct polarographic determinations were made with DPA solutions (100 ml.) containing 16 ml. of 0.2 M-citric acid and 4 ml. of M-Na₂HPO₄ at pH 3.3. The start potential was -1.0 v.

Method 2. DPA solutions (100 ml.) containing 5 N-HCl (10 ml.) and nutrient broth (50 ml.) were prepared and polarographed by using a start potential of -0.50 v and the 50 msec. derivative circuit.

The peak potentials occurred at about -1.3 v with method 1 and -0.8 v with method 2. Concentrations of DPA between 0.25 and 80 µg./ml. were used. Calibration curves (five points) for concentrations between 2 and 32 µg./ml. were linear with both methods (method 1: $r=0.998$, $P>0.999$; method 2: $r=0.988$, $P>0.999$). Each calibration point represented the mean of five

Table 1. *Polarographic estimation of DPA in the presence of (a) spores (method 1) and (b) vegetative cells in broth (method 2)*

Supporting electrolyte for method 1 was citric acid-phosphate buffer, pH 3.3, and for method 2 was 0.5N-HCl.

	Concn. of DPA ($\mu\text{g./ml.}$)		Difference (%)
	Weighed	Estimated	
(a)	2.5	2.55	2.0
	6.0	6.15	2.5
	12.0	12.13	1.2
	17.0	17.20	1.1
	29.0	29.30	1.0
(b)	2.5	2.60	4.0
	5.5	5.70	3.6
	10.5	10.30	1.9
	12.0	11.90	0.8
	27.0	27.30	1.1

determinations with fresh solutions made from crystalline DPA for each determination.

Effect of spores, nutrient broth and vegetative cells on DPA determination. *Bacillus stearothermophilus* N.C.I.B. 8919 spore suspensions in water (Cook & Brown, 1964) to a final concentration of about $5 \times 10^7/\text{ml.}$ were added to known concentrations (weighed) of DPA and assays were made by method 1.

An equal volume of a broth culture of vegetative cells was added to known concentrations (weighed) of DPA with HCl to 0.5N and assayed by method 2.

Table 1 shows that the assay remained sensitive

in the presence of spores, broth and vegetative cells. In no case was the difference between the assay estimate and the weighed amount greater than 4%. A direct comparison of the two methods was made by autoclaving (5 hr., 25 lb./in.²) a spore suspension of total (chamber) count $5 \times 10^8/\text{ml.}$ to release DPA (Janssen *et al.* 1958) and assaying by methods 1 and 2. This procedure was repeated to test complete release of DPA with the same suspension. There was no significant difference between the methods for DPA release by autoclaving. The mean value ($\mu\text{g./ml.}$ of spore suspension) was 68.4 ± 0.8 (8). Twin cell subtractive operation confirmed that spores did not interfere with the DPA determination with either method.

Spores release DPA in the presence of HCl (Janssen *et al.* 1958) and consequently method 2 was found to be less useful than method 1 in the presence of spores still retaining some DPA. Delay in polarographing the solutions under these circumstances led to gradually increasing readings, owing to DPA release. Both methods were satisfactory when used in the presence of spores from which the DPA was totally released. Each calibration graph was prepared in about 40 min. and a single determination took about 6 min.

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The Pathway of Synthesis of Mammary-Gland Nicotinamide-Adenine Dinucleotide

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Although it is now generally thought that the main pathway of NAD synthesis is that described by Preiss & Handler (1958), the existence of this pathway has been demonstrated in relatively few tissues [liver and erythrocytes (Preiss & Handler, 1958); *Escherichia coli* (Ismande, 1961)]. More recently,

interest has revived in the alternative route by which nicotinamide is converted into NMN and hence into NAD, and evidence has now been produced (Grunicke *et al.* 1966) that this latter route is indeed the main mechanism for NAD synthesis in ascites-tumour cells.