fast rate of change comparable only to that of the period preceding the 17th day of age. The 90th day approximates about the age of 20 years in man and the appearance in the liver of an octaploid DNA class[21].

The data and relationship for the two organs and the whole rat have been chosen only as an illustration. Similar plots for other organs and tissues for a variety of species may prove useful not only as basic information but may have practical implications. A few which come to mind immediately are the determination of optimal organ and tissue-specific radiosensitivity since the latter is known to vary with phases of the mitotic cycle[22]. A relation could also exist between animal mortality and the state of the animal's cellular populations[23]. Another use may be the calculation of optimal timing for the administration of certain classes of drugs and specifically of cytostatic compounds[24].

Zusammenfassung. Das Verhältnis zwischen Lebensdauer und stoffwechselbedingter Körpergrösse ($kg^{3/4}$) wird zur Analyse des Differenzialwachstums einzelner Gewebe und Organe herangezogen.

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PRO EXPERIMENTIS

Quantitative N-Terminal Amino Acid Analysis by Thin Layer Chromatography

The fluorodinitrobenzene reagent is widely used to detect the N-terminal amino acid in peptides and proteins[1]. A variety of column chromatographic procedures have been applied to identify and estimate the dinitrophenyl (DNP) derivatives[2–8], but these methods are laborious and time consuming or need expensive equipment. For these reasons the paper chromatographic technique described by LEVY[9] has gained in favour for quantitative determinations.

Recently, BRENNER et al.[10] applied thin layer chromatography to identify DNP-amino acids. This method has more sensitivity, gives better separation and consumes less time than paper chromatography.

Taking advantage of these convenient features we have developed a procedure for quantitative determination of N-terminal amino acid in proteins and peptides using chromatography on thin layers of silicagel.

Experimental. Since DNP derivatives are light sensitive, most operations must be performed in the dark.

0.05 to 1 μmole of the protein is dissolved in 5 ml of 0.1 *M*, pH 8.7, ammonium carbonate. If the analysis is carried out on small peptides, 1% aqueous trimethylamine[3] is used as solvent. Fluorodinitrobenzene (0.2 ml) is added and the mixture is vigorously stirred for 2 h at 40 °C. At the end of this period the excess of reagent is extracted with peroxide-free ether. To remove the remaining dinitrophenol, the extracted reaction mixture is dialyzed against distilled water and freeze-dried. This step is replaced by the sublimation technique of MILLS[6] when the method is applied to small peptides.

The purified DNP derivative is dissolved in glacial acetic acid avoiding any excess of solvent and the exact concentration of this solution is found by weight analysis[11]. The number of DNP groups present is estimated by ultraviolet absorption measurements. The following formulas are used:

$$C_1 = (1.67\ E_{290} - 0.51\ E_{370}) \times 10^{-4}$$

$$C_2 = (9.55\ E_{370} - 0.73\ E_{290}) \times 10^{-5}$$

where E_{290} and E_{370} are the absorbances measured at 290 and 370 mμ of the acetic acid solution and C_1 and C_2 are the molar concentrations of O-DNP-tyrosine and ε-NH_2-DNP-lysine respectively.

A known volume of the solution is hydrolysed with 5.7 *N* hydrochloric acid in a vacuum sealed ampoule at 100–110 °C. The acetic acid is eliminated in a flash evaporator before addition of hydrochloric acid to the ampoule. The destruction of the N-terminal amino acid derivative during the hydrolysis step is variable in each particular case[3]. The proper correction factors are estimated in parallel recovery experiments in which the unsubstituted protein is hydrolysed in the presence of a known amount of the adequate DNP-amino acid.

The N-terminal amino acid is extracted from the hydrolysate and isolated by thin layer chromatography according to BRENNER et al.[10]. The DNP-derivatives of aspartic and glutamic acids which run together in the original procedure, are resolved employing benzene:pyridine:glacial acetic acid (80:20:10) as second system of solvents.

The elution of the DNP amino acid from the chromatographic plate is performed as follows: the silica gel in the area of the DNP-amino acids spot is peeled off about 0.5 cm

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Protein	Amount employed μmoles	Conditions of hydrolysis	DNP amino acid recovery %[a]	N-terminal amino acid mole/mole	
				Present work[b]	Results from literature
Insulin (MW: 5.733)	0.24	100°C 8 h	60	Gly (0.9)	Gly (2.2)
		110°C 16 h	90	Phe (0.8)	Phe (1.8)[1c]
Pepsinogen (MW: 42.000)	0.14	110°C 16 h	70	Leu (0.8)	Leu (1.2)[13]
	0.046		61	Leu (0.8)	
Bovine growth hormone (MW: 45.000)	0.022	110°C 16 h	77	Ala (0.8)	
			79	Phe (0.9)	
	0.045	110°C 16 h	72	Ala (0.8)	Ala (0.8)
			71	Phe (1.0)	Phe (0.9)[14]
	0.081	110°C 16 h	68	Ala (0.8)	
			68	Phe (0.9)	
Aspartylamide 1-valine 5-angiotensin II (MW: 1031)	0.33	110°C 10 h	64	Asp (0.7)	Asp (0.5)[15]
Human γ-globulin (MW: 160.000)	0.056	110°C 24 h	46	Asp (1.0)	Asp (app. 1.0)
			45	Glu (1.0)	Glu (app. 1.0)[16]
	0.076	110°C 16 h	60	Asp (0.8)	
			62	Glu (0.9)	

[a] This column shows the recoveries of the corresponding free DNP-amino acids when submitted to hydrolysis conditions, in the presence of native protein. [b] Values corrected for actual recovery. [c] For 12.000 MW.

beyond its visible periphery. The powder is suspended in 1 ml of Tris buffer, pH 8.6, 0.05 *M* in a glass stoppered volumetric flask which is shaken for 5 min at room temperature. The suspension is centrifuged and the absorbance of the supernatant is determined at 360 mμ (385 mμ in the case of DNP-proline) against a blank prepared in the same way but using a similar area of silica gel from a zone of the plate where no DNP-derivative had been present at any time during the run. The absorbance is converted to μmoles using a molar extinction coefficient determined with a sample of chromatographically pure DNP-amino acid (Mann Research Laboratories).

Results and discussion. Since most DNP-proteins are soluble in glacial acetic acid, it is possible to estimate the DNP-groups attached to them by absorbance measurements at two wavelengths, without previous hydrolysis. This procedure was originally introduced by SMITH[12] for mixtures of free DNP-amino acids dissolved in 1 *M* hydrochloric acid. We have observed that the absorption curves of solutions of O-DNP-tyrosine or ε-NH_2-DNP-lysine in glacial acetic acid show a blue shift and consequently have measured the absorbance of the DNP-protein solution at slightly shorter wavelengths. The formulas used to estimate the molar concentration of O-DNP-tyrosine and ε-NH_2-DNP-lysine were established after a preliminary study of the absorption curves of acetic acid solutions of the free amino acid derivatives.

The Table shows the results obtained with five different proteins and one octapeptide. Different times of hydrolysis were applied to each sample, according to the nature of the N-terminal amino acid. Although recoveries between 97 and 100% were obtained when pure DNP-amino acids were chromatographed and eluted, their recoveries were regularly lower when submitted to the whole procedure.

All values obtained are in good agreement with those existing in the literature. It is interesting to point out that the growth hormone was a specially purified sample[17] with a biological activity almost double compared to that of LI[14].

The human γ-globulin was purified by DEAE cellulose chromatography[18] in 0.01 *M* phosphate buffer pH 8.1 and had a sedimentation coefficient of 7 S[19,20].

Zusammenfassung. Eine Methode für die quantitative Bestimmung N-endständiger Aminosäuren, Proteine und Peptide mittels Dünnschichtchromatographie wurde entwickelt.

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