Effect of D(+)-Leucine on the Hydrolysis of Glycil-L-Leucine in Normal and Neoplastic Tissues

In a previous paper[1], describing the beginning of a series of investigations on the action of D-amino-acids on L-peptidasic activity, it was observed that the effect of D(+)-leucine on hydrolysis of glycil-L-leucine by raw extracts of bovine intestinal mucosa clearly differed according to the *p*H of the reaction and to the final concentration of the D-amino-acid. D(+)-Leucine inhibited hydrolysis of glycil-L-leucine at a *p*H near the neutral point; it had no influence at an alkaline *p*H (8·5), while at an acid *p*H (6·2) it activated the reaction slightly.

Subsequent research showed that, when raw extracts of normal and pathological non-neoplastic human tissues were used in place of bovine mucosa extracts, the same results were obtained. The study of the effects produced by D(+)-leucine on the hydrolysis of glycil-L-leucine in human neoplastic tissues is therefore of considerable interest.

The material used in the present research consisted of twenty-five neoplasms (eight adenocarcinomas of the breast, three carcinomas (scirrhus) of the breast, one carcinoma of the gluteal zone, six carcinomas of the uterine cervix, two lymphosarcomas, two scapular sarcomas, two carcinomas of the rectum and one carcinoma of the penis). In all these cases it was definitely observed that D(+)-leucine affected hydrolysis of glycil-L-leucine in an entirely different way from that when normal and pathological but non-neoplastic human tissues were used; that is, from *p*H 6·2 to *p*H 8·5 it exerts a very marked activating influence. Such influence does not alter noticeably on varying the concentration of the D-amino-acid from 0·04 to 0·01 *M*; while at still lower molarity the activating effect decreases gradually, and ceases at a final concentration lower than 0·001 *M*. Control experiments with D(+)-leucine and extract alone were regularly made and no modifications at all were observed. When L(−)-leucine is used in place of its antipode, inhibitory action is, naturally, noted.

The method for the preparation of raw enzymatic extracts and for the tests of activity is described elsewhere[2], hydrolysis was determined by the measurement of liberated carboxyl groups[3], and in some experiments the liberated amino-groups were also determined (Van Slyke). For all tests, the temperature was 38° C., 0·1 *M* veronal buffer was employed and the *p*H controlled by means of a Cambridge *p*H meter, and the substrate solution was 0·025 *M* in the final reaction mixture.

Effect of D(+)-Leucine (L) on Hydrolysis of Glycil-L-Leucine (GL) by Human Non-neoplastic and Neoplastic Tissues

Conc. of D(+)-leucine	Time (min.)	*K* GL	*K* GL+L	Hydrolysis (per cent) GL	Hydrolysis (per cent) GL+L	Tissue
0·04 *M*	15	0·00305	0·00832	10	25	breast cancer
	30	0·00287	0·00780	18	42	
	60	0·00290	0·00824	33	68	
0·04 *M*	10	0·00315	0·00289	7	5	normal skin
	30	0·00305	0·00201	19	13	
	60	0·00319	0·00227	35	25	
0·01 *M*	10	0·00155	0·00706	3·5	15	uterine cancer
	30	0·00152	0·00602	10	34	
	60	0·00156	0·00562	19	54	
0·01 *M*	10	0·0173	0·0173	33	33	uterus, chronic metritis
	20	0·0173	0·0173	59	59	
	40	0·0159	0·0159	77	77	
0·01 *M*	5	0·0131	0·0309	14	39	adeno-carcinoma
	15	0·0147	0·0303	39	65	
	30	0·0136	0·0294	61	85	

Some of the results are shown in the table, where the values for hydrolysis are expressed as the first-order velocity constant calculated as a decimal logarithm (*K*); the *p*H is 7·2, since at this value D(+)-leucine (final concentration, 0·04 *M*) has a distinct inhibitory influence when non-neoplastic tissues are used.

Hence it is clear that the activating influence of D(+)-leucine on the hydrolysis of glycil-L-leucine (at *p*H 7·2) in the case of neoplastic tissues is just the opposite to the inhibitory one observed in normal and pathological non-neoplastic tissues.

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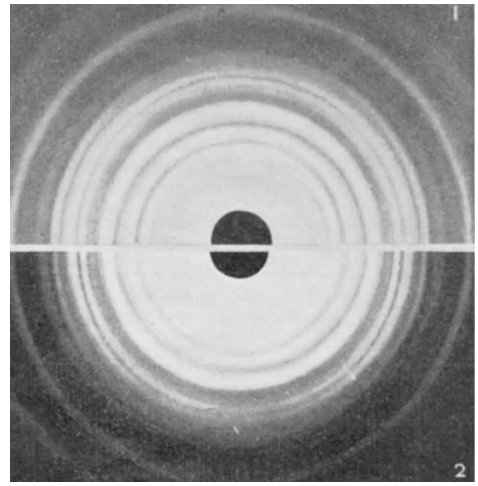
Isolation of Phosphothreonine from Bovine Casein

Lipmann has shown[1] that the phosphorus in casein is bound to serine, and this has been further confirmed by the isolation of crystalline phosphoserine in this Institute[2]; but it is also conceivable that a part of the phosphorus may be bound to threonine.

To investigate this, bovine casein was hydrolysed and a barium complex prepared, according to Lipmann. The barium was removed with a cation-exchanger ('Dowex 50'), and further purification was obtained by displacement on an anion-exchanger ('Dowex 2'), by the method of Partridge and Brimley[3]. For the final separation elution with 0·01 *N* hydrochloric acid from a 'Dowex 50' column was used. One of the fractions consisted, after evaporation, of lustrous stout prisms that yielded threonine after hydrolysis.

These prisms were compared with DL-phosphothreonine, synthesized according to Plimmer[4]. They had the same R_F value in paper chromatograms run with different solvents and the same melting point, 194° (decomp.). The crystals from the hydrolysate showed an optical rotation $[\alpha]_D^{24} - 7{\cdot}37°$, whereas the synthetic compound was inactive.



X-ray powder diffraction diagrams from (1) DL-phosphothreonine and (2) L-phosphothreonine