Verbindung im Vergleich zum Imidazol erheblich absinkt. Denselben Effekt beobachtet man auch bei Reaktion mit *p*-Nitrophenylacetat[5].

(3) Von den Verbindungen, die eine Hydroxylgruppe und den Imidazolring im *selben* Molekül enthalten, zeigt das 4-Hydroxyäthyl-imidazol die *höchste Acetylierungsgeschwindigkeit*. Die Werte für das 4-Hydroxymethyl-imidazol sind nicht zu ermitteln, da in alkalischer Hydroxylaminlösung eine Eliminierungsreaktion neben der Hydroxylaminolyse abläuft[6]. Die hohe Acetylierungsgeschwindigkeit des 4-Hydroxyäthyl-imidazols ist auf eine intramolekulare Acylübertragung zurückzuführen, die durch die mögliche Ausbildung eines Sechsringes begünstigt ist. Für diese intramolekulare Reaktion spricht die Unabhängigkeit der Acetylierungsgeschwindigkeit von der Konzentration. Die o-Acetylierungsgeschwindigkeit des 4-Hydroxyäthyl-imidazols ist 14mal grösser als die des c-his-ser und 32mal grösser als die des c-his-thr. Die Acetylierungsgeschwindigkeit der letztgenannten Peptide ist proportional ihrer Konzentration, d.h. es findet keine intramolekulare Acetylierung statt. Das zeigt auch die Übereinstimmung der Acetylierungsgeschwindigkeit des c-his-ser mit derjenigen von Acetyl-serinamid in Gegenwart von Acetyl-histidin. Gegenüber *cis* cyclo-his-ser ist die Acylierungsgeschwindigkeit des *cis* cyclo-his-thr stark erniedrigt.

Geschwindigkeitskonstanten sowie Halbwertzeiten der o-Acetylierung in Essigsäure/Acetanhydrid

	$k \cdot 10^5$ min^{-1}	$t \cdot 10^{-2}$ min
Hydroxyäthyl-imidazol[7]	973,0	0,71
Hydroxymethyl-imidazol[8]	nicht messbar	–
N-Acetyl-DL-serinamid[9]	2,3	300,0
N-Acetyl-DL-serinamid + Imidazol	165,0	4,2
N-Acetyl-DL-serinamid + Acetyl-histidin[10]	70,5	9,8
N-Acetyl-DL-serinamid + 2-Methyl-imidazol[11]	21,0	33,0
c-his-ser[1]	70,0	98,5
N-Acetyl-DL-threoninamid[12]	0,9	770,0
N-Acetyl-DL-threoninamid + Imidazol	30,1	23,0
N-Acetyl-DL-threoninamid + Acetyl-histidin	14,2	48,8
c-his-thr[1]	3,3	208,0
c-asp-ser[12]	2,0	346,0
c-asp-ser + Imidazol	115,8	6,0
c-asp-ser + Acetyl-histidin	50,3	13,7

c = cyclo

Summary. o-Acetylation of cyclo-his-ser and cyclo-his-thr in acetic acid/acetanhydride proceeds via intermolecular, and in the case of 4-hydroxyethyl-imidazole via intramolecular, acyl transfer. The highest rate of acetylation is observed with 4-hydroxyethyl-imidazole, and the most effective catalyst in the case of intermolecular acyl transfer is imidazole; derivatives of imidazole are less effective. Derivatives and peptides of serine are generally more reactive than those of threonine.

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Use of *Escherichia coli* A.T.C.C. 9637 for the Asymmetric Hydrolysis of Amino Acid Derivatives

In the course of research work carried out with *Escherichia coli* A.T.C.C. 9637 cells[1], we made the following observations.

The enzymatic system present in *E. coli* cells acts on some amides producing the hydrolytic cleavage[2] of the amide linkage. Among the compounds tried, the following were hydrolysed[3]: phenylacetamide, desthiobenzylpenicillin, D-α-desthiobenzylpenicilloic acid[4], phenylacetyl-L-alanine, phenylacetyl-L-valine, phenylacetyl-L-α-amino-n-butyric acid, phenylacetyl-L-leucine, phenylacetyl-L-phenylalanine, chloroacetyl-L-phenylalanine, chloroacetyl-L-alanine, chloroacetyl-L-leucine.

In the case of the phenylacetyl derivatives, we followed the cleavage by determining the phenylacetic acid produced (see Table).

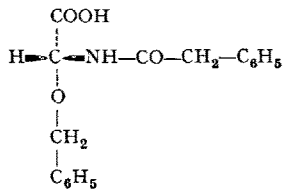
Among the compounds tested, the following did not undergo any appreciable cleavage of the amide linkage: phenylacetyl-D-alanine, phenylacetyl-D-valine, phenylacetyl-D-α-amino-n-butyric acid, chloroacetyl-D-alanine, chloroacetyl-D-phenylalanine, chloroacetyl-D-leucine,

[1] While this work was in progress, M. COLE published a note concerning substrates hydrolysed by the acylases present in *E. coli* B.R.L. 1040 cells. Nature *203*, 519 (1964).
[2] All the hydrolysis experiments were carried out at pH 7.5 and at 37°C. For one milli-mole of substrate we used 100 mg lyophilized cells of *E. coli* in 40 ml of buffer solution.
[3] It should be noted that all the experiments made refer to *E. coli* cells, in which it is not yet known if there is more than one acylase present.
[4] The asymmetric carbon atom to which the phenyl acetamido group is linked has the L-configuration. A. ROMEO and G. DI MAIO, Ann. Chim. (Rome) *47*, 676 (1957).

phenylacetyl-α-amino isobutyric acid, DL-α-benzyloxy-α-phenylacetamidopropionic acid[5], DL-α-methoxy-α-phenylacetamidopropionic acid methyl ester[5], chloroacetyl-α-amino isobutyric acid.

With the amino acid derivatives tested, it was possible to observe cleavage only in those belonging to the L-series, the acyl group being phenylacetic or chloroacetic acid and the carbon atom not being completely substituted.

For the above-mentioned compounds, which undergo enzymatic hydrolysis and have the asymmetric carbon atom containing the amide group, the configuration at asymmetric centre is known. We, however, also utilized α-benzyloxy-phenaceturic acid[5], whose configuration is not known. In our experiments with the DL-compound, one of the two forms was completely hydrolysed after 65 h, while the other was unchanged. This latter gave m.p. 106–108°, $[\alpha]_D = -2.05°$ (c = 3.8%)[6]. The configuration of the asymmetric carbon atom present in the isolated optically active compound is probably as follows:



Amino acid derivatives	Time in h	% of phenylacetic acid produced
Phenylacetyl-L-alanine	48	77
Phenylacetyl-L-valine	100	10
Phenylacetyl-L-leucine	48	50
Phenylacetyl-L-α-amino-*n*-butyric acid	48	66
Phenylacetyl-L-phenyl-alanine	48	48

Riassunto. L'azione delle acilasi presenti nelle cellule di *E. coli* A.T.C.C. 9637 è stata studiata utilizzando diversi substrati. Si è così trovato che alcuni cloroacetil e fenilacetil amino acidi subiscono un'idrolisi asimmetrica.

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[5] We shall report on the preparation of the α-alkoxy-α-phenylacetamido acids in a further note.

[6] Satisfactory elemental analyses were obtained for each new compound and optical rotation values were determined in ethanol solution.

Synergism Between Ionizing Radiation and a Cytotoxic Methylhydrazine Derivative: Effect on DNA-Degradation

The degradation of deoxyribonucleic acid (DNA) by methylhydrazine derivatives has recently been described[1]. It has been pointed out that the mechanism of action of these antitumour agents seems to show a similarity with the indirect effect of ionizing radiation[2], e.g. formation of strongly oxidizing and reducing free radicals. It therefore appeared desirable to investigate the effect of the combined action of the above mentioned compounds and of ionizing radiation on deoxyribonucleic acid.

The experiments were carried out with a 0.05% solution of sodium deoxyribonucleate prepared from calf thymus glands in 1/30 *M* phosphate buffer of pH 7, with the addition of 10% sodium chloride[3] as well as 0.002 moles/l sodium pyrophosphate to eliminate iron ions[4]. The cytotoxic compound[5] N-isopropyl-*p*-(2-methylhydrazinomethyl)benzamide hydrochloride (Natulan®) was added in solid form. The degradation of the DNA was checked by viscosity measurements with an Ostwald type viscometer (sheer stress about 200 to 500 sec^{-1}) and by the determination of sedimentation constants (extrapolation to concentration limes zero) with a Spinco analytical ultracentrifuge type E. Further experimental details have been described in previous papers[1]. For the irradiation experiments a cobalt-60 γ-ray source of 112 curies was used[6]. 10 ml of the solutions in cylindrical flasks were placed at a distance of 11.5 cm from the radiation source, the dose rate being 11,000 rad/h. The irradiation was carried out at room temperature. About 30 min after termination of the irradiation, the sampels were put in a thermostat and kept at 37°C.

Figure 1 presents the viscosity decrease of the DNA solution initiated by the following treatments: I = irradiation with 11,000 rad, II = addition of 0.0005 moles/l Natulan, III = irradiation with 11,000 rad followed immediately by addition of 0.0005 moles/l Natulan. According to the experimental results, the decrease in specific viscosity caused by the combined action of irradiation and of Natulan is substantially greater than that expected from a simple addition of both treatments. The maximum effect was obtained when Natulan was added immediately after termination of the irradiation. The effect decreased gradually as the interval between the termination of irradia-

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