residues 3 amino acids apart exhibit the maximal effect. (3) The neighbourhood of the stabilizing sequence does not influence the increase in the T_m value.

Compared with the results published by PHILLIPS and SIMPSON[4], the pronase-liberated telopeptides are very close to the tryptic digestion of arginine-rich histones, from which sequences like gly(2glu,arg).arg or lys.(pro,his).arg were isolated. For this reason, the stabilizing effect of telopeptides upon DNA is to be expected. Speculation on the possibility of an inhibitory effect of these peptides upon survival of diploid fibroblasts in a tissue culture seems to form an attractive basis for further investigation.

Zusammenfassung. Es wird festgestellt, dass die von dem Tropokollagenmolekül abspaltbare Telopeptidmischung das T_m der Thymusdesoxyribonukleidsäure erhöht. Für die Erhöhung erweisen sich die folgenden Peptide verantwortlich:

arg.glu.lys.gly.asp.gly.lys.
gly.arg.gly.arg.ser.
arg.glu.lys.gly.
gly.ala.arg.gly.arg.
lys.gly.asp.gly.lys.

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Tryptophan Production by Mutant Strains of *Escherichia coli* K 12

The purpose of this research is to investigate the development of a general method of obtaining strains able to accumulate amino acids in the medium.

It is well known that strains of *Escherichia coli* resistant to 5-methyl-tryptophan (5-MT) excrete small amounts of tryptophan and indole into the medium[1,2]. 5-MT is an analogue of tryptophan which is not incorporated into proteins but which represses the formation of the enzymes involved in tryptophan synthesis. Tryptophan and indole production has generally been attributed to the fact that strains resistant to 5-MT are non-sensitive to repression by either 5-MT or tryptophan[1,2].

Materials and methods. An Hfr strain of *E. coli* has been chosen as a starting strain. Throughout the work a minimal medium (MM) and a complete medium (CM) described by JACOB and WOLLMAN[3] have been used.

5-MT resistant mutants were selected by seeding cells of *E. coli* on solid MM supplemented with 5-MT to a final concentration of $4.10^{-4}M$.

UV- and X-rays were used as mutagenic agents at doses giving about 10% survival. The X-ray treatment was carried out in liquid medium with a Gilardoni-Neoderma Be apparatus (V = 3.3 70 Kvp, 5 uA). The depth of the medium was 1.6 mm and the dose was approximately 10^5r.

Some resistant colonies produce a small turbid halo of non-resistant bacteria. These are colonies that excrete small amounts of indole or tryptophan into the medium which permits the non-resistant cells to grow. Such colonies were picked up and streaked on MM + 5-MT; finally they were plated on the same medium to purify the mutants.

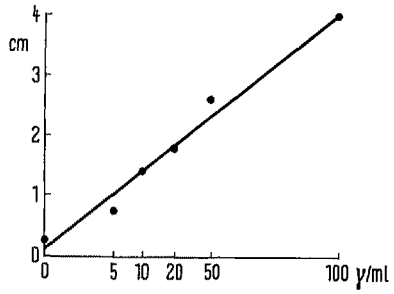
Tryptophan production has been measured microbiologically by the 'fish-spine-bead' technique by using a general method described in [4]. This technique utilizes the growth-stimulating action of tryptophan on 7-aza-tryptophan inhibited cells. The toxic action of 7-aza-tryptophan is overcome by tryptophan but not by indole (Figure). Therefore indole production was measured by the Kovacs colorimetric method[5]. For the assays the mutant strains were cultured in bottles on MM that was neither aereated nor stirred. A preliminary test has shown that either aereation or stirring do not improve production of tryptophan or indole.

The medium with the bacterial suspension was heated 1 h at 70 °C in order to kill cells and then the production titrated. Heating for 1 h at 70 °C does not destroy tryptophan; destruction of indole is approximately 10%.

Results. 60 spontaneous, 220 X-ray induced and 35 UV-induced mutants were cultured in liquid MM and the production of tryptophan and indole assayed.

Among the 315 mutants examined production varied from traces non-measurable to a maximum of 4 γ/ml and that of indole from 0–0.2 γ/ml.

Cells from 5 mutants resistant to 5-MT (code numbers D, E, Z, 3, 63) were plated on MM supplemented with 5-fluoro-tryptophan (5-FT) at the final concentration of $7.10^{-4}M$. 5-FT is an analogue of tryptophan which is probably incorporated into the proteins[6]. The 5 mutants, sensitive to 5-FT, spontaneously and after mutagenic



The dose-response curve of L-tryptophan on MM supplemented with 7-aza-tryptophan ($1.65 \cdot 10^{-3}$). On the abscissa scale the log doses of the amino acid, on the ordinate scale the diameter of growth zones in cm. The bacterium used was *E. coli* K 12. Indole is not titrated with such a system and does not disturb tryptophan titration.

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Tryptophan production in *E. coli* double mutants resistant to 5-MT and 5-FT.

	No. of mutants	Tryptophan production γ/ml	No. of mutants	Tryptophan production γ/ml	No. of mutants	Tryptophan production γ/ml	No. of mutants	Tryptophan production γ/ml	No. of mutants	Tryptophan production γ/ml
From	D[a]	2	E[a]	2	Z[a]	4	63[a]	2	3[a]	2.5
Mutagen	1	17	2	12	2	12	1	10	2	20
	1	15	1	11	1	10			1	18
	2	7	1	10	2	11			1	17
	1	5	1	7					1	12
									2	10
X-rays					1	15	1	10	1	17
					2	10	2	7	1	12
					1	7	1	5	1	11
UV-light	1	25	1	17	2	18	1	60	4	23
	1	17	1	15	1	17	1	17	1	20
	1	15	2	12	2	15	1	10	1	18
	2	12	7	10	3	12			2	17
	7	10	2	9	4	10			1	7
	1	7	2	7	3	7			1	5

[a] Strains resistant to 5-MT.

treatment, produce resistant colonies, some of which present a small turbid halo due to the growth of non-resistant cells. This type of colony has been isolated and purified with the procedure previously described.

96 strains resistant to 5-FT derived from D, E, Z, 3 and 63 have been cultivated on liquid MM and production of tryptophan and indole was titrated after 48 h of fermentation. Data are reported in the Table, and it can be seen that the second mutation for resistance to 5-FT increases the production of tryptophan to a factor of about 10.

A similar production of tryptophan by *E. coli* has been obtained by LIM and MATELES[7] with a much more complicated method and only in one instance.

In a parallel experiment we tried to produce strains resistant to 5-FT directly (by the Hfr strain non-resistant to 5-MT). No resistant and tryptophan-producing strains have been obtained by plating 10^9 cells. Also, plating 10^9 cells which survived treatment with UV- or X-rays was unsuccessful in obtaining tryptophan-producing mutants.

Evidently the mutation for resistance to 5-FT with the concomitant production of tryptophan is possible only in a strain which is derepressed in the synthesis of the enzymes of the tryptophan pathway. We therefore suggest that the second mutation gives a strain which is not only derepressed but is also insensitive to the feedback inhibition of enzyme activity induced by tryptophan on the first enzyme of the biosynthetic pathway.

This method could be a general one to obtain strains which have lost both enzymatic repression and inhibition and hence are relatively higher producers of metabolites.

Riassunto. In questa ricerca è stata messa a punto una tecnica per ottenere ceppi di *E. coli* produttori di quantità relativamente alte di triptofano. In un primo passo, sono stati selezionati mutanti di *E. coli* resistenti al 5-metil-triptofano, capaci di produrre triptofano in quantità misurabili. In un secondo passo mutativo si è aumentata la produzione di triptofano per una successiva selezione di mutanti resistenti al 5-fluoro-triptofano. La quantità di triptofano prodotta dai ceppi doppi mutanti è di circa dieci volte maggiore di quella dei singoli mutanti.

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Die postnatale Entwicklung der Bursttätigkeit im Bulbus olfactorius des Kaninchens

Nachdem seit 1892 bekannt ist, dass die elektrische Tätigkeit des Gehirns unter dem Einfluss olfactorischer Reize eine Veränderung erfährt[1], untersuchten ADRIAN[2,3], OTTOSON[4] u.a. die Form der Aktivität des Bulbus olfactorius unter verschiedenen Versuchsbedingungen. Sie fanden, dass bei Atmung von Zimmerluft atemsynchronen Schwankungen des Elektrogramms schnellere Oscillationen um 60 Hz überlagert waren, die jeweils auf der Höhe der Inspiration auftraten. Diese Olfactoriusbursts (OB) erwiesen sich in der Amplitude von der Durchströmungsgeschwindigkeit der Nase und von Geruchsreizen abhängig. Nach Tracheotomie kamen sie nicht mehr zur Beobachtung; Hypoxie brachte sie zum Ver-

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