Table 1. Presence of ε-(γ-glutamyl)lysine in enzymic hydrolyzates of cross-linked fibrin; its absence in soluble fibrin prepared by removing essential calcium with EDTA; its reduction in soluble fibrin prepared with competitive inhibitor GEE.

Sample	ε-(γ-Glutamyl) lysine (moles per mole of fibrin*)
Polymerized fibrin	1.8
Polymerized fibrin	1.2
Soluble fibrin with EDTA	0.07
Soluble fibrin with GEE	0.7
Control (no fibrinogen)	0

*Based on a fibrinogen molecular weight of 330,000.

water, and put on a column (1.1 by 23 cm) of Bio-Rad AG 2-X8 (100 to 200 mesh, acetate form) which was then washed with water (about 35 ml) until virtually all the radioactive material had been eluted. This eluate was freeze-dried, dissolved in pyridine acetate buffer (0.20*M* pyridine, *p*H 3.23), and chromatographed in a Spinco model 120C amino acid analyzer on a column (18 cm) packed with PA-35 resin prepared according to instructions for treatment prior to chromatography with pyridine acetate buffers (*15*). Fractions (1 ml) were collected; those containing ε-(γ-glutamyl)lysine (fractions 51-57) were pooled, freeze-dried, and chromatographed in the amino acid analyzer by the accelerated procedure for acidic and neutral amino acids (*16*), with the exception that a 0.20*M* sodium citrate buffer (*p*H 3.83) was used without a buffer change. Amounts of ε-(γ-glutamyl)lysine, which were eluted after 88 minutes under these conditions, were calculated by the isotope dilution method.

A direct demonstration (Table 1) of ε-(γ-glutamyl)lysine was substantiated by the values for ε-amino-cross-linked lysine determined by subjecting a portion of each sample to cyanoethylation. The procedure (*17*) may offer a generally applicable method for detecting ε-lysyl linkages in proteins, especially insoluble proteins such as fibrin and collagen. The technique consists essentially of treating the protein with acrylonitrile, which reacts with residues having free amino groups to form cyanoethyl amino acids (*18*). When the protein is subsequently hydrolyzed with acid, these are converted to carboxyethyl derivatives (such as carboxyethyllysine). Lysine residues bound in ε-amino cross-links have no free amino group to react with acrylonitrile, hence upon hydrolysis they appear as free lysine. Lysine measured in the amino acid analyzer then serves as an index of the number of cross-links. This procedure not only confirmed the above results but also proved of great value in supporting experiments. It revealed that little cross-linked lysine was formed when the substrate was human fibrinogen free of factor XIII (*19*), whereas 1.3 moles per mole of fibrin were found when a preparation of human factor XIII (*11*) was added to this substrate.

Cross-linking of fibrin is usually determined by its solubility in acid. Now it is apparent that this is not a reliable test as evidenced by the fact that even though the amount of glycine ethyl ester used was sufficient to yield an acid-soluble clot ("non-cross-linked" fibrin by this criterion), the amounts of ε-amino–cross-linked lysine and of ε-(γ-glutamyl)lysine were reduced by only half. Similarly, data obtained with the analytical ultracentrifuge showed that cross-linked fibrin may be acid soluble (*20*).

The good agreement between the amount of cross-linking measured by enzymic and chemical methods indicates that lysine is cross-linked only to glutamate and not to any other acceptor (such as aspartate). The possibility of other, completely different types of cross-links is not ruled out, nor is it certain that transamidation is the only reaction occurring during fibrin polymerization (*20*). Nevertheless, our results provide the first direct demonstration that ε-(γ-glutamyl)lysine constitutes a cross-link of polymerized fibrin (*21*).

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19 January 1968

Mutant of Bacterium paracoli 5099 with an Altered DNA: Identification as a Flavobacterium

Abstract. *A difference in the base composition of the DNA of* Bacterium paracoli *5099 and of a "mutant" (No. 1975) derived from it was found. This is in accordance with the finding of others. However, biochemical tests revealed that the "mutant" was a* Flavobacterium, *whereas the parent strain belonged to a species of* Escherichia. *The base composition of the DNA of the "mutant" is similar to that reported for the DNA of* Flavobacterium.

Our current concepts of the chemical basis of heredity equate a mutation with a change in a single nucleotide in the material (DNA or RNA) which constitutes the genome of the system under observation. The present-day techniques are not discriminatory enough to permit us to differentiate between the DNA's isolated from a wild bacterial strain and its mutants, although a start in this direction was made by the demonstration of a slight shift in buoyant density of the DNA of an *Escherichia coli* mutator strain (*1*). Therefore, the report by Gause and his collaborators (*2, 3*) of significant differences in base composition of the DNA derived from *Bacterium paracoli* 5099 and some of its mutants was of considerable interest and warranted confirmation by other laboratories. We now

Table 1. Biochemical characteristics of *Bacterium paracoli* 5099 (ATCC 23280) and its "mutant" (ATCC 23281).

Reaction characteristic	ATCC 23280	ATCC 23281	*Flavobacterium* sp.
	Primary differences (8)		
Reaction in O–F medium*	Fermenter	Oxidizer	Oxidizer
Gas from dextrose	Produced	None	None
Oxidase	Negative	Positive	Positive
Pigment	None	Yellow	Yellow
	Secondary differences		
Motility	Motile	Nonmotile	
Dextrose	Acid and gas	Acid	
Maltose	Acid and gas	Negative	
Mannitol	Acid and gas	Negative	
Nitrate reduced	Positive	Negative	
Lysine decarboxylase	Positive	Negative	
Growth on EMB† agar	Amber colonies, becoming bluish with dark centers	No growth	
Growth on MacConkey's agar	Present	No growth	
	Similarities		
H_2S	Negative	Negative	
Indole	Negative	Negative	
Methyl red	Negative	Negative	
Voges-Proskauer	Negative	Negative	
Gelatin (48 hr)	Negative	Negative	
Lactose	Negative	Negative	
Sucrose	Negative	Negative	
Catalase	Positive	Positive	
Citrate utilized	Negative	Negative	
Malonate	Negative	Negative	
Phenylalanine deaminase	Negative	Negative	
	Identification		
	Escherichia sp.	*Flavobacterium sp.*	

* Oxidation-fermentation medium (Hill-Leifson). † Eosin methylene blue.

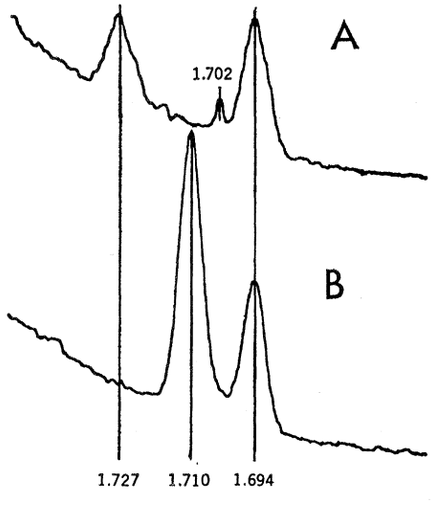

Fig. 1. Buoyant densities of DNA's isolated from ATCC 23280 and ATCC 23281. Portions of the DNA's together with a reference sample (DNA from *Clostridium perfringens*, 1.694 g/cm³) were placed in a CsCl solution (density, 1.70 g/cm³). The samples were centrifuged at 44770 rev/min for 24 hours, and photographs were then taken. The buoyant densities of the specimens were calculated (*6*) by comparison with the position of the reference DNA (*Clostridium perfringens*). In the tracings, the band to the right represents the position of the marker DNA. (A) ATCC 23281 ("mutant"); (B) ATCC 23280 (*Bacterium paracoli* 5099).

report some of our findings with *B. paracoli* 5099 and a mutant (No. 1975) derived from it (*3*).

Specimens of *B. paracoli* 5099 and of its mutant No. 1975 were obtained from the American Type Culture Collection as strains ATCC 23280 and ATCC 23281, respectively [see (*2*) and (*3*)]. Strain ATCC 23280 gave rise to white colonies on Columbia agar (*4*), whereas the mutant (ATCC 23281) yielded yellow colonies as described by Gause *et al.* (*3*). We also observed that the growth rate of ATCC 23281 was much slower than that of the parent strain in both liquid and solid media.

Bacteria were grown in nutrient broth and their DNA was isolated by the procedure described by Marmur (*5*). The DNA's so obtained were analyzed in gradients of cesium chloride to determine their buoyant densities.

The tracings reproduced in Fig. 1 represent the banding properties of these nucleic acids. The DNA derived from the wild strain (ATCC 23280) has a buoyant density of 1.710 g/cm³, whereas that isolated from the mutant has a value of 1.727 g/cm³. If the buoyant density of DNA is related to its base composition [see (*6*)], then these density values should correspond to guanine-cytosine contents of 51 and 68 percent respectively; this is in fair agreement with the values of 48 and 70.5 percent determined by Gause *et al.* (*3*) from thermal helix-coil transitions. It should be noted that the DNA from the mutant strain contains a minor component of density 1.702 g/cm³. The significance of this band is unknown; it may reflect the presence of an episome.

The two strains were subjected to a series of biochemical tests; the results are shown in Table 1. It can be seen that the two strains differ extensively. The parent strain (ATCC 23280) can be placed in the coliform group, but the "mutant" strain (ATCC 23281) can be identified as a *Flavobacterium*. For comparison, the fundamental properties of *Flavobacterium* are listed in Table 1. It is interesting and perhaps significant that the guanine-cytosine content of members of the *Flavobacterium* group falls within the range of 66 to 70 percent (*7*).

We do not wish to speculate here as to the nature of the bacterial mutants studied by Gause *et al.* (*2, 3*). Our findings indicate, however, that the specimen supplied to us by the American Type Culture Collection under code ATCC 23281, which presumably originated in Gause's laboratory (*2, 3*), is not a coliform but a member of the *Flavobacterium* group.

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