electrophoresis in urea-starch gel. The electrophoretic patterns obtained with peptides from the 2  $\beta$  chains were essentially identical, confirming the impression obtained from electrophoresis of the whole chains. The difference in mobility of the whole hemoglobins (components A and B) is thus not believed to represent a difference in amino acid sequence.

The isolated  $\beta$  chain of component C showed a major band with more cathodal mobility in urea-starch gel than that of component B (Figure 2), consistent with the mobility difference between the whole hemoglobins. The patterns obtained after cyanogen bromide cleavage of the  $\beta$  chains of components B and C were markedly different suggesting that there are multiple differences in their amino acid sequence. These findings are consistent with the results of HUTTON et al. 8 who isolated 2 components from hemolyzates of the 'diffuse' type (AKR and FL mice) by Amberlite CG-50 column chromatography. These workers identified at least 3 points of difference between the  $\beta$  chains of these 2 components by 'finger-printing' the soluble tryptic peptides.

Despite the multiple banded nature of the hemoglobin electrophoretic pattern obtained with hemolyzates from mice homozygous for 'diffuse', there appear to be only 2 components with differing amino acid sequences. These components have identical  $\alpha$  chains and vary only in their  $\beta$  chains <sup>18</sup>.

Résumé. L'hémoglobine des souris de type DBA/1J apparaît à l'électrophorèse sous forme de 4 bandes. En les séparant par l'électrophorèse en gel d'urée-amidon, on ne trouve qu'une seule ligne de globine  $\alpha$  et deux lignes de globine  $\beta$ . Les taches se montrant à l'électrophorèse après traitement au bromide cyanogène sur les chaînes peptides  $\beta$  suggèrent plusieurs différences entre les 2 séquences d'acides aminés.

E. SCHWARTZ and P. S. GERALD

Department of Pediatrics, Harvard Medical School and Children's Hospital Medical Center, Boston (Massachusetts 02115, USA), 9th June 1967.

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## Correlation between Sensitivity to Trypaflavine and DNA Base Composition in Mutants of Bacterium paracoli 5099

A few rare mutants of Bacterium paracoli with the hereditary impairment of respiration were induced by urethane and by UV-radiation. The functional inefficiency of their respiratory system was accompanied by characteristic alterations in cytochromes of the mutant cultures1. One of these mutants was later studied in greater detail, and it was observed that guanine-cytosine content in DNA (% GC) of the mutant attained 70%, as compared with 55% of the parent culture2. Some other mutants, which form small colonies but do not reveal any defects in respiration, also do not reveal any alterations in GC content of DNA as compared with the parent strain. Another important observation is concerned with the fact that the mutant with altered GC content is about 100 times more sensitive to the action of trypaflavine, whereas mutants with small colonies and normal GC content do not differ significantly from the parent culture in their sensitivity to this aminoacridine. If this correlation between drastic increase of sensitivity to trypaflavine and DNA base composition in mutants of *B. paracoli* has some real significance, it could then be exploited for the development of a new technique for isolation of mutants with altered GC content, which represent extremely rare events. This possibility was explored by us in experiments described below.

Mutants with small colonies were induced by UV-radiation at 2540 Å, with a radiation intensity of 11.53 erg/sec transmitted to each mm². B. paracoli 5099 was obtained from Type Culture Collection of U.S.S.R. (State Control Institute of Medical Biological Preparations of the Ministry of Health, Moscow). Bacteria grown on nutrient agar at 37°C for 24 h were suspended in water to the density 10° cells/ml, poured into dishes in layers 1 mm thick, and irradiated for various intervals of time between 45 and 105 sec. Under given conditions the

- <sup>1</sup> G. F. GAUSE, Nature 182, 97 (1958).
- <sup>2</sup> G. G. GAUSE, N. P. LOSHKAREVA, I. B. ZBARSKY and G. F. GAUSE, Nature 203, 598 (1964).
- <sup>8</sup> G. V. Kochetkova, M. K. Kudinova, L. P. Zimenkova and M. V. Bibikova, Mikrobiologiya 33, 587 (1964).

Table I. Growth of some small colony mutants of B. paracoli 5099 on gradient agar plates with trypaflavine

Strain	Irradiation time (sec)	Concentrations of trypaflavine in the upper layer of gradient agar plate, $\mu g/ml$										
		1000	500	250	125	62	31	16	8	4	2	1
Parent	None		+	+	+	+	+	+	+	+	+	+
Mutants												
168	75	_	-		_	_		_	_	_	+	+
975	45	_	_	-	_	_	_	_	_	+	<u>.</u>	+
1008	75	_	-	_	_		_	_	~	_	+	+
1041	90	_	_	_	_	_	_	-	_		_	+
1055	60		_	_		_	4000	-	_	_	+	+

Table II. The effect of addition of glucose (0.5%) upon respiration in the parent culture of B. paracoli 5099 and in small colony mutants susceptible to trypaflavine

Strain	Q <sub>O</sub> , (endogenous)	Q <sub>O3</sub> (exogenous)	Increase in the presence of glucose
Parent culture	13.1	54.9	4.2
Mutant 168	27.0	55.9	2.1
Mutant 975	20.5	60.7	2.9
Mutant 1008	28.3	46.6	1.6
Mutant 1041	22.9	23.1	1.0
Mutant 1055	23.1	72.3	3.1

the oxidative rate of mutants is more refractory to stimulation by glucose, and increases only 1.0-3.1 times, while in parent cells under similar conditions it increases 4.2 times.

An investigation of the effect of various inhibitors upon the growth of parent and mutant cultures of *B. paracoli* 5099 in the nutrient broth has produced results which are shown in Table III. It is clear that small colony mutants susceptible to trypaflavine are also more susceptible to 2 other inhibitors affecting DNA (streptonigrin and mitomycin C), and also to 2 inhibitors of protein synthesis in the bacterial cell (chloramphenicol and tetracycline).

For the examination of DNA base composition we isolated DNA from bacterial cells according to Marmur's procedure<sup>4</sup>. Base composition was calculated from the

Table III. Minimal inhibitory concentrations in  $\mu$ g/ml for growth in nutrient broth of various inhibitors for the parent culture B. paracoli, 5099 and its mutants

Inhibitor	Parent culture	Mutant 168		Mutant 975		Mutant 1008		Mutant 1041		Mutant 1055	
	С	С	s	С	S	С	s	С	S	С	s
Trypaflavine	250	0.5	500	0.6	416	0.5	500	0.4	624	0.6	416
Streptonigrin	4,2	0.43	10	0.63	6.7	0.43	10	0.36	11.7	0.4	10.5
Mitomycin C	10	2	5	3	3.3	2	5	0.2	50	2	5
Chloramphenicol	10.5	1.9	5.5	2.2	4.8	2.3	4.6	2.3	4.6	2	5.2
Tetracycline	15	0.6	25	0.9	17	0.8	19	0.6	25	1.1	14

C, minimal inhibitory concentrations; S, increase of sensitivity.

Table IV. GC content in DNA of the parent culture B. paracoli 5099 and of small colony mutants susceptible to trypaflavine as determined from the melting temperature

Strain	Melting temperatures $(T_m)$ of various independently purified samples of native DNA	Average $T_m$	% GC	
Parent	88.9; 89.0; 89.0	89.0	48.0	
Mutant 168	97.1; 97.0; 97.2	97.1	67.8	
Mutant 975	97.8; 97.8; 97.6	97.7	69.3	
Mutant 1008	97.6; 97.8	97.7	69.3	
Mutant 1041	97.7; 97.8	97.7	69.3	
Mutant 1055	97.7; 97.7	97.7	69.3	

survival of bacteria was of the order of 0.01%. Samples of irradiated suspensions were plated upon the nutrient agar containing 1% glucose and incubated at 37 °C for 7 days.

In these experiments 1310 mutants with small colonies were isolated and studied for their sensitivity to trypaflavine on gradient agar plates. In 1305 mutants the susceptibility to trypaflavine did not differ significantly from that of the parent culture, but in 5 mutants the sensitivity to trypaflavine was drastically increased, on the average by 200 times, as it can be seen from the data given in Table I. These susceptible mutants appear with the frequency of 0.4% among small colony mutants of B. paracoli 5099, and 5 such mutants possessing yellowish colonies (168, 975, 1008, 1041, 1055) will be described in this communication.

The respiratory capacity of the mutants with small colonies listed in Table I was studied with the aid of the Warburg technique at 37 °C, and the results of these measurements are given in Table II. It can be seen that

melting temperature  $(T_m)$  of purified samples of native DNA according to the formula<sup>5</sup>:  $T_m = 69.3 + 0.41$  (G + C)%. Table IV gives information on GC content in DNA of the parent culture B. paracoli 5099 and of small colony mutants susceptible to trypaflavine. It is of considerable interest that in mutants GC content in DNA is increased to 68-69% as compared with 48% of the parent culture.

The study of antigenic relationships has shown that there is cross-agglutination of parent culture *B. paracoli* 5099 and of mutants listed in Table IV by rabbit sera, immunized either by parent culture *B. paracoli* 5099 or by culture of mutant 168. It is therefore possible to conclude that drastic increase of sensitivity to trypaflavine can be instrumental in the isolation of some very rare mutants with altered GC content, which are induced by UV-radiation in cultures of *B. paracoli* 5099.

Выводы. Весьма редкие мутанты Bacterium paracoli 5099 с измененным нуклеотидным составом ДНК, индуцированные путем воздействия ультрафиолетовой радиации, могут быть выделены с помощью градиентных агаровых пластинок содержащих трипафлавин.

G. F. GAUSE, A. V. LAIKO, YU. V. DUDNIK, E. M. NETYKSA and G. V. KOCHETKOVA

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6 The parent culture (ATCC 23280) as well as one of the mutants (ATCC 23281) are now available in the American Type Culture Collection.