533. *Induced and Other Variations in Bacterial Cultures. Part V.* The Effect of Ultra-violet Irradiation on* Bact. coli *with Special Reference to Nutritional Mutants.*

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The effect of ultra-violet light on a strain of *Bact. coli* is studied, with special reference to the formation of nutritional mutants.

From a series of experiments on strains both fully adapted and unadapted to a simple synthetic medium, 1092 strains were isolated by various techniques. Seventy-eight of these strains had nutritional requirements of varying degrees of complexity, which have been classified.

Nutritional requirements appeared more readily than in the corresponding experiments with *Bact. lactis aerogenes* and there is an almost continuous spectrum of behaviour.

The preceding papers in this series (*J.*, 1951, 1157—1177) were concerned with a study of the changes induced in *Bact. lactis aerogenes* by ultra-violet light and some other mutagenic agents. By this action certain biochemical characters were lost and after appropriate treatment these lost abilities were regained in a gradual and quantitative manner. Specific amino-acid requirements seldom appeared and were never absolute.

In the present paper experiments in which a strain of *Bact. coli* was irradiated with ultra-violet light in an attempt to obtain nutritional mutants are described. *Bact. coli* differs from *Bact. lactis aerogenes* in that it is more reluctant to grow in a simple synthetic medium containing ammonia as nitrogen source when introduced for the first time and requires a period of training of about 20—30 subcultures before the optimal growth rate is obtained. In comparison, the growth rate of a normal *Bact. lactis aerogenes* has usually reached the optimal value within 5—10 subcultures. It seemed to be a reasonable assumption that an organism which grew less readily in the synthetic medium than *Bact. lactis aerogenes* might develop nutritional requirements more easily when irradiated. This was one reason why *Bact. coli* was chosen; other reasons were that of the experiments described in the literature in which the isolation of nutritional mutants has been claimed, many have been carried out on this organism, and also the two techniques for concentrating nutritional mutants, namely, the "screening" method (Lederberg and Tatum, *J. Biol. Chem.*, 1946, **165**, 381) and the penicillin method (Davis, *J. Amer. Chem. Soc.*, 1948, **70**, 4267), were devised by using this organism.

The investigation described in this part is more extensive than in Parts I—IV in that a much larger number of strains has been isolated and tested, but these strains have been examined less intensively in that the emphasis has been entirely on nutritional requirements. Strains both fully adapted and unadapted to a simple synthetic medium containing ammonia as nitrogen source were irradiated.

At the same time the effect of the degree of irradiation on the frequency of mutation and the efficiency of various techniques for the isolation of mutants were examined. These are discussed in Part VI.

The General Characteristics of the Isolated Strains.—From a series of irradiation experiments which are summarised in Table 1, 1092 strains were isolated by various techniques and were tested for their growth requirements by methods to be described later.

These strains may be classified into several groups:

(1) Strains which grew in the standard synthetic medium on inoculation from broth. (2) Strains which grew in the standard synthetic medium when it was supplemented with asparagine. (3) Strains which grew in asparagine–glutamic acid medium. (4) Strains which required other amino-acids as supplements.

Group 4 may be further subdivided: (*a*) Growth took place when the synthetic medium was supplemented by any one of several amino-acids. (*b*) One specific amino-acid was

* Part IV, *J.*, 1951, 1173.

TABLE 1. *Summary of irradiation experiments.*

Expt. no.	Fraction surviving	Method of isolation *	No. of strains tested	Mutants †
1	$1{\cdot}3 \times 10^{-5}$	D	3	1
	$8{\cdot}0 \times 10^{-9}$	C	2	2
	$2{\cdot}0 \times 10^{-9}$	C	2	0
2	$2{\cdot}0 \times 10^{-7}$	C	4	0
3	$1{\cdot}7 \times 10^{-7}$	C	4	0
	,,	D	2	0
4	$1{\cdot}6 \times 10^{-7}$	C	6	0
	,,	A	75	1
	,,	B	80	1
5	$2{\cdot}3 \times 10^{-7}$	C	4	0
	$2{\cdot}0 \times 10^{-8}$	C	1	0
	,,	A	6	2
	,,	B	60	0
	,,	D	5	0
6	$2{\cdot}6 \times 10^{-8}$	A	157	0
7	$8{\cdot}4 \times 10^{-8}$	C	23	0
	,,	A	131	3
8	$1{\cdot}6 \times 10^{-1}$	E	89	1
	$1{\cdot}0 \times 10^{-2}$	E	45	0
	$6{\cdot}0 \times 10^{-3}$	E	49	0
	$6{\cdot}0 \times 10^{-4}$	E	16	0
	$4{\cdot}0 \times 10^{-5}$	E	28	3
9	$0{\cdot}76 \times 10^{-1}$	E	91	16
	$0{\cdot}84 \times 10^{-5}$	E	75	16
	$1{\cdot}0 \times 10^{-7}$	E	19	0
	$0{\cdot}6 \times 10^{-8}$	E	15	0
10	$1{\cdot}74 \times 10^{-3}$	E	50	15
	$1{\cdot}40 \times 10^{-4}$	E	50	17

Total number of strains tested : 1092
,, .. mutants : 78

* A, Direct plating on heart broth agar. B, Direct plating on heart broth agar after growth in "Lemco" broth. C, "Screening." D, "Screening" after a period of growth in "Lemco" broth. E, Penicillin method.

† Mutants—the strains which did not grow in the standard synthetic medium on inoculation from "Lemco" broth :

Trained strain irradiated : Expts. 1, 2, 3, 4, 5, 6, 9 & 10.
Untrained ,, Expts. 7 & 8.

necessary. (*c*) Several specific amino-acids together were necessary. (*d*) Casein hydrolysate—a mixture of amino-acids and growth factors—was necessary.

The number of strains falling into each group is :

			Group 4				
Group 1	Group 2	Group 3	*a*	*b*	*c*	*d*	Total
1014	7	47	8	14	1	1	1092

The 1014 strains in Group 1 were not examined further. The remaining 78 strains appear to have a wide range of requirements of varying degrees of complexity. A list is given in Table 2. Whether these requirements are of a permanent or transitory nature is discussed in a later section. In drawing up the classification the following procedure was adopted. If a strain grew with asparagine it was included in Group 2, even although it would also grow with asparagine–glutamic acid and single amino-acids as alternatives. Likewise, a strain which grew in the presence of asparagine–glutamic acid or single amino-acids was included in Group 3. None of the strains in Group 4 grew in the presence of either asparagine or asparagine–glutamic acid alone.

Normal strains of *Bact. coli* can always utilise asparagine as nitrogen source, although the ability to utilise ammonia varies from strain to strain. A training process is usually necessary before growth takes place with optimal efficiency when an ammonium salt is the nitrogen source. The strain used in the present investigation was capable of growth in the

standard synthetic medium (ammonium sulphate as nitrogen source) on inoculation from broth, but about 20 subcultures were necessary before the optimal growth rate was obtained. Of the 1092 strains isolated, 78 had lost this ability to grow in ammonia, and of these all but 7 (Group 2) were even unable to utilise asparagine. It is interesting to note here that of the remaining 71 strains, 47 (Group 3) grew in asparagine–glutamic acid medium. In the experiments on *Bact. lactis aerogenes* reported in the previous paper (*J.*, 1951, 1159)

TABLE 2. *Nutritional requirements of mutant strains.*

Strain no.	Supplements with which growth took place	Lag (hr.)	Lag (hr.) in standard synthetic medium * Sub. 1	Sub. 2	Sub. 3
P 34/1	Asparagine + glutamic acid	15—24	<15	—	—
	Leucine	15—24	<15	—	—
	Asparagine	6—15	<15	<7	<13
	All amino-acids	65—85	<15	—	—
P 34/2	Tryptophan	6—15	<22	<15	<14
	Cysteine	55—65	<22	—	—
	Asparagine + glutamic acid	55—65	44—48	—	—
	Leucine	111—120	<22	—	—
P 34/7	Asparagine + glutamic acid	28—42	28—42	<19	4—7
	Valine	28—42	No growth		
	Leucine	28—42	28—42	<19	—
	Tyrosine	28—42	No growth		
	*iso*Leucine	28—42	No growth		
	Threonine	28—42	28—42	<19	—
	Hydroxyproline	28—42	28—42	<19	—
	Tryptophan	42—49	28—42	<19	—
	Histidine	42—49	28—42	<19	—
	Serine	70—77	20—24	<15	—
	Cysteine	112—120	20—28	<16	—
	Asparagine	124—139	<16	<15	—
P 34/80	Leucine	30—46	15—24	<15	<15
	Lysine	10—21	<19	8—24	9—24
P 34/136	Asparagine + glutamic acid	<9	No growth		
	All amino-acids	9—21	No growth		
	Serine	39—48	No growth		
	Valine	48—57	16—24	24—39	<14
	Asparagine	48—57	40—48	15—24	16—24
	Lysine	48—57	No growth		
	Leucine	48—57	No growth		
P 34/187	Asparagine + glutamic acid	8—22	No growth		
	Lysine	8—22	39—47	<17	14—20
P 34/190	Leucine	46—54	<15	15—24	16—25
P 34/426	Asparagine + glutamic acid	<16	No growth		
	All amino-acids	<16	<30	40—48	<16
P 34/509	All amino-acids and growth factors	<15	No growth		
	Casein hydrolysate	<15	No growth		
P 34/529	Valine	16—37	<14	—	—
	Threonine	16—37	15—23	16—24	<15
P 34/628	Valine	24—42	35—44	<15	<16
	*iso*Leucine	72—88	<15	—	—
	Threonine	96—111	<15	—	—
	Asparagine	168—192	<24	—	—
P 34/768	Histidine	24—42	120—136	—	—
	Valine	24—42	120—136	<15	<15
	Leucine	64—72	96—120	—	—
	Cysteine	96—111	48—72	—	—
	*iso*Leucine	96—111	48—72	—	—
	Tryptophan	130—154	Not tested		
	Tyrosine	130—154	Not tested		
	Methionine	130—154	Not tested		
P 34/778	Asparagine + glutamic acid Not tested in other amino-acids	4—24	24—38	<15	<15
P 34/789	Asparagine + glutamic acid Not tested in other amino-acids	4—24	24—38	8—24	<15

TABLE 2—*continued.*

Strain No.	Supplements with which growth took place	Lag (hr.)	Lag (hr.) in standard synthetic medium * Sub. 1	Sub. 2	Sub. 3
P 34/1011	Asparagine + glutamic acid	<15	<24	<15	<15
1028	,, ,,	,,	<24	<15	<15
1059	,, ,,	,,	<24	<15	<15
1061	,, ,,	,,	No growth		
1067	,, ,,	,,	<24	<15	<15
1072	,, ,,	,,	49—64	<24	<15
1073	,, ,,	,,	24—48	<15	<15
1074	,, ,,	,,	No growth		
1080	,, ,,	,,	<24	<15	<15
1104	,, ,,	,,	<24	<15	<15
1108	,, ,,	,,	<24	4	2
1110	,, ,,	,,	No growth		
1113	,, ,,	,,	72—96	<24	<15
1134	,, ,,	,,	15—24	<15	<15
1143	,, ,,	,,	No growth		
1149	,, ,,	,,	No growth		
1167	,, ,,	,,	No growth		

The strains in this group were only tested with asparagine as supplement and in asparagine–glutamic acid medium.

P 34/1026	Tryptophan	<15	72—87	<15	<15
1047	,,	,,	24—39	<15	<15
1084	,,	,,	<24	<17	3
1106	,,	,,	57—72	24—39	<15
1107	,,	,,	15—24	<15	3
1112	,,	,,	72—87	24—39	<15
1114	,,	,,	24—39	<15	<15
1154	,,	,,	144—159	<24	<15
P 34/1045	All amino-acids	<15	144—159	72—96	24—39
P 34/1055	Tyrosine	24—39	<16	<17	<15
	Tryptophan	<15	24—44	<24	<15
P 34/1064	Asparagine	53—68	<15	<15	<15
1157	,,	<15	<15	<15	<15
1161	,,	<16	<15	<15	<15

These strains were only tested in asparagine.

P 34/1091	Tryptophan	<15	<15	<15	<15
	*iso*Leucine	144—159	<15	<15	<15
P 34/1156	All amino-acids singly	<15	—	—	—
	All amino-acids together	<15	<15	<15	<15
P 34/1206	Asparagine + glutamic acid	<15	<15	<15	<15
1207	,, ,,	,,	No growth		
1208	,, ,,	,,	<15	<15	<15
1210	,, ,,	,,	No growth		
1212	,, ,,	,,	<15	<15	<15
1222	,, ,,	,,	<15	<15	<15
1223	,, ,,	,,	<15	<15	<15
1225	,, ,,	,,	<15	<15	<15
1228	,, ,,	,,	No growth		
1237	,, ,,	,,	No growth		
1254	,, ,,	,,	No growth		
1263	,, ,,	,,	24—39	<15	<15
1274	,, ,,	,,	No growth		
1277	,, ,,	,,	48—62	15—24	<15
1280	,, ,,	,,	No growth		
1283	,, ,,	,,	No growth		
1285	,, ,,	,,	No growth		
1287	,, ,,	,,	<15	<15	<15
1289	,, ,,	,,	No growth		
1290	,, ,,	,,	No growth		
1294	,, ,,	,,	No growth		
1296	,, ,,	,,	<15	<15	<15
1298	,, ,,	,,	<15	<15	<15
1300	,, ,,	,,	No growth		
1302	,, ,,	,,	No growth		

The strains in this group were only tested with asparagine as supplement and in asparagine–glutamic acid medium.

TABLE 2—*continued.*

Strain no.	Supplements with which growth took place	Lag (hr.)	Lag (hr.) in standard synthetic medium * Sub. 1	Sub. 2	Sub 3
P 34/1211	Arginine	72—87	<15	<15	<15
P 34/1233	Tryptophan	<15	<15	<15	<15
1252	,,	,,	No growth		
1256	,,	,,	<15	<15	4
P 34/1240	Tryptophan	<15	<15	<15	<15
	Leucine	24—39	<15	—	—
P 34/1258	Hydroxyproline	<15	No growth		
P 34/1265	Tryptophan	<15	<15	<15	<15
	Leucine	48—63	<15	—	—

* A dash (—) indicates that no test was made.
Sub. 1 means the first subculture after growth in the supplemental medium.

no strain was isolated which did not grow readily in the presence of asparagine and glutamic acid.

Group 4 presents an almost continuous spectrum of growth requirements. For example, in Subgroup (*a*), although asparagine and glutamic acid are not able to provide sufficient stimulus to initiate growth, other amino-acids may do so. The requirement is not specific, and there is a gradation within the subgroup. Thus strain P 34/1156 (Table 2) grew in the standard synthetic medium when supplemented singly by any of the 16 amino-acids tried, and strain P 34/768, when supplemented singly with 8 of these 16 amino-acids. Strain P 34/529 seemed to require either valine or threonine, and strain P 34/1055 either tyrosine or tryptophan. From this subgroup we pass to Subgroup (*b*), where one specific amino-acid is necessary, and from there to Subgroup (*c*), where more than one specific amino-acid is necessary. In Subgroup (*c*) the exact combination of amino-acids required was not worked out, *i.e.*, if growth was not obtained with a single amino-acid, a mixture of all the amino-acids was tried. It is possible that several combinations may have sufficed. With Subgroup (*d*) we reach the extreme of the spectrum; the member of this group, P 34/509, was incapable of growth in a mixture of all the amino-acids. Neither did it grow in a mixture of the growth factors biotin, aneurin, nicotinic acid, pyridoxine, inositol, and calcium D-pantothenate, but grew when all the amino-acids and growth factors were combined or when a commercial casein hydrolysate was used as supplement. That the requirement was not a single amino-acid together with one or more growth factors was shown by testing synthetic mixtures containing these combinations. So far the exact requirements have not been ascertained, but it seems likely that they are complex and comprise a combination of several amino-acids and growth factors.

In some of the cases in which several amino-acids could each serve as the supplement the lag with one amino-acid was shorter than with the others. To assume, as is sometimes done, that the amino-acid with which the lag is shortest constitutes the nutritional requirement would be erroneous, since in most cases the amino-acids were quite unrelated.

Many reports in the literature on the irradiation of *Bact. coli* with ultra-violet light do not give a clear indication of the state of the strain used, *i.e.*, whether the strain was fully adapted to a specific synthetic medium or not. There are other researches in which asparagine was the nitrogen source used, with the result that the bacteria would not be fully adapted to ammonia. It is possible that the degree of adaptation might have an influence on whether or not nutritional requirements were induced on irradiation. To investigate this possibility experiments were carried out with a strain of *Bact. coli* not trained to ammonia. The classification above may now be subdivided further as follows:

	Group 1	Group 2	Group 3	Group 4 *a*	*b*	*c*	*d*
Trained strain	640	6	44	6	14	1	0
Untrained strain	374	1	3	2	0	0	1
	1014	7	47	8	14	1	1

Whilst the incidence of mutants totally unable at the first subculture to grow in the standard synthetic medium was actually lower in the experiments on untrained bacteria, it is interesting that the strain with the most complex growth requirements was obtained from one of these experiments. Since other factors, such as the method used for isolating the mutants, and the degree of irradiation, are also involved in any comparison between trained and untrained bacteria, further discussion will be deferred to Part VI, in which these factors are specifically examined.

It is possible that a strain of bacteria, not trained to a synthetic medium, may contain cells unable to grow in that medium, which would be eliminated during the training process. In other words, it is necessary in experiments with untrained bacteria to prove that any variants isolated were not present before the application of the deleterious agent. To investigate this point the untrained strain was plated out in a complete agar medium (to give normal and deficient cells equal opportunity for growth) and 200 colonies were isolated and tested. They all grew in less than 15 hours in the standard synthetic medium and hence it can be concluded that the variants isolated in the earlier experiments were produced by the action of ultra-violet light.

Experimental

Organism.—A strain of *Bact. coli* was used. Its biochemical reactions were: M.R. +, V.P. −, indole +, citrate −. This strain fermented D-arabinose, xylose, glucose, mannose, galactose, maltose, lactose, sorbitol, and rhamnose, producing acid and gas but neither acid nor gas was produced from raffinose, sucrose, cellobiose, inositol, or dulcitol.

Cultivation of Organism.—The organism was trained to the standard synthetic medium by serial subculture at 40°, a stream of sterile air being passed through the medium. Training was considered to be complete when the optimal growth rate corresponding to a mean generation time of 40 minutes was reached; this generally required 20—30 sub-cultures. In experiments on untrained bacteria, 3 loopfuls of a " Lemco " broth culture were inoculated into the standard synthetic medium and the latter kept at 40° until growth had ceased.

Media.—The standard synthetic, asparagine–glutamic acid, minimal agar, and complete agar media have been described in Part II (*J.*, 1951, 1159). Amino-acid media were prepared by supplementing the standard synthetic medium with the following amino-acids: arginine, asparagine, cysteine, glutamic acid, histidine, hydroxyproline, *iso*leucine, leucine, lysine, methionine, proline, serine, threonine, tryptophan, tyrosine, and valine, either singly or in admixture and at a concentration of 0·1 g. of each amino-acid per l. The amino-acids were in most cases D + L-mixtures.

Production and Isolation of Mutants.—The methods used were those described previously (*J.*, 1951, 1159).

Testing of Mutants.—One loopful of the appropriate broth culture (of the order of 10^5 cells) was inoculated into 10 ml. of the standard synthetic medium, and the latter incubated at 37° for 2 weeks. If no growth took place the culture was inoculated (1 loopful) into 10 ml. of asparagine–glutamic acid medium and also into 10-ml. portions of the standard synthetic medium supplemented by the various amino-acids. These cultures were also incubated at 37° for 2 weeks.

When growth took place in a culture, that culture was inoculated into the synthetic medium (10 ml.) and if growth again took place serial subculture was carried out twice more to determine whether the strains would now grow in the absence of the growth requirement. In all cases the inoculum was 1 loopful.

Some strains were only tested in the synthetic medium supplemented with asparagine and in asparagine–glutamic acid medium. This is indicated in Table 2.

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