ANALYSIS OF THE *su-leuA* LOCUS IN *SALMONELLA TYPHIMURIUM*

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1. INTRODUCTION

WE have been studying a leucine auxotroph of *Salmonella typhimurium*, designated *leu*-151, which when plated on minimal medium spontaneously reverts to wild type and to a type which, in the absence of leucine, grows more slowly than the wild type. Those slow growing reversions have been shown to be due to an additional mutation at a locus linked to the leucine locus. (Smith-Keary 1960). This second mutation partially suppresses the requirement for leucine, and it has been given the symbol *su-leuA*. Thus *su-leuA*-1, *su-leuA*-2 etc. stand for independently isolated suppressors of *leu*-151.

A locus that controls the ability to ferment arabinose (*araB*) is known from transduction experiments to be linked to *leu*. We have previously shown that *su-leuA* is linked to *leu* and that the order is *su-leuA*, *leu*, *araB* (Smith-Keary, 1960). These linkages permit the genetic analysis of independently isolated *su-leuA* mutants and it is possible to show that these mutants specifically suppress *leu*-151 and not some other *leu* mutants and that they are mutations at different sites in the *su-leuA* locus. We have been able to deduce the relevant linkage map of the *su-leuA* region.

2. MATERIALS AND METHODS

The wild type and all the mutants we used were of the LT 2 strain of *Salmonella typhimurium*. Genetic material was transduced with the temperate phage PLT 22, or when sensitive transductants were required, with the H 4 mutant of this phage.

All stocks of *leu*-151 also carried *meA*-22 (requires methionine) and *tryB*-2 (requires tryptophane). In all the relevant experiments adequate amounts of methionine and tryptophane were added to the media and in the following account we shall omit references to these genes and to these requirements.

The mutants *leu*-8, *leu*-10, *leu*-20, *leu*-32, *leu*-38, *leu*-39 and *araB*-9 were obtained from the Carnegie Department of Genetics, Cold Spring Harbor, Long Island, New York.

The preparation of the phage, the method of performing the transductions and the media we used have been previously described (Smith-Keary, 1960). In this account we use the following abbreviations for the different media:

MM	minimal medium.
EMM	minimal medium enriched with broth.
MM+L	media supplemented with 0·002 per cent. dl-leucine.
EMM(arab)+LL	EMM containing 0·2 per cent. arabinose in place of glucose and supplemented with 0·004 per cent. dl-leucine.
EMB	eosin-methylene blue agar.

We characterised the colonies obtained in transduction experiments in one of two ways. When selection was made for colonies that did not require leucine, this was confirmed by streaking or plating each colony on MM and MM+L, and their ability to ferment arabinose was determined by streaking on EMB. When selection was for those colonies that could ferment arabinose, the colonies were first streaked on EMB and, from each streak, a colony that fermented arabinose was streaked or plated on MM and on MM+L to determine whether it required leucine. This sequential streaking is necessary to isolate pure *ara+* clones from the transductant colonies as these are often a mixture of *ara+* and *ara−* cells.

3. EXPERIMENTAL ANALYSIS

Before we describe the genetic analysis of the suppressor locus it is necessary to develop the argument of the previous paper in which the linkage order *su-leuA leu*-151 *leu*-39 *araB*-9 was established so as to decide whether *leu*-151 falls within or without the known limits of the *leu* locus and also whether the *su-leuA* sites are outside or inside these limits. If the *su-leuA* sites are within the *leu* locus we are probably investigating complementation within a locus; if outside we are probably investigating two separate loci.

(i) *Order of* leu *sites*

It is known that *leu*-39 is near the end of the *leu* locus nearest to *araB* and that *leu*-32 is near the end furthest from *araB*. We know that *leu*-151 is further from *araB* than *leu*-39 and we now have to show that it lies between *leu*-32 and *leu*-39. As neither *leu*-32 nor *leu*-151 is available in combination with an *araB* marker the most satisfactory tests (transducing either *leu*-151 *araB−* with phage raised on *leu*-32 *araB+* or *leu*-32 *araB−* with phage raised on *leu*-151 *araB+*, selecting for *leu+* transductants and scoring for *ara+* and *ara−*) cannot be made. Instead we used the following methods.

(*a*) *Transductions between* leu-151, leu-32 *and* leu-39. The frequency of prototrophs from transductions between *leu*-32, *leu*-151 and *leu*-39 in all possible combinations are set out in table 1. That the frequency obtained from the transduction *leu*-39 (×) *leu*-32 is higher than from the transduction *leu*-39 (×) *leu*-151 indicates that *leu*-151 is probably located between *leu*-32 and *leu*-39. The only comparison inconsistent with this conclusion is the higher frequency of prototrophs from the transduction *leu*-151 (×) *leu*-39 than from the transduction *leu*-32 (×) *leu*-39. This inconsistency is probably attributable to the greater effectiveness of *leu*-151 than *leu*-32 as recipient. This greater effectiveness is clearly shown when transductions are made with phage grown on a wild type strain (table 1, last column).

(*b*) *Transductions between* araB-9 *and* leu-32, leu-151 *and* leu-39. Phage grown on each of the leucine mutants was used to infect *araB*-9. *ara+* transductants were selected by plating on EMM(arab)+LL and these were then tested for their ability to grow in the presence and absence of leucine. The results are set out in table 2. The results of reciprocal transductions, selecting for leucine independence and

testing the transductants for their ability to ferment arabinose, are set out in table 3.

TABLE 1

Wild type transductants per 10^8 survivors in reciprocal transductions between different leu auxotrophs

Recipient \ Donor	*leu*-32	*leu*-151	*leu*-39	Wild type
leu-32 . . .	0	185	189	3250
leu-151 . .	17·5	0	283	5048
leu-39 . . .	617	314	0	2989

TABLE 2

Relative frequencies of leu⁺ *and* leu⁻ *transductants from the transductions* araB-9 (×) leu-32, araB-9 (×) leu-151 *and* araB-9 (×) leu-39, *selecting for* ara⁺ *transductants*

	Donor		
	leu-32	*leu*-151	*leu*-39
leu⁺ . . .	148	100	35
leu⁻ . . .	76	68	81
Total . .	224	168	116
Per cent. *leu*⁻	33·93	40·48	69·83

TABLE 3

Relative frequencies of ara⁺ *and* ara⁻ *transductants from the transductions* leu-32 (×) araB-9, leu-151 (×) araB-9 *and* leu-39 (×) araB-9, *selecting for* leu⁺ *transductants*

	Recipient		
	leu-32	*leu*-151	*leu*-39
ara⁺ . . .	228	162	145
ara⁻ . . .	135	100	260
Total . . .	363	262	405
Per cent. *ara*⁻	37·19	38·17	64·20

In both series of experiments the frequency of joint transduction of the selected and unselected marker (per cent. *leu*⁻ in table 2, per cent. *ara*⁻ in table 3) was highest with *leu*-39, lowest with *leu*-32 and intermediate with *leu*-151. As the frequency of joint transduction usually decreases with increase in the distance between the markers, though not necessarily proportionately, these results confirm the position of *leu*-151 between *leu*-32 and *leu*-39. Further confirmation

of the relative positions of *leu*-32 and *leu*-151 is provided by the results of the transductions described in section 3.2(*b*).

Recently Margolin has sent us representative mutants of the 4 complementation groups which he has discovered within the *leu* locus: *leu*-121, *leu*-129, *leu*-126 and *leu*-128. Margolin has shown that these complementation groups correspond to the linear arrangement of the sites (Margolin, 1959) and that their linkage order is (personal communication):

121	129	126	128	araB
I	II	III	IV	

complementation groups

From the results of the transduction *leu*-121 *araB*-9 (×) *leu*-151 and its reciprocal we have found that *leu*-121 is nearer *araB* than is *leu*-151. Thus the order of these sites and *leu*-32 is *leu*-32, *leu*-151, *leu*-121.

No abortive transductions occurred when *leu*-121, as recipient, was transduced with either *leu*-151 or *leu*-32, but these did occur when *leu*-129 or wild type was used as the donor. We conclude that *leu*-151 and *leu*-32 are in the same complementation group as *leu*-121. This is consistent with the linkage data and suggests that there is unlikely to be an additional complementation group to the left of Margolin's group I.

(ii) su-leuA *and* leu *as separate but linked loci*

We have already shown that the first isolated suppressor of *leu*-151 (*su-leuA*-1) is linked to *leu* and *araB*. By the following two series of experiments five other independently isolated suppressors have been shown to be linked, and no suppressor of *leu*-151 has been found which is not linked to *leu* and *araB*.

(*a*) *Linkage of suppressors with* leu *and* araB. Phage grown on each of the six suppressed mutants (*su-leuA*-1 *leu*-151, *su-leuA*-2 *leu*-151 etc.) was used to infect *leu*-39 *araB*-9 and selection made for leucine independent transductants. Most of these transductants were slow growing. These slow growing colonies were tested for their ability to ferment arabinose and the frequency of joint transduction of *su-leuA-x leu*-151 and *araB*+ showed non-significant variation between 53·1 per cent. and 57·2 per cent. with the different suppressors. Significant variation is not expected as this figure measures the relative frequencies of cross-overs in regions *a* and *b* and in regions *a* and *c* and this is independent of the position of the *su-leuA* site.

	su-leuA-x		leu-151	ara+
Donor				
	a		*b*	*c*
Recipient				
	+		leu-39	araB-9

These results show that the suppressor site in each of the six suppressed mutants is linked to the *leu* and *araB* loci.

(*b*) su-leuA *and* leu *as separate loci.* Four different suppressed mutants were used as donors in transductions with *araB*-9 as recipient. *ara*⁺ transductants were selected and then characterised for their ability to grow in the absence of leucine. These transductants must arise, ignoring quadruple cross-overs, by one crossover in region *a* and the second in region *b* (wild type for leucine) or in region *c* (auxotroph for leucine) or in region *d* (suppressed mutant—grows slowly in absence of leucine).

	su-leu-A		leu-151		+	
Donor						
	d	*c*		*b*		*a*
Recipient						
	+		+		ara-B9	

As all three classes can be detected, the results (table 4) provide an estimate of linkage of *su-leuA* with *araB*-9. These results give an average of 45·28 per cent. joint transductions of *leu*-151 and *araB*-9 and an average of 43·67 per cent. joint transductions of *su-leuA* and

TABLE 4

Relative frequencies of fast growing, slow growing and leucine auxotroph transductants from the transduction araB-9 (×) suleuA-x leu-151 araB⁺ *using different* su-leuA *mutants, selecting for* ara⁺ *transductants*

su-leuA allele of donor (*x*)	ara⁺ clones tested	Fast		Slow		Auxotroph	
		Observed	Per cent.	Observed	Per cent.	Observed	Per cent.
1	108	63	58·33	45	41·67	0	0
4	331	184	55·59	139	41·99	8	2·42
2	214	113	52·80	98	45·79	3	1·40
6	89	46	51·69	42	47·19	1	1·12
Totals	742	406	54·72	324	43·67	12	1·62

araB-9. The suppressor locus is thus quite close to *leu*-151. Because of this close linkage it is necessary to consider the possibility that the suppressor sites are within the limits of the leucine locus.

Table 2 shows that there is 33·93 per cent. joint transduction of *leu*-32 with *araB*-9, but, while it is reasonable to take this with other evidence as showing that the order is *leu*-32 *leu*-151 *araB* the data are not sufficiently extensive to decide whether *leu*-32 is relatively far from or very close indeed to *leu*-151. This latter possibility is indicated

Y 2

by the similarity in the percentage of joint transductions of *leu*-32 and *araB*-9 and of *leu*-151 and *araB*-9 when selection is for *leu*⁺ (table 3).

The best available evidence that the suppressor locus is beyond the known limits of the leucine locus, that is further from *araB* than *leu*-32, is from the transduction *su-leuA*-1 *leu*-151 *araB*-9 (×) *leu*-32 using H4 phage and selecting for *ara*⁺ transductants. If the order is *su-leuA*-1 *leu*-32 *leu*-151 *araB*-9 this transduction can be represented as follows:

	+	*leu*-32		+		+	
Donor							
	e	*d*		*c*		*b*	*a*
Recipient							
	su-leuA-1		+		*leu*-151	*araB*-9	

Crossovers in regions *a* and *b* will give *su-leuA*-1 *leu*-151 colonies.
Crossovers in regions *a* and *c* will give *su-leuA*-1 *leu*⁺ colonies.
Crossovers in regions *a* and *d* will give *su-leuA*-1 *leu*-32 *leu*-151 colonies.
Crossovers in regions *a* and *e* will give *su-leuA*⁺ *leu*-32.

If the order is *leu*-32 *su-leuA*-1 *leu*-151 *araB*-9 this transduction can be represented as follows:

	leu-32		+		+		+	
Donor							
	e	*d*		*c*		*b*	*a*
Recipient							
	+	*su-leuA*-1		*leu*-151		*araB*-9	

Crossovers in regions *a* and *b* will give *su-leuA*-1 *leu*-151 colonies.
Crossovers in regions *a* and *c* will give *su-leuA*-1 *leu*⁺ colonies.
Crossovers in regions *a* and *d* will give *su-leuA*⁺ *leu*⁺ colonies.
Crossovers in regions *a* and *e* will give *su-leuA*⁺ *leu*-32 colonies.

Thus, omitting genotypes arising by quadruple crossovers, all the colonies which grow as wild type in the absence of leucine will, if the first order is correct, be *su-leuA*-1 *leu*⁺; if the second order is correct they will be either *su-leuA*-1 *leu*⁺, or, *su-leuA*⁺ *leu*⁺. Whether both these genotypes arise can be investigated by taking phage sensitive transductants that grow like wild type in the absence of leucine, raising phage PLT 22 on them, and using this phage to infect *leu*-151. If such a transductant is *su-leuA*-1 *leu*⁺ both " fast " and " slow " colonies will be produced; five such transductants were scored in this way and all were *su-leuA*-1 *leu*⁺. Two transductants from a similar cross involving *su-leuA*-4, one from a cross involving *su-leuA*-5 and one from a cross involving *su-leuA*-2 were also found to be *su-leuA*-4 (or 5 or 2) *leu*⁺.

We conclude that while there remains the slight possibility that *su-leuA* is between *leu*-32 and *leu*-151 (being much closer to *leu*-32 than to *leu*-151) it is more likely that *su-leuA* is outside the known limits of the *leu* locus. This likelihood is increased by the discovery that one of the suppressors (*su-leuA*-1) is a multisite mutant (see next section) and it would seem unlikely that a single site mutant in the leucine locus could be suppressed by such a major disturbance within the same complementation group of the locus.

These transductions are further evidence for the relative positions of *leu*-32 and *leu*-151. If *leu*-32 were nearer to *araB* than *leu*-151, the initial transductants would be unlikely to carry the suppressor mutation and no slow growing colonies would arise in the second transduction with *leu*-151 as recipient.

(iii) Linkage order of the suppressor sites

If the independently isolated suppressed mutants arise by mutations at different sites within the *su-leuA* locus it should be possible to plot the linkage order of these sites. Consider a transduction *su-leuA-x leu*-151 *araB*-9 ($\times$) *su-leuA-y leu*-151 where x and y are mutations at different sites of the *su-leuA* locus. If y is distal to x it should be possible to discover leucine auxotrophs among the *ara*+ transductants: these are unlikely to be discovered if y is proximal to x.

	su-leuA-y		+		*leu*-151		+	
Donor								
		d		*c*		*b*		*a*
Recipient								
		+		*su-leuA-x*	*leu*-151		*araB*-9	
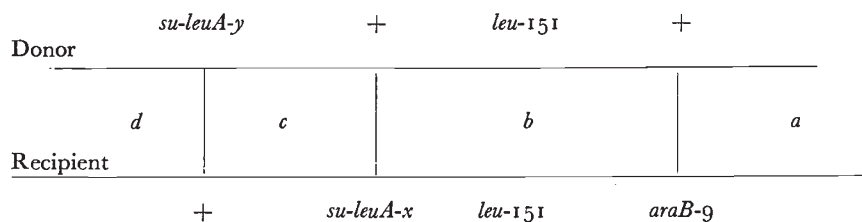
Those *ara*+ transductants that arise by crossovers in *a* and *c* will be leucine auxotrophs.

	+		*su-leuA-y*		*leu*-151		+	
Donor								
		d		*c*		*b*		*a*
Recipient								
		su-leuA-x		+		*leu*-151		*araB*-9
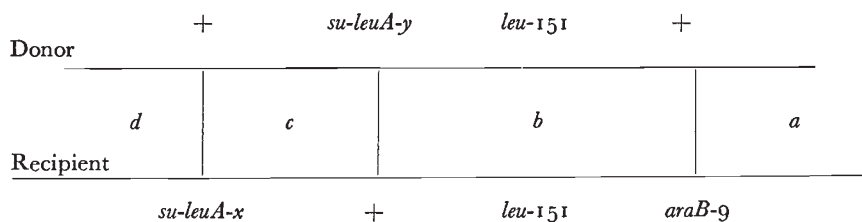
Only those very rare transductants that arise by quadruple crossovers in *a* and *b*, and in *c* add *d* will be leucine auxotrophs.

Such an analysis of the six independently isolated suppressed mutants requires that each of these *su-leuA-x leu*-151 genotypes be introduced into the *araB*-9 stock. This was done by transducing

su-leuA-x *leu*-151 into *leu*-39 *araB*-9 with H4 phage and selecting for transductants that grow in the absence of leucine. From among these phage sensitive, arabinose non-fermenting colonies that only grow slowly in the absence of leucine were chosen. Since *su-leuA-x* does not suppress *leu*-39 (see next section) the strains obtained must be *su-leuA-x* *leu*-151 *araB*-9.

The proportions of leucine auxotrophs that were found among the *ara*+ transductants when the six suppressed mutants were crossed in all possible ways are set out in table 5. It is conspicuous, and in

TABLE 5

su-leuA-x leu-151 araB-9 (×) su-leuA-y leu-151, *selecting for* ara+ *transductants.*
Number of leucine auxotrophs among ara+ *transductants tested*

		su-leuA allele in donor (su-leuA-y)					
		1	2	3	4	5	6
	1	0/100	0/58	0/80	0/90	0/92	0/110
su-leuA	2	0/92	0/75	0/75	1/104	0/105	0/73
allele in	3	0/92	1/64	0/60	2/30	1/143	0/80
recipient	4	0/103	0/70	0/60	0/60	0/30	0/60
(*su-leuA-x*)	5	0/94	2/82	0/153	8/144	0/30	4/192
	6	0/91	1/68	0/192	4/90	0/160	0/130

accord with the above argument, that auxotrophs are never found in both of the two possible transductions involving any two suppressed mutants.

su-leuA-1 differs from the other suppressed mutants in yielding no auxotrophs in any of the crosses. We conclude that this is a multisite mutant (Demerec *et al.*, 1956) extending over the whole distance marked by the mutant sites of the other suppressed mutants. If this were interpreted as a deletion extending close to the end of the *leu* locus it would explain the absence of auxotrophs from the transduction *araB*-9 (×) *su-leuA*-1 *leu*-151 (table 4).

Excluding *su-leuA*-1 and transductions with homologous phage we can make the following deductions of the order of the *su-leuA* sites, with reference to *araB*, from each of the crosses:

su-leuA donor	su-leuA recipient	Deduced order	su-leuA donor	su-leuA recipient	Deduced order
2	3	2-3-*araB*	4	5	4-5-*araB*
2	4	4-2-*araB*	4	6	4-6-*araB*
2	5	5-2-*araB*	5	2	2-5-*araB*
2	6	2-6-*araB*	5	3	5-3-*araB*
3	2	2-3-*araB*	5	4	4-5-*araB*
3	4	4-3-*araB*	5	6	6-5-*araB*
3	5	5-3-*araB*	6	2	2-6-*araB*
3	6	6-3-*araB*	6	3	3-6-*araB*
4	2	4-2-*araB*	6	4	4-6-*araB*
4	3	4-3-*araB*	6	5	6-5-*araB*

With the exception of the transduction where *su-leuA*-6 was in the donor and *su-leuA*-3 in the recipient, from which about two auxotrophs were expected and none found, all the results agree with the order of the sites being:

$$su\text{-}leuA \qquad 4 \text{-} 2 \text{-} 6 \text{-} 5 \text{-} 3 \text{———————————} araB$$
$$\longleftarrow \text{—} \text{I} \text{ —} \longrightarrow$$

This order is in full agreement with the crude estimates of the degree of linkage of *su-leuA*-4, *su-leuA*-2 and *su-leuA*-6 with *araB* previously obtained (table 4).

The proportion of auxotrophs from the transduction *su-leuA-x leu*-151 *araB*-9 ($\times$) *su-leuA-y leu*-151 (table 5), which is a rough measure of the distances between the *su-leuA* sites, and the proportion of auxotrophs from the transductions *araB*-9 ($\times$) *su-leuA-x leu*-151 (table 4), which is a rough measure of the distances between the *su-leuA* sites and *leu*-151, are of the same order of magnitude. Thus *su-leuA* and *leu* are probably adjacent loci, and site 151 is close to the end of the *leu* locus nearest the *su-leuA* locus, but not quite as close as *leu*-32.

(iv) *Specificity of suppressors*

We have tested three of the suppressors to discover whether they will suppress other leucine mutants than *leu*-151. The three suppressors tested were *su-leuA*-1, 2 and 5, and the leucine mutants were *leu*-32, 151, 121, 8, 10, 20, 38 and 39. Of these leucine mutants the first three are known to be in the same complementation group; we do not know the complementation groups of the other leucine mutants.

Phage PLT 22 grown on each of 3 stocks separately carrying the 3 mutant suppressors in combination with *leu*+ (obtained from the experiments described in section 3 (ii) (*b*)) was used to infect each of the leucine mutants. Leucine independent transductants were selected and these were examined to discover whether they included any which had slow growth in the absence of leucine. If slow growing transductants were not found we concluded that the *su-leuA* mutant site did not suppress that particular leucine mutant. Slow growing transductants were recovered only when *leu*-151 was infected.

su-leuA-1, 2 and 5 therefore specifically suppress *leu*-151 and do not even suppress other mutant sites in the same complementation group as *leu*-151.

4. NOTE ON METHODOLOGY

We know very little about many of the factors that influence the outcome of a transduction experiment with *Salmonella typhimurium*. For example we are not certain whether all transduced bacterial genetic fragments that include a particular locus are of identical length. The use of some strains as recipient inexplicably results in a larger number of transductants than the use of other strains that are very similar

genetically. We know too little about the degree of negative inter-
ference between crossovers to be able to take satisfactory account of this
in our predictions. A transduced fragment of bacterial genome may
undergo a number of successive incorporations in the recipient cell
and its descendants. These complications make us wary of reaching
decisive conclusions from particular experiments. For example, the
experiments described in section 1 indicate that *leu*-32 is further from
araB than is *leu*-151 but they do not rule out the possibility that the
reverse is true. The additional evidence in section 3 (ii) (*b*), makes
the reverse order more unlikely. We can only conclude that, on the
available evidence the order *leu*-32 *leu*-151 *araB* is the most likely. All
our conclusions are simply the most probable explanations of the data,
after ignoring complications that we have no means of assessing.

We would emphasise two technical problems in the present study.
First, when leucine-independent transductants are selected by plating
on media without leucine, the distinction between slow growing (sup-
pressed mutants) and fast growing (wild type) transductants cannot
be made on the transduction plates. Each colony must be picked off
and replated on media without leucine so as to give isolated colonies.
This involves considerable labour and accounts for some of the data
not being more extensive than they are. Second, it is known that a
transduced fragment carrying *araB* may undergo successive incorpora-
tions in the recipient cell and its descendants. Thus when we select
for transduction of *leu*+ and then streak out the colonies to score for
arabinose fermentation some may be scored *ara*+ which, if it had been
possible to score only the result of the incorporation that introduced the
leu+, would have been scored *ara*-. There may therefore be in many
experiments an excessive proportion of *ara*+ and a deficiency of *ara*-.
There is, however, no good reason for supposing that this bias varies
significantly from experiment to experiment, as we used the same
arabinose marker in all the experiments.

5. DISCUSSION

The *su-leuA*, *leu* and *araB* factors are linked and this has permitted
a genetical analysis of the *su-leuA* locus. We have shown that in-
dependently isolated suppressed mutants of *leu*-151 arise by mutations
at different sites within the suppressor locus. It seems that any of a
number of mutations, even a multisite mutation, within *su-leuA* will
specifically suppress *leu*-151 and will not suppress seven other *leu*
mutants, two of which are in the same complementation group as
leu-151. It might be thought possible formally to restate these con-
clusions in terms of complementation and not suppression and say
that many mutational changes within *su-leuA* complement *leu*-151.
However the term " complementation " is usually reserved for a
situation where a wild type phenotype only occurs when either both or
neither mutant sites are present, and not when only one is present,

and here the single mutant *su-leuA leu+* is not an auxotroph but has a nearly wild type phenotype, *su-leuA leu-151* an intermediate phenotype, and only *su-leuA+ leu-151* is an auxotroph. While it is convenient to maintain this distinction between " suppression " and " complementation " it is by no means certain that the biochemical basis of these phenomena will necessarily always be of a different type. That *su-leuA* and *leu* are probably adjacent regions is perhaps a hint that the biochemical basis of this suppression may be similar to those mechanisms which are invoked to explain complementation within a functional unit. Thus *su-leuA* may be part of the same functional unit as the *leu* locus, having the property that at least some of the possible mutational changes within it only slightly inhibit leucine synthesis and complement a small minority of mutations within the *leu* part of the unit.

Among other microorganisms site specific suppressors are known in *Neurospora* (Giles and Partridge, 1953; Yanofsky and Bonner, 1955) and *Aspergillus* (Käfer, 1958), but in these and other previous studies a genetic analysis has either not been possible or has not been attempted. It may well be that site specific suppression will be found that has its basis in mutation at a single site in a suppressor locus, but the present study has demonstrated that here at least, such suppression arises by many different site, and multisite, mutations within the one locus.

6. SUMMARY

1. Slow growing reversions of *leu-151* have been shown to be due to mutations at a suppressor locus, *su-leuA*, linked to *leu* and *araB*.

2. *su-leuA* is probably adjacent to and does not overlap the *leu* locus.

3. Five separate sites within the *su-leuA* locus have been plotted. *su-leuA-1* is probably a multisite mutant.

4. *su-leuA-1, 2* and *5* suppress *leu-151* but do not suppress *leu-8, 10, 20, 32, 38, 39* or *121*.

5. A linkage map of the *su-leuA leu araB* region has been constructed and the probable order of the markers shown to be:
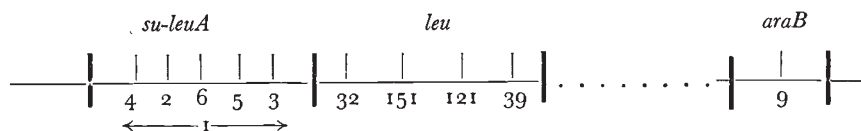
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