UNCLASSIFIED

AD 404 540

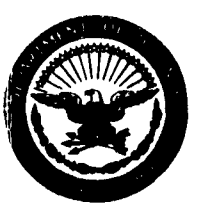
Reproduced
by the

DEFENSE DOCUMENTATION CENTER

FOR

SCIENTIFIC AND TECHNICAL INFORMATION

CAMERON STATION, ALEXANDRIA, VIRGINIA



UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

63 3-4

CATALOGED BY ASTIA
AS AD NO. 404540

CONTRACTOR:- G.W.P. Dawson,
Department of Genetics,
Trinity College,
Dublin.

REPORT:- Final Technical Report
September, 1961

SUBJECT:- To investigate the variety of bacterial mutants occuring spontaneously and produced by various radiations and chemicals.

CONTRACT NUMBER:- DA-91-591-EUC-1863.

PERIOD:- 1st October, 1961 - 30th September, 1962.

The research reported in this document has been made possible through the support and sponsorship of the U.S. Department of Army, through its European Research Office.

G.W.P. DAWSON.

DDC
MAY 23 1963
TISIA A

ASTIA AVAILABILITY NOTICE
QUALIFIED REQUESTORS MAY OBTAIN COPIES OF THIS REPORT FROM ASTIA.

PERSONNEL.

Mr. G.W.P. Dawson (Reader in Genetics)	- part time.
Dr. P.F. Smith-Keary.	- full time.
Miss S. Riyasaty.	- full time. (retired 31/3/62)
Mr. R. W. Hedges.	- full time. (from 1/4/62)
Technician & Secretary	- part time.

EXPENDITURE:

For Sept. 1st 1961 to Aug. 31st 1962.

Salaries	£1,200
Technician & Secretary	320
Overheads	200
Materials	280
	£2,000

The man hours involved in the carrying out of this contract were approximately:

G.W.P. Dawson	10 hr./week	(part time).
P.F. Smith-Keary	45 Hr./week	(full time)
S. Riyasaty	45 hr./week	(full time to 31/3/62)
R.W. Hedges	45 hr./week	(full time from 1/4/62)
Technician & Secretary	20 hr./week	(part time).
	120 hr./week.	

ABSTRACT.

The main body of the report is an account of studies on strains of Salmonella typhimurium which show instability at particular loci. The more extensive study was on the locus that suppresses the requirement for leucine of strain leu 151. This suppressor locus is adjacent to the leucine locus. Instability was shown to occur at different sites in this suppressor locus in different strains. The site of instability can undergo forward and back mutation - the instability being retained at the site through these successive mutations. The instability can change from one locus to another - for example the aquiring of instability by a locus affecting requirement for proline usually, but not always, is accompanied by the loss of instability from the suppressor of leucine requirement. These and related observations are interpreted in terms of units that attach to the loci - by analogy with, on one hand, controlling episomes in maize and, on the other hand, episomes in bacteria they are called controlling episomes. From a study of loci affecting tryptophane requirement there is some evidence that controlling episomes, recognised by the induction of instability, may depress the effeciency of gene expression.

1. Episomic control of instability in Salmonella typhimurium.

G.W.P. DAWSON and P.F. SMITH-KEARY.

In a previous annual report we have demonstrated that (i) unstable slow growing strains derived from the leucine requiring auxotroph leu-151 are due to attachment of a controlling episome at a mutant site within a suppressor locus (su-leuA) which is closely linked to leu; (ii) the controlling episome can be retained at a su-leuA site through successive back and forward mutations at that site, (iii) the controlling episome can be transposed from su-leuA to other known loci, resulting in stability at su-leuA and instability at the new site of attachment; (iv) different strains contain different frequencies of controlling episomes.

These controlling episomes are in many ways similar to other bacterial episomes, whose characteristic feature is that they can exist either free in the cytoplasm (autonomous state) or integrated with the chromosome. The existance of a controlling episome as a unit integrated with the chromosomes is adequately demonstrated by its high frequency of transduction with the affected locus, while its transposition from one locus to another shows that it can exist in an autonomous state, however transitory this may be. Evidence that supports our comparison of the transposition state with the autonomous state is provided by the following experiments, which show that controlling episomes can be partly eliminated by treatment with acridine orange, a chemical known to 'eliminate' episomes when in the autonomous state.

The first series of experiments was as follows:-

1. A single colony (A) of leu-151 (araB-9 genome) [a strain believed to contain a relatively high number of available controlling episomes] was inoculated into 5 broth and into 5 broth + 0.001% acridine orange cultures. The pH of all the cultures was adjusted to 7.6 with N.NaOH.
2. After the cultures had grown to saturation, 0.1 ml. samples, each containing 1×10^8 surviving bacteria, were plated on enriched minimal medium.
3. After 4 days incubation every reversion was characterised by subculturing onto medium without leucine.
4. The experiment was repeated using 4 further single colony inocula (B,C,D,E).

The results of these experiments are shown on Table 1, and may also be compared with the results of similar control experiments (i.e. no Acridine orange) using 5 single colony inocula (FGHIJ) of leu-151 (a strain containing a lower frequency of available controlling episomes). As in the previous experiment each inoculum was used to inoculate 5 cultures, and the reversions characterised 4 days after plating samples on enriched minimal medium (Table 2).

Comparing the numbers of stable slow (S) reversions in the control and treated cultures (col III; Table 1) it is apparent that there is no significant deveation from the 1:1 ratio expected on the assumption that these stable

reversions are not episome induced, either within each group of individual cultures or within the set of groups of cultures. [deviation $X^2 = 0.925$]. Furthermore the heterogeneity X^2 (3,0.5) has a high probability (.3 - .5), so that the groups agree both with a 1:1 ratio and with one another.

On the other hand, when the numbers of unstable slow (US) reversions are similarly compared each group except E shows a striking departure from a 1:1 ratio, there being a large deficiency of US reversions in the treated cultures. The data are homogeneous, having a heterogeneity $X^2 = 4.87$, $P = .1 - .2$.

When the group totals of the treated cultures of leu-151 (araB.9 genone) are compared with the group totals of control cultures of leu-151_e, both S:S and US:US are in agreement with a 1:1 ratio. These results are best interpreted as follows: The US reversions arise by (1) the transposition of a controlling episome from an unknown location to a su-leuA^+ site and (2) the induction of a mutation at that site.

The effect of the acridine orange has been to eliminate controlling episomes during their transposition, thus decreasing the frequency of US reversions. The frequency of F and S reversions are unaffected since the mutations involved are not induced by controlling episomes.

The effect of the acridine orange treatment has been to reduce the frequency of available controlling episomes to approximately the same frequency with which they occur in the more stable leu-151_e strain.

In a second series of experiments an unsuccessful attempt was made to produce an 'episome-free' strain. As in the previous experiment a suspension of leu-151 (araB.9 genome) was used to inoculate 3 broth CK.LM and 5 broth + NOPCR .001% acridine orange cultures. After growing to saturation, a sample of each culture was deleeted and used to inoculate a fresh culture (either broth or broth + acridine orange). This was repeated for 5 such serial passages through either broth or broth + acridine orange, and after each passage samples of each culture were plated on 5 EMM plates and the reversions characterised after 4 days' incubation. These results are shown in Table 3.

While these data are highly heterogeneous it appears that there is (I) a decrease in the frequency of US reversions after 1 passage through broth + acridine orange and (II) no further significant decrease after repeated passages through broth + acridine orange.

TABLE 1.

Frequencies of leucine independent reversions of leu-151 (ara8.9 genome) after growth in broth and in broth + 0.001% acridine orange. Groups of 5 cultures.

Reversions	Total	F	S	US	Total	F	S	US	X^2	P	X^2	P
Inoculum												
A	47	3	18	16	25	1	17	7	0.03	.8-.9	8.39	.001-.01
B	47	0	33	14	26	1	21	4	2.67	.1-.2	5.55	.01-.02
C	64	3	27	34	41	2	33	16	0.32	.7-.8	6.48	.01-.02
D	64	1	22	41	34	1	30	3	1.23	.2-.3	32.82	.001
E	46	4	29	13	30	0	23	7	0.69	.3-.5	1.82	.1-.2
					total X^2				4.94 (5)		55.06 (5)	
TOTALS	268	11	129	128	156	5	114	37	0.925	.3-.5	50.19	.001
					Heterogeneity X^2 (4)				3.015	.5-.7	4.87	0.3

TABLE 2.

Frequencies of leucine independent reversions of $leu\text{-}151_e$ after growth in broth. Groups of 5 cultures.

	Reversions observed.			
enoculum	Total	F	S	US
F	33	3	19	11
G	38	1	23	14
H	29	2	17	10
I	31	1	21	9
J	32	2	22	9
total	164	9	102	53

Comparison with leu-151 (ara8.9 genome) treated cultures.

S:S = 1:1 X^2 0.66 P .3-.5

US:US = 1:1 X^2 2.84 P .05-.1

TABLE 3.

Effect of repeated passage through broth or broth + acridine orange on the frequency of reversion of leu-151 (araD.9 genome).

a) Groups of 3 control cultures (K,L,M) grown in broth:

	Reversions Observed			Mean reversions/culture		
	F	S	US	F	S	US
After 1 passage	4	29	20	1.33	9.66	6.66
2	2	26	38	0.66	8.66	12.66
3	1	16	35	0.33	5.33	11.66
4	0	8	12	0.0	1.66	4.00
5	20	9	42	6.66	3.0	14.00
Total	27	88	147	1.80	5.86	9.80

b) Groups of 5 treated cultures (N,O,P,Q,R) grown in broth + acridine orange:

	Reversions Observed			Mean reversions/culture		
	F	S	US	F	S	US
After 1 passage	6	44	20	1.20	8.80	4.00
2	10	33	34	2.00	6.60	6.80
3	5	50	47	1.00	10.00	9.40
4	1	57	21	0.20	11.40	4.20
5	3	27	30	0.60	5.40	6.00
Total	25	217	152	1.00	4.68	6.04

C. The genetics of sexuality in E. coli: R. W. HEDGES.

Now that F' strains have been isolated in which the sex factor carries markers derived from the main chromosome it is possible to detect the presence of the episome in a strain and to select directly for retention of the factor. This being so one can attempt to isolate genetic modifications of the F factor and by studying these by genetic means.

In the experimental programme begining the F' is a Flac derived from W3747 and the recipient strains are derived from W4132 - which latter has a deletion for the 'lac' region and is streptomycin resistant. Thus W4132 is non-reverting lac^- and the F' strain (duced) is lac^+. It is known that the 'chromosomal' material of an F' strain will recombine with the homologous material of the chromosome. In this case, however, it seems plausible that the deficiency in the chromosome might be sufficient to prevent such exchange. To test this an F duced W4132 was prepared. This stock, termed W4132F', was grown overnight in nutrient broth with acridine. The resulting bacteria were, of course, almost exclusively $(F^-)lac^-$ as tested on EMB but a very small proportion of Lac^+ colonies were detected. If recombination between F' and chromosome occurred with any appreciable frequency it would seem certain that a high proportion of the selected colonies should be the result of such recombination (and hence the lac^+ character would be AO stable). All the lac^+ colonies (14) were grown and treated with AO. All were found to loose the lac^+ character when treated with AO. Thus it seems likely that very little recombination between episome and chromosome occurs in this system.

Since selection for the lac^+ character is a main feature of this programme it is obviously desirable to gain experience of the strains, effect of chemicals, etc., in lactose-salts medium. Difficulties arose and to remove any special features of lactose metabolism glucose-salts medium has also been used.

In both media there seems to be little or no ability for the W4132F' strain to support growth of 2 inders f2 phage and two Hfr strains behaved in similar fashion. Thus when plated on F' er Hfr bacteria on minimal plates f2 gives rise to no plaques. When similarly plated on minimal plates to which a single amino acid has been added (phenylalanine, isoleucine and valine have been used) plaques are observed.

Adsorption, never extremely efficient (compare Detton, R., Maccacaro G.A. & Piccinini, G.L. J. Microbiol. 9, 1961) was found to be too low in minimal or supplemented minimal to allow selection for Vf^r and lactose utilisation simultaneously. Thus selection for this mutant was contraindicated.

Acridine orange proved to have much reduced Feliminatory powers in minimal medium so selection for A.O. resistance of the F was a failure.

These technical difficulties, the discovery that other investigators at Stanford and London were interested in the same topic and the isolation of defective Fs by workers in France suggest that it will be best to abandon this research topic.

2. Estimation of tryA locus.

S. Riyasaty.

The lines of research pursued during the year concerned the mutant tryA- 47 and its primary and secondary reversions.

1. In certain transduction tests with various tryA mutants in which tryA-47 is used as donor (singly) and as recipient (with cysB-12 as the additional marker), it is found that when tryA-50 is the donor, tryA-47cysB-12 the recipient, the average percentage of prototrophic clones recovered is 1.9. In the transduction tryA-50cysB-12(x) tryA-47 one would expect a much higher or a much lower percentage of prototrophic clones, whereas three repeat experiments gave an average value of 1.75%. One of the possible models, namely the intralocus cross-suppression of the two mutants tryA-50 and tryA-47, was investigated. According to this model, the genotype of the prototrophic recombinants in one of the reciprocal transductions must be tryA-50tryA-47. Transduction of such recombinants to cysB-12 recipient with selection for cys^+ would result in the selection of approximately 20% $tryA^-$ clones.

Prototrophic clones were selected from the transductions tryA-47cysB-12(x)try A-50 and tryA-50cysB-12(x) tryA-47 and used as donors to cysB-12 with selection for cys^+ on minimal medium supplemented with anthranilic acid 200 clones from each set of transduction tests were then characterized and found to be prototrophs. Allele cross-suppression cannot therefore be responsible for the results obtained. However, allele-specific behaviour in recombination has been repeatedly reported in the literature and the results obtained with the above system may well represent the influence of structural changes at certain sites on recombination values.

2. In the genetic analysis of reverse mutations at the tryptophan A region of Salmonella typhimurium, it is found that the slow growing reversion (SxA-47)of try A-47 all map at the try A locus. Although on transduction all such reversions map at the tryA-47 site, it is hard to escape the conclusion that some at least of these SxA-47 reversions represent base-par changes other than that responsible for the tryA-47 phenotype, for consistent growth rate differences can be shown to exist between the different reversions. Since strains with enhanced growth rates (SxA-47-Ix) were obtainable from the SxA-47, it was hoped to find non-identity between the slow-growing strains (and hence demonstrate the intralocus suppressor nature of these reversions by studying their intermediate derivatives. The intermediates may represent the manifestations of the controlling effect of certain alleles on mutations at adjacent sites - the genotype of the slow-growing reversions may be: A-47-S_1, A-47-S_2 etc., and the genotype of the intermediates $A\text{-}47^+\text{-}S_1$, $A\text{-}47^+\text{-}S_2$, and the presence of S_1 and S_2 suppress the full expression of $A\text{-}47^+$. Since S_1 and S_2 would be at separate base-pairs, their action on $A\text{-}47^+$ might be different. For each slow-growing reversions, then, only one type of intermediate would be recognisable and moreover, none would be separable from the A-47: SxA-47, site.

Intermediates isolated for S_1A-47 and S_2A-47 were examined for growth rates and distinct phenotypic diversity was shown between them. The site of reversion is therefore more likely to be unlinked, linked or intralocus rather than at the A-47 site. On transduction of the intermediate reversions to the double auxotrophic

recipient, tryA-47cysB-12 with Selections for cys^+ intermediate recombinants would not be recoverable if unlinked suppressor reversion is involved. If the site of Ix corresponds to SxA-47, then no slow growing recombinants would be expected. Both intermediate and slowgrowing recombinants are expected if the site of Ix is linked or intralocus and on the left of tryA-47 site.

Experiments with 21 intermediate reversions from S_1A-47 and S_2A-47 resulted in the recovery of slowgrowing recombinants only for 11 reversions. Of the remaining 10 reversions, 9 yielded both intermediate and slow-growing recombinants. The percentage recovery of slow-growing recombinants varied from 0.80 to 0.17, strongly suggesting very closely linked or intralocus nature of the Ix mutations. The alternative models for the recovery of the slow-growing recombinants in this set of transductions namely, instability of Ix-sites and the possibility that the genotype A-47-Ix (which could result in these transduction tests if Ix site is th the right of A-47) may be slow-growing, were ruled out by appropriate tests. It is of considerable importance to know whether the Ix mutations have occurred within the tryA locus or just adjacent to it (probably within tryB locus). No satisfactory models could be constructed if Ix mutations turn out to be linked rather than intralocus mutations (unless one assumes a 'gap' between tryA and try B loci) for try A and B locus instruct the formation of separate enzymes and it is not immediately apparent as to why mutations within one locus should affect the functioning of the other in the given circumstances.

Two member strains: tryB-13 - the nearest B mutant to try A locus - and try A-52, a mutant furthest to the left of the A locus were used as recipients with intermediate reversions as donors. If Ix sites are outside either of these markers, intermediate and slow-growing recombinants would be expected, otherwise only intermediate clones would be recovered. Results obtained were compatible with the model of intralocus suppression.

Recalling that no site separation could be established between the various Ix and A-47 sites in the slow growing reversions of tryA-47 and that there is some evidence that they may not all represent recurrent mutations at the one site it is possible to test the non-identity of the slowgrowing reversions by examining the specificity of the unlinked intermediate suppressors. Such suppressors of S_1A-47 for example, could only be specific if S_1A-47 is different from S_2A-47 etc. On the other hand unspecificity may be shown whether the various slow-growing reversions are identical or not. Experiments using several slowgrowing reversions have shown that the action of unlinked suppressor mutations is probably non-specific and it is thus not possible to draw any conclusions concerning the non-identity of the SxA-47 reversions in this system.

3. The slowgrowing reversions of tryA-47 not only revert to the type referred to as intermediate, but also to strains with very slow growth rates (VS). Such derivatives were isolated in the course of some transduction tests of SxA-47 to cysB-12 and tryB-4 and were examined for their characteristics and origin. Plated on enriched minimal medium, the majority of the colonies have the slow-strain growth characteristics and evidently represent reversions to the original slowgrowing strains. The events which give rise to the VS strains

can be shown to originate on the chromosome portion which carries the try loci since the transduction of such strains to a tryA-47 cysB-12 recipient results in the recovery of both Sx and VS prototrophic recombinants, whereas only Sx would be expected if VS strains were due to unlinked modifiers. The importance of the VS strains is in the elucidation of the possible involvement of episomic systems in the modification of gene activity at the tryA locus and investigations designed to demonstrate properties in common between this system and known episome behaviour are in progress.
