ing these tasks are due to changes in accommodative convergence. Hess and Polt (*3*) had concluded that changes of accommodation do not account for the effects of mental activity on the pupil. However, we were impressed by the subjective feeling reported by many subjects that the visual field apparently becomes blurred during those stages of memory tasks where pupillary diameter is at its maximum. These reports appeared to justify a further study of the role of accommodation.

It may be observed that the fixation conditions of the main experiment were such as to induce a high level of accommodation. If the pupillary response in fact depends on loss of accommodation, initial fixation to a near object should produce very large responses, which would be expected to disappear under different fixation conditions. Immediately after completion of the main experiment, the fixation card containing the faint gray fixation circle was replaced by another card, on which a 1-inch (2.5 cm) black cardboard cross was pasted, providing a high degree of figure-ground contrast for the fixation target. Four recall trials were run with strings of seven digits. The fixation card was then removed, and a white surface was exposed at a distance of 6 feet (about 2 m), with a 12-inch black fixation cross upon it. The two fixation surfaces were matched in both luminance and the visual angle subtended by the fixation distance. Four additional trials were run at this greater fixation distance. Finally, the original fixation card was replaced, and four trials were run under conditions identical to those of the main experiment. The data for these three conditions are shown in Fig. 2, which also includes the results for strings of seven digits in the main experiment. A strong effect of accommodation distance is evident in Fig. 2, where the pupils average 10 percent larger when the subject fixates at 6 feet than when she fixates at 6 inches. However, the experimental effect is clearly present even at the largest distance, which approximates the limit of accommodation effects (*6*). Finally, a further control experiment was conducted, in which the conditions of the initial study were replicated in full, except that the fixation target was now presented at a distance of 6 feet during the entire session. The results for two subjects were very similar to those of the initial experiment. These results confirm Hess's conclusion that pupillary changes in mental activity are not mediated by changes of accommodation.

Another conclusion which may be drawn from Fig. 2 concerns the effects of repeated performance of the same task. In the last block of trials, which was performed under fixation conditions identical to those of the main experiment, the pupillary response appears to be markedly reduced. One of our subjects, initially the least responsive, failed to show this reduction in the slope of the loading function, which is only of marginal significance in these data ($t = 2.28$, 4 df, $p < .10$). We draw attention to this effect after having observed it on several other occasions in which a single procedure was repeated for several trials, as was the case in Fig. 2. On the other hand, we observed no habituation effect over the successive blocks of the main experiment, where several different procedures were interspersed in each block. The data suggest that the adoption by subject of a consistent performance set will tend to reduce both the subjective difficulty of the task and the pupillary response to it. The appearance of such practice effects in the pupillary response appears to provide additional evidence for the validity of this response as an indicator of processing load.

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References and Notes

1. E. Hess and J. Polt, *Science* **132**, 349 (1960).
2. E. Hess, *Sci. Am.* **212**, 46 (1965).
3. ——— and J. Polt, *Science* **140**, 1190 (1964).
4. D. E. Broadbent, *Perception and Communication* (Pergamon, New York, 1958); I. Pollack, *Can. J. Psychol.* **17**, 380 (1963).
5. J. I. Lacey, in *Research in Psychotherapy*, E. A. Rubenstein and M. B. Parloff, Eds. (National Publishing Co., Washington, D.C., 1959); J. I. Lacey, J. Kagan, B. Lacey, H. Moss, in *Expressions of the Emotions of Man*, P. H. Knapp, Ed. (International Univ. Press, New York, 1963); P. A. Obrist, *Psychosomat. Med.* **25**, 450 (1963); J. Kagan and M. Lewis, *Merrill-Palmer Quart. Behavior Develop.* **11**, 95 (1965).
6. M. Alpern, G. L. Mason, R. E. Jardinico, *Am. J. Ophthalmol.* **52**, 330 (1961).
7. This research was supported by grant NIMH 08847-02 to G. S. Blum. It was conducted while the senior author (D.K.) was Visiting Scientist at the University of Michigan under the auspices of the Institute of Science and Technology. We thank M. Alpern, G. S. Blum, and I. Pollack for suggestions and assistance.

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Tryptophan Operon of Escherichia coli: Regulatory Behavior in Salmonella typhimurium Cytoplasm

Abstract. *Hybrids hemizygous for the tryptophan genes were prepared by episomal transfer of an* Escherichia coli *element into* Salmonella typhimurium. *Regulation of enzyme production by hybrids carrying wild-type* E. coli *genes in response to changes in the growth medium occurs in precisely the same manner as in haploid* E. coli *wild type. Mutant alleles of the anthranilate synthetase gene of* E. coli *which prevent derepression in* E. coli *function identically in* S. typhimurium. *At least one* Salmonella *tryptophan regulatory gene unlinked to the structural genes is known. Any differences which may exist between the tryptophan regulatory genes of* E. coli *and* Salmonella *have little effect on the regulation of enzyme formation in hybrids.*

There is a high degree of gross homology in both genetic structure and function between *Salmonella typhimurium* and *Escherichia coli* (*1*). Nevertheless, considerable divergence in the molecular architecture of analogous genes has been revealed by fine structure analysis with transduction techniques (*2*). These genetic results complement earlier experiments of nucleic acid hybridization in vitro (*3*) which showed poor homology between the two genera, despite close similarities in guanine and cytosine content (*4*).

The cluster of tryptophan structural genes has been studied in both *Salmonella* and *E. coli* (*5*). In both organisms the tryptophan genes are localized within a short segment of the chromosome in an order which corresponds to the biosynthetic sequence. However, the fine structures of corresponding genes vary considerably, as indicated by the low recombination frequencies observed in transduction crosses between the two genera (*2*). Fine structure differences between *E. coli* and *Salmonella* have also been inferred from comparisons of the peptide patterns after treatment by trypsin

and chymotrypsin of the α-subunits (formerly designated A proteins) of tryptophan synthetase. Only about half of the peptides in a two-dimensional separation by chromatography and electrophoresis showed similar mobilities (*6*).

Because the genetic code is probably universal (*7*), one may expect a given gene to yield the same polypeptide chain regardless of the cytoplasm in which it functions. For instance, it has been shown that the *A* gene of *E. coli* is translated identically in cyptoplasms of both *Salmonella* and *E. coli* (*6*).

The regulation of gene function is less clear. There are some indications that the elements of the regulatory systems for alkaline phosphatase in *Serratia marcescens* and *E. coli* interact with less than optimum efficiency (*8*). The regulation of the formation of β-galactosidase in *E. coli* is impaired when the lac *z* gene of this organism functions in *Proteus* cytoplasm (*9*); yet the *i* cistrons of *E. coli* and *Shigella dysenteriae* are homologous in location and function despite differences in their lac *z* genes (*10*).

Hybrids between *S. typhimurium* and *E. coli* have been prepared by episomal transfer of the *try* operon from *E. coli* into *Salmonella* recipients whose tryptophan genes had been deleted. I now describe the properties of these hybrids with respect to the functioning of the tryptophan regulatory system.

Mutants of *S. typhimurium,* carrying deletions of the entire tryptophan operon, which arose concurrently with the loss of the *sup X* locus (formerly termed *su leu* 500) (*11*) were used as recipients in standard F′ episomal transfers (*12*) with donor strains carrying the wild-type or mutant tryptophan operon of *E. coli*. The F′ episome, which also carries determinants for the production of colicines V and B, cysteine biosynthesis, and phage T1 receptor substance, was isolated originally by Fredericq (*13*). All *E. coli* donors were auxotrophic for histidine and proline and were counterselected in crosses by the omission of these supplements.

A summary of the phenotypes of these mutants and hybrids is given in Table 1. The *E. coli* episome carries a number of genes which are functional in *Salmonella* cytoplasm. In addition to the entire cluster of five tryptophan genes, wild-type alleles for the *cys B* locus, the *chr* (formerly *car*) locus (*15*) and the *sup X* (formerly *su leu* 500) locus (*11*) are carried on the episome and function efficiently. Furthermore the hybrids produce colicines V and B as a result of acquisition of the episome, but sensitivity to bacteriophage P22 remains unchanged. Bacteriophage sensitivity is of interest in this connection because in *E. coli* a locus controlling the production of T1 receptor substance is adjacent to the tryptophan genes (*14*) and is carried by the F′ episome which was used. Thus the *chr* gene of *Salmonella* is probably related to the phage T1 receptor gene of *Escherichia coli* (*15*).

The F′ *try* episome is transferred from *E. coli* to *Salmonella* at very low frequencies (Table 2). Somewhat higher efficiencies of transfer are observed when the hybrids themselves serve as F′ donors (Table 2, experiments 3 and 4). The resulting hybrids are extremely stable. No endogenotic segregants were found among 10^4 cells which had been grown under nonselective conditions, plated on nonselective media, then checked after 24 hours by plating and replication to minimum agar.

For the study of enzyme formation in hybrids, cultures were grown overnight in appropriately supplemented minimal medium (*16*), with 0.2 percent glucose as carbon source. The cells were sonically disrupted, and extracts were assayed for the tryptophan synthetase α-subunit (*17*). Anthranilate synthetase was assayed spectrophotofluorometrically by a modification of the procedure of Gibson and Gibson (*18*). A unit of tryptophan synthetase is that amount of protein which converts 0.1 μmole of indole to tryptophan in 20 minutes at 37°C under standard assay conditions. A unit of anthranilate synthetase catalyzes the formation of 1 nanomole of anthranilate from chorismate in 1 minute. Before assay, all extracts were dialyzed overnight in 0.1*M* tris, *p*H 7.8 containing 0.001*M* 2-mercaptoethanol and 0.0001*M* EDTA. Protein was determined by the Lowry procedure (*19*).

Table 3 gives the results of experiments designed to examine the effects on enzyme production of various growth conditions for hybrids harboring wild-type *E. coli* episomes. In high concentrations of tryptophan, efficient repression of both anthranilate synthetase and tryptophan synthetase occurred. Derepression resulted when the hybrids were grown in minimal media, and it was greatly enhanced when 3-

Table 1. Phenotypes of *Salmonella* mutants and of hybrids derived from them by episome transfer from *Escherichia coli*. Abbreviations used: *sup X* (formerly termed *su leu* 500) (*11*): suppressor of *leu* 500 mutation; leu, cys, try: requirements for leucine, cysteine, and tryptophan; chr (formerly termed car) (*14*) ability to utilize glucose, galactose, mannose, mannitol, sorbitol, and maltose on agar medium in the absence of citrate; col V, col B: determinants for colicines V and B; P22: sensitivity to phage P22. Mutants *sup X*30, *sup X*43, *sup X*24 and *sup X*26 were originally designated 1T1, 15T1, 8CT1, and 9CT1, respectively (*5, 6, 11, 15*).

Mutant or hybrid	leu	cys	try	chr	col V, col B	P22	Isolates studied (No.)
*sup X*30	+	+	−	−	−	S	1
*sup X*43	+	+	−	+	−	S	1
*sup X*24	+	−	−	+	−	S	1
*sup X*26	+	−	−	−	−	S	1
*sup X*30/F′ +	−	+	+	+	+	S	2
*sup X*43/F′ +	−	+	+	+	+	S	16
*sup X*24/F′ +	−	+	+	+	+	S	11
*sup X*26/F′ +	−	+	+	+	+	S	12

Table 2. Transfer frequencies for the F′ *try* episome with different donor and recipient genera. The frequencies given are the number of recombinants observed per donor cell when a mating mixture consisting of 2×10^9 donor cells per milliliter and excess recipient cells was incubated for 1 hour at 37°C, plated on media selective for recombinants, and incubated at 37°C for 48 to 72 hours. In experiment 3, the recipient was a streptomycin-resistant derivative of *sup X*43 (Table 1). Selection for recombinants was carried out on minimal media containing leucine (20 μg/ml) and streptomycin (200 μg/ml).

Expt.	Donor	Recipient	Frequency
1	*E. coli*/F′ *try*$^+$	*E. coli F*$^-$	10^{-2}
2	*E. coli*/F′ *try*$^+$	*S. typhimurium*	10^{-7} to 10^{-8}
3	*S. typhimurium*/F′ *try*$^+$	*S. typhimurium*	10^{-5}
4	*S. typhimurium*/F′ *try*$^+$	*E. coli F*$^-$	10^{-3}

Table 3. Formation of anthranilate synthetase and the α-subunit of tryptophan synthetase by hybrids of *S. typhimurium* and *E. coli* carrying wild-type *E. coli* tryptophan genes. Values given are specific activities. The β_2-protein components of tryptophan synthetase were also measured and gave values essentially identical to those found for the α-protein. Leucine (20 μg/ml) was added as a supplement to all media.

Hybrid	Synthetase specific activities	
	Anthranilate	Tryptophan
Minimal media + L-*tryptophan* (*50 μg/ml*)		
sup X30/F′ +	0.27	1.5
sup X24/F′ +	.24	1.2
sup X26/F′ +	.28	1.2
sup X43/F′ +	.28	1.2
Minimal media		
sup X30/F′ +	2.7	4.0
sup X24/F′ +	1.4	3.6
sup X26/F′ +	2.0	4.2
sup X43/F′ +	2.2	4.4
Minimal media + 3-methyl anthranilate (*20 μg/ml*)		
sup X30/F′ +	23.0	39
sup X24/F′ +	12.0	28
sup X26/F′ +	9.2	20
sup X43/F′ +	12.1	27

methylanthranilate was included in the growth flasks. This compound causes derepression of the tryptophan pathway enzymes (*20*), presumably because it reduces the intracellular tryptophan concentration by inhibiting the action of indole-3-glycerolphosphate synthetase (*21*). The specific activities of the α-subunit of tryptophan synthetase observed in this experiment are identical to those found for *E. coli* strains with a chromosomal deletion of the tryptophan genes and with the wild-type episome being carried (*22*). Similarly, the extent of derepression noted in cells grown in high concentrations of tryptophan compared with cells grown under conditions of tryptophan limitation agrees well with other observations in *E. coli* (*12*).

Certain *E. coli* anthranilate synthetase mutants have regulatory defects (*12*) in that they are unable to derepress under conditions of tryptophan limitation. It was of interest to determine whether this type of regulatory lesion would manifest itself when the *E. coli* alleles were present in *S. typhimurium* cytoplasm. Four different alleles of the anthranilate synthetase gene were transferred into *Salmonella*. Enzyme formation in these hybrids was measured after growth under repression and derepression conditions (Table 4). Mutants 5927 and 6185 exerted the same regulation effect upon tryptophan synthetase derepression in *Salmonella* cytoplasm as observed in *E. coli*; enzyme activities did not rise significantly above that observed for wild-type cells grown in minimal medium. In contrast, mutants T3 and 5984 derepressed efficiently, yielding extracts whose tryptophan synthetase specific activity was higher by an order of magnitude.

A tenfold difference in specific activity of tryptophan synthetase was also found between wild-type and mutant hybrids for growth under repression conditions (Tables 3 and 4). It is not apparent why hybrids carrying a mutant form of the anthranilate synthetase gene should repress so much more efficiently than hybrids carrying the wild-type tryptophan operon.

Probably *E. coli* and *S. typhimurium* arose from a common ancestor. Despite the considerable degree of structural divergence between the genomes of the two organisms (*2, 3*) strong selective pressure for the maintenance of similar regulatory systems for the tryptophan enzymes must have existed during evolution. There is at present no way of assessing the degree of homology required of regulatory elements from different genera to obtain effective control of enzyme synthesis in hybrids. My work suggests that extensive interaction is possible between the tryptophan regulatory systems of *E. coli* and *Salmonella*.

In *E. coli* a tryptophan regulatory gene, R^-_{try}, has been identified and mapped; it is near the *thr* locus (*24*). Since a homologous regulatory gene almost certainly exists in *Salmonella* (*11*), the product of the R^-_{try} gene of *Salmonella* must interact efficiently with the tryptophan operon of *E. coli to* modulate gene expression.

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Table 4. Formation of the tryptophan synthetase α-subunit in hybrids of *Salmonella typhimurium* and *Escherichia coli*. The hybrids carry mutant alleles of the *E. coli try* E anthranilate synthetase) gene, and require either tryptophan, indole, or anthranilate for growth. The 6185 and 5984 alleles are nonsense mutations of the *ochre type* (*23*); since the hybrids have the mutant phenotype it is unlikely that *S. typhimurium sup X24* carried an *ochre* suppressor. Repressed cells were grown in the presence of excess L-tryptophan (50 μg/ml); depressed cells were grown in limiting tryptophan (4 μg/ml) and then harvested after sufficient time had elapsed for expression of derepression potential (18 to 20 hours).

Hybrid	*Try* E allele type (haploid *E. coli*)	Tryptophan synthetase α-subunit (specific activity)	
		Repression	Derepression
sup X24/F′ 5927	Pleiotropic	0.073	7.8
sup X24/F′ 6185	Pleiotropic	.12	5.2
sup X24/F′ T3	No effect	.13	65
sup X24/F′ 5984	No effect	.081	49

References and Notes

1. A. L. Taylor and M. S. Thoman, *Genetics* **50**, 659 (1964); K. E. Sanderson and M. Demerec, *ibid.* **51**, 897 (1965).
2. M. Demerec and N. Ohta, *Proc. Nat. Acad. Sci. U.S.* **52**, 317 (1964); M. Demerec, in *Evolving Genes and Proteins*, V. Bryson and H. J. Vogel, Eds. (Academic Press, New York, 1965), p. 505.
3. C. L. Schildkraut, J. Marmur, P. Doty, *J. Mol. Biol.* **3**, 595 (196 → J. M. McCarthy and E. T. Bolton, *Proc. Nat. Acad. Sci. U.S.* **50**, 156 (1963).
4. C. L. Schildkraut, J. Marmur, P. Doty, *J. Mol. Biol.* **4**, 430 (1961); J. Marmur and P. Doty, *ibid.* **5**, 109 (1962); A. N. Belozersky and A. S. Spirin, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 1960), vol. 3, p. 147.
5. C. Yanofsky, *Bacteriol. Rev.* **24**, 221 (1960); I. P. Crawford and L. M. Johnson, *Genetics* **49**, 269 (1964); O. H. Smith, *Bacteriol. Proc.* **65**, 29 (1965); M. Demerec and Z. Hartman, in *Genetic Studies with Bacteria* (Carnegie Institution of Washington Publ. No. 612, 1956), p. 5; E. Balbinder, A. J. Blume, H. Tamaki, *Bacteriol. Proc.* **65**, 95 (1965); P. Margolin, *Science* **147**, 1456 (1965).
6. T. E. Creighton, D. R. Helinski, R. L. Somerville, C. Yanofsky, *J. Bacteriol.* **91**, 1819 (1966).
7. G. von Ehrenstein and F. Lipmann, *Proc. Nat. Acad. Sci. U.S.* **47**, 941 (196 → J. H. Schwartz, J. M. Eisenstadt, G. Brawerman, N. D. Zinder, *ibid.* **53**, 195 (1965).
8. E. R. Signer, *J. Mol. Biol.* **12**, 1 (1965).
9. S. Falkow, J. A. Wohlhieter, R. V. Citarella, L. S. Baron, *J. Bacteriol.* **87**, 209 (1964).
10. S. Sarkar, *ibid.* **91**, 1477 (1966).
→ F. H. Mukai and P. Margolin, *Proc. Nat. Acad. Sci. U.S.* **50**, 140 (1963); R. H. Bauerle and P. Margolin, *ibid.* **56**, 111 (1966).
12. R. L. Somerville and C. Yanofsky, *J. Mol. Biol.* **11**, 747 (1965).
13. P. Fredericq, in *Genetics Today* (Pergamon Press, New York, 1963), vol. 1, p. 42.
14. C. Yanofsky and E. Lennox, *Virology* **8**, 425 (1959).
15. L. M. Corwin, G. R. Fanning, F. Feldman, P. Margolin, *J. Bacteriol.* **91**, 1509 (1966).
16. H. J. Vogel and D. M. Bonner, *J. Biol. Chem.* **218**, 97 (1956).
→ C. Yanofsky and J. Stadler, *Proc. Nat. Acad. Sci. U.S.* **44**, 245 (19 → I. P. Crawford and C. Yanofsky, *ibid.*, p. 1161.
18. M. J. Gibson and F. Gibson, *Biochem. J.* **90**, 248 (1964).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
20. G. Lester and C. Yanofsky, *J. Bacteriol.* **81**, 81 (1961).
21. F. Gibson and C. Yanofsky, *Biochim. Biophys. Acta* **43**, 489 (1960).
22. H. Stetson, personal communication.
23. C. Yanofsky and J. Ito, personal communication.
24. G. H. Cohen and F. Jacob, *Compt. Rend.* **248**, 3490 (1959).
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