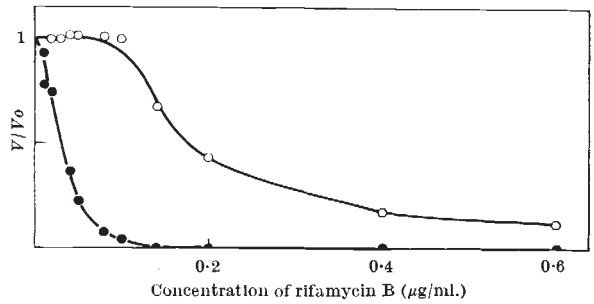

Fig. 1. Comparative effect of rifamycin B on wild type and on mutant purified RNA polymerases. *V/Vo* is the ratio of the velocity in the presence and absence of the drug. *V/Vo* is plotted against the concentration of rifamycin B. Rifamycin B and the enzymes were mixed together and after 5 min a mixture containing T4 DNA, the four naturally occurring ribonucleotide triphosphates (among them ATP was labelled) and ions[5] was added and the incubation carried out at 30° C for 10 min. To obtain 50 per cent inhibition of the wild type enzyme (●) 0·03 μg/ml. of rifamycin B were required. To inhibit the mutant enzyme (○) to the same degree 0·18 μg/ml. of the drug were needed.

met⁻ rif^R RC^{rel}) with an F⁻ (PA340 B_1⁻ T⁻ L⁻ His⁻ Arg8⁻ Asp⁻ Lac⁻ Gal⁻ Malt⁻ Xyl⁻ Mtl⁻ Sm^R obtained from Professor L. Gorini) selecting for Arg⁺ Sm^R recombinants. Out of sixty-two recombinants examined, fifty-five were resistant to rifampicin. We therefore conclude that a gene for RNA polymerase is located on the *E. coli* map between the origin of Hfr Cavalli and arginine H or slightly distal to the latter marker.

The fact that rifamycin promptly blocks the synthesis of all cellular RNA in the wild type[10] seems to indicate that at least the part of RNA polymerase which reacts with rifamycin is involved in the synthesis of all types of RNA. We have also found and reported elsewhere[14] that the growth of RNA phages f2 and MS2 in the wild type is sensitive to the drug, when added before infection. The RNA phages, however, grow normally in the presence of rifampicin on the mutant which possesses an altered polymerase. These results are compatible with the idea that at least a part of the host DNA-dependent RNA polymerase has a role in the replication process of viral RNA.

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Mutations affecting RNA Polymerase associated with Rifampicin Resistance in *Escherichia coli*

The rifamycins and their semi-synthetic derivatives such as rifampicin inhibited the RNA polymerase of *E. coli*[1]. Instead of interacting with the DNA, they appear to act on the RNA polymerase itself[2], inhibiting the initiation of RNA synthesis after the RNA polymerase has already combined with the DNA[3,4].

It might therefore be possible to isolate RNA polymerase mutants by selecting for rifampicin-resistant mutants. We mutagenized *E. coli* strain K100 cells with N-methyl-N′-nitro-N-nitrosoguanidine[5], grew them overnight to allow phenotypic expression and treated with 0·01 M EDTA in 0·3 M *tris*, *p*H 8, hoping to minimize the survival of mutants resistant to rifampicin because of reduced permeability[6], and plated in the presence of 50 μg/ml. rifampicin. There were about 200 colonies per 10^7 cells plated. After purification, cultures were grown up, broken by sonic oscillation and centrifuged at low speed and at 81,000*g* (avg.) for 90 min. The high speed supernatants were tested for RNA polymerase activity in the presence and absence of rifampicin.

All extracts examined exhibited RNA polymerase activity with increased resistance to rifampicin. In our assay conditions, almost complete inhibition of the enzyme of the parental strain is obtained at 2 μg/ml. A mutant culture chosen for further testing, labelled strain K100R3, exhibited RNA polymerase activity resistant to 250 μg/ml. rifampicin (Table 1). The enzyme activity of the mutant strain is insensitive to rifamycin SV and largely insensitive to the compounds present in a fresh aqueous solution of rifamycin B not protected from the atmosphere (Table 2). When mutant and normal enzyme were mixed, we found no evidence of destruction or binding of the rifampicin by factors in the mutant extract (Table 3). Pre-incubation of 10 μg/ml. of rifampicin with the mutant extract at 37° C for 15 min does not destroy the capacity of the drug to inhibit the wild type enzyme (Table 4). There is no significant difference in the rate of heat inactivation of the two enzyme preparations at 56° C (Table 5).

The growth rate of this strain in tryptose phosphate broth was unaffected by the mutation, but the cells are considerably fatter in shape than those of the parent strain. As in the parent (relaxed) strain, starvation of amino-acids does not markedly affect the rate of RNA synthesis.

Rifampicin-resistant mutants of four other strains derived from K12, isolated by this procedure, have been

Table 1. EFFECT OF RIFAMPICIN ON RNA POLYMERASE OF *E. coli* K100 AND K100R3

Rifampicin (μg/ml.)	μμMole UTP-^{14}C incorporated K100	K100R3
0	31	22
2	3	25
50		21
100		16
250		18

The assay was performed according to Chamberlin and Berg[7], except that 2 mM spermidine and 100 μg bovine albumin were added, the DNA was reduced to 7 μg, and the UTP-2-^{14}C, 22 mCi/mmole, was present at 1·14 μM. Samples were incubated at 30° C for 10 min, chilled, mixed with 0·25 mg RNA and 5 per cent TCA, plated on 'Millipore' filters, and counted by liquid scintillation. Control values (not subtracted) without added GTP, CTP and ATP or with ribonuclease or without DNA and with deoxyribonuclease ranged from 1·5 to 5 μμmole. Enzyme samples tested throughout this paper are 0·05 or 0·1 ml. of high speed supernatant of crude extract.

Table 2. EFFECT OF RIFAMYCINS ON RNA POLYMERASE OF *E. coli* K100 AND K100R3

Rifamycin (Control)	Concentration (μg/ml.)	μμMole UTP-^{14}C incorporated K100	K100R3
	—	45	48
SV	2	3	42
	60	2	31
B	2	3	27
	60	6	19

Assay performed as in Table 1.

Table 3. EFFECT OF RIFAMPICIN ON RNA POLYMERASE ACTIVITY OF MIXED EXTRACTS OF *E. coli* K100 AND K100R3

	$\mu\mu$Mole UTP-^{14}C incorporated	
	Control	Rifampicin (2 μg/ml.)
K100 extract	21	2
K100R3 extract	23	22
1 : 1 mixture	43	23

Assay performed as in Table 1. In one mixing experiment, samples were taken at 5, 10 and 15 min. Incorporation was linear with time in all tubes.

Table 4. EFFECT OF PRE-INCUBATION OF RIFAMPICIN WITH MUTANT EXTRACT

Pre-incubation mixture	Wild type enzyme added at 15 min	c.p.m. UTP-^{3}H incorporated
Mutant enzyme	No	2,840
Mutant enzyme	Yes	7,190
Mutant enzyme plus rifampicin	No	2,050
Mutant enzyme plus rifampicin	Yes	1,690

Assay as in Table 1, except that UTP-^{3}H, 140 mCi/mmole, was present at 72 μM, instead of UTP-^{14}C. All components except substrates were incubated with 0·05 ml. K100R3 extract with or without rifampicin for 15 min at 37° C. Then the samples were chilled, and substrates, a second aliquot of DNA and 0·05 ml. K100 enzyme (where required) were added. The samples were incubated for 10 min at 30° C, and processed in the usual fashion. The rifampicin concentration was approximately 20 μg/ml. during pre-incubation and 10 μg/ml. during incubation.

Table 5. HEAT DENATURATION OF RNA POLYMERASES

Min at 56° C	c.p.m. UTP-^{3}H incorporated	
	Rifampicin-sensitive	Rifampicin-resistant
0	4,340 (1·00)	3,240 (1·00)
0·5	2,810 (0·65)	1,960 (0·60)
1·0	1,770 (0·41)	1,360 (0·42)
2·0	1,210 (0·28)	950 (0·29)

Assay as in Table 4. A mixture of 0·6 ml. K100 extract and 1·2 ml. K100R3 extract was incubated at 56° C. Samples (0·1 ml.) were withdrawn and assayed with and without 2 μg/ml. rifampicin to measure the disappearance of sensitive and resistant enzyme in the same milieu. The numbers in parentheses represent the fraction of the original activity remaining. Similar results were obtained with the extracts tested separately.

examined. One of them, derived from strain JHM 544, yielded an extract displaying 70 per cent inhibition of RNA polymerase at 10 μg/ml. rifampicin. The other three were all completely resistant at that level. Mixing experiments and heat inactivation experiments with the four strains produced results parallel to those illustrated in Tables 3 and 4.

One of the three completely resistant mutants has been partially mapped. We propose the name *rna* for genetic loci affecting RNA polymerase, *rna*-1 for the mutation in strain P4X6R1, derived from strain P4X6, and Rif-r and Rif-s for the rifampicin-resistant and sensitive phenotypes. P4X6R1 is a Met B^{-}, streptomycin-sensitive Hfr strain injecting in the order *origin leu thi metB str*. It was mated on 'Millipore' filters with W678 (Thre^{-}, Leu^{-}, Thi^{-}, Str-r), the mating interrupted with a Vortex mixer at 30, 60, 90 and 120 min, and Thre^{+} Leu^{+} Str-r recombinants selected. These recombinants were tested for *rna* by replica plating on 100 μg/ml. rifampicin–streptomycin plates, and Rif-r colonies tested (without purification) for *met* by spotting and for *thi* by diluting and streaking. Rifampicin resistance was present in 6 out of 111 colonies at 30 min and 42 out of 237 at 60 min. At 30 min, 18 out of 46 Rif-r colonies were Met^{-}, while at 90 min, 19 out of 30 were. At 30 min, 3 to 6 out of 28 Rif-r Met^{+} colonies were Thi^{-}. At 90 min, 2 out of 28 Rif-r colonies were Thi^{-}. Evidently the *rna* locus is located very close to *thi*. More detailed and extensive mapping is in progress.

It seems likely that the mutants described are altered in a structural gene for RNA polymerase. It will be interesting to examine the effects of such mutations on the regulation of RNA synthesis by amino-acids and by enzyme repression. Definitive establishment of the nature of this mutation would also serve as additional evidence that the commonly studied RNA polymerase is responsible for all, or virtually all, of the RNA synthesis in the *E. coli* cell.

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Note added in proof. The isolation of similar mutants has also been reported by Wehrli, W., Knüsel, F., and Staehelin, M., *Biochem. Biophys. Res. Commun.*, **32**, 284 (1968), and by Babinet, C., and Condamine, H., *CR Acad. Sci. Paris*, **267D**, 231 (1968).

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Metabolic Behaviour of Isozymes of Acetylcholinesterase

CHOLINESTERASE (ChE) from various sources can be separated into different molecular forms or isozymes[1] and we have resolved several bands with ChE activity in extracts of rat neural tissue using disc electrophoresis (Fig. 1). To gain more information about these isozymes, we have studied their physiological properties, particularly their turnover, and have detected an isozyme which seems to have a rapid rate of turnover.

In order to obtain high resolution of the ChE isozymes, some modifications were applied to a standard disc electrophoretic technique[2]. Glass tubes with an internal diameter of 3 mm were used. The separation gel, with *tris* buffer (*p*H 8·1), was cast in two parts, a lower gel (*B*) with 8·0 per cent acrylamide and an upper gel (*A*)

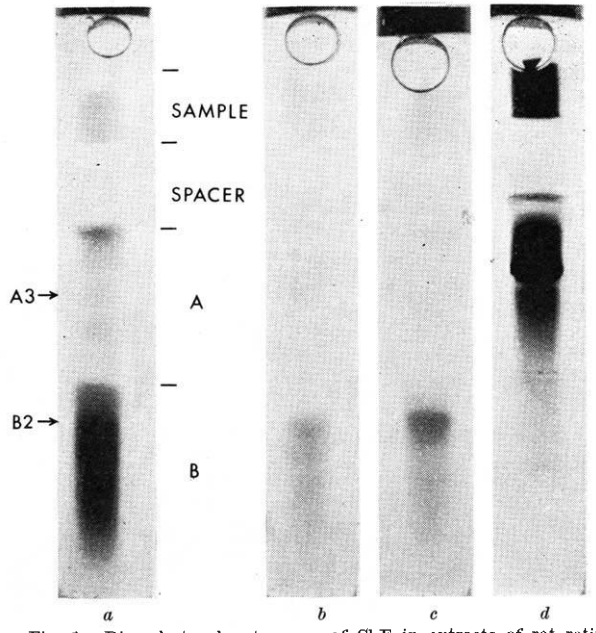

Fig. 1. Disc electrophoretograms of ChE in extracts of rat retina. The gels were incubated in Cu-AcThCh solution for 1 h, and after washing, treated with dithio-oxamide. (*a*) Detergent ('Myrj 53') extract of control retina. (*b*) 'Myrj' extract 2 h after DFP. (*c*) 'Myrj' extract 6 h after DFP. (*d*) Detergent ('Triton *X*-100') extract of the 'Myrj' residue from a control retina.