Isolation of Substances That Stimulate RNA Synthesis from Red Kidney Bean: Their Activities in Lymphocytes and *Escherichia coli*

(phytohemagglutinin/*Phaseolus*/proteins/rifampicin)

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ABSTRACT An extract from red kidney beans (*Phaseolus vulgaris*) was fractionated with respect to RNA synthesis-stimulating activity. The activity of the fractions was tested in chicken-spleen lymphocytes cultured in serum-free medium and in plasmolyzed *E. coli* cells. Lymphocyte-stimulating activity was found in a fraction called F III. This fraction contained protein and orcinol-positive carbohydrate. The protein (called F III p) responsible for the observed activity was separated from the contaminating carbohydrate by acid precipitation. F III p appears to belong to the group of well-known mitogens of high molecular weight present in red kidney beans. A bacteria-stimulating factor was found in a fraction called F V. This factor strongly stimulated RNA synthesis in the bacterial system, and had a weak stimulating effect in the lymphocyte system. In plasmolyzed *E. coli* cells, F V counteracted the inhibiting effect of rifampicin on RNA synthesis.

From observations of an early increase of RNA synthesis in phytohemagglutinin (PHA)-stimulated lymphocytes (1-5), the importance of activation of RNA synthesis in the stimulation of resting cells has been discussed (6). After the partial separation by Rivera and Mueller (7) of RNA synthesis-stimulating and mitogenic activities from the leukoagglutinating activity of PHA, Goldberg *et al.* (8) have shown that these factors are completely separable.

However, in work aiming at the separation of components of PHA with different actions on cell metabolism, the conventional lymphocyte system that contains serum seems to be a somewhat unreliable biological guide. In this communication we report the separation of factors stimulating RNA synthesis from seeds of *Phaseolus vulgaris*, using a test system consisting of *Escherichia coli* cells with increased permeability (to be published) in parallel with chicken-spleen lymphocytes cultivated in serum-free synthetic medium.

MATERIALS AND METHODS

Chemicals. [5-³H]Uridine, [methyl-³H]thymidine, and L-[ring-4-³H]phenylalanine were from Radiochemical Centre (Amersham); phytohemagglutinin-P (PHA-P) and casamino acids from Difco; Medium 199, Waymouth's MB 752/1, and penicillin/streptomycin solution (5000 IU of each per ml) from Flow Laboratories; rifampicin from CIBA; acrylamide, *N,N'*-methylenebisacrylamide (Bis), and *N,N,N',N'*-tetramethylethylenediamine (Temed) from Fluka; ammonium persulfate from Riedel; Sephadex gels from Pharmacia; inorganic chemicals for analytical purpose from Merck (Darmstadt).

Abbreviation: PHA, phytohemagglutinin.

All other chemicals and biochemicals were purchased from Sigma Chemical Co.

Polyacrylamide Gel Electrophoresis was performed by the technique of Fromageot and Zinder (9), with gels of 8-cm length, 0.6-cm diameter. The samples to be analyzed were mixed 1:1 with 10 mM sodium phosphate (pH 7.2), containing 0.1% sodium dodecylsulfate, 1.0% 2-mercaptoethanol, and 50% sucrose. Electrophoresis was performed towards the anode for 7 hr at room temperature at 8 mA per gel. The gels were stained with 1% amido black in 7% acetic acid for 5 min, destained with 7% acetic acid, and scanned in a Joyce Loeb ME 12 MK III photometer.

Assay for Stimulation of RNA or DNA Synthesis in Chicken-Spleen Cells Under Serum-Free Culture Conditions. The technique described here is a modified procedure of that described by Weber (10). Unless otherwise indicated, all manipulations were done in the cold (about 4°) under sterile conditions. All the solutions were sterilized by filtration with 0.45- μ m Millipore filters. Disposable polystyrene pipettes and polystyrene tubes (16 $\times$ 125-mm plastic screw-top tissue culture tubes, Falcon Plastics Co.) were used.

The spleen of an exsanguinated chicken (White Leghorn, 8-14 weeks old) was washed with PSS solution at 0° and minced with scissors in 6.0 ml of PSS solution. (PSS solution contains 14 mg of CaCl₂, 40 mg of KCl, 20 mg of MgSO₄·7H₂O, 800 mg of NaCl, and 100 mg of glucose in 100 ml of distilled water; the pH of the solution was adjusted by NaOH to 7.5). The suspension was mixed; after 1 min, 5 ml of tissue-free lymphocyte suspension was separated by a pipette. The cell suspension was centrifuged for 10 min at 300 $\times g$; the pellet was suspended in 6.0 ml of PSS solution, mixed well, centrifuged again, and suspended in 6.0 ml of PSS solution. The number of lymphocytes present in the suspension was determined by a Bürker chamber with Türk's diluting fluid. Finally, a standard lymphocyte suspension was prepared containing 3 $\times$ 10⁷ cells per ml of PSS solution.

Samples to be tested for stimulating activity were dissolved in PSS solution and pipetted into the culture tubes (0.3 ml of assay solution per tube). Then 0.2 ml of standard lymphocyte suspension was added, and the tubes were incubated in a 37° water bath for 10 min. After incubation, 1.5 ml of Medium U or Medium T was added, and the tubes, with loosened screw caps, were placed at 38° in an atmosphere of air with 5% CO₂. Medium U (100 ml) was prepared by mixing 32 ml of Medium 199, 65 ml of Medium 752/1, 1.0 ml of 0.2 M glutamine, 1.34 ml of penicillin/streptomycin solution, 0.22 ml of 0.4

FIG. 1. Effect of PHA-P (Difco) on RNA synthesis in chicken-spleen lymphocytes and in plasmolyzed *E. coli* Q 13 cells. Incubation time was 150 min for *E. coli* Q 13 and 40 hr for chicken-spleen lymphocytes. (The content of one vial of PHA-P, Difco, was dissolved in 5 ml of distilled water.)

mM uridine, and 0.44 ml of [³H]uridine containing 100 μCi/ml (specific activity 24 Ci/mmol). Medium T was the same, but contained 0.22 ml of 0.4 mM thymidine instead of uridine and 0.44 ml of [³H]thymidine (100 μCi/ml, 14.1 Ci/mmol) instead of [³H]uridine.

At the appropriate time (usually after 40–45 hr), the tubes were chilled in ice. For determination of the amount of incorporated uridine or thymidine, the assay suspension was treated as described by Goldberg *et al.* (8). The radioactivity was measured in a Nuclear Chicago Liquid Scintillation Counter model 6850 with an efficiency of 16%. The results obtained were expressed as pmol of uridine or thymidine incorporated in 10⁶ cells.

Assay for Stimulation of RNA, Protein, or DNA Synthesis in Bacteria. The bacterial system developed for study of cell stimulation consists of *E. coli* cells subjected to plasmolysis. The treatment of the cells is related to the procedure applied by Heppel in order to increase permeability of the bacterial cell wall (11, 12). *E. coli* Q-13 (kindly provided by Dr. J. T. August, Albert Einstein College, Bronx) was grown in a medium containing Na₂HPO₄·2H₂O (8.8 g), KH₂PO₄ (3.0 g), NaCl (0.5 g), NH₄Cl (1.0 g), glycerol (3.0 g), casamino acids (technical) (6.0 g), and MgSO₄·7H₂O (1.25 g) per liter. The medium was sterilized by filtration through a Sartorius membrane filter, pore size 0.2 μm. The culture was aerated by shaking at 37° until the bacteria reached a density of 10⁸ cells per ml. It was then cooled rapidly to 0°, and the bacteria were collected by centrifugation at 2° for 15 min at 12,000 × *g*. The cells were resuspended in the original volume of 30 mM Tris·HCl buffer (pH 8.1) at 0°. After they were slowly shaken for 5 min at 0°, the centrifugation was repeated. The collected cells were then suspended at 0° in SMG solution (containing 10% sucrose, 0.1% MgSO₄·7H₂O, and 0.1% glucose) to yield a cell suspension of 6.0 × 10⁸ cells per ml. The cell suspension was stored at 2° and was used for experiments after 12 hr and up to 1 week. During storage for 1 week, the rate of incorporation of uridine usually drops by a factor of 2, although the ability to become stimulated relative to the controls remains practically unchanged. Before use, an aliquot of the suspension was centrifuged as above, the supernatant was discarded, and the cell pellet was suspended at 0° in a new

SMG solution to yield a standard bacterial cell suspension of 3.0 × 10⁷ cells per ml.

In order to measure the stimulation of RNA, protein, or DNA synthesis, the cells were incubated with the factor to be assayed at 35° in the presence of a suitable reaction mixture containing [³H]uridine, or [³H]phenylalanine, or [³H]thymidine; incorporation of these precursors was measured. The reaction mixture, derived from the one generally used in cell-free protein-synthesizing systems, was freshly prepared every day. The composition of the reaction mixture was as follows: Tris·HCl buffer (112 mM) (pH 7.8), NH₄Cl (0.1 M), KCl (3.7 mM), Mg acetate (2.6 mM), ATP (Na salt) (2.5 mM), GTP (Na salt) (0.6 mM), creatine phosphate (Na salt) (26 mM), creatine phosphokinase (0.13 mg/ml), sucrose (0.34 M), uridine (15 μM), each of 19 amino acids (except phenylalanine) (70 μM), phenylalanine (15 μM), thymidine (15 μM), 2-mercaptoethanol (10 mM), and [³H]uridine (7.5 μCi/ml; 24 Ci/mmol) or [³H]phenylalanine (7.5 μCi/ml; 18 Ci/mmol) or [³H]thymidine (7.5 μCi/ml; 14.1 Ci/mmol). The incubation mixture for one experiment consisted of 134 μl of reaction mixture to which was added the sample to be tested dissolved in 66 μl of distilled water. The incubation was started by addition of 50 μl of the standard bacterial cell suspension and placement of the tube in a 35° water bath. After an appropriate time, an aliquot of the incubated mixture was mixed with an equal volume of 10% sodium dodecyl sulfate, and after 15 min incubation at 35°, when the cells were lysed, 50 μl was transferred to the middle of a glass-fiber filter (Whatman GF/C, 2.5 cm). The filter was dried for 1 min at room temperature and immersed in ice-cold 10% trichloroacetic acid. For investigation of amino acid incorporation, the filters were further processed to hydrolyze aminoacyl-tRNA and to eliminate unincorporated radioactivity (13). When incorporation of uridine or thymidine was measured, hydrolysis with hot trichloroacetic acid was omitted, and the filters were washed several times with 5% ice-cold trichloroacetic acid, followed by washing with alcohol and ether. Finally the filters were dried, placed in scintillation vials, and incubated at 55° for 30 min with 0.5 ml of Soluene (TM 100, Packard). Then 10 ml of scintillation fluid (0.5% PPO and 0.005% POPOP in toluene) was added, and radioactivity was measured in a Nuclear Chicago Liquid Scintillation Counter model 6850 with an efficiency of 19%. The data obtained were expressed as pmol of uridine (or thymidine or amino acid) incorporated in 10⁶ cells.

Quantitative Presentation of Activity of Stimulators. In the figures and tables, the amount of stimulator present in 1 ml of incubation mixture is given. In calculations of yields, one bacterial unit (B.U.) and one lymphocyte unit (L.U.) is taken to mean the amount of stimulator that has to be added to 1 ml of the bacterial or lymphocyte test suspension, respectively, in order to induce 50% of the maximum (saturation) stimulation with regard to uridine incorporation during 150 min of incubation for *E. coli* Q 13 and 40 hr for chicken spleen lymphocytes (compare ref. 14).

RESULTS

Effect of Commercial PHA in Lymphocytes and *E. coli*. Fig. 1 demonstrates the RNA synthesis stimulating activity of commercial PHA in *E. coli* cells, as well as in chicken-spleen lymphocytes. The same batch of PHA-P (Difco) was used in both systems. 1 L.U. corresponds to 2.7 μl of PHA-P

solution, and 1 B.U. to 9 μ l of PHA-P solution. The usual inhibitory action of a high concentration of PHA appears in the lymphocyte system.

Extraction and Purification of Active Fractions from Beans. PHA-P (Difco) is a rather complex extract of red kidney beans containing about 17 different proteins, as revealed by polyacrylamide gel electrophoresis (14). In order to isolate from the extract the substances that stimulate RNA synthesis responsible for the effects shown in Fig. 1, the following purification procedure was used: Red kidney beans (*Phaseolus vulgaris*) were extracted for preparation of "dry powder" (15). Fraction I (F I) was prepared by extraction of 6.0 g of "dry powder" (derived from 500 g of beans) with 100 ml of PM buffer (8). The cloudy suspension was centrifuged, the precipitate was discarded, and the supernatant was filtered through a Sartorius membrane filter, pore size 0.22 μ m. Unless otherwise indicated, all centrifugations were at 2° for 20 min at 30,000 $\times$ g.

To each ml of F I, 0.16 ml of 11.5 M HClO₄ was added at 0°, as described (8). After 40 min at 0°, the suspension was centrifuged. The precipitate was discarded; the supernatant was neutralized at 0° by addition of about 0.2 ml of 8 M KOH to each ml of supernatant until pH 7.6 was reached. After 20 min at 0°, the suspension was centrifuged and the precipitate was discarded. To each 1 ml of supernatant, 0.43 ml of cold absolute ethanol was added at 0°. After 50 min, the suspension was centrifuged, and both the precipitate (p3) and supernatant (s3) were kept. The p3 was washed twice with 25 ml of ice-cold ethanol (30%) and was extracted at 0° with 50 ml of 5 mM potassium phosphate buffer (pH 7.5). The turbid suspension was centrifuged, the precipitate was discarded, and the supernatant was kept and called F III.

Besides protein (about 0.5–0.7 mg of N per ml), F III contains a large amount of some polysaccharide containing about 6.0 μ mol of ribose-equivalent pentose per ml, measured by the orcinol reaction (16). Most of the lymphocyte-stimulating activity present in the bean extract is associated with the protein fraction of F III. The protein fraction can be sepa-

FIG. 2. Gel filtration of fraction F IV on Sephadex G-50 fine. Bed diameter: 3.2 cm; bed volume: 320 ml; flow rate: 10 ml/hr; eluant: 1 mM phosphate buffer (pH 7.6). Sample size was 4 ml of F IV. Fraction size was 5 ml. Temperature 5°. For calibration of the column, Blue Dextran 2000 and tritiated water were used. K_{av} is calculated from the formula $K_{av} = (v_e - v_0)/(v_t - v_0)$, where v_e is the elution volume, v_0 the void volume, and v_t the total volume. RNA synthesis-stimulating activity in *E. coli* is expressed in B.U./ml. For definition of B.U. see *Methods*, and compare Table 1. Pentose (++++); absorbance (—); bacterial units (---).

FIG. 3. Polyacrylamide gel electrophoresis of (a) fraction F III, sample size 40 μ l; (b) fraction F III p, sample size 60 μ l; (c) fraction F V, sample size 30 μ l.

rated from the polysaccharide by precipitation at pH 2.5–3.0. This principle was used for the further purification of F III: 50 ml of F III was mixed with 1.0 ml of concentrated acetic acid at 0°, and after 30 min at 0° the suspension was centrifuged. The supernatant was freeze-dried, dissolved in 50 ml of distilled water with a drop of diluted NaOH (to pH 7.8), and was called F III s. The precipitate was washed with 10 ml of ice-cold 1 M acetic acid, then freeze-dried, dissolved in 50 ml of distilled water with a drop of diluted NaOH (to pH 7.8), and was called F III p.

Most of the bacteria-stimulating activity remained in s3, which was further purified as follows: s3 was mixed with an equal volume of 95% ethanol, and the mixture was boiled (about 80°) for 5 min under reflux and in N₂ atmosphere. The warm suspension was centrifuged (20,000 $\times$ g, room temperature, 10 min), the precipitate was discarded, and the supernatant was evaporated at 50° under reduced pressure to a final volume of 25 ml. The solution was centrifuged (20,000 $\times$ g, room temperature, 10 min), and the resulting 24-ml supernatant was mixed with 1.2 ml of Pronase solution containing 10 mg of Pronase per ml in 0.1 M NaCl–0.05 M Tris-HCl buffer (pH 7.8). After incubation at 40° for 4 hr the solution was mixed with 2 volumes of 95% ethanol, boiled for 5 min in N₂ atmosphere and reflux, and centrifuged (20,000 $\times$ g, room temperature, 10 min). The supernatant was concentrated at 50° under reduced pressure to a final volume of 6–7 ml, and was then dialyzed against three changes at 4-hr intervals of 1 liter of distilled water at 2°. The dialyzed solution was centrifuged; the final volume of the supernatant was

FIG. 4. Effect of rifampicin per ml test. (●) Control; (■) 0.5 μ g; (▲) 2.0 μ g; (◆) 4.0 μ g. Incubation time, 150 min.

adjusted to 10 ml and was called F IV. F IV was further purified by gel filtration on Sephadex G-50. A typical pattern of gel filtration of 4 ml of F IV is shown in Fig. 2. Fractions no. 40–52 were combined (48 ml), freeze-dried, dissolved in 4 ml of distilled water, and called F V.

Biological Activity of Fractions from Bean Extract. Tests for the ability of different fractions to stimulate RNA synthesis in the two biological systems are presented in Table 1. The lymphocyte-stimulating activity of F I was recovered with a 93% yield in F III from which F III p was prepared by acid precipitation without further loss of lymphocyte-stimulating activity. The orcinol-positive fraction F III s had neither lymphocyte- nor bacteria-stimulating activity. Bacteria-stimulating activity was found in F V, which was obtained from F I with 12% yield.

Electropherograms of the biologically active fractions (Fig. 3) indicate that the lymphocyte-stimulating fraction, F III p, consists of two main proteins. The bacteria-stimulating fraction, F V, contains one protein. Typical experiments demonstrating the lymphocyte- and bacteria-stimulating properties of F III p and F V are shown in Table 2. At a concentration of 2.7 μ g N/ml, F III p gives the strong stimulation of lymphocytes characteristic of phytohemagglutinins. This concentration of F III p is without effect in the bacterial system, and even at a 50-times higher concentration, only a weak effect is obtained. F V gives a strong stimulation,

TABLE 1. Biological activity and content of nitrogen and pentose* in different fractions of the bean extract

Fraction (ml)	N (mg/ml)	Activity in			Nitrogen per unit	
		Pentose (μ mol/ml)	<i>E. coli</i> (B.U./ml)	Lymphocytes (L.U./ml)	<i>E. coli</i> (μ g N/B.U.)	Lymphocytes (μ g N/L.U.)
F I (100)	2.3	28.0	230	3600	10.0	0.64
F III (50)	0.60	5.4	4	6700	(150)	0.09
F III s (50)	<0.05	4.8	0	0	—	—
F III p (50)	0.54	<0.2	2.5	6700	(220)	0.08
F V (10)	0.67	<0.2	270	—†	2.5	—†
For comparison: PHA-P	1.30	1.2	100	500	13.0	2.6

* Ribose equivalents were determined with orcinol (16).

† A slight activity was observed; see text and Table 3.

especially of RNA synthesis in the bacteria, but its action in the lymphocyte system is limited to a small, although significant, increase of nucleic-acid synthesis, probably a consequence of a delayed dying of the cells.

The mechanism of action of the bacteria-stimulating fraction, F V, was studied with rifampicin and chloramphenicol, inhibitors of RNA and protein synthesis, respectively. Table 3 shows that F V increased RNA synthesis in rifampicin-blocked cells. Incorporation of phenylalanine was also increased by F V in rifampicin-blocked cells, whereas, in the presence of chloramphenicol, only a minute effect of F V on incorporation of phenylalanine could be observed. The RNA synthesis of chloramphenicol-blocked cells could be stimulated proportionally to that of the uninhibited control. The decreased incorporation of uridine in chloramphenicol-treated cells shows that the RNA synthesis was promoted by products of protein synthesis. The dose-response curves of F V in the bacterial system at different rifampicin concentrations (Fig. 4) suggest that F V in some way counteracted the RNA synthesis-inhibiting effect of rifampicin.

DISCUSSION

Using chicken-spleen lymphocytes and plasmolyzed *E. coli* cells, we isolated RNA synthesis stimulating factors, here called F III p and F V from an extract of red kidney beans, the source of commercial phytohemagglutinins. The lymphocyte-stimulating component of the bean extract was found in a fraction, here called F III, that consists mainly of two proteins and carbohydrates. The biologically active proteins, F III p, which appear to be the well-known mitogens for lymphocytes, could be separated from contaminating carbohydrates by acid precipitation. The property of stimulation of lymphocytes and the preliminary determination of the amino acid composi-

TABLE 2. Effect of F III p and F V on RNA and DNA synthesis in lymphocytes and in bacteria*

				F III p (140 μg N/ ml)	F V (8.3 μg N/ml)	F III p + F V (2.7 μg N/ml + 8.3 μg N/ml)
	Hr	Con- trol	F III p (2.7 μg N/ml).			
<i>Lymphocytes</i>						
RNA syn- thesis	18	0.85 (100)†	6.70 (789)	—	1.18 (139)	6.71 (790)
	42	0.29 (100)	19.1 (6,660)	—	0.53 (187)	19.5 (6,800)
DNA syn- thesis	18	0.14 (100)	2.02 (1,470)	—	0.26 (188)	2.48 (1,610)
	42	0.028 (100)	6.50 (23,400)	—	0.051 (183)	6.25 (22,400)
<i>Bacteria</i>						
RNA syn- thesis	2.5	88 (100)	89 (100)	201 (228)	448 (510)	440 (500)
DNA syn- thesis	2.5	1.40 (100)	1.35 (96)	1.52 (113)	2.82 (202)	2.89 (206)

* Given as pmol of uridine and thymidine incorporated, respectively, per 10^6 cells.

† Incorporation in % of the respective control is given in parenthesis.

TABLE 3. RNA, DNA, and protein synthesis and effects of rifampicin and chloramphenicol on incorporation of [3 H]uridine and [3 H]phenylalanine in *E. coli*.

	Uridine		Phenylalanine		Thymidine	
	Control	F V	Control	F V	Control	F V
Control	81 (100)	398 (490)	15.4 (100)	48 (320)	1.32 (100)	2.75 (208)
Rifampicin (9 μ g/ml)	2.47 (100)	53 (2150)	0.83 (100)	7.7 (930)	—	—
Chloram- phenicol (4 μ g/ml)	17.0 (100)	95 (633)	1.82 (100)	2.48 (136)	—	—

Inhibitors were added before the start of the test, the duration of which was 150 min. F V was added to a final concentration of 6 μ g N per ml test. Incorporation of precursors is given in pmol per 10^6 cells, and, in parenthesis, as % of the respective control.

tion of F III p show that it is identical or similar to the pooled proteins, L-PHAP + H-PHAP, described by Allen *et al.* (14).

The orcinol-positive fraction, F III s, was inactive in the tests applied. This fraction contains no protein. By comparing the electropherograms of F III and F III p (Fig. 3), one can see that removal of this component did not influence the electrophoretic patterns.

The bacteria-stimulating component of the bean extract was found in a fraction, called F V, which gives one band in polyacrylamide gel electrophoresis.

RNA synthesis in the bacterial system cannot be stimulated by F III p, the slight effect observed being assignable to the small contaminating amount of F V revealed by polyacrylamide gel electrophoresis. On the other hand, F V gives only a weak stimulation of RNA synthesis in the lymphocyte system. The slight increase in uridine incorporation and the better survival of lymphocytes caused by F V is not comparable to the characteristic lymphocyte-stimulating effect of F III p.

These observations lead us to conclude that the lymphocyte-stimulating substance (F III p) and the bacteria-stimulating substance (F V) in the bean extract are different entities that exert their actions by different mechanisms. Preliminary data indicate that the amino acid composition of F V is different from that of F III p. F V contains about 20 mol % cysteine, and the molecular weight seems to be 9,500–10,000, as measured by ultracentrifugation or gel filtration (to be published). F V and the components of F III p agree, however, with respect to the N-terminal amino acid, only serine being found in this position.

The uniqueness of a protein, here called F V, with regard to its specificity to stimulate RNA synthesis in the bacterial system, unusually high cysteine content, low molecular weight, and presence in red kidney beans, the source of phytohemag-

glutinin, is here stated. Pokeweed mitogen is the only plant protein that contains a similarly high amount of cysteine (17); this protein activates lymphocytes to cell division as well as to large-scale production of immunoglobulins (18). Under the conditions in the present study, F V had only a weak RNA synthesis-stimulating effect on lymphocytes. This effect was not comparable to the characteristic activating effect induced by F III p.

The particular stimulating effect of F V on RNA synthesis in the bacterial system is not yet understood. The antagonism of F V and rifampicin (see Fig. 4) indicates that F V might act on the level of DNA-dependent RNA polymerase. However, this hypothesis was not supported by *in vitro* studies [done according to Sippel *et al.* (19), kindly performed by Dr. G. R. Hartmann, Institute of Biochemistry, University of Würzburg, Würzburg] of the action of F V on the purified enzyme in the presence and absence of rifampicin. This result leads to the assumption that F V modifies the rate of RNA synthesis on some other level.

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