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## TRANSLATION OF ONCOGENIC VIRAL RNA AND EUKARYOTIC MESSENGER RNA IN THE *E. COLI* CELL-FREE SYSTEM

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### 1. Introduction

Recently several studies have been published which describe the faithful translation of animal messenger and viral-RNA in eukaryotic cross systems [1–12].

Although stimulation of amino acid incorporation by viral RNA or eukaryotic messengers in bacterial cell-free systems has been reported, reliable translation has only been described incidentally [13–19].

We compared the translation of eukaryotic- and oncogenic viral-RNA's in the cell-free system of *E. coli* with that of the reticulocyte lysate. Our results provide evidence that in the bacterial system the viral RNA's are translated into several distinct polypeptides, whereas no indication for the translation of eukaryotic messengers could be obtained. Moreover, addition of the viral RNA's to the mammalian systems described, appeared not to result in translation.

### 2. Materials and methods

Rauscher leukemia virus (RLV) was isolated from the plasma of leukemic mice as described [20]. Purified mouse mammary tumor virus (MTV) from mammary tumors of (C3H/HeA × O20)F<sub>1</sub> mice was a generous gift of Dr. P. Hageman. After incubation of the virus with pronase in the presence of SDS the viral RNA's were extracted with chloroform–phenol [21, 22]. The 60 S component of RLV-RNA was isolated by centri-

fugation in isokinetic glycerol gradients (15.0–30.2 w/v). Denaturation of RNA was performed in 3 mM KCl for 3 min at 65° [23]. After heating and rapid cooling the RNA preparations were immediately tested for their messenger activity. Globin 9 S and  $\alpha$ -crystallin 14 S mRNA were a gift of Dr. A. Berns. Mono layers of mouse spleen- and thymus-cells (JLS-V5) were used for the propagation of radioactive-labeled RLV. The JLS-V5 cell line was a generous gift of Dr W. Schäfer. The preparation of phage M12 RNA and of the cell-free system of *E. coli* has previously been described [24]. The incubation mixture contained p ml: 50 nmoles of each of the added unlabeled amino acids, 10  $\mu$ moles magnesium acetate, 50  $\mu$ moles Tris HCl, pH 7.8, 70  $\mu$ moles NH<sub>4</sub>Cl, 8  $\mu$ moles 2-mercapt ethanol, 0.25 mg *E. coli* tRNA, 5  $\mu$ moles ATP, 0.3  $\mu$ moles GTP, 5  $\mu$ moles PEP, 8  $\mu$ g pyruvate kinase, and 0.24 ml of pre-incubated S-30. The amounts of RNA and radioactive label added to the system are given in the legend of table 1. After 30 min of incubation at 37°, 10  $\mu$ l aliquots from the incubation mixture were removed for the estimation of total protein synthesis by precipitation with TCA. The remainder was treated with pancreatic RNAase and analyzed by electrophoresis on 12.5% SDS–polyacrylamide gels [25].



### 3. Results and discussion

#### 3.1. Effect of viral RNA and eukaryotic mRNA on amino acid incorporation

Addition of either RLV-RNA or MTV-RNA to the cell-free system of *E. coli* resulted in a significant stimulation of the amino acid incorporation (table 1). When equal amounts of RLV- and MTV-RNA were tested, the stimulatory effect of the latter was always more pronounced. An even higher stimulation could be obtained if instead of total RLV-RNA the purified 60 S RNA component was added to the system. This is probably due to an inhibitory effect of the low molecular weight viral RNA (4–5 S), present in the virion, on the translation of 60 S RLV-RNA. Evidence for this assumption was derived from the fact that this low molecular weight RNA fraction also inhibited the translation of phage M12 RNA (cf. [26]). Denaturation of RLV-RNA consistently resulted in an additional stimulation of the amino acid incorporation, while denaturation

of MTV-RNA was much less effective. For comparison the effect of several distinct eukaryotic mRNA preparations on the amino acid incorporation was studied. Addition of globin 9 S mRNA resulted in a stimulation of the amino acid incorporation which could be enhanced by denaturation. The effect of lens 14 S mRNA, coding for the A<sub>2</sub>-chain of  $\alpha$ -crystallin, was almost negligible.

No significant stimulation could be observed after addition of either 18 S or 28 S ribosomal RNA to the cell-free system.

#### 3.2. Analysis of the *in vitro* products by SDS polyacrylamide gel electrophoresis

From the results presented in fig. 1 A it can be concluded that addition of native RLV-RNA to the cell-free system gives rise to the synthesis of a number of distinct polypeptides. Co-electrophoresis of these polypeptides with the proteins derived from the virion indicated that at least those migrating in the low molecular weight range (slice no. 52–71) coincide with the native viral proteins (fig. 1 B). Furthermore additional polypeptides are synthesized which migrate in the 30,000 dalton region (slice no. 32–40). The effect of denaturation of RLV-RNA on the translation is striking. This resulted in the appearance of a high molecular weight polypeptide (slice no. 24–27) which is only present in trace amounts in the control experiment. In order to obtain more accurate data about the size and number of the synthesized polypeptides, autoradiographic analysis on dried gels was performed. In fig. 2 A, the autoradiogram of the polypeptides synthesized under the direction of denatured RLV-RNA is shown (b). For comparison, the gel pattern of labeled viral proteins is also depicted (c). It may be concluded that denatured RLV-RNA mainly gives rise to the synthesis of two polypeptides, RE<sub>0</sub> and RE<sub>2</sub>, with molecular weights of approx. 45,000 and 15,000 daltons, respectively. The electrophoretic mobility of the polypeptide RE<sub>2</sub> is identical to that of the native protein R<sub>2</sub>. It might be possible that the polypeptide RE<sub>0</sub> is identical to one of the minor viral proteins present in the 45,000 daltons region. No clear indication could be obtained whether a polypeptide of the same size as the viral protein R<sub>1</sub> (gs 1) was synthesized. Furthermore several faint bands are visible of which at least some seem to have no corresponding polypeptide syn-

Table 1

Stimulation of amino acid incorporation by viral RNA and eukaryotic RNA preparations in the cell-free system of *E. coli*.

| Exp. I    |   | Exp. II              |   |
|-----------|---|----------------------|---|
| RNA added | [ <sup>14</sup> C]amino acid (cpm incorporated) | RNA added            | [ <sup>35</sup> S]methionine (cpm incorporated) |
| None      | 558   | None                 | 5820  |
| Phage M12 | 12,693  | Phage M12            | 138,866   |
| RLV       | 1058 (2010)*                                    | RLV                  | 8784 (14,398)                                   |
| RLV 60 S  | 1928 (2749)                                     | Globin 9 S           | 8960 (10,102)                                   |
| MTV       | 2033 (2386)                                     | $\alpha$ -crystallin |   |
| Ribosomal |   | 14 S                 | 6506 (7290)                                     |
| 18 S      | 604   |                      |   |
| Ribosomal |   |                      |   |
| 28 S      | 613   |                      |   |

To each 60  $\mu$ l incubation mixture, in experiment I, 4  $\mu$ g RNA and 5  $\mu$ Ci [<sup>14</sup>C]amino acid mixture (54 Ci/mg-atom of carbon) was added. In experiment II, to each 60  $\mu$ l incubation mixture 5  $\mu$ g RNA and 8  $\mu$ Ci [<sup>35</sup>S]methionine (32 Ci/mmol) was added. After 30 min of incubation at 37° the reaction was terminated by the addition of 60  $\mu$ l of 0.025 M EDTA and TCA precipitable radioactivity was determined in 10  $\mu$ l aliquots of the incubation mixture.

\* Values obtained after denaturation of the RNA are given in parentheses.

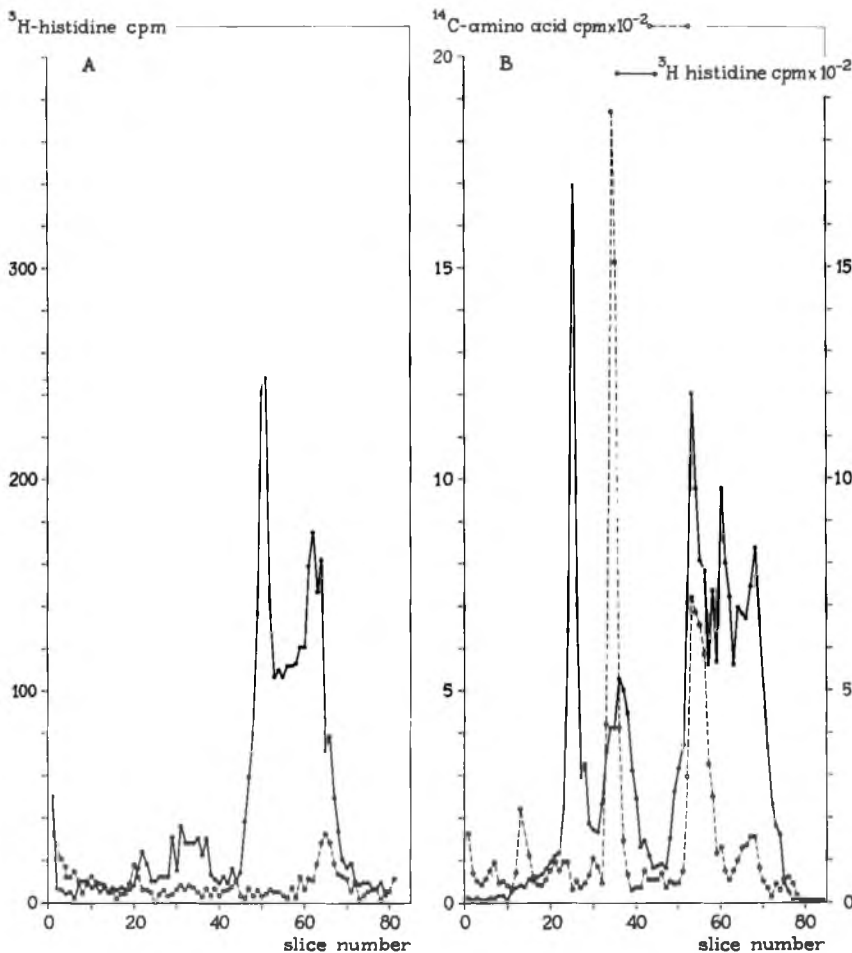


Fig. 1. SDS gel analysis of the polypeptides synthesized under direction of RLV-RNA. A) Polypeptides synthesized in the absence (○-○-○) and presence (●-●-●) of RLV-RNA. B) Comparison by co-electrophoresis of the polypeptides synthesized *in vitro* under the direction of denatured RLV-RNA (●-●-●) with the viral proteins labeled with [<sup>14</sup>C] amino acids (○-○-○). Incubation in the presence of [<sup>3</sup>H]histidine. After electrophoresis the gels were sliced in 1 mm sections. To measure the radioactive labels separately the dried gel slices were processed with the aid of the Tri-Carb Sample Oxidizer (Packard Model 305).

thesized in the endogenous system.

As the RNA present in the RLV virion consists of several RNA species, experiments were performed to investigate whether there exists a difference between the polypeptides synthesized under the direction of total RNA and the purified 60 S RNA component. Analysis of the *in vitro* products showed that both RNA preparations directed the synthesis of the same polypeptides (fig. 2 B, a-c). From these results it may be concluded that the RNA component sedimenting in the 60 S region contains all the genetic information

necessary to code for the polypeptides synthesized in the *in vitro* system.

Evidence for the reliability of the *in vitro* translation of RLV-RNA can be derived from the observation that under direction of RNA isolated from the non-related mouse mammary tumor virus (MTV) quite different polypeptides are synthesized. MTV-RNA directs the synthesis of at least one polypeptide ME<sub>1</sub> which coincides with one of the native MTV proteins (P<sub>6</sub>), (fig. 3 A, a-c). This polypeptide migrates slightly faster than the RLV-RNA directed polypeptide RE<sub>2</sub>.

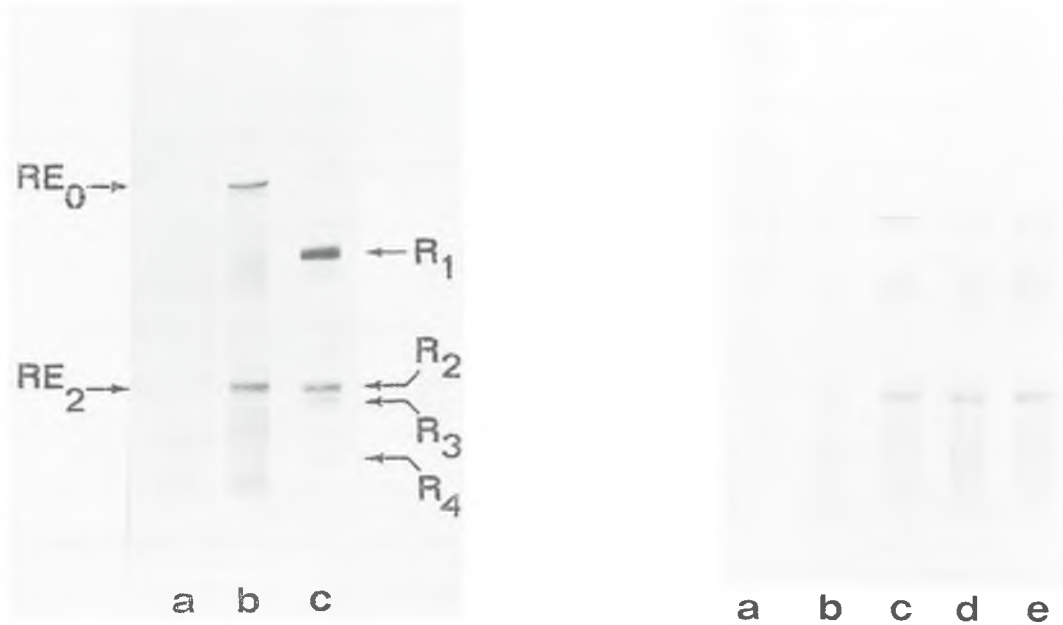


Fig. 2. Autoradiograms of the polypeptides synthesized under the direction of RLV-RNA and separated by SDS gel electrophoresis. A) Products of the endogenous *E. coli* system (a); with denatured RLV-RNA (b); RLV proteins labeled with [<sup>14</sup>C] amino acids (c). B) Products of the endogenous *E. coli* system (a); with RLV-RNA (b); with denatured RLV-RNA (c); with the 60 S RLV-RNA component (d); with the denatured 60 S RLV-RNA component (e). Incubation in the presence of [<sup>14</sup>C] amino acids.

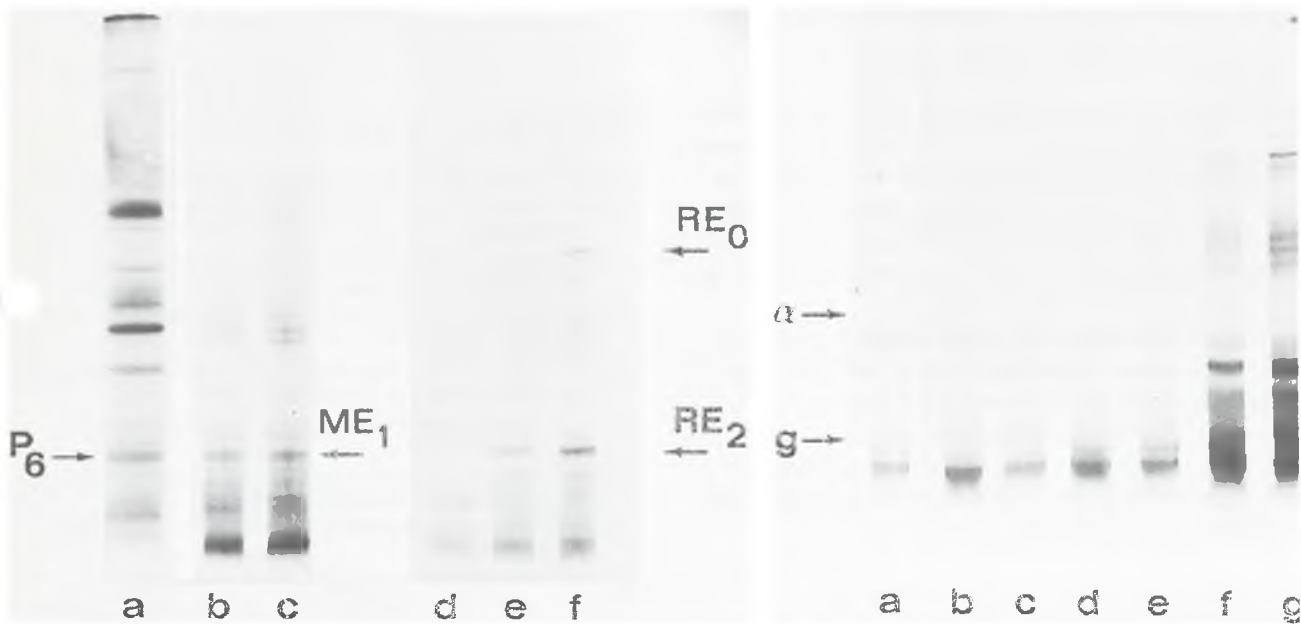


Fig. 3. A) Autoradiograms of the polypeptides synthesized under the direction of MTV- or RLV-RNA and separated by SDS gel electrophoresis. Native MTV proteins stained with Coomassie blue (a); *in vitro* system supplemented with MTV-RNA (b); with denatured MTV-RNA (c); without RNA (d); with RLV-RNA (e); with denatured RLV-RNA (f). Incubation in the presence of [<sup>14</sup>C] amino acids. B) Autoradiograms of the polypeptides synthesized under the direction of globin 9 S and α-crystallin 14 S mRNA. Products of the endogenous *E. coli* system (a); with globin 9 S mRNA (b); with denatured globin 9 S mRNA (c); with α-crystallin 14 S mRNA (d); with denatured α-crystallin 14 S mRNA (e); with native RLV-RNA (f); with denatured RLV-RNA (g). α stands for α-crystallin, g stands for globin. Incubation in the presence of [<sup>35</sup>S]methionine.

Denaturation of MTV-RNA does not result in the synthesis of an additional polypeptide (c) as has been observed in the case of RLV-RNA (f). There is a striking discrepancy between the distribution of radioactivity of polypeptides synthesized under the direction of MTV-RNA as compared with those synthesized under the direction of RLV-RNA (compare b, c with e and f).

Furthermore, in case of MTV-RNA a pronounced synthesis of polypeptides which migrated with the buffer-front could be observed (b, c). The reason for this may be that, although there is a high rate of initiation, the completion of polypeptides is inefficient.

In order to examine whether eukaryotic mRNA preparations are faithfully translated we studied the effect of addition of  $\alpha$ -crystallin 14 S and globin 9 S mRNA to the *E. coli* cell-free system. From fig. 3 B it may be concluded that both mRNA fractions do not give rise to the synthesis of their encoded proteins (b-e). Only low molecular weight polypeptides seem to be synthesized. As suggested above this may be due to the fact that proteins are initiated but, for reasons still unknown, not completed. In this connection it should be mentioned that under conditions in which globin- and  $\alpha$ -crystallin-mRNA are translated very efficiently in a reticulocyte cell-free system and in oocytes, until now in both systems no evidence could be obtained for the translation of RLV-RNA (experiments in collaboration with Dr. J.B. Gurdon, Unpublished). In the reticulocyte system only a strong inhibition of globin synthesis, up to 85%, could be observed. Although a 0.5 M KCl-wash derived from polysomes of leukemic spleens stimulated the endogenous globin and  $\alpha$ -crystallin synthesis, no indication could be obtained whether this fraction has a positive effect on the translation of RLV-RNA as well.

From our results the conclusion seems to be justified that in the cell-free system of *E. coli* under the direction of the different viral RNA preparations tested at least some virus-specific polypeptides are synthesized. No specific polypeptides, however, are synthesized when this cell-free system is programmed with eukaryotic mRNA's.

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## INHIBITORY EFFECT OF RIFAMPICIN ON RAUSCHER-VIRUS-INDUCED MURINE LEUKAEMIA

by

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*Rifampicin has a marked inhibitory effect on Rauscher-virus-induced leukaemia. Up to 90% inhibition of splenomegaly can be achieved when the drug is administered immediately after infection. It is suggested that these results may have implications for a rational leukaemia therapy.*

All the RNA tumour viruses contain an enzyme that transcribes RNA to DNA (Schlom *et al.*, 1971a). This finding provides direct evidence for Temin's hypothesis that RNA tumour viruses need a DNA intermediate for virus replication (Temin, 1964). Synthesis of new DNA, possibly virus-specific, appears in the cytoplasm of cells within a short time after infection (Hatanaka *et al.*, 1971). Chronically infected cells do not show such a DNA synthesis. Whether the newly-formed DNA becomes integrated in the host cell genome remains to be determined.

An RNA-dependent DNA polymerase has been detected in lymphoblasts of patients with acute and chronic lymphoblastic leukaemia (Gallo *et al.*, 1970, and Penner *et al.*, 1971). DNA polymerase activity seems to be present in a particulate fraction of plasma from patients with chronic lymphatic leukaemia (Kießling *et al.*, 1971). Reserve transcriptase activities have also been observed in a cell line derived from leukocytes of a patient with a myeloid leukaemia (Ackermann *et al.*, 1971) and in virus-like particles isolated from human milk (Schlom *et al.*, 1971b).

*In vitro* the RNA-dependent DNA polymerase can be inhibited partly by rifampicin and to a much greater extent by rifampicin derivatives,

with modified aminopiperazine side chains and streptovaricins (Gallo *et al.*, 1970; Gurgo *et al.*, 1971; Brockman *et al.*, 1971). Rather contradictory results have been published concerning the effect of rifampicin and rifampicin derivatives on virus production and cell transformation after infection of tissue culture cells with an RNA tumour virus (Diggelmann and Weissmann, 1969; Robinson and Robinson, 1971; Richert and Balduzzi, 1971; Calvin *et al.*, 1971; Carter *et al.*, 1971; Lancini *et al.*, 1971). This article describes the inhibitory effect of rifampicin on Rauscher-virus-induced leukaemia *in vitro*. The Rauscher virus elicits primarily a rapid proliferation of erythroblastic elements which results in spleen enlargement. The rate of splenomegaly can be used as an indicator of the progression of the disease (Rauscher and Allen, 1964). Drug-induced inhibition of spleen enlargement can be considered as an easily-detectable manifestation of a therapeutic effect (Chirigos, 1963).

### MATERIAL AND METHODS

In all experiments mentioned below, 3-week-old random-bred female Swiss mice were used. Mice were infected intraperitoneally with 0.1 ml of a 10% spleen homogenate as described before (Burghouts *et al.*, 1970). Unless otherwise indicated, treatment with rifampicin commenced

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7 days after infection. Rifampicin powder (Lepetit, Milan, Italy) in various amounts as described in the "Results" section was mixed with 2.5 g of pulverized mouse feed granules per mouse. Control animals received the same amount of feed. Mice were killed 4 weeks after infection, *i.e.*, 3 weeks after commencing treatment. The spleen weight was determined immediately.

#### RESULTS

The relation between spleen weight and appropriate time intervals after infection is demonstrated in Figure 1. Already 7 days after infection a marked increase in spleen weight can be observed. This means that treatment was started when all mice had overt leukaemia.

The results of some experiments performed with rifampicin are given in Table I. It can be seen from this Table that administration of 5 mg of rifampicin, 7 days after Rauscher virus infection, results in a reduction of spleen enlargement of 61.7%. This experiment was repeated five times. In all these different experiments the inhibitory effect of the drug (approximately 50-60%) was obvious. The inhibition of spleen enlargement is even more pronounced when treatment is started 4 days (85.1%) or immediately (90.3%) after infection. We have evidence that pretreatment of the mice with rifampicin for 7 days does not result in an enhanced effect.

The inhibitory effect of rifampicin depends on the amount of the drug used in each experiment (Fig. 2). A higher concentration finally results in a lower spleen weight. The drug concentration generally used (5 mg rifampicin/mouse/24 h) was not toxic since uninfected

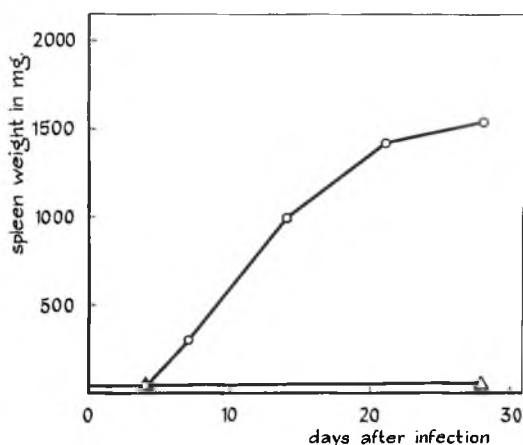


FIGURE 1

*Relation between spleen weight and time after infection*

Random-bred Swiss mice were infected with the Rauscher virus as described in the text. Uninfected mice served as controls. At appropriate time intervals mice were killed with ether anaesthesia and their spleens were weighed immediately. Each point represents the average spleen weight of at least 10 mice. ○: spleen weights of Rauscher-virus-infected mice. △: spleen weights of uninfected control mice.

control animals which were treated similarly had exactly the same spleen weights and body weights as the untreated control mice. In mice the LD<sub>50</sub> of the drug *per os* was approximately 1,400 mg/kg body weight (Gruppo Lepetit S.p.A. Milan, Italy, unpublished results). In our experiments 1,250 mg/kg body weight given in a single dose orally and mixed with food was not lethal. For comparison, the effect of tetracycline was also studied. The latter treatment did not reveal any inhibitory effect.

TABLE I  
INHIBITORY EFFECT OF RIFAMPICIN ON SPLEEN ENLARGEMENT IN LEUKAEMIC MICE<sup>1</sup>

| Number of mice | Treatment mg drug/mouse/24h | Duration of treatment in days after infection | Average spleen weight in mg | Standard error in mg | Inhibition of splenomegaly (%) |
|----------------|-----------------------------|---|-----------------------------|----------------------|--------------------------------|
| 35             | None                        | —   | 1531.4                      | 176.9                | —                              |
| 9              | 5 mg Rifampicin             | 0-28  | 174.9                       | 40.1                 | 90.3                           |
| 10             | 5 mg Rifampicin             | 4-28  | 254.3                       | 66.9                 | 85.1                           |
| 9              | 5 mg Rifampicin             | 7-28  | 604.1                       | 163.2                | 61.7                           |

<sup>1</sup> Random-bred Swiss mice were infected with the Rauscher virus as described in the text. Mice were treated at appropriate dates with drugs as specified. After 4 weeks the mice were killed by ether anaesthesia and their spleens were weighed immediately. The results in this Table are a typical example of five different experiments performed. Spleens of uninfected mice of the same age weighed 30.4 mg.

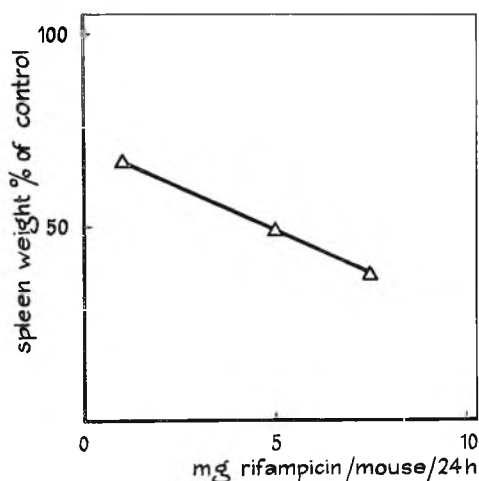


FIGURE 2

Relation between spleen weight and rifampicin concentration during treatment

Four groups of random-bred Swiss mice were infected with the Rauscher virus as described in the text. One week after infection rifampicin in specified concentrations was administered to three groups of mice. After 4 weeks, all the mice were killed by ether anaesthesia and their spleens were weighed immediately. Spleens of the untreated group of mice served as a 100% control. Inhibition of spleen enlargement is dependent on the concentration of Rifampicin used for each group of mice.

#### DISCUSSION

The influence of rifampicin (an inhibitor of the RNA-dependent DNA polymerase *in vitro*) on the development of Rauscher-virus-induced leukaemia *in vivo* was studied. The drug displayed an inhibitory effect which was more pronounced when treatment was started immediately after virus infection. This effect can be explained by the assumption that rifampicin prevents the infection of (previously) uninfected cells. Cells already involved in virus production and transformation may not be sensitive to the drug. This means that rifampicin inhibits either an early event, necessary for virus replication and cell transformation, *i.e.*, it blocks the reverse transcriptase (thus preventing provirus DNA formation) or it prevents some of the sequences leading to the fixation of the provirus DNA in the host cell genome (Richert and Balduzzi, 1971). Apparently in our experiments the

rifampicin block does not seem to be absolute since complete inhibition could not be obtained. This may be explained by the fact that rifampicin itself is not a potent inhibitor of the RNA-dependent DNA polymerase *in vitro* (Gallo *et al.*, 1970; Gurgo *et al.*, 1971). Furthermore the route of administration (mixing with food) probably does not result in optimal serum rifampicin levels during the full 24 h. More potent inhibitors of reverse transcriptase AF/AP, AF/ABP and AF/ABDP could be tested only in rather low concentrations. The preliminary results of these experiments indeed indicate an inhibitory effect.

As the inhibitory effect of rifampicin *in vitro* is rather low, our results obtained with the drug *in vivo* are surprising. Experiments performed with streptovaricins, which are more potent inhibitors of the reverse transcriptase (Brockman *et al.*, 1971; Carter *et al.*, 1971), resulted in an inhibition of spleen enlargement only for approximately 20% when administered 8 days after infection (Borden *et al.*, 1971). This result may probably be ascribed to the low absorption rate of the streptovaricin complex when administered orally and the duration of the treatment (one week). Serum levels of 1-3  $\mu\text{g/ml}$  can be obtained with 1,500 mg streptovaricin complex/kg body weight/24 h. In our experiments with 5 mg rifampicin/mouse/24 h (approximately 250 mg/kg body weight/24 h) we obtained serum levels of 100  $\mu\text{g/ml}$ . Our results also admit an alternative explanation recently proposed by Vaheri and Hanafusa (1971). The authors suggest that rifampicin may be more toxic to transformed cells than to normal cells.

If the effect observed is as selective as it seems to be, then it may have important implications for a rational leukaemia therapy.

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EFFET INHIBITEUR DE LA RIFAMPICINE SUR LA  
LEUCÉMIE MURINE INDUITE PAR LE VIRUS DE RAUSCHER

*La rifampicine a des effets inhibiteurs prononcés sur la leucémie induite par le virus de Rauscher. L'inhibition de la splénomégalie peut atteindre 90% si le produit est administré tout de suite après l'infection. Nous pensons que ces résultats peuvent avoir des implications quant à un traitement rationnel de la leucémie.*

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LETTER TO THE EDITORS

**Evidence for a "Non-genetic" Origin of the A1 Chains of  $\alpha$ -Crystallin**

It is now firmly established that  $\alpha$ -crystallin is an aggregate of different polypeptides. Calf lens  $\alpha$ -crystallin consists of basic and the acidic polypeptide chains (Bloemendal, 1969) which separate on basic urea-polyacrylamide gels into  $\alpha$ A1,  $\alpha$ A2,  $\alpha$ B1 and  $\alpha$ B2 (Bloemendal, Berns, Zweers, Hoenders and Benedetti, 1972).

Schoenmakers and Bloemendal (1968) were able to show that in embryonic calf lenses the  $\alpha$ A1 chain is lacking. This polypeptide, however, appears gradually with increasing age of the embryo. Palmer and Papaconstantinou (1969) confirmed this observation and suggested that the  $\alpha$ A1 chain is not formed by *de novo* synthesis but by a chemical or enzymic conversion from  $\alpha$ A2. Delcour and Papaconstantinou (1970) demonstrated that in fiber cells all four chains are present whereas adult lens epithelial cells contain mainly  $\alpha$ A2 and  $\alpha$ B2.

Schoenmakers, Gerding and Bloemendal (1969) demonstrated that both acidic chains have an identical amino acid content. Peptide mapping after chymotryptic digestion of the A chains revealed the occurrence of one "difference-peptide" of similar amino acid composition. The difference was ascribed to the fact that  $\alpha$ A2 had a glutamine or asparagine residue instead of a glutamic acid or aspartic acid in  $\alpha$ A1. The latter observation has recently been confirmed and extended by analysis of tryptic peptides from A1 and A2. We have strong evidence that actually a glutamine-glutamic acid conversion is responsible for the difference, in which Gln in position 9 of the cysteine peptide described by Corran and Waley (1971), is involved (van der Ouderaa, de Jong and Bloemendal, unpublished data). This difference explains the higher mobility of  $\alpha$ A1 in gel electrophoresis at alkaline pH. We have recently isolated messenger RNA species from calf lens (Berns, de Abreu, van Kraaikamp, Benedetti and Bloemendal, 1971). These messengers were precisely translated in two different cell-free systems (Berns, Strous and Bloemendal, 1972; Mathews, Osborn, Berns and Bloemendal, 1972) and in an *in vivo* system (Berns, van Kraaikamp, Bloemendal and Lane, 1972).

Our recent results clearly show that there is no detectable messenger RNA species in calf lens coding for  $\alpha$ A1. This finding may explain the preliminary experiments of Palmer and Papaconstantinou (1969) who pulse-labeled fetal calf lens with radioactive amino acids and found no radioactivity in  $\alpha$ A1. The absence of a messenger priming the synthesis of  $\alpha$ A1 in post-natal calf lens renders gene activation as an explanation very unlikely. The possibility, however, that a repression mechanism blocks the translation of a messenger coding for the  $\alpha$ A1 chain cannot be ruled out completely. At any rate the appearance of  $\alpha$ A1 in the adult lens can easily be understood in terms of deamidation of one glutamine in the  $\alpha$ A2 chain.

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