water, alcohol, ether and all the usual organic solvents, soluble in alkaline media (aqueous) and in alkaline organic solvents, such as pyridine and liquid amines. Acute toxicity (LD_{50}) for mice was found to be 800 μg/g.

Coagulation times were measured by the usual method of Sabrazes (capillary tube), using blood collected from the marginal vein of the ear of rabbits, before and at different times after the intravenous injection of slightly alkaline (pH 7.5) aqueous solutions of the hydrazone. Control animals were injected with the same volumes of alkaline solutions of identical pH and bled simultaneously.

Results and Discussion. Upon the examination of more than 60 rabbits, we found the normal coagulation time to lie between 30 sec and 1 min. About 20% of the animals had coagulation times as high as 4 min, though being apparently healthy, and among them we selected 12 of the ones presenting the highest values. Two of them we kept as controls, and the rest was injected with different amounts of the hydrazone.

The coagulation time was measured every 30 min during 3 h, and typical examples of the results so obtained are shown in the Figure, where we compare the values found for the controls with the ones of 2 rabbits injected with 5 mg/kg IHA.

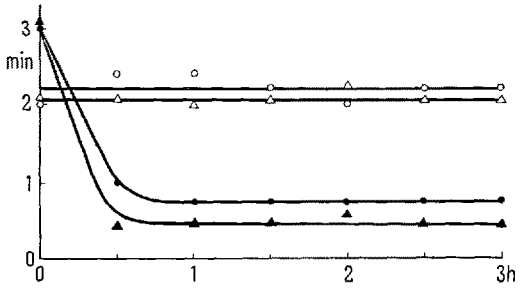
As can be seen, under these conditions the coagulation time drops at a very fast rate during the first 30 min after the injection of the drug, till the lower normal values are reached. These lower values were found to be stable during all the period of observation, the rate of that decrease being roughly proportional to the amount of drug injected.

The Table shows the initial values found for the coagulation times of all the animals used in the present experiment, as well as the lower ones found after the injection of different amounts of the drug.

No explanation of the hemostatic activity of IHA can be offered at the present moment, though the possibility exists of its being due to the interference of the drug on the acetylation of thrombin, leading to a higher clotting power[6].

Coagulation times before and after the intravenous injection of different doses of IHA

Animal No.	Coagulation time (min) initial	final	Dose mg/kg
1	2:45	1:05	2
2	4:00	1:30	2
3	2:50	0:40	4
4	2:50	1:20	4
5	2:35	0:30	4
6	3:00	1:00	5
7	3:00	0:45	5
8	2:30	0:30	5
9	3:05	0:25	10
10	3:00	0:30	10



Coagulation times of two rabbits injected intravenously with 5 mg/kg of IHA (black marks) compared with the values found for two untreated animals (empty marks).

Résumé. Les auteurs ont étudié l'activité hémostatique de l'isonicotinyl hydrazone de l'aldéhyde acétique, utilisant des lapins normaux à temps de coagulation sanguine relativement long (2–4 min). L'injection intraveineuse de 5 mg/kg de la drogue cause une chute très rapide du temps de coagulation vers les valeurs normales (0,5–1 min), valeurs qu'ils ont trouvées constantes pendant toute la période d'observation (3 h).

R. C. R. BARRETO, D. B. MANO, and A. M. CUTRIM

Institute of Phthisiology and Pneumology, University of Brazil, Rio de Janeiro (Brazil), November 7, 1962.

[6] R. H. LANDABURU and W. H. SEEGERS, Can. J. Biochem. Physiol. *37*, 1361 (1959).

[7] *Acknowledgments.* The present work was carried on at the Central Laboratory of Tuberculosis, in collaboration with the Institute of Phthisiology and Pneumology of the University of Brazil. Two of the authors (R.C.R.B. and D.B.M.) had grants from the National Research Council of Brazil.

Selective Inhibition of the Multiplication of Phage T1 in *E. coli* K12

During a screening of antibiotics from Actinomyces, we isolated a product which is chemically correlated with Netropsin[1] and with Congocidin[2]. In previous communications[3,4] we reported some preliminary data about the activity of the antibiotic named by us Distamycin A on experimental tumours.

This compound (Distamycin A)[5] shows the interesting ability to inhibit the multiplication of phage T1 in *E. coli* K12 in a selective way, at concentrations quite harmless to the growth of the host microorganism. From tests carried out on solid medium, determining the number of plaque-forming units present in a preparation of phage T1, it appeared that the inhibiting activity of the product was proportional to the dose. In fact, in the presence of this compound, the number of plaque-forming units decreased

[1] A. C. FINLAY, F. A. HOCHSTEIN, B. A. SOBIN, and F. MURPHY, J. Amer. chem. Soc. *73*, 341 (1951).

[2] R. DESPOIS and L. NINET, VI° Congresso inter. Microbiol. Roma *1*, 241 (Settembre 1953).

[3] A. DI MARCO, M. GAETANI, P. OREZZI, T. SCOTTI, and F. ARCAMONE, Cancer Chemotherapy Reports 18, May (1962), p. 15.

[4] A. DI MARCO, M. SOLDATI, and A. FIORETTI, VIII. International Cancer Congress, Moscow, July (1962).

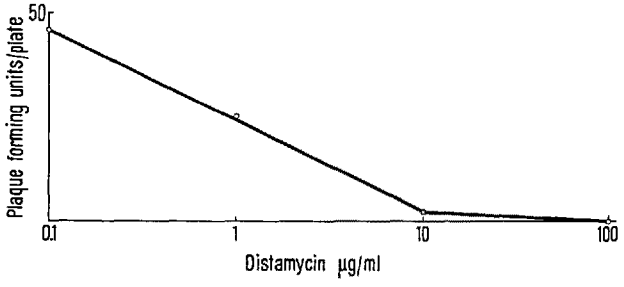
[5] F. ARCAMONE, F. BIZIOLI, G. CANEVAZZI, and A. GREIN, Chem. Abstr. *55*, 2012 (1961).

as a direct function of the antibiotic concentration (Figure).

There was evidence of the activity of the compound on phage multiplication also in other experimental conditions. In fact a culture of *E. coli* K12 in liquid medium was completely protected from phage lysis when one or more μg/ml of antibiotic were added to the mixture before infecting it with phage T1. A more detailed investigation of the mode of action of the antibiotic on the phage infection could be obtained from the study of results collected by the premature-lysis technique, according to the following experimental scheme.

Culture of *E. coli* K12 in logarithmic growth phase were infected with phage T1. At different times after infection, cultures were treated with 10 μg/ml of Distamycin A. Immediately before each treatment a sample was taken, on which the following determinations were carried out: turbidimetric determination; determination of phage free particles present in the culture broth after the bacterial cells had been removed by centrifugation; determination of total phage particles after lysis of bacterial cells by KCN and glycine. Similar determinations were carried out in each culture after 60 and 180 min incubations. The results are shown in the Table.

From the data obtained, it is possible to note that between 5 and 10 min after phage infection the adsorption and eclipse periods were completed and the latent period had started. In fact 10 min after infection the number of total present phage particles was ten times larger than the number of phage particles present in the inoculum; all particles were intracellular. Between 10 and 15 min the latent period was completed. The lysis period, which can be detected turbidimetrically because of the decreasing of the turbidity in the bacterial culture, begins 20 min after infection.



In the presence of the antibiotic, changes in the course of phage multiplication were observed, which are grouped under the following paragraphs for clearness:

(1) By administering Distamycin A at the time of infection or within 5 min, the bacterial culture was completely protected from phage lysis. The phage particles of the inoculum were found in the culture broth, i.e. outside the bacterial cells. The bacterial culture protected by Distamycin A was able to reach the same growth as a non-infected culture.

(2) The administration of the compound either 10 min after infection, i.e. when most phage particles were in a latent period, or after 15 min, i.e. at the beginning of the lysis period, prevented the lysis from occurring and caused a 90% decrease of the amount of phage particles in the bacterial cell. 65 min after infection, the bacterial culture protected by Distamycin A was able to reach a growth only slightly lower than the growth of non-infected cultures (controls).

(3) If the compound was administered 25 min after infection, i.e. when the bacterial lysis was starting, this phenomenon was prevented. First, an increase in the number of total phage particles was observed, with an increase in the number of intracellular phage particles, and later a strong decrease of intracellular phage particles and a resumption of the growth of the bacterial culture were observed.

Phenomena similar to the ones described under paragraphs (1), (2) and (3) were observed when administering the compound 25 to 55 min after infection, i.e. during subsequent cycles of phage multiplication occurring in the bacterial cells survived to the first infection.

Conclusion. The antibiotic inhibits the multiplication of phage T1 in *E. coli* K12 at concentrations devoid of activity against the host. The antibiotic does not inactivate the phage particles, which are able to resume the infection process as soon as the compound is removed. When the compound is present at the time of infection, it prevents the phage from penetrating the bacteria without affecting either phage or bacteria irreversibly. If the adsorption has already taken place, the addition of antibiotic within the first few minutes, while phage is intracellular, causes the disappearance of the intracellular phage already formed. If the treatment is further delayed, the number of the intracellular phage first increases, but subsequently decreases.

It is not possible for us to suggest any hypothesis on the mechanism of action of this antibiotic, as the number of our experimental data is not yet large enough. It can be

Gap between infection and treatment with Distamycin A 10 μg/ml	At treatment			60 min after infection			180 min after infection		
	Optical density	Total phages $\times 10^6$	Phages extrac. $\times 10^6$	Optical density	Total phages $\times 10^6$	Phages extrac. $\times 10^6$	Optical density	Total phages $\times 10^6$	Phages extrac. $\times 10^6$
0 min	89	1.0	1.0	135	0.97	0.94	197	0.5	0.4
5 min	98	1.27	1 2	140	1	1 08	203	0.6	0.47
10 min	101	10 1	0.59	122	0.95	1.15	207	0.43	0.48
15 min	106	10.8	6	119	1.82	1.07	183	0.39	0.2
20 min	103	10	10.7	116	18.3	6.3	163	1.42	0.57
25 min	97	7.5	1.45	103	75	10	133	1.43	1
30 min	83	7.7	1.46	93	22.2	9.5	133	7.5	0.7
35 min	83	9.8	7.4	90	15.6	17	127	10.5	6.8
45 min	83	45	7.7	78	207	40	114	16.1	8.2
55 min	67	131	8.5	57	300	25	97	124	74
Culture *E. coli* K12 infected with phage				43	198	140	30	134	158
Culture *E. coli* K12 non-infected (control)	89			140			200		

observed, however, that the interference must act on a mechanism which is required for the establishment of two different stages of infection, i.e. for the phage penetration into the cells and for its release from the infected bacterial cells.

Riassunto. Un nuovo antibiotico, la Distamicina, inibisce selettivamente lo sviluppo del batteriofago T1 in *E. coli* K12 pur non esercitando alcuna azione sullo sviluppo del microorganismo ospite. L'antibiotico può inibire sia la penetrazione del batteriofago nelle cellule batteriche sia la sua liberazione.

A. DI MARCO, M. GHIONE, A. SANFILIPPO, and E. MORVILLO

Farmitalia, Laboratori Ricerche Microbiologiche e Chemoterapiche, Milano (Italy), October 19, 1962.

Die Wirkung von Eiweissfraktionen normaler menschlicher Sera auf die Thrombocytenzahl der Maus

Immer mehr Angaben sprechen dafür, dass in der Regulation der Thrombopoese ein – oder mehrere – humorale Faktoren eine Rolle spielen. Einen humanen, höchstwahrscheinlich spezifischen thrombopoetischen Serumfaktor haben als erste 1958 KELEMEN et al.[1] beschrieben. Sie waren es auch, die an einem grösseren hämatologischen Krankengut die Thrombocytose verursachende Eigenschaft des Serums untersuchten und in gewissen Fällen – namentlich bei pathologisch veränderter Thrombocytopoese – das Serum im Mäusetest als wirksam befanden. Nur bei zwei von 31 hämopoetisch normalen Personen zeigte das Serum bei gleichartiger Untersuchung eine mässige Aktivität[2]. SCHULMAN et al.[3] berichteten 1960 über klinische Beobachtungen, auf Grund derer sie annehmen, dass das normale Plasma einen die Thrombopoese stimulierenden Faktor enthält.

Bei der eingehenderen Untersuchung einiger thrombopoetisch wirksamer Sera beobachteten wir, dass die Aktivität unter den mittels Papier- bzw. Stärke-Gel-Elektrophorese getrennten Eiweissfraktionen an die β-Globuline gebunden ist bzw. mit diesen wandert[1,4].

In der vorliegenden Mitteilung berichten wir über die Wirkung von mittels kontinuierlicher Elektrophorese separierten Eiweissfraktionen humaner Sera auf die kreisende Thrombocytenzahl weisser Mäuse.

Experimenteller Teil. Gegenstand der Untersuchungen waren die Blutsera hämatologisch gesunder Personen. Die Proteine aus 20–30 ml nach spontaner Gerinnung gewonnenem Serum wurden in einem selbstbereiteten kontinuierlichen Elektrophoreseapparat vom Typ GRASSMAN separiert. Verwendet wurde 30×30 cm Whatman-Filterpapier Nr. 3, Natrium-Veronalacetatpuffer vom pH 8,6 mit einer Ionenkonzentration (μ) von 0,03. Stromquelle: 450–500 V Gleichstrom. Laufdauer ungefähr 16 h bei maximal $+10°$C. Die erhaltenen Fraktionen wurden mittels Immunelektrophorese mit der Scheideggerschen Modifikation der von GRABAR und WILLIAMS eingeführten Technik kontrolliert. Als Antigen dienten einzelne Fraktionen und gemischte Humansera und als Antikörper Anti-Human-Immunserum. Wir benutzten Veronalpuffer mit einem pH von 8,4 und einer Ionenstärke (μ) von 0,05. Die Spannungsdifferenz betrug 5–6 V/cm. Nach dem Trocknen wurden die Präparate mit Fuchsin gefärbt.

Bei Anwendung dieser Methode erhielten wir gewöhnlich neben den Albumin- und den γ-Globulinfraktionen zwei verschiedene α- und β-Globulinfraktionen. Zunächst

[1] E. KELEMEN, I. CSERHÁTI und B. TANOS, Acta haemat. (Basel) *20*, 350 (1958).
[2] K. RÁK, D. LEHOCZKY, F. KRIZSA, I. CSERHÁTI und E. KELEMEN, Folia haemat. N.F., im Druck.
[3] I. SCHULMAN, M. PIERCE, A. LUKENS und Z. CURRIMBHOY, Blood *16*, 943 (1960).
[4] K. RÁK, I. CSERHÁTI und E. KELEMEN, Med. exp. *1*, 125 (1959).

Die Wirkung von mittels Elektrophorese getrennten Eiweissfraktionen menschlicher normaler Sera auf die Thrombocytenzahl der Maus. Es sind die Mittelwerte der Ausgangs- und der am 5. Tage gefundenen Thrombocytenzahlen angegeben, in Klammern die Zahl der untersuchten Mäuse.

Nr.	Vollserum	Albumin	α-Globuline	β-Globuline	γ-Globuline
1.	610[a] → 570 (4)	—	645 → 605 (2)	600 → 680 (4)	640 → 605 (4)
	80[b] 27		42 7	18 16	22 35
	−7% negativ		−6% negativ	+15% *mässig positiv*	−5% negativ
2.	—	620 → 700 (3)	560 → 560 (5)	660 → 830 (5)[c]	710 → 690 (5)
		85 67	74 95	92 80	57 86
		+12% negativ	0% negativ	+25% *mässig positiv*	−3% negativ
3.	—	650 → 590 (3)	—	543 → 724 (11)	580 → 600 (4)
		92 81		43 31	92 81
		−9% negativ		+33% *positiv*	+3% negativ
4.	540 → 610 (3)	640 → 615 (5)	660 → 850 (2)	560 → 850 (7)	590 → 790 (4)
	76 52	60 48	28 14	24 15	61 34
	+13% negativ	−4% negativ	+29% *mässig positiv*	+52% *positiv*	+34% *positiv*
5.	640 → 660 (4)	600 → 590 (4)	600 → 620 (4)	500 → 660 (6)	560 → 640 (3)
	19 40	48 42	91 47	14 80	60 42
	+3% negativ	−2% negativ	+3% negativ	+32% *positiv*	+14% negativ

[a] Thrombocyten × 1000. [b] Standard Deviation. [c] Isoliertes Globulin mit β_2-Mobilität.