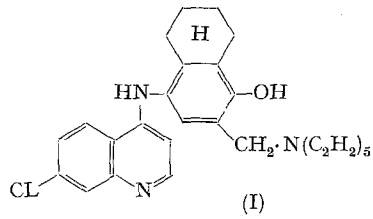
practical applications without facing the problems of skin colouring and insolubility. Chemically, compound I could be prepared through condensation of both moieties, 2-diethylaminomethyl-4-amino-5, 6, 7, 8-tetrahydro-1-naphthol and 4, 7-dichloroquinoline. The former is accessible through a Mannich reaction (formaldehyde/diethylamine) with 4-acetamino-5, 6, 7, 8-tetrahydro-1-naphthol followed by acid hydrolysis.



(I)

Biological testing was based on the response to the product by *Plasmodium berghei* in mice. For this purpose mice of NMRI-SPF-Hann. strain (males) were used of weights 18–22 g. Infection was induced in the mice groups through i.p. inoculation of heparinized infected blood diluted with physiological saline solution. Inoculum for each mouse was 0.3 ml with ca. 10^7 infected erythrocytes with K173 strain of *Plasmodium berghei*.

Oral treatment with the compound in water solution followed 2 h after infection. The various doses in the different groups of infected mice were given daily once for 4 consecutive days. Regular blood microscopical examinations were carried out (Giemsa staining), 3 days after infection for a period of 4 weeks post-infection. Criteria for a given dose to be curative: when no parasites could be microscopically detected in the blood samples withdrawn from the treated animals. The same animals were submitted to further reinfection and if the new infection is established as in controls, then the animals that had been initially treated with these doses were considered to be cured and the given doses as curative. The re-exposure to infection was to secure the rationalization of the absence of the parasites in the treated animals as due to treatment and not due to preimmunity factors. In these studies dosages range of 5 mg/kg body weight through to 100 mg/kg were found to be curative. Lower doses of 1 mg/kg were inactive and of 2.5 mg/kg the activity was poor.

Further the compound I was tested for its repository effect. For this purpose single oral doses of 100 mg/kg body weight and 250 mg/kg were given to mice 1 and 2 weeks before infection with Schizontes bearing erythrocytes. This oral application was shown to be inactive to protect the animals against infection. On the other hand, s.c. applications of the same single doses, 100 mg and 250 mg/kg body weight could provide complete protection against infection with the parasite for a period of at least 2 weeks. Inspite of the exposure to massive infection, no parasitemae could be observed for a period of 4 weeks post infection exposure.

In addition, compound I was evaluated for its antimalarial activity in another group of experiments. This was based on comparison of response to compound by *Plasmodium berghei* KG173 malaria in mice as expressed in mean survival times and the mean survival times of untreated controls[5]. A single dose at the desired level is given 72 h after mice infection with *P. berghei*. To consider the tested compound as active, a minimum survival time of 13 days is required. If the treated mice lived 60 days or more, the compound is considered as curative. In these experiments, doses of 5 mg/kg body weight gave an increase in survival days of 3.7. Doses of 10 mg caused increase in the survival days by 12.3. Cures were affected (5/5) at doses of 40 through 350 mg/kg body weight. At 20 mg/kg cure rate was 4/5.

These biological findings show that the compound I possesses both curative and prophylactic effect against malaria infection in mice. This may offer a lead for future syntheses of compounds bearing structural features of biologically active groupings of antimalarials as for the substituted naphthoquinones and nitrogen-heterocyclics[6].

Zusammenfassung. Ein potentielles Malariamittel, welches Strukturelemente wirksamer Heterocyclen mit denjenigen der Naphtochinone vereint, wurde in orientierenden Versuchen als wirksam gefunden.

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[6] Acknowledgment. The author wishes to express his thanks to Prof. Dr. R. GÖNNERT, Dr. HABERKORN and the group of Farbenfabriken Bayer AG., Wuppertal-Elberfeld, W. Germany, for their distinguished help and cooperation for the compound tests. Also, his gratitude to Prof. Dr. J. BURCKHALTER, University of Michigan, and the Walter Reed Institute of Research, USA for their cooperation for the biological evaluation of the product.

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Behaviour of a Guanidine-Dependent Strain of Poliovirus 1 in Sucrose Density and pH Gradients

Results of recent researches indicate that guanidine invalidates the functions of newly-formed Enterovirus proteins[1,2]. The guanidine marker is thought to be a part of the nucleotide sequence of viral RNA which codes for protein synthesis[3]. The researches reported here have been carried out to establish if a state of guanidine dependence is accompanied by modifications in the density and in the isoelectric point of the viral particle.

Materials and methods. Researches have been carried out by using a concentrated, Genetron-treated suspension in Hank's BSS of both a Brunenders strain of poliovirus 1 and its guanidine dependent variant G 200, which requires 200 µg/ml of guanidine HCl for optimal growth.

To study viral density, 0.5 ml of viral suspension were layered on the top of a preformed 4.5 ml sucrose gradient (42–30%) in *Tris* mg buffer (0.05 *M Tris*, 0.025 *M* KCl, 0.005 *M* $MgCl_2$ mg; pH 7.45) in 5 ml tubes of cellulose nitrate, and centrifuged in Spinco (rotor SW 50) at 35,000 rpm × 100 min at + 4 °C. Fractions were collected from the bottom of the tubes. Plaque forming units of either viruses were titrated according to the DULBECCO and VOGT technique[4] taking advantage of the fact that 60 µg/ml of

guanidine inhibited the sensitive virus while the guanidine-dependent strain failed to grow in drug-free medium.

The isoelectric point was determined by utilizing the capacity of ampholite mixtures (Ampholine, LKB Instruments Inc.) to create pH gradients between 10 and 3. The electrofocusing technique previously adopted by others[5] for poliovirus was followed. The apparatus (Fig. 1) consisted of 2 common 5 ml pipettes, 36 mm long, placed in vertical position and connected at the bottom with a tube interrupted by a stopcock in the centre. The whole right pipette, the connecting tupe and the lower part of the left pipette up to the 4 ml notch were filled with a 56% sucrose solution in H_2O containing 1% of H_2SO_4. A standard concentration of 2% ampholine, divided into 8 fractions of 0.5 ml containing decreasing sucrose concentrations (26.5; 23.2; 20.0; 16.8; 13.2; 10.3; 7.1; 3.9% of H_2O) was layered in the left pipette, up to the O notch. The left pipette was then filled up to its mouth with 2% triethanolamine in H_2O. Virus suspension (0.1 ml) was incorporated, in the course of the above operations, in the 6th fraction (sucrose 10.3%). Platinum wire electrodes (positive on the right and negative on the left pipette) were placed and electric current was regulated so to obtain 2 mA with about 500–600 Volts. During the 7 h operation, the left pipette was maintained at 20 °C by a continuous flow of water in its external lining. 40 fractions of 0.1 ml, representing the ampholine 'zone', were then collected from the upper part of the left pipette. The pH of them was measured and the content in plaque-forming units established as indicated above.

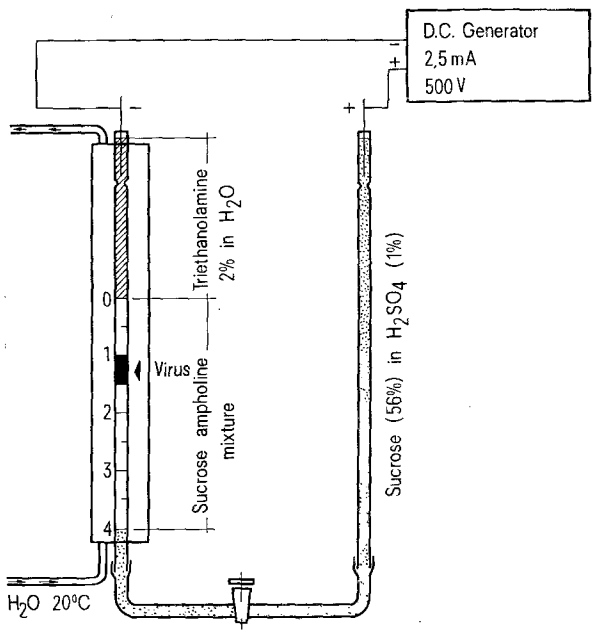

Fig. 1. Electrofocusing apparatus.

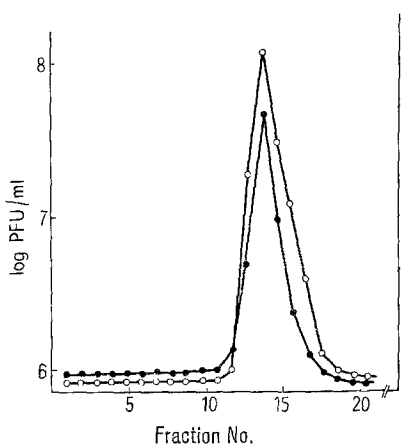

Fig. 2. The distribution of infectivity of sensitive poliovirus (○—○) and its guanidine-dependent mutant (●—●) in sucrose (30–42%) density gradient after 100 min at 35,000 rpm in Spinco SW 50. Virus titers were evaluated in drug-free medium (for the sensitive strain) and in the presence of guanidine HCl 200 μg/ml (for the guanidine-dependent mutant) (the bottom of the tube is on the left). (PFU = plaque forming units).

Results. Both the sucrose and ampholine gradient tests were repeated 3 times. Since the results obtained from these tests were practically identical, only data obtained from 1 test of each method are reported here.

Figure 2 shows that the peak of maximum infectivity for the 2 viral strains coincides perfectly in the sucrose gradient. As for the tests with ampholine (Figure 3), the peak of maximum infectivity was localized for both viruses at a pH 7.4. Both strains present a second peak rather lower at pH 3.8, a fact which others have already observed for the sensitive virus and have hypothetically attributed to transition states at different reactive points in the virus particles[5].

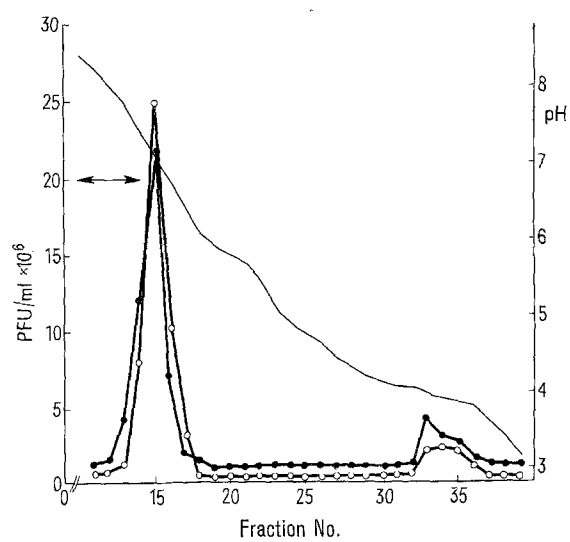

Fig. 3. The distribution of infectivity after electrofocusing of a mixture of guanidine sensitive (○—○) and guanidine-dependent (●—●) poliovirus. Virus mixture was placed in the column in the zone indicated by the double arrow. pH is also recorded in the figure (—). (PFU = plaque forming unit).

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All of these results led to the conclusion that if modifications are induced by a guanidine-dependence to the viral capsid, these modifications do not noteably affect the density and isoelectric point of the virion[6].

Riassunto. La densità in gradiente di saccarosio ed il punto isoelettrico delle particelle virali di poliovirus guanidino-dipendenti non differiscono da quelle del virus guanidino-sensibile di origine.

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Thiopyrimidines: Specific Inhibitors of Poliovirus Induced Early Cell Damages

It has been observed that several thiopyrimidine derivatives inhibit specifically the in vitro development of polioviruses[1-4]. The researches reported here show that one of these compounds is also able to prevent the virus-induced blockade of cell protein synthesis.

Materials. Ethyl-2-methylthio-4-methyl-5-pyrimidine carboxylate (S-7) was kindly provided by Dr. Masuzo Kanamori of the Toyama Chemical Co., Tokyo. Guanidine HCl[5] (Eastman Kodak), D-penicillamine[6] (Dista) and α-hydroxybenzylbenzimidazole[7] (HBB, kindly given by Dr. Igor Tamm, Rockefeller University, New York) were also used. Experiments were carried out by employing human aneuploid HEp2 cells (American type culture collection, Rockville, USA), a strain of poliovirus 1 Brunenders and a S-7 resistant variant of the same strain, obtained through serial transplants in the presence of the thiopyrimidine, up to a maximum of 250 μg/ml.

Method. Cell cultures (10^6 cells/petri dish, 3 petri dishes/sample) were infected with 50 plaque forming units (PFU) of either viruses at +4 °C × 1 h. Cell monolayers were then washed three times with Hank's BSS (pH 7.2) and incubated at 37 °C in the same buffer containing 2 μg/ml of actinomycin D, in order to inhibit nuclear transcriptions. The inhibitors were added, soon after infection, at the maximum concentration which had been previously found to be inactive on the incorporation of RNA and protein precursors in uninfected cells. Infectious progeny was titrated as PFU, according to the Dulbecco and Vogt method[8]. Overall protein synthesis and virus RNA replication were established by measuring, respectively, the uptake under acidinsoluble form of H^3 leucine (Amersham, 15.2 Ci/m*M*, 0.1 μCi/ml, 1 h pulses) and of H^3 uridine (Amersham, 25 Ci/m*M*, 0.2 μCi/ml, from time 0).

Cytopathic effect (CPE) was evaluated by measuring the ability of cell to incorporate vital stains (neutral red 100 μg/ml of medium, 90 min pulses). Cells were then solubilized with sodium deoxycholate 1% in H_2O and neu-

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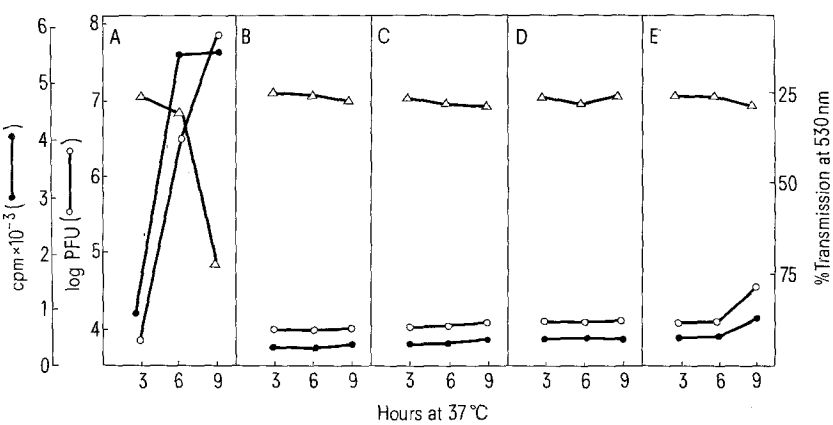

Fig. 1. Effect of several inhibitors on polivirus synthesis and CPE. HEp2 cell monolayers were infected with 50 PFU/cell and incubated at 37 °C in Hank's BSS supplemented with AMD 2 μg/ml (A) or in the same medium + guanidine HCl 300 μg/ml (B), + D-penicillamine 150 μg/ml (C), + HBB 150 μg/ml (D), + S-7 250 μg/ml (E). Infectious virus yield (○—○) was titrated as PFU produced, according to the Dulbecco and Vogt[8] method; viral RNA synthesis (●—●) was measured on the basis of H^3 uridine uptake (0.3 μCi/ml from time 0); CPE was evaluated on the basis of intracellular incorporation of neutral red, solubilized with DOC 1% and read at 530 nm (△—△).