gehend gegen Peroxydation und gelbbraune Färbung des Fettgewebes, nicht aber gegen die Depigmentierung der Inzisoren von Ratten. Diese Beobachtung deutet darauf hin, daß Sulfaguanidin *in vivo* ähnlich wie das Vitamin E als Antioxydans wirkt.

Effect of Stilbamidine on Ascites Tumor of Mice

SNAPPER's studies on the treatment of multiple myeloma with stilbamidine[1] have suggested the possibility of its application to other malignant tumors. We have therefore investigated its influence on the ascites tumor of mice.

We have inoculated 5 mice (18–24 g) with 2 million ascites cells intraperitoneally and then treated them with 0·6 mg stilbamidine-diisethionate intraperitoneally daily until death occurred. This was the maximum daily dose which did not produce toxic effects. No influence on survival time was observed (Fig. 1). The ascites fluid

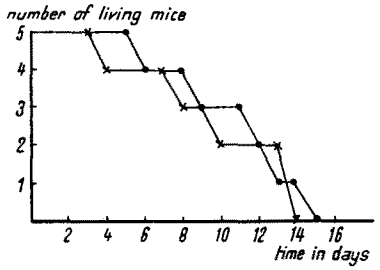

Fig. 1. – Number of living mice.
• stilbamidine, × control

collected upon the death of the animal revealed occasional cells with bright greenish fluorescent granules resembling those described in myeloma cells[2]. These granules stained deep blue with toluidine blue (Fig. 2).

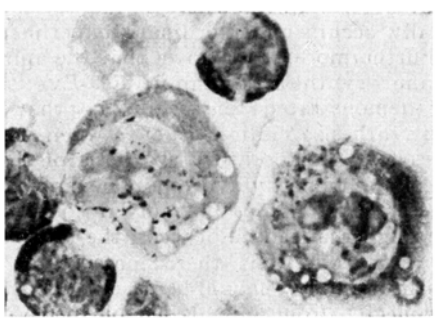

Fig. 2. – Smear of ascites cell after *in vivo* stilbamidine treatment, stained with aqueous 1% toluidine blue.

If the animals were pre-treated for 10 days with 0·3 mg stilbamidine injected intraperitoneally daily before the inoculation of 800,000 ascites cells, and stilbamidine injections continued daily until death, almost all cells contained small granules as described above. Nevertheless no effect on survival time was observed (Fig. 3).

Ascites tumor cells incubated for 30 minutes in the cold in 0·2% stilbamidine-diisethionate in glucose developed granules in most of the cells (0·5 cc ascites

[1] I. SNAPPER, J. Mt. Sinai Hosp. *13*, 119 (1946).
[2] I. SNAPPER, A. E. MIRSKY, H. RIS, B. SCHNEID, and M. ROSENTHAL, Blood *2*, 311 (1947).

Wave-length mμ	Extinction coeff.
250	0.40
257	0.45
280	0.27
300	0.33
330	0.50
380	0.28

fluid +15 cc 0·2% stilbamidine-diisethionate). These granules are visible as slightly refractile bodies in the light microscope, as dark granules in the phase contrast

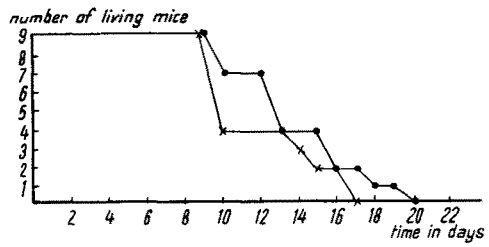

Fig. 3. – Number of living mice.
• stilbamidine, × control

microscope and as bright fluorescent granules in the fluorescent microscope. Photographed at 257 mμ and 330 mμ in the UV they showed marked absorption while at 280 mμ they absorbed very little (Table). The inoculation of 800,000 such cells intraperitoneally (without washing, so that 0·5 mg stilbamidine was included) resulted in no appreciable difference in survival

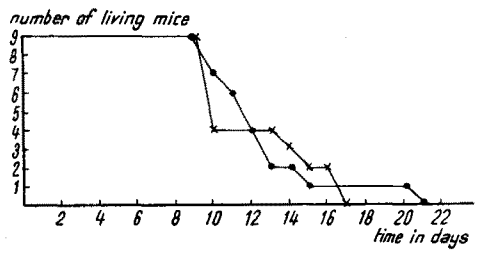

Fig. 4. – Number of living mice.
• stilbamidine, × control

when compared with untreated cells (Fig. 4). However upon storage overnight in the cold with stilbamidine a marked reduction in virulence was observed as compared

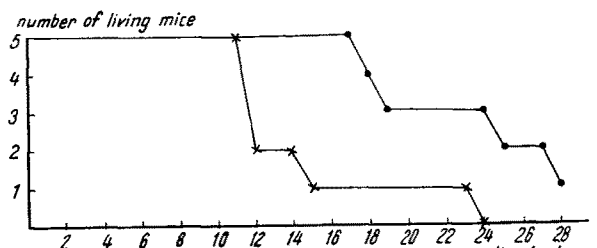

Fig. 5. – Number of living mice.
• stilbamidine, × control

with similarly stored but not stilbamidine-treated cells (Fig. 5). The same effect is noted if the unbound stilb-

amidine is removed by washing the cells with Tyrode's solution after 30 minutes before storing overnight in Tyrode's.

The observation on basophilic staining, fluorescence and marked absorption at the wave-lengths characteristic for nucleic acid and stilbamidine of the cytoplasmic granules induced in ascites tumor cells indicate that these granules, like those described by SNAPPER and coworkers in myeloma cells, consist of stilbamidine ribonucleate. That most of the cytoplasmic RNA is also affected by stilbamidine but without the production of granular precipitates is indicated by the observation that whereas a few minutes of UV irradiation in the KÖHLER microscope at 257 mμ reduced the originally high cytoplasmic absorption at 257 mμ of untreated ascites tumor cells to practically zero, stilbamidinized cells show no appreciable change in absorption on prolonged UV irradiation. However, the combination of the cytoplasmic RNA with stilbamidine appears to be without immediate effect on the virulence of the tumor. A delayed effect is noted in that cells allowed to remain *in vitro* after stilbamidine treatment show a much more rapid loss in virulence then untreated cells. It may be that the noxious effect of stilbamidine on the cells can be manifest only in the presence of additional unfavourable conditions which exist on *in vitro* storage, which it has been observed results in much more gradual loss of RNA and virulence in untreated cells[1].

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Department for Cell Research, Caroline Institute, Stockholm, Sweden, December 15, 1949.

Zusammenfassung

In *in-vitro*- und *in-vivo*-Versuchen wurde die Wirkung von Stilbamidin auf die Tumorasziteszellen der Maus untersucht. Intrazytoplasmatische körnige Präzipitate konnten durch die Behandlung hervorgerufen werden, die wahrscheinlich aus Stilbamidinribonukleat aufgebaut sind. Das Auftreten der Präzipitate hat keinen unmittelbaren Effekt auf die Virulenz des Tumors.

[1] E. KLEIN, N. B. KURNICK, G. KLEIN, Exp. Cell Research *1*, 127 (1950).

[2] This work was done in part (N. B. KURNICK) under a fellowship granted by the American Cancer Society on the recommendation of the National Research Council, Committee on Growth.

"Potentiating" Effect of Repeated Penicillin Treatments in *B. novyi* Infection

Analysing the factors which could account for the excellent clinical[1] and experimental[2] therapeutic results obtained without the continuous presence of penicillin in the host organism, we expressed the opinion[3] that the *additive effect* of repeated treatments probably plays an important role in this picture. In order to test this assumption, an infection was required which would allow us to follow the fate of the infective organism—as well as that of the drug—in each phase of the therapy. We found the experimental *Borrelia novyi* infection of mice, which is very sensitive to penicillin dosage variations[1], applicable for this purpose.

Experimental. The infection used in these experiments is similar to that described by R. J. SCHNITZER and coworkers[1], to whom we are indebted for the strain of *Borrelia novyi*. Material for infection was prepared by suspending a few drops of blood from an infected mouse in heparinized tryptose broth and diluting to obtain 3–6 spirochætes per dark field (x 900). Intraperitoneal injection of 0·4 cc. of this suspension into albino mice weighing 16–22 grams produces an infection with 1–4 parasites per dark field in the blood after 18–22 hours.

All treatments were by the subcutaneous route. Mice were observed repeatedly the first day of treatment, then daily, for a period of 3 weeks; most of them were reinfected after this time and the take of this reinfection considered further circumstantial evidence of cure. Groups of 3 to 10 mice for each dosage or treatment schedule were used in these experiments.

Table I

Effect of pretreatment with "ineffective" doses of penicillin in *B. novyi* infection of mice.

Schedule		Hours after treatment	Ineffective (50-100%)[1]	Slight eff. (25–40%)	Signif. red. (0–15%)
24 hours after infection	48 hours after infection		in number of mice		
Pretreatment: 150 μ/20 g (ineffective)	Treatment: 300–400 μ/20 g	3½	1	2	7
		6	1	1	8
		10	1	1	8
Pretreatment: none	Treatment: 300–400 μ/20 g	3½	5	2	3
		6	1	4	5
		10	3	2	5

[1] Percentage of the initial spirochæte count.

Results. The minimal subcutaneous dose administered the day after initial infection, which *significantly* reduces the parasite count within 3 to 5 hours, is 500 μ/20 g. Fractions of this dose varying from 75 to 150 μ, which had no immediate effect on the parasite count, were used for "pretreatment". (A) *One pretreatment with ineffective doses.* In one set of experiments pretreatment with these fractions was made the first day and was followed, the next day, by the determination of the minimal clearing dose. In one experiment, No. 50, one pretreatment with 100 μ, followed by 300 μ the next day, resulted in significant reduction (80 and 98%) in the parasite count in $^2/_3$ mice and complete clearance in the third. The 3 non-pretreated mice injected with the 300 μ showed no response. A somewhat similar experiment (No. 55) is presented in more detail in Table I. (B) *Repeated pretreatments with ineffective doses.* A number of pretreatment schedules were used. In Experiment 48, 2 pretreatments with 100 μ, on the first and second days after infection, had no effect on the parasite count. The subsequent treatment was given the third day following the infection. 400 and 800 μ cleared 3 out of 6 mice, while in 2 other mice significant reduction took place. In contrast, in the non-pretreated group 800 μ were

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[1] M. BUCK, A. C. FARR, R. J. SCHNITZER, Science *104*, 370 (1946).