Im Magensaft von 8 gesunden Personen und 7 Patienten mit verschiedenen Magenerkrankungen einschließlich Magengeschwür wurden nur in geringen Mengen Alanin, Leucin, Histamin, Oxyprolin, Valin, Phenylalanin und Glukosamin nachgewiesen. Wahrscheinlich enthalten diese Magensäfte auch eine oder zwei Aminosäuren aus der Cystingruppe.

Herrn Prof. Dr. PL. A. PLATTNER sind wir für die Ermöglichung dieser Arbeit zu großem Dank verpflichtet.

B. CAGIANUT, K. ZEHNDER und U. NAGER[1]

Medizinische Poliklinik der Universität Zürich, Organisch-chemisches Laboratorium der ETH., Zürich, den 15. Dezember 1949.

Summary

By means of the paper partition chromatographic method, it was shown that gastric juice of patients with carcinoma ventriculi contains glutamic and aspartic acid. Besides a higher amino acid level, we found a few lower peptides. The content of basic amino acids in the stomach is higher in carcinomic patients than in normal persons.

[1] Commercial Solvent Corp Terre Haute, Ind., USA.

Influence of Sulfaguanidine on Certain Symptoms of Vitamin E Deficiency in Rats[1]

In previous papers[2] we have reported on the influence of certain nutrients, among them protein, cystine, nordihydroguaiaretic acid and manganese, in the development and progress of peroxidation and yellow-brown coloration of the adipose tissue, and incisor depigmentation of vitamin E-deficient rats. In order to further these studies we have tested the action of certain sulfonamide compounds against the above-mentioned symptoms. The present paper reports the finding that sulfaguanidine possesses a marked antioxidant effect *in vivo*, similar to that of vitamin E.

Forty-eight newly weaned male rats were distributed into four equally large groups, and were fed the diets presented in Table I, as follows: Group 1, a vitamin E-deficient diet; group 2, a vitamin E-deficient diet containing 1% sulfanilylguanidine; group 3, a vitamin E-rich diet; and group 4, a vitamin E-rich diet containing 1% sulfanilylguanidine. The animals were fed the rations as well as tap water *ad libitum* for a 70-day experimental period, and were weighed weekly. From the 3rd experimental week the degree of enamel depigmentation in the various groups was recorded using a method previously described[3]. At the end of the experiment the animals were killed with chloroform, and the colour of their lumbar fat deposits was recorded using a scale from 0 to 5, 0 indicating no colour, 1 pale yellow, 2 yellow, 3 dark yellow, 4 yellow-brown, and 5 dark yellow-brown. Then samples of lumbar fat were taken for the determination of peroxides by HARTMANN and GLAVIND'S method[4], and by the method of KING and co-workers[5] as modified by DAM and GRANADOS[6].

Sulfaguanidine at the level given was not apparently toxic. Figure 1 shows the average growth curves of the four groups. It can be seen that the growth rate was not reduced by including 1% sulfaguanidine in either the vitamin E-deficient (group 2) or in the vitamin E-rich diet (group 4) as compared, respectively, with the growth of the rats fed the vitamin E-deficient (group 1) or the vitamin E-rich diet (group 3 alone). Under different

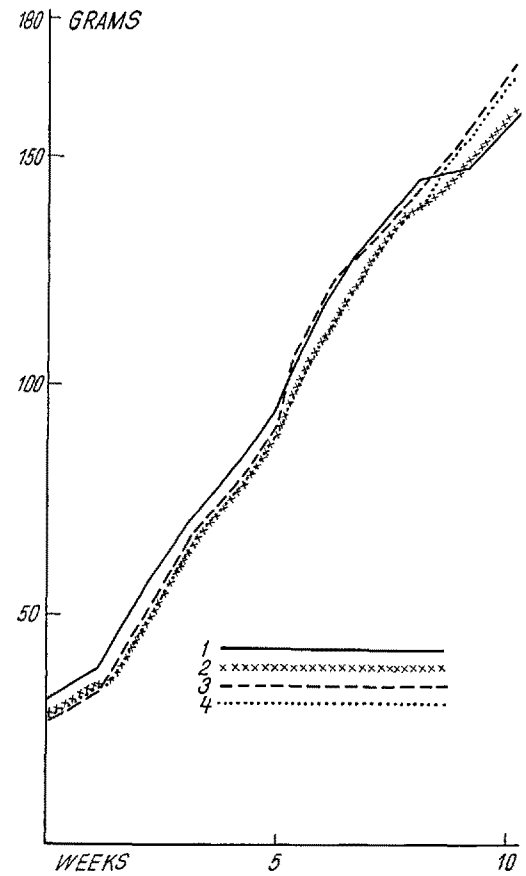

Fig. 1. – Average growth curves of the four groups.

Table I

Diets, in percentage, fed to the four groups

Ingredients	Group 1	Group 2	Group 3	Group 4
choline chloride	0·4	0·4	0·4	0·4
vitamin mixture[1]	0·5	0·5	0·5	0·5
salt mixture[2]	5	5	5	5
casein[3]	20	20	20	20
sucrose	54·10	53·10	54·10	53·10
cod liver oil[4]	20	20	20	20
sulfanilylguanidine		1		1
d,l-α-tocopherol acetate			0·02	0·02

[1] The 0·5 g of vitamin mixture contained: biotin 20 μg, folic acid 400 μg, thiamine hydrochloride 5 mg, riboflavin 5 mg, pyridoxine hydrochloride 5 mg, calcium pantothenate 5 mg, nicotinic acid 7·5 mg, p-aminobenzoic acid 100 mg, inositol 100 mg, vitamin K substitute 3 mg, and sucrose 269·080 mg.

[2] The salt mixture used was McCollum's Salt Mixture No. 185.

[3] The casein used was Vitamin-Test Casein GBI, from General Biochemicals, Inc., Chagrin Falls, Ohio, U.S.A.

[4] The cod liver oil was added fresh every day to the basal diets.

[1] This work was supported by a grant from Rask-Örsted Fondet.

[2] H. GRANADOS, E. AAES-JÖRGENSEN, and H. DAM, Brit. J. Nutrition, *3*, 320 (1950); Acta pathol. et microbiol. Scand., in press.

[3] H. GRANADOS and H. DAM, Odontologisk Tidskrift *56*, 457 (1948).

[4] S. HARTMANN and J. GLAVIND, Acta chem. Scand. *3*, 954 (1949).

[5] A. E. KING, H. L. ROSCHEN, and W. H. IRWIN, Oil and Soap *10*, 105 (1933).

[6] H. DAM and H. GRANADOS, Acta physiol. Scand. *10*, 162 (1945).

Table II

Average degrees of enamel depigmentation and of coloration and peroxidation of fat in the four groups.

Groups	Diets	Incisor depigmentation							Colour of lumbar fat	Peroxide values	
		3.week	4.week	5.week	6.week	7.week	8.week	9.week	10. week	H-G[1]	K-R-I[2]
1	vitamin E-free	1	3	4	4	5	6	6	7	3	37	24
2	vitamin E-free and 1% sulfaguanidine	1	2	4	4	6	5	3	4	1	7	5
3	vitamin E-rich	1	2	3	3	3	3	3	3	0	2	0·3
4	vitamin E-rich and 1% sulfaguanidine	1	2	1	1	2	2	2	1	0	2	0

[1] The letters H–G indicate Hartmann and Glavind's method for the chemical determination of fat peroxides.

[2] The letters K–R–I indicate King, Roschen and Irwin's method for the chemical determination of peroxides.

experimental conditions, i. e. by omitting certain water-soluble vitamins from the diet, SPICER and co-workers[1] have found a marked reduction of growth through the supply of 1% sulfaguanidine.

We thank F. Hoffmann-La Roche & Co., Basle, Switzerland, for the kind supply of the *d,l*-α-tocopherol acetate (Ephynal "Roche") used in these experiments.

Table II presents the average degrees of enamel depigmentation and of coloration and peroxidation of fat in the four groups. This table shows the following results: (1) The incisor depigmentation of the rats fed the vitamin E-free diet (group 1) did not decrease markedly by adding 1% sulfaguanidine to the vitamin E-deficient ration (group 2). (2) Both the yellow-brown coloration and the peroxide values of the fat from the vitamin E-deficient rats (group 1) decreased markedly by adding 1% sulfaguanidine to the vitamin E-free ration (group 2). This protection was comparable to that offered by vitamin E (groups 3 and 4). Thus these experiments demonstrate that sulfanilylguanidine possesses a definite antioxidant action *in vivo*, similar to that of vitamin E and certain other compounds. The mechanism whereby sulfaguanidine acts as an *in vivo* antioxidant remains to be studied.

These studies also show once more that the mechanisms whereby vitamin E-deficient diets rich in highly unsaturated fatty acids induce dental depigmentation are, at least partially, different from those which bring about peroxidation and coloration of the adipose tissue, since a number of substances protect markedly against the fat changes without altering significantly the degree of dental depigmentation, or vice versa.

A relationship between the antibiotic effect of sulfonamide compounds on the intestinal flora and the production of vitamin E deficiency in the rat has recently been claimed: PINDBORG[2] reared a group of rats on a vitamin E-deficient diet to which was added feces from rats fed a normal stock ration, and found no incisor depigmentation or histopathological changes in the enamel organ. Thus it was assumed that vitamin E synthesized by the intestinal flora and excreted in the feces had been responsible for the protection against the dental changes. Therefore he concluded that bacterial synthesis of vitamin E occurs in the intestinal tract of the rat. This conclusion is unwarranted since he overlooked the possibility that a part of the natural tocopherols usually present in abundance in the stock diets may not be absorbed by the digestive tract and may be excreted in the feces. In such a case the vitamin E present in the feces, and which was supposed to have been responsible for the protection against the dental changes, may have been of dietary origin. On the other hand, it should be taken into consideration that the excrement from normal animals may contain substances, other than vitamin E, which have been shown to protect[1] against the dental and other changes brought about by vitamin E-deficient diets. Moreover, that no significant microbial synthesis of vitamin E takes place in the intestinal tract may be concluded from the fact that all the known symptoms of vitamin E deficiency have been found and reproduced without feeding antibiotics together with the vitamin E-dificient diets, and without preventing the coprophagy that usually occurs under ordinary experimental conditions. Furthermore, it is known that the animal organism cannot synthesize vitamin E[2]. EVANS and co-workers[3] demonstrated ten years ago that rats are unable to synthesize vitamin E even when the starting substances for the laboratory synthesis of this factor, i. e. phytol and trimethylhydroquinone, are fed to them. Nevertheless, it is obvious that any rational atempt to study, through the feeding of feces, the possibility that under certain conditions there may occur bacterial synthesis of vitamin E, should be based upon the feeding of feces collected from animals fed purified Vitamin-E-deficient diets.

H. GRANADOS, E. AAES-JÖRGENSEN, and H. DAM

Department of Biology, Polytechnic Institute, Copenhagen, January 10, 1950.

Zusammenfassung

Zugabe von 1% Sulfaguanidin zu einer Vitamin E-armen Nahrung mit 20% Dorschlebertran schützt weit-

[1] S. S. SPICER, F. S. DAFT, W. H. SEBRELL, and L. L. ASHBURN, Public Health Reports *57*, 1559 (1942).

[2] J. J. PINDBORG, Nature *164*, 493 (1949).

[1] H. GRANADOS, E. AAES-JÖRGENSEN, and H. DAM, Brit. J. Nutrition, *3*, 320 (1950); Acta pathol. et microbiol. Scand., in press.

[2] H. R. ROSENBERG, *Chem. and Physiol. of the Vitamins*, p. 448 (Interscience Publishers, Inc., New York, 1945).

[3] H. M. EVANS, O. H. EMERSON, G. A. EMERSON, L. I. SMITH, H. E. UNGNADE, W. W. PRICHARD, F. L. AUSTIN, H. H. HOEHN, J. W. OPIE, and S. WAWZONEK, J. Org. Chem. *4*, 376 (1939).

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

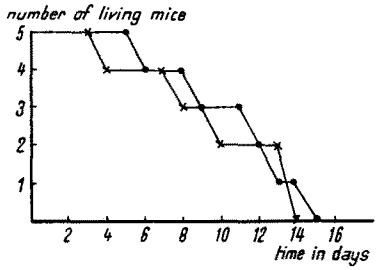

Fig. 1. – Number of living mice.
• stilbamidine, × control

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).

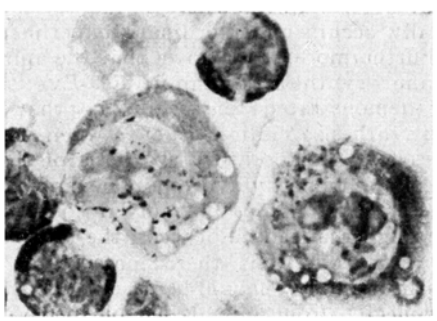

Fig. 2. – Smear of ascites cell after *in vivo* stilbamidine treatment, stained with aqueous 1% toluidine blue.

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

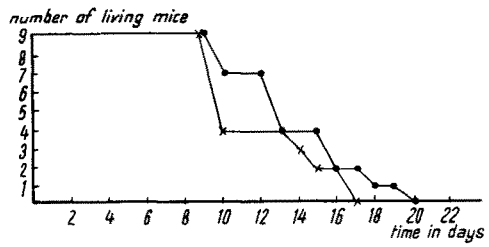

Fig. 3. – Number of living mice.
• stilbamidine, × control

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

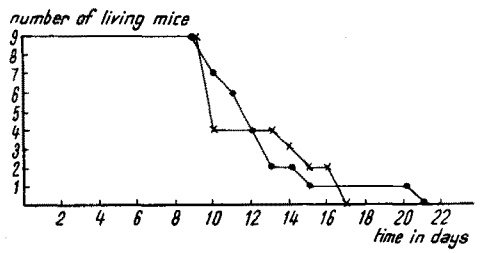

Fig. 4. – Number of living mice.
• stilbamidine, × control

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

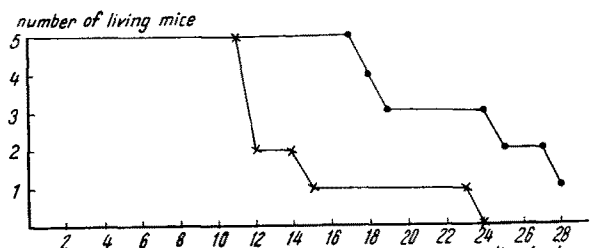

Fig. 5. – Number of living mice.
• stilbamidine, × control

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