and it is possible that permeability changes play a role. It has been shown recently that the thin ascending limb of Henle's loop lacks any active transport mechanism but has a selective high permeability to Cl. As a result, NaCl difuses from the lumen of this tubular segment to the interstitium following its concentration gradient and a hypotonic tubular fluid is thus created by pure physical forces[10]. Inhibition of these passive movements by furosemide would be expected to contribute to its natriuretic effect. This possibility, which still awaits experimental verification, certainly merits investigation[11].

Summary. Furosemide added to the Ringer solution bathing the external side of the isolated skin of *Leptodactylus ocellatus* increased the PD and SCC and inhibited both active chloride influx and passive chloride efflux. The action on chloride permeability is thought to contribute to the diuretic effect of the drug.

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Increased Sensitivity to Pentobarbital in Rats Fed a Diet Lacking Flavonoids

It has been shown that feeding rats a diet lacking flavonoids gives rise to cerebral oedema[1], due to the increased permeability of the blood-brain-barrier[2]. Similarly, it has been demonstrated that their deficiency in the diet greatly increases lymphoedema of the face and neck after cervical lymphatic obstruction[3]. In rats fed a diet lacking flavonoids (which had supplementary vitamin C) definite fine structural alterations were found in blood capillaries and tissues. These were quite different from those reported in C-avitaminosis[4].

Composition of the flavonoid-free diet (for 100 kg)

Isolated soya protein	22.96	kg
Maize starch	33.62	kg
Saccharose	10.00	kg
Soya oil	3.97	kg
Cellulose powder	5.16	kg
"Hostalen PP"	1.86	kg
$CaCO_3$	13.70	g
$CaHPO_4$	2831.20	g
NaCl	457.30	g
K_2SO_4	1113.00	g
MgO	90.30	g
Ferrous fumarate	34.00	g
MnO	6.40	g
$CuSO_4$	1.30	g
ZnO	7.60	g
$NaMoO_4$	0.025	g
KJ	0.650	g
NaF	0.11	g
Vitamin A	5.20	g
D_3	0.25	g
E	1.00	g
K_3	5.30	g
B_1	5.00	g
B_2	5.00	g
B_6	7.00	g
B_{12}	24.00	g
Panthothenic acid	10.0	g
Nicotinic acid	10.0	g
Folic acid	2.5	g
Biotin	5.0	g
Inosite	10.0	g
Vitamin C	25.0	g
Choline	100.0	g
Methionine	384.0	g
Lysine	120.0	g

In the course of these studies, it has been observed that rats fed a diet lacking flavonoids when anesthetized with pentobarbital sodium[5] slept longer than those fed a normal diet. The present paper deals with this phenomenon.

Materials and methods. In December 1974, Pentobarbital – induced sleeping time was estimated in 10 ♂ Sprague-Dawley rats (body weight 470 ± 18 g) fed for 118 days a diet lacking flavonoids[6] (Table) and in 10 ♂ rats of the same species (body weight 415 ± 9 g) fed a normal diet (Herilan® RM 204). Pentobarbital sodium50 mg/kg was injected i.p. The interval elapsing until the animal, lying on his back, was able to hold its head up was regarded as sleeping time.

In June 1975, the same study was performed, using 16 ♂ Sprague-Dawley rats (body weight 480 ± 36 g) fed for 160 days the diet lacking flavonoids and 16 ♂ rats (body weight 462 ± 13) of the same species fed Herilan® R/M 204. Statistical analysis was performed by the *t*-test[7].

Results. In the study performed in winter, sleeping time in control rats amounted to 4487 ± 777″, in rats fed the diet lacking flavonoids 6386 ± 859″. The difference is highly significant ($p \ll 0.0001$). In the study performed in summer, control rats slept 4247 ± 1192″, rats fed the diet lacking flavonoids 5539 ± 1239″. This difference is also highly significant ($p < 0.005$).

Discussion. Since BENTSÁTH, RUSZNYÁK and SZENT-GYÖRGYI[8] first described vitamin P, it has been the subject of considerable debate[9]. The results described in this

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paper clearly demonstrate that the sensitivity of the rat against pentobarbital is greatly increased by feeding a diet lacking benzopyrones and furnishes additional evidence to the fact that, in this species, benzopyrones should be regarded as vitamins.

Wheter metabolism of pentobarbital is altered, or sensitivity of the brain is increased by benzopyrone deficiency, must be elucidated by further experiments.

Summary. Pentobarbital-sensitivity is highly increased in rats fed a diet lacking flavonoids; sleeping time was found to be increased by 42 and 30% as compared with rats fed a normal diet. These studies confirm our previous statement according to which for the rat, benzopyrones are vitamins.

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Effect of Vitamin A on Tumor Development in Burned, Unburned, and Glucocorticoid-Treated Mice Inoculated with an Oncogenic Virus

High doses of vitamin A have been reported to decrease the incidence and severity of tumor development in mice inoculated with a murine sarcoma virus of the Moloney strain (MSV)[1]. This effect was also noted when vitamin A was administered to inoculated mice who were subjected to partial body casting a, mild physical stress[1]. Several findings suggested that the anti-tumor effect of vitamin A might be mediated through an inhibition of 'stress'-either that caused by the virus or that caused by the virus plus the added physical stress. First, physically stressed animals have been shown to have an increased susceptibility to viral oncogenesis[2]. Second, thymic involution occurs after inoculation of mice with MSV[3], after physical stress[4], or after treatment with glucocorticocoid hormones[5]; and the stress-initiated thymic changes require either endogenously produced or exogenously administered adrenal corticoid hormones[6]. That the effect of glucocorticoid suppression of certain immune functions is mediated through interference with thymic function is suggested by reports that thymic extracts can partially reverse the suppression of cellular immunocompetence which follows exogenous glucocorticoid administration[7]. Third, vitamin A administration to mice partially prevents the thymic involution associated with a physiologic stress[8]. Further, large doses of vitamin A have been reported to reverse some of the effects of glucocorticoids on other physiologic processes such as wound healing[9,10].

If the anti-tumor effects of vitamin A are mediated through a partial reversal of the changes associated with physical stress, then an augmentation of the anti-tumor action would be anticipated in severely stressed animals or animals treated with exogenous glucocorticoids. This study compares the effect of high dose vitamin A on burn-stressed, glucocorticoid-treated, and normal mice inoculated with MSV.

Methods. Seven-week-old male CBA mice of 20–25 g were used for all experiments. They were fed Purina Lab Chow[11] (containing 12 IU vitamin A per g), housed 10 per cage, and allowed to acclimate to their surroundings for 1 week before commencement of the study.

Standardized burns covering 25% of the body surface area of the mouse were produced by heating brass blocks with a 2×5 cm surface to 98.5 °C and placing the blocks in contact with the animals for 4 sec per burn. 2 contact burns (each 10 cm^2) were placed on the right and left dorsal surface of each mouse, resulting in 20 cm^2 burns. The burns were full thickness by histological determination at 24 and 72 h postburn. Sham-burning was performed by placing unheated brass blocks on anesthetized animals for the same periods of time in the same areas. Anesthesia was maintained with pentobarbital, 0.05 mg/g body weight.

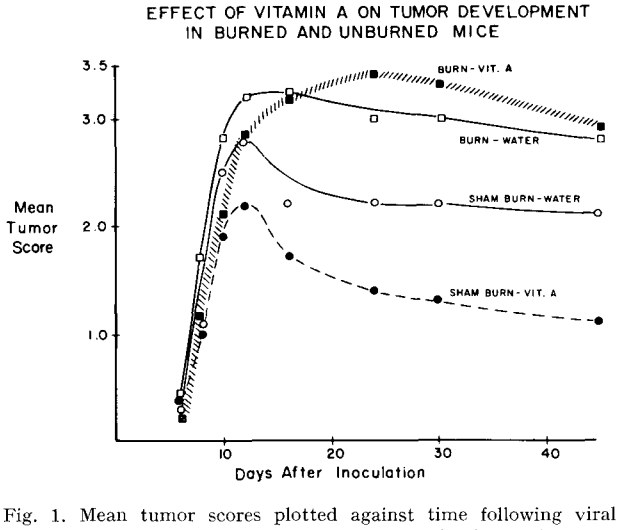

Fig. 1. Mean tumor scores plotted against time following viral inoculation. Tumor size was greater in burned animals than in sham-burned animals, from Day 16 and thereafter. Although viramin A appeared to accelerate tumor regression in the sham-burned animals, it had no effect on the increased tumor growth in the burned animals.

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