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.

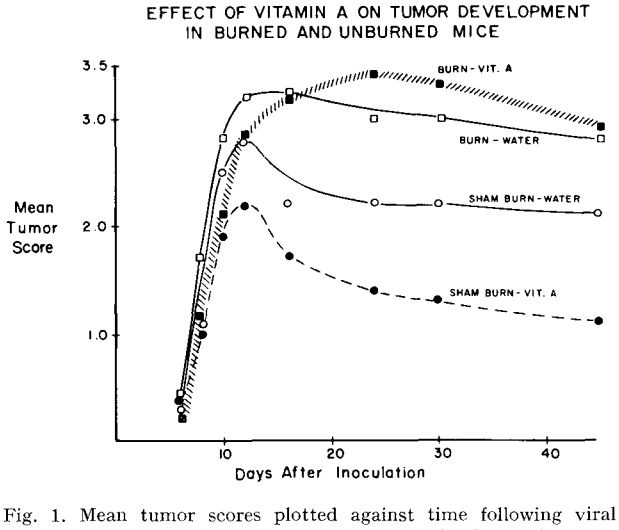

Fig. 1. Mean tumor scores plotted against time following viral inoculation. Tumor size was greater in burned animals than in sham-burned animals, from Day 16 and thereafter. Although viramin A appeared to accelerate tumor regression in the sham-burned animals, it had no effect on the increased tumor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

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Table I. Effect of vitamin A on tumor growth in burned and unburned mice inoculated with the Moloney sarcoma virus

	Day 12[a]				Day 30[a]			
	Sham-burned		Burned		Sham-burned		Burned	
Tumor score	Vitamin A	Water	Vitamin A	Water	Vitamin A	Water	Vitamin A	Water
0	1	0	0	0	5	0	1	0
1	4	3	2	0	6	8	0	1
2	6	4	2	3	4	2	2	1
3	9	8	13	4	2	8	4	5
4	0	5	2	5	1	2	9	2
Analysis[b]								
Overall	$\chi^2(12) = 18.39, p\ 0.104.$				$\chi^2(12) = 34.50, p\ 0.0006.$			
Burn vs. sham-burn	$\chi^2(4) = 4.71, p\ 0.32.$				$\chi^2(4) = 17.62, p\ 0.0015.$			
Sham-burned:								
Vitamin A vs. water	$\chi^2(4) = 6.6017, p\ 0.15.$				$\chi^2(4) = 9.81, p\ 0.0438.$			
Burned:								
Vitamin A vs. water	$\chi^2(3) = 7.0281, p\ 0.071.$				$\chi^2(4) = 5.35, p\ 0.2524.$			

[a] Number of animals with designated tumor scores are listed for Day 12 (no significant difference between groups) and Day 30 (after significant differences were noted. Only 25 of 80 burned animals survived for 30 days after thermal injury. [b] By factorial χ^2 analysis of variance[15].

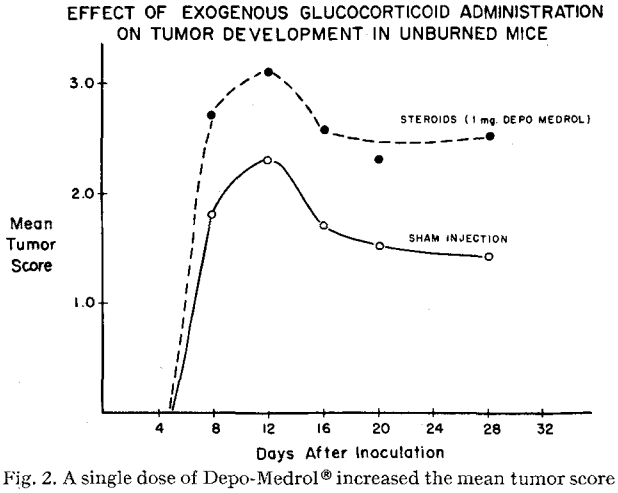

Fig. 2. A single dose of Depo-Medrol® increased the mean tumor score from 8 days after inoculation and thereafter.

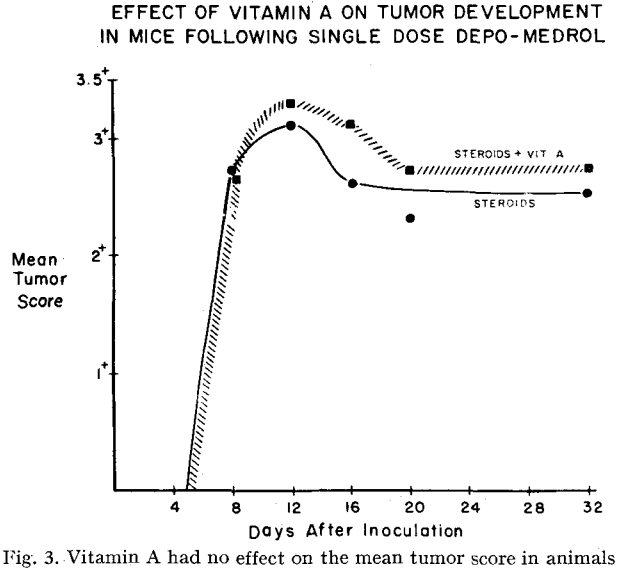

Fig. 3. Vitamin A had no effect on the mean tumor score in animals treated with Depo-Medrol®.

40 burned and 20 sham-burned animals were fed drinking water containing Aquasol A®[12] 150 units per cm^3. The water bottles were covered with brown paper to avoid decomposition of the vitamin on contact with light. 40 burned and 20 sham-burned animals were fed drinking water which contained no vitamin A. Vitamin A feeding was commenced immediately after burning and was continued throughout the duration of the experiment (40 days).

The design of the first experiment, therefore, included 4 groups of animals: Group I (40 animals) – burned, vitamin A fed; Group II (40 animals) – burned, water fed; Group III (20 animals) – unburned, vitamin A fed; and Group IV (20 animals) – unburned, water fed.

48 h after burning, 0.1 cm^3 of a uniform inoculum of MSV was administered i.m. to the medial aspect of the right thigh of every mouse. Tumor size was graded by an adaptation of the scale of BLUMENSTEIN and MALONEY[13]. A Grade 1 tumor indicated a palpable swelling in the thigh muscle at the site of inoculation. A Grade 2 tumor corresponded to a palpable mass involving the entire thigh muscle. A Grade 3 tumor indicated extension of the tumor over the pelvic brim and into the abdomen. A Grade 4 tumor involved the entire leg and had extended across the midline of the abdomen.

In the second experiment, 20 animals were given a single dose of glucocorticoid (Solu-medrol®[14] 1 mg s.c. in 0.25 cm^3 saline) 48 h prior to inoculation. A control group of 20 animals was given a s.c. injection of 0.25 cm^3 saline 48 h prior to inoculation. Tumor growth in the Solu-Medrol® treated animals was compared to that in the animals which did not receive exogenous glucocorticoid.

In the third experiment, 2 groups of 20 animals each were used. Both groups received 1 mg Solu-Medrol® s.c. 48 h prior to inoculation. One group was fed Aquasol A®, 150 U/cm^3 in drinking water. The other group was fed drinking water without vitamin A. The effect of vitamin A on glucocorticoid-augmented oncogenesis was studied.

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Table II. Effect of steroids on tumor development

	Day 8[a]		Day 28[a]	
Tumor score	Saline injection	Depo-Medrol®	Saline injection	Depo-Medrol®
0	0	1	7	0
1	28	5	32	13
2	12	9	14	9
3	16	39	4	21
4	0	5	0	11
Analysis[b]	$\chi^2(4) = 32.02, p < 0.0001$		$\chi^2(4) = 38.62, p < 0.0001$	

[a] Number of animals with designated tumor score are presented. [b] By factorial χ^2 analysis of variance[15].

Table III. Inefficacy of vitamin A on tumorigenesis in mice treated with Depo-Medrol®

	Day 8		Day 28	
Tumor score	Depo-Medrol® + Water	Depo-Medrol® + vitamin A	Depo-Medrol® + Water	Depo-Medrol® + vitamin A
0	0	1	0	0
1	3	2	7	6
2	6	3	5	4
3	18	21	12	9
4	3	2	4	7
Analysis[b]	$\chi^2(4) = 2.6$ N.S.		$\chi^2(3) = 1.36$ N.S.	

[a] Number of animals with designated tumor score are listed. [b] By factorial χ^2 analysis of variance[15]

Results. The arithmetic means of the tumor scores for all 3 experiments are useful graphically (Figures 1, 2, and 3), but are not valid for statistical comparison of an ordinal tumor grading system. For statistical comparison, a factorial χ^2 analysis was performed on the tumor score populations[15] (Tables I–III).

Experiment 1. The difference in tumor size between burned and sham-burned mice was highly significant ($p < 0.01$ from Day 16 and thereafter). Vitamin A administration significantly reduced peak tumor size and accelerated tumor regression in the sham-burned animals ($p < 0.025$ from Day 16 and thereafter). However, vitamin A no had significant effect on tumor size or regression in the burned animals.

Experiment 2. Mice receiving a single dose of Depo-Medrol® 48 h prior to viral inoculation had larger tumors ($p < 0.001$) than did mice which did not receive steroids. This difference was significant on Day 8 ($p < 0.001$), and the level of significance persisted for the remainder of the experiment.

Experiment 3. Vitamin A administration had no effect on tumor growth in rats which had previously received a single injection of Depo-Medrol® (Figure 3).

Discussion. Our data indicate that, although vitamin A effectively inhibited murine viral oncogenesis in animals stressed only with the viral inoculation and the sham-burn, it had no effect on tumorigenesis in the severely stressed, burned animals. Similarly, vitamin A did not appear to alter glucocorticoid-augmented tumorigenesis.

Although high doses of vitamin A have been shown to prevent thymic involution attributed to physiologic stress in mice[8], the same studies also suggest that vitamin A administration to unstressed mice causes some increase in thymic size. It is possible that, for the mouse, insufficient vitamin A is provided by the laboratory diet to maintain lymphoid elements at optimum levels of function. Such an explanation is compatible with COHEN's[16] observation that i.p. vitamin A increased the ability of mice to survive a number of bacterial and fungal infections where no additional physiologic stress was applied. The ability to combat bacterial infection involves lymphoid and leukocytic mechanisms beyond those which are commonly thought to be under thymic control. Indeed, several reports of vitamin A inhibiting the severity of viral infections and viral-induced oncogenesis have been reported, and positive effects of vitamin A on epithelial cell function, membrane permeability, and integrity of connective tissue have been proposed[17–20]. In some of these studies, animals were deliberately made vitamin A deficient, and comparison was made to minimally supplemented animals.

An alternate explanation for our findings is that the physiologic stress of burning was so severe and the dose of exogenous Depo-Medrol® was so high that the lymphotrophic effects of vitamin A were completely overridden in both cases. The data presented in this report cannot clearly refute this possibility. In this series of

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experiments, the effect of vitamin A was observed only in healthy animals exposed to no stress other than that of the viral inoculation, anesthesia, and sham-burn injury. When burn disease was induced or when biochemical stress (Depo-Medrol®) was administered, a salutary effect of vitamin A was not observed.

[21] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

[22] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care.", as promulgated by the Committee on the guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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Summary. High doses of vitamin A decreased the severity of tumor development in mice inoculated with a murine sarcoma virus; the same doses of vitamin A had no effect on the increased tumorigenesis seen in animals severely stressed with thermal injury or the increased tumorigenesis induced by exogenous glucocorticoid administration[21].

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Embryonic Death in Mouse due to Lead Exposure

Lead exposure represents a particular hazard to pregnancy. Sterility, abortions and high fetal and neonatal loss in female lead workers were observed more than 100 years ago[1], and for a time, lead enjoyed popularity as a mean to induce criminal abortions, a procedure which quite often gave raise to serious intoxication[2]. The data on experimental animals are contradictory in that some authors observed reduced fertility even after exposure to low doses, whereas others did not. Thus, SCHROEDER et al.[3,4] supplied lead at a level of 25 ppm to rats and mice over a period of 3 generations and reported fewer litters and early death at tissue levels which were only 2 to 3 times greater than those in controls. On the other hand, LÉONARD et al.[5] giving up to 1000 ppm of lead in the drinking water for 9 months, could not find a significant difference in fertility of mice, and this agrees with the results of AZAR et al.[6] and JESSUP[7] in dogs and rats.

Similar disagreements exist with respect to a possible teratogenic action of lead in experimental animals. Hamsters[8] display an increased rate of malformations, particularly in the tail region, when the mothers are injected with 50 mg/kg of lead on the 8th day of pregnancy. FOURNIER and ROSENBERG[9] could, however, not note any malformation in rabbits or rats after somewhat lower doses (a total of 16 mg/kg), and similar observations have been reported for the cow[10] and the sheep[11]. In view of these disagreements, we have investigated the effects on the pregnancy of different doses of lead acetate added to the diet.

Material and methods. Young adult mice of the C57Bl strain raised in our laboratory were utilized. 3 females were caged with 1 male from the beginning of the week and examined daily for the presence of vaginal plugs. Females with vaginal plug were removed and immediately

Table I. Results of the dissections (16–18 days after the vaginal plug)

Lead [% in diet]	Pregnant females[a]	Corpora lutea	Live embryos	Dead embryos	Loss before implantation
0	26	352	216 [8.31]	32 [1.23]	104 [4.00]
0.125	28	381	231 [8.25]	41 [1.46]	109 [3.89]
0.250	11[c]	138	91 [8.27]	21 [1.91]	26[b] [2.36]
0.500	8[c]	92	60 [7.50]	19[b] [2.38]	13[c] [1.63]

[a] 50 mice with vaginal plug were utilized in each group. Values in brackets are mean numbers per pregnant female. [b] Significant at $p \leqslant 0.05$ level. [c] Significant at $p \leqslant 0.01$ level compared to the controls in the χ^2 test.

Table II. Weight of the embryos (mean in mg $\pm$ SE)

Lead [% in diet]	Days of pregnancy 16	17	18
0	389 ± 11	617 ± 16	978 ± 21
0.125	375 ± 14	605 ± 15	922 ± 15[b]
0.250	406 ± 11	587 ± 23	850 ± 17[c]
0.500	312 ± 36[b]	331 ± 15[a, c]	793 ± 33[c]

[a] These embryos were obtained from only 1 mouse. [b] Significant at $p \leqslant 0.05$ level. [c] Significant at $p \leqslant 0.01$ level compared to controls in t-test.

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