bond is not a sufficient alteration of an unsaturated fatty acid molecule to give positive results in the newt test.

Negative results were also obtained for 2 of the substances containing conjugated double-bonds, sorbic acid and β-carotene. However, as mentioned above, both substances are sparingly soluble in arachid oil and β-carotene could be tested only in concentrations that were low in comparison with those required for positive results of a number of other conjugated compounds.

Substance	Concentration % in arachid oil	No. of experiments	Results Positive reactions/ Effective No. of animals
Oleum arachidis		12	0/68
Tung oil	0.5	1	1/6
Tung oil	1.0	3	0/18
Tung oil	2.0	1	1/5
Tung oil	5.0	3	6/15
Tung oil	10.0	4	14/20
Tung oil	25.0	1	5/5
Ethyl linoleate	100.0	1	0/6
Conjugated ethyl linoleate	50.0	2	2/12
Conjugated ethyl linoleate	100.0	3	11/18
Ethyl elaidate	50.0	2	1/13
Ethyl elaidate	100.0	2	2/12
Sorbic acid	1.0	3	1/18
Sorbic acid	2.0	2	0/12
Sorbic acid	saturated (< 5.0)	1	0/6
β-Carotene	0.1	1	1/6
β-Carotene	0.2	1	0/6
β-Carotene	0.5	1	0/6
Vitamin A-acetate	1.0	1	2/6
Vitamin A-acetate	5.0	2	4/12
Vitamin A-acetate	10.0	2	2/12
Vitamin A-acetate	20.0	1	3/6
Ergosterol	1.0	3	8/18
Ergosterol	2.0	3	10/18
Ergosterol	5.0	1	9/12
3-Methylcholanthrene	0.5	9	23/53
Dibenz(a,h)anthracene	0.1	2	5/12
Dibenz(a,h)anthracene	0.5	1	1/6

Results in the newt test with a trans-fatty acid and a number of compounds containing conjugated double-bonds. Each experiment (column 3) consisted of a group of newts, generally 6 animals, which were injected with the substance indicated in column 1. On the same day a series of experiments on a number of test substances, on pure arachid oil (negative controls) and on methylcholanthrene or dibenzanthracene (positive controls) was started. The effective number of animals (column 4) was the number remaining after, in certain experiments, a few animals dying before the third day or showing severe necrosis, cadaverosis or skin inflammation had been discarded.

Positive results were obtained for vitamin A-acetate, ergosterol, tung oil, and conjugated ethyl linoleate. Since the 4 compounds all contain conjugated double-bonds, the results seem to support the hypothesis that the presence of conjugated double-bonds in a lipid brings about newt-positive properties. It should be mentioned, however, that conjugated ethyl linoleate, although highly active when tested undiluted, was only weakly active in 50% dilution whereas tung oil was active in much lower dilutions. Higher activities were also reported, in our first paper, for ethyl linoleate hydroperoxide and ethyl hydroxyoctadecadienoate. Structurally the 2 compounds are supposed to be conjugated linoleic acids containing a hydroperoxide- and an alcohol-group, respectively. Present results therefore do not permit us finally to decide whether other aspects of the chemical structure than the presence of conjugated double-bonds are essential for positive results.

The substances positive in the newt test are carcinogen-suspect. Immediately it seems improbable that a substance like vitamin A could be carcinogenic. On the other hand, vitamin A influences the growth of epidermal and other types of cells, and recently it has been claimed that excessive vitamin A increases the incidence of avian leukosis[6], and that topical application increases the incidence of DMBA-induced carcinoma in the hamster cheek pouch[7,8].

Zusammenfassung. Mittels der Salamander-Schnellmethode für Karzinogen-Prüfung wurde eine Reihe von Fettprodukten und verwandten Verbindungen untersucht. Äthylelaidat erwies sich als unwirksam, während Vitamin A, Ergosterol, Holzöl und konjugiertes Äthyllinoleat wirksam waren. Die Ergebnisse weisen auf eine grössere Bedeutung der konjugierten Doppelbindungen hin, während der quantitative Vergleich zeigt, dass auch andere Aspekte der chemischen Struktur von Bedeutung sein können.

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12 February 1968.

[6] B. E. MARCH and J. BIELY, Nature *214*, 287 (1967).
[7] A. POLLIACK and I. S. LEVIJ, Nature *216*, 187 (1967).
[8] This work was supported by a grant from the Danish Anti-Cancer League.

Effect of Magnesium Pemoline on the Survival of Mice with Ehrlich Ascites Tumor

Magnesium pemoline, a central nervous system stimulant, has been reported to be a good radioprotective agent[1] for both short- and long-terms[2]. Some of the stimulants in this class such as dextroamphetamine, do not exhibit this property[3]. Recent studies have also indicated that the effect of magnesium pemoline in enhancing radioresistance in mice seems to be independent of drug dosages (up to 2 weeks after irradiation) whether the animals are exposed to lethal amount of X-irradiation before or after the drug administration[4]. These interesting properties of magnesium pemoline suggest the potential application of the drug in both radio-diagnosis and therapy and its possible use in civil defense and industrial accidents involving radiation exposures. The implications mentioned above have encouraged our explicit pilot study of the effect of this drug on the survival of Ehrlich ascites tumor mice with and without the effect of X-rays.

Methods. 360 CF_1 male mice, 50–60 days old (20–22 g) were used in 2 experiments. In the first experiment, 180 animals were divided randomly into 3 groups of

Table I. Post transplantation survival % of Ehrlich ascites tumor mice treated with magnesium pemoline

Days after tumor transplantation	Group I control (tumor only)	Group II tumor + 0.7 mg/kg Mg pemoline	Group III tumor + 14 mg/kg Mg pemoline
1	100	100	100
2	100	100	100
3	100	100	100
4	100	98[a]	100[a]
5	98	95[a]	100[a]
6	93	93[a]	100[a]
7	86	93[a]	98[a]
8	84	91[a]	98[a]
9	81	91	98
10	76	88	98
11	69	88	98
12	62	86	98
13	57	83	96
14	57	81	94
15	41	76	81
16	37	65	68
17	21	62	57
18	16	57	50
19	14	55	50
20	11	55	41
21	6	43	39
22	4	41	37
23	4	38	37
24	0	33	35

[a] Daily i.p. injection of magnesium pemoline from day 4 through day 8 after tumor transplantation.

Table II. Post transplantation survival % of Ehrlich ascites tumor mice treated with magnesium pemoline and high doses of X-irradiation

Days after tumor transplantation	Group I control (tumor + radiation)	Group II tumor + 0.7 mg/kg Mg pemoline + radiation	Group III tumor + 14 mg/kg Mg pemoline + radiation
1	100	100	100
2	100	100	100
3	100	98	100
4	98	95[a]	98[a]
5	95	93[a]	98[a]
6	95	90[a]	94[a]
7	93	85[a]	92[a]
8	93	85[a]	88[a]
9	90	85	88
10	90	80	88
11	90	80	85
12	65	80	85
13	33	78	85
14	20	75	74
15	10	70	74
16	5	54	46
17	3	36	33
18	0	27	27
19		22	24
20		15	24
21		13	24
22		8	24
23		5	24
24		5	24

[a] Daily i.p. injection of magnesium pemoline and daily exposure to 100 R of X-irradiation from day 4 through day 8 after tumor transplantation.

60 mice each. The mice were housed in standard plastic cages, 10 mice in a cage, and placed in an air-conditioned quarters. All the mice were transplanted with Ehrlich ascites tumors 10 days after their arrival from the supplier. Approximately 100,000 cells (0.2 cm^3) of Ehrlich ascites tumor were injected into the peritoneal cavity of each mouse under sterilized conditions. Measures were taken to handle all the animals in the same way.

72 h after the tumor transplantation, each mouse in the first group (control) was injected i.p. with 0.6 cm^3 of bacteriostatic water (0.3% tragacanth suspension); each animal in the second group and the third group was injected with 0.7 mg/kg and 14 mg/kg of magnesium pemoline respectively. Preparation technique and drug concentration were described previously[1-4]. The daily injection of tragacanth and drug was repeated at the same time from day 4 through day 8 after tumor transplantations. Thus, a total of 3.5 mg/kg of magnesium pemoline was accumulated for each mouse in group II and 70 mg/kg for each animal in group III after 5 days of drug injection. In the second experiment, 180 mice were also randomly divided in 3 groups. All the experimental procedures were duplicated from the first experiment, except that in addition to daily tragacanth or drug injections between the 4th and the 8th day after tumor transplantation, the animals in all 3 groups were exposed to a daily total-body dose of 100 R (after injection) of X-rays at approximately 80 R/min during these 5 days. Post tumor transplantation survival was observed and recorded daily, and random specimens were collected for cytological examinations. A separate report dealing with the cytological findings is in preparation. This paper concerns only the effect of magnesium pemoline on the post transplantation survival of the tumor animals.

Results. Table I shows the survival % for 3 groups of the first experiment. In group I, the control mice died quite rapidly as compared to the mortality in groups II and III. All the control animals were dead by the 24th post transplantation day against 67% in group II and 65% in group III. The mice in group II were injected daily for 5 days with very low drug dosage (0.7 mg/kg daily). The amount received by the animals in group III was 20 times this dose. After the first injection, the daily mortality rate in group III was significantly lower than in group II. This trend stopped about 1 week after the 5th injection for both groups. There was no significant difference in the survival % in these 2 groups after the 15th day after transplantation (12 days after the first injection).

Table II presents the data obtained from the second experiment in which all the mice were exposed to 100 R of X-irradiation daily for 5 days. The accumulated dose (500 R) was still sublethal but the additional stress on the tumor animals was undeniable. By the 18th day after transplantation the control mice in group I reached their 100% mark in mortality while 27% in group II and 27% in group III still survived. To compare these 2 groups with group II and group III in the first experiment, daily mortality was recorded up to the 24th day after transplantation. For the benefit of our cytological studies, further observations were made up to 30 days after transplantation or about 3 weeks after the final drug injection and radiation exposure. By this day 28% of the mice in

[1] H. LeVan, Experientia *23*, 1058 (1967).
[2] H. LeVan, Experientia *24*, 477 (1968).
[3] H. LeVan and D. L. Hebron, J. Am. pharm. Ass., in press.
[4] H. LeVan, Int. J. Pharm. Ther. Toxic., in press.

group II and 24% in group III survived in the first experiment against 3% for group II and 22% in group III in the second experiment.

Discussion. Comparing the survival % between the control and 2 experimental groups in both Table I and Table II one notes that magnesium pemoline not only slows down the mortality in the experimental groups but also prolongs the survival of the tumor mice injected with this drug either with or without the additional effect of radiation. The additional damage caused by radiation was clearly noticed in the mortality of the control groups in both experiments. The life span of the control tumor mice shown in Table II was shortened about 1 week. Again, the data shown in Table II for group II and group III confirm once more that short-term protection of magnesium pemoline against radiation was independent of the drug dose; but for long-term protection, the effect was more pronounced with higher drug dose. Until we have the final report from our cytological studies on the tumor taken from these 2 experiments, it is too early to speculate about either the tumorstatic or tumoricidal effect of magnesium pemoline. The results shown above, however, strongly indicated another important and interesting property of this drug[5].

Zusammenfassung. Magnesium Pemolin, ein zentralnervöses Reiz- und gutes Schutzmittel gegen Radiumbestrahlung, verlängert die Lebensdauer von Ehrlich-Ascites-Tumor-Mäusen, und zwar mit oder ohne zusätzlichen Einfluss einer Dosis nichttödlicher Bestrahlung (500 R).

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The Influence of *Bordetella pertussis* on the Kinetics of Antibody Production to Sheep Red Blood Cells in NMRI Mice

The adjuvant activity of *Bordetella pertussis* has been described repeatedly[1-5], but the mode of action of these bacteria and all other types of immunological adjuvants is still unknown. In previous studies a significant enlargement of the spleens of pertussis-treated mice was found[6,7]. This was caused by an increase in tissue substance and characterized by a multiplication of the number of cells up to about 100% and an increased protein synthesis of the individual cell. Furthermore the additional injection of pertussis organisms (PO) into NMRI mice immunized with sheep red blood cells (SRBC) resulted in an accelerated, increased and prolonged formation of γM antibody producing spleen cells[8]. Thus it was of interest to find out whether the injection of PO also leads to an increased formation of 7 S antibody producing spleen cells and 7 S serum antibodies.

Method. NMRI male mice (20–25 g) were i.p. immunized with 4×10^8 SRBC. A second group of mice was given simultaneously with the SRBC an i.p. injection of 2×10^9 PO (phase I, heat-killed and not absorbed). At different intervals after immunization 4 mice of each group were killed, their spleens removed aseptically and their sera collected. For the quantitative determination of plaque-forming spleen cells the direct[9,10] and indirect[11,12] 'localized hemolysis in gel' assay (LHG) were employed using Oxoid agar No. 3 and diethylaminoethyl-dextran (DEAE-dextran) as described[8]. The rabbit anti-mouse immunoglobulin antiserum was prepared by immunizing rabbits with a mouse 7 S-serum globulin obtained by elution of NMRI mouse serum from a Sephadex 200 column with 0.15 *M* phosphate-buffered NaCl, pH 7.2. Its optimal concentration was found at a 1:200 dilution. Serum hemolysin activity was determined spectrophotometrically at 530 nm according to the 50% hemolysis method[13] on serum samples pooled from 4 identically treated mice. Hemolysin concentration is given in 50% hemolysis units (HU)/ml of serum. The lowest value determined was 10 HU, because dubious results were obtained if the 7 S hemolysin titer was below 10 HU[14]. In addition hemagglutination tests were performed. Concomitantly those fractions of the total hemolysin and agglutinin activity resistant to treatment with 0.1 *M* 2-mercaptoethanol (2-ME) were determined. According to DEUTSCH and MORTON[15] it is justified to assume that antibodies resistant to 2-ME are 7 S antibodies.

Results. In pertussis-treated mice increased spleen indices (mg wet spleen weight/g body weight) up to about 120% were demonstrable between the third and fourteenth day after immunization. As can be seen from Figures 1 and 2 the formation of direct plaque-forming cells (PFC) apparently producing 19 S antibodies and the formation of developed PFC apparently producing 7 S antibodies are increased and prolonged in pertussis-treated mice. Taking into consideration that the elevation of the spleen weights demonstrated 7 and 10 days after the immunization of mice with bovine serum albumin and PO was

[1] L. GREENBERG and D. S. FLEMING, Can. publ. Hlth J. *38*, 27 (1947).
[2] L. GREENBERG and D. S. FLEMING, Can. publ. Hlth J. *39*, 131 (1948).
[3] J. R. FARTHING, Br. J. exp. Path. *42*, 614 (1961).
[4] J. MUNOZ, J. Immun. *90*, 132 (1963).
[5] L. S. KIND, Bact. Rev. *22*, 173 (1958).
[6] H. FINGER, G. BENEKE and P. EMMERLING, Z. med. Mikrobiol. Immun. *154*, 23 (1968).
[7] H. FINGER, G. BENEKE and P. EMMERLING, Z. Naturf. *23b*, 288 (1968).
[8] H. FINGER, P. EMMERLING and H. SCHMIDT, Experientia *23*, 591 (1967).
[9] N. K. JERNE and A. A. NORDIN, Science *140*, 405 (1963).
[10] N. K. JERNE, A. A. NORDIN and C. HENRY, in *Cell Bound Antibodies* (Wistar Institute Press, Philadelphia 1963), p. 109.
[11] D. W. DRESSER and H. H. WORTIS, Nature *208*, 858 (1965).
[12] H. H. WORTIS, R. B. TAYLOR and D. W. DRESSER, Immunology *11*, 603 (1966).
[13] D. H. CAMPBELL, J. S. GARVEY, N. E. CREMER and D. H. SUSSDORF, *Methods in Immunology* (W. A. Benjamin, New York, Amsterdam 1964).
[14] J. S. HEGE and L. J. COLE, J. Immun. *96*, 559 (1966).
[15] N. F. DEUTSCH and J. I. MORTON, Science *125*, 600 (1957).