

THE INFLUENCE OF THE LIPID COMPOSITION OF THE FEED GIVEN TO MICE ON THE IMMUNOCOMPETENCE AND TUMOUR RESISTANCE OF THE PROGENY

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In inbred CBA mice, the immunocompetence of adult progeny from breeding pairs fed three different diets was compared. Substitution of soy oil for animal fat in the feed of the mice during gestation or lactation significantly decreased the PFC response to SRBC in the adult offspring. Addition of 2-methoxy-substituted glycerol ethers to the feed of mothers deprived of animal fat during lactation partly restored the PFC response of the male offspring. In the adult mice fed differently pre- and perinatally the resistance to a transplanted syngeneic sarcoma was similar. The growth of offspring from mice fed the three diets was similar. In mice deprived of animal fat at weaning and for the following 21 days the immune reactivity to SRBC, tested about 3 months after stopping the diet, was not influenced. However, the resistance to a transplanted tumour in similarly fed mice was increased and this resistance was brought approximately to the control level by methoxy-substituted glycerol ethers.

2-Methoxy-substituted glycerol ethers have previously been shown to be oral adjuvants. The compounds incorporated into the feed in concentrations of 0.1%, 0.25% or 0.5% stimulated the plaque-forming cell (PFC) response to sheep red blood cells (SRBC) in CBA and C57BL/6J mice. Further, the ability of spleen cells to induce graft-versus-host reaction (GVHR) in (C57BL/6J × DBA/2J)_F₁ hybrids was increased after pretreatment of the spleen-cell donors for 44 days with 0.1% of the compounds (Boeryd *et al.*, 1978).

Methoxy-substituted glycerol ethers occur in human red bone marrow, human milk, cow's milk and sheep's milk (Hallgren *et al.*, 1974). The findings of glycerol ethers in the milk of different species and of their effects as oral adjuvants gave rise to the question whether these compounds in the milk might influence the immune reactivity of the progeny.

The aim of this investigation was to study the immune reactivity of the adult progeny from mice given 2-methoxy-substituted glycerol ethers in the feed and of adult mice given these substances during a period after weaning. Further, the resistance of similarly fed mice against the growth and spread of a transplantable tumour was investigated.

MATERIAL AND METHODS

Inbred mice of CBA strain were used. At an age of 10-12 weeks brothers and sisters were paired and these pairs were differently fed. Three different diets were used: (1) Astra Ewos' commercial maintenance feed for rats and mice, containing animal fats in the form of fish meal, meat-bone meal and bone fat (AE). (2) A corresponding feed with soy oil instead

of animal fat (AE-AF). The content of protein, vitamins and lipotropes and the caloric value of these two feeds were similar. (3) The AE-AF feed with 0.1% synthetic 1-0-(2-methoxy-hexadecyl) glycerol (MGE) added (AE-AF+MGE).

The breeding pairs were divided into three groups, each of which was fed one of the three diets during the following periods: (1) from the day of mating to delivery, *i.e.* during the gestation period, and (2) from the day of delivery and for the following 21 days, *i.e.* during lactation.

In another series of experiments, offspring from breeding pairs fed AE during gestation and lactation were divided into three groups at weaning, each group being given one of the three diets for 3 weeks (weeks 4-6).

Before and after the dietary periods all mice studied were given the control feed, *i.e.* AE. All litters were weighed every week from the 1st to the 5th and in the 7th week; the litters from the AE groups were also weighed in the 6th week.

The 19 S PFC response to SRBC was tested 5 days after *i.v.* injection of 10⁸ SRBC according to the method of Cunningham and Szenberg (1968). The PFC were assayed by incubation of lymphoid cells together with target erythrocytes and complement as monolayer without jellying of the medium.

At the time of SRBC injection the mice from differently fed breeding pairs were intended to be about 3 months of age and those differently fed during weeks 4-6 about 4.5 months. This plan could not be completely fulfilled. However, in the separate experiments the age of the mice was similar. Each experiment comprised three groups, AE, AE-AF and AE-AF+MGE, and each group contained five mice of the same sex. On a few occasions one group consisted of six or four mice and on one occasion only three mice were available in one group. All experiments were repeated four times.

The growth and spread of a transplantable tumour was studied in groups of mice fed as above. The age of the mice at tumour transplantation was planned to be the same as in the tests of the PFC response. However, this could not be completely fulfilled. The tumour used, a weakly antigenic syngeneic methylcholanthrene-induced sarcoma MCG12 (Suurkula and Boeryd, 1975), was transplanted subcutaneously on the tail. The tails with the tumours were amputated after 17-18 days and the experiments terminated 43-47 days after the tumour transplantation. At

autopsy macroscopic metastases were noted. The lungs were prepared for histological investigation as described previously (Boeryd, 1965).

Statistical methods

Data obtained from tests of the PFC response were subjected to an analysis of variance. Results are reported mainly with reference to the outcome of Student's t-test. The average standard deviation of observations in each of the four experiments on mice from the same diet groups is also presented.

RESULTS

Litter size and mortality

Fifteen breeding pairs given AE diet had altogether 113 young and four of these died before 7 weeks of age.

During gestation 12 breeding pairs were fed the AE-AF diet and they produced 93 young, 5 of which died before 7 weeks of age. Nine pairs given the AE-AF+MGE diet produced 63 young, 3 of which died before 7 weeks of age.

TABLE I

NUMBER OF LITTERS AND YOUNG FROM BREEDING PAIRS FED DIFFERENT DIETS DURING GESTATION OR LACTATION AND THE NUMBER OF YOUNG WHICH DIED BEFORE 7 WEEKS OF AGE

Groups	Period fed different diets	No. of litters	No. of young	No. of young which died before 7 weeks of age
AE		15	113	4
AE-AF	Gestation	12	93	5
AE-AF+MGE	Gestation	9	63	3
AE-AF	Lactation	19	92	2
AE-AF+MGE	Lactation	16	112	5

Differences in tumour weight between groups were analyzed with the aid of Wilcoxon's two-sample rank test and differences in the incidence of metastases by means of the fourfold table test. Values of $p < 0.05$ were accepted as significant.

Nineteen pairs given the AE-AF diet during lactation had 92 young, 2 of which died before 7 weeks of age. The 16 pairs given the AE-AF+MGE diet had altogether 112 young, 5 of which died before 7 weeks of age (Table I).

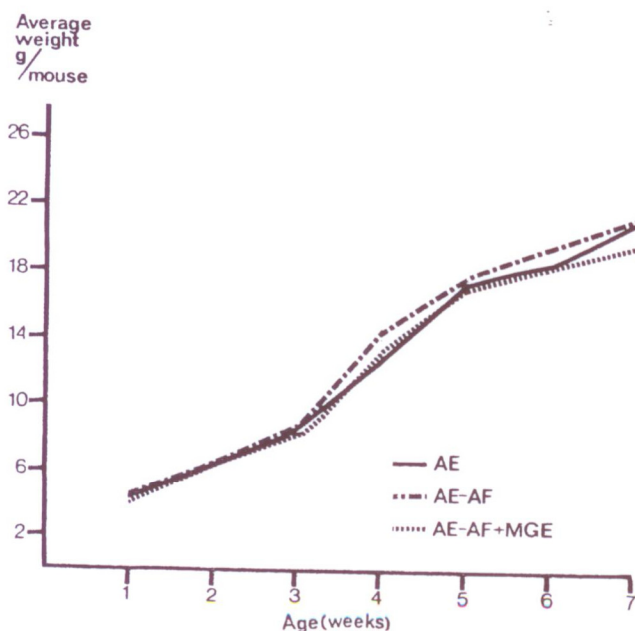


FIGURE 1 — Growth of offspring from groups of six breeding pairs differently fed during gestation. The AE group consists of 44 young, the AE-AF group of 38 and the AE-AF+MGE group of 37 young.

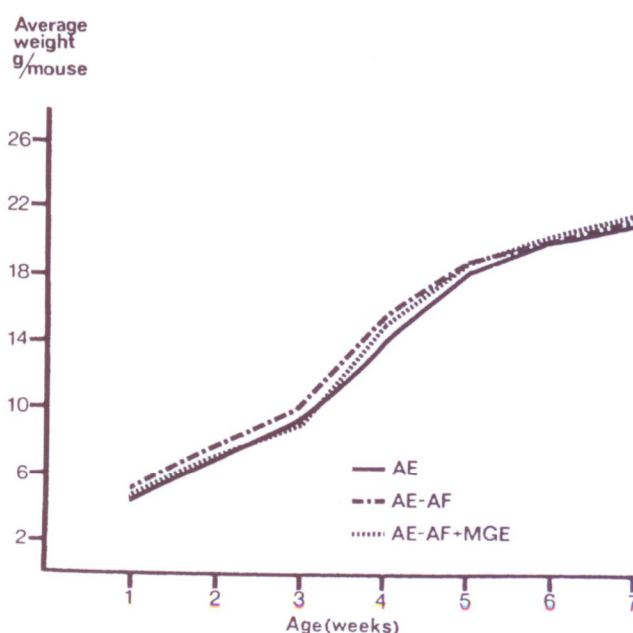


FIGURE 2 — Growth of offspring from groups of four breeding pairs differently fed during lactation. The litters in each group consist of 4, 5, 6 or 8 young.

TABLE II
NUMBER OF MICE USED, THEIR AGE AND WEIGHTS AND THE WEIGHT OF THE THYMUS AND SPLEEN AS A PERCENTAGE OF BODY WEIGHT AT THE TIME OF TESTING FOR PFC RESPONSE. DATA FOR BOTH SEXES COMBINED.

Groups	Period fed different diets	No. of mice	Age of mice, days (range)	Average weight of mice (g)	Average weight of thymus (% of body weight)	Average weight of spleen (% of body weight)
AE	Gestation	20	108-89	25.8	0.17	0.47
AE-AF	Gestation	20	103-77	24.3	0.18	0.46
AE-AF-MGE	Gestation	19	102-93	25.0	0.16	0.44
AE	Lactation	19	109-74	25.7	0.14	0.47
AE-AF	Lactation	20	103-70	25.7	0.11	0.43
AE-AF+MGE	Lactation	20	103-98	25.7	0.13	0.39
AE	weeks 4-6	21	139-111	27.2	0.14	0.44
AE-AF	weeks 4-6	19	137-116	28.9	0.14	0.45
AE-AF+MGE	weeks 4-6	18	137-119	29.6	0.13	0.41

Growth of progeny

The growth of offspring from breeding pairs fed the AE-AF or AE-AF+MGE diet was not inhibited compared to the controls, whether the diets were given during gestation or during lactation (Fig. 1, 2). The weights of mice at the time of testing for the PFC response in the separate groups from the same periods of diet were similar, and the weights of the thymus and spleen did not differ significantly between these groups (Table II).

The growth of mice differently fed from weaning and during the following 3 weeks was similar, irrespective of the diet fed.

PFC response

The statistical analysis comprised results for both sexes together and for males and females separately. In offspring from mice differently fed during gestation, the AE-AF group as well as the AE-AF+MGE group had a lower number of PFC than the controls ($p < 0.001$). These differences were valid for

both sexes together. The statistical analysis also disclosed that the differences found in the whole material also seemed to be valid for each sex separately; no clear sex difference in reactivity was found. In offspring from mice differently fed during lactation, the AE-AF group and the AE-AF+MGE group had a lower number of PFC than the controls ($p < 0.001$ and $p < 0.01$ respectively). However, the males in the AE-AF+MGE group had a significantly higher number of PFC than the males in the AE-AF group ($p < 0.01$) (Table III). The same difference was found in one of the experiments in females, but not in the other one.

In mice differently fed during the 4th to 6th weeks there were no differences in the number of PFC between the three groups (Table III).

Tumour growth and spread

There were no significant differences in the growth of MCG12 or its dissemination in the adult offspring from mice fed three different diets during gestation or lactation (Table IV, V).

TABLE III
19 S PFC/SPLEEN 5 DAYS AFTER I.V. INJECTION OF 10^8 SRBC IN OFFSPRING FROM BREEDING PAIRS FED DIFFERENT DIETS DURING GESTATION OR LACTATION AND IN MICE FED DIFFERENT DIETS FROM 4 TO 6 WEEKS OF AGE

Groups	Sex	Periods fed different diets		
		Gestation	Lactation	Weeks 4-6
AE	♂	78,900	76,400	75,300
AE-AF		60,000	39,100	79,900
AE-AF+MGE		57,700	58,600 ³	72,700
AE	♀	54,400	77,800	96,400
AE-AF		38,000	47,500	104,900
AE-AF+MGE		22,300	44,200	83,200
AE	♂ + ♀	66,700	77,100	86,300
AE-AF		49,000 ¹	43,300 ⁴	91,800
AE-AF+MGE		40,900 ²	51,400 ⁵	78,500
		SD: $\pm 12,700$	SD: $\pm 18,800$	SD: $\pm 18,400$

¹ 49,000 vs 66,700, $p < 0.001$. ² 40,900 vs 66,700, $p < 0.001$. ³ 58,600 vs 39,100, $p < 0.01$. ⁴ 43,300 vs 77,100, $p < 0.001$. ⁵ 51,400 vs 77,100, $p < 0.01$.

TABLE IV

GROWTH AND SPREAD OF MCG12 IN ADULT OFFSPRING FROM CBA MICE DIFFERENTLY FED DURING GESTATION. TUMOUR AMPUTATION 17 DAYS AFTER TRANSPLANTATION. OBSERVATION PERIOD 47 DAYS

Groups ¹	Age of mice at tumour transplantation days (range)	Average tumour weight (g. \pm SEM)	Incidence of metastases to		
			Lymph nodes	Lungs	
AE	♂	101-81	0.87 \pm 0.10	5/13	11/13
	♀	101-81	1.23 \pm 0.12	5/15	13/15
	♂+♀			10/28	24/28
AE-AF	♂	79-67	1.00 \pm 0.09	4/12	8/12
	♀	79-67	1.09 \pm 0.13	4/13	8/13
	♂+♀			8/25	16/25
AE-AF +MGE	♂	79-71	1.13 \pm 0.10	6/9	4/9
	♀	79-58	1.17 \pm 0.11	8/15	11/15
	♂+♀			14/24	15/24

¹ In the AE groups one male and in each of the other groups one female were missing at autopsy.

In mice differently fed from weaning, the tumours were smaller in both sexes in the AE-AF group than in the AE group ($p < 0.01$) and smaller in the AE-AF males than in the AE-AF+MGE males ($p < 0.01$). The tumours tended to be smaller in the AE-AF+MGE females than in the AE females ($p < 0.10$). The incidence of metastases to lymph nodes was lower in the AE-AF group than in the AE group ($p = 0.05$) and lower in the AE-AF females than in the AE females ($p = 0.05$). Further, the incidence of lung metastases was lower in the AE-AF group than in the AE group ($p < 0.05$) (Table VI).

DISCUSSION

The neutral fat and phospholipid fractions of animal fat contain glycerol ethers, unsubstituted as well as methoxy-substituted. This fat was therefore excluded from the AE-AF diet and replaced with soy oil so that the effect of addition of glycerol ethers could be studied.

It is well known that malnutrition of animals influences their immunocompetence. Feeding a deficient

diet to female mice during gestation and/or lactation has been shown to reduce the body weight of the progeny (Dubos *et al.*, 1969). Pre- and perinatal deprivation of protein and lipotropes, methionine and choline, or vitamin B₁₂, reduced the body weight of the animals and/or their immunocompetence, as measured by the PFC response to SRBC (Gebhardt and Newberne, 1974; Newberne, 1977). From the present investigation it is obvious that pre- or perinatal deprival of animal fat or its replacement with soy oil without restriction of the caloric value, lipotropes or vitamins, greatly impairs the development of the immunocompetence of the progeny without affecting body weight.

Addition of MGE to the diet of mothers deprived of animal fat during lactation partly restored the PFC response to SRBC. Glycerol ethers, including methoxy-substituted ones, occur in the milk of different species (Hallgren *et al.*, 1974) and the methoxy-substituted glycerol ethers are oral adjuvants (Boeryd *et al.*, 1978). The partial restitution induced by one synthetic compound occurring in the mixture of methoxy-substituted glycerol ethers suggests that

TABLE V

GROWTH AND SPREAD OF MCG12 IN ADULT OFFSPRING FROM CBA MICE DIFFERENTLY FED DURING LACTATION. TUMOUR AMPUTATION 18 DAYS AFTER TRANSPLANTATION. OBSERVATION PERIOD 45 DAYS

Groups	Age of mice at tumour transplantation days (range)	Average tumour weight (g. \pm SEM)	Incidence of metastases to		
			Lymph nodes	Lungs	
AE ¹	♂	81-64	0.90 \pm 0.10	1/14	10/14
	♀	127-116	1.07 \pm 0.12	3/14	11/14
	♂+♀			4/28	21/24
AE-AF	♂	106-60	1.03 \pm 0.06	3/17	11/17
	♀	106-60	0.95 \pm 0.12	4/14	9/14
	♂+♀			7/31	20/31
AE-AF +MGE	♂	104-60	1.13 \pm 0.11	0/13	8/13
	♀	104-60	1.19 \pm 0.10	5/15	9/15
	♂+♀			5/28	17/28

¹ In the groups AE one male, AE-AF one female and AE-AF+MGE one male and one female were missing at autopsy.

these glycerol ethers, or their metabolites, in the milk may influence the immunocompetence of the progeny.

Malnutrition of animals can also change their reactivity to tumours. In rats fed a diet deficient in casein the cytotoxic cell-mediated immune response to tumours was intact but the specific antibody response profoundly depressed (Jose and Good, 1971). Restricted protein-caloric intake of mice aged 32-35 days increased the cytotoxic activity against allogeneic tumours while the serum blocking activity was absent (Jose and Good, 1973a). Similar changes of immune reactivity to allogeneic tumours were noted in mice fed diets deficient in different essential amino-acids from 5 weeks of age (Jose and Good, 1973b). Protein deprivation of male mice for a period of 2 weeks, commencing at weaning, decreased cytotoxic cellular immunity up to 8 weeks after the restricted diet was stopped (Jose *et al.*, 1973). The body weights in these calorie-restricted mice were decreased.

Whether the ratio between cellular and humoral antibodies against the tumour was changed in our differently fed animals, as in protein-restricted animals, has not been tested.

However, in rats fed a diet enriched with animal fat from weaning, the 1,2-dimethylhydrazine-induced colonic tumours appeared earlier and at a higher frequency and metastasized more often than in the other groups (Bansal *et al.*, 1978). In rats fed a diet low in animal fat and enriched with carbohydrate, the number of tumours was reduced. The pattern of immune response, including depressed levels of serum immunoglobulin G, was similar in all groups of rats fed the different diets. The authors interpret their results as demonstrating a correlation between the serum cholesterol level and the incidence of colonic tumours.

These results suggest that animal fat contains substances whose presence in the feed of young mice is of importance for the development of their immune reactivity and their resistance to tumours. Some of

TABLE VI
GROWTH AND SPREAD OF MCG12 IN ADULT CBA MICE DIFFERENTLY FED FROM WEANING AND THE FOLLOWING 3 WEEKS (WEEKS 4-6). TUMOUR AMPUTATION 18 DAYS AFTER TRANSPLANTATION. OBSERVATION PERIOD 43 DAYS

Groups	Age of mice at tumour transplantation days (range)	Average tumour weight (g. \pm SEM)	Incidence of metastases to		
			Lymph nodes	Lungs	
AE ¹	♂	107-103	1.40 \pm 0.09	5/13	
	♀	107-100	1.35 \pm 0.10	8/12	12/13
	♂+♀			13/25	9/10
AE-AF	♂	89-61	0.78 \pm 0.09 ^{2,4}	5/20	
	♀	89-79	0.81 \pm 0.15 ³	2/14 ⁶	11/20
	♂+♀			7/34 ⁷	9/14
AE-AF +MGE	♂	89-77	1.23 \pm 0.13	6/16	
	♀	89-77	1.03 \pm 0.12 ⁵	5/17	14/16
	♂+♀			11/33	12/17
					26/36

¹ One male and four females were missing at autopsy or their lungs were not available for investigation. - ² 0.78 vs 1.40, $p < 0.01$. - ³ 0.81 vs 1.35, $p < 0.01$. - ⁴ 0.78 vs 1.23, $p < 0.01$. - ⁵ 1.03 vs 1.35, $p < 0.10$. - ⁶ 2/14 vs 8/12, $p = 0.05$. - ⁷ 7/34 vs 13/25, $p = 0.05$. - ⁸ 20/34 vs 21/23, $p < 0.05$.

In this experiment pre- or perinatal deprivation of animal fat impaired the T-cell-dependent (Cantor, 1972) response to SRBC but the resistance to the transplanted tumour and its dissemination was not significantly inhibited. Addition of MGE to the diet of the mothers did not affect this resistance. However, deprivation of animal fat for only three weeks from weaning increased the resistance of the adult mice to the transplanted tumour and its dissemination, and the addition of MGE to the diet tended to bring the resistance to the tumour back to control level. The PFC response to SRBC did not differ between these groups of mice. Thus, there was no correlation between the T-cell-dependent immune response and the resistance to the tumour.

The immune effect on tumours may be a result of interplay between cellular and humoral antibodies (Prehn, 1971; Klein, 1972), where the ratio between these types of antibodies determines the effect.

these substances might be methoxy-substituted glycerol ethers. Further, it may be of importance that the composition of lipids in breast milk substitutes imitates that of human milk.

In this study only one compound of methoxy-substituted glycerol ethers, the synthetic 1-O-(2-methoxyhexadecyl) glycerol, has been incorporated into the feed. The effect of the whole mixture of methoxy-substituted glycerol ethers on the development of immunocompetence will also be investigated and well-defined synthetic diets will be used. The effect of non-substituted, ordinary glycerol ethers also needs to be studied.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Cancer Society (Project Number 156-B78-12X). We wish to thank Miss Barbro Melin for skilful technical assistance.

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