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Received November 6, 1957. P.S.E.B.M., 1958, v97.

Potentiation of Smooth Surface Caries by Sodium Dehydroacetate Variously Administered to the White Rat. (23727)

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In a previous study(1) dehydroacetic acid (DHA) markedly potentiated the severity of smooth surface caries in the rat when incorporated in a cariogenic diet at the 0.1% level. To extend this finding and to evaluate the possible extra-oral systemic effect of the compound on caries, DHA was administered by intubation and intraperitoneal injection as well as in the diet and drinking fluid.

Methods. The water soluble sodium salt DHA-S* was used throughout but the dosage levels are given in terms of DHA† to conform with the study previously reported(1). Litter-mated weanling, female, Sprague-Dawley rats individually housed in screen-bottomed cages received the cariogenic diet of McClure and Folk containing an autoclaved skim milk powder(2) for 60 days. The experimental design of the study is shown in Table I. Twenty-four litters of rats were used in Exp. A and B, and 40 litters in Exp. C. The diets were fed *ad libitum* in Exp. A and B. In Exp. C fresh diet was offered daily on a paired feeding basis. Distilled water intake was *ad libitum*. The DHA drinking fluids in Experiment A varied between 0.25, 0.50, and 0.75 mg/ml, and the volumes were controlled in order to equilibrate fluid DHA intake with dietary DHA. The diet and weight gain were measured routinely. The solutions used for intubation and intraperitoneal injection contained 10.0 mg DHA/ml (12.4 mg DHA-S/ml) and were given in 2 divided doses daily except on week ends when half amounts were administered. The mean daily DHA intake by these extra-oral routes in Exp. B and C is shown in Table I. Since 0.10% DHA in the diet had markedly potentiated caries in 3 previous trials(1) and Exp. A, no dietary DHA control was included in Exp. B. To further substantiate the caries potentiating effect of parenterally administered DHA, the regimen of injection used in Exp. B was followed in Exp. C. Amounts of DHA varying from 0.60 to 1.0 ml/day were injected and control rats were injected with equal volumes of distilled water. In Exp. B and C, the rats were kept on the cariogenic diet for one week before intubation and injection of DHA, since weanling rats did not tolerate DHA by these routes of administration. After 60 days on experiment, the rats were sacrificed and the molar teeth were scored for smooth surface

* Obtained from Gaines Chemical Works, Carlstadt, N. J. Calculated for $C_8H_7NaH_2O$: C, 46.16; H, 4.36. Found: C, 46.20; H, 4.44.

† Molecular weights of sodium salt monohydrate and free acid are 208.1 and 168.1 respectively, so that an 0.124% solution of the former is equivalent to 0.10% solution of the free acid DHA.

TABLE I. Caries Experience* of White Rats Receiving DHA† in the Diet, in the Drinking Water, by Intubation and by Intraperitoneal Injection.

Exp. No.	No. rats	Mode of DHA admin.	DHA intake (mg/day)	Carious rats (%)	Caries score/rat
A	23		.0	73.9	3.5 ± .9
	18	0.10% in diet	5.3 ± .1	100.0	13.8 ± 2.2
	18	Drinking water	4.5 ± .3	88.9	9.3 ± 2.0
B	21	0.10% in diet	6.4 ± .2	100.0	15.9 ± 1.0
	17	Intubation	6.2 ± .1	100.0	13.9 ± 2.3
	17	Intraper. inj.	6.2 ± .1	100.0	13.0 ± 1.8
C	40	(H_2O by intraper. inj.)	.0	47.5	1.4 ± .3
	22	Intraper. inj.	6.3‡	86.4	9.9 ± 2.3

All values are expressed as mean ± S.E.

* Smooth surface caries on molar teeth. † Provided as sodium salt, DHA-S. ‡ All animals received identical amounts.

caries according to McClure *et al.*(2). Occlusal surface caries was very limited or nonexistent as seen by low power magnification. The chi square test as previously employed (3) was used to assess the difference in caries incidence, and Fisher's "t" test was used to measure the significance of the difference of all other comparisons. All estimates of errors of the means are expressed as the standard errors calculated according to Mantel(4).

Determination of DHA in Rat Saliva. Although DHA was administered parenterally, it was possible that appreciable excretion of DHA in the saliva could produce an intraoral effect. Hence, the content of DHA in pooled rats' saliva was measured at various intervals of time following intubation and intraperitoneal injection of 5.0 mg DHA (as DHA-S) in 0.5 ml H_2O. In addition, a pooled sample of plasma was also analyzed. To determine the DHA content in the saliva following its oral administration, diet 636 containing 0.10% DHA was offered to 12 rats and the intake recorded. At the end of 14 days, the rats' mouths were swabbed under nembutal to remove diet residuum. Adequate controls and recoveries were carried out. Salivas were collected according to Benarde *et al.*(5) with minor modifications‡ and assayed for DHA by the procedure of Woods *et al.*(6).

Results. In Exp. A, the rats receiving 0.10% DHA in the diet§ or the DHA drinking fluid showed significant increases in caries scores when compared with the controls ($p < 0.01$ and $p < 0.02$ respectively). In Exp. B, DHA given by intubation or by intraperitoneal injection appeared as effective in potentiating caries as an equivalent amount of dietary DHA. Although the experimental regimen in Exp. C results in a low caries score as previously observed(7), nevertheless both the caries incidence and severity were again significantly increased ($p < 0.01$) when DHA was injected.

The diet intakes for the experimental rats of Exp. A and B were significantly lower than those for the controls (6.5 ± 0.2 g *vs.* 7.8 ± 0.2 g and $p < 0.01$). The weight gains were also similarly significantly reduced (1.3 ± 0.04 g *vs.* 0.9 ± 0.08 g and $p < 0.01$).

Concentration of DHA in rat saliva. The concentration of DHA in rat saliva was 3.5, 4.1, 5.5, 5.2 and 4.6 mg % at 1, 2, 3.5, 4 and 5 hours after injection of 5 mg DHA. Saliva collected from another group of rats 3.5 hours after injection of DHA contained 5.2 mg % DHA, and the blood plasma contained 11.1 mg % DHA. Rats intubated with 5 mg DHA had 5.6 mg % DHA in the saliva after

‡ The following solutions were administered by intraperitoneal injection:

1. DHA-S Solution: 12.4 mg DHA-S/ml; equivalent to 10 mg DHA/ml.
2. Nembutal solution: Abbott, Veterinary Grade 60 mg/ml. Used 1 ml of a 1:10 dilution.
3. Picrotoxin solution; K and K Laboratories, Long Island City, N. Y. Used 0.5 ml of solution containing 1 mg/ml to counteract depressant effect of nembutal on respiratory center.
4. Pilocarpine nitrate—Gane and Ingram, N. Y. City. Used 1 ml of solution containing 1 mg/ml.

§ No significant change in either incidence or severity (caries score) of caries was observed in an additional group of rats receiving 0.05% DHA in diet.

4 hours which was similar to that found after injection (5.2 mg %) of the same amount. Twelve rats receiving diet 636 containing 0.10% DHA for 14 days had a mean daily DHA intake of 10.9 ± 0.4 of DHA. Three pooled samples of saliva collected from these animals contained 15.0, 16.4 and 17.6 mg % DHA. No absorption at 310 mμ (wave length at maximum density) was found in the control samples, and 95% of the DHA added to rat saliva and blood could be recovered.

Discussion. The present findings corroborate previous studies(1) that 0.10% DHA in diet 636 has a pronounced potentiating effect on the incidence and severity of smooth surface caries. These additional studies have indicated that DHA also has a marked caries potentiating effect when administered by intubation or by intraperitoneal injection.

Of primary interest is the possibility that an extra-oral systemic effect of DHA may be involved in its caries potentiation. Although administration of DHA by intubation and injection precludes any direct contact of the compound with the oral cavity, it is apparent that its secretion in the saliva is an important consideration. Rather extensive studies demonstrated that appreciable concentrations of DHA in rat's saliva resulted from dietary as well as intubated and injected DHA. Hence, it appears that the caries potentiating effect of DHA by these routes may be indirectly mediated through its secretion in the saliva, even though the rate of flow would be presumed to be less under normal physiological conditions than under pilocarpine stimulation.‖

Our results, nonetheless, do not eliminate the possibility that systemic factors may be operative in the potentiation of caries. The idea that systemic factors may be concerned in experimental dental caries has received further attention in this laboratory. Thus with a lysine deficient cariogenic diet, intubated lysine has been shown to be cariostatic although no significant elevation of lysine was noted in the rats' saliva(8). In addition, intubated EDTA also potentiated caries. However, its concentration in saliva was not determined(9).

Summary. 1. The ingestion of DHA in the diet, drinking water, by intubation and by intraperitoneal injection all had a pronounced potentiating effect on the smooth surface caries produced in white rats by a heat processed skim milk powder diet. 2. Considerable concentrations of DHA were found in stimulated rat saliva following its administration by diet, by intubation and by intraperitoneal injection. 3. It appears that the potentiating effect of DHA on smooth surface rat caries cannot be attributed unequivocally at this time to an extra-oral systemic effect.

‖ Under pilocarpine stimulation approximately 1 ml of saliva was collected in 20 minutes, after which little saliva was secreted.

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