

TEST METHOD #38

DETERMINATION OF ANIMAL CARIES REDUCTION

RATIONALE

The rat caries test is a model designed for the study and the prevention of human caries. Although using animals it remains a laboratory test which has many features that are relevant to the carious environment found in human beings. It permits the production of acid from dietary sugars by oral bacteria resident in the oral cavity of the rat, and the acid is harboured in plaque in sheltered regions of the teeth. The prolonged contact-time of the acidic plaque with the tooth enamel causes the loss of mineral phase and the onset of caries. The rat model permits other interactions to take place such as the constant bathing of the teeth in saliva, the replenishment of bacterial inoculum on the teeth from other oral environments and also the dilution and removal of a treatment from a site all of which are difficult to effect in tests carried out in vitro.

The mean pH is higher and the buffering capacity, especially with respect to phosphate, is lower in the rat saliva than in human saliva. Thus, the saliva could regulate the carious challenge differently in the two species. The rat flora is almost certainly modified through the use in the test of the cariogenic diet which contains up to 66% sucrose. This level of sugar is given to ensure the onset of caries over a short period of time. The caries occurs most frequently in the rat fissures which are proportionally larger than their human counterpart.

The treatment administered to the rat is greater than that given to the human subject on a unit body-weight basis; additionally, none of the treatment material is expectorated and so the treatment may have, and in the case of fluorides apparently does have, further benefit by a systemic route after ingestion and absorption through the alimentary tract.

The protocol for the rat test has been determined predominantly to evaluate the activity of fluoride, which reduces caries in human beings and also reduces caries in rats over a much shorter time scale. A judgement of the ability of the test on rats to anticipate the effectiveness of a treatment in human clinical studies rests on the results

obtained after appropriate refinement of the protocol in any study undertaken.

In general, however, it can be said that in our experience similar fluoride-containing products can be compared, and results show a close parallel between availability of fluoride and monofluorophosphate ions.

PROTOCOL

Animals (Management)

The method is basically that described by König (König, Marthaler, Muhlemann; Dent. Zahn - Mund - Kiefer heilk. 29, 99-128; 1958) but certain minor modifications have been incorporated to achieve closer reproducibility between experiments.

Osborne-Mendel rats are interbred in a closed colony on site, and the animals mated randomly. The same mating pairs are used to produce up to five litters.

The breeding animals are maintained on pelleted UAR No. 113 diet, but for 12 days prior to mating are given the richer diet of UAR No. D.03. (UAR Villemoisson-sur-Orge, cf appendix). After mating the males are returned to the original diet, UAR No.113. Weaning occurs when the progeny are 21-26 days of age (generally 22-24 days old to allow experimental work to start on Mondays), after which the dams are returned to diet UAR No.113.

The animals from one litter are randomly assigned one, or preferably two to each of the various treatments and the total number of animals per treatment is generally between sixteen and twenty. Each animal is weighed, numbered and caged separately in a stainless steel wire cage which restricts coprophagy.

The animals are then given diet MIT 2000 (Keyes & Jordan Archs.oral Biol. 9, 377, 1964) supplemented with 2% Gerval (R) (Lederle Lab. Cyanamid International) or Stephan diet 580 (Stephan, J.dent.Res. 30, 484, 1951) fed ad libitum and water (F<0.2ppm) ad libitum. (It has been our experience that these two diets produce the same caries challenge).

Treatments

Treatment is by dipping a small sable-haired brush (Harriet Hubbard Ayer) into undiluted toothpaste which is applied in a rotating fashion to the left mandible whilst the animal's mouth is held open for 15 seconds. The application is repeated on the right mandible with a fresh quantity of paste. Following the treatment, water and food are withheld for one hour. These procedures are applied twice daily on each work day (5 days) for the first two weeks and once daily for the final week. Total period of treatment is 21 days.

Preparation of Tissues

At the end of the treatment period the animals are killed with chloroform and weighed. The two separate mandibles and the complete upper jaw are then excised and the mandibles defleshed by means of a scalpel and dental chisel after immersion in neutralised formal saline for 48 hours. The molar teeth are mounted in thermoplastic wax and sectioned longitudinally to give five to six sections about 200 - 250 μ m thick using initially the NMRI type of machine as described in the Art and Science of Dental Caries Research p.300 (Ed. R.S. Harris, New York, Academic Press 1968) and finishing the cut with a scalpel.

Dried sections are stained with Schiff's reagent* for 15 seconds and rinsed with water. The stained sections are laid-out in succession from the lingual to buccal aspects on a microscope slide such that the corresponding fissures are aligned vertically down the slide. The left and right molar teeth are placed side by side.

The sections are fixed with a clear varnish.

Using a microscope at X40 magnification, ten fissures are scored on each slide, three from each of the first molars and the two big fissures on each of the second molars. The most severe type of lesion found in any section of the fissure determines the state of caries in that fissure.

The method of scoring lesion severity is as follows:-

- 0 No coloration in the enamel or dentine of the fissure.
- A Pink coloration in the enamel within the fissure.
- T Pink coloration at the enamel-dentine junction but without loss of dentinal material.
- B Coloration and loss of material at the enamel-dentine junction. This category is divided into three sub-groups:
 - B1 Coloration and slight loss of dentine (darkened area) without a hole through the entire section
 - B2 Physical separation of part of the dentine from the enamel and/or increased area of coloration.
 - B3 More extensive coloration and/or more extensive separation of the dentine and enamel.
- C Loss of enamel material from within the fissure (interpretation here requires particular care because of possible artifacts caused by mechanical vibration during the sectioning process).

*0.5g pararosaniline (acridine-free) in 15 ml. N hydrochloric acid + 0.5g potassium metabisulphite in 85 ml. water, decolorised with activated charcoal for 2 minutes after standing for 24 hours. The solution is diluted 1 : 4 with water before use.

At the beginning of an experiment the rats have no caries and at the end of the experiment they have a severity distribution through the various grades of caries. Thus, in computing the severity of caries, the lesions of a particular grade in all the fissures are cumulated with all those lesions of greater severity also, on the premise that lesions progress through the states 0, A, T, B1, B2, B3 to C.

The following example demonstrates the effect. The ten fissures of the mandible have been assessed for caries severity as shown on the first two lines in the Table. These have been totalled on the third line and the cumulated total for each grade is given on the last line. Thus, if one is considering the B1 lesions the value 7 would be used, whereas for B2 it would be only 3.

	0	A	T	B1	B2	B3	C
Left mandible	0	0	2	1	2	0	0
Right "	0	0	1	2	1	0	1
Total lesions	0	0	3	3	3	0	1
Cumulative total:	10	10	10	7	4	1	1

The mean value for each type of lesion over all the rats within one treatment is determined. In our experience, on evaluating toothpastes containing up to about 1000 ppm F, the useful cumulative indices have been the (T + B1 + B2 + B3 + C) lesions, the (B1 + B2 + B3 + C) lesions and the (B2 + B3 + C) lesions. Each of these indices is analysed statistically on a two-way ANOVA for litter and treatment.

Statistical Analysis

The maximum separation of lesions between treatments in a particular severity scale is ten, i.e. the number of fissures scored. For the comparison of treatments, it is desirable that they give the greatest difference on an appropriate scale and that the standard deviation between animals within the treatment is small.

Based on more than 25 tests, the difference between treatment means of toothpastes with or without 1000 ppm of fluoride is 1.6 lesions on the (T + B1 + B2 + B3 + C) scale, 3.2 lesions on the (B1 + B2 + B3 + C) scale and 3.4 lesions on the (B2 + B3 + C) scale. The separation of the treatment means required for a significant difference to occur depends upon the number of animals in each group but, in our experience, for 16-20 animals the separation must exceed 0.6 - 0.9, 0.8 - 1.1 and 0.8 - 1.1 lesions on the respective scales to achieve a 95% confidence level. Thus, it is desirable to use the (B1 + B2 + B3 + C) or the (B2 + B3 + C) scales for comparison of our treatments with our protocol. With other conditions, another scale might be more appropriate.

The response of the test to different fluoride concentrations on the various scales is shown in the appendices. From these graphs it can be seen that the increased level of fluoride or monofluorophosphate in the product does progressively reduce the level of caries during the experimental period.



U. A. R.

FICHE TECHNIQUE U. A. R. N° 1,13

Aliment Complet pour Rats et Souris Stérilisable

Bouchon, diam. 20 %, longueur 25 %
Bouchon, diam. 10 %, longueur 20 %
Farine.

Ration journalière du Rat : suivant l'âge et le poids de 18 à 25 g. — Eau à volonté.
Ration journalière de la Souris : suivant l'âge et le poids, de 8 à 12 g. — Eau à volonté.

FORMULE

Céréales	59 %
Issues	4,5 %
Tourteaux Expellers	10,5 %
Farine d'origine Animale	15 %
Activateurs de croissance	6 %
Composé Minéral Vitaminisé	5 %
(A. D. groupe B Méthionine).	

GARANTIES

Energie productive (en cal/kg)	1.950
Matières sèches	86
Protéines digestibles	20
Humidité	12
Matières Minérales	6,8
Matières Cellulosiques	3
Matières azotées totales	23
Matières Grasses	5,4
Extr. non azoté (sucre, amid.)	49,8
	100

TENEURS EN ACIDES AMINES

Arginine	12.700 mg/kg
Cystine	3.300 >
Lysine	12.500 >
Méthionine (suppl. synth.)	14.400 >
Tryptophane	2.400 >
Glycine	11.700 >

TENEURS des MINÉRAUX TOTAUX

Phosphore	8.500 mg/kg
Calcium	12.700 "
Potassium	6.500 "
Sodium	2.100 "
Magnésium	1.350 "
Manganèse	115 "
Far.	470 "
Cuivre	11 "
Zinc	43 "
Cobalt	0,09 "
Iode	0,10 "

VITAMINES (calculées aux cent kg)

	Apport Nat.	App. Synthet.	TOTAUX
Vitam. A	21.000 UI	3.980.000 UI	4.190.000 UI
Vitam. D ₃	1.000 UI	2.800.000 UI	2.801.000 UI
Vitam. B ₁	560 mg	120 mg	680 mg
Vitam. B ₂	300 "	900 "	1.200 "
Vitam. PP	6.000 "	3.600 "	9.600 "
Vitam. B ₆	1.300 "	100 "	1.400 "
Vitam. K ₁	25 "	450 "	475 "
Vitam. B ₁₂	3,5 "	3 "	6,5 "
Vitam. E	2.300 "	5.275 "	7.575 "
Choline	130.000 "	54.600 "	184.600 "
Vitam. B ₄	160 "	100 "	260 "
Acide Folique	50 "	0 "	50 "
Biotine	5 "	0 "	5 "

Les vitamines sont calculées largement pour tenir compte des traitements divers de stérilisation.

Cet aliment est destiné aux laboratoires qui possèdent une animalerie E.O.P.S. Sa forme (bouchon de 10 %) permet une stérilisation de l'aliment directement par les méthodes classiques.

Présentation : Sac papier 50 kg et 25 kg.

7, Rue du Maréchal-Galliéni, Villemoisson-sur-Orge (S.-&-O.) -- Tél. 921-13-69

FICHE TECHNIQUE U. A. R. N° D 03



U. A. R.

Aliment Complet Rats et Souris Stérilisable Elevage

Bouchons 15 %.
Farine.

Ration journalière du Rat : suivant l'âge et le poids, de 16 à 22 g. — Eau à volonté.
Ration journalière de la Souris : suivant l'âge et le poids, de 6 à 10 g. — Eau à volonté.

FORMULE

Céréales et Produits Hydrocarbonés	59	%
Tourteaux Expellers	7	%
Farine d'origine Animale	24	%
Activateurs de croissance	5	%
Composé Minéral Vitaminisé	4	%
(A. D. groupe B).		

ANALYSE MOYENNE

Energie (en cal kg)	3.800
Matières sèches	88
Protéines digestibles	22,4
Humidité	12
Matières Minérales	6,1
Matières Cellulosiques	2,7
Matières azotées totales	24
Matières Grasses	8
Extr. non azoté (sucre, amid.)	47,2
100	

TENEURS EN ACIDES AMINES (calculées)

Arginine	12.800	mg/kg
Cystine	3.500	"
Lysine	16.500	"
Méthionine	5.600	"
Tryptophane	2.300	"
Glycine	12.300	"

TENEURS en MINÉRAUX TOTAUX

	App. Nat. (moy.)	App. par C.M.	TOTAUX
			mg kg
Phosphore	6.700	3.500	10.200
Calcium	5.700	4.600	10.300
Potassium	5.900	0	5.900
Sodium	1.700	800	2.500
Magnésium	1.400	137	1.537
Manganèse	29	40	69
Fer	95	150	245
Cuivre	12	15	27
Zinc	23	45	68
Cobalt	0,07	1,5	1,6
Iode	apporté, sous forme assimilable par algues marines.		

VITAMINES (calculées aux cent kg)

	App. Nat. (moy.)	App. Synthét.	TOTAUX
Vitamine A	20.000 UI	2.500.000 UI	2.520.000 UI
" D ²	4.500 "	700.000 "	704.500 "
" B ¹	700 mg	250 mg	950 mg
" B ²	500 "	1.000 "	1.500 "
" B ³	1.500 "	2.400 "	3.900 "
" B ⁴	500 "	400 "	900 "
" B ¹²	2,1 "	2,5 "	4,6 "
" E	2.100 UI	10.000 UI	12.100 UI
" K ¹	25 mg	500 mg	525 mg
" PP	5.700 "	3.500 "	9.200 "
Acide Folique	75 "	25 "	100 "
Acide P. A. B.	0 "	50 "	min. 50 "
Biotine	15 "	10 "	25 "
Choline	156.000 "	80.000 "	236.000 "
Meso-Inositol	0 "	100 "	min. 100 "

Les teneurs en vitamines sont indiquées avant stérilisation.
Une surcharge par rapport aux besoins pallie les destructions subies pendant la stérilisation.

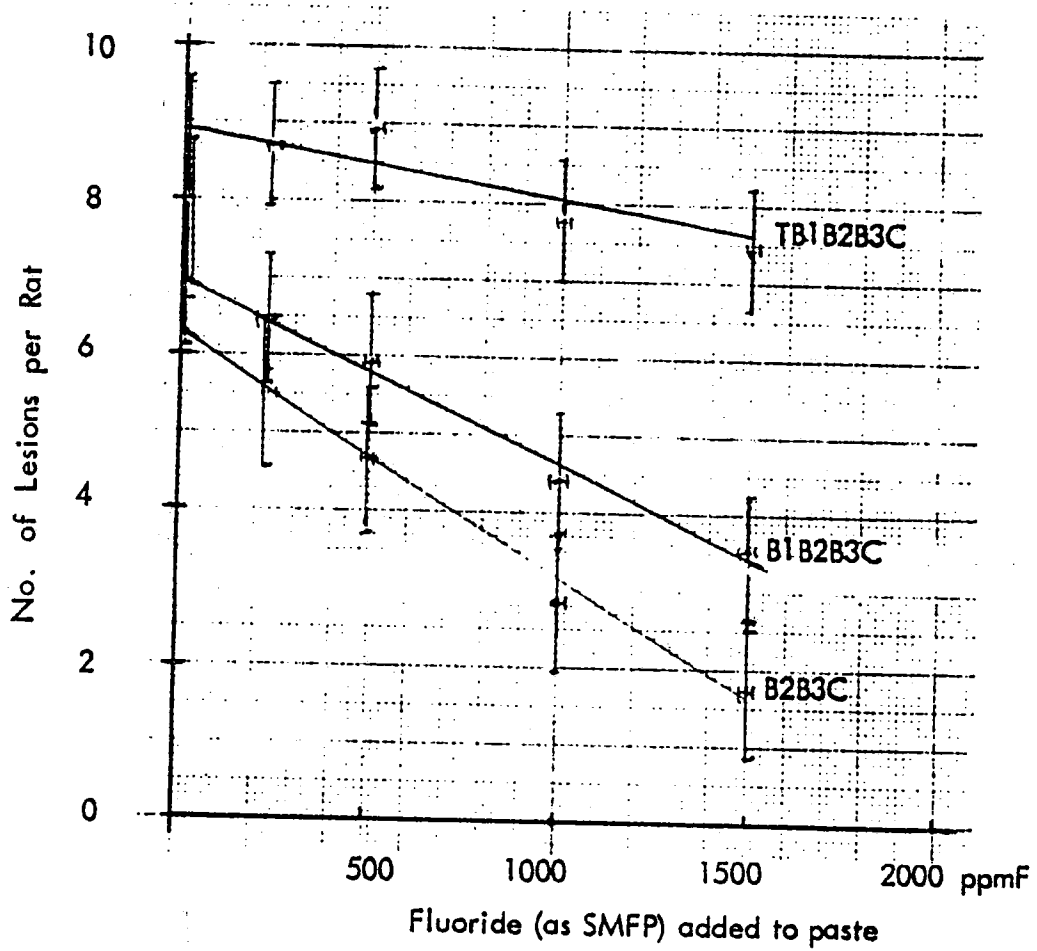
Aliment spécialement conditionné pour permettre une stérilisation directe en sac.

Autres présentations : B 03 - Aliment complet Rats et Souris Autoclavé Elevage.
C 03 - Aliment complet Rats et Souris Stérilisé Elevage.

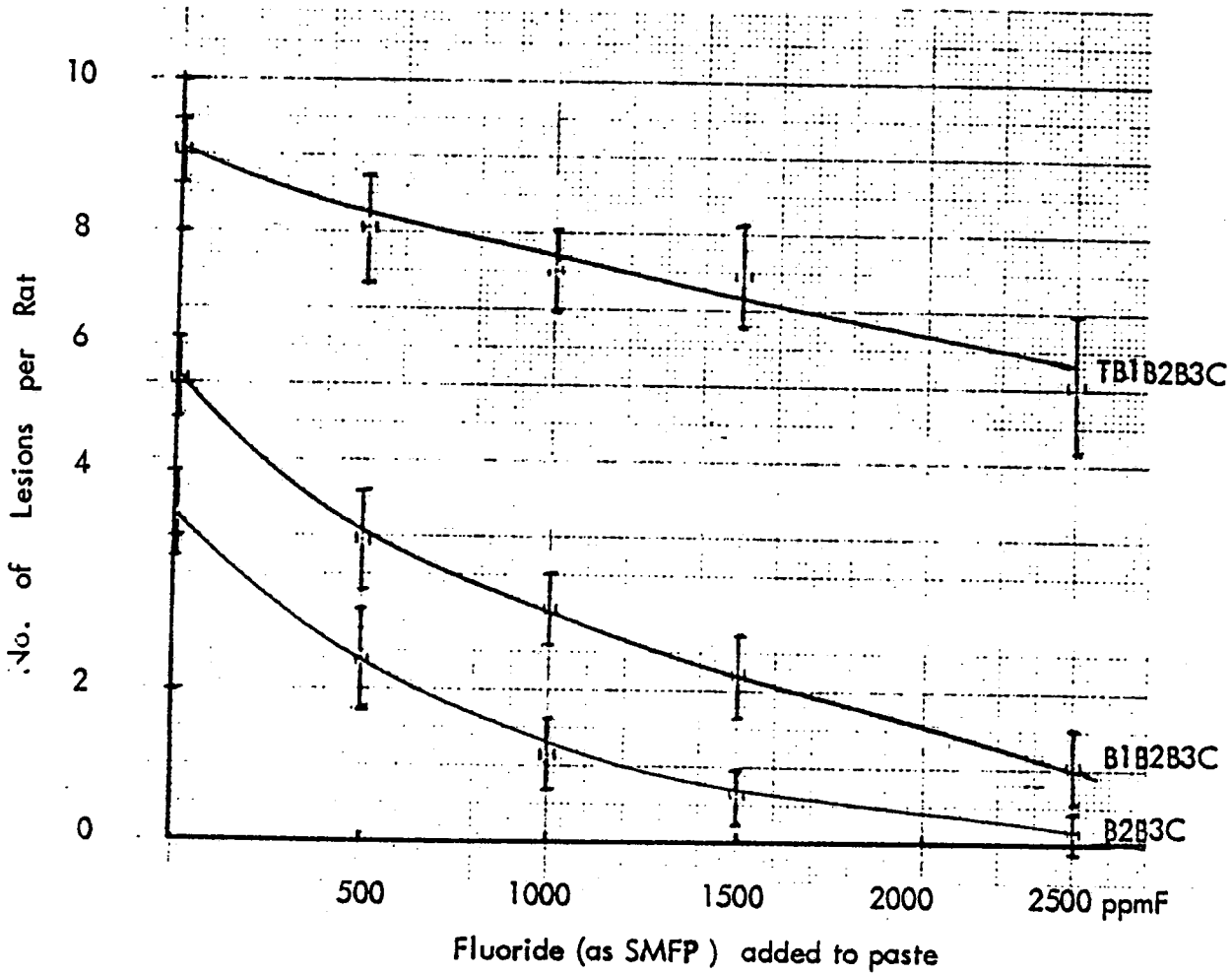
(imp. Larot, Epina)

7, Rue du Maréchal-Galliéni, 91 - Villemoisson-sur-Orge -- Tél. 921-13-69

Effect of Fluoride (SMFP) in a Silica Abrasive
Toothpaste on the Caries Incidence in Rats on the
T + B1 + B2 + B3 + C, B1 + B2 + B3 + C and B2 + B3 + C
Scales (showing the 95% Confidence Limits).



Effect of Fluoride (SMFP) in an Alumina Abrasive Toothpaste on the Caries Incidence in Rats on T + B1 + B2 + B3 + C, B1 + B2 + B3 + C and B2 + B3 + C Scales (showing 95% Confidence Limits).



Effect of Fluoride (SnF_2) in a Silica Abrasive Toothpaste
on the Caries Incidence in Rats on the T + B1 + B2 + B3 + C,
B1 + B2 + B3 + C and B2 + B3 + C Scales (showing 95% Confidence Limits.)

