EXPERIMENTS WITH VARIOUS FATS IN A CARIOGENIC DIET

IV. Experimental Dental Caries in Golden Hamsters.

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INTRODUCTION

It has been previously shown in animal experiments that the presence of fat in a cariogenic diet has the effect of decreasing the incidence of caries.

Rosebury and *Karshan* (1939) found that an increasing percentage of corn oil (from 0.5 -5 %) added to both corn and rice diets resulted in a decrease in caries incidence. They state that this effect seems to be exerted locally in the mouth rather than through nutritional or systemic channels. The presence of fat may possibly interfere with the impaction of food by its lubricating action, coating both food particles and tooth surfaces with an insoluble oily film. It will thus limit the penetration of oral and bacterial enzymes into the food particles and will also protect the tooth surfaces against the action of acids produced by fermentation.

Schweigert et al. (1946) have, in experiments with the cotton rat, shown that a diet containing 35 % fat offered almost total protection against caries. This investigation was, however, performed with only 2 -6 animals in each experimental group.

In their investigation in 1948 *Granados et al.* compared the cariogenic effect of a purified diet with and without dietary fat (7 %). They found a lower incidence and extent of carious lesions on the fat-containing diet but the difference was of a low statistical significance.

It has been suggested that the content of unsaturated fatty acids with their vitamin character might give this effect. This suggestion has been studied in an investigation by *Gustafson, Stelling* and *Brunius* (1953), who compared the effect of incorporating in a purified diet three different fats at a 7 % level with varying contents of unsaturated fatty acids.

The fats used were cocoa fat, which has a low content of these acids (iodine number 8), olive oil with a medium (iodine number 86) and poppy seed oil with a high content (iodine number 130). No difference in cariogenicity between the various diets could, however, be detected after an experimental period of 110 days.

EXPERIMENTAL

Composition of diets

If the cause of the caries-reducing effect of fats is of a physical nature there should be a difference between fats varying in physical properties. In the following experiment we have used arachis oil (melting point below body temperature), hydrogenated arachis oil (melting point 40—42°C.) and lard (melting point below body temperature). It was considered necessary to use a fairly high content of fat in the diets, although the chosen level of 25 % may still be found in natural diets. Our stock diet has, for example, a fat content of 21.6 %.

One group without fat was also included in the experiment. The diet of this group contained, instead of fat, wheat starch, which has been previously shown to have no caries-producing effect (*Gustafson, Stelling* and *Brunius*, 1952).

2 % soy-bean oil was added to all diets as a vehicle for vitamins A and D.

The composition of the diets is seen in Table 1.

Table 1

Experimental diets

Diet number	147	148	149	150
Casein	20 g	20 g	20 g	20 g
Saccharose, finely powdered	45	45	45	45
Wheat starch	25	—	—	—
Arachis oil	—	25	—	—
Hydrogenated arachis oil	—	—	25	—
Lard	—	—	—	25
Soy-bean oil "ADE"	2	2	2	2
Cellulose flour	3	3	3	3
Salt mixture "6"	4	4	4	4
Vitamin mixture "20" with cystine	0.8	0.8	0.8	0.8
Vitamin mixture "22"	0.2	0.2	0.2	02.
	100 g	100 g	100 g	100 g

Soy-bean oil "ADE":

dl-α-tocopherol ..... 1 g
Vitamin A ..... 1,600,000 I.U. of synthetic all-trans vitamin A acetate
Vitamin D_2 ..... 160,000 I.U.
Soy-bean oil to ..... 400 g

Vitamin mixture "20" with cystine:

Choline chloride	120 g
l-Cystine	30 »
Inositol	60 »
p-Aminobenzoic acid	30 »
	240 g

Vitamin mixture "22":

Thiamine mononitrate	1.50 g
Riboflavin	1.50 »
Nicotinamide	2.25 »
Pyridoxin hydrochloride	1.50 »
Calcium-d-pantothenate	1.50 »
Pteroylglutaminic acid	1.20 »
Biotin	0.01 »
Cyanocobalamin-sodium chloride 1:1000	3.00 »
Menadiol sodium phosphate	0.90 »
Ascorbic acid	6.00 »
Wheat starch	40.64 »
	60.00 g

Salt mixture "6":

NaCl	43.3 g	$Ca(H_2PO_4)_2.H_2O$	135.0 g
$Mg\ SO_4.H_2O$	76.5 »	Calcium lactate	325.0 »
$Na\ H_2PO_4.H_2O$	86.8 »	Ferric citrate	29.5 »
$K_2H\ PO_4$	238.5 »	KJ	0.125 »
$CuSO_4.5H_2O$	1.25 »	$MnSO_4.H_2O$	3.79 »

Distribution and Experimental Periods

Upon weaning, 145 animals from our stock colony were divided as evenly as possible with regard to litter and sex and put on the different experimental diets. Three animals died during the experiment.

As this experiment was considered to be of a preliminary character we only used one experimental period of 150 days instead of three (70, 110 and 150 days) as used in earlier experiments.

After this time the animals were sacrificed, decapitated and the heads placed in a 9 % solution of formaldehyde till the caries examination could be made.

Growth

There are no significant differences in growth between the animals on the different diets and the growth for all diets must be considered normal.

Caries Examination

The incidence of caries was recorded and scored according to the method used in our earlier experiments, which is described in detail in a paper by *Gustafson, Stelling* and *Brunius* (1952). Only the main points need be mentioned here.

1. The outlines of the carious lesions are drawn on a special scorecard with photographic reproductions of the occlusal, buccal and lingual surfaces of the hamster molars.

2. The carious area on each tooth is measured with a planimeter so calibrated that it is possible to read off directly the size of carious areas as a percentage of the areas that can be attacked. One carious unit thus indicates that 1 % of the total area is affected.

3. Only three pairs of molars, the upper 2nd and 3rd and the lower 3rd are used for registration as they have been shown to be representative of the caries activity of the whole molar set.

4. The figure for the caries incidence of an experimental group thus indicates in carious units the size of the average carious area for each of the six teeth recorded.

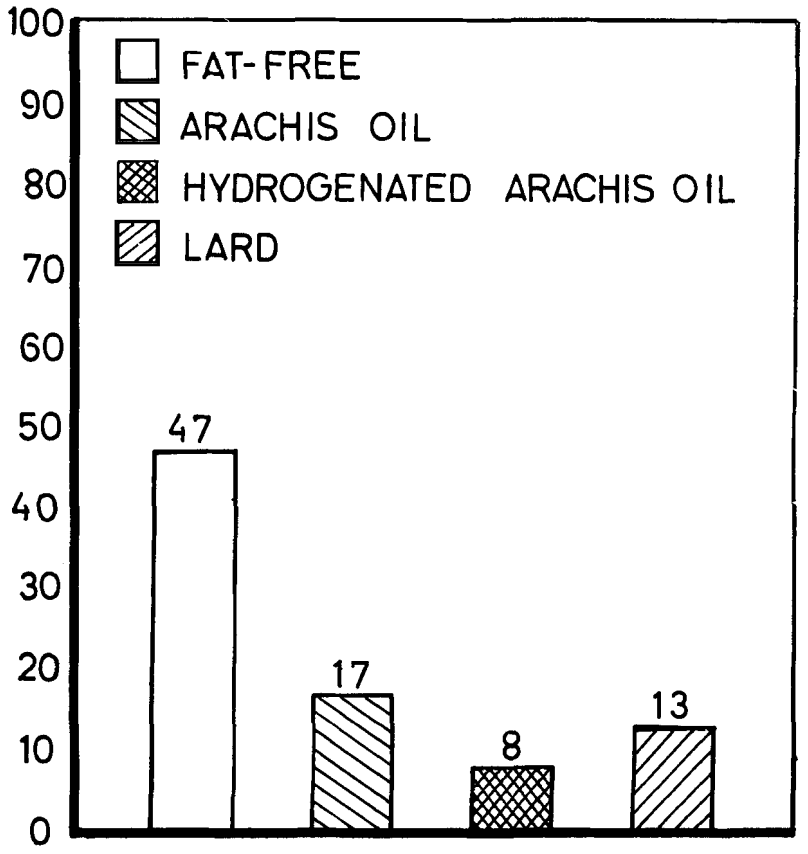

Figure 1. Number of carious units for males and females.

Results

The results of the caries-examination are given in Table 2. The scores are given for males and females separately as we have found in our material that there may be a difference in caries susceptibility between the two sexes, the males being more susceptible than the females. A paper on this question is under preparation.

The groups with males and the groups with females can be considered as two different parallel experiments independent of each other. As the animals are divided as evenly as possible with regard to litter and sex it should, however, be possible to combine them in groups and in Table 2 and in Figure 1 such combined values are also given.

Table 2

Number of carious units on the various diets

Males and females

Fat-free (147)	Arachis oil (148)	Hydrogenated arachis oil (149)	Lard (150)
$47 \cdot 1 \pm 3 \cdot 2$ (36)[a]	$16 \cdot 7 \pm 2 \cdot 4$ (36)	$8 \cdot 1 \pm 1 \cdot 3$ (35)	$12 \cdot 9 \pm 2 \cdot 1$ (35)

Number of animals = 142

Males

Fat-free (147)	Arachis oil (148)	Hydrogenated arachis oil (149)	Lard (150)
$50 \cdot 5 \pm 5 \cdot 0$ (18)	$24 \cdot 4 \pm 3 \cdot 7$ (18)	$9 \cdot 8 \pm 2 \cdot 1$ (18)	$15 \cdot 9 \pm 3 \cdot 4$ (17)

Number of males = 71

Females

Fat-free (147)	Arachis oil (148)	Hydrogenated arachis oil (149)	Lard (150)
$43 \cdot 8 \pm 4 \cdot 0$ (18)	$9 \cdot 0 \pm 2 \cdot 0$ (18)	$6 \cdot 8 \pm 3 \cdot 1$ (17)	$11 \cdot 4 \pm 2 \cdot 6$ (18)

Number of females = 71

(a) = mean, standard error of the mean, and number of animals.

The fat-free diet is associated with a very high caries incidence both in males and in females, whereas animals on the fat-containing diets have a considerably lower one. This supports earlier suggestions and experiments by other investigators. The difference found here is particularly marked. As the number of animals in our experiments is sufficient to allow definite conclusions, the caries-decreasing effect of fat in this type of diet may be taken as proven.

There seems to be a real difference in cariogenicity between the diets containing fats with melting points lower than body temperature on one side, and the diet containing hydrogenated oil with a melting point above the body temperature on the other.

The differences in the male group are great whereas the differences in the female group are not so pronounced. These results are interesting and further experiments on this line are now being carried out. As stated before this experiment was of a preliminary character with experimental periods of only 150 days' duration.

Unsaturated Fatty Acids

The present investigation also completes the study of the rôle of unsaturated fatty acids mentioned above. The arachis oil used in this experiment has a high content of these acids and lard a very low one. The cariogenic activity of both diets was the same. This confirms the conclusions of the earlier study and shows them to be valid even for 25 % of fat in the diet and an experimental time of 150 days.

Rate of Solubility of the Sucrose Constituent of the Diets

In order to find out if there is any real background to the suggestion that, on a fat diet, the film of fat around the food particles protects them to some extent from penetration by mouth fluids, we have made an evaluation of the amount of sucrose that is during a very short time dissolved by water from the diets used in this experiment. We found that, in 15 seconds, 86.7 % sucrose was dissolved from the fat-free diet, while a significantly lower amount was dissolved from the fat-containing diets.

The various fat-containing diets also showed differences in this respect. Thus for the diet with arachis oil the solubility was found to be 65.9 % and for the diet with arachis fat 61.5 %. This difference is not statistically significant. The value for the lard diet was, however, significantly lower being only 44.4 %. These differences are not parallel to the respective caries scores. However, these solubility experiments are only preliminary and have to be followed by more detailed investigations.

SUMMARY

The cariogenic effect of three purified diets containing different fats at a 25 % level has been compared with that of a fat-free diet. The fats used were arachis oil, hydrogenated arachis oil and lard. The experimental period was 150 days and 34 to 36 animals were used in each group.

The experiment has shown that:

1. The addition of 25 % of fat to a cariogenic diet causes a very marked decrease of its cariogenic effect.

2. This decrease is about the same for diets containing arachis oil and lard.

3. The decrease seems to be greater for a diet containing hydrogenated arachis oil with a melting point above body temperature.

4. This caries-reducing effect is not dependent on the content of unsaturated fatty acids of the various fats.

5. Solubility experiments performed in vitro for a very short time have shown that a greater quantity of the sucrose is dissolved from the fat-free diet than from the fat-containing diets.

6. The rate at which the sucrose is dissolved from a fat-containing diet seems also to depend on the type of fat that is included in the diet.

7. There seems to be no difference in caries-reducing effect between diets with the animal and vegetable fats used here.

LES EFFETS DE DIFFÉRENTES GRAISSES SUR UN RÉGIME GÉNÉRATEUR DE CARIES

Carie dentaire chez le hamster doré. IV

Les effets de trois régimes purifiés, générateurs de caries chez le Hamster doré et contenant diverses graisses dans une proportion de 25 %, ont été comparés à ceux produits par un régime dépourvu de corps gras.

Les graisses utilisées ont été l'huile d'arachide, l'huile d'arachide hydrogénée et le saindoux. Les animaux ont été soumis a ces régimes pendant une période de 150 jours: 34 à 36 Hamsters furent utilisés dans chaque groupe.

Les résultats suivants ont été obtenus:

1. 25 % de graisse ajoutée à un régime générateur de caries provoque une nette diminution dans le nombre de caries observées.

2. Cette diminution est la même pour des régimes contenant soit de l'huile d'arachide soit du saindoux.

3. Cette diminution est par contre plus marque pour un régime contenant de l'huile d'arachide hydrogénée, dont le point de fusion est supérieur à la température corporelle de l'animal.

4. Les acides gras non saturés, contenus dans ces diverses graisses, ne semblent pas intervenir dans l'activité génératrice de caries de ces régimes.

5. Des essais de solubilité dans l'eau, réalisés in vitro pendant 15 secondes, ont montré que, dans un régime dépourvu de graisse, une quantité importante de sucrose est dissoute. Par

contre dans un régime contenant des graisses, la proportion de sucrose dissoute pendant le même temps est nettement plus faible.

6. La quantité de sucrose dissoute dans un régime renfermant des graisses dépend de la nature des graisses utilisées.

7. Il ne semble pas y avoir de différence entre les graisses animales et végétales utilisées dans nos régimes, en ce qui concerne le nombre de caries ainsi produites chez le Hamster.

KARIESVERSUCHE AN HAMSTERN MIT VERSCHIEDENEN FETTEN IN EINER ZUCKERHALTIGEN KOST

Die karieserzeugende Wirkung von drei Kostarten, die verschiedene Fette enthalten (25 %), wurde mit der Wirkung einer fettfreien Kost verglichen. Der Zuckergehalt betrug 45 %. Als Fette gelangten 1) Erdnussöl (oleum arachidis), 2) gehärtetes Erdnussöl und 3) Schweineschmalz zur Verwendung. Die Versuchszeit erstreckte sich auf 150 Tage, und jede Versuchsgruppe bestand aus 34–36 Tiere.

Es wurden folgende Resultate erzielt:

1. Der Zusatz von 25 % Fett zu einer fettfreien, zuckerhaltigen Kost ergab eine deutliche Verminderung der kariogenen Wirkung im Vergleich zu derjenigen der fettfreien Kost.

2. Erdnussöl und Schweineschmalz hatten ungefähr die gleiche kariesreduzierende Wirkung.

3. Gehärtetes Erdnussöl mit einem Schmelzpunkt, der höher liegt als die Körpertemperatur des Tieres, verursachte eine stärkere Kariesreduktion als gewöhnliches Erdnussöl und Schmalz.

4. Die kariesreduzierende Wirkung war nicht vom Gehalt der Kost an ungesättigten Fettsäuren abhängig.

5. Kurzzeitige Löslichkeitsversuche in vitro ergaben, dass von der fettfreien Kost eine grössere Menge Zucker als von den fetthaltigen Kostarten abgegeben wurde.

6. Die Löslichkeitsgeschwindigkeit des Zuckers hängt anscheinend von der Art der Fette ab.

7. Zwischen der Wirkung animaler und vegetabiler Fette war kein Unterschied festzustellen.

Die Karies wurde an Hand einer eigenen Methode, nach der die kariöse Fläche berechnet wird, registriert.

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