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CARIES EXPERIENCE OF RATS FED VARIOUS STARCHES FOR STUDY OF EXPERIMENTAL CALCULUS FORMATION

by
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INTRODUCTION

Diets containing large quantities of starch have been found to enhance the formation of dental calculus when fed to weanling Sprague-Dawley rats (*Baer & White,* 1967). In some of the experimental groups rampant dental caries was observed. The purpose of the present investigation was fourfold:

1) to determine the ability of various starches to induce dental caries,

2) to determine whether pregelatinization of the starches influences their cariogenicity.

3) to determine whether amylopectin, the insoluble fraction of starch, or amylose, the soluble fraction, is the most effective in inducing dental caries,

4) to determine whether there is a correlation between calculus formation and dental caries experience.

I. EFFECT OF DIFFERENT STARCHES

MATERIALS AND METHODS

The experiments on calculus formation have been described in detail by *Baer & White* (1967). Eleven groups of weanling Sprague-Dawley rats of

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Table I
*Diets**)

Unmodified starch (66 %)	Pregelatinized starch (66 %)
Wheat (a)	Wheat (a)
Rice (b)	Potato (c)
Arrowroot (b)	Tapioca (d)
Tapioca (c)	Amioca (d)
Amioca (d)	
Potato (c)	
Cornstarch (e)	

*) In addition, all diets contained 32 % skim milk and 2 % liver powder

Sources: (a) Hercules Powder Co., New York: (b) Morningstar Paisley, Inc., Clifton, N. J.; (c) Stein Hall Co., New York; (d) National Starch & Chemical Co., New York; (e) Clinton Corn Processing Co., New York.

both sexes, containing 60 animals per group, were fed a powdered diet containing 32 per cent skim milk, 2 per cent liver powder and 66 per cent of one of the commercially available starches listed in Table I. Distilled drinking water was administered *ad libitum*.

Usually 24 animals in each group were sacrificed after a 30-day experimental period and an additional 24 per group after 60 days. The remainder were kept for study of calculus formation only and were not available for caries scoring. Upon termination of the experimental period the animals were decapitated, defleshed by the use of dermestid beetles and the molar teeth scored for calculus (*Baer, Stephan & White*, 1961) and for caries (*Keyes*, 1958). The total number of carious enamel areas was determined for each animal.

RESULTS

After a 30 day experimental period there were no statistically significant differences in the total number of carious enamel areas between animals on the regular corn starch and those on unmodified rice. Animals on unmodified amioca and unmodified potato starch had a tendency to have higher caries rates ($P < 0.05$). (Table II, Fig. 1.)

After 60 days (Table IIIa) there were no statistically significant differences between the animals fed the regular corn starch and the unmodified wheat, unmodified rice, unmodified tapioca and unmodified arrowroot. For the

Table II

Effect of various starches on calculus formation and dental caries. 30 day experimental period

Diet No	Diet Regimen (Starch)	Calculus scores N	$\bar{X}$	S.E.	Caries scores N	$\bar{X}$	S.E.	Vs Control t	P	Level of significance
S—1	Unmodified wheat	24	3.13	0.519	—	—	—			
Control	Cornstarch, regular	24	3.67	0.428	18	0.72	0.351			
S—2	Pregelatinized arrowroot	—	—	—	12	17.17	2.763			
S—3	Pregelatinized potato	24	1.63	0.300	12	34.67	5.855			
S—4	Unmodified rice	23	2.65	0.473	11	3.72	1.830	2.0211	0.10>P>0.05	—
S—5	Unmodified arrowroot	23	1.57	0.402	—	—	—			
S—6	Unmodified tapioca	23	2.70	0.442	—	—	—			
S—7	Pregelatinized amioca	22	0.77	0.130	10	25.00	3.952			
S—8	Pregelatinized tapioca	23	1.61	0.265	12	18.25	3.099			
S—9	Unmodified amioca	24	3.38	0.496	12	7.00	2.871	2.6564	0.05>P>0.01	*
S—10	Pregelatinized wheat	23	1.78	0.398	11	24.18	5.784			
S—11	Unmodified potato	15	0.47	0.369	9	3.67	1.607	2.4157	0.05>P>0.01	*

Table III a

Effect of various starches on calculus formation and dental caries. 60 day experimental period

Diet No	Diet Regimen (Starch)	Calculus scores N	$\bar{X}$	S.E.	Caries scores N	$\bar{X}$	S.E.	Vs Control t	P	Level of significance
S—1	Unmodified wheat	24	4.79	0.478	24	2.50	0.812	0.5343	0.6>P>0.5	—
Control	Cornstarch, regular	23	4.57	0.555	12	1.83	0.694			
S—2	Pregelatinized arrowroot	—	—	—	12	24.58	3.253			
S—3	Pregelatinized potato	23	2.48	0.338	23	33.52	3.491			
S—4	Unmodified rice	24	4.67	0.476	24	0.50	0.458	1.6378	0.20>P>0.10	—
S—5	Unmodified arrowroot	23	2.17	0.528	23	2.87	0.737	0.9119	0.40>P>0.30	—
S—6	Unmodified tapioca	21	3.71	0.594	21	4.14	0.874	1.8126	0.10>P>0.05	—
S—7	Pregelatinized amioca	22	1.77	0.436	22	18.05	3.708			
S—8	Pregelatinized tapioca	24	2.67	0.238	24	32.25	2.653			
S—9	Unmodified amioca	24	4.63	0.339	24	4.79	0.866	2.2339	0.05>P>0.01	*
S—10	Pregelatinized wheat	24	2.83	0.280	24	6.42	1.268			
S—11	Unmodified potato	19	0.37	0.114	18	16.67	5.334	2.2452	0.05>P>0.01	*

*) ?

Table III b

Effect of pregelatinization of various starches on their caries-inducing properties. Values from Table II and IIIa.

Substance	Day	Unmodified N	$\bar{X}$	S.E.	Pregelatinized N	$\bar{X}$	S.E.	Vs Control t	P	Level od significance**)
Wheat	60	24	2.50	0.812	24	6.42	1.268	2.6028	0.05>P<0.01	*
Arrowroot	60	23	2.87	0.737	12	24.58	3.253	8.5624	P<0.001	***
Potato starch	30	9	3.67	1.607	12	34.67	5.855	4.4578	P<0.001	***
	60	18	16.67	5.334	23	33.52	3.491	2.7364	0.01>P>0.001	**
Tapioca	60	21	4.14	0.874	24	32.25	2.653	9.5096	P<0.001	***
Amioca	30	12	7.00	2.871	10	25.00	3.952	3.7620	0.01>P>0.001	**
	60	24	4.79	0.866	22	18.05	3.708	3.6213	P<0.001	***

**) If calculated on the number of lesions all the differences were significant on the 0.1 per cent level (***).

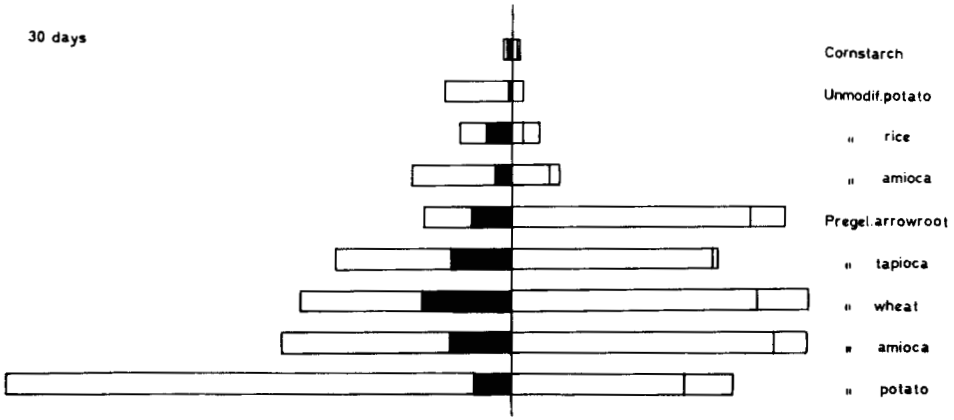
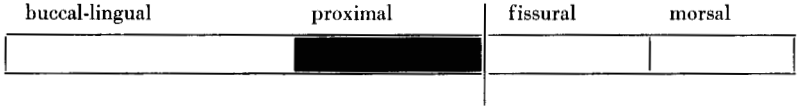


Fig. 1. Bar graph showing variations in the pattern of smooth-surface (buccal, lingual, proximal) and sulcal (fissural, morsal) lesions in rats fed various starches. for 30 days.



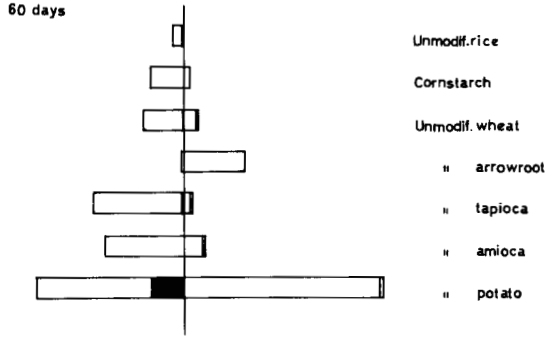


Fig. 2 a. Bar graph showing variations in the pattern of smooth-surface and sulcal caries in rats fed various unmodified starches for 60 days.

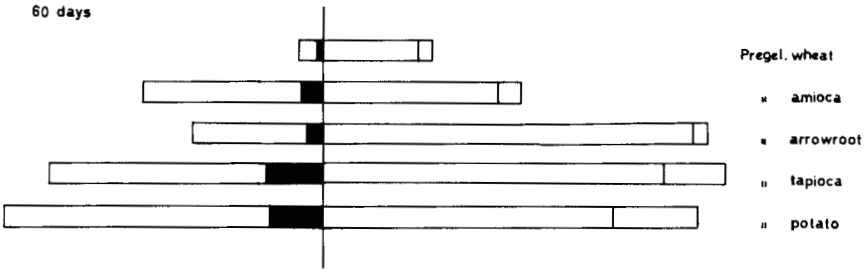


Fig. 2 b. Bar graph showing variations in the pattern of smooth-surface and sulcal caries in rats fed various pregelatinized starches for 60 days.

unmodified amioca and the unmodified potato starch diets there was a tendency towards a higher number of carious areas ($P<0.05$).

Animals on all the pregelatinized starch diets (arrowroot, potato, amioca, tapioca and wheat) had several times more carious enamel areas than those fed the regular corn starch diet and those fed the unmodified starches. The differences were significant or highly significant, (Table IIIb), except for wheat for which there was a strong tendency (Figs. 2a and b.)

II. EFFECTS OF AMYLOSE AND AMYLOPECTIN

MATERIALS AND METHODS (A.)

Four groups of weanling Sprague-Dawley female rats, containing 20—25 animals per group, were fed for an experimental period of 30 days, one of the four diets shown in Table IV. Other experimental conditions were the same as in part I.

RESULTS

Animals on the corn amylose and the corn amylopectin diets (Table V) had more carious enamel areas than those animals on the high amylose corn starch. For amylopectin the difference was significant on the 1 per cent level.

Animals on the corn amylopectin diet had more carious enamel areas than animals on the corn amylose diet, but the difference was not statistically significant ($P>0.10$). The calculus scores are given in Table VI. For additional information about the weight gain of the animals see *Baer & White* (1967).

Table IV
*Diets**)

Diet*) (55 %)	Percent amylose or amylopectin contained in each starch fraction	
	Amylose	Amylopectin
a. Regular cornstarch (1)	28	72
b. High amylose cornstarch (2)	70	30
c. Corn amylose (3)	100	—
d. Corn amylopectin (3)	—	100

*) In addition, each diet contained 32 % skim milk and 2 % liver powder.

Sources: (1) Clinton Corn Processing Co., New York; (2) National Starch & Chemical Co., New York; (3) A. E. Staley Mfg. Co., Decatur, Ill.

Table V

Effect of various starches and starch constituents on the caries experience of rats

Substance	Day	Unmodified caries-scores N	$\bar{X}$	S.E.	Amylose caries-scores N	$\bar{X}$	S.E.	Amylopectin caries-scores N	$\bar{X}$	S.E.
Cornstarch,	30		—	—	20	7.60	1.297	20	10.85	2.071
regular	60	20	3.10	0.767		—	—		—	—
	90	40	5.50	0.914		—	—		—	—
Cornstarch,	30	17	2.71	1.325		—	—		—	—
high amylose	60	16	3.38	1.221		—	—		—	—
Potato starch	30	19	5.78	1.420	20	12.45	1.991	19	30.00	4.447
	30	26	13.42	1.992		—	—		—	—
	60	28	16.43	2.954	19	34.00	4.438	17	47.29	5.535

	Day	t	P	Level of significance
Corn starch				
Regular — corn starch, high amylose	60	0.2018	P>0.1	—
Amylose — corn starch, high amylose*)	30	2.6234	0.05>P>0.01	*
Amylopectin — corn starch, high amylose*)	30	3.1794	0.01>P>0.001	**
Amylopectin — amylose	30	1.3295	P>0.1	—
Potato starch				
Amylose — unmodified potato starch	30	2.7013	0.05>P>0.01	*
		0.3383	P>0.1	—
	60	3.4331	0.01>P>;0.001	**
Amylopectin — unmodified potato starch	30	5.1830	P<0.001	***
		3.7255	P<0.001	***
	60	5.3788	P<0.001	***
Amylopectin — amylose	30	3.6591	P<0.001	***
	60	1.8885	0.10>P>0.05	—

*) If calculated on the number of lesions the difference was highly significant.

Table VI

Effect of amylose vs. amylopectin on calculus scores

Diet	N	Calculus Scores $\bar{X}$	S.E.
Corn starch, regular	40	4.48	.41
High amylose cornstarch	40	1.45	.30
Corn amylose	40	0.65	.18
Corn amylopectin	37	2.89	.37

The difference between groups on the regular corn starch diet and those on the high amylose corn starch diet was not statistically significant ($P>0.10$).

MATERIALS AND METHODS (B.)

Groups of weanling Sprague-Dawley female rats, containing approximately 20 animals per group, were fed for an experimental period of 30 and 60 days, unmodified potato starch, potato amylose and potato amylopectin diets, respectively. Other experimental conditions were the same as in previous experiment.

RESULTS

Animals on the potato amylopectin diet for 30 days had significantly more carious enamel areas than those on the potato amylose diet ($P<0.001$). (Table V). After 60 days there was still a difference, but this difference was not statistically significant ($0.10>P>0.05$).

Animals fed the potato amylopectin diets had significantly more carious areas than animals fed the unmodified potato starch diets ($P<0.001$).

There was no statistically significant difference in the number of carious enamel areas between animals on the potato amylose diet for 30 days and those on the unmodified potato starch diet, although there was a tendency for animals on the amylose diet to have more caries. After 60 days the difference was statistically significant ($0.01>P>0.001$).

III. CORRELATION BETWEEN CALCULUS FORMATION AND CARIES EXPERIENCE

MATERIAL AND METHODS

Since group means for both calculus formation and caries experience were available for some of the different dietary groups, a study was made of the

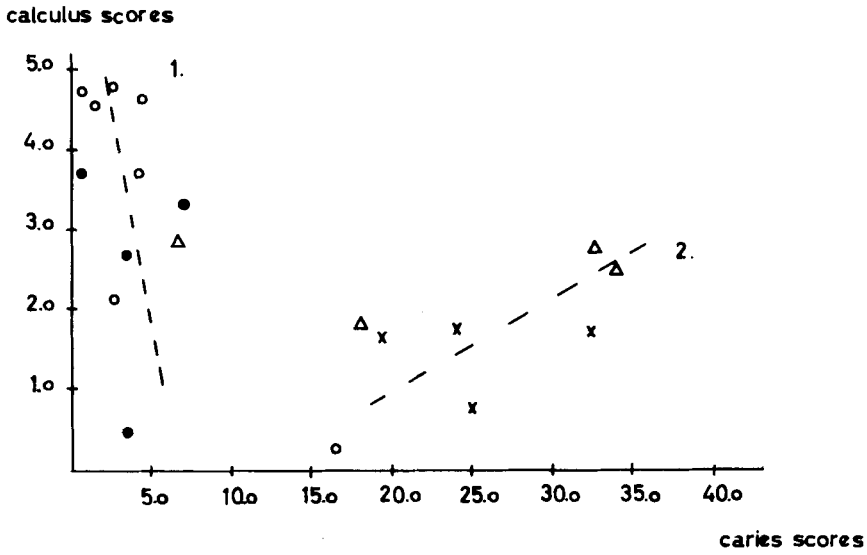


Fig. 3. Distribution of group means of calculus scores and caries scores.

1. Unmodified starches. 2. Pregelatinized starches.
O unmodified starches 30 days
● unmodified starches 60 days.
△ pregelatinized starches 30 days.
+ pregelatinized starches 60 days.

correlation between calculus formation and caries experience. At the time the scoring of dental caries was finished the scores for the individual values of calculus formation in the different animals were not available. Thus, it was not possible to carry through a detailed statistical study of the correlation between calculus formation and caries experience in the individual animal.

For those groups in which group means for calculus formation and caries experience were available, a correlation diagram was set up.

RESULTS

No clear correlation was found between calculus formation and caries experience (Fig. 3). In general, those animals fed the unmodified starches had very high calculus scores and very low caries scores. However, in experiments in which the animals were fed pregelatinized starches, there was a tendency for those groups with high caries scores also to have high calculus scores. Thus, those groups fed the pregelatinized starches usually had high caries scores and high calculus scores.

DISCUSSION

Since all the diets contained 32 per cent skim milk, which contains lactose, the lactose content of the diet may to some extent have contributed to the caries experience of the animals. Lactose, however, is considered to be only moderately cariogenic, a belief which is confirmed by the experiments with the unmodified starches.

Pregelatinization, however, caused a significant increase in cariogenicity of all the starches tested in all the experimental series. This finding is in accordance with the results of *Neff* (1967), who showed that cooked starch caused a considerable decrease in pH of dental plaques whereas raw starch did not. His results have been confirmed (*Frostell,* in press).

Amylopectin, the insoluble, branched constituent of starch, was generally more cariogenic than amylose, the soluble, linear constituent of starch. The cariogenicity of amylose was of the same order of magnitude as that of pregelatinized starch.

Thus, the results tend to show that the processing of starch increases its cariogenicity considerably and that this property is mainly ascribable to its amylopectin content and to a lesser extent to its amylose content, since regular starch contains much more amylopectin than amylose and since amylopectin was somewhat more cariogenic than amylose. Since in these experiments no polysaccharide production from sucrose was possible and still considerable plaque accumulation on smooth surfaces occurred, the question is raised whether amylopectin — which is not completely hydrolyzed by alfa-amylase and which chemical composition resembles that of the bacterial dextrans — may replace bacterial dextrans and favour plaque formation.

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SUMMARY

Groups of weanling Sprague-Dawley rats were fed a diet containing 66 per cent starch, amylose or amylopectin. Twelve different types of unmodified or pregelatinized starches were studied. The diets were administered for 30 or 60 days in most experiments and caries and calculus were scored.

There was no statistically significant difference in the number of carious enamel areas between animals fed the regular corn starch control diet and those fed unmodified wheat, unmodified rice, unmodified arrowroot and unmodified tapioca, whereas animals fed the unmodified potato starch and

unmodified amioca had a tendency to have more carious areas. Animals on the pregelatinized starches (arrowroot, potato, amioca, tapioca and wheat) had several times more carious areas than those on the regular corn starch diet.

Animals fed corn amylose or corn amylopectin had significantly more carious areas than those on unmodified corn starch diets. Animals on amylopectin diets had more caries than animals on amylose. For potato amylose and amylopectin the difference was highly significant after 30 days on the diets, but not after 60 days.

No clear correlation was found between calculus formation and caries experience. In general, animals fed the unmodified starches had very high calculus scores and very low caries scores. However, in experiments in which the animals were fed pregelatinized starches there was a tendency for groups with high caries scores to have high calculus scores also.

RÉSUMÉ

CARIE DENTAIRE CHEZ DES RATS NOURRIS AVEC DIFFÉRENTES FÉCULES AU COURS D'UNE ÉTUDE SUR LA FORMATION EXPÉRIMENTALE DU TARTRE

Un régime contenant 66 % de fécule, d'amylose ou d'amylopectine a été administré à des groupes de rats Sprague-Dawley venant d'être sevrés. Douze types différents de fécule non modifiée ou prégélatinisée ont été étudiés. Les régimes ont été administrés pendant 30 ou 60 jours; la carie et le tartre ont été enregistrés.

Il n'y avait pas de différence statistiquement significative dans le taux de la carie entre les animaux ayant reçu le régime témoin à la fécule de maïs ordinaire et ceux qui étaient nourris à l'amidon de blé non modifié, la fécule de riz non modifiée, la fécule d'arrowroot non modifiée et la fécule de tapioca non modifiée, tandis que les animaux nourris à la fécule de pomme de terre non modifiée avaient un nombre significativement plus élevé de zones carieuses. Les animaux recevant les fécules prégélatinisées (arrowroot, pomme de terre, amioca, tapioca et blé) avaient plusieurs fois plus de zones carieuses que ceux qui recevaient le régime à la fécule de maïs ordinaire.

Les animaux nourris à l'amylose de maïs ou à l'amylopectine de maïs avaient un nombre significativement plus élevé de zones carieuses que ceux qui recevaient le régime à la fécule de maïs ordinaire. Les animaux recevant les régimes à l'amylopectine avaient plus de caries que les animaux recevant l'amylose. En ce qui concernait l'amylose et l'amylopectine de pomme de

terre, la différence était hautement significative après 30 jours au régime, mais pas après 60 jours.

ZUSAMMENFASSUNG

KARIESBEFALL VON RATTEN DENEN VERSCHIEDEN STÄRKESORTEN GEGEBEN WURDEN UM EXPERIMENTELLE ZAHNSTEINBILDUNG ZU STUDIEREN

Gruppen von Sprague-Dawley-Ratten, die eben abgewohnt waren, wurde eine Diät gegeben, die 66 Prozent Stärke, Amylose oder Amylopektin enthielt. Zwölf verschiedene Sorten von nichtmodifizierten oder pregelatinizierten Stärken wurden untersucht. Die Diäten wurden den Tieren während 30 oder 60 Tagen gegeben und Karies und Zahnsteinbildung wurden registriert.

Die Verschiedenheit im Kariesbefall zwischen Tieren, denen die nichtmodifizierte Kontrollstärke (Maisstärke) gegeben wurde und derjenigen die nichtmodifizierte Weissestärke, nichtmodifizierte Reisstärke, nichtmodifizierte Arrowrootstärke und nichtmodifizierte Tapiokastärke gegeben wurde war nicht statistisch signifikant, aber Tiere die nichtmodifizierte Kartoffelstärke und nichtmodifizierte Amiokastärke bekommen hatten einen Tendenz nach mehr Karies als die Kontrolltiere. Tiere, denen pregelatinizierten Stärkesorten gegeben wurde, hatten vielmals mehr kariierte Zahnflächen als Tiere der Kontrastdiätgruppe.

Tiere, denen Maisamylose oder Maisamylopektin gegeben wurde, hatten signifikant mehr Karies als Tiere der Kontrastdiätgruppe (High amylose corn starch). Tiere der Amylopektindiätgruppen hatten mehr Karies als Tiere der Amylosediätgruppen. Zwischen Kartoffelamylose und Kartoffelamylopektin war der Unterschied stark signifikant nach dreissig Tagen aber nicht nach sechzig.

Man konnte keine klare Korrelation zwischen Zahnsteinbildung und Kariesbefall finden. Tiere, denen nichtmodifizierte Stärkesorten gegeben wurde, wiesen starke Zahnsteinbildung und niedrigen Kariesbefall auf. Doch wenn Tieren pregelatinizierte Stärkesorten gegeben wurde, fand man bei Gruppen mit starkem Kariesbefall auch eine Tendenz starker Zahnsteinbildung.

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