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EXPERIMENTAL DENTAL CARIES[1]

III. A System of Recording Corn Meal Caries in Rats

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We have used corn meal[2] caries in rats for the study of three phases of nutrition in its relation to dental caries, namely, "1) nutrition during the period of formation of the teeth, 2) foods which initiate caries, and 3) factors which affect the rate of progress of decay" (2).

Our first use of corn meal caries in rats was in an investigation of rate of decay as affected by foods. Our premise was that regardless of the origin of the lesions, if the corn meal ration was replaced by diets that do not initiate decay, any increase in the size of the cavities would be by a true carious process. In the course of such studies we observed a significantly lowered incidence of decay ("macroscopic" or "fracture" caries as described by others) in rats whose mothers had been on a ration with a high content of fat; we also found lesions (the opaque type described below) that resemble beginning human caries and could scarcely have originated in gross fracture of the teeth. These observations suggested that corn meal caries could be used for the study of dietary factors operative in the formation of teeth resistant to decay and that the findings would be applicable to man.

The results of our studies using corn meal caries in rats are not in conflict with well-authenticated facts of nutrition as related to human caries. For example, we have found that fermentable carbohydrates promote decay in rat molars. We have observed a lower incidence of caries in rats whose mothers had an adequate supply of fluorine during pregnancy and lactation. These examples and other observations

[1] Presented in part at the Fifteenth General Meeting of the International Association for Dental Research, Baltimore, Maryland, March 13, 1937 (*J. D. Res.*, **16**: 302, 1937).

[2] We have simplified the ration of Hoppert, Webber and Canniff (*J. D. Res.*, **12**: 161, 1932) using yellow corn meal 66, whole milk powder 30, alfalfa meal 3, sodium chloride 1.

lead us to believe that corn meal caries in rat molars can provide much information for the prevention of human dental caries by formation of caries-resistant teeth and for the reduction of rate of decay in existing caries. Furthermore, rat molars may be satisfactory for investigation of the pertinent factors in initiation of decay in susceptible teeth.

Based on our premise of independence of origin of cavities and their subsequent development after removal of caries-initiating factors, we developed a system of study of rat caries using cavities visible under 15 diameters magnification in teeth which were not sectioned. Our first attempts to record incidence and intensity of caries dealt with individual teeth. We soon found, however, that no comparisons could be made between teeth comparable numerically but decayed in different areas. We therefore worked out a procedure of recording incidence in areas empirically observed to be subject to corn meal caries. Later, when we had devised an efficient method of sectioning teeth (3), we were able to make an extensive study of rat molars following the system of scoring devised by Rosebury, Karshan, and Foley (8).

SCORING METHODS

Scoring of Occlusal Caries: We prefer to designate as "occlusal" cavities the lesions visible in unsectioned rat teeth after a ration containing corn meal. "Fissure" cavities are those lesions seen in ground sections, such as described by Rosebury, Karshan, and Foley (7, 8).

Occlusal cavities, as produced by corn meal in our experience, appear in 17 pairs of areas in the 12 rat molars. Our system of recording occlusal lesions (and also the fissure cavities) provides for 1) description, 2) location, 3) number, and 4) size. Description of the cavities is related to location and size, so that a record of a cavity of given size in a certain area indicates a definite type of cavity, as presented below.

1. In *CENTER* lesions the initial damage to the enamel occurs on the line of the anterior-posterior axis. The areas are in the occlusal edge of distal walls of sulci in the first and second lower molars.

2. *FUSED CUSP* cavities occur lingual to the anterior-posterior axis of the upper first and second molars at the occlusal edge of the mesial walls of the sulci at a line of cusp fusion. In the lower first and second molars they appear in shallow longitudinal buccal fissures in the occlusal surface of the major distal cusps.

3. *OPAQUE* lesions appear initially as white areas which have lost the normal translucency of the enamel. In later stages surface continuity is lost. The sites are at the buccal end of the floor of the mesial sulcus of the lower first molar and similarly in the main sulcus

of the third lower molar; also on the gingival line of the buccal-distal angle of the first lower molar and similarly on the buccal-mesial angle of the second lower molar.

4. *LATERAL CUSP* lesions appear as strippings of the enamel or gross fractures of the mesial-buccal minor cusps of the second and third lower molars. Also lesions appear in the sulcus lying between the minor mesial-lingual cusp of the second upper molar.

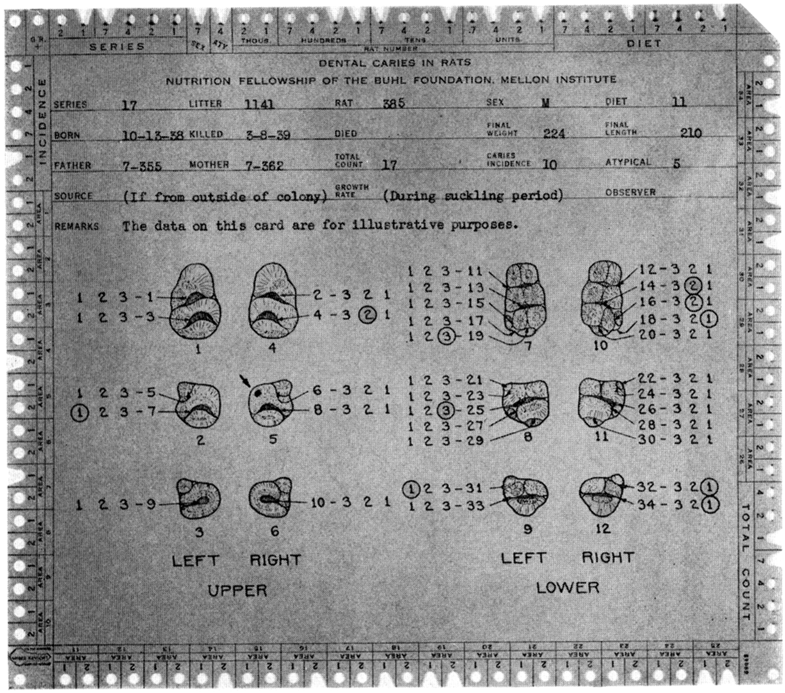
5. Cavitation of the third upper molars occurs so infrequently that description of this lesion is omitted. It is possible that some conditions which we have not yet encountered may lead to more frequent caries in these molars, so we have provided for recording decay if it does occur.

Fig. 1 shows on a punched card[3] the 12 molars of the rat. The upper molars are normally examined inverted and for convenience these sketches are transposed. The areas subject to decay, numbered and indicated by arrows in this illustration, are specifically located as described in the summary below. Areas up to and including 10 are in the maxillary molars; the remainder are in the teeth of the mandible.

Areas	*Type*	*Molar*	*Location in Tooth*
1, 2	Fused cusp	1st	Anterior wall, mesial sulcus
3, 4	Fused cusp	1st	Anterior wall, distal sulcus
5, 6	Lateral cusp	2nd	Posterior buccal wall, mesial-lingual minor cusp
7, 8	Fused cusp	2nd	Anterior wall, distal sulcus
9, 10	Uncertain	3rd	Not definite, probably main sulcus
11, 12	Opaque	1st	Floor of buccal end of mesial sulcus
13, 14	Center	1st	Occlusal edge, posterior wall, main sulcus
15, 16	Fused cusp	1st	Buccal, occlusal and posterior to main sulcus
17, 18	Opaque	1st	Buccal, proximal angle at gingival line
19, 20	Center	1st	Mesial wall, distal cusp
21, 22	Opaque	2nd	Mesial, buccal, proximal angle at gingival line
23, 24	Lateral cusp	2nd	Mesial, buccal minor cusp
25, 26	Center	2nd	Occlusal edge, posterior wall, main sulcus
27, 28	Fused cusp	2nd	Buccal, occlusal, posterior to main sulcus
29, 30	Center	2nd	Mesial wall, distal cusp
31, 32	Side cusp	3rd	Mesial, buccal minor cusp
33, 34	Opaque	3rd	Floor of buccal end of main sulcus

The sketches in *fig. 1* are provided with scores of 1, 2, and 3. The score appropriate to the cavity is encircled, a score of 1 being the first damage to the enamel, 2 indicates involvement of dentin, and 3 extensive cavitation, frequently being complete destruction of the area. If 2 or more adjacent areas have cavities which are merged, each area is given a score of its degree of cavitation. We have found it necessary to remove debris from cavities by means of dissecting needles

[3] The Keysort card of the McBee Company, Athens, Ohio.


DENTAL CARIES IN RATS
NUTRITION FELLOWSHIP OF THE BUHL FOUNDATION, MELLON INSTITUTE

SERIES 17 LITTER 1141 RAT 385 SEX M DIET 11

BORN 10-13-38 KILLED 3-8-39 DIED FINAL WEIGHT 224 FINAL LENGTH 210

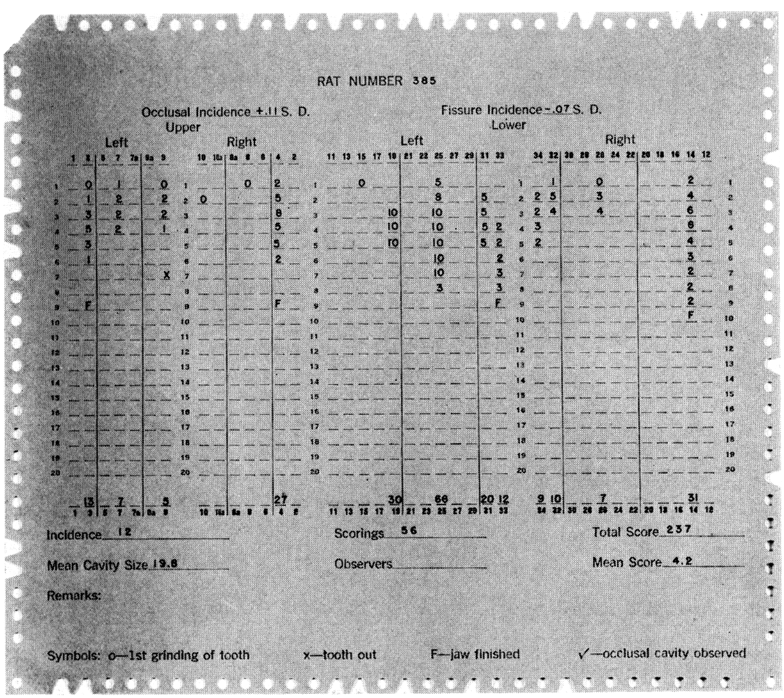
FATHER 7-355 MOTHER 7-362 TOTAL COUNT 17 CARIES INCIDENCE 10 ATYPICAL 5

SOURCE (If from outside of colony) GROWTH RATE (During suckling period) OBSERVER

REMARKS The data on this card are for illustrative purposes.

LEFT RIGHT UPPER
LEFT RIGHT LOWER

Fig. 1. Form for recording occlusal cavities


RAT NUMBER 385

Occlusal Incidence +.11 S. D. Fissure Incidence -.07 S. D.

Upper Lower

Incidence 12 Scorings 56 Total Score 237

Mean Cavity Size 19.8 Observers Mean Score 4.2

Remarks:

Symbols: o—1st grinding of tooth x—tooth out F—jaw finished ✓—occlusal cavity observed

Fig. 2. Form for recording fissure cavities

(made from fine explorers) as we score, in order to make proper evaluation of the size of the cavities.

Scoring of Fissure Caries: Fig. 2 gives our record form for fissure caries. We have used the reverse side of the card, shown in *fig. 1*, thus keeping the records together and utilizing the punched card feature for sorting the data. We have given numbers to areas which may be susceptible to decay. These numbers are the same as for adjacent occlusal areas with the addition of 7a–10a, inclusive. Certain of these designated areas later proved to be identical with those recorded for occlusal areas and are therefore not used in fissure scoring.

The description of the cavities is that of Rosebury, Karshan, and Foley (8). In general, these cavities appear in their early stages as crescent-shaped areas along the dentino-enamel junction of *each* sulcus, except in areas 7a and 8a, as will be related. Cavities in these areas were not originally included in our occlusal scoring and hence were transferred to the fissure records when they appeared.

The following list contains the locations of the fissure cavities.

Areas		*Molar*	*Location in Tooth*
1, 2	Upper	1st	Mesial sulcus
3, 4		1st	Distal sulcus
5, 6		2nd	Sulcus distal to minor lateral cusp
7, 8		2nd	Main sulcus
7a, 8a		2nd	Distal cusp summit
9a, 10a		3rd	Sulcus distal to minor lateral cusp
9, 10		3rd	Main sulcus
11, 12	Lower	1st	Mesial sulcus
13, 14		1st	Distal sulcus
15, 16		1st	Identical with occlusal lesion
17, 18		1st	Identical with occlusal lesion
19, 20		1st	Mesial to distal minor cusp
21, 22		2nd	Identical with occlusal lesion
23, 24		2nd	Distal to minor cusp, mesial-buccal angle
25, 26		2nd	Main sulcus
27, 28		2nd	Identical with occlusal lesion
29, 30		2nd	Mesial to distal minor cusp
31, 32		3rd	Distal to minor cusp, mesial-buccal angle
33, 34		3rd	Main sulcus

The teeth are prepared, scored for occlusal caries, mounted, and ground in successive planes (3). As various features develop in successive sections, they are recorded for the respective areas in the space opposite the number for the grind, the vertical column of numbers 1 to 20, inclusive. We record the beginning of grinding of any tooth with *0;* and if the tooth is ground away before completion of others, and the thin shell of remaining enamel drops out, we show its disappearance with *x*. Other symbols are presented in *fig. 2*.

The score of fissure caries is in the system of Rosebury, Karshan, and Foley (8). The numerical score for each carious area is recorded for each section and when the record is complete the scores are totaled, the *number* of cavities being counted as the incidence. Though we have provision on our record card for reduction of these values to other forms, so far we have used only the *incidence* in studies of prevention of decay and initiation of caries and the *total score* for individual areas for size comparison with cavities in litter mates in the study of arrest of caries.

STATISTICAL INTERPRETATION OF DATA

Our use of a punched card for recording dental caries in rats, especially the occlusal scorings, greatly facilitates study of the data. The examination of frequency of decay in any area, the association of decay in one area with lesions in any other area, or the elimination of any type of decay from the data are readily done. Likewise easy to perform are the routine analyses described below.

In order that our data may be comparable among all experimental groups, we have 1) begun feeding of the corn meal ration at weaning of the young rats at 21 days, 2) continued the ration for eight weeks, and, 3) if progress of caries is to be studied, used the non-caries producing diet for an additional 8 weeks. The standardization of time eliminates one important variable from a situation already too complex. If the time element is being studied, we have of course departed from these schedules.

Incidence of Caries: For the study of prevention of caries by dietary means during the period of formation of the teeth or of factors which initiate decay, we use as our criterion the mean number of cavities per animal, designated in *fig. 1* as "caries incidence." The mean incidence in an experimental group of rats is compared with that of a control group, using the standard error of the difference of the means as the unit of measurement.

We have found by the χ^2 test of Pearson (4) for a group of 63 animals of uniform dietary history that the distribution of the individual incidences of occlusal caries has a probability of 0.22 for a fit to the normal error function; for the fissure caries for a different group of 180 rats the probability was 0.90. Because it is known that the distribution of *means* tends more toward the normal than single observations, and, it is with the *means* that we are concerned, there is adequate justification for use of the integral tables of the normal error function in evaluating the data of both occlusal and fissure caries in rats.

By formula for difference of incidence of caries we evaluate t in

$$t = \frac{M_a - M_b}{\sqrt{\sigma_{M_a}^2 + \sigma_{M_b}^2}}$$

in which M_a and M_b are the respective mean incidences and σ_{M_a} and σ_{M_b} their standard errors derived from the respective standard deviations, σ_a and σ_b, by division by n_a and n_b, in which n_a and n_b are the number of rats in series a and b, respectively. In general, if t exceeds 2, the difference of the means is regarded as significant. The odds against the chance occurrence of a given difference, as measured in terms of its standard error, can be found from tables of the normal distribution curve integral for the value of t.

In a series of 443 rats we found between the *scores* of occlusal and fissure caries a correlation coefficient of +0.64. The positive correlation suggests a common etiology of the two types of lesions. Regression equations were also derived but, since they suggest causal relationship between the lesions, they are omitted. But in each case the equations indicate that as the teeth are improved the occlusal caries disappears first. This is in agreement with some cases several years ago in which we discerned no occlusal caries but Rosebury (6) found fissure caries by his technique.

We have observed that the standard deviation of a single observation of incidence of decay in rats on the corn meal ration is about 4.7. If no preponderance of zero incidence occurs, such as in a series on good preventive pre-eruptive diets, the standard deviation is statistically constant and independent of the number of rats. The standard error of the mean, upon which our test of significance is based, is inversely proportional to the number of rats. Small groups of rats can be used in studies of incidence of decay, but we have preferred to use enough animals so that a difference of 2 between the means would be significant, i.e., would yield a value of $t \geqq 2$. This condition is provided for a standard deviation of 4.7, if about 67 rats are used in each series.

We prefer to interpret separately our findings on initiation of decay in terms of occlusal and fissure caries parameters. We have found that tests of significance using the combined scores are less sensitive, probably because there is association between some fissure and occlusal cavities of the same area number.

Size of Cavities: For the study of alteration of rate of decay, either arrest or promotion of caries, we use rats weaned by mothers on a stock diet. The young are placed at 3 weeks age on the standard caries-producing diet for 8 weeks. Pairs of the same sex from the same litters are then put on 2 diets differing generally in a single constituent. After a further period of 8 weeks, all animals are killed and comparisons made of the size of cavities in identical areas.

The separation of the rats into 2 groups is by random selection to keep the mean weights statistically equal. It is therefore justifiable to assume that the 2 resulting groups have statistically equal numbers and sizes of cavities at the beginning of the experimental period. If the 2 rations do not differ in promoting the carious process, and neither initiates new cavities, statistical equivalence of number and size of cavities will continue throughout the experimental period. But, if the rations which do not initiate decay, differ in accelerating the carious process, the size of cavities of one group will increase and, at the end of the experiment, a greater number of cavities than that expected by chance will exceed the size of similarly located cavities in the litter mate. We test this deviation from that expected by chance by reference to the binomial distribution.

The formula for t used for comparison of rate of decay is

$$t = \frac{m - b}{\sqrt{npq}}$$

in which b is the number of pairs of cavities in which the one on ration B is greater, m the mean of a and b, n the number of pairs of cavities,

p the probability that a will be greater than b, and q the probability that b will exceed a.

If we are without knowledge of the power of either of 2 rations A or B to promote decay, then the *a priori* probability, p, is 0.5 that a cavity in a molar of a rat on ration A is greater than a similarly located cavity in a rat on ration B. It follows that if a cavities are greater on ration A than similarly located cavities from ration B and b cavities are greater on ration B, we would expect a and b to be nearly equal, their most probable values being their mean, $\frac{a + b}{2}$, which is half the number of comparisons, designated by n.

The standard deviation of observations in the binomial distribution $\sqrt{npq}$, reduces to $\frac{\sqrt{a + b}}{2}$, when $n = a + b$ and $p = q = 0.5$. The equation for t then reduces to $t = \frac{a - b}{\sqrt{a + b}}$. If t, as calculated by this formula, exceeds 2 the odds are that some other factor than chance has caused the deviation. Odds against chance can be found for specific values of t from tables of the normal curve integral.

The data for a study of rate of decay can be considerably increased if lesions in teeth of one side of a rat can be compared for size with lesions in the opposite jaw of the litter mate. For such cases, which we term *contralateral* comparison, the *a priori* probability 0.5 as used for *homolateral* studies cannot be assigned, inasmuch as asymmetric susceptibility to decay may occur. But *a posteriori* probability can be determined by study of incidence and cavity size in the left and right teeth of a sufficient number of rats.

We have studied 1344 rats for the number and size of occlusal cavities in their left and right jaws. We found a greater tendency for initiation of decay in the molars in the right jaw for both males and females, but not a sufficient deviation from that within the normal chance expectation to be significant. We noted a total of 5243 occlusal cavities in the left molars and 5313 in the right.

We observed in all cases of pairs of cavities (including the "pair" in which no decay occurred in one area) 2604 cavities larger on the left and 2808 cavities larger on the right. Here is a significant trend, the odds against its chance occurrence being 177 to 1. Consequently the probability of decay on the right being greater than on the left is $\frac{2808}{2604 + 2808}$, or 0.519; the probability of the left cavity being greater is 0.481. However, as the values are so nearly equal and, if the number of rats on each of the two diets is equalized to offset the bias, probabilities of 0.5 can be used with consequent simplification of the analysis. By similar reasoning the probabilities of 0.5 are used for comparison of fissure caries.

Effect of Inclusion of False Cavities: The variety of tooth injuries which we include as caries suggests that some types may not be caries in any sense. Until, however, the nature of such "lesions" is known we will record and include them in the analysis of the data. Our system of punched card records readily enables us to exclude any occlusal cavities if in the future they are found to be of non-carious nature.

If false cavities are included in the data of incidence, the random selection of the animals tends to distribute caries-like formations so that they are equally likely to appear in the experimental groups. The effect of their inclusion is to increase the mean in each group without affecting the difference of the means. The standard deviation, if affected, will be increased and such effect would be to increase the standard error of the difference of the means. Consequently, in the formula above for evaluation of the difference of means, t would be unaltered or diminished by false cavities.

Inclusion of false cavities in the data of size of cavities would add lesions not subject to decay, and, as they would be of equal likelihood of occurrence, their number may be considered as 2n′. Half of them would increase a in the formula for t and half would increase b. The result would be

$$t = \frac{a + n' - (b + n')}{\sqrt{a + n' + b + n'}}$$

$$= \frac{a - b}{\sqrt{a + b + 2n'}}$$

The effect therefore of inclusion of false cavities in the data of either incidence or alteration of rate of decay is to render more conservative the conclusions drawn from evaluation of t.

DISCUSSION

Rosebury, Karshan, and Foley (7, 8) have expressed their observations of dental caries in rats in terms of a "caries index"; most other workers have reported only percentage of caries-free rats or teeth without reference to size or position of the lesions.

We early discarded the rat and the tooth as the unit in favor of carious areas in order to have a finer scale of measurement. With respect to the types of damaged enamel which we record as caries, we would find few caries-free animals and no distinction could be made between experiments. An animal with a single small cavity would be ranked equal with one with molars completely decayed away.

If the tooth is used as the unit much better grading of incidence of decay is obtained. But for the purpose of comparison of size of cavities between 2 animals for study of rate of decay, the lesions should exist in identical tissue, a condition best approximated by using identical sites. Thus, in our system, decay in area 1 is not to be compared with caries in area 3 in another animal, although these empirical sites are in the upper left first molar.

The caries index of Rosebury, Karshan, and Foley involves the use of number and size of cavities. Furthermore, it is derived from observation of a single cavity in part of the molars of each rat. Rosebury, Karshan, and Foley have used caries index in evaluating what we view as combined initiation and progress of decay for which we provide separate experiments interpreted, respectively, by 1) number and 2) size of cavities. Their caries index uses a restricted number of teeth and cavities which we feel entails loss of information.

We are aware that both number and size of cavities are indices of susceptibility to dental caries. Caries index includes both. Our system apparently uses only *number* of cavities as an index of susceptibility. But *size* of cavity is represented by counting a single large cavity as 2 or more, (1) depending on the number of areas involved. Thus a first lower molar completely decayed counts as 5 occlusal and 3 fissure cavities. If such a procedure were not adopted, an animal with several molars decayed away would show a smaller score than one with a number of small cavities in the same molars. We have conjectured that a tooth so susceptible to decay that such large cavities can develop in a short time must have been attacked in the several areas involved. Thus our omission of size of cavity from the measure of susceptibility is partially compensated.

We have provided a "total count" (see *fig. 1*) which is the sum of the sizes of the cavities. We have hesitated to use total count, as it involves the summation of numbers that are non-linear; e.g., a cavity of size 2 is not twice the size of one with a score of 1. Furthermore, use of the parameters of total count has not given as fine a distinction between experiments as use of the parameters of incidence. We have used total count in a system of checking our punched card records for accuracy.

Miller (5) has recorded the number of lesions on a per hundred basis as a means of expressing caries incidence without reference to size.

Our system resembles that of Bödecker and Bödecker (1) for recording caries in man, in that it uses as its basis susceptible surfaces.

In our analysis of the data of incidence we have routinely prepared tables of the frequency and size of cavities in the specific areas. These tables serve as part of our check on the accuracy of our punched card record; but as the pertinent conclusions are drawn from mean incidence and standard error of the mean, we will not in future papers include tables of such distributions unless some significant differences in the sites of decay occur.

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