EFFECTS OF ALCOHOL ON DENTIN FORMATION IN HAMSTER AND RABBIT INCISOR*

BY

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ABSTRACT

Although no effect on dentin formation was seen following voluntary alcohol consumption in the hamster for a period of 11 weeks, the dentin that was formed and calcified during the several days after abrupt withdrawal of alcohol was weakly stainable with hematoxylin.

Similar qualitative changes of a lesser degree were also observed in the dentin formed after repeated administration of an intoxicating dose of alcohol. It was suggested that the effects of alcohol on the dentin formation were causally related to the non-specific stress induced by an intoxicating dose of alcohol and/or the withdrawal of alcohol.

INTRODUCTION

Little information is presently available with regard to the effects of alcohol on the tooth. The exploratory experiments reported here were designed to determine whether or not prolonged drinking of alcohol could produce any effect on the dentin formation.

Rodent incisors grow continuously to keep pace with their constant attrition, and dentin is formed along most of their pulpal surfaces at a remarkably constant rate. Consequently, thick or faint patterns, such as seen in the annual rings of trees, surrounding the pulp cavity are observed when the decalcified specimen of a rodent incisor is stained with hematoxylin. If lead salts, such as lead acetate, were administered at fixed time intervals to the rodent, the lead is localized exclusively in areas undergoing mineralization at the time of each injection.

By an application of this phenomenon to the vital staining of hard tissues, Okada and Mimura succeeded in obtaining distinct stained images in decalcified specimens[1–11]. The availability of this vital staining method has

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made it possible to introduce a time element into this study.

METHODS

Three separate experiments were carried out to test the possible influence of acute and chronic alcohol administrations to hamsters and rabbits.

In the first experiment, the Golden hamster was selected as the experimental animal, since under normal conditions hamsters show a high preference to alcohol offered as an alternative of water. The animals weighed between 70 and 160 g. at the beginning of the experiment. Animals were kept in an air-conditioned room, and five animals of the same sex were confined together in a single cage. Commercial food (Clea Co., Ltd.) was available to all animals used ad libitum. After the initial water control period, animals were given a free choice between tap water and a 15% alcohol solution. Upon completion of the free-choice period (for 2, 3, 4 or 11 weeks), animals were withdrawn from alcohol. One control group of 5 animals was given only water throughout the entire course of the experiment. The volume of liquid consumed was measured every day. Weights of the animals were recorded weekly.

In the second experiment, after a suitable control interval hamsters received relatively large doses (3 g/kg) of alcohol as a 20% solution by oral intubation either once or twice (10:00 A.M. and 4:00 P.M.) a day for 3 consecutive days. The control hamsters received an equal volume of a 20% glucose solution or water, instead of the alcohol solution. Ten hamsters received intraperitoneal injections of pentobarbital (40 mg/kg) either once or twice a day for 2 consecutive days.

In the third experiment, rabbits with an initial weight of 3 to 4 kg were employed. Rabbits received oral administrations of alcohol (3 g/kg) either once or twice a day for 3 consecutive days.

Additionally, all animals in each experiment were injected intraperitoneally with lead disodium ethylenediamine-tetraacetate as a 2% solution in doses of 30 mg/kg at appropriate intervals, in order to mark the level of dentin that was mineralizing at the time of injection. A few days after the last injection of the lead disodium ethylenediamine-tetraacetate solution, the animals were anesthetized with pentobarbital and sacrificed. The lower incisors were then excised and fixed in 10% formaline buffered with phosphate at pH 7.3 for several days. The incisors were next demineralized for two or three weeks in 1% hydrochloric acid saturated with hydrogen sulphide. The specimens were then made into frozen sections imbedded in gelatin and stained with hematoxylin[8–11].

Results

The first experiment. The total amount of liquid consumed by the hamster during the free-choice period showed a considerable variance from day to day. The mean daily alcohol consumption of the hamster was about 5 ml and consequently the mean daily amount of alcohol imbibed was about 5.4 g/kg of body weight. The animals appeared to tolerate alcohol well and only mild signs of intoxication were seen throughout the free-choice period. Some hamsters were relatively motionless and some exhibited slight ataxia. No definite differences were found at any given time between body weights of the hamster consuming alcohol and those of the control group.

No histopathologic changes were observed in the dentin formed during the free-choice period, as compared with that of the control hamster or that formed during the initial water control period.

Characteristic changes occurred in the dentin of the hamster when alcohol was abruptly withdrawn after the free-choice period, though hamsters did not show any evidence of the abstinence syndrome. Fig. 1 is a microphotograph of a transverse section in the longitudinal mid-region of maxillary incisor of the hamster consumed alcohol for 2 weeks. Fig. 2 is a microphotograph of the lingual portion of a transverse section of maxillary incisor of the hamster consumed alcohol for 4 weeks. These hamsters received 5 injections of lead at one week intervals (Pb_{1-5}). Fig. 1 and Fig. 2 show three defined lead lines (Pb_{3-5}) in the dentin due to vital staining. One of which (Pb_4), a distinct black ring in the mid-portion of the dentin, marks the level of dentin formed and calcified at the beginning of alcohol withdrawal. A conspicuous change in the dentin formation was seen after the abrupt withdrawal of alcohol. The dentin that was formed and calcified after the alcohol withdrawal (an internal part of Pb_4) was weakly stained with hematoxylin, in contrast with that which was formed during the free-choice period (an external part of Pb_4). Thus, the lead line (Pb_4) formed the boundary between the normally stained layer and the weakly stained layer in dentin. The other lead line (Pb_5) adjacent to the pulp cavity indicated that these qualitative changes in the dentin formed after the withdrawal of alcohol continued over one week, because the lead was injected one week after alcohol withdrawal. The formation of normal dentin resumed within 10 days after the withdrawal of alcohol. It was also of interest that the changes observed in the dentin were most pronounced within the lingual portion of the incisor (Fig. 1). These staining reactions observed in the dentin formed after alcohol withdrawal were interpreted to be indicative of an arrest of predentin maturation. These changes observed in dentin were also common

to the hamster consuming alcohol for 2, 3 or 4 weeks.

In some hamsters consuming alcohol for 3 or 4 weeks, one narrow band, about 20 μ in width, relatively intensely stained with hematoxylin, was observed in the middle of the unstained zone formed after alcohol withdrawal. (Fig. 2).

An interesting appearance was also seen in the incisor of one hamster consuming alcohol for 4 weeks. As seen in Fig. 3, periodic patterns which were usually indistinct in the normal dentin of hamsters were found clearly in the lingual dentin formed after the withdrawal of alcohol. These patterns produced by hematoxylin staining were confined exactly within a period of one day with a white striate formation during the day and blue-stained striations at night. The rhythm of these patterns were identical to the normal diurnal rhythm described for rabbits and rats by Okada and Mimura[1–7].

On the other hand, no qualitative or quantitative changes in the dentin formation were observed during the period of alcohol consumption in the hamster, even if a free-choice period was extended to 11 weeks.

The second experiment. Hamsters were given relatively large doses of alcohol by oral intubation. Most of hamsters receiving alcohol showed marked ataxic symptoms within 30 minutes and appeared to be almost unconscious for 2 hours. The signs of intoxication completely disappeared, however, 5 hours after alcohol administration.

The dentin that was formed and calcified during and after repeated administrations of alcohol was less deeply stained than normal dentin. Thus, the staining reactions observed in the dentin formed after alcohol administration were similar in appearance to those observed after the withdrawal of alcohol. However, the changes observed after alcohol administration were not so great and continuous as those observed after alcohol withdrawal, even if larger total doses (three doses of 3 g/kg daily) of alcohol were administered to the hamster. The responses produced by the repeated administrations of alcohol while showing a hypomineralization showed no hypermineralization. These changes observed in the alcohol-treated hamster were pronounced in the lingual portion of the incisor.

Similar changes of a lesser degree were also observed in all hamsters receiving intraperitoneal injections of pentobarbital.

The third experiment. In this experiment rabbits were given a relatively large dose of alcohol by oral intubation for 3 days. Most of the ribbits showed ataxic symptoms within an hour after the administration of alcohol and appeared to be almost unconscious for at least one hour. The signs of intoxication completely disappeared, however, 3 hours after alcohol administration. The rabbit showed no weight changes in this short period.

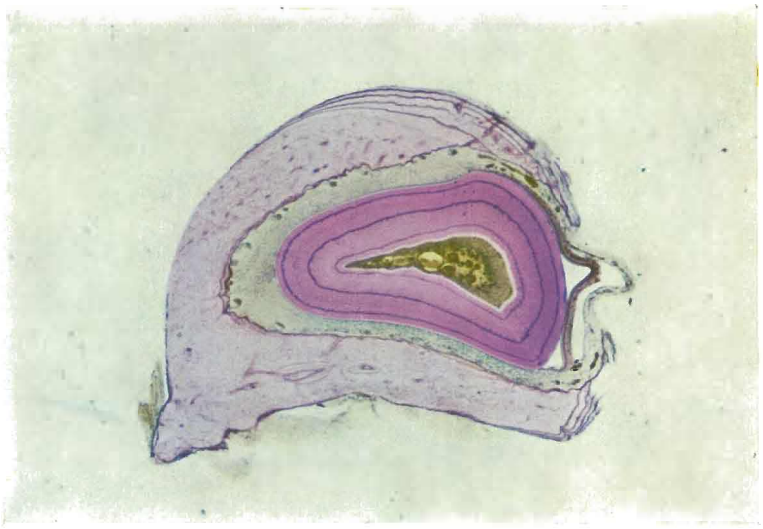

Fig. 1. Photomicrograph of a transverse section of hamster maxillary incisor. (30×).
The dentin formed and calcified after alcohol withdrawal is weakly stained with hematoxylin as compared with that formed during the free-choice period. Lead incorporation (Pb) occurs at sites mineralizing at the time of each injection.

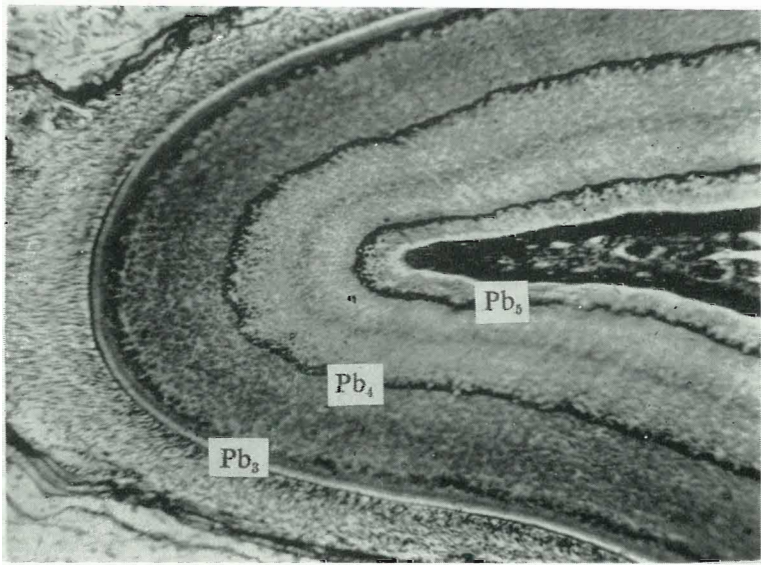

Fig. 2. Photomicrograph of the lingual portion of a transverse section of hamster maxillary incisor. (100×).
One narrow band relatively intensely stained with hematoxylin is shown in the middle of the unstained zone (Pb_4–Pb_5) formed after alcohol withdrawal.

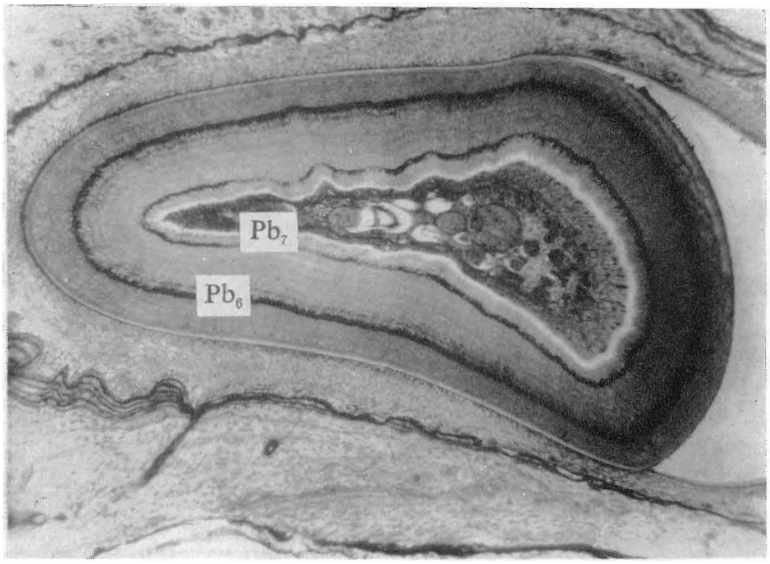

Fig. 3. Photomicrograph of a transverse section of hamster maxillary incisor. (50×).
Periodic patterns are shown in the lingual dentin formed after the withdrawal of alcohol (Pb_6–Pb_7). Lead was injected at 7 day intervals.

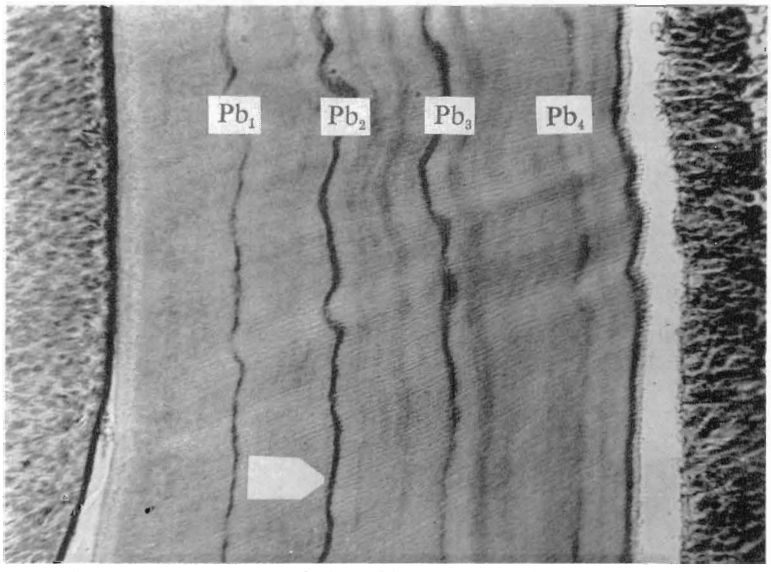

Fig. 4. Photomicrograph of the labial portion of a transverse section of rabbit maxillary incisor. (160×).
An unusual increment of periodic patterns is shown in dentin formed after alcohol administrations. Lead was injected at 3 day intervals. Normally mineralized pre-experimental dentin is shown on the left of the arrow (Pb_2).

As seen in Fig. 4, the dentin formed during (Pb_2–Pb_3) and after (Pb_3–Pb_4) the administration of alcohol showed abnormal staining patterns, which consisted of intensely stained layers and weakly stained layers. Weakly stained layers corresponded to the periods of intoxication, during the period of alcohol administration in so far as the present experiment is concerned. The rhythm of these patterns showed a similarity to the normal circadian rhythm of dentin formation, which was, however, indistinct under normal conditions. These abnormal increments of periodic patterns continued for several days after alcohol administration. These characteristic patterns in dentin occurred in a focal rather than a diffuse manner, and these patterns were most pronounced in the labial dentin. The control rabbits receiving water instead of the alcohol solution did not show any changes such as seen in the alcohol-treated rabbit.

Discussion

In the experimental situations studied so far, the results obtained clearly indicate that acute withdrawal of alcohol causes a definite pathological change in dentin, though the dentin formation may be capable of responding in a normal manner to the prolonged voluntary intake of alcohol in hamsters.

None of the histochemical staining methods available are precise indicators of the degree of mineralization. However, it appears that hematoxylin staining is closely related to mineralization. Thus, it is surmised that areas having a greater affinity to hematoxylin contain more mineral prior to demineralization and thus weakly stained areas in dentin become hypomineralized[12–14]. Hence, the suggestion arises that the changes observed in dentin after the withdrawal of alcohol might be associated with the disturbances of calcification, because the dentin that was formed and calcified after the withdrawal of alcohol is weakly stained with hematoxylin, when compared with the normal dentin.

On the other hand, it is likely that no effects on the dentin formation result from the prolonged consumption of alcohol in hamsters. However, it should be considered that the conditions of the present experiment permitted the amount of alcohol consumed to be distributed over a 24-hour period, which may have afforded a significant degree of protection from the effects observed under acute alcohol intoxication. Thus, it is reasonable to conclude that the continued intake of small quantities of alcohol does not result in disturbance of calcification.

The results of the second experiment demonstrate that changes observed in the dentin formed after acute alcohol intoxication or pentobarbital

anesthesia are not essentially different from those after alcohol withdrawal.

On the other hand, the effect of an intoxicating dose of alcohol on the dentin formation in the intact rabbit is unique and not similar to those observed with alcohol in the hamster. The chief difference noted was a tendency for an increase in amplitude of the diurnal rhythm in dentin; hypermineralized and hypomineralized layers developed alternately after alcohol administration. It was ascertained that weakly stained zones were produced corresponding to the period of intoxication and these erratic changes last several days after the period of alcohol administration. The reasons for the difference of histological changes obtained between rabbits and hamsters are not clear. However, the data obtained so far in these experiments indicate that there was no fundamental difference between rabbits and hamsters. Although normal periodic patterns are found in the hard tissues of the animal body, such as bones, teeth, just as there are annual rings in trees, the causes of formation of periodic patterns in dentin are still unclear[13]. From the present experiment, it seems unlikely that alcohol at the doses used, inhibits mineralization of rabbit incisors directly or amplifies the circadian rhythm present in dentin as a whole[14].

Okada and Suga[15] observed similar alterations in rat incisors after ACTH treatment. Forbes and Duncan[16] reported that a single intoxicating dose of alcohol caused a marked stimulation of the pituitary-adrenal system in the intact rat and guinea pig as indicated by the reduction in the levels of cholesterol and ascorbic acid in the adrenal glands. The fact that similar changes may arise in dentin under various conditions does not justify the conclusion that the pathological mechanism is one and the same. However, these changes obtained appears at present to be highly non-specific. The results obtained suggest that changes in staining reaction of dentin following the administration of an intoxicating dose of alcohol were related to the ACTH release induced by alcohol-produced stress, and not causally related to a direct depressant action of alcohol on the central nervous system[17]. Similarly, it is also possible that the effects of alcohol withdrawal on dentin were somehow related to the ACTH release.

Zarrow et al.[18] have reported that animals treated with alcohol for 14 days and then suddenly withdrawn from alcohol showed maximal hyperexcitability on the second day but this did not subside until an entire week had elapsed. Such hyperexcitability is the experimental equivalent of the abstinence state seen so frequently in the alcohol addict upon withdrawal. It seems therefore that the effect of alcohol withdrawal on ACTH release is probably secondary to the central nervous system effect rather than to a direct action on the anterior pituitary[19]. In the present experiment, the qualitative changes in the dentin formation after the withdrawal of alcohol

continued for several days. Thus, it is assumed that hamsters withdrawn from alcohol would be under a non-specific stress and consequently the production of ACTH would not have returned to its physiological baseline for several days. However, it is still surprising that the withdrawal of alcohol after a few weeks alcohol consumption would have a more intensive and persistent effect on the dentin formation than by severe acute alcohol intoxication.

We are not in a position at present to suggest any explanation of the relation between the abstinence syndrome and non-specific stress induced by alcohol withdrawal.

Histological study of comparable zones of dentin in the labial and lingual portions of the incisor indicates a possible difference in degree of their sensitivity to the same systemic disturbance.

Although evidence favors the probability that non-specific stress in primarily involved, an explanation of the mechanism of action of alcohol in producing the responses in dentin formation is limited by the chemical uncertainty of the histologic observations.

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