大韓齒科醫學會誌 第二卷

原 著

弗化曹達投與에 依한 齒牙硬組織形成과 石灰代謝에 關한 硏究

第一報 : 弗化曹達를 短期投與한 白鼠에 있어 齒牙硬組織의 組織學的所見

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I. 緖 言

自然界에 널리 分布되여 있는 弗素는 그 量은 鹽素의 折半이나 地下水中에도 含有되여 있고 또 齒牙形成에도 必要한 元素이며 自古로 一般原形質毒으로서도 重要한 藥物이다.

弗素가 人體에 미치는 影響에 關한 初期의 硏究는 1891年 Brandle[1]이 骨組織에 對하여서 1932年 Smith 및 Lanz[2]는 斑狀齒의 原因으로서 報告한 以來 斑狀齒 및 人體의 各臟器에 미치는 影響에 關한 業蹟은 許多[3]하였으나 아직도 各器管의 變化의 本態와 發生에 關하여는 不分明하다.

또한 弗素가 齒牙에 對한 作用은 石灰期에 全身的으로 作用하는 境遇와 齒牙崩出後 그 表面으로부터 局所的으로 作用하는 境遇와는 先進學者의 硏究에 依하면 相反된 機序가 있지 않은가 思料되는 바이다.

弗化物投與에 依하여 齒牙硬組織形成障害가 된다는 것은 平田, 今尾, 野坂, 瀧澤等에 依하여 이미 旣知된 事實이며 特히 琺瑯質 및 象牙質의 變質層板이 出現함은 Schour and Smith[8]. 岡田[9], Euler and Eichler[10] 및 Irving[11]等에 依하여 報告되고 있다.

이번 著者는 齒牙硬組織이 投與된 弗化物로 말미암아 石灰代謝機態을 攪亂하여 形成障害를 如何히 惹起하는가는 興味있는 問題임으로 爲先 白鼠에다 弗化曹達를 經口的으로 短期間 投與하면 齒牙硬組織에 如何한 影響을 미치는가를 檢索할려고 本實驗을 試圖한 것으로서 그 成績을 玆에 報告하는 바이다.

II. 實驗材料 및 實驗方法

體重 20.0 gram 內外의 健康한 白鼠를 使用하여 一定한 期間 飼育한後 異常없음을 確認하고 實驗에 供試하였다.

白鼠46頭를 七群으로 區分하였는데 10頭를 一群으로 하야 正常對照群으로하고 36頭를 6頭式 6群으로 區分하여 弗化曹達를 經口的으로 40日間에 걸쳐 每日各各 0.05 mg/F(第一群), 0.1mg/F (第二群), 0.5 mg/F(第三群), 1.0 mg/F(第四群) 2.5 mg/F(第五群), 5.0 mg/F(第六群)을 投與하고 斃殺한後 切除한 顎骨齒牙切片을 通法에 依하여 標本을 製作한後 Heamatoxylin-eosin 二重染色法을 實施하고서 檢鏡하였다.

III. 實驗成績

實驗白鼠의 各群은 弗化曹達의 經口的인 一回投與量의 差異別로 投與量, 投與前과 實驗終了 및 死亡後의 體重, 生存期間等을 表示하면 第一表로부터 第六表와 같다.

正常對照群

白鼠番號	實驗期間 (4291)	實驗前體重 (g)	實驗後體重 (g)	生存期間 (日)
1	8月7日→9月15日	24.50	25.00	40
2	〃	18.00	19.00	〃
3	〃	23.00	24.00	〃
4	〃	21.50	22.00	〃
5	〃	19.00	20.00	〃
6	〃	22.00	24.00	〃
7	〃	22.00	23.50	〃
8	〃	21.00	22.50	〃
9	〃	23.00	24.50	〃
10	〃	19.00	20.50	〃
平均(値)	〃	21.30	22.50	〃

第一群 第一表 (0.05 mg/F 投與群)

白鼠番號	實驗期間 (4292年)	投與量 mg/F	實驗前體重 (g)	實驗後體重 (g)	生存期間(日)
1	1月21日→3月1日	2.0	14.30	15.20	40
2	1月21日→1月23日	0.2	15.50	15.10	4
3	1月21日→3月1日	2.0	16.40	15.90	40
4	〃	2.0	14.80	15.70	40
5	〃	2.0	15.00	16.20	40
6	〃	2.0	15.30	15.10	40
平均(値)		1.7	15.21	15.85	34

第二群 第二表 (0.1 mg/F 投與群)

白鼠番號	實驗期間 (4292年)	投與量 mg/F	實驗前體重 (g)	實驗後體重 (g)	生存期間(日)
1	1月21日→3月1日	4.0	18.90	21.40	40
2	〃	4.0	14.60	21.30	40
3	〃	4.0	16.70	20.70	40
4	1月21日→1月30日	1.0	20.90	19.10	10
5	1月21日→3月1日	4.0	16.80	21.20	40
6	〃	4.0	17.20	22.00	40
平均(値)		3.5	17.52	20.93	35

第三表 第三群 (0.5 mg/F 投與群)

白鼠番號	實驗期間 (4291年)	投與量 mg/F	實驗前體重 (g)	實驗後體重 (g)	生存期間(月)
1	8月7日→9月15日	20.0	22.00	18.50	40
2	〃	20.0	22.00	19.00	40
3	〃	20.0	22.00	17.50	40
4	〃	20.0	24.00	19.50	40
5	〃	20.0	23.00	18.50	40
6	〃	20.0	22.00	19.00	40
平均(値)		20.0	22.50	19.00	40

第四表 第四群 (1.0 mg/F 投與群)

鼠白號番	實驗期間 (4291年)	投與量 mg/F	實驗前體重 (g)	實驗後體重 (g)	生存期間(月)
1	8月7日→9月15日	40.0	22.00	21.00	40
2	〃	40.0	21.00	20.00	40
3	〃	40.0	23.00	21.50	40
4	〃	40.0	22.00	19.50	40
5	〃	40.0	22.00	20.00	40
6	〃	40.0	25.00	22.50	40
平均(値)	〃	40.0	22.50	20.75	40

第五表 第五群 (2.5 mg/F 投與群)

白鼠番號	實驗期間 (4292年)	投與量 mg/F	實驗前體重 (g)	實驗後體重 (g)	生存期間(月)
1	1月21日→1日28日	20.00	20.30	19.00	8
2	1月21日→1月22日	5.00	21.10	19.30	2
3	1月21日→1月30日	25.00	19.90	16.40	10
4	1月21日→1月22日	5.00	18.90	18.00	2
5	1月21日→1月25日	12.50	23.30	22.50	5
6	1月21日→1月27日	17.50	19.50	17.30	7
平均(値)		14.17	20.20	18.75	5.6

第六表 第六群 (5 mg/F 投與群)

白鼠番號	實驗期間 (4292年)	投與量 mg/F	實驗前體重 (g)	實驗後體重 (g)	生存期間(日)
1	1月21日→1月25日	25.00	19.50	17.00	5
2	1月21日→1月22日	10.0	18.50	17.10	2
3	1/21→1/22	10.0	19.60	17.90	2
4	1/21→1/22	10.0	19.30	16.80	2
5	1/21→1/25	25.0	19.70	17.70	5
6	1/21→1/22	10.0	18.90	16.70	2
平均(値)		15.0	19.25	17.20	3

白鼠의 發育을 보건대 一般的으로 對照正常群보다 不良하며 齒牙는 灰白色으로 軟弱하고 磨耗가 强度이며 投與量의 增加에 따라 死亡한 것이 많다.

實驗白鼠切齒의 所見은 特有한 縞紋様의 形成이 認定되었으니 即固有한 黃色調의 琺瑯質과 弗化曹達로 因한 色素脫失한 白色 琺瑯質이 出現함을 觀察하였다.

또한 臼齒에 있어서의 變化는 切齒에 比하여 顯著하지는 않었다.

組織學的으로는 切齒에 있어서는 琺瑯質에 있어서 Ameloblasts는 正常의 配列을 喪失하였고 또한 輕度의 萎縮性이 出現하였고 象牙質에 있어서는 그發育線에 一致하여 明層과 暗層이 形成되었으며 Odontoblast의 變化는 明確치 않었다. 이에 反하여 臼齒에 있어서는 顯著한變化를 觀察하지 못하였다. 切齒및 臼齒에 걸쳐서 白堊質에 있어서는 形成障害를 檢鏡하기 困難하였다.

IV. 總 括

弗素에 依한 齒牙의 變化에 關한 實驗的硏究는 1925年 McCollum[12] 以來 多數[13][14][15][16]의 業蹟이 있어 그實驗結果는 琺瑯質의 形成異常이 强度로 發生하며 象牙質 및 白堊質의 變化는 比較的輕度이라한다. 그러나 本實驗에 依하면 切齒琺瑯質에 高度의 變化가 出現함은 認定하였으나 臼齒琺瑯質에서는 그 變化가 輕度임을 觀察하였다.

그리고 切齒琺瑯質이 白色을 띄움은 琺瑯質上皮에서 形成되는 小顆粒이 琺瑯質表層으로 移行하는것이 障害되는 것에 起因하는것으로 생각된다.

上記實驗成績을 綜合하건대 著者는 健常白鼠에다 經口的으로 弗化曹達를 弗素含有量에 따라 第一群(0.05mg/F), 第二群(0.1mg/F), 第三群(0.5mg/F), 第四群(1.0mg/F), 第五群(2.5mg/F), 第六群(5.0mg/F)을 四十日間에 걸쳐 投與하고 齒牙硬組織의 變化를 觀察한結果 다음 같은 成績을 얻었다.

1) 第一群및 第二群에서는 齒牙硬組織에 있어서 이렇다할 變化를 觀察하기 困難하였다.
2) 第三群및 第四群에서는 齒牙硬組織에 있어서 顯著한 變化를 招來함을 觀察하였다.
3) 第五群에서는 齒牙硬組織에 있어서 輕度의 變化를 觀察하였다.
4) 第六群에서는 齒牙硬組織에 있어서 顯著한 變化를 認定하지 못하였다.
5) 各群을 通하여 臼齒에 比하여 切齒의 琺瑯質에 있어서 形成障害가 顯著함을 檢鏡하였다.

Resume;——

Using the mice as the experimental animals. I have partly observed the effects of fluoride on the relationship of calcium metabolism and formation of dental hard tissue.

Thirty six mice are devided into sixgroups and daily administered the sodium fluoride containing 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, mg of fluoride for fourty days After which the Animals were sacrificed and the dental hard tissues are observed microscopically.

From the results obtained it appeared that:

No changes were observed in the dental hard tissues of the 1st and 2nd groups.

Significant changes were observed in the dental hard tissues of the 3rd and 4th groups.

No appreciable changes were observed in the 5th and 6th groups.

It was appearantly observed in each groups that the formation of incisor enamels were more disturbed than in the molar enamels.

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A STUDY ON THE LOCALIZATION OF A FEW HISTOCHEMICALLY DEMONSTRABLE ENZYMES IN LYMPH NODES

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INTRODUCTION:

This is a part of my study to demonstrate various enzymatic activities in lymphoid organs

histochemically, which is being done along with the electron microscopic observation, in an effort of understanding the differentiation of cell populations in the organs.

Since every cellular activity or differentiation, in a sense, is a reflection of complicated enzyme activities within the cell, it is a great help to know the changes in the amout and the state of activities of enzymes histochemically in comprehending cellular physiology and differentiation. The number of histochemically demonstrable enzymes are very limited and fewer than that of the undemonstrable ones, but it still thraws a light on the future, as more of the enzymes are uncovered to be demonstrable with the advance of histochemical techniques.

For the fact that it is diffcult to distinguish various cell types of the lymphoid organs without counterstaining which is not favorable for clear-cut demonstration of enzyme reactions, it has been thought that the use of phase-contrast microscope would be of some help for this approach. Although it is still diffcult to pinpointall of the cell types in which enzymes are active due to the multitude of different cells in this particular organ. yet some of the activities can successfully be localized.

Enzymes studied are alkaline and acid phosphatases, succinic dehydrogenase and nonspecific esterases.

MATERIALS AND METHODS:

Sprague-Dawley rats of 200 gm average body weight were used for this experiment. Mesenteric lymph nodes were taken out under either inhalation anaesthesia, and directly frozen with dry ice, then cut into sections of 5 to 10 μ thickness in a cryostat. After a few minutes of air drying, mounted sections were incubated in appropriate Azo-coupling media as following;

1. For alkaline phosphatase, Gomori's Azo dye method was employed with sodium α-naphthyl phosphate used as the substrate. Red RC was the dye coupled. Incubation time was 20 to 30 minutes at 37°C.
2. For esterases 1% naphthyl ASacetate in acetone propylene glycol was used as the substrate, and coupled with Garnet G.B.C. Incubation time was 15 to 30 minutes at 37°C.
3. For acid phosphotase a modified Azo-dye techniqne by Grogg and Pearse was employed using sodium α-naphthyl phosphate as the substrate, incubating for 12 hours at 37°C. Garnet G.B.C. was the dye used. The incubation media were preheated to the temperature.
4. For succinic dehydrogenase the new nitrobluetetrazolium was used, with the incubation time of 10 to 15 minutes at 37°C.

Controls were run without substrate or by heating the sections for 5 minutes or more, at nearly 100°C.

Sections of kidney were studied for controlling the negative results in the case of alkaline phosphotase, esterase and succinic dehydrogenase. For acid phosph otase a piece of lateral prostate of a ratwas studied as the control.

Slides incubated were mounted in glycerol jelly. seeled with commercial nail polish in usual way, and studied with a phase cotrast microscope.

Leitz Ortholux with phase optics was the microscope of use. Photomicrographs were taken with a Microibso by Leica, using $3\frac{1}{4} \times 4\frac{1}{4}''$ Kodak M plates. Pictures were photographically

enlarged from 1.5 to 3 times. Actual caliberation of the magnification was done with a stage micrometer.

OBSERVATIONS AND DISCUSSIONS:

1. **Alkaline phosphotase.**

The lymphocytes in normal mesenteric lymph nodes show little alkaline phosphotase activity regardless their sizes. Activities are, however, observed in many of the cells surrounding the blood capillaries, whereas little activity appears in most of the lymphatic capillaries.

Cells with positive activity are scattered in the germinal centers as well as well as in the medullary region, showingno particular difference in their large and irregular cell shapes, although less number of these active cells are present in germinal centers.

The morphology of the cells is alike to some of the reticular cells forming the sinusoids. Since, however, not all of the reticular cells are positive, it seems to be necessary to distinguish the type of reticular cells which are positive from the others. Although some efforts have been made to make distinction of positive cell types during this experiment, for the fact that lymph node has such a magnitude of tremendously variable cell types, and that the classification of this has not been done to a satisfactory point, it appears to be needed a considerably long time and effort before one can bring about an answer.

The kidney sections run asa control showed intensive activity at the brush border of the convoluted tubules, while the glomeruli showed no activity atall. It is of interest that cells of small arterioles lying outside of the smooth muscle layer also show positive reaction.

2. **Esterases activity.**

There are not many cells showing intensive positive reaction of esterases activity for the method employed. It is true, however, that there are some reticular cells with the demonstrable activity in the germinal centers, as well as in the medullary regions.

These cells show more regular cytoplasmic contour than that of the alkaline phosphotase positive ones, while they are too large to be lymphocytes. It has been observed that macrophage in subcapsular sinus are reactive. Lymphocytes within the germinal center seem to have small amount of activity in their cytoplasm. No pericapillary cells were positive.

Control sections of kidney showed again a good reaction in the cytoplasm of the convoluted tubule cells, which havea rather interesting feature of contrast to, in as much as the comparative localizations of the two enzymes are concerned, that alkaline phosphotase was positive more at the surface of the cells along the brush borders—a characteristic for a membrane where active fluid transport is being carried, while the sites of esterase activity is perinuclear.

3. **Acid phosphotase activity.**

Again lymphocytes have not shown reactivity for acid phosphotase in the centrai area of germinal centres, although there are scattered reticular cells, morein number than in the case of alkaline phosphotase or esterases, which showed intense activity. It is of interest to note that the cells in the medullary region as well as some of the cells in the mantle area of germinal centers present so tremendous acid phosphotase activity that the sections of lymph nodes seem to have stronger reactivity than the control sections of the prostate to gross eyes.

Prostate showed beautiful reaction for acid phosphotase, as is expected, within the eytoplasm

of the glandular cells. Basal portions of the cytoplasm are more reactive.

4. Demonstration of the succinic dehydrogenase activity.

Succinic dehydrogenase activity was appeared in most of the lymphocytes to a weaker degree while some of the reticular cells were more intensely reactive, which corresponds to the electron microscopic distribution of the mitochondria.

As stated already it is very difficult to make a distinction between various reticular cell types of a lymph node at the moment, not only because of their similarity of morphology among cells of diffierent functions and at different degrees of differentiation, but also of the diffculty that correlation of the functional significance of the enzymatic activities shown in different cell types is almost impossible at the present phase. More diffcult to know is whether one cell is reacting to several kinds of enzyme substrates or different cells are reactive to different enzyme sustrates. But it might well be that both cases are true, as is known in many tissues.

It is suspicious that immunohistological studies, electron microscopy and tissue culture methods along with biochemical techniques will be of great aid in the future clearification of these problems.

RESUME :

1. A study on the histochemical localization of some enzymes has been made; i.e., on alkaline and acid phosphotases, esterases and succinic dehydrogenase, with the aid of phase contrast microscopy.

2. Although it is farfrom be complete some of the results are that;

a) Alkaline phosphotase activity is localized in reticular cells of the sinusoids which show more irregular shape, as well as in pericapillary cells.

b) Reaction of esterases activity is similarly localized in some of the reticular cells as in the alkaline phosphotase, although they seem to be different cells, for they have more regular cell contours.

c) The activity of acid phosphotase is much more intense and wide apread among reticular cells than the two before-mentioned enzymes, particularly in the medullary area with many of the lymphocytes showing the activity at the periphery of germinal centers.

d. Localization of the succinic dehydrogenase activity in lymph nodes corresponds with mitochodrial distribution of the tissue.

3. Cell typing of reticular cells reactive to various enzyme substrates has not been made.