# STUDIES ON THE MECHANISM OF GINGIVAL HYPERPLASIA INDUCED BY DIPHENYLHYDANTOIN.

by

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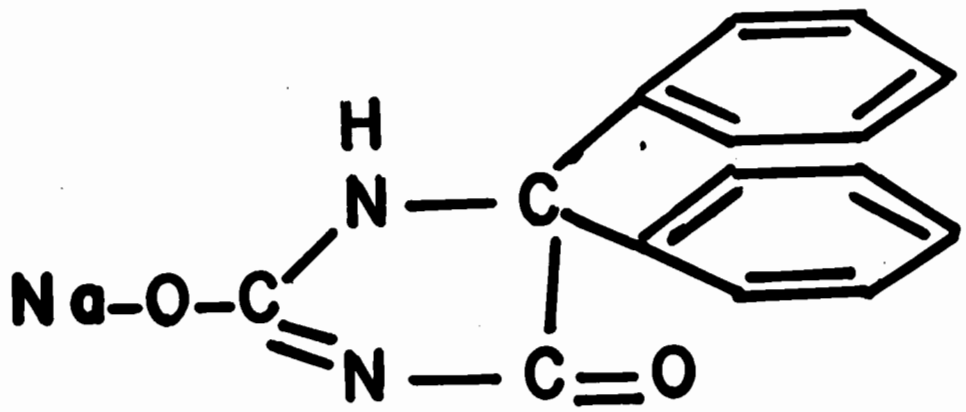
## INTRODUCTION

Since the discovery of the anti-epileptic action of diphenylhydantoin, it has been repeatedly observed that some patients undergoing treatment develop gingival changes. These changes appear superficially like a low grade inflammatory reaction, showing irregularity of the gingival surface, but progressing slowly to a firm, painless overgrowth of the gums. Histological examination of sections of the affected gums have revealed a proliferation of capillaries and connective tissue associated with a thickening of the epithelial surface, with marked increase in cell numbers or hyperplasia. It has also been observed that stopping the administration of the drug leads slowly to restitution to normal. The hyperplasia appears therefore to be of a benign or non-malignant type. There is no specific therapy for this condition. Good dental care appears to help but does not prevent it, and in cases of excessive hyperplasia, surgical excision by the dentist has often been resorted to. This, however, only affords temporary relief and with continued therapy remission ensues.

The drug is employed in the form of diphenylhydantoin sodium, which is chemically, 5,5-diphenylhydantoin sodium, or 5,5-diphenyl 2,4-imidazolidinedione sodium, or 5,5-diphenyl hydantoinate or phenytoin sodium. It is also known under various proprietary names as Dilantin, Dilantin sodium, Dihydan soluble, Alepsin, Epanutin, Eptoin, Hydantal, Solantoin, Solantyl and Zentropil. Its chemical structure is represented hereunder:




It is prepared by the condensation of benzil with urea followed by treatment with sodium hydroxide. It is a white, somewhat hygroscopic powder, which on exposure to air gradually absorbs carbon dioxide with the liberation of diphenylhydantoin. One gram of diphenylhydantoin sodium dissolves in about 66 ml. of water, but the solution is incomplete and turbid unless alkali is added to raise the pH above 11.7.

Despite the many theories which have been advanced during the past 20 years concerning the possible mechanism of production of gingival hyperplasia associated with diphenylhydantoin therapy in man, there has been surprisingly little experimental investigation of this problem. Undoubtedly, one of the main reasons for this was the early report, that even following prolonged administration of the drug to experimental animals, no "gingival changes" ensued. However, the possibility arises that in many of the earlier studies in this field no particular attention was directed to the gingiva. Indeed, in Drill's Pharmacology in Medicine, Second Edition, 1958, it is stated

that "prolonged administration of anti-convulsant doses of the drug (diphenylhydantoin) in animals, fails to produce pathologic changes in the tissues". While this is still current opinion, it should be stated that in 1952, King reported that following prolonged administration of the drug to ferrets, both gross and microscopic changes resembling those occurring in man, could be observed. These workers however, suggested that this occurred only under special dietary and other conditions, as will be pointed out later.

In view of the above it appeared worthwhile to reinvestigate the effects of administration of diphenylhydantoin upon tissue changes in various laboratory animals, with special reference to those occurring in gingival tissue. It was soon apparent that in certain instances, despite the absence of any observable gross changes, varying degrees of histological alterations could be detected in the gums and lungs of some of the experimental animals. This suggested that diphenylhydantoin might induce local chemical changes in some tissues, especially gingiva, and that such changes might act as "trigger mechanisms" leading progressively to microscopic and later characteristic macroscopic changes.

Since the lungs, skin and mucous membrane are rich in histamine, on the basis of the above hypothesis, it was decided to undertake a comparative study of the "histamine content" of normal gingiva and the gingiva obtained from diphenylhydantoin-treated animals. Although the exact role of histamine in tissues is still obscure, evidence exists

that it plays some part in anaphylactic and allergic responses. Although it has been repeatedly suggested (see Historical Data) that the gingival changes associated with dilantin therapy in man, might be of an allergic nature, there have been no previous experimental studies in the literature concerning histamine changes in the gingiva in this condition.

It is now well established that histamine is found in mast cells, and Riley and West (1953) have shown that there appears to be a close parallel between the histamine, heperin, and mast cell contents of several tissues, although there have been no systematic studies on this question in respect to gingiva. The possible physiological and pathological roles of histamine in the gingiva appear therefore to warrant investigation.

Another important tissue substance which has recently been identified and which occurs in mast cells is 5-hydroxytryptamine or serotonin. In conjunction with this study it was therefore of interest to compare the "serotonin content" of normal gingival tissue and that obtained from diphenylhydantoin-treated animals. There are again no previous studies in the literature on this question.

In many of these experiments attempt was also made to obtain histological data, with the hope of correlating these with observed changes in histamine and 5-hydroxytryptamine in the tissue. However, the findings concerning this aspect of the problem are by no means complete, and are largely presented to illustrate the gingival changes induced experimentally under the conditions employed. A number of incidental

histological observations on other tissues are also noted.

## HISTORICAL DATA

Merritt and Putnam (1938) first reported that dilantin can protect experimental animals from electrically induced convulsions. The drug was found to be as effective as bromides and phenobarbital. It however, possessed some advantage in that it did not show the characteristic central nervous system sedative effects of the latter agents. The results of these early animal experiments, using cats, dogs and rats, and giving massive single and long-continued daily doses, showed that the drug was well tolerated by the ordinary laboratory animals, and there appeared to be no toxic reactions observed at that time. No details of the exact dosage or duration of administration were given by these workers.

In connection with the above observations, Merritt and Putnam also reported studies on two hundred patients suffering from epilepsy, using a dose of 0.1 gm. three times daily. These patients were treated for periods varying from three weeks to eleven months. Despite the apparent lack of toxicity of the drug in animals, two types of toxic reactions were observed in some of the early treated cases. These consisted of what the authors refer to as: (1) various minor toxic symptoms, such as dizziness, ataxia, tremors, blurring of vision, diplopia and nausea - symptoms in this group occurring in about 15% of the cases, and (2) more serious effects, such as purpura and dermatitis of varying degrees, which developed in about 5% of cases. However, in this first series of patients there is

no mention of gingival hyperplasia or any oral disturbances. It is possible that since many changes within the oral cavity occur from poor hygiene, any gingival changes observed at that time might not have been associated with the administration of the drug, and therefore not considered of any significance.

Kimball (1939) reported a similar study on 152 children receiving sodium diphenylhydantoinate therapy. It was found that 68 showed varying degrees of what was described as hyperplasia and of the group 17 showed advanced hyperplasia. In some of these cases, the changes were extreme enough to cover completely some of the crowns of both the mandibular and maxillary teeth. In this series 57% of the patients, who had used the drug for two months or longer, showed some changes in the gingiva. It was also noted by the author that "there appeared to be no relationship between the dosage of the drug and the extent of the hyperplasia found". Thus, some patients who had been on the drug for as long as a year showed normal gingiva, while others on the drug for as little as two months showed definite changes. In addition, it was considered by the author, that the gum changes represented a "hyperplasia associated with vitamin C deficiency", and a study of the relationship of the ascorbic acid content of the blood serum and the degree of hyperplasia occurring in different patients, was also undertaken. It was observed that in the "normal group" the average blood ascorbic acid concentration was 1.14 mg. per 100 ml. of blood, but in the groups showing

hyperplasia the average ascorbic acid content ranged from 0.55 to 0.58 mg. per 100 ml. of blood. Although the data of these workers showed that there was generally a slightly lower ascorbic acid level in the more severe cases, this was not invariably so. It was concluded that there was a definite relationship between the severity of the hyperplasia and the degree of the ascorbic acid deficienty, associated with the treatment of epilepsy with diphenylhydantoin.

Kimball and Horan (1939) reported further observations on the use of dilantin in the treatment of epilepsy, and again, they refer to the most serious complications arising as "gum changes and repeated attacks of gastric irritation". Indeed, the authors stated that "gastric irritation" only occurred when there was hyperplasia of the gingiva, and further, that both appeared to be related to vitamin C deficiency in some way. It was however, observed by these workers that when the drug was stopped, the gingiva gradually returned to normal within three months, even though the diet was not changed and no vitamin C was added. Moreover, the patients showed no other effects of vitamin C deficiency, and administrations of large doses of vitamin C did not influence the course of the hyperplasia. It is of some interest that these workers observed that all cases complained of 'sore mouth' two to four weeks after starting the treatment, but after a period of another two weeks the soreness disappeared and was replaced by a swelling of the gingiva.

Merritt and Foster (1940) investigated 257 ambulatory patients, treated with dilantin. They reported that about 22% of these cases showed some gingival changes, but only 3% showed sufficiently severe changes to cause discomfort. These workers also studied the vitamin C content of the plasma in (a) a group of 31 normal patients, (b) a group of 44 patients treated with phenobarbital, and (c) a group of 182 patients treated with dilantin. No significant difference was observed in the three groups. Again, it was noted that some of the patients affected had been on the drug for two years, and showed little difference from those on the drug for a short period of time.

Merritt and Foster could also find no correlation between the degree of gingival hyperplasia and the vitamin C content of the blood in a second series of patients similarly studied. It was felt that the lowered ascorbic acid content previously reported by Kimball (1939) was due to the low economic groups from which his patients were drawn, and therefore was only an associated condition. It was therefore concluded that the "hypertrophic gingivitis developing in patients under treatment with dilantin sodium is not related to the vitamin C content of the plasma or to the utilization of vitamin C," but was rather a "benign hyperplasia".

Since patients suffering from gingival changes frequently consult their dentists, it is not surprising that there are many reports on this condition in the dental literature,

particularly from orthodontists. In 1940 McIntosh noted that although there were already twenty-nine clinical studies in the medical literature, there was at that time no mention of any observations by dentists. Since varying degrees of gingival changes are frequently seen by dentists, it is possible that in many cases in which the drug might be involved the cause might not have been known, and many errors in diagnosis were reported in the earlier literature. Indeed, in some of these cases the etiology was ascribed to "infection" or "traumatic occlusion", and it is of some interest to note that the hyperplasia was even considered in one of McIntosh's cases, as due to "crowding of molars and an excessive overbite". The detailed clinical course of the patient was, however, typical of that described earlier by Kimball (1939) and Merritt (1940), in epileptic patients treated with dilantin.

Thompson and Gillespie (1941) first reported the use of surgical intervention in the hyperplasia. A variety of other local palliative treatment was attempted by these workers but no details are given by them. In some patients local applications of 100 mg. of cevitamic acid daily were made and a high vitamin diet was prescribed. However, there was apparently no response to either of these forms of treatment. Furthermore, follow-up therapy by the use of X-radiation of the gingiva, led to no improvement in the degree of the hyperplasia. Since all previous treatment appeared to have been ineffective, gingivectomy was performed on both jaws. Following excision

of large amounts of the excessive tissue, the usual obtundant dressings were applied. It is noteworthy, that the writers state "very little pain was experienced" by the patient. The pathological report on the tissue was that of a "chronic inflammatory tissue". The conclusion was reached that this was a method of restoring the gingival tissue to a normal appearance, although no follow-up or re-examination of the gingival condition was reported. However, Robinson (1942) in another report concerning dental surgical excision, states that while it gave temporary relief, the tissue returned to its hyperplastic state when the dilantin therapy was continued.

In many of the earlier reports in the literature the terminology is rather vague, and the term 'hypertrophy' has often been applied, as synonymous with the hypertrophy seen in vitamin C deficiency. However, it is now clear that in the latter there is no increase in cell number in the tissue. In the Classification of Periodontal Diseases by Orban (1942) and which was recommended by the Nomenclature Committees of The American Dental Association (1943) and The American Academy of Periodontology (1943), the term 'hypertrophy' was still the principal one used to designate gross overgrowth of the gingival tissue, which was characteristic of certain cases, including the effects of dilantin.

Miller (1944) has emphasized that 'hypertrophy' is an increase in the size of the individual cells as a result of which an organ may become enlarged, while 'hyperplasia' is an increase in size, resulting from an increase in the number of

cells of an organ. It was felt by this author that hyperplasia, unlike hypertrophy, is often the result of irritation, and that probably either hyperplasia or/and hypertrophy occurred in the observed diphenylhydantoin sodium gingival overgrowth.

Orban (1947) also described 'hypertrophy' as an overgrowth of an organ by enlargement of its specific tissue elements - an enlargement which serves a useful function. However, he considered 'hyperplasia' as an enlargement of an organ caused by multiplication of its structural elements or by an accumulation of foreign elements, such as fluid and inflammatory cells - an enlargement which does not necessarily serve an increased and useful function in the organ. It is clear from this and the preceding comments that considerable confusion exists in the literature regarding the proper terminology to be applied to this condition. The term hyperplasia however appears to be preferable, and is employed throughout this study.

In regard to the frequency of the occurrence of clinical gingival hyperplasia following dilantin therapy, Reader (1957) has recently summarized the data from 35 publications in the literature on this question during the period from 1938 to 1956. In Table I, taken from the publication of Reader, are shown the number of cases and the calculated percent of these which showed clinical evidence of gingival changes.

TABLE I

| | AUTHORS | NUMBER OF PATIENTS | % SHOWING GINGIVAL CHANGES |
|---|---|---|---|
| 1 | Merritt & Putnam (1938) | 142 | No mention of hyperplasia |
| 2 | Philips (1939) | 12 | " " " |
| 3 | Kimball (1939) | 119 | 57 |
| 4 | Kimball & Horan (1939) | 220 | 51 |
| 5 | Blair, Bailey McGregor (1939) | 75 | 25 |
| 6 | Steel & Smith (1939) | 20 | 15 |
| 7 | Frost (1939) | 9 | No mention of hyperplasia |
| 8 | Weaver, Harnell & Arnold (1939) | 14 | " " " |
| 9 | Pratt (1939) | 52 | 50 |
| 10 | Williams (1939) | 91 | No mention of hyperplasia |
| 11 | McCarton & Carson (1939) | 20 | 5 |
| 12 | Hodgson & Reese (1939) | 44 | 7 |
| 13 | Merritt & Putnam (1939) | 350 | 6 |
| 14 | Fetterman (1940) | 28 | 25 |
| 15 | Weinberg & Goldstein (1940) | 15 | 33 |
| 16 | Robinson & Osgood (1940) | 100 | No exact figure |
| 17 | Allen (1940) | 65 | No mention of hyperplasia |
| 18 | Frankel (1940) | 48 | 62.5 |
| 19 | Ross & Jackson (1940) | 73 | No exact figure |
| 20 | Morgan (1940) | 8 | " " " |
| 21 | Johnson (1940) | 20 | No mention of hyperplasia |
| 22 | Merritt & Foster (1940) | 182 | 22 |
| 23 | Butler (1940) | 43 | 2 |
| 24 | Seklotthaver (1940) | 32 | 13 |
| 25 | Lowery (1941) | 34 | 29 |
| 26 | Glickman & Levitus (1941) | 76 | 21 |
| 27 | Millhon & Osterberg (1942) | 30 | 40 |
| 28 | Robinson (1942) | 143 | 19 |
| 29 | Macfarlane, Baxter & Mitchel (1942) | 67 | 55 |
| 30 | Prudhomme (1942) | 57 | 30 |
| 31 | Palk (1942) | 20 | 50 |
| 32 | McLendon (1943) | 29 | 3.5 |
| 33 | Stern, Eisenbud & Klatell (1943) | 50 | 52 |
| 34 | Esterberg & White (1945) | 244 | 54 |
| 35 | Baskinski (1956) | 30 | No exact figure |

It is clear from the table that there is considerable variation in the reported frequency of the development of this condition. Some of these differences might of course be due to individual differences in diagnostic standards by different workers, regarding the degree of involvement particularly in view of the fact that most of these observations were reported by physicians (neurologists mainly), who are unfamiliar with normal periodontal tissue (Klatell 1943).

In regard to the underlying cause of the hyperplasia during dilantin therapy, most of the reports have been concerned with its possible relationship to: (a) vitamin C deficiency, (b) changes in oral hygiene, or (c) traumatic occlusion (Kimball 1939, McIntosh 1940, and Dobin 1951). The surgical removal of the tissue with follow-up home care, has been emphasized by Levey (1950) and Blake and Blake (1953). These workers conclude that while surgery is not a cure, it is clear that it lessens subsequent superimposed gingival conditions that might occur. However, if the dilantin therapy is continued the gingival hyperplasia will return. None of these forms of treatment therefore, appears to lead to prevention or cure of the condition.

In respect to the possible role of oral hygiene, Dobin (1951) states that "poor oral hygiene does not play a part in the incidence of the hyperplasia, but it is an important factor in the degree of complicating conditions such as secondary infection. The hyperplastic tissue that occurs in clean mouths is smooth and firm, while more inflammation and bleeding is noted in those

mouths with poor oral hygiene". Furthermore, the author adds, "individual tolerance or sensitivity to the drug determines the incidence of gingival reaction. The lack of definite correlation is demonstrated by the absence of changes in many of the most neglected mouths, and the presence on the other hand of advanced changes in many relatively clean mouths."

Glickman and Levitus (1941) did not observe hyperplasia in edentulous areas, and after the removal of teeth which were surrounded by hyperplastic tissue, normal healing occurred with disappearance of the gingival enlargement.

Esterberg and White (1945) have reported that the presence on the lingual surfaces of the lower incisors of large deposits of calculus (which could certainly be considered a source of irritation) caused no marked overgrowth, while the same teeth showed frequently extensive hyperplasia on the labial surfaces, where little or no calculus was present. The regions of the upper and lower teeth show hyperplasia to equal degrees, but the condition appears more frequently on the labial aspect of anterior teeth than on the lingual aspect. In the presence of partial dentures with teeth abutting directly into the gingival ridge, Dobin (1951) states that hyperplasia appeared around the dentures as around the natural teeth. It would also appear that in the case of fixed bridges a marked overgrowth appeared above the facings, similar to the growth around natural teeth.

Van der Kwast (1956) investigated 23 patients showing gingival hyperplasia from dilantin therapy, with special reference to the possible role of some underlying allergic mechanism.

Biopsies of the tissue were examined histologically, and showed that the squamous epithelium of the mucosa, as well as the fibrillary connective tissue of the submucosa, were markedly hyperplastic. In the connective tissue layer numbers of mononuclear cells were found, and these the author states were "practically all plasma cells". Since plasma cells have been thought to be producers of antibodies, it was felt by the author that these represent "the formation of allergic antibodies in the hyperplastic gingival tissue". This author also carried out serum electrophoresis and found an increased gamma globulin (19 to 32.5% of the total protein) in 20 of the 23 cases. An increase in serum gamma globulin was also found in 4 out of 7 cases on dilantin, but not showing hyperplasia. All patients also received "patch tests" using dilantin dissolved in saline in order to investigate skin sensitization. These however repeatedly proved negative. From the above findings it was concluded by Van der Kwast that gingival hyperplasia may be regarded as an allergic reaction.

On the assumption of a possible allergic basis of the hyperplasia, Gaillard (1957) has reported some preliminary observations on 2 cases showing extreme hyperplasia and treated with oral administration of doses of 12 mg. of the antihistamine agent chlorprophenpyridamine (Teldrin), in sustained release capsules twice daily. The author states that "one case returned to normal within two weeks, and in the other case two months were necessary". Furthermore, when the drug was stopped the hyperplasia returned. No histological studies of the

gingival tissue were however carried out by this worker. Martin (1957) has also reported some preliminary observations with the use of this agent in 20 cases undergoing treatment with dilantin. This worker however, observed only some regression of the superimposed gingivitis, but no significant changes in the hyperplastic overgrowth.

Previous Experimental Studies

As already pointed out in the original studies of Merritt and Putnam (1938) no evidence of gingival or other tissue changes were observed in cats, dogs and rats, which received massive single or long-continued daily doses of the drug. However, no details were given by these workers. Although the drug was extensively investigated experimentally during the next decade in regard to its anti-convulsant action, in none of these studies was any evidence for the occurrence of gingival alteration in animals reported. Shafer (1948) could find no evidence of either gross or microscopic gingival changes in 72 albino rats given oral doses of dilantin which were considerably above those corresponding to human therapeutic dosages, despite administration for periods of 13 to 14 weeks. No details are given by the author.

King (1952) appears to have been the first to undertake a systematic investigation of the effects of dilantin on various animal species. In that paper he states that in "pilot experiments begun by the writer in 1947, 18 weanling rats and 10 adult hamsters were each given 1.6 mg. epanutin daily in their food

for 29 and 15 weeks respectively". The main diet for the rats was oatmeal or unground oats, and for the hamsters crushed "rat cubes". No gross lesions occurred in any of these animals. However, histological examinations revealed some "slight proliferation of the connective tissue of the gum corium and periodontal membrane, but no pedicle formation was observed."

It was also first reported by King that following prolonged administration of epanutin to ferrets, gingival changes resembling those previously reported in man developed. In these experiments a total of 69 ferrets were used. The animals were kept on the following diet: Bread: 60-70 g.; whole milk powder: 3.5 g.; baker's yeast: 2.0 g.; salt mixture (McCallum 185): 1.0 g.; vitamin A (1000 i.u.) and vitamin D (100 i.u.) in peanut oil: 2 ml.; all mixed and moistened with water. Doses of dilantin ranging from 8 to 16 mg. daily were added to the diet for periods ranging from 14 to 29 weeks, and in some experiments injected intramuscularly in a finely dispersed suspension in distilled water. In some experiments also supplements of short lengths of rib-bone (about 15 grams) with some attached muscle were added to induce gnawing. Both gross and histological examinations of the gums were made. Following oral administration it was observed that all 7 animals on dilantin <u>without bone supplement</u>, showed "gingival hyperplasia" of some degree, but pedunculated multilobular growths did not appear until the fourteenth week or later. " The addition of the bone supplement apparently prevented any detectable paradontal abnormalities,

but did not prevent the development of the characteristic histological hyperplasia. Following intramuscular injections, with or without bone supplement, similar results were observed in 8 ferrets. It was also noted that 2 ferrets receiving no dilantin but only bone supplement for 15 weeks, showed some inflammatory changes in the gums. Nevertheless, with continued administration of the bones to 2 other controls for 43 weeks, no gingival lesions of any type were noted. The author concludes that the hyperplasia induced by dilantin differs from that following local irritation, such as produced by calculus, and the fact that the effect developed following intramuscular injections suggested that the growth stimulus was probably not associated with the oral administration of the drug leading to some local disturbance.

Swenson (1954) has also attempted to induce gingival changes in albino rats by daily administrations of dilantin. The animals were kept on a diet consisting of 2/3 ground whole wheat, 1/3 powdered milk, and 1% sodium chloride. The dilantin sodium in solution (1 mg./cc in water) was injected intraperitoneally daily in a 1 mg. dose (1 cc.). This dose was calculated to be about twice the human dosage on a weight basis. The experiment was continued for 90 days on 36 rats. In order to induce local irritation, a stainless steel wire was wrapped around the lower anterior teeth in 18 of the animals. At the end of the administration the gingiva were examined grossly and microscopically. There was a slight increase in round cell infiltration in the

rats with the wires, and no systemic effects from the drug were noted. Local irritation did not appear therefore to be a precipitating cause of the condition.

Vichi and Masi (1955) using rabbits have reported both gross and microscopic gingival changes in this species. These authors used the stomach tube method of administration and employed doses of 10-50 mg. daily in two divided doses for 20 days in 12 animals. The diet employed was made up of grass, wheat and corn. The histological changes shown by these workers however, do not appear to demonstrate the typical subepithelial cellular changes generally considered characteristic of diphenylhydantoin gingival hyperplasia. These observations therefore need confirmation,

From the above review of the literature, it is clear that although the occurrence of gingival hyperplasia in a variable proportion of epileptic patients under diphenylhydantoin therapy has been well established, the mechanism of its production is still obscure. However, the possibility that both gross and microscopic gingival changes similar to those observed in man, can be elicited in ferrets,would appear to be definitely established. It is also of some interest, that on some other species (rats and ginuea pigs) variable indications of histological changes have been reported, despite the absence of gross hyperplasia.

## EXPERIMENTAL OBSERVATIONS

### 1. Preliminary studies on various laboratory experimental animals.

Despite the negative findings of earlier workers concerning the production of gingival hyperplasia in ordinary laboratory animals, it appeared of interest to re-investigate the problem, using somewhat higher doses of the drug than those previously employed, and studying both young and old animals. In many of these experiments more prolonged administrations were also employed than hitherto. Attempt was also made to induce the condition by local injections into the gingival regions. In these and all subsequent experiments diphenylhydantoin (Dilantin) powder kindly supplied by Parke Davis and Co., was used.

#### (a) Effects of oral administration.

Using 24 hooded rats (6 retained as controls), 12 albino rats (3 retained as controls), 12 guinea pigs (3 retained as controls), the drug was administered by stomach tube. The rats and guinea pigs received a dose of 20 mg. in 2 ml. of saline, three times a week, while the control animals received only 2 ml. of saline. A dose of 2 mg. in 2 ml. saline of the drug was also given similarly to 6 hamsters and 3 controls received 2 ml. of saline. Administration was continued for a period of three months. All animals were kept on a normal Purina Chow Diet.

The results of the oral administration in these animals were essentially negative and can therefore only be summarized. During the entire period of observation there were no significant systemic changes in the treated or controls groups of animals. All, either continued to gain weight or maintained their weights satisfactorily. At the end of the observation period there was no gross difference detectable in the gingiva of the treated or control groups.

As these were only orienting experiments, no sections were made of the tissue of the hamsters, but in the case of the rats, all animals were killed with ether, and the mandibles removed. Small sections were then taken from the buccal-molar region of each animal, and placed in a 10% solution of formalin. Longitudinal strips were removed and after suitable embedding in paraffin blocks, were sectioned horizontally. All sections in these and other experiments were stained with haematoxylin and eosin, in the usual manner.

In none of these animals was any clear hyperplasia observed. However, occasionally the pathologist reported that mild hyperplastic tissue could be seen, but their exact significance were rather questionable. Since these changes occurred only in some animals and not in others and in view of the prolonged periods (3 months) of administration required, no further studies of this type were undertaken.

In a second group of experiments, weanling rats, both male and females, were placed on a rather high (10 mg.) dosage of dilantin, given three times weekly by stomach tube. During

the first two months the dose was further increased by 5 mg. up to 20 mg. three times per week. At the end of the three-month period there was no significant difference in the weights of the treated and control animals. In view of the difficulty of observing clearly the gingiva in these small animals, they were killed with ether, and the mandibles dissected out. Close examination of the gingiva revealed no gross abnormalities, and no significant difference in the two groups. No histological studies of these animals were also undertaken.

In a third group of experiments, 6 young rats weighing from 90-125 grams were maintained on massive doses (50 mg.-200 mg.) of dilantin suspended in saline. The suspension was given three times weekly by stomach tube for a period of one month. During the initial week, the animals received 50 mg. of the drug, during the second, third and fourth weeks - 200 mg. For several hours after the administration of these high doses the rats appeared in a stupor and occasionally showed "washing movements", but all survived the experiment. At the end of the period the animals were sacrificed and after removal of the mandible the gingiva examined grossly. The tissue appeared entirely normal.

In view of the high doses employed in these experiments, there is little doubt that the gingival changes associated with diphenylhydantoin are not simply acute toxic effects of the drug. It is also apparent that in rats, guinea pigs and hamsters, no gross gingival changes could be detected

after periods of one to three months of oral administration with the doses employed. Finally, young or old rats showed no significant differences in their responses to the drug.

(b) Effects of Local Injections.

Twenty-four adult albino rats weighing from 200-500 grams were used in these experiments. In view of the relative insolubility of dilantin and the fact that complete solution could only be obtained by addition of alkali to increase the pH to 11 or more, such solutions could not be employed, and consequently the drug was used as a suspension. A 2% suspension was made up in saline, and 0.2 ml. injected into the mucobuccal fold in the lower molar region on alternate sides, three times weekly. Eighteen animals received the drug and six were retained as controls, and received similar injections of 0.2 ml. of normal saline. Care was taken not to injure the gingiva itself, in order to prevent any cellular changes due to trauma. Despite the fact that a suspension of the drug was employed, there was no gross evidence of any local tissue reaction, although the experiment was continued for a period of four months. At the end of that time all animals (24) were sacrificed and the gingivae examined macroscopically after excision of the mandibles. Biopsies were taken from the gingiva, skin, kidney and spleen of the animals for histological study.

Microscopically, the gingiva and all the other tissues examined appeared to be quite normal in both groups of animals.

Microscopically, also all the tissues appeared normal with the exception of the gingival tissues, which showed varying degrees of what appeared to be hyperplastic changes.
In Figures 1 and 2 are shown microphotographs of sections taken from a normal untreated control saline-injected rat (Fig.1) and from a dilantin-injected animal (Fig.2). The reports of the pathologist on these were as follows: Figure 1, shows that "the epithelium is in orderly arrangement with cells of uniform size and shape. A few mitoses are present in the basal zone. The epithelium is supported by a thin rim of loosely arranged connective tissue, which is resting upon a muscle coat, and is essentially quite normal."
Figure 2 shows that " there is a thickening of the corium and epithelial tissue with an increase in number of superficial cells and more prominent mitoses in the basal zone. Rete pegs are elongated and ramify throughout the connective tissue. There is a patchy edema and focal collections of round cells in subepithelial areas. Finally, there appears to be a hyperplasia of the epithelium as evidenced by acanthosis of the epithelium, overcrowding of cells and possible increase in mitotic activity". While some of these differences might be due to non-specific changes resulting from the injections, this appears to be rather improbable, since the injections were not given directly into the gingiva, but only in the nearby muco-buccal fold. This technique therefore, appeared to offer some promise, but in view of the possibility of

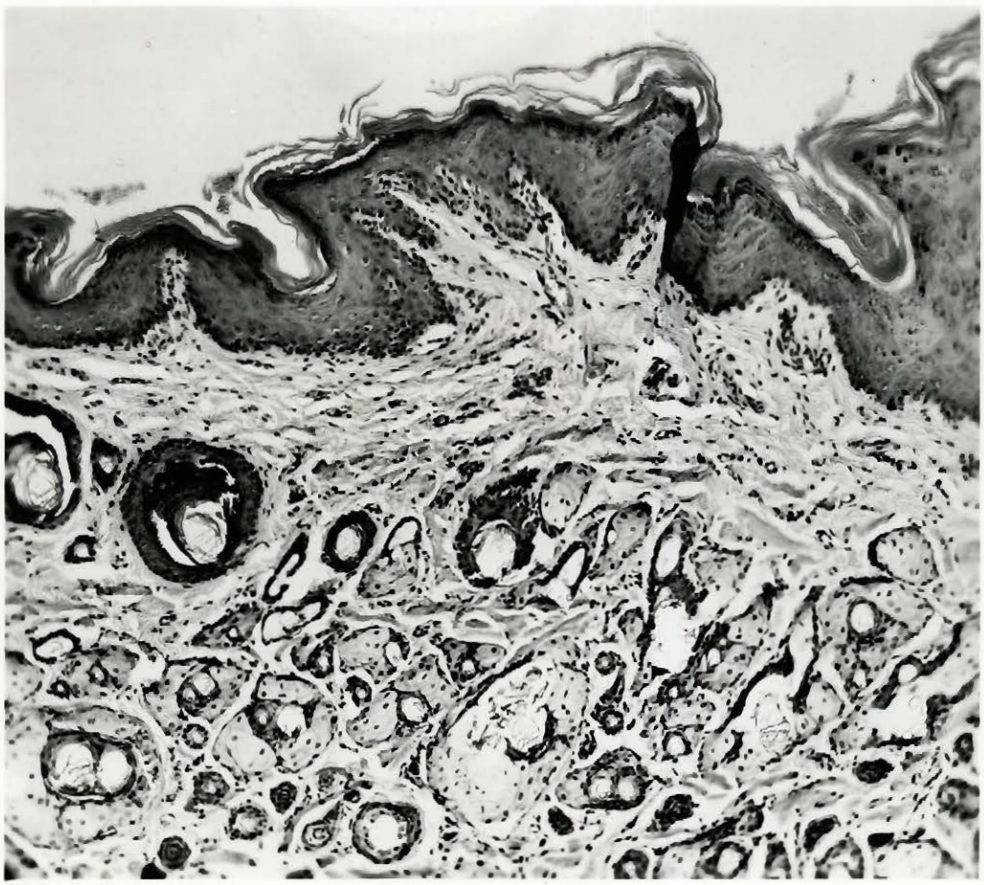


Fig. 1: Microphotograph of section of normal control albino rat gingiva, showing normal appearance of epithelium and underlying stroma.

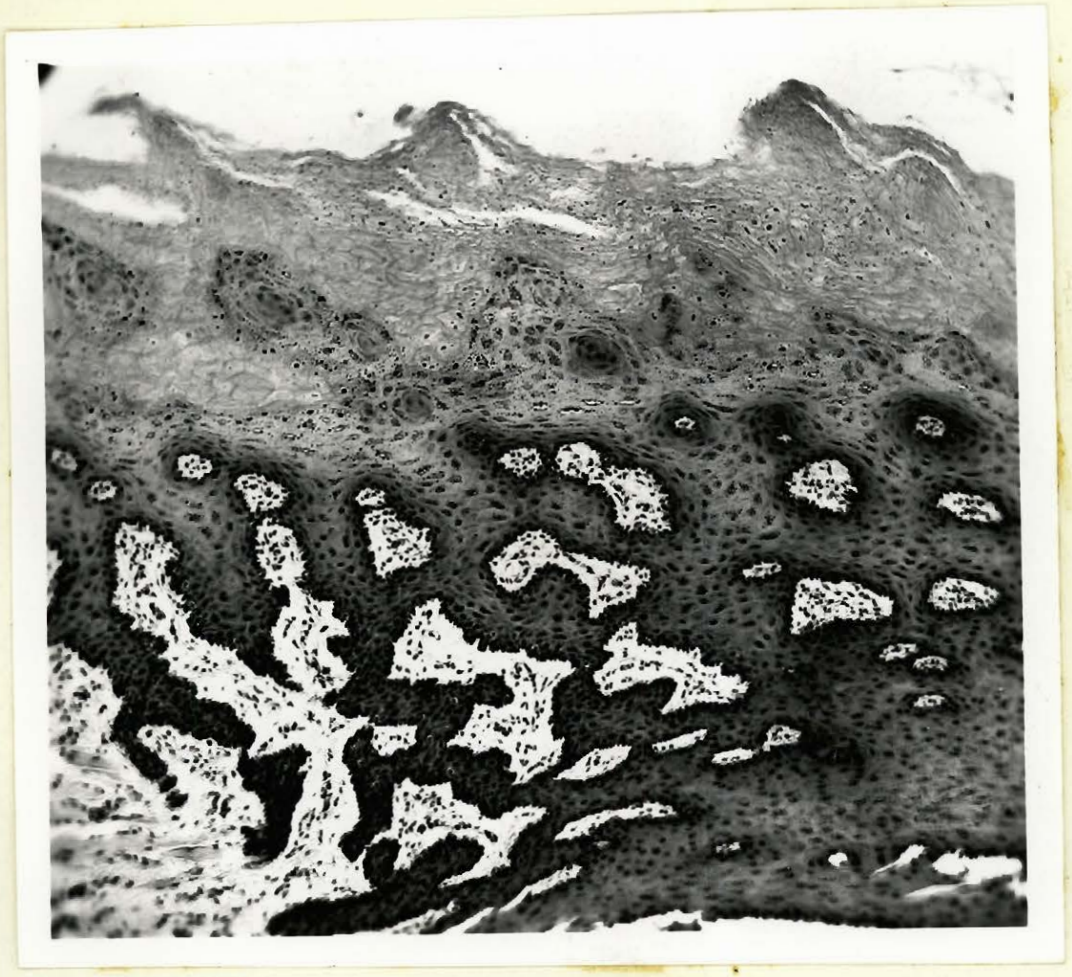


Fig. 2: Microphotograph of section of hyperplastic gingival tissue of dilantin-injected albino rat.

introducing repeated local irritations of the gums and since no gross hyperplasia developed, this route of administration was not pursued further.

II Studies on Ferrets.

A total of 22 ferrets of varying ages, sex, and weight were used. The ferrets were kept on a balanced diet consisting of Purina Chow Diet with Purina Chick Startina supplement, along with powdered milk. The amount of food necessary was calculated on a weight basis. From a calorimetric determination done on a sample of the diet*, it was estimated that one gram of food contained 1.67 Cals, and that 200 grams were necessary to maintain the weight and general good health of the animals. This allowed each adult ferret approximately 190 grams of Purina Chow and 10 grams of the supplement.

(a) General and Gross observations.

In the first series of studies 12 adult animals, consisting of 10 males and 2 females, were used. Each animal was kept in a separate cage in the laboratory for a preliminary period of 6 weeks prior to the beginning of the experiment, in order to establish a stable condition, and to make sure that no loss of weight ensued with the diet. The animals were divided into two groups of 6 each (5 males and 1 female). Five in each group were treated and one (male) kept as control. Group 1 received 40 mg. of dilantin per day in milk for a period of 60 weeks, while Group 2 received 200 mg. per day for a period of 20 weeks.

Results.

Group I. All animals remained in good health, with the exception of one in the treated group. This animal showed a

* (Courtesy of Dr. E.W. Crampton, MacDonald College)

sudden progressive loss in weight and hemorrhagic stools for some days before it died, after 12 weeks of drug administration. At autopsy, the stomach and intestines contained large amounts of blood. Whether or not this was due to the drug cannot be stated. In general, the dilantin-treated animals showed no evidence of any depression and were ostensibly no different in their appearance or behaviour from the untreated controls.

Group 2. During the nineteenth week the animals on the high dose level, began to lose weight and became rather lethargic. The drug was therefore stopped for one week, during which the animals appeared to return to normal. The drug was then continued for a further fortnight. All the animals, both treated and controls were alive and appeared normal at that time.

In regard to the gross changes in the gums, all the dilantin-fed animals in the above groups of experiment showed progressively increasing gross evidence of varying degrees of swelling of the gingiva around the maxillary molars. These changes began about the 32nd week of treatment in Group I, and culminated in visible overgrowth of the gingival tissue, as the experiment continued. The changes, however, began somewhat earlier (about the 12th week) in the animals on the higher dose (Group 2), but progressed quite similarly in both groups.

At the termination of the above experiments, all the animals were killed rapidly by a fatal dose of pentobarbital sodium (60 mg./kg.,). Gross examination of the gingiva, which

could now be more thoroughly done, revealed in the control animals a normal healthy appearance of the gingiva, and both molar and premolar teeth could be clearly seen. A typical example of the gross appearance of the teeth and gums of one of these ferret, photographed in situ, is shown in Figure 3. As can be seen the dense and sharp outline of the teeth are clearly discernable and the adjacent gums show a uniform appearance.

On the other hand, in the dilantin-fed animals, there was definite overgrowth of the gingiva in all cases. A typical example of this is shown in Figure 4. This was obtained from an animal receiving 40 mg. of the drug daily for 60 weeks. As can be seen the adjacent tissue also presents an irregular pattern but there is a clear demarcation between this area and the normal healthy tissue. There is a partial obliteration of the molar tooth and almost complete obliteration of the premolar, only the dense cusps of the teeth are seen. Around the molar and premolar, the overgrowths of the gums also showed definite lobulations.

A still more advanced stage in the gross gingival changes is shown in figure 5. This was obtained from one of the animals receiving a dose of 200mgm. daily for 20 weeks. Here, again it is seen that the overgrowth of gingiva adjacent to the teeth presents an irregular bulbous appearance and both molar and premolar are partly obliterated. The area between the teeth also strikingly shows excessive downgrowths of gingival tissue.

Since gingival hyperplasia has been reported to be more

Fig. 3: Photograph of maxilla of normal untreated ferret, showing normal appearance of molar and premolar teeth, and gingiva.

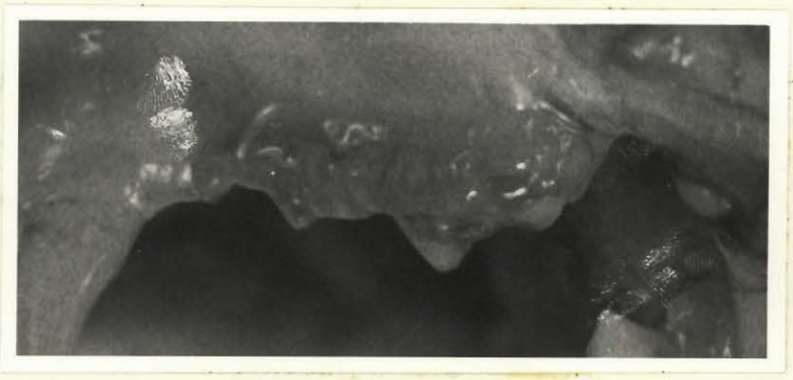


Fig. 4: Photograph of maxilla of dilantin-fed ferret, showing molar tooth partially covered and premolar tooth completely covered with hyperplastic gingival tissue. A dose of 40 mg. of dilantin sodium was administered orally daily for 60 weeks previously.

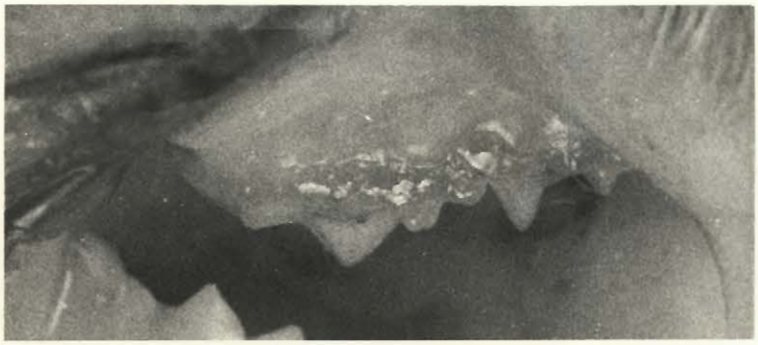


Fig.5: Photograph of maxilla of dilantin-fed ferret, showing partial obliteration of the crown of the molar tooth with lobulations of hypoplastic tissue between the molar and the premolar, and partial obliteration of the premolar tooth.

frequent in children and young adults than older people, in the second series of studies, ten young ferrets, approximately 8 weeks old and weighing from 500 to 700 grams, were used. After a preliminary 2-week period of observation, 8 of the animals were given the drug and 2 kept as controls. The initial dose of dilantin was 10 mg. per day, gradually increasing over a period of 2 months to 40 mg. daily. This dose was then maintained to the end of the experiment (60 weeks). The treated animals again showed no significant difference in general health or appearance from the controls. All of both groups showed similar growth curves.

In regard to the gross changes observed in the gums in this group of animals, these were rather similar to those previously described in the adult animals, and started after about 12 weeks of treatment. However, the changes progressed rather similarly to irregular bulbous overgrowths of the gingiva.

Figure 6 shows a grossly enlarged photograph of the upper and lower jaws of one of these animals. As can be seen there is a crowding of the molar and premolar, and the teeth are in essentially poor alignment. The teeth are surrounded by hyperplastic tissue, with downgrowths of gingiva distal to the upper molar, and between the cuspid and premolar teeth. A similar picture was observed in all of the treated animals on the other hand, as in the normal adult ferrets, the untreated controls in this series of experiments all showed regular dentition with normal

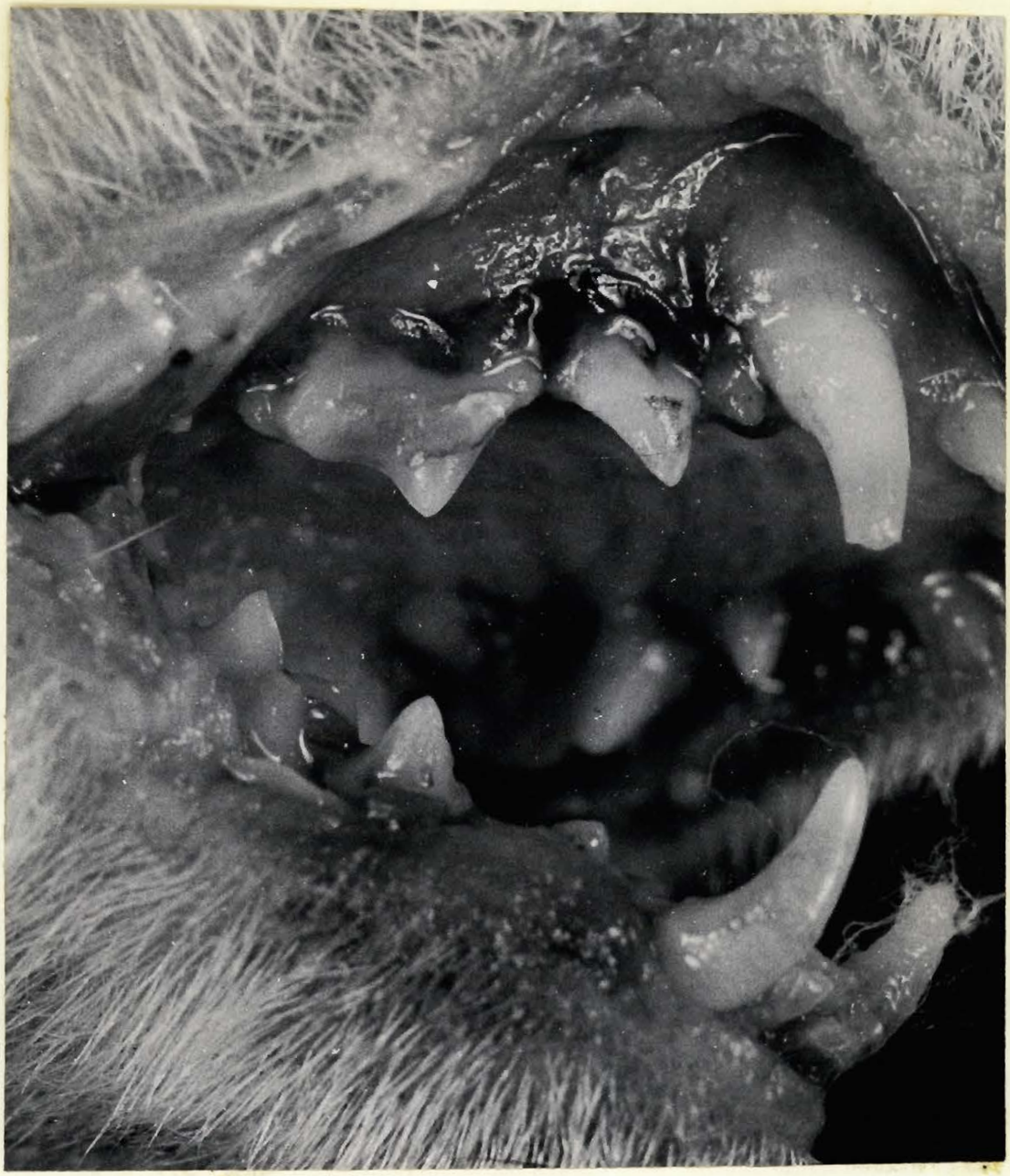


Fig. 6: Photograph (grossly enlarged) of jaws of ferret, showing crowding and poor alignment of teeth with hyperplastic gingiva, Dilantin started at age of 8 weeks, and continued for 60 weeks.

appearance of the gums. These observations would rather suggest that the fibrous state of the gingival tissue might be responsible for this apparent malocclusion.

(b) Histological observations on tissues.

Immediately after killing the animals, as indicated in the previous section (a) specimens of gingiva, skin, lung, liver, spleen, and kidney were obtained for histological studies.

Typical microphotographs obtained from sections of gingiva, obtained from these animals are reproduced in Figures 7 and 8. Figures 7 shows a microphotograph (90x) of a gingival section made from a normal untreated control ferret, as previously shown grossly in Figure 3. As can be seen, the normal stratified squamous epithelium shows characteristic downgrowths, but fairly regular demarcation between the epithelium and underlying connective tissue can be made out. Special attention is drawn to the relatively few cells scattered throughout the subepithelial tissue layer.

In contrast, figure 8 is a microphotograph (45 x magnification) of gingival tissue obtained from a dilantin-fed ferret (same animal as in Fig. 5). It can be seen that there is a complete invasion of the underlying connective tissue by epithelium masses, which appear as isolated islands. The superficial layer of epithelium is also obviously relatively thickened and in some instances this was as much as 6 or 8 times that of the normal.

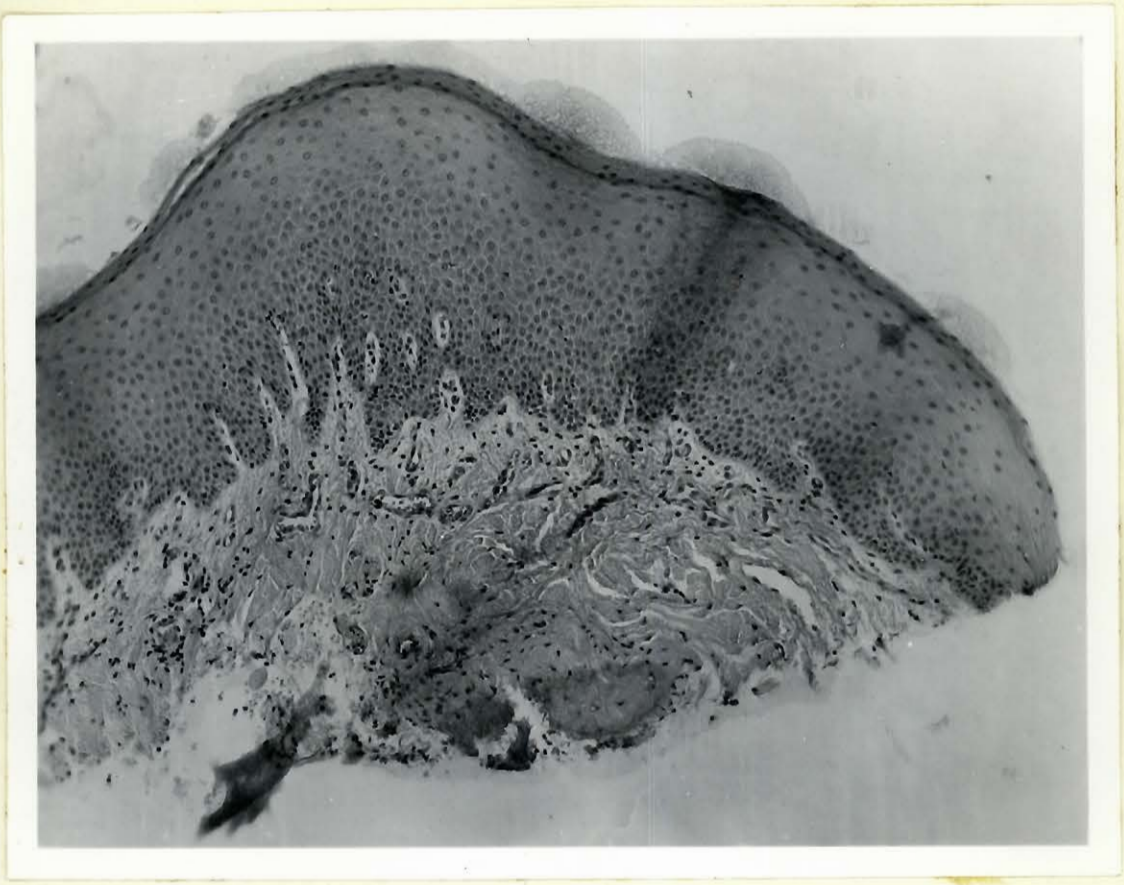


Fig. 7: Microphotograph of section of normal untreated ferret gingiva (same animal illustrated in Fig. 3), showing normal appearance of epithelium and deeper tissue. Magnification 90 X. (See text.)

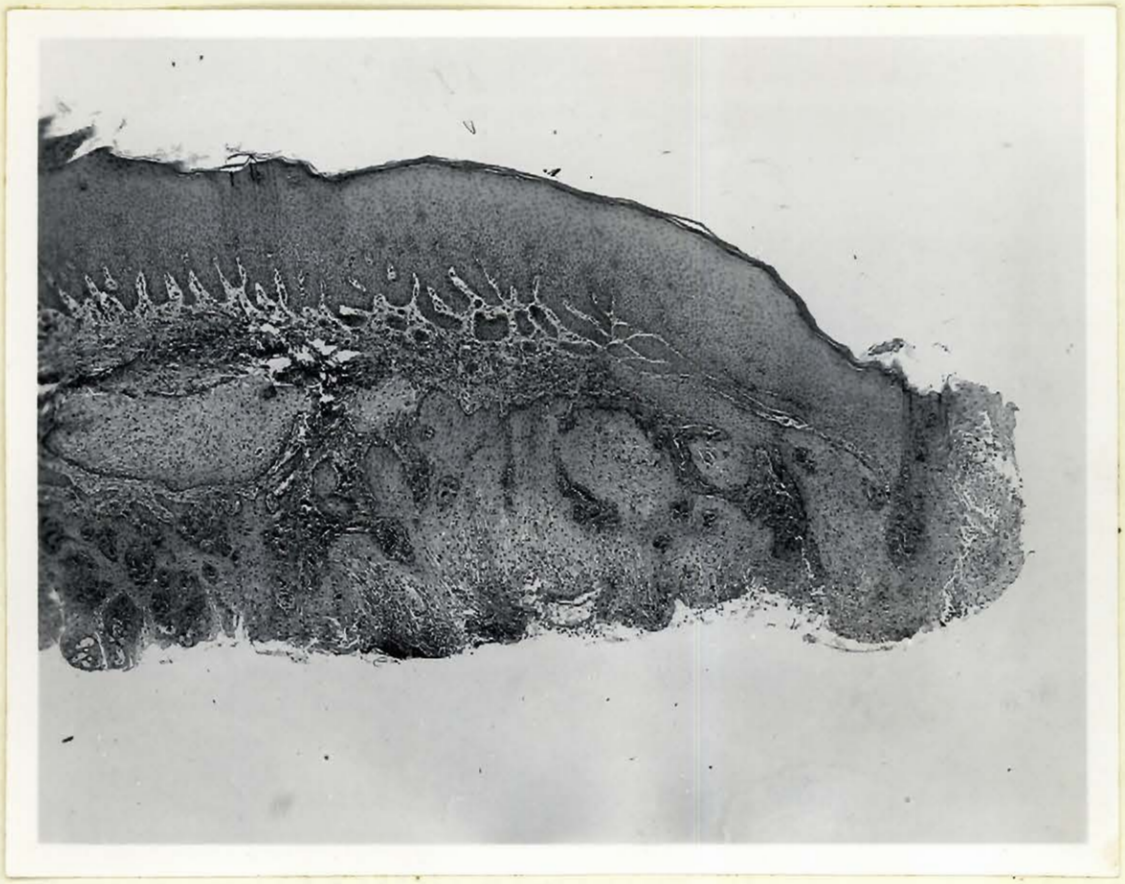


Fig. 8: Microphotograph of section of hyperplastic gingival tissue of dilantin-fed ferret (same animal illustrated in Fig. 5) Magnification 45 X. (See text.)

Indeed, in one of the sections obtained from this group of dilantin-fed animals, the histopathological report stated that "the complexity of the interdigitations of the rete pegs with the papillae of the underlying connective tissue is so complex that the tissue bears a superficial resemblance to a low grade squamous cell carcinoma." However the tissue was not considered neoplastic.

In regard to the microscopic examinations of the other tissues, the skin, liver, spleen, and kidney showed normal histological appearances. On the other hand, the pathological report on the lung was as follows:"the tissue shows patchy areas of atelectasis. The pulmonary tissue itself is also mildly hyperaemic, but shows no abnormality, except for the presence of scattered histiocytes containing moderate quantities of very finely divided brown granular pigment. The bronchi and bronchioles in the section show no abnormalty, except for occasional areas showing an apparent increase in both the number and size of the lining epithelial cells. In these instances, the bronchial epithelial cells are markedly vacuolated but their nuclei appear normal and are not encountered in mitosis. There is no evidence of inflammation." The question arises whether or not these changes are of any real significance. More will be said about this later.

It is however clear, that with prolonged administration of dilantin sodium to the ferret, gingival hyperplasia similar to that observed in man on dilantin therapy, can be

induced experimentally. The skin, liver, spleen and kidney of these animals show no gross or histological changes. However, some suggestion of hyperplasia in the lining epithelium of the bronchi and bronchioles was occasionaly observed in these animals. It therefore appeared that ferrets would be a satisfactory species for the study of gingival hyperplasia, but the prolonged periods of administration of the drug necessary was a decided disadvantage in attempting any widespread pharmacological investigation of the mechanism of the phenomenon.

(c) Observations on changes in extractable gingival histamine in normal and dilantin-fed ferrets.

For reasons already pointed out, it was of interest to make a comparative study of the histamine content of gingival tissue extracts, prepared from the normal and the dilantin-fed ferrets, which were employed in the foregoing sections (a and b).

In view of the small quantities of tissue (only about 25 mg.) obtainable from a ferret it was necessary to pool the tissue from four ferrets in each group. Immediately after the animal was killed, the gingiva was excised from around both the maxilla & mandibles. The total quantity of tissue used in each experiment was approximately 100 mg.

The procedure employed for extraction and assay of histamine in these and all other similar experiments was that described by Barsoum and Gaddum (1935), and was essentially as follows:- The excised tissue was placed on a weighed tinfoil plate, which was reweighed on a torsion balance, and quickly transferred to a mortar. Within a period of one minute, 0.5 ml. of HCl was added to cover the tissue. Granulated sea sand (approximately 1 gram) was added, after the tissue was cut into small pieces. The material was then ground with a pestle until a homogeneous mass was formed. The contents of the mortar were then transferred to a 40 ml. tube and washed in with 10 ml. of saline. The tube was placed in a boiling water bath for a period of 60 minutes, occasionaly washing down the sides with saline. The contents were then centrifuged for 5 minutes at 2700 r.p.m., or until the supernatant fluid was clear. After transferring the

supernatant to a 25 ml. volumetric flask, the solution was neutralized to pH 7 with N/3 NaOH, the pH being determined with a pH meter. The solution was then made up to a volume of 25 ml. with normal saline, except where otherwise stated.

The assays for histamine were carried out on the excised guinea pig ileum (approximately a one inch strip) suspended in a 4 ml. bath containing atropinized Tyrode solution of the following composition: NaCl-0.8%, KCl-0.02%, $CaCl_2$-0.01%, $NaHCO_3$-0.1%, Glucose-0.1%, $MgCl_2$-0.01%, $NaH_2PO4$-0.005%, and atropine sulfate-0.00002%. The bath was kept at a temperature of between $37^0$ and $39^0$ C and aerated continually throughout the experiments. Histamine dihydrochloride (Hoffman-La Roche) in aqueous solution was used as the standard. Two standard solutions were usually employed, the one containing 10 micrograms per ml. and the other containing 1 microgram per ml. At the end of most experiments the effects of additions of the antihistamine agent mepyramine (0.1 ml. of $1.2 \times 10^{-6}$ concentration), upon the responses to both the standard and the unknown extract, were determined. Figure 9 shows the results of an assay of gingival extracts obtained from normal untreated and dilantin-treated ferrets, which had previously developed gingival hyperplasia. These extracts were prepared from the gingiva of animals showing the gross changes described earlier in fig. 4 and 5. The responsivness of the gut was first

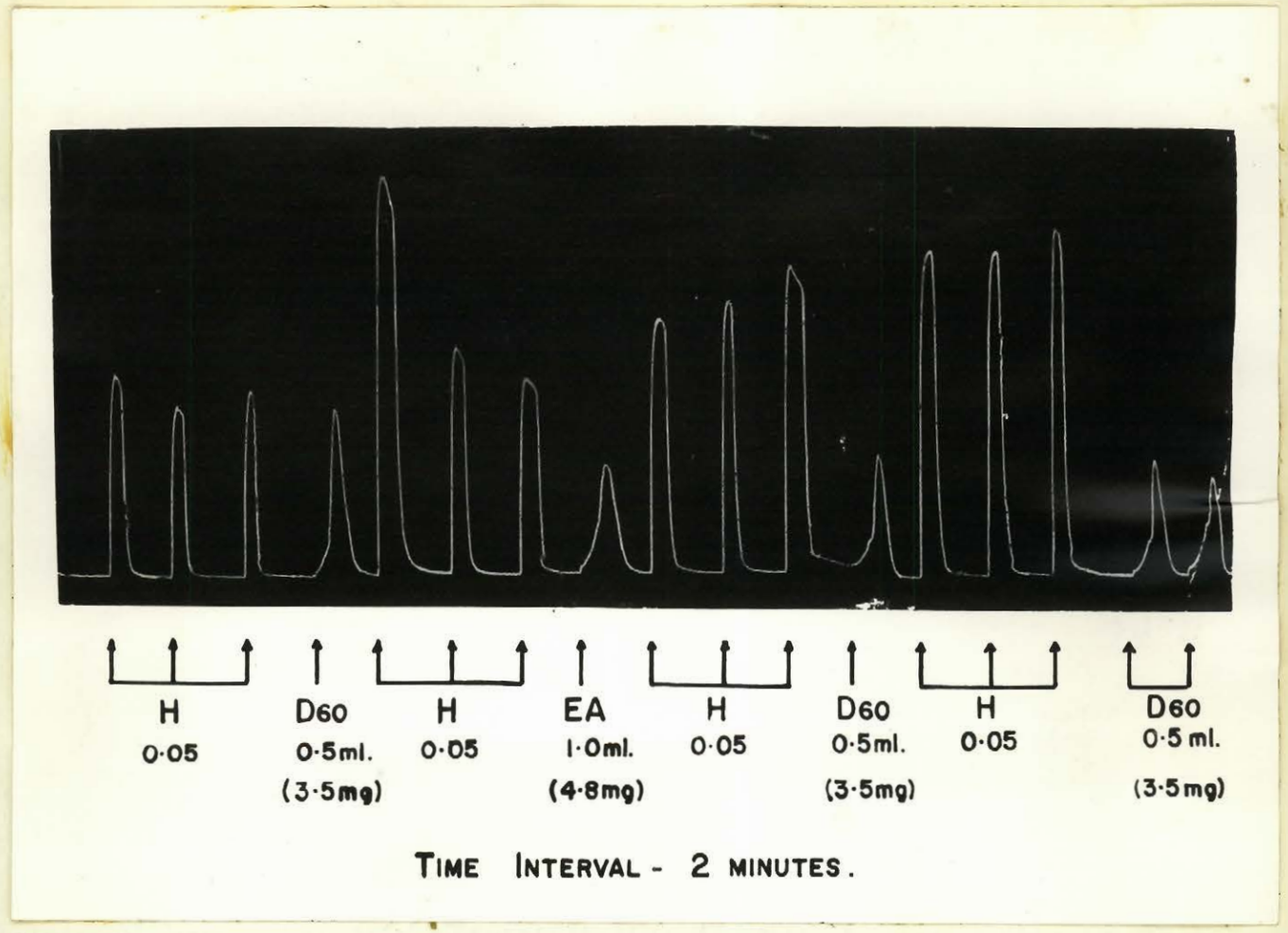



Fig. 9: Histamine assay on guinea pig ileum, showing effects of dilantin-fed ferret extracts (D60), and responses to 0.05 microgram of histamine (H/0.05), also, responses to extracts from normal control ferrets (EA). In these and all succeeding figures on histamine assays, an interval of 2 minutes elapsed between each test of extract or histamine.

standardized, using a 0.05 microgram dose of histamine (H/0.05) the injection was repeated 3 times and led to approximately the same responses. The gingival extract from 60-weeks dilantin-fed hyperplastic ferrets (D 60) in a dose of 0.5 ml. containing the equivalent of 3.5 mg. of tissue, led to a somewhat similar contraction, as that to the preceding histamine (0.05 microgram ) and appeared to be a typical histamine response. On the basis of this observation it was calculated that the gingival tissue of the dilantin-fed ferret contained approximately 14 micrograms per gram of histamine or histamine-like substance.

It is also shown in the figure (Fig. 9) that following the initial test of the extract, the next subsequent test response to the standard histamine (H/0.05) is greatly potentiated. After 2 additional standard tests of the same dose, responses similar to those previously observed however, were obtained. At this point an injection of the extract prepared from the normal untreated animals was tested, using 1.0 ml.(EA) of the extract, this contained the equivalent of 4.8 mg. of tissue. It can be seen from the slope of the contraction that this response was somewhat delayed, and slightly less intense, although the quantity of tissue from which it was obtained was greater than that used in the extract (D60) prepared from the dilantin-fed animals. On the basis of this response the normal gingival tissue of the ferret was estimated to contain approximately 5 μg./ gm. of histamine or histamine-like substance. When the standard histamine dose (H/0.05) was again tested there

was also no potentiation, although succeeding responses were somewhat gradually increased. This latter effect might however, be due to a progressive change in the response of the strip to histamine, since throughout the remainder of the experiment no restoration to normal sensitivity was observed. It must be added that it is difficult to draw conclusions from responses obtained in long experiments of this type, but all of the records clearly show that the responses to histamine after the tests with the extracts from the treated animals, were rather different from those observed with the control extracts. The record also shows that two later repetitions (end of tracing) of tests of the extract from the dilantin-fed animals (D 60) , induced quantitatively reproducible responses.

In conclusion, it is shown that (a) the extract obtained from normal gingival tissue of ferrets contains histamine or some histamine-like substance, (b) that similarly prepared extracts from dilantin-fed animals showed a relatively higher concentration of this substance, and (c) that after the test doses of the latter extracts the responsiveness of the preparation to histamine appears to be potentiated. In view of the limited supply of the gingival tissue obtainable from ferrets, and the prolonged period of administration of the drug necessary to induce hyperplasia in the animals, it was felt that some other animal species might be more suitable for extension of these observations.

## III Studies on Dogs.

Due to the difficulty in obtaining adequate amounts of gingival tissue from the animals used in the preceeding experiments, it was felt that more extensive study of the problems might be achieved using the dog as the experimental animal. A total of 47 dogs were used in these experiments. Both males and females were employed and the animals ranged in weight from 6.8 - 17.8 kilos. Each animal was kept in an individual cage and observed for at least one week in the laboratory before any treatment was started. The animals were kept on a Purina Chow diet with water ad. lib. The drug was administered mixed with a small quantity of meat, which the animal ate each morning before the remainder of the food for the day was placed in the cage. Total doses of dilantin varying from 40 to 200 mg. were studied and the duration of administration was from 7 to 63 days in different groups of experiments.

### (a) Observations on changes in extractable gingival histamine in normal and dilantin-fed dogs.

None of the dogs which were given the drug showed any gross changes in the gingiva or other tissues, which could be considered abnormal. The general procedure employed for obtaining the tissue in ferrets as described above (Section 1c) was again employed in these experiments. However much larger quantities of gingiva were available and amounts varying from 250-570 mg. were excised from different animals. The animals were also simply anesthetized with pentobarbital

sodium (35 mg./kg.) intravenously and the mouth swabbed out with physiological saline. In most experiments after the removal of the section of gingiva the animal was cared for and allowed to survive, and could then be employed in later studies.

In order to compare the histamine content of the gingiva with that of other tissues in the same animals, comparable amounts of ileum and lung tissue were excised at the same time, and similarly extracted and assayed. In addition in this group of experiments, the extracts were also compared with histamine for their effects upon the blood pressure of the etherized cat. The blood pressure was recorded directly from a carotid artery on smoked paper with a kymograph in the usual manner.

Figure 10 is an example of an assay of gingival extract obtained from a normal untreated control dog. The contractions obtained from 10 microgram doses (H/10) and 2 microgram doses (H/2) of the standard histamine solutions, are first shown. The preparation gave no response to a histamine dose of 0.3 micrograms (H/0.3). As can be seen two injections of doses of 0.3 ml. of normal gingival tissue extracts (E/19), induced a greater contraction than that obtained from 2 micrograms of histamine (H/2), but less than that obtained from 10.micrograms (H/10). The second response $t_0$ the extract was however somewhat less than the first.

Following the exposure of the ileum to these gingival extracts, the figure also shows that for some time, there was

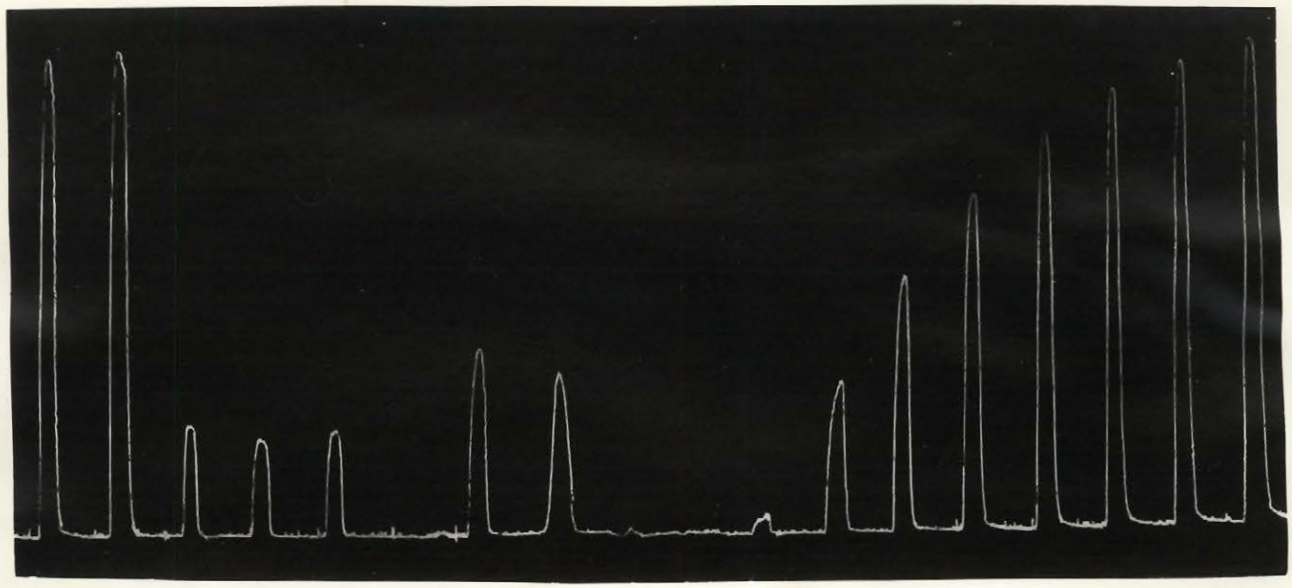



Fig. 10: Histamine assay on guinea pig ileum, showing effects of normal untreated dog gingival extract (E19), and response to 10 and 2 micrograms of histamine (H10 and H2) before, and 10 micrograms of histamine (H10) after, testing the extract.

no response to even the 10 microgram dose of histamine (H/10). However with repeated washings and repetitions of the standard histamine there was progressive restoration of the normal responsivemess of the gut to histamine (end of tracing).

It should also be added that in all of the tests in which extracts of normal gingival tissue were studied, the initial response generally developed somewhat more slowly than the response to histamine and was generally decreased on repeated testing. In some instances, the effect was even completely abolished on repetition, and concomitantly the control responses to histamine were similarly diminished or prevented.

In figure 11 is shown an example of another similar assay from an extract obtained from normal gingival tissue. Following four successive repeated tests of the same extracts (E/13), there was a progressive lessening of the response. In addition the record also shows two successive responses from an extract (E 13a) prepared from the gingivae taken from the opposite side of the mouth of the same animal. These again showed a peculiar decreased response on repetition. Again, the preparation was almost completely non-responsive to histamine but on repetition there was a progressive increased responsiveness, as in the previous figure (Fig. 10). Similar results to those shown above have been observed in a large majority of experiments.

From the assay data obtained from 12 normal dogs, it was calculated that the quantities of histamine or histamine-

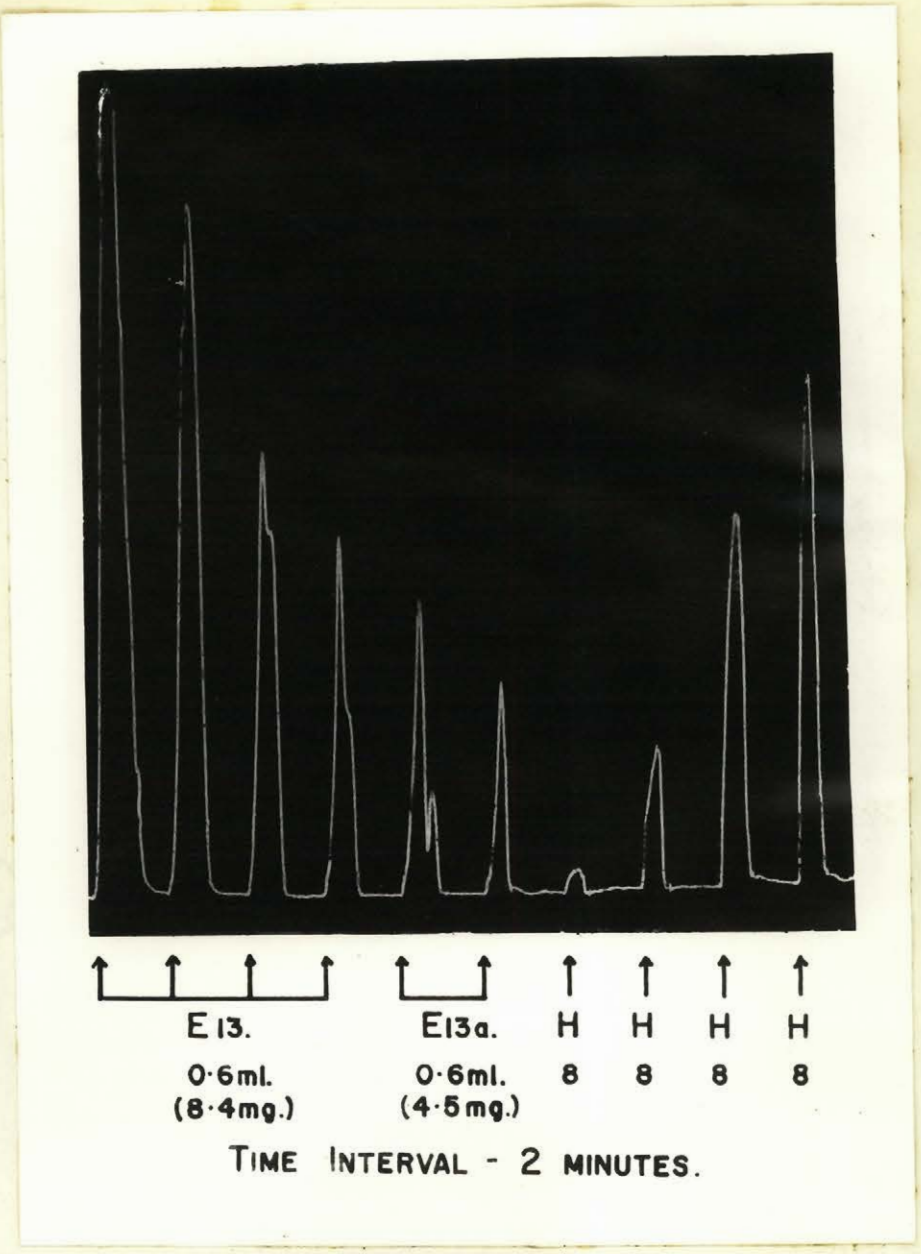



Fig. 11: Histamine assay on guinea pig ileum, showing diminishing effects of repeated tests of normal dog gingival extracts (E13 and E13a), also responses to repeated 8 micrograms test doses of histamine (H/8).

like substance present, ranged from 3 to 89 micrograms per gram of gingival tissue, with a mean of 22.4 micrograms per gram. These calculations were all based upon the initial response to the extract, and it is obvious that, since the response decreased with subsequent repetition, they may not represent true values, but were nevertheless of some interest when compared with similar data obtained from the dilantin-fed animals, as will be pointed out later.

Figure 12 shows an example of an assay of an extract obtained from the gingiva of a dilantin-treated animal which had received 200 mg./ day for 22 days.The responsiveness of the gut was again initially standardized, using a 1 microgram dose of histamine (H/1). This injection was repeated three times and led to identical responses. The first test of the gingival extract from the dilantin fed dog (D/22) in a dose of 0.6 ml. led to the striking response shown. Indeed, on repetition even a much smaller dose (0.2 ml.) of the extract elicited a considerably greater response than the standard histamine doses of 1 microgram as previously shown (H/1).

It can also be seen that following these tests of the extract the responses to the standard histamine (H/1) are greatly potentiated.This is in marked contrast to the diminished effects to histamine observed after tests of extracts made from normal gingival tissue. Indeed at the end of the experiment the histamine responses were still increased and considerable time was generally required before return of the gut to its normal sensitivity to histamine in such experiments.

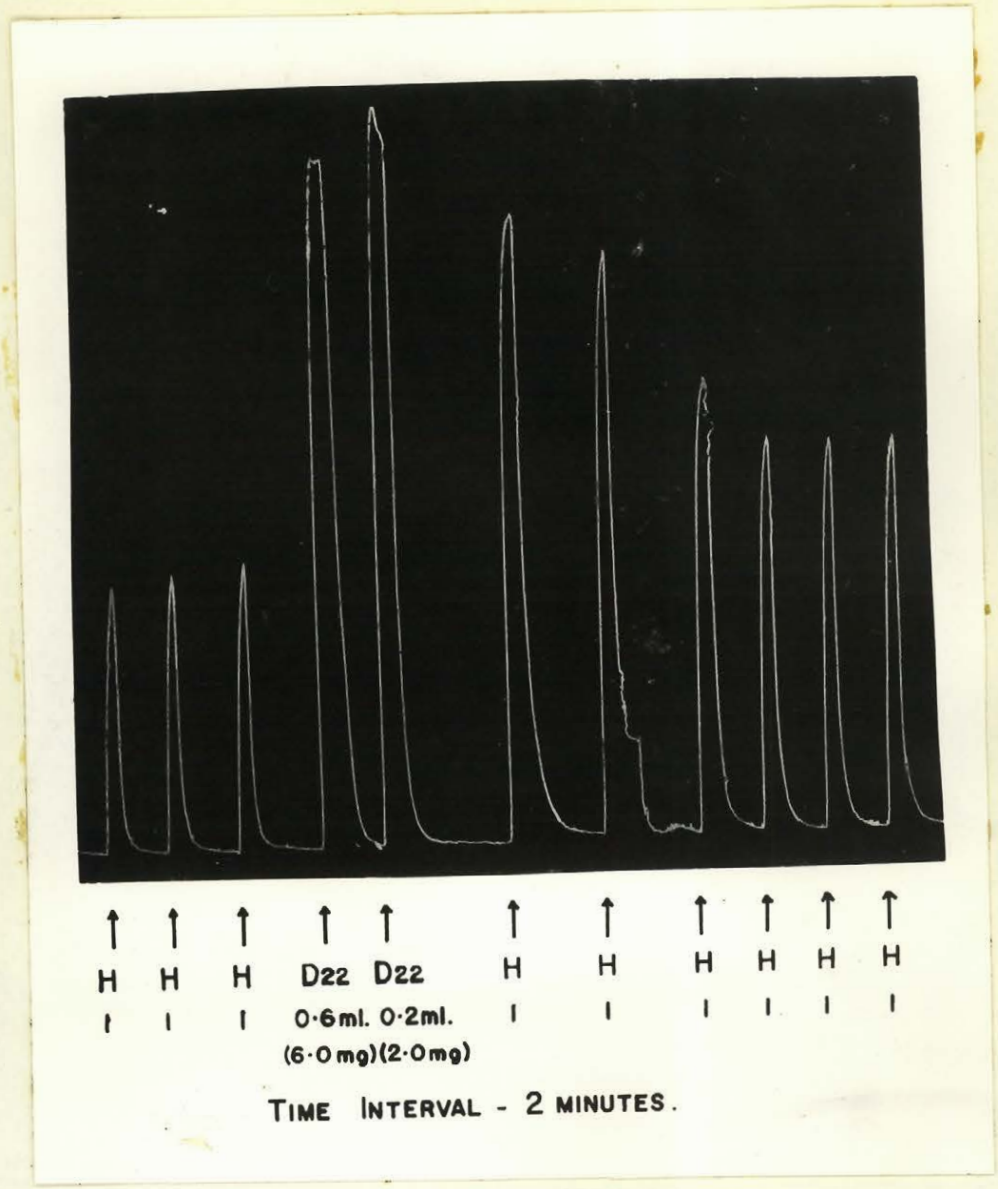



Fig. 12: Histamine assay on guinea pig ileum, showing effect of dilantin-fed dog gingival extract (D 22), and responses to 1 microgram doses of histamine (H/1) before and after testing the extract. A dose of 200 mg. of dilantin sodium was administered orally daily for 22 days previously.

It was of some interest to compare the effects of different gingival extracts obtained from dogs treated with dilantin for varying periods of time. In Figure 13 is shown the assay result with an extract obtained after only 14 days of dilantin. As can be seen again, there is a strongly positive repeatable histamine response, followed by potentiation of the effects of the standard histamine doses. This figure needs no further comment.

Figure 14 shows the responses to an extract obtained after 46 days of dilantin. As can be seen, the responses obtained from 4 initial successive tests of the extract (D/46) were quantitatively repeatable. A later addition to the bath of the same quantity of extract (D/46), towards end of record) also showed an augmented response. In this experiment, the histamine responses recorded throughout are all identical and obviously maximal, so that no evidence of potentiation is demonstrable. It is, however, clear that unlike extracts from normal gingiva, the successive histamine responses are definitely not diminished.

The question arises whether or not these histamine-like responses of the extract are really due to histamine or some other substance in the extracts. There seems to be little doubt on this point. At the end of the assays in numerous experiments it could readily be demonstrated that the response to the extract like that to histamine was blocked by the antihistamine agent, mepyramine (see Figs. 16 and 21). Moreover, as can be seen from figure 15, where

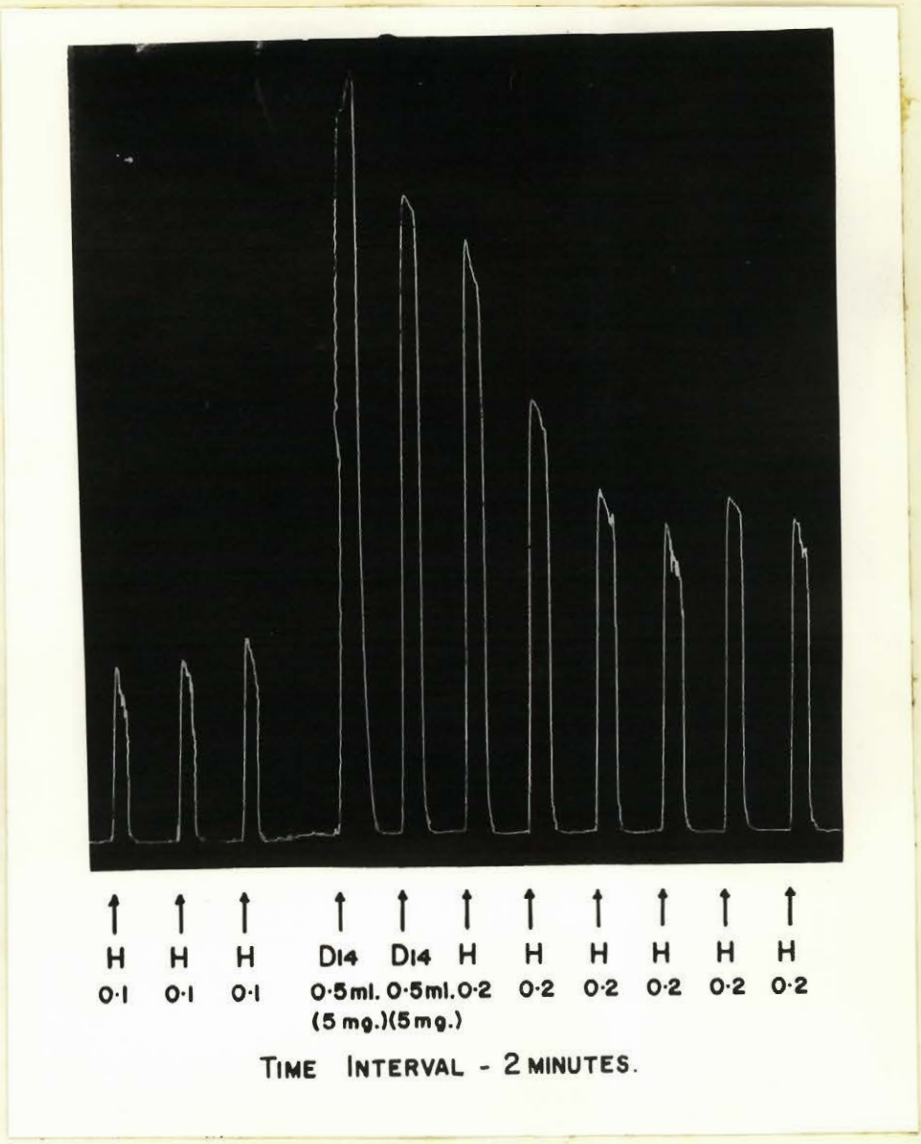



Fig. 13: Histamine assay on guinea pig ileum of extract prepared from a dog treated with dilantin for 14 days (D14) and showing potentiation of the effects of standard histamine doses (H/0.2).

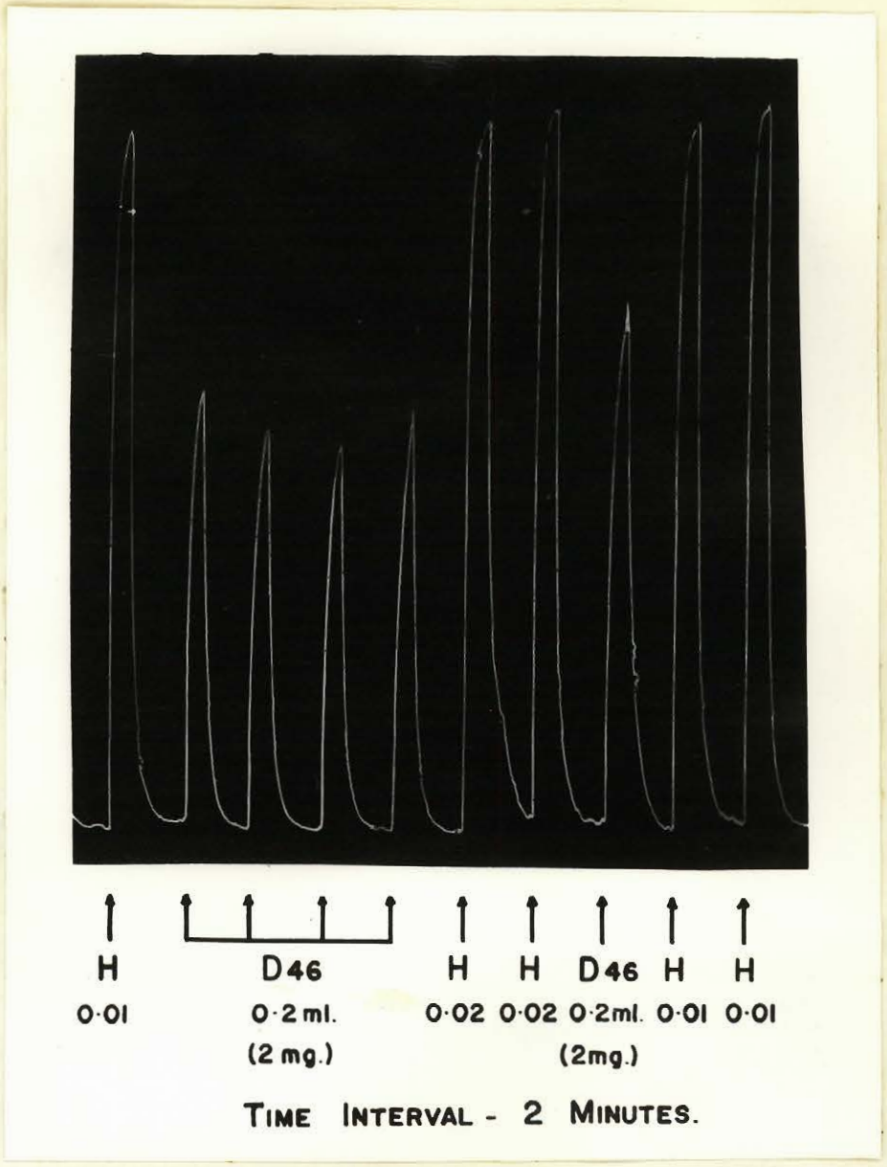



Fig. 14: Histamine assay on guinea pig ileum, showing repeated effects of a gingival extract from a dilantin-fed dog (D 46) and responses to 0.01 and 0.02 microgram doses of histamine (H/0.01 and H/0.02).

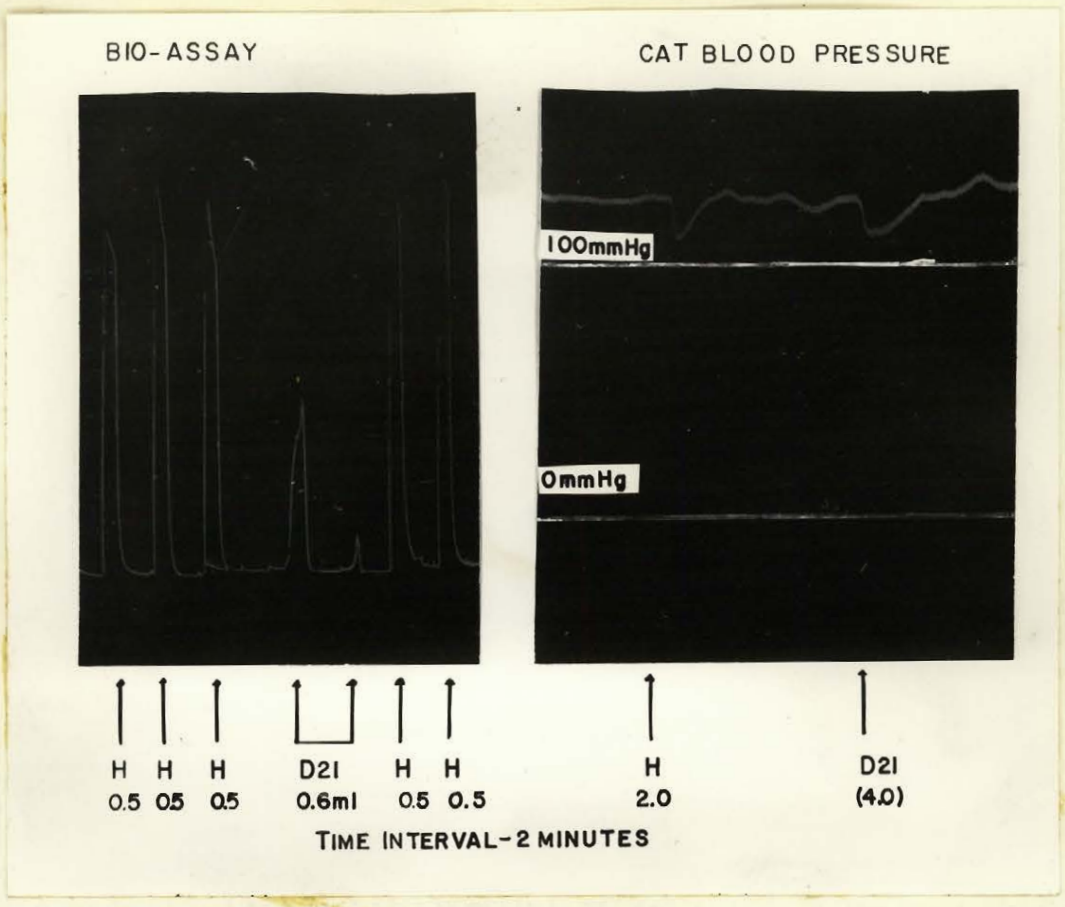



Fig. 15: Left: Histamine assay on guinea pig ileum showing repeated effects of a dilantin-fed dog gingival extract (D 21), and responses repeated to histamine (H/0.5). Right: Blood pressure record of etherized cat, showing depressor responses to the same extract (D21/4.0) and to 2 micrograms histamine (H/2), injected intraveneously.

the effects of the same extract (D/21) are compared, using two different procedures - the cat blood pressure and the guinea pig ileum. In both tests the responses were similar to those of histamine. Although the tests were performed several days apart, the calculated amounts based on the data shown were quite comparable (2 micrograms/gm on the ileum and 1.46 microgram/gm. on the blood pressure). Obviously, absolute identification of the extracted material as histamine, would require chemical identification. However, the quantities involved are so small, that their estimation presents a problem, which it was not considered essential to embark upon at this time. In addition, the extraction procedure and assay methods employed in this study have been extensively used by numerous workers and are generally accepted for quantitative estimations of the histamine content of tissues.

Similar results to those shown above have been observed in most experiments. However, varying quantitative degrees in the changes described, were noted in different animals, although on dilantin for the same periods of time. It did not appear therefore that these changes were related or dependent upon the dosage and duration of treatment. Extracts obtained from animals treated for shorter periods (up to 14 days) also showed no significant difference from those of the control group.

Finally, from the assay data obtained in tests performed on extracts from 12 dilantin-treated dogs, it was calculated that the gingiva contained quantities of histamine or histamine-like substances varying from 6 to 240 micrograms per gram of tissue, with a mean of 87.7 micrograms per gram. As in the control experiments, these calculations were based upon initial responses to the extract, which were usually quantitatively repeatable. The figures therefore appear to represent real values. It may be concluded therefore that there is a significant increase in extractable histamine in these animals as compared with the controls.

In summary, extracts of the gingival tissue obtained from dogs treated with dilantin for periods of at least 14 days, show (a) a relative increased concentration of extractable histamine as compared with controls, (b) that the test responses (guinea pig ileum) to these extracts are also repeatable, in contrast to those obtained from the controls, and (c) that previous addition of the extract to the preparation leads in general, to potentiation of the response to histamine, in contrast to antagonism following normal gingival tissue extract.

(b) Observations on changes in extractable gingival histamine in dogs fed either mesantoin or phenobarbital.

In view of the close chemical relationship of mesantoin (3-methyl-5-ethyl-5 phenylhydantoin) to dilantin (diphenylhydantoin) and in view of the claim that this agent does not induce gingival hyperplasia in man, it was of interest to compare the histamine extracts of the gingiva from dogs treated with this agent, with those observed with dilantin.

Figure 16 shows an example of an assay of the extract (M/32) obtained from a dog maintained on mesantoin (100 mgm. daily orally for 32 days).

As can be seen after the initial histamine standardization (H/0.1) an injection of 0.6 ml. of the gingival extract (M/32 - 0.6 ml.), led to a slight response, while a second and third similar injection showed no response at all. Immediately following this, six administrations of the standard histamine (H/0.1) were repeated. On the initial of these there was no response, but on repetition progressively increasing responses were observed. Even after six such repetitions the original responsiveness of the gut was still not completely restored (H/0.1 at end of section 7). Indeed, another period of ten minutes was necessary to restandardize the gut. These results are essentially similar to those previously seen with the use of normal gingival tissue, and need no comment.

In the second section of the figure (after 10 mins)., the normal responsiveness to histamine (H/0.1) was again elicited. The next three injections show responses to 0.6 ml. of an extract (L-M32 0.6 ml.) of <u>lung</u> obtained from the same dog, at the same time as the gingiva, and similarly extracted. As can be seen, the responses to the lung extract are almost quantitatively repeatable, and compare fairly well with the three succeeding test injections of the standard (H/0.1, middle of section).

The tracing also shows similar effects from three repeated administrations of an extract (I-M32 0.6 ml.) prepared from the ileum of the same dog. However, there was a slight initial de-

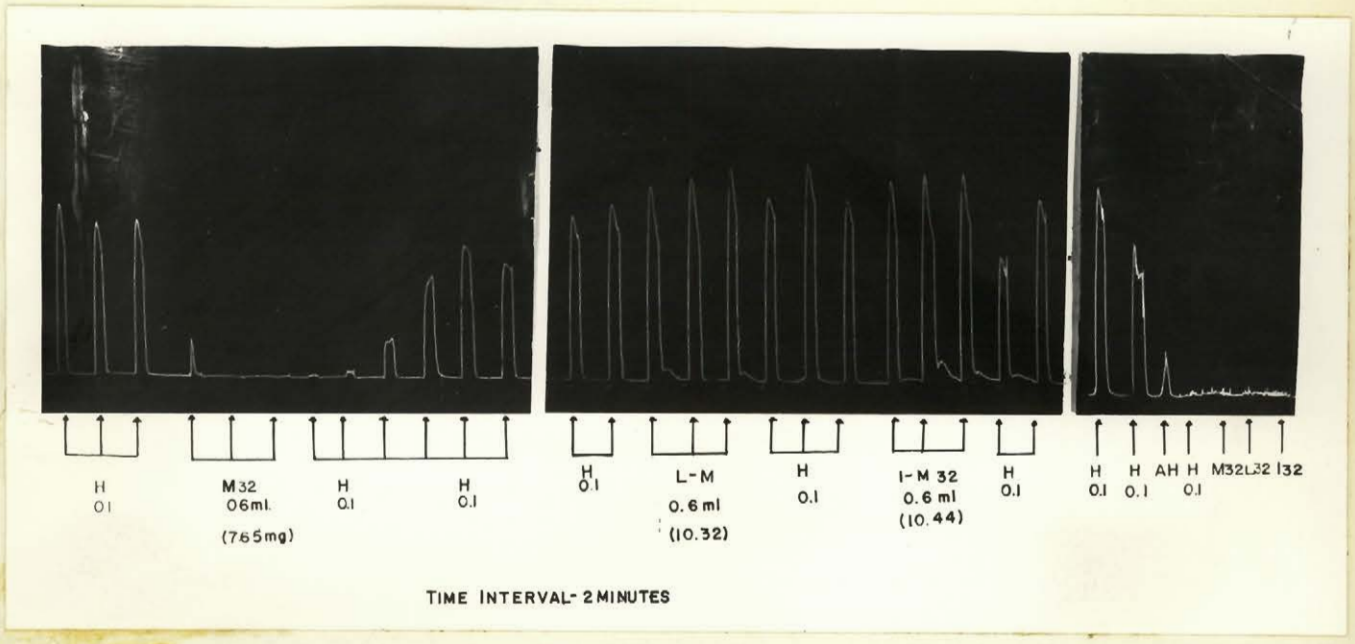



Fig. 16: Histamine assay on guinea pig ileum showing in the first section the effects of a mesantoin-fed dog gingival extract (M 32) and the responses to 0.1 microgram doses of standard histamine (H/0.1). In the second section can be seen the responses to the standard (H/0.1) and to similarly prepared lung extracts (L-M32) and ileum extracts (I-M 32). The last section shows the lack of responses to all three extracts (M32, L32, and I32) to the standard histamine (H/0.1), after treating the gut with mepyramine maleate (AH).

pression of the first standard histamine injection following these,as shown at the end of the section. Finally, 5 mins. later (third section of record) good responses to histamine (H/0.1) were still observed, but following mepyramine, all four subsequent administrations shown, that is, of standard histamine (H/0.1), gingival extract (M/32), lung extract (L/32), ileum extract (I/32 ), led to no responses. Similar results were obtained in four different experiments.

It is concluded therefore that the closely related agent, mesantoin, which has not been shown to lead to hyperplasia in man, also leads to no significant change in histamine or histamine-like substance extractable from the gingiva.

However, the other tissues examined (lung and ileum) showed their characteristic high histamine contents. It would therefore appear that gingival tissue is rather different in its response to dilantin than the other tissues. However more data would be required before a definite conclusion on this point can be drawn.

Figure 17 shows a typical example of an assay of an extract obtained from the gingiva of a dog given phenobarbital sodium (100 mgm. daily for 36 days). Again the gut was initially standardized using a 1 microgram dose of histamine. This led to three similar repeatable contractions. The gingival extract from the phenobarbital-fed dog (P/36) in a dose of 0.6 ml., repeated three times, led to no response of the gut. In addition, following this the ileum was completely irresponsive to the standard histamine (H/0,1 mg.) twice repeated. Using a higher dose (3 micrograms) of the standard histamine (H/3) elicited a small contraction.

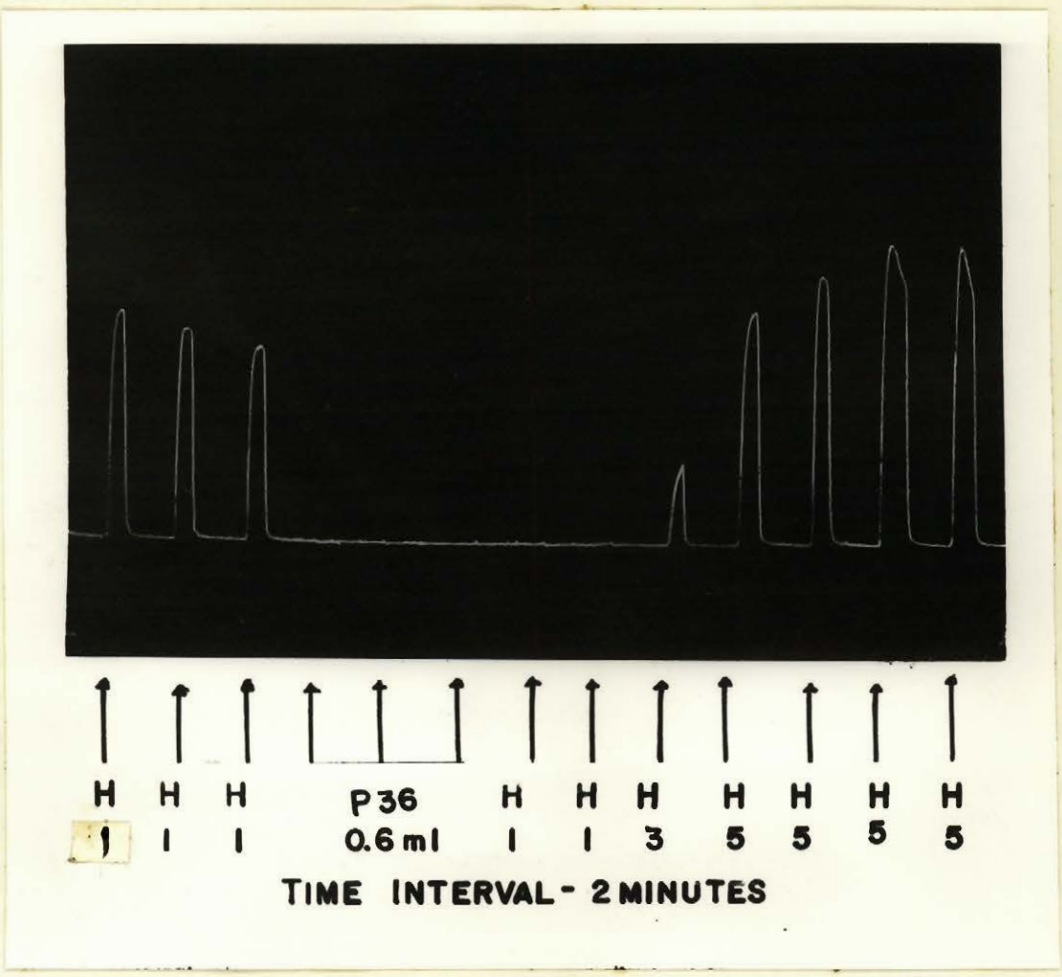



Fig. 17: Histamine assay on guinea pig ileum showing the effects of gingival extracts obtained from a phenobarbital-fed dog (P36) and responses to successive test doses (1, 3 and 5 micrograms) of standard histamine (H/1, H/3 and H/5).

The dose of histamine was then increased to 5.0 micrograms (H/5) before good repeatable contractions were elicited. Results of a similar type were obtained on three dogs.

It would therefore appear that little or no extractable histamine is to be found in the gingiva of the chronic phenobarbitalized dog, but the gingival extract appears to contain definite histamine-antagonizing action. Indeed, the consistent occurrence of this type of activity in normal gingival extracts of both ferrets and dog, suggest that this might be an important constituent in this tissue. However, its identification and significance are problems for future study.

(c) Some preliminary observations on changes in extractable 5-hydroxytryptamine from the gingiva of the normal and dilantin fed dog.

Since it is now well known that many cells and tissue of the body contain both histamine and 5-hydroxytryptamine or serotonin, it was of interest to investigate possible changes in extractable serotonin under the influence of dilantin. In these experiments, again the animals were anesthetized with pentobarbital sodium, and the tissue excised and weighed as in the histamine experiments. The extraction procedure employed was that described by Gaddum (1953), which consists essentially of the following:- The weighed tissue was covered with cold acetone (10 ml.) in a mortar and after cutting it up into small pieces was ground with sand. This was left in the refrigerator at 0°C for 24 to 48 hours with occasional agitation. The material was then filtered into a 50 ml. graduated cylinder, washing the residue with small quantities of acetone to make a total volume of 20 ml. to 25 ml. of filtrate. The acetone was then removed by placing the cylinder in a warm (35°C)

water bath and blowing compressed air over the solution. The remaining aqueous phase was then extracted twice with one-half its volume of petroleum ether each time, and twice with one half its volume of ethyl acetate, using a separatory funnel. The dissolved ethyl acetate was then extracted from the water layer, by addition of peroxide free ether, after standing for about an hour. Finally, the dissolved ether was removed in vacuo, and the residual material dissolved with saline to make a volume of 10 ml.

The extracts so prepared were then assayed by two different procedures as described by Dagliesh (1953) for serotonin assays. I. The Rat's Colon Method. A strip of colon of approximately 1 inch, freshly removed from the rat, was suspended in a 18 ml. bath containing the following solution: NaCl, 9.0 gm.;KCl, 0.4 gm.; $CaCl_2$,0.03 gm.; $NaHCO_3$, 0.9 gm.; and glucose 1 gm.; per litre with added atropine sulfate (0.002%). The temperature was maintained between 22 and 24 degrees centigrade and the solution was continually aerated. The contractions were recorded on a smoked drum with a lever. The contractions observed after additions of a standard serotonin solution to the bath were compared with those recorded after the additions of the unknown extract. Each injection was left in the bath for 1.25 minutes, before washing and an interval of 4.5 minutes was allowed to elapse between successive tests. II. The Rat's Uterus Method. In this method rats weighing between 116 and 200 gms. were prepared by the subcutaneous injection of stilboestrol (0.5 mg.) on the day before the experiment. One horn of the freshly removed uterus was then suspended

in a 4 ml. bath kept at 30°C, and the same solution as employed in the rats colon method was used. The general procedure of recording and adding the standard or unknown, were the same as that described above in the histamine assays.

Using the rat colon method, Figure 18 shows the effects observed in comparative assays of extracts obtained from the gingiva of a dog which had been maintained on a daily oral dose of 200 mg. of dilantin for 41 days, (EDA (2 mls.) upper tracing) and from the gingiva of a normal dog. (EN (2 mls.) lower tracing). As can be seen following three initial 5 microgram administrations of the standard serotonin (5 HT/5 µg.) almost equal contractions were recorded in both assays. Whereupon, little or no response was observed (upper tracing) following three successive additions of 2 ml. of the extract from the dilantin-fed animal (EDA 2 mls.), while two successive additions (lower tracing) of 2 ml. of extract (EN, 2mls.) from normal gingiva showed definite response. Subsequently, good responses to the standard serotonin solutions were also elicited in both experiments.

It is clear from the above findings that the normal gingiva of the dog contains measurable quantities of extractable serotonin, and that following prolonged dilantin administration this cannot be detected.

When the rat uterus method was used, Figures 19 and 20 show the results obtained with two similarly prepared extracts. Fig 19 shows an assay using an extract obtained from a dog maintained on a dose of 200 mg. of dilantin for 34 days before the gingiva was excised. Again, it can be seen that following each of three initial

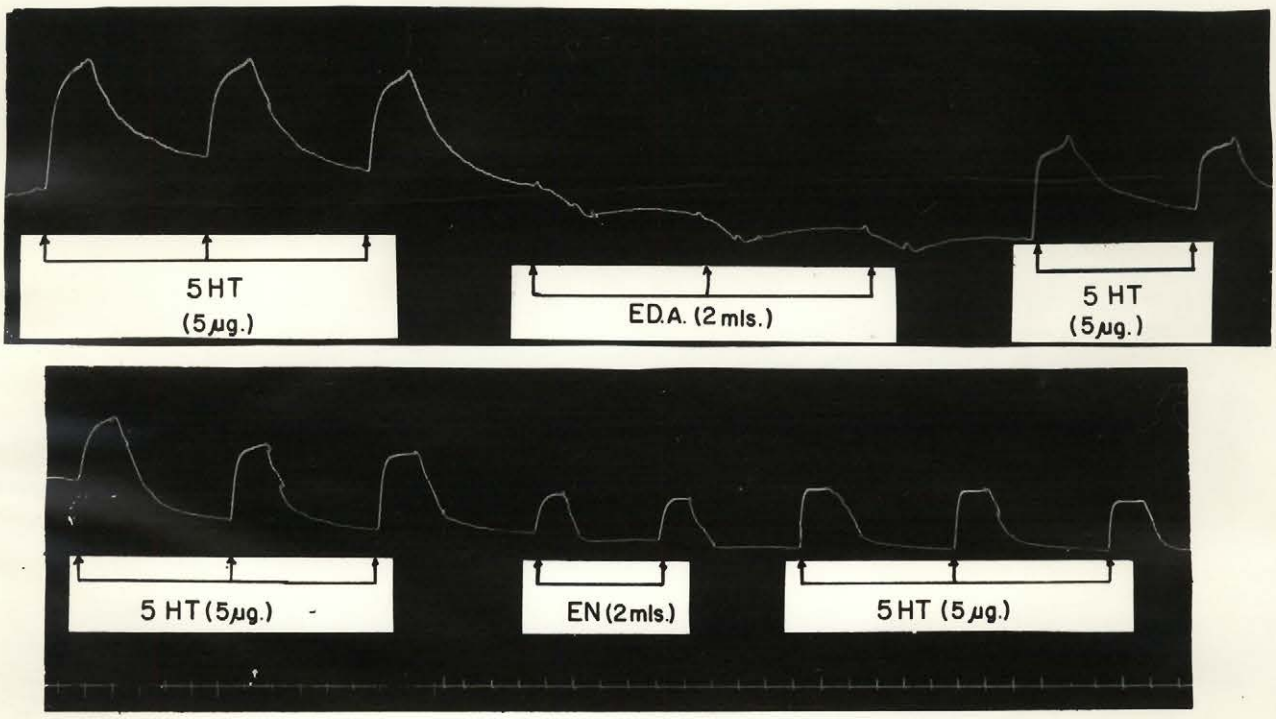



Fig. 18: Above: Serotonin assay on albino rat colon, showing responses to 5 microgram doses of standard serotonin (5-HT/5-µG), and the absence of responses to 2 ml. quantities of extracts from a dilantin-fed dog (EDA). Below: Serotonin assay on albino rat colon, showing responses to 5 microgram doses of serotonin (5-HT/5-µG) of normal untreated dog, (EN) and the standard (5-HT).

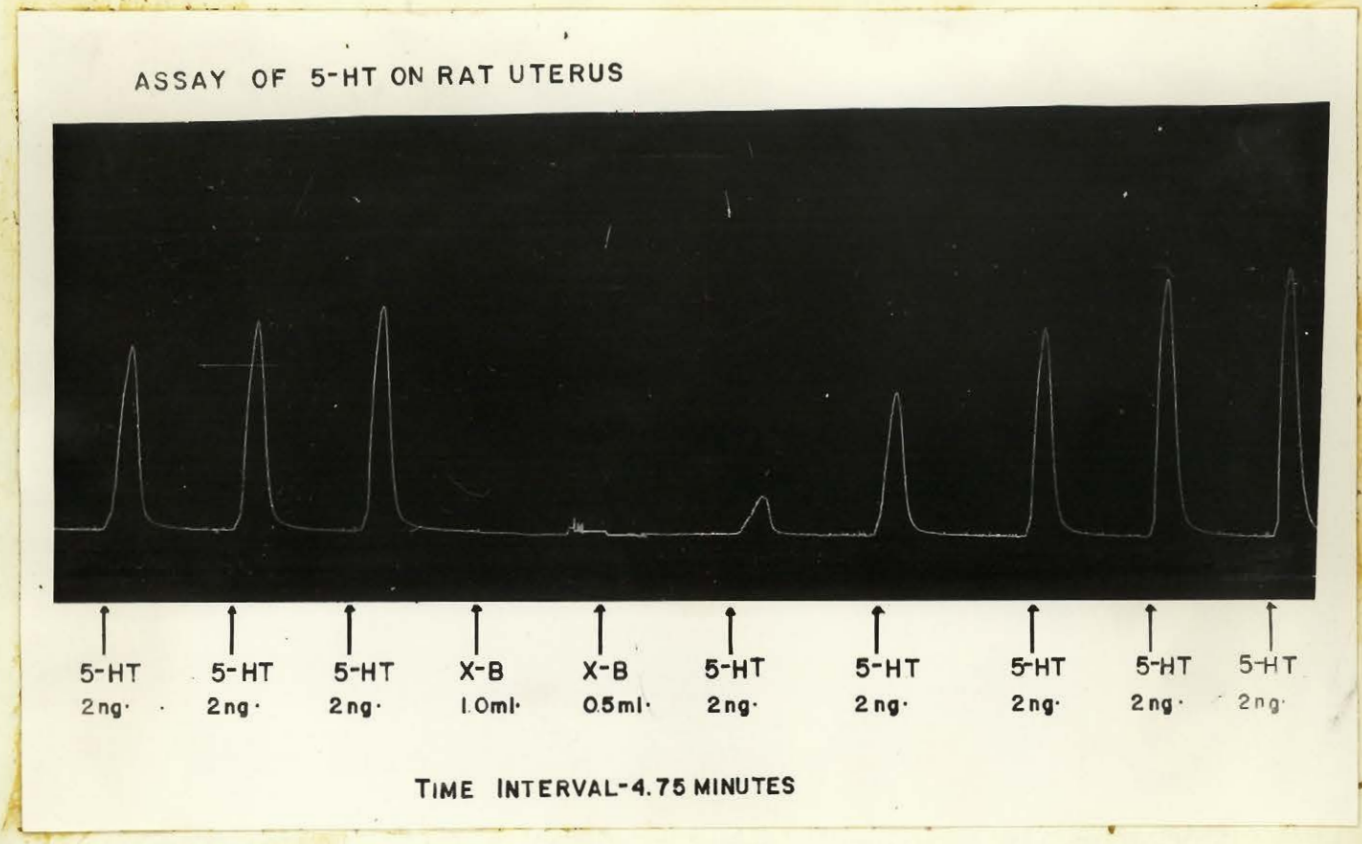



Fig. 19: Serotonin assay on the albino rat uterus, showing absence of responses to two successive tests of extracts obtained from gingiva of a dilantin-fed dog (X-B) and responses to 2 nonogram doses of standard serotonin (5HT/2 ng) before and after.

administrations of 2 nanograms (0.002 μg.) of serotonin, there was an almost identical good contraction recorded ( 5 HT/2 ng.) in each instance. However, following two successive tests using 1 ml. and 0.5 ml. of the extract ( X B) from the dilantin-fed dog, no responses were elicited. In view of the high sensitivity of the preparation to serotonin, it must be concluded that following dilantin there is a complete disappearance of serotonin from the gingiva.

The record however, shows that following the tests with the extracts there was a temporary diminished responsiveness of the uterus to serotonin, and the tissue was only slowly restored to normal sensitivity. This effect may be due to some type of serotonin-antagonism induced by the extract and possibly related to the complete absence of any response to the extract itself. This hypothetical material would however be extractable by the same procedure employed for serotonin. This aspect of the problem again requires further study.

Finally, in Fig. 20 are shown results of a typical experiment using the rats uterus, and in which the responses to an extract prepared from the gingiva of a normal untreated dog was tested. Again it is seen that three initial injections of a dose of 10 ng. (0.010 μg.) of a standard serotonin solution led to rather similar responses. However, following the addition of two successive doses of 0.2 ml. and 0.05 ml. of the extract (X/0.2 ml. and X/0.05 ml.) the contractions of the uterus were so intense that in both instances the record extended above the upper limit of the recording drum. Again by this procedure, it is clear that normal dog gingiva contains considerable quantities of serotonin.

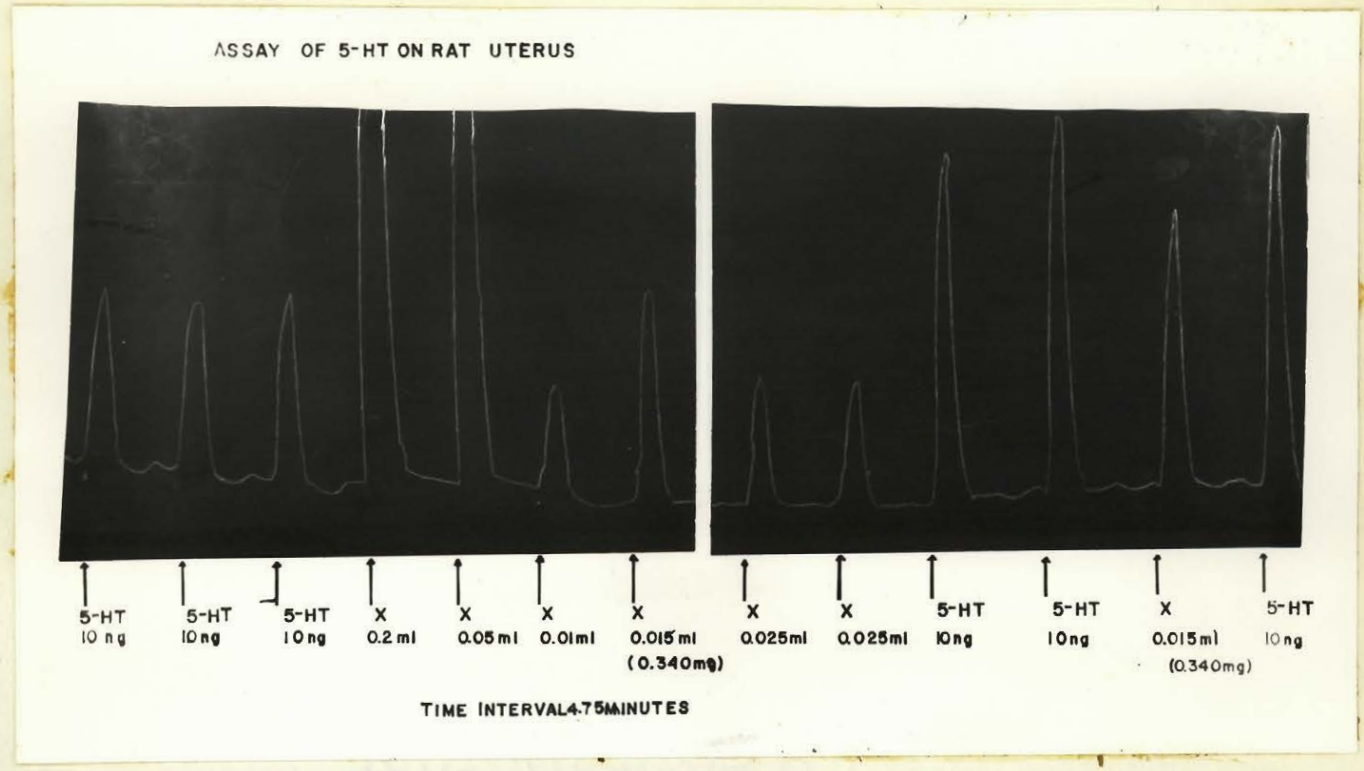



Fig. 20: Serotonin assay on albino rat uterus, showing responses to repeated tests of normal untreated dogs gingival extracts (X) and to repeated 10 nonogram doses of standard serotonin (5-HT/10 ng.)

In order to quantitate the effects of the extract smaller doses were then tested, and as can be seen a dose of 0.01 ml. of the extract (X/0.01 ml.) gave a response which can be differentiated from that to a dose of 0.015 ml. of the extract (X/0.015 ml.) After a further interval of some minutes, uniform responses were then elicited by doses of 0.025 ml. of the extract (X/0.025 ml.) Following these additions to the bath, the uterus showed much greater responses to the standard serotonin, and the effects of two successive 10 nonogram doses (5-HT/10 ng.) were clearly greater than were observed at the begining of the experiment. However, the response to the extract (X/0.015 ml.) was also somewhat increased but the exact significance of this apparent potentiation of serotonin action is not clear.

From the above findings, there is a little doubt that normal dog gingiva contains a high concentration of extractable serotonin, and that following dilantin administration, this extractable gingival serotonin disappears completely. This latter effect also appears to be associated with development of some temporary antagonism to subsequent responses to serotonin. In conclusion, the data available at present are too scant to permit any proper assessment of the exact quantities of serotonin in the normal gingiva, and this aspect of the problem is being further investigated.

## IV - PRELIMINARY OBSERVATIONS ON EXTRACTABLE HISTAMINE FROM HUMAN GINGIVAL TISSUE.

In the course of this work, three samples of gingival tissue that were surgically excised from patients were also studied. These were removed under local anesthesia (procaine) from patients showing excessive gingival inflammatory changes, but not receiving dilantin. *

After excision the tissue was immediately refrigerated and extracted as in the earlier studies. Figure 21 shows that following initial standardization of the gut response to histamine (H/0.1), addition of 0.2 ml. of the extract (HG/0.2 nl.) prepared from excised human gingival tissue (200 mg. extracted in 25 ml. volume) gave a positive contraction which was only about one half of that obtained from 0.1 microgram of standard histamine. This response moreover, was quite repeatable quantitavely, as shown at subsequent points marked (HG/0. 2 and HG/0.4). It is also quite clear that extracts obtained from the tissue did not antagonize the effects of the histamine (H/0.1). Finally, as can be seen at the end of the experiment both the responses to the extract (HG/0.2) and that to histamine (H/0.2) are blocked by previous treatment with mepyramine (AH).

In order to assess the relative histamine content of the gingiva in comparison with the guinea pig lung (which is known to be rich in histamine), an extract prepared from guinea pig lung tissue was also tested as shown at (L). As can be seen, after addition of 0.5 ml. of lung extract (L/0.5), prepared from

* The samples of human gingiva were kindly supplied by Dr. Robert Harvey.

200 mg. of tissue extracted in a volume of 25 ml., the contraction recorded was somewhat higher than that from 0.2 ml. of the gingival extract. However, the latter response was almost equal to that observed after addition of 0.2 ml. of the lung extract as shown (L/0.2). From these findings it may be concluded that the histamine content of inflamed human gingival tissue is at least twice that of the normal guinea pig lung tissue.

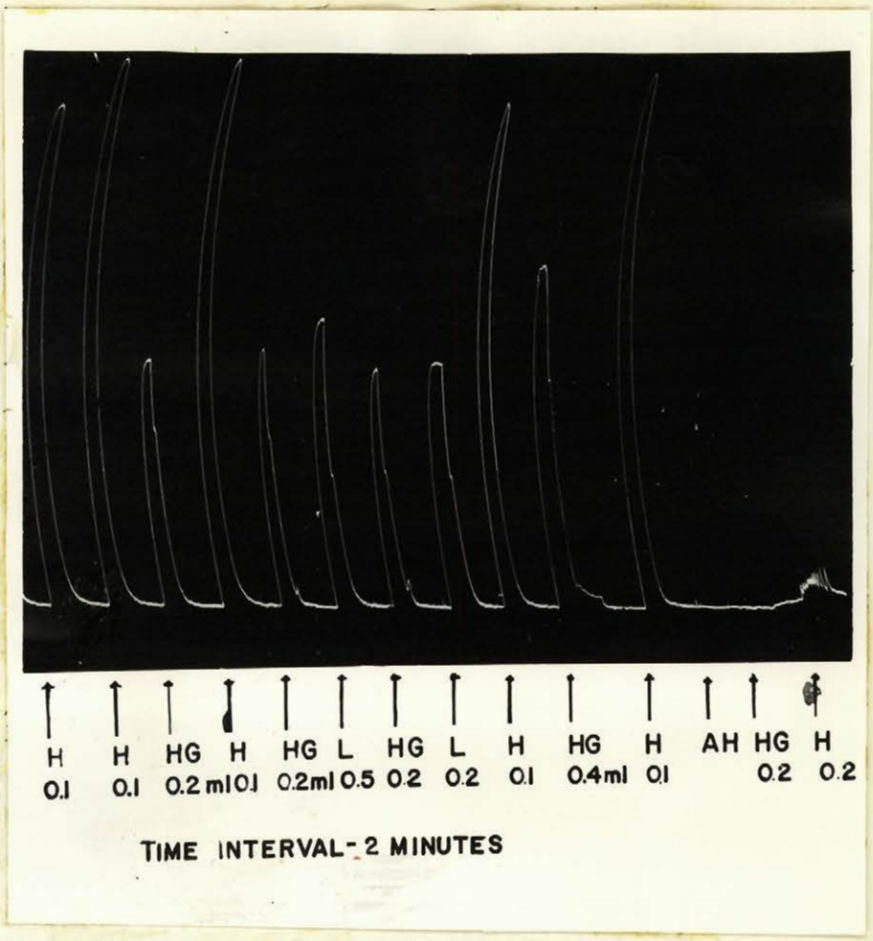



Fig. 21: Histamine assay on the guinea pig ileum, showing the responses to human gingiva inflamed tissue extracts (HG/0.2 and H/0.4 ml.) and comparative responses to pig lung extracts (L/0.5 and L/0.2) and to standard histamine (H/0.1 and 0.2). At the end of the record, the absence of the usual responses to the extract (H /0.2) and to histamine (H/0.2) after mepyramine (AH) are shown.

## DISCUSSION

The experiments presented above show clearly that no characteristic gingival changes develop in rats, hamsters, guinea pigs or dogs, maintained on prolonged oral administrations of dilantin. However, occasional microscopic hyperplastic changes may occur in the gingiva of rats. On the other hand, both gross and histological gingival hyperplasia can be induced in ferrets following prolonged oral administration of the drug. The observed changes are rather similar to those described in man following dilantin therapy, and the findings confirm the earlier observations of King (1952).

In addition, it is clear that in very young ferrets the excessive preformed hyperplastic tissue can induce altered dentition leading to malocclusion. As already pointed out in some of the earlier theories concerning the cause of gingival hyperplasia, it was suggested that this effect of the drug might be associated with or precipitated by malocclusion. The changes observed in the young ferrets would rather indicate that the malocclusion is a result rather than a precipitating cause of hyperplasia.

The findings also show that extracts of gingival tissue from normal ferrets and dogs, contain varying quantities of histamine or histamine-like substance. Such extracts apparently also exert an unusual antihistamine or histamine-antagonizing effect upon the guinea pig ileum following repeated testing. On the other hand, similarly prepared extracts of gingival tissue from dilantin-fed ferrets or dogs contain relatively higher

histamine or histamine-like activity, and such extracts induce quantitatively repeatable responses on the guinea pig ileum. Indeed, the extracts from the dilantin-fed animals appear to exert rather a histamine-potentiating action upon the ileum.

In similar types of experiments in which changes in 5 - hydroxytryptamine-content were investigated, it was observed that normal gingiva shows relatively large amounts of extractable serotonin, and the test responses to such extracts were quantitatively repeatable. On the contrary gingival extracts from dilantin-fed dogs showed no evidence of 5-hydroxytryptamine, when tested by two different assay methods. Such extracts also show clearly 5-hydroxytryptamine-antagonizing activity on both isolated guinea pig ileum and uterus preparations of the rat. Finally, preliminary data indicate that chronically inflamed excised human gingiva tissue contains high concentrations of histamine.

It is concluded from the above findings that normal gingiva of ferrets and dogs contains both histamine and serotonin (5-hydroxytryptamine), and these substances are inversely altered in their concentrations following dilantin administration. It would therefore appear that the amines might be of some physiological significance in the regulation of normal gingival function, and the question naturally arises whether or not the observed changes after dilantin are related in any way to the gingival hyperplasia? The experimental findings so far do not permit a definite answer to this question, nor do they permit any definite conclusions regarding the exact

physiological role played by the two substances in the gingiva. However, as already pointed out, it is generally accepted that changes in tissue histamine are in some way or other involved in allergic reactions. The increased concentration of extractable histamine in the gums following dilantin would therefore suggest that the hyperplastic changes may be associated with allergic or hypersentization phenomena.

It has, however, been reported by Humphrey and Jaques (1953) that both histamine and serotonin are released in association with an in vitro antigen-antibody reaction in rabbit blood. Moreover, Feldberg and Smith (1953) have shown that 5-hydroxytryptamine can lead to the release of histamine from perfused skin. It is therefore conceivable that alterations in both agents might be involved in allergic reactions, although their interrelationship is obscure.

Furthermore, as already pointed out both histamine and 5-hydroxytryptamine have been shown to be present in mast cells. Although the exact function of these cells in the body has not been determined, Dewar (1958) has recently reported that "normal gingival tissue of man is rich in mast cells, and that the number of these increases significantly when the tissue becomes inflamed". The same worker has also shown that inflamed gingiva is likewise rich in histamine, but states that "normal tissue (gingival) was unfortunately not available in significant quantity for analysis of histamine". However, comparative figures of the histamine content of other types of normal tissues:-

skin of the dog (12.3 to 23 micrograms per gram) epidermis of man (16 to 23 micrograms per gram) and lung of the dog (9 micrograms per gram) showed clearly that there was a much higher concentration of histamine (average 60 micrograms per gram) in inflamed human gingival tissue. Our findings also are in agreement with this observation.

Mast cell counts of the inflamed tissue were also shown by Dewar to be significantly higher (47 per sq.mm.S.D.17) than in normal tissue (18.3 per sq. mm. S.D. 14.3). These figures were obtained from counts carried out on specially fixed and stained sections (7 $\mu$ thickness), and are expressed as the number of mast cells in the connective tissue, which was relatively richest. Van Der Kwast (1956) has also concluded that hyperplastic gingival tissue obtained from dilantin-treated patients, shows large number of what the author considers to be 'plasma cells', in the connective tissue layer. This worker has further demonstrated significant increase in the serum gamma globulin in 20 out of 23 patients showing dilantin gingival hyperplasia.

In regard to the question as to whether the observed changes in histamine and serotonin are primary or secondary to the development of the hyperplasia, the fact that they are demonstrable in dogs only after 14 days of treatment, and that this occurs even in the absence of gross gingival changes, show clearly that they precede the development of these changes, and may therefore be in some way or other related to the initiation of the condition. However some histological examinations of gingival tissue excised

from 2 dogs after 40 days of dilantin and recently reexamined in detail showed definite evidence of microscopic hyperplastic changes. Further confirmation of this is however necessary, but the findings would suggest that the alterations in the histamine and 5-hydroxytryptamine are intimately related to and probably the cause of the cellular hyperplasia.

The above observations lend strong support to the concept that gingival changes following dilantin are due to some allergic-type of phenomenon, which leads to changes in histamine and 5-hydroxytryptamine in the tissue, but the exact role of each is unknown. These changes are also presumably associated histologically with increased mast-cell formation. The question therefore arises as to whether or not similar changes in histamine and serotonin occur in other body tissues and if not, why is gingival tissue particularly susceptible to these changes?

In regard to the possible roles played by the amines, nothing definite can be said. It is however of interest to note that Cambel and Sgouris (1952) have reported that after repeated subcutaneous injections of histamine dihydrochloride (0.04 mg. daily) in rats, there was "a progressive thickening of the gastric mucosa due to an increase in size and number of parietal cells." Thus, after 4 weeks of histamine "enlarged parietal cells often replaced peptic cells which were much decreased in number, and were also compressed by them." The authors conclude that histamine can induce experimental hyperplasia of the gastric mucosa. However, as the authors pointed out there were many associated local and systemic changes, e.g. cell hypertrophy, decrease in

peptic cells, decrease in size of spleen and some atrophy of the pancreas. The direct causal relationship of histamine to this hyperplasia cannot therefore be stated.

Histamine is also well-known to be released into tissues following irritation and inflammation, and it is difficult to assess the role of such factors in this type of experiment. However, it has recently been reported by Gaillard (1957) from preliminary observations on 2 cases of dilantin hyperplasia, that administration of the anti-histamine agent chlorpropheny-pyridamine (Teldrin) led to some favourable effects upon the hyperplastic overgrowth. Martin (1957) however, observed that while this agent appeared to lessen the associated superimposed gingivitis, it did not influence the tissue overgrowth. The possible usefulness of this and other known types of anti-histamine and anti-allergic drugs upon experimental gingival hyperplasia remains to be investigated and assessed.

The preceding scanty data in the literature while they do not confirm the results presented above, are certainly not opposed to the view that histamine is in some way or other related to the hyperplasia. In some of our animals it was also noted that, during the initial period of dilantin administration, there was what appeared to be a mild reddening of the gums. However this apparently disappeared later as the experiment progressed, and was replaced by the characteristic hyperplastic changes. It is conceivable that this initial effect might represent the initial sensitization period of the gums to dilantin. However this would require more detailed study.

In regard to the possible significance of the disappearance of 5-hydroxytryptamine from the gums after dilantin, it is of some interest to note that in clinical cases of dilantin hyperplasia, pain is frequently absent. High histamine and serotonin concentrations have been reported to be associated with the local vasodilator and irritant effects (itchiness and pain) induced by certain materials like nettle sting (Emmelin and Feldberg 1947), wasp venoms (Jaques and Schacter 1954), etc. Armstrong et. al. (1953) have also suggested that while the "itchiness might due to the histamine liberation, the pain might well be due to the 5-hydroxytryptamine." The absence of pain in this condition might therefore be due to the disappearance of the serotonin, but obviously this again requires further study.

It is also of some interest to note that studies concerning the influence of dilantin upon brain serotonin levels (Bonnycastle et al 1956) have shown contrarily that in rats treated with dilantin (100 mg/Kgm twice daily, intraperitonely), for two days there is an increased concentration to twice the normal brain level. During this short period of treatment no gingival changes would of course be expected, but these observations tend to confirm some possible relationship between serotonin-changes and the effects of dilantin on the body tissue.

Finally, since little or no hyperplastic changes or alterations in histamine-content appear to occur in most of the other body tissues examined (except lung) it is possible that some local physiological condition in the gum and perhaps to a lesser extent

in the lung, might be responsible for the apparent selective involvement of these organs. Moore (1958) has stated that "discrete pulmonary lesions which may be hypertrophy of the small pulmonary vessels and/or the bronchioles," have been observed at autopsy on individuals previously treated with dilantin. However there has been no systematic investigation of this aspect of the problem. It also is not clear why there are apparent species differences in the gingival responses to dilantin. Both the ferret and the dog, however, showed similar changes in histamine and 5-hydroxytryptamine, so that the gross differences are probably more apparent then real. This problem again needs more detailed study.

In conclusion, it is postulated as a working hypothesis that under the influence of dilantin the observed histamine and serotonin changes in gum resulting from some allergic mechanism, might conceivably "trigger" cell processes concerned with mitosis. It is quite clear that the histamine and serotonin changes do not arise directly from the presence of dilantin in the body, but requires some time (14 days in dogs) before the initiation of these changes. Study of the fundamental biochemical changes associated with hyperplasia may indeed throw further light upon the obscure processes concerned with local inflammatory reactions and cell multiplication. These and other aspects of the problem are being further studied.

## SUMMARY

In summary, experiments are presented to show the following:

1 - Repeated oral administrations of diphenylhydantoin (Dilantin) to albino rats, hooded rats, guinea pigs, hamsters and weanling rats for 3 months in relatively high non-toxic doses, lead to no gross gingival changes. Histological examination of the rat's gingiva showed however, occasional mild hyperplastic changes.

2- Repeated local injections of a dilantin suspension (0.2%) into the muco-buccal fold of rats lead to no gross gingival changes nor marked local visible inflammatory reactions. Microscopically, the gingiva showed varying degrees of hyperplastic changes, but skin, kidney and spleen appeared to be quite normal.

3 - Repeated daily oral administrations of dilantin to growing young and adult ferrets for periods ranging from 20 to 60 weeks, in doses of 20 to 200 mg., lead to gross and microscopic gingival hyperplasia of varying degrees. Some microscopic hyperplastic changes were also seen in the lungs, but none of the other tissues examined (skin, liver, spleen and kidney) showed any abnormal changes. The intensity of the gingival changes did not appear to depend upon the dosage, but required at least 12 weeks before any gross changes in growth were detectable.

4 - In all of the growing young ferrets, the dentition erupted in poor alignment, in association with the extreme hyperplasia.

5 - Extracts obtained from gingival tissue of ferrets, both normal and dilantin-fed, contain histamine, or some histamine-like substance, and the mean concentration is significantly higher in the dilantin-fed group, as judged by assays on the guinea pig ileum.

6 - Following "test doses" of extracts obtained from normal ferrets, the responses of the guinea pig ileum to histamine are temporarily diminished, but conversely, following extracts from dilantin-fed animals, the histamine responses are potentiated.

7 - Following repeated oral daily administration of dilantin to dogs for periods ranging from 7 to 63 days, in doses ranging from 40 to 200 mg., no gross gingival changes developed. Some preliminary microscopic studies of gingiva excised after 42 days, however, showed some hyperplasia.

8 - Extracts of gingival tissue prepared from both normal and dilantin-fed dogs contain histamine in variable amounts, but the mean concentration is definitely higher in the dilantin-fed animals.

9 - Following repeated 'test doses' of extracts from normal dogs, the responses of the guinea pig ileum to the extract diminish strikingly and the effects of standard histamine are also temporarily diminished or prevented. Conversely, with repeated 'test doses' of extracts from dilantin-fed dogs, the subsequent responses of the ileum to the extract itself or to histamine, are either unchanged or potentiated.

10 - Following prolonged administration of mesantoin or phenobarbital sodium to dogs, in doses of 100 mg. per day, similarly prepared extracts show identical responses to those observed with extracts from the normal untreated animals.

11 - Extracts of normal gingiva of the dog contain relatively high quantities of extractable 5-hydroxytryptamine and following prolonged dilantin administration no evidence of 5-hydroxytryptamine is detectable in the extracts using two assay methods.

12 - Extracts prepared from inflamed human gingival tissue after surgical excision (3 patients) contain approximately twice the concentration found in similarly prepared extracts from guinea pig lung tissue.

The possible significance of these findings is discussed, and it is concluded, (a) that the gingival hyperplasia induced in ferrets following diphenylhydantoin shows considerable gross and histological similarities to that observed in man; (b) that the condition appears to be an 'allergic' or 'hypersensitization' effect induced by the drug on the gingiva, although some histological evidence of hyperplastic changes has also been detected in the lung; (c) that histamine and 5-hydroxytryptamine appear to be of considerable physiological importance in gingival function, and characteristic changes in the concentrations of these can be detected in both ferrets and dogs fed diphenylhydantoin; (d) that mesantoin and phenobarbital sodium do not appear to lead to any abnormal gingival changes nor changes in histamine or serotonin

and that the histamine content of inflamed human gingiva is higher than that in lung.

It is postulated that the above-described gingival changes are not direct effects of the drug, but rather result from secondary hypothetical "trigger mechanisms" concerned with mitosis, and probably involving alterations in histamine and 5-hydroxytryptamine. The problem requires further investigation.

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