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The Effect of Cortical Steroids on the Rate of Eruption of the First Maxillary Molar in the Albino Rat

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Loyola University Chicago

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THE EFFECT OF CORTICAL STEROIDS ON THE RATE OF ERUPTION OF THE FIRST MAXILLARY MOLAR IN THE ALBINO RAT

by

Stephanie Jean Zayachek

A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

June 1969

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Stephanie Jean Zayachek was born in Granville, New York, on January 23, 1942.

After graduating from Granville Central High School in June, 1959, she attended the State University College of Education at Albany, New York, and The Catholic University of America in Washington, D.C., from which institution she received the Bachelor of Arts degree in absentia in October, 1963. In September, 1963, she began graduate work at the University of Chicago, Chicago, Illinois, as a United States Public Health Service Fellow in Anatomy. In September, 1965, she transferred to Loyola University Stritch School of Medicine, Chicago, where she continued her graduate studies in Anatomy.

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ABSTRACT

This study is concerned with the effect of adrenal cortical steroids on the eruption of the first maxillary molar of the albino rat. The rate of eruption was determined by means of measurements on roentgenogram exposures, and histological observations were made on hematoxylin and eosin stained sections of the molar area.

The treated rats received varying dosages (0.1 to 2.0 mg) of either cortisone (cortone acetate) or hydrocortisone (hydrocortone acetate). Litter mate controls received equivalent volumes of saline. Five consecutive daily injections were given beginning on day 11. The rats were exsanguinated by decapitation on day 16, and the heads immediately placed in cold 80% alcohol for subsequent gross and histological examination. Radiological measurements were made from exposures taken on days 11 and 16 in order to measure the rate of eruption and bone growth.

The cortical steroids administered elicited a gradient decrease in weight gain proportional to dosage and brought about a decrease in growth of body hair. Upon gross examination, precocious eruption of the first maxillary molar appeared to have occurred in all steroid treated animals.

Histological examination of hematoxylin and eosin stained sections of molar regions of high (1.0mg) and low (0.1mg)
dosage animals showed no effect on dental tissues. Bone resorption was extensive at the 1.0 mg dosage. Depletion of loose connective tissue was seen in all the steroid treated animals, as well as marked hypoplasia of bone marrow cells with evidence of fat storage in the marrow and adjacent to muscle.

Radiological measurements revealed no significant bone growth in the 0.1 mg cortisone treated rats while in those receiving 1.0 mg there was significant resorption at the molar alveolar crest.

In contrast to the initial gross observations, radiological measurements showed that the eruption rate of the first maxillary molar was accelerated only at the lower dosages of cortisone and hydrocortisone. The effect was inversely proportional to dosage; accelerated 39 and 32% respectively at 0.1 mg; 25 and 20% at 0.5 mg; and retarded at the 1.0, 1.5 and 2.0 mg dosages.
INTRODUCTION

Tomes ('14), in discussing the evolution of teeth, states that the various types of teeth seen in vertebrates were probably derived from a common form, a mono-cusp, mono-rooted, conical form which, in the course of time, became modified through natural selection. A living example of this type of tooth is seen in the ungulate, Anoplotherium. According to Tomes, Gervais (1854) postulated that molars, as we know them today, evolved from conical teeth which fused as the jaw shortened in length. Tomes further cites Virchow as having observed a molar made up of separate conical denticles each with a root of its own. Virchow viewed this as a case of atavism. Ness ('56) concluded that eruption of the different types of vertebrate teeth may be considered to be fundamentally the same.

This paper reports the results of an investigation on the effects of cortical steroids on the first maxillary molar of the rat. It has been shown by a number of investigators that cortisone accelerates the rate of eruption of the continuously erupting rat incisor (Parmer et al., '51; Domm and Marzano, '54; Goldsmith and Ross, '54; Domm and Leroy, '55; Leroy and Domm, '55; Domm and Wellband, '60; Garren and Greep, '60). Domm and Wellband ('60, '61) reported that cortisone elicits this reaction not only in normal rats but also in adrenalectomized
and thyroidectomized animals. However, these studies did not explain the actual mechanism of incisor eruption or how cortisone acts to produce accelerated eruption.

Eruption of the rat molar is believed to be continuous throughout the life of the rat, but is so slow as to be undetectable (Schour and Massler, '49). We were interested in determining, if possible, whether the mechanism operative in the limited eruption of the molars is similar to that in the continuously erupting incisors. In order to shed light on this problem, we studied the effects of cortical steroids on the first maxillary molar of the rat. Specifically, we were interested in determining any change in rate of eruption or histologically apparent modification of growth following injection of these steroids. If the molars, under the influence of cortical steroids also reveal accelerated eruption, it would indicate that this effect is common to the teeth of the rat and would seem to lend support to the theory that both types had a common origin. Conversely, if cortisone does not accelerate eruption, it can be assumed either, that the mechanism of eruption involved in the two types of teeth is different, or that cortical steroids are not effective on molars at the dosages administered.
II REVIEW OF LITERATURE

A. Theories of Eruption

Constant (1900) theorized that tooth eruption is caused by blood pressure "pushing" the tooth into the oral cavity. A parallel to this theory was proposed by Addison and Appleton ('15) who attributed tooth movement to the pressure exerted by dividing cells at the basal or proliferative zone of the tooth.

The blood pressure theory was supported by Schour and Van Dyke ('32a) who hypophysectomized rats and noted that the rate of eruption was more seriously affected than the growth of dental tissues. These investigators suggested that the vascular disturbances involved in hypophysectomy may be a primary factor in the observed decrease in eruption rate. Massler and Schour ('41), in an evaluation of eruption theories, supported this proposal and suggested that other factors may also be involved. Bryer ('57) concluded that the tooth movement, inherent in eruption, is the direct result of hydrostatic tissue pressure in the pulp and periodontal membrane.

Sicher ('42a, '42b) described a "cushioned hammock ligament" slung beneath the root of the tooth against which the proliferating cells push, thus forcing the tooth occlusally. Scott ('53) also observed this structure and attributed to it the role proposed by Sicher. Baume et al. ('54a, '54b, '54c)
reported a decrease in vascularity correlated with a retardation in rate of eruption of incisors in rats following hypophysectomy and thyroidectomy. These investigators concluded, however, that eruption is the direct result of dental and periodontal tissue growth. Domm and Kiely (’68) correlated increase in the number of colchicine-arrested cells in the basal proliferative loop of the rat incisor with acceleration in eruption rate following the administration of cortisone.

Taylor and Butcher (’51) cut the inferior dental nerve in a sympathectomized rat and showed that the incisor eruption rate is not sensitive to even large changes in blood flow. Miller (’57) changed blood flow in the dental area of the rat and rabbit by carotid ligation or sympathectomy and observed no correlation between blood flow and eruption rate. Sturman (’57) injected the vasodilator drug, Priscoline, and the vasoconstrictor drug, Levophed, and observed fluctuations in the eruption rate of the rat incisor. However, he questioned this effect and described an "excitability factor", needle injections, which produced similar results. The vasoconstrictor maintained eruption at the normal rate for one week and then accelerated it. From this, Sturman concluded that the actual eruptive force, though locally affected by blood pressure, is the manifestation of mitotic activity in the odontogenic epithelium and pulp cells. Main (’61) and Main and Adams (’62, ’66) produced a hypotensive state in rats with hydralazine and
guanethididine and noted no modification in incisor eruption rate.

The persistence of eruption following the removal of both the pulp and Hertwig's sheath (odontogenic epithelium) demonstrated that cell proliferation at these loci is a process separate from that of the eruptive movement (Herzberg and Schour, '41; Bryer, '57; Kostlan et al., '60). Main ('65) disavowed the existence of a functional hammock-like ligament as the result of an extensive histological examination of actively-erupting teeth in rats, dogs, cats, sheep, pigs, calves, guinea pigs, rabbits and mice. Main and Adams ('66) repeated their 1962 experiments with hypotensive drugs and did a parallel experiment with the nucleotoxic drugs, demecolcine and triethylene melamine (TEM). They measured the effect of these drugs on the growth of incisors cut out of occlusion, thereby removing the variable of attrition. They found no fluctuation in the eruption rate of the treated when compared with that of untreated rats. Hence, they concluded that, neither blood pressure nor cellular proliferation is the source of the eruptive force.

The only proposed concept left to explore is that of the collagen fibers of the periodontal membrane, which by their contraction are believed by some investigators to pull the tooth into the oral cavity. The first to suggest that eruption may be due to factors inherent in the periapical tissues was
Underwood cited by Tomes ('14). Verzar ('63) noted that normal collagen under tension will contract as it ages. The nature of this contraction is due to the accumulation of intermolecular cross-linkages. These cross-linkages are believed to consist of hydrogen bonding, covalent bonding, and (possibly) ester linkages. It is thought that the hexose molecules gained from the mucopolysaccharide ground substance are the source of this bonding. Levene and Gross ('59) and Van den Hooff et al. ('59) noted that the drug, alpha-aminopropionitrile, interfered with the normal (cross-linking) maturation process of collagen. Martin et al. ('62) produced lathyritic rats (a condition in which the teeth do not erupt) by injecting this drug. From the results of this study on collagen extraction they came to the conclusion that these rats showed a defect in the cross-linking of collagen, specifically a failure in chain-pair linking. They therefore concluded that the collagen of the periodontal membrane normally contracts and, in this manner, produces occlusal tooth movement. Thomas ('64, '65, '67) demonstrated this phenomenon more completely. In the 1965 experiment he showed that the collagen cross-linkage breaking drug, beta-aminoacetonitrile, retarded molar eruption and slowed the eruption rate of the continuously erupting incisor. He showed that while dentin, bone, and vascularity of the pulp and periodontium were normal, the collagen fibers of the periodontal membrane were palisaded.
rather than obliquely oriented. Eruption in normal rats, said Thomas, is initiated by growth of the tooth germ away from the oral cavity. Fibroblasts orient themselves along the lines of stress (Weiss, '59), set up by this growth. The collagen fibers are consequently laid down in a functional manner between the cementum and alveolar bone. Thomas maintained that as the root is established, its growth away from the oral cavity causes a stretching of the collagen fibers leading to an increase in cross-linkages, subsequent contraction of the fibers, and consequent tooth movement. For more information on the nature of collagen see Verzar ('63), Piez et al. ('61), and Bear ('44).

For the above phenomenon to occur, the connective tissue fibers would have to connect the alveolar bone to the tooth and the points of tooth connection would have to move with the erupting tooth. This, in fact, they do. Melcher ('67) made a lesion with a slow-running burr (size 6) in the mandibular incisor of the adult rat. This lesion included the alveolar bone, periodontal membrane, cementum, and dentin. After four days of healing, histological examination showed that the intact periodontal fibers and the adjacent cementum moved into the area of the lesion marked by the absence of intact bone. This would not have been possible had the fibers not been carried there by the erupting tooth. Ramos and Hunt ('67), using tritiated proline, demonstrated a similar movement.
of the periodontal fibers in the continuously erupting guinea pig molar.

It is of interest here to note the relevance of the discussion on eruption theories by Massler and Schour (141). They noted a consistent correlation between increase in vascularity and positive tooth eruption. For instance, they observed that when a denture irritates a submerged tooth, or if the tooth is rubbed, it will erupt; they observed increased hyperemia in such conditions and correlated this with the increase in rate of eruption. This data could be applied in the light of the information on connective tissue contraction. Such downward pressure from a denture could stimulate the fibroblastic and collagen fiber orientation and place the fibers under tension. With subsequent maturation (increase in cross-linkages), the teeth would erupt. With this increased movement, chain reaction events could occur, such as dental tissue growth, increased vascularity of the physiologically active tissue and fibroblastic replacement of cells and periodontal fibers. Biting forces in increased attrition would stimulate the process. In incisors cut out of occlusion, the incisor eruption rate is increased above normal and the enamel and dentin are decreased in amount (Noe, '02; Addison and Appleton, '15; Dalldorf and Zall, '30; Hunter and Sawin, '42; Schour and Medak, '51; Taylor and Butcher, '51; Ness, '56; Main, '65; Ness, '67). Under similar conditions the molar
eruption rate is also increased (Sognnaes, '41). In such a tooth the accelerated rate of eruption would reflect the rate of collagen maturation and cell proliferation, and the decrease in amounts of enamel and dentin (hard substance apposition) would indicate that formation of these substances would have to keep pace with the accelerated eruption.

B. Hormones and Eruption

Hoskins ('27) reported that as little as 0.1 mg of acetyl thyroxine stimulated precocious eruption of incisors in newborn rats. Growth of the nasal bone, otherwise normal, was accelerated. No observable effect was apparent in the molars. A decrease in the eruption rate of the incisors in hypophysectomized rats was reported by Schour and Van Dyke ('31). The incisors, two-thirds the size of controls, showed degeneration of Hertwig's epithelial sheath (odontogenic epithelium), the periodontal membrane and the labial alveolar periosteum. Examination of the dentin and enamel revealed foldings at the basal end of the tooth and a thickening of cementum. A decrease in calcification of the dentin was evident. No changes were seen in the molars which had just come into occlusal contact at the start of the experiment. In 1932, Schour and Van Dyke repeated this experiment, confirming their earlier findings, and attempted replacement therapy with hypophyseal extract. They reported an acceleration in rate of eruption and a restoration of a more normal histological picture. They noted that hypophysectomy had a
more pronounced effect on rate of eruption than on growth rate of the dental tissues and attributed this to vascular disturbances (Schour and Van Dyke, '32a). That same year they also reported the effect of growth hormone on the dentition of hypophysectomized rats. This hormone, in addition to replacing lost body weight, also accelerated the depressed eruption rate (Schour and Van Dyke, '32b). Their work was confirmed by Collins et al. ('49). Schour and Rogoff ('36) reported the additional observation that growth hormone did not accelerate the eruption of incisors in normal rats. They, in turn, adrenalectomized rats and observed, in addition to disturbed dentin calcification and degeneration of cells, a decrease in incisor eruption rate. They concluded that the causative factor was the effect of the adrenal on calcification.

Parmer ('47) noted that when DOCA (desoxycorticosterone) was administered to thiouracil-treated (thyroxin-inhibited) animals, the impaired incisor eruption rate and delayed eye-opening returned to normal. However, this hormone did not restore the thyroid to normal, suggesting that the process of eruption is not necessarily mediated through this gland. Cortisone, found to be the most potent growth inhibitor and the most potent stimulus for eruption of incisors, eye-opening and gingival development, was compared in its effect on newborn rats with ACTH, aqueous adrenal extract, corticosterone, desoxycorticosterone, and pregnenolone (Parmer et al., '51).
These investigators concluded that the mode of activity of cortisone was probably quite specific since pregnenolone, which is of a similar configuration, had no effect. Domm and Marzano (’54) observed that the incisors of newborn rats treated with cortisone erupted three days earlier than those of the controls. Growth hormone had little or no effect on the eruption rate of the incisors in normal rats. Administered in hypophysectomized rats, cortisone brought about a complete recovery of the incisor eruption rate. Garren (’55), in studies on rats, confirmed the work of Domm and Marzano. Goldsmith and Ross (’54), also observed a precocity in the rate of incisor eruption in cortisone-treated normal rats. They reported a stimulation of gingival keratinization, osteodentin formation, amelogenesis and odontogenesis. However, growth of the periodontal connective tissue appeared to be depressed. Domm and Leroy (’55) and Leroy and Domm (’55) administered cortisone to pregnant rats and to rat fetuses in utero. In both instances they observed a precocious eruption of the incisors in the neonatal rats. Goldsmith and Ross (’56) administered different dosages of cortisone to pregnant rats and observed accelerated incisor development in the 18 and 20 day old fetuses. Cortisone treatment in newborn rats elicited a 2.9 day precocity in incisor eruption. In addition to an increase in alkaline phosphatase and RNA content of the tooth bud cells and odontoblasts, a disorganization of the periodontal membrane and alveolar bone was
seen. These effects of cortisone administration were believed to be responsible for the early eruption observed. Domm and Wellband ('60) observed a decrease in the incisor eruption rate in adrenalectomized rats. The administration of cortisone brought about an acceleration in the rate of eruption of 21% above normal. In 1961, these investigators, in their studies on adrenalectomized, thyroidectomized and thyro-adrenalectomized rats, reported that the thyroid gland was also involved in stimulating eruption. Garren and Greep ('60) reported that cortisone had the effect of normalizing the rate of incisor eruption in adrenalectomized as well as in hypophysectomized rats. Wellband and Domm ('64) reported an experiment showing that cortisone accelerates the rate of eruption in incisors cut out of occlusion. Domm and Kiely ('68), by application of the colchicine metaphase arrest technique, reported an increase in the number of mitoses in the basal or proliferative zone of the rat incisor following cortisone administration. On the basis of their observations, they concluded that mitotic activity was probably one of the factors responsible for the acceleration in the rate of eruption observed in cortisone-treated rats.
III MATERIALS AND METHODS

Pregnant, multiparous, albino rats of the Sprague-Dawley strain, in about the fifteenth day of pregnancy, were purchased commercially. They were housed in separate cages and maintained on Rockland Chow pellets and tap water ad libitum. On the sixteenth day of gestation, the wire mesh floors of the cages were removed and the rats placed into the attached pans and provided with appropriate nesting materials. This measure was taken so that the newborn would not be subject to injury from contact with the coarse wire mesh floors. Except for removal of dead newborn, all young remained untouched for four days after delivery. This precaution insured maximum survival of the young since it minimized the danger of cannibalism.

The newborn were weighed daily, beginning on day four, at which time each animal was marked with picric acid for identification. Their condition, date of incisor appearance and date of eye opening were recorded.

Injections were begun on day 11 and continued to the day of sacrifice, day 16. All animals were injected intraperitonally with either 0.1, 0.5, 0.75, 1.0, 1.5 or 2.0 mg of cortisone or 0.1, 0.5, 0.75, 1.0, 1.5 or 2.0 mg of hydrocortisone.2

1Purchased from the Hormone Assay Laboratories, Inc., Chicago, Illinois.
2The cortisone (cortone acetate) and hydrocortisone (hydrocortone acetate) were generously supplied to Dr. L.V. Domm by Sharpe and Dohme, Division of the Merck and Company, Philadelphia, Pa.
Litter mates served as controls and were administered equivalent volumes of saline to simulate the trauma of injection. A second series of controls without injection or X-ray was also maintained.

The degree of molar eruption was determined by means of measurements on roentgenogram exposures. At the completion of experiments, the animals were exsanguinated by decapitation. The heads were immediately placed in cold 80% alcohol for not less than 24 hours. Subsequently, gross observations on the molar areas were recorded.

All animals were anaesthetized before each of two X-ray exposures with 32.5 mg/kg of nembutal, injected intraperitoneally. A cephalostat was designed and constructed to hold the head in a fixed position during exposure. It was found that placing the ear posts of this instrument into the ears was unsatisfactory since the ears of the newborn rat are very flexible and movable over the bony skull. A more suitable method for head stabilization was to place the ear posts in the temporal fossae.

Kodak ultra-speed, fine emulsion, dental occlusal film was placed in a fixed cassette in the cephalostat beneath the animal's head. Lead foil letters were placed on the cassette for identification of exposed film. Roentgenograms were taken in a Universal V-U X-ray machine at 3 M.A., 60 K.V., for an exposure time of one second at a distance of 16 inches. The
films were placed in standard X-ray developer (D-19) for $3\frac{1}{2}$ minutes, rinsed in clean water for seven seconds, then placed in standard fixer for 10 minutes and washed in clean running water for another 20 minutes. The films were processed at $73^\circ$ F. during each step.

The measurements were made on roentgenograms that had been enlarged about 4 X. The distance between the most mesial (rostral) cusp of the first maxillary molar and its alveolar crest was measured. The difference between the day 11 measurement and that of day 16 was calculated as the distance the tooth had erupted into the oral cavity. By this method, the effect of the steroid on eruption can be compared with that of the control.

To insure that the data obtained from measurements represent actual eruption and not merely bone resorption, the following additional measurements were made on the roentgenograms (fig. 7). A line was drawn connecting the lingual alveolar crest of the maxillary incisor (I) with the molar alveolar crest (M). A line perpendicular to this line was constructed connecting the midpoint of the intercrestal line to the superior aspect of the nasal bone (N). The perpendicular line, representative of the extent of bone growth, was then measured. Values on experimental and control animals were compared. To determine the possible physiological effect of X-ray as a variable, the weights of the X-rayed and non-X-rayed animals
were compared and the standard error of difference (s.e.d.) test of significance applied. These weights were then tested against those of a second control group.

In preparation for histological examination, specimens of the entire molar region were cut and placed in a sodium citrate-formic acid decalcifying agent. Complete decalcification was determined by the negative oxalate test (Humason, '62). The specimens were then dehydrated and double-imbedded in celloidin benzoate and paraffin. The blocks were sectioned at 8µ, stained with Harris hematoxylin and eosin Y. In order to compare growth effects, a series of measurements were made on histological sections by means of an ocular micrometer. The measurements included connective tissue thickness, the distance between the most superior portion of the alveolar bone and the dentino-enamel junction, trabecular thickness, height of the Haversian space and diameter of the major pulpal vessels. The measurements were expressed in high and low power units.
IV EXPERIMENTAL RESULTS

True precocious eruption in this study is defined as an earlier than normal appearance in the oral cavity of any part of the molar crown before that of a control animal. Our initial gross observations on the effects of cortical steroids in the molar area led us to the conclusion that eruption of the first maxillary molar had been accelerated in the 1.0, 1.5 and 2.0 mg injected animals. However, radiological measurements showed that its eruption was, in fact, retarded. The gross appearance of the molar area was probably due to shrinkage or a thinning of the gingivae so that the molar cusps appeared precociously (figs. 10, 11).

A. Gross Observations

In our study on the effects of cortical steroids on the molar eruption rate, we observed precocious eruption of the molars (fig. 2) early eye-opening, a decrease in hair growth and a decrease in weight gain and body size.

These effects, with the exception of hair growth, varied directly with increase in dosage administered. After five days of cortisone treatment, the 16 day-old rats appeared to have somewhat less than a normal growth of hair when compared with their litter mate controls. The decrease in body weight tended to increase with an increase in dosage.
(fig. 6, table 1). This decrease in weight, following five days of cortisone injection, went from 4% at the 0.1 mg dosage to 66% at 1.0 mg and 83% at 2.0 mg. Hydrocortisone was found to have a more severe effect on body weight. Following five days of injection with this steroid, total weight loss went from 31% at 0.1 mg to 93% at 1.0 mg and 88% at 2.0 mg when compared with the weight of controls.

B. Radiological Observations

The validity of the use of X-ray as an experimental tool was determined by a comparison of body weight gain of the rats during this experiment. By the absence of statistical significance in the groups compared, it was demonstrated that X-ray did not introduce a variable into the experiments (table 6).

The mean measurement of all control molar eruption rates was calculated to be 0.201 mm per day in the period between age 11 and 16 days. Corrected for magnification intrinsic to the X-ray procedure (1.038X), the normal rate of eruption of the first maxillary molar during this five day period is 0.194 mm per day. Cortisone accelerated this rate to 0.244 mm per day.

Daily administration for five consecutive days of 0.1 mg of cortisone and 0.1 mg of hydrocortisone produced an increase in the rate of eruption of the first maxillary molar of 39 and 32% respectively while the rate was accelerated 25
and 20% at the 0.5 mg dosage. This effect tended to decrease as the dosage was increased (fig. 5, table 2). Cortisone accelerated the molar eruption rate 26% at the 0.75 mg dosage whereas the rate was retarded 2% at the 1.0 mg and 25% at the 1.5 mg dosages. It should also be noted that the eruption rate of the first mandibular molar measured at the 0.1 mg dosage of cortisone and hydrocortisone also exhibited acceleration in eruption (table 3).

In the 0.1, 0.5 and 0.75 mg injected groups, the first maxillary molars of the hydrocortisone treated animals appeared to have erupted somewhat earlier than those of the cortisone treated animals. This observation is based on gross examination of the molar cusps and the gingivae immediately following exsanguination. However, X-ray measurements showed that the molars of the cortisone-treated animals had in fact erupted before those of rats treated with an equal concentration of hydrocortisone. This data would seem to show that a given concentration of hydrocortisone has a more severe wasting or dehydrating effect on the gingivae than does an equal concentration of cortisone.

The extent of eruption of the first maxillary molar was determined from measurements between the most rostral molar cusp (C) and its adjacent alveolar crest (fig. 7, (M)). Neither cortisone nor hydrocortisone produced a change in bone growth in the 0.1 mg injected animals as shown by X-ray
measurements. Therefore, decreased bone growth can not be regarded as a factor in the measured precocious (accelerated) molar eruption in these animals. The absence of bone apposition in the 1.0 mg injected rats when compared with control measurements, indicated that the observed retardation in eruption is not a manifestation of alveolar bone apposition. In fact, a decrease was observed in molar alveolar bone growth in measurements on experimental rats when compared with litter mate controls. These findings were determined from radiological measurements (fig. 7,(line N to IM), table 5).

C. Histological Observations

Specimens were prepared from all animals for histological study. Sagittal sections, taking every 12-15th section, of the entire maxillary molar region were made at 8µ. The customary exposure to the decalcifying agent, sodium citrate-formic acid, accounts for the absence of enamel and removal of the dentinal matrix.

In order to determine histological growth effects on dental structures, a series of measurements was taken on 0.1 and 1.0 mg cortisone treated rats, by means of an ocular micrometer. The measurements included: connective tissue thickness, the distance between the most superior portion of the alveolar bone and the dentino-enamel junction, trabecular thickness, height of the Haversian space and diameter of major pulpal vessels (table 4).
The treated rats showed a histological picture very similar to that of their litter mate controls (figs. 3,4,8,9). The basal loop (Hertwig's sheath or odontogenic epithelium) consisted of two rows of cells forming a loop at the base of the pulpal chamber. The inner row of cells gives rise to the dentin producing odontoblasts, and the outer may give rise to the enamel forming ameloblasts (Sicher, '66). The odontoblasts of the steroid treated animals appear to be normal as judged by the morphology of the cells, regularity of arrangement and thickness of odontoblastic row. These cells have a columnar body with an oval nucleus. Some of the bodies are long, others short. Their irregular arrangement higher up in the pulpal cavity is normal.

In the hematoxylin and eosin stained sections, dentin forms the heavily eosin-stained region surrounding the pulpal chamber. The dentinal tubules run outward and curve occlusally from the odontoblastic row of cells. In the living animal, the dentin consists of an organic and an inorganic part. The inorganic component (hydroxyapatite) has been removed in these sections by decalcification. The dentin was measured for thickness to determine whether or not the rate of dentinal apposition was affected by cortisone treatment. This measurement was made at the dentino-enamel junction at a plane perpendicular to the plane of growth. Its apposition appears to be unaffected by the administration of either
dosage of cortisone.

Vascularity of the pulpal region was closely examined and no consistent variation was seen in either the low or high dosage treated animals (0.1-1.0 mg). At times, hypertrophied vessels were seen as was an increase in the number of vessels. However, this condition was also seen in control animals. Hence, the variations in vascular caliber and number were normal. Our lack of positive findings in the dental tissues (odontoblasts and ameloblasts) of the steroid treated animals concurs with the work of others (Goldsmith and Ross, '54; Garren, '55; Wellband, '61; and Kiely, '67).

Active resorption and apposition is the normal condition of the molar alveolar bone. These processes are only considered pathological when one process for outweighs the other resulting in an abnormal loss or gain in bone. In the steroid treated animals, resorption was characterized by a greater than normal number of osteoclasts on all surfaces of the trabeculae. This finding indicates pathology which agrees with observations of Frost ('66). Measurements of the trabeculae showed a marked decrease in thickness most evident in the alveolar bone of rats treated with the higher dosage (1.0 mg).

The height of the alveolar bone when measured from the dentino-enamel junction was decreased in the steroid treated animals. In order to determine whether this was due to an increase in eruption or a decrease in bone growth, we examined
our X-ray data. Roentgenographic bone measurements revealed an accelerated eruption at this dosage. Hence, we concluded that the decreased height of the alveolar bone, as measured from the dentino-enamel junction, is a manifestation of an acceleration in rate of eruption in this group. Roentgenographic bone measurements in the 1.0 mg cortisone injected animals revealed a decrease in bone growth at the alveolar crest, while measurements on eruption revealed a retardation in rate. We therefore concluded that the decreased height of the alveolar bone in this group is a manifestation of bone resorption.

Osteoporosis is defined as a condition characterized by a decrease in the normal amount of bone, i.e., negative skeletal balance (Frost, '66). The presence of this condition was determined by measurements on the size of the Haversian space (bone marrow space) and by the thickness of its trabecular envelope. The Haversian space was found to be larger and the trabecular wall markedly thinner than that of controls in both 0.1 and 1.0 mg cortisone treated rats (table 4). This represents an enlargement of the Haversian space at the expense of the surrounding matrix and in the case of the 1.0 mg treated rats, in a net loss of alveolar bone.

The number of hematopoietic cells in the marrow was reduced in all steroid treated (cortisone and hydrocortisone) rats when compared with controls (figs. 3, 4, 8, 9).
The most remarkable effect noticed in the histological sections of all our steroid treated rats was the striking accumulation of fat in the bone marrow, filling the spaces vacated by the blood cell precursors. Normally, (note control) only a few scattered fat cells are found in the area. Furthermore, in the muscle seen adjacent to the alveolar process, we also noted an increase in the accumulation of fat cells surrounding the muscle belly (fig. 4). These conditions were seen in animals treated with either hydrocortisone or cortisone regardless of the dosage administered.

These observations on the presence of osteoporosis, hypoplasia of blood cell precursors, and replacement of these cells by fat cells confirm the results of Baker and Ingle ('48) who injected adult rats with 1.0 to 3.0 mg of ACTH for a period of 21 days. The occurrence of osteoporosis correlates with the findings of Becks et al. ('44) who observed a decrease in the amount of bone in cortisone treated rats, and Ragan et al. ('49) who observed this condition in ACTH treated normal rats.

The connective tissue of the oral epithelium had decreased in thickness in the 0.1 and 1.0 mg cortisone treated animals: the decrease in thickness was determined by direct histological measurements (table 4). This finding supports our conclusion that the "apparent" precocious eruption observed in our high dosage treated rats is not due to an accelerated
movement of the tooth. Rather it may be due to a thinning of
the overlying gingivae, thus leading to its rupture over the
molar cusps (figs 10, 11).
V. DISCUSSION

A. Cortisone and Molar Eruption

The effects of cortical steroid administration on molar eruption in the albino rat show that five consecutive daily injections begun on eleven day old rats will accelerate the eruption rate. The effect was inversely proportional to the dosage administered showing that this steroid has an optimal dosage preference. An acceleration in rate of eruption of the first maxillary molar was observed at 0.1 and 0.5 mg and a retardation at the 1.0, 1.5 and 2.0 mg dosages.

An acceleration in the rate of eruption of incisors has been observed by other investigators following the administration of cortisone in neonatal rats (Parmer et al., '51; Leroy and Domm, '51; Goldsmith and Ross, '54; Domm and Leroy, '55; Goldsmith and Ross, '56) and in adult rats (Domm and Marzano, '54; Garren, '55; Domm and Wellband, '60; Garren and Greep, '60).

To our knowledge there have been no studies on the effects of cortical steroids on molar eruption in the rat. Indirect evidence was furnished by the work of Schour and Van Dyke ('31) who noted that hypophysectomy, which induces a decrease in adrenal function in rats, caused no observable gross change in the molar. However, their study was begun on day 34 when the
molars had already achieved functional occlusion and without a reliable method of measurement, they could not have detected fluctuations in the molar eruption rate.

Bundgard-Jorgensen et al. (1958) administered 1.0 mg of cortisone daily for the first two weeks of life and 2.0 mg thereafter until day 49. They observed inflammation and ulceration in the molar areas which they attributed to trauma from biting on foreign bodies found in the lesions of this area.

In the light of our experiments, eruption of the molars would have been retarded with the administration of such a dosage, and the anti-repair effects (inhibition of protein synthesis) of cortisone would have been severe enough to explain their observations on inflammation and ulceration. Johannessen (1964) administered 1.0 to 2.0 mg of cortisone daily via food intake for 41 days. The first mandibular molars were removed and the growth of dentin measured. He found that dentinogenesis had been inhibited and was not restored in animals kept intact for later examination. Furthermore, in experiments on semi-starvation of normal rats, he noted that somatic growth was more severely affected than dentinogenesis.

Sobkowksi and Smithgall (1941), Garren (1955), and Garren and Greep (1960) observed that even at high dosages (8.0-10.0 mg) cortisone accelerated the eruption rate of incisors in adult rats. However, in our experiments, a comparably high dose per
kilogram of body weight (1.0 and 1.5 mg in newborn rats) induced a retardation of molar eruption. We attributed this to a lack of tolerance in the younger rat to the toxicity induced by the cortisone. If weight gain is taken as a parameter of intensity of the cortisone effect, our results show that the weight loss at higher dosages is greater than at lower dosages in animals of the same age. Smith ('64) reported, using body weight change and skin collagen per total skin area as parameters of toxicity, that weanling rats are less tolerant to cortisone treatment than young adult or adult rats. He used a 10 mg/kg dosage, the same dosage employed by us. Therefore, from his data, it can be deduced that the retardation of molar eruption at 1.0 and 1.5 mg is the manifestation of a toxic effect of cortisone and hydrocortisone in the young animal.

It occurred to us that cortisone may be accelerating the rate of molar eruption in an indirect manner, that is, it may be exerting its effect on the overlying connective tissue rather than directly on the eruptive mechanism of the molar. Massler and Schour ('41) in their review on theories of eruption pointed out that surgical "release" (incision of the overlying tissues) of an unerupted tooth often precipitates its eruption. Cortisone may well have an analogous effect on the overlying tissues through which the unerupted molar must pass before attaining its future occlusal position. It has
been reported that cortisone exerts a protein catabolic effect and therefore an anti-repair effect on connective tissues. (Clark, '53). In an environment of trauma from nursing and other masticatory activity and even trauma from the force of the erupting tooth, cortisone, because of its anti-repair effect, could release the erupting tooth from restraint. If this were the case, the molar could erupt at a faster rate similar to a molar released from occlusal contact (Soggnaes, '41).

B. Cortisone and the Cellular Proliferation Theory

The observed acceleration in the rate of eruption of the rat molar under the influence of cortisone could be due to an acceleration of cellular activity (differentiation and/or mitosis) in the basal or proliferative zone of the tooth. This view gains support because of earlier observations on the proliferative area in incisors under the influence of cortisone. Goldsmith and Ross ('53) administered 15 and 20 mg of cortisone daily to pregnant rats and observed that the fetuses at twenty days showed accelerated ameloblastic and odontoblastic differentiation. Goldsmith and Ross ('54) reported histologic precocity in three day old rats given 0.1 mg of cortisone in two daily injections beginning at 24 hours of age. The ameloblasts and odontoblasts, though they appeared normal, were producing above normal amounts of enamel and dentin. These investigators, in conjunction with another experiment, confirmed
their earlier observation on acceleration of ameloblastic and odontoblastic development with a 0.1 mg daily dosage (Goldsmith and Ross '56). Anneroth and Bloom ('66) noted that 50 mg of cortisone administered daily for eight days in adult rats caused Hertwig's epithelial sheath (odontogenic epithelium) to be abnormally proliferated. They also noted an increase in the number of pulpal cells. Domm and Kiely ('68) correlated an increase in colchicine-arrested (dividing) cells in the basal or proliferative loop with accelerated eruption of incisors in young adult rats following daily administration of 0.5 and 1.0 mg of cortisone.

Although cortisone generally depresses mitotic activity, (Baker et al., '51; Thiersch et al., '52; Wellington and Moon, '60; Bass and Snell, '61) and growth of cells (Kaufman et al., '53; Wellington and Moon, '60) stimulatory effects on mitosis following cortisone administration have been reported. For example, cortisone accelerated mitotic activity of bone marrow erythroblasts (Fruman, '55). In an in vitro study, Aujard and Chany ('63) found that although cortisone usually depressed mitotic activity, low dosages sometimes stimulated an increased yield in tissue culture. Such seemingly contradictory results may perhaps be explained in terms of specific actions confined to specific dosages. It should be considered that cells may have different types of mitotic triggers, some of which may be sensitive to cortisone only at
certain dosages (Mazia, '60).

C. Cortisone and the Vascular Theory

In view of the reported effects of cortisone on blood vessels and blood pressure there is some support for the vascular theory of eruption, if in fact increased vascularity or blood pressure is the source of the eruptive force. Anneroth and Bloom ('66) recorded an increase in pulpal vascularity with cortisone administration. Wellband ('61), working under the supervision of Dr. L. V. Domm in this laboratory, reported an increase in vascularization of the pulp correlated with injections of 1.5 mg of cortisone in adult hypophysectomized, thyro-adrenalectomized, thiouracil-fed, and normal rats.

Also of interest is the observation that some glucocorticoids cause sodium retention, that is, they induce an exchange of extracellular sodium for intracellular potassium. The potassium is excreted by the kidney and the sodium is mobilized from connective tissue into the extracellular compartment. There is a concomitant increase in extracellular water, inducing a rise in blood pressure and a diuresis (White et al., '64). These factors may effect an increase in molar eruption if vascularity and blood pressure are causative agents in this phenomenon.

D. Cortisone and the Connective Tissue Theory

In any discussion on the collagen contraction theory of
tooth eruption, the role of cortisone must be considered. Thomas ('67), a proponent of this theory, suggested that cortisone may specifically affect the connective tissue of the periodontal membrane, i.e., induce catabolism of the collagen and thereby speed up eruption. Collagen shortening has been shown to be a manifestation of increased hydrogen, covalent, and (possibly) ester bonding (cross-linking) of the molecule (Verzar, '63). We assume that Thomas is implying that cortisone increases the maturation (cross-linking) of collagen fibers, thus inducing their contraction and thereby accelerating eruption. This has not as yet been shown.

Thomas ('67) has also suggested that cortisone may elicit an accelerated eruption by its dehydration effect on collagen fibers. This is supported by the work of Bear ('44) who by means of X-ray diffraction studies reported collagen contraction as a result of dehydration.

E. Cortisone and Other Hormones

Schour and Rogoff ('36) noted that adrenalectomy in the rat caused a decrease but not a complete cessation in incisor eruption rate. Their finding suggests that there may be factors other than adrenal influences that regulate the eruption rate. Schour and Van Dyke ('32b) reported that hypophysectomy completely stopped eruption of the incisors in the adult rat. At the same time, they noted that growth hormone (GH) replacement therapy brought about a partial recovery of the eruption rate.
Of interest is the fact that in their experiment, growth hormone had no effect on the eruption rate in normal animals. Collins et al. ('49) also administered growth hormone to hypophysectomized rats and observed that the eruption rate returned to one-half that of normal. At the same time they also reported that the incisors were larger than those of the hypophysectomized controls. Enamel formation had ceased altogether in the apical one-third leaving the dentin in direct contact with the periodontal membrane. They also observed oral epithelial degeneration, scattered dentin deposits in the pulp and a general increase in bone accretion in the jaw and calvarium. Domm and Marzano ('54) while reporting cortisone stimulation of incisor eruption rate in newborn rats, confirmed the findings of Schour and Van Dyke ('32b) noting that growth hormone had no effect on the eruption rate in normal rats.

Paralleling the above research, were studies being performed concerning the effect of thyroid hormone on eruption. By 1927, it had been shown that acetyl thyroxin could stimulate incisor eruption in the newborn rat (Hoskins, '27), and, by 1947, that thiouracil, a thyroid gland inhibitor, caused an inhibition in the rate of eruption of the incisor in the rat. (Parmer, '47).

Baume et al. ('54a, '54b, '54c) in a series of three consecutive papers reported initially that in a group of 60 thyroidectomized rats, the incisor eruption rate was decreased
45%; growth hormone stimulated this rate to 10% over that of control thyroidectomized rats; thyroxin to 27%; and a combination of growth hormone and thyroxin stimulated it slightly more than 27%. The histology of the incisors in the thyroidectomized, thyroxin-treated animals did not return to normal whereas that of the growth hormone thyroxin-treated ones had returned to normal.

In their second paper ('54b) they compared incisor eruption rates of normal and hypophysectomized rats and found that the normal rate was 2.53 mm per week, while the hypophysectomized rate had decreased 76% to 0.55 mm per week. The fact that hypophysectomy decreases the eruption rate more than thyroidectomy may indicate that another pituitary factor(s) is involved.

In their third paper ('54c) Baume's group reported the effects of growth hormone and thyroxin in hypophysectomized rats. Growth hormone, while increasing the size of the incisor, had no effect on the eruption rate whereas thyroxin administration stimulated the rate by as much as 46% over that of hypophysectomized controls. The simultaneous administration of growth hormone and thyroxin restored the eruption rate to normal. In evaluating their data these investigators postulated that growth hormone and thyroxin synergistically regulate the eruption process; i.e., growth hormone affects the basic growth process, while thyroxin controls maturation and differentiation.

Goldsmith and Ross ('56) injected 0.1 mg of cortisone into
newborn rats and observed an acceleration in the rate of eruption of incisors. They also noted from the work of Schour and Rogoff ('36) that adrenalectomy only blocks eruption partially. Extrapolating from the data in the literature, they concluded that growth hormone and thyroxin directly control eruption and, on the basis of their own study, they concluded that cortisone is vital in normal eruption because of its effects on RNA and alkaline phosphatase levels.

Domm and Wellband ('61) measured eruption rates in thyroidectomized, adrenalectomized, thyro-adrenalectomized, and normal adult female rats. Each of these groups were then divided; one was treated with cortisone, while the other was kept as an operated control. Thyroidectomy decreased the eruption rate of incisors 19%; adrenalectomy 33%; and thyro-adrenalectomy 53%. The administration of 1.5 mg of cortisone restored the eruption rate to normal in each of these groups. They concluded on the basis of percentage results in operated animals that normal eruption is synergistically controlled by the thyroid and the adrenal glands.

For purposes of clarification of the many facts presented on hormones and tooth eruption, we have constructed the table shown below. On the basis of this data, we believe we can make certain tentative conclusions and predictions.

In view of the wide application of cortisone as replacement therapy in many hormonally-deficient states (row three) we could
## The Effect of Hormones on Eruption Rate in the Rat

<table>
<thead>
<tr>
<th>Normal Hormone</th>
<th>Hypophysectomy</th>
<th>Thyroidectomy or Thiouracil</th>
<th>Adrenalectomy</th>
<th>Thyro-adrenalectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No GH, T, C*</td>
<td>No T; normal GH &amp; C</td>
<td>No C; normal GH &amp; T</td>
<td>No T, C. normal GH</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Growth Hormone</th>
<th>Thyroxin</th>
<th>Cortisone</th>
<th>GH &amp; Thyroxin</th>
<th>No therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4 or 2,3,9</td>
<td>7</td>
<td>11,12</td>
<td>9 Predict</td>
<td>2,8,12</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>11,12</td>
<td>9 Predict</td>
<td>6,7,11</td>
</tr>
<tr>
<td>11,12</td>
<td>7</td>
<td>11,12</td>
<td>9 Predict</td>
<td>1,11,12</td>
</tr>
</tbody>
</table>

* GH Growth Hormone; T Thyroxin or acetyl thyroxin; C Cortisone.
WNL Within Normal Limits.

1. Schour and Rogoff (1936)
2. Schour and Van Dyke (1932)
3. Collins et al. (1949)
4. Domm and Marzano (1954)
5. Hoskins (1927)
6. Parmar (1947)
7. Baume et al. (1954a)
8. (1954b)
9. (1954c)
10. Goldsmith and Ross (1956)
12. Wellband (1961)

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Postulate that the use of pharmacological doses is sufficient for the maintenance of normal tooth eruption in rats whether they are hypophysectomized, thyroidectomized, chemically-thyroidectomized, adrenalectomized, thyro-adrenalectomized, or normal. Noting that growth hormone and thyroxin combined (row four) can restore the eruption rate to within normal
limits, we might question whether cortisone is necessary at all. In the normal animal in which only physiological doses of each of these hormones are acting, we may tentatively suggest that these three hormones, growth hormone, thyroxin, and cortisone, all act synergistically to control tooth eruption; or, that cortisone potentiates the other two hormones. The studies performed on animals, not receiving replacement therapy (row five), that were hypophysectomized (eruption stops), thyroidectomized (eruption rate slows), and adrenalectomized (eruption rate slows) would seem to support this assumption.

The question marks in the table indicate research which to our knowledge has not yet been reported. Our predictions are based on the publications cited. We believe that these missing links are the factors that, once reported, may explain the hormonal mechanisms acting in normal tooth eruption.

F. Cortisone and Body Weight

The degree of severity of the cortical steroid effect was obvious. Our gross observations included weight loss, diarrhea, loose yellow stools, and decreased hair growth. These observations confirm the report of Parmer et al. (51) who administered daily, for five consecutive days, 0.1 mg injections of cortisone to one day-old rats. In our experiments we began 0.1 mg injections on day 11. Our gross
observations did not include effects so severe as those reported by Farmer's group, but in a previous experiment using the same dosage (0.1 mg) with injections beginning on day 4, our observations correlated very well.

In this discussion, weight gain refers to the total gain from the onset of hormone injection to the day of sacrifice, a five day period. The immediate effect of the cortical steroids was reflected in all treated animals as a weight loss at the end of the first day and a gradual recovery on subsequent days. As shown in table 1, weight gain decreased as the dosage increased. Hydrocortisone had a slightly greater effect in this regard than did cortisone. A decrease in weight gain has been reported by several investigators in work on newborn rats (Leroy and Domm, '51; Parmer et al., '51) and adult rats (Garren, '54; Bundgaard-Jorgensen et al., '58; Johannessen '64; Anneroth and Bloom '66); and in newborn mice (Stock et al., '51).

Parmer et al. ('51) explained the weight loss in terms of dehydration due possibly to decreased nursing by the newborn rats under the influence of cortisone. This explanation is not confirmed by the data of Johannessen ('64) who administered 1.0 mg of cortisone per day to adult rats and also observed weight loss with decreased food intake. However, upon the discontinuance of treatment, the increase in food intake was not sufficient to explain the observed
gain in weight. Russell and Wilhelmi ('54) note that in humans as well as in animals there tends to be an increase in appetite with the administration of cortical steroids. These varying reports suggest that the weight loss observed in cortisone-treated rats is not due to a decrease in food intake but rather to a decrease in the utilization of metabolites at the cellular level, or to a change in weight due to some other factor such as water balance.

Cortisone does affect water balance. This hormone may directly affect kidney function (Harrison and Darrow, '38), however, extrarenal effects were also observed. Cortical steroids may alter water balance by decreasing the serum potassium in adrenalectomized and nephrectomized rats (Ingle et al., '37). In this way, these steroids may affect electrolyte balance. Conn et al. ('51) reported that hydrocortisone induces sodium retention (and, therefore, water retention) in normal people. In other investigations, cortisone induced sodium excretion and diuresis (Thorn et al., '41; Dorfman, '49). This difference may be due to the dosages used. Dehydration due to transfer and/or excretion of water could be a factor to consider in the weight loss in cortisone-treated rats.

Weight loss in the cortisone-treated rat may be explained by the effect of cortisone on carbohydrate, lipid, and/or protein metabolism. Cortisone administration in rats
increases the carbohydrate content of blood and tissues, much of this increase being stored as liver glycogen. The effect of cortisone in "diabetogenic", i.e., there is an increase in blood glucose and urinary nitrogen (Long et al., '40). The increase in urinary nitrogen excretion is a reflection of protein catabolism and is referred to as a negative nitrogen balance. Cortisone administration also leads to a breakdown or a blockage in the synthesis of proteins in rats and man which has been reported to be the source of the excess urinary nitrogen (Clark, '53). This protein is altered metabolically and released as carbohydrate into the blood and tissues. The liver, under the effect of cortisone, stores the blood carbohydrate as hepatic glycogen (Greengard et al., '63a, '63b) by stimulating the enzymes necessary for storage. In the second paper ('63b), they reported on injections of cortisone into starved adult Sprague-Dawley rats. Puromycin (an inhibitor of cortisone-induced hepatic enzymes, i.e., tyrosine transaminase and tryptophane Pyrrolase) were injected in these rats to block hepatic glycogen storage. These antimetabolites did inhibit the cortisone-induced storage of glycogen. From this, they concluded that cortisone stores liver glycogen by stimulating an increase of the necessary hepatic enzymes.

In balance experiments in which adrenal corticoids were administered, the increase in excretion of glucose far
exceeds the amount of nitrogen excreted. This implies that the source of this glucose is not entirely protein catabolism but that the normal utilization of carbohydrate may be blocked as well (Ingle and Thorn, '41; Russell and Wilhelmi, '54). Weight loss in rats on cortical steroid treatment could therefore be explained in terms of a tissue breakdown and repair inhibition which, specifically, is a manifestation of protein catabolism and blockage of carbohydrate utilization at the cellular level.

Since, under the effects of corticoids, the body is suffering a protein catabolism and a blocked utilization of blood carbohydrates, it seems logical to assume that the body would tap fat stores for required energy. Cahn and Houget ('64) noted that in the cortisone-treated doe-rabbit, the fat stores are mobilized to compensate for the decreased combustion of glucides (carbohydrates and glycosides). Cortisone enhances the storage of hepatic fat and muscle fat and burns the blood lipids for energy. According to Jeanrenaud and Renold ('66), the glucocorticoid action on rat adipose tissue in vitro is that of increasing the output of free fatty acids. But they do not act by breaking down (lipolysis) fat stores as such, but by decreasing the rate of re-esterification of free fatty acids. The most likely explanation is that the decreased glucose metabolism and the inhibition of re-esterification are somehow directly linked, possibly through a decreased avail-
ability of glycerophosphate. Whether this glucose metabolism is at the cell membrane or in the Kreb's cycle itself is not known.

G. X-ray as an Experimental Procedure

The use of the X-ray as an experimental procedure was evaluated by application of the standard error of difference (s.e.d.) test of significance to comparisons of body weights (significant deviation in body weight was chosen as an indicator of procedural variation (Smith, '64)). Compared were the groups outlined on table 6. Statistical significance is present if D (the difference between the means of the compared groups) exceeds 3 s.e.d. There was no statistical significance in any of the groups compared. Therefore, use of the X-ray procedure did not introduce a variable into these experiments.

H. True or Apparent Precocious Eruption

Our gross observations on the gingivae overlying the first maxillary molar, initially led us to conclude that we were observing true precocious eruption of this molar in rats injected with 1.0 and 1.5 mg of cortisone. However, measurements on roentgenograms showed that molar eruption was in fact retarded. What we had viewed grossly was the molar cusps through a dehydrated or thinner-than-normal gingivae.

We have already discussed cortisone-induced dehydration. In this case, we may simply have been observing a dehydrated
gingivae that weakened and separated over the advancing molar cusps. We also discussed the data on cortisone-induced protein catabolism. With this in mind, the gingival condition may be due to a thinning of the subjacent connective tissue due to loss of protein.

I. Cortisone and Gingival Keratinization

We did consider the effect of cortisone on keratinization of the molar gingivae. Goldsmith and Ross ('54) noted that the oral epithelium in the incisor area became completely keratinized following the administration of 0.1 mg of cortisone in newborn rats. Farmer et al, ('51) found that in addition to accelerated eruption and early eye-opening, cortisone induced an accelerated gingival development. Mangine ('65), working under the supervision of Professor L. V. Domm of this department, did a careful study on the effects of cortisone on the gingivae and oral mucosa of the hard palate. She noted that 0.5 and 1.5 mg of cortisone stimulated keratinization in these areas in the neonatal rat. Accompanying this increase in keratinization there was a decrease in the thickness of the non-keratinized epithelial layer. She concluded that, in consequence, an erupting tooth has a thinner layer of stratum corneum in its path to occlusion which may, in part, account for the precocious eruption observed in cortisone-treated neonatal rats. Our observations of "apparent" precocious eruption of the rat molar under the effect of cortisone may be explained
in terms of the above data. Cortisone may have stimulated the keratinization of the molar gingivae and reduced the thickness of the underlying non-keratinized layers in our experiments. The thinned gingivae may then have been pulled down over the molar cusps, rupturing the gingivae, thus giving the appearance of accelerated growth of the molar and precocious eruption.

J. Cortisone and Hair Growth

In our experiments we observed a decrease in hair growth as a result of the administration of cortisone and of hydrocortisone. This failure of growth was consistent and tended to be of greater severity when the hormone treatment was begun on younger rats. Other investigators have reported decreased hair formation following cortisone administration (Baker et al., '48; Baker and Whitaker, '48; Castor and Baker, '50) but the mechanism of action is unknown. Capillary fragility and permeability increase with adrenalectomy and are correlated with precocious hair growth, (Kozam, '52). Therefore, it would seem that cortisone administration would decrease this fragility and permeability. This could deprive the hair follicle cells of needed nutrients, of metabolite exchange, or of other growth factors (Chase, '55). Cortisone is known to deprive body cells of needed carbohydrates, lipids, and proteins that may be necessary for hair growth. It has been reported by many authors that cortisone, minerals, vitamins, and sulfhydryls affect hair growth (Ralli and Graef, '43,
'45; Anderson et al., '51; Hundley and Ing, '51; Meites, '51 and others).

In our results, decreased hair growth tended to be more severe in the younger rats treated with cortical steroids. Mohn ('58) found in his work on rats that only follicle growth is affected by cortisone. This would explain our observations of a more severe decrease in hair growth in the younger rats treated with either cortisone or hydrocortisone.

K. Cortisone and Bone Growth

The degree of eruption was measured as the distance between the molar alveolar crest and the most mesial (rostral) molar cusp (fig. 7). Radiographical bone measurements were made on the roentgenograms of 0.1 and 1.0 mg cortisone-treated and control animals to determine the validity of the radiological measurements which indicated acceleration and retardation of molar eruption in these groups. In this way, the effect of cortisone on bone growth could be measured and compared with control values.

We found no significant difference in the amount of bone growth between the 0.1 mg cortisone-treated and control animals. Therefore, since there was no manifestation of bone resorption at the molar alveolar crest we concluded that accelerated molar eruption had actually occurred. We observed a retardation in eruption of the 1.0 mg cortisone-treated rats. This finding is not a manifestation of bone apposition at the
molar alveolar crest. We did in fact observe a statistically significant decrease in bone growth at the alveolar crest.

Schour and Van Dyke ('32a) noted that hypophysectomy (inducing an adrenal insufficiency) led to a decrease in alveolar bone growth in adult rats. Specifically, they noted a thinning and degeneration of the periosteum. The effect of cortisone in such rats was not known is 1932. Cortisone when administered to growing rats in various dosages from 0.25 to 100 mg/kg (1 to 10 mg/kg in our experiments) induces the formation of a dense zone of calcified cartilagenous spicules in long bones (Urist et al., '48; Ragan et al., '49). Leroy and Domm ('51) injected 5 mg of cortisone into normal pregnant rats and noted that X-ray examination of the skull in the ten day-old rat showed a decrease in calcification. Goldsmith and Stahl ('53) injected 3.0 mg of cortisone in adult rats and reported an increase in growth of alveolar bone. Goldsmith and Ross ('56) administered 0.1 mg of cortisone in newborn rats and observed a marked overgrowth of alveolar bone and a precocity in the rate of incisor eruption of 2.9 days. These studies reporting an increase in bone growth lead us to conclude that cortisone may possess a specificity for accelerating bone growth at certain dosages.

The observations of Garren and Greep ('60) are in partial agreement with our results. They administered 1 to 10 mg/kg in adult rats (the dosage employed by us) and reported no
change in alveolar bone. In our studies, newborn rats receiving the lower dosage (0.1 mg) revealed no significant change in alveolar bone growth. At the higher dosage, however, we observed a resorption of bone (determined by radiologic measurements).

It has been reported that the alveolar crest is more stable in an environment of physiological change than the gingivae for the purpose of measuring rate of eruption (Ness, '54; Miller, '57). The choice of the alveolar crest as a reference point in the measurement of molar eruption is reinforced by the consistency of our results (table 2) in each dosage grouping.

L. Histological Observations

The molars of rats treated with cortisone and hydrocortisone showed no morphologic modifications with respect to odontoblasts, ameloblasts, dentin formation or pulpal cellularity and vascularity. It was not possible to make observations on enamel on the decalcified sections. Since amelogenesis is complete at 11 days (Schour and Massler, '49) we did not expect any change in enamel formation as a result of the experimental procedures which began on day 11.

Our findings confirm those of Kiely ('67) who found no significant histological modifications in his control rats, 0.25, 0.5 and 1.0 mg cortisone-treated rats, or normal untreated controls. Wellband ('61) also noted no histological
differences between his 0.25, 0.5 and 1.5 mg cortisone-treated and control rats.

Of significance in our study was the increase in alveolar bone resorption in the 1.0 mg treated rats. This was first observed as an increase in the number of multinucleated osteoblasts on the walls of all trabeculae. Actual measurements on the thickness of the trabeculae supported this observation by indicating a marked decrease in thickness in the treated rats. Measurements on the height of the alveolar crest from the dentino-enamel junction also gave an indication that a resorption process was taking place. These measurements, (table 4) combined with the radiological observations on bone growth (table 5) and eruption (table 2) indicated that bone resorption increased noticably in the 1.0 mg cortisone-treated rats when compared with those receiving 0.1 mg. It has been shown by Follis ('51) and Stanisavljevic et al., ('62) that cortisone suppresses bone formation more than bone resorption in the rat. Hence, resorption quickly overcomes formation, resulting in a net bone loss.

This resorption in the alveolus of the rat may be a response by bone to the cortisone-induced alteration in calcium and phosphorus metabolism (Storey, '60). Because of this alteration, the bone matrix cannot be mineralized and it is subsequently broken down (resorbed).

This resorption brings about "osteoporosis" defined by
Frost ('66) as the condition resulting in the net loss of bone. A more popular theory accounting for this extensive bone resorption holds that cortisone brings about a "negative skeletal balance" caused by the immediate increase in number of osteoclasts (Frost, '66). However, the mechanism of action is unknown. Robbins ('64) suggests that in hyperadrenalism (Cushing's syndrome) there is an increase in protein catabolism leading to a breakdown of the organic matrix of bone. Since there is then no place where calcium can be deposited, the resorption-formation balance is upset—resulting in a net loss of bone.

The marrow space of the molar alveolar bone was marked by a hypoplasia of the blood cell precursors and an increase in fat cells. Baker and Ingle ('48) injected 1.0 to 3.0 mg of ACTH in adult rats and observed red marrow atrophy and replacement of these cells with fat cells.

The presence of histologically detectable fat storage was a noticeable difference between experimentals and controls in all our cortical steroid treated rats. This accumulation of fat was seen in the marrow spaces of the alveolar bone and surrounding the muscle in these section. As mentioned above, fat stores are usually mobilized to compensate for decreased combustion of carbohydrates; moreover, cortisone is known to enhance the storage of hepatic and muscle fat (Cahn and Houget, '64).
A deficiency in the loose connective tissue of the molar gingivae was observed in all cortisone-treated rats. This would seem to lend support to our conclusion that gingival rupture over the molar cusps brings about an apparent precocious eruption rather than a true eruption. We believe that the connective tissue deficiency is in some way related to protein catabolism. Ragan et al. ('49) also observed an inhibition in the formation of connective tissue in rabbits treated with 12.5 mg of cortisone twice daily for three days, and Castor and Baker ('50) reported a decrease in the collagenous part of connective tissue in rats treated with cortisone.
VI SUMMARY AND CONCLUSIONS

1. The administration of cortisone and hydrocortisone in five consecutive daily doses brought about precocious eruption of the molars (gross observation). The injection of 1.0 mg of either of those steroids did not result in an increase in the eruption rate as shown by measurements on roentgenograms. It is concluded that shrinkage or thinning of the gingivae over the erupting cusps rather than acceleration in eruption facilitated this appearance in the molar region. Hydrocortisone at comparable dosages was somewhat more effective than cortisone in this response.

2. A reduced gain in body weight and a decrease in the density of hair were observed regardless of the steroid employed or the dosage administered.

3. The molar eruption rate was accelerated by both cortisone and hydrocortisone at dosages of 0.1 and 0.5 mg while it was actually retarded at the 1.0, 1.5 and 2.0 mg dosages. Cortisone accelerated eruption slightly more than hydrocortisone (roentgenographic observation).

4. The measurements on rate of eruption which were obtained radiographically are considered to be valid and are not manifestations of increased or decreased alveolar bone growth.

5. The radiographical measurements on bone growth showed that there was little fluctuation in growth at a dosage of 0.1 mg.
of cortisone whereas at the 1.0 mg dosage, the molar alveolar bone revealed a resorption when compared with controls.

6. Neither cortisone nor hydrocortisone had any histologically observable influence on basal loop proliferation, odontoblastic and ameloblastic morphology, dentin formation, or pulpal vascularity. However, connective tissues were depleted. Extensive decrease in growth of the molar alveolar crest was observed with high (1.0 mg) dosages of cortisone while on the contrary it was minimal at the lower (0.1 mg) dosage.

Cortisone caused a hypoplasia of blood cell precursors in the bone marrow. Accompanying this there was extensive storage of fat in the bone marrow.

7. The X-ray procedure, as employed in this study, is a valid experimental tool. Using body weight as a measure of variation, it was shown that the use of X-ray did not introduce a variable into the experiments.

8. Both cortisone and hydrocortisone accelerated the rate of eruption of molars. These observations support the hypothesis that similar mechanisms operate in the eruptive process of both the continuously erupting incisors and the limited erupting molars of the rat.


---


TABLE 1

Body Weight Changes in Rats

Injected Between Days 11 and 16

<table>
<thead>
<tr>
<th># of Rats</th>
<th>Treatment</th>
<th>Weight (gm)</th>
<th>gm Gain</th>
<th>S.D.</th>
<th>S.E.</th>
<th>S.E.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 11</td>
<td>Day 16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Control*</td>
<td>20.7</td>
<td>31.5</td>
<td>10.8</td>
<td>3.72</td>
<td>1.52</td>
</tr>
<tr>
<td>11</td>
<td>0.1 mg E*</td>
<td>24.4</td>
<td>34.8</td>
<td>10.4</td>
<td>1.72</td>
<td>0.81</td>
</tr>
<tr>
<td>7</td>
<td>0.1 mg F*</td>
<td>22.4</td>
<td>29.8</td>
<td>7.4</td>
<td>2.49</td>
<td>1.02</td>
</tr>
<tr>
<td>13</td>
<td>Control</td>
<td>22.7</td>
<td>33.7</td>
<td>11.0</td>
<td>1.90</td>
<td>0.53</td>
</tr>
<tr>
<td>9</td>
<td>0.5 mg E</td>
<td>23.3</td>
<td>30.1</td>
<td>6.8</td>
<td>3.21</td>
<td>1.07</td>
</tr>
<tr>
<td>6</td>
<td>0.5 mg F</td>
<td>21.8</td>
<td>22.9</td>
<td>1.1</td>
<td>2.24</td>
<td>0.85</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>23.4</td>
<td>34.3</td>
<td>10.9</td>
<td>4.18</td>
<td>2.30</td>
</tr>
<tr>
<td>7</td>
<td>0.75 mg E</td>
<td>21.3</td>
<td>25.7</td>
<td>4.4</td>
<td>3.68</td>
<td>1.84</td>
</tr>
<tr>
<td>5</td>
<td>0.75 mg F</td>
<td>22.2</td>
<td>23.9</td>
<td>1.7</td>
<td>1.34</td>
<td>0.95</td>
</tr>
<tr>
<td>17</td>
<td>Control</td>
<td>18.4</td>
<td>28.2</td>
<td>9.8</td>
<td>1.48</td>
<td>0.38</td>
</tr>
<tr>
<td>13</td>
<td>1.0 mg E</td>
<td>18.8</td>
<td>22.1</td>
<td>3.3</td>
<td>1.70</td>
<td>0.54</td>
</tr>
<tr>
<td>11</td>
<td>1.0 mg F</td>
<td>19.3</td>
<td>20.0</td>
<td>0.7</td>
<td>1.17</td>
<td>0.35</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>20.8</td>
<td>31.7</td>
<td>10.9</td>
<td>1.62</td>
<td>0.72</td>
</tr>
<tr>
<td>7</td>
<td>1.5 mg E</td>
<td>19.9</td>
<td>22.8</td>
<td>2.9</td>
<td>1.20</td>
<td>0.69</td>
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<tr>
<td>6</td>
<td>1.5 mg F</td>
<td>20.8</td>
<td>21.7</td>
<td>0.9</td>
<td>1.85</td>
<td>0.92</td>
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<tr>
<td>6</td>
<td>Control</td>
<td>21.3</td>
<td>33.6</td>
<td>12.3</td>
<td>1.98</td>
<td>0.70</td>
</tr>
<tr>
<td>5</td>
<td>2.0 mg E</td>
<td>19.3</td>
<td>21.3</td>
<td>2.1</td>
<td>1.60</td>
<td>0.54</td>
</tr>
<tr>
<td>5</td>
<td>2.0 mg F</td>
<td>2.01</td>
<td>21.6</td>
<td>1.5</td>
<td>2.12</td>
<td>0.75</td>
</tr>
<tr>
<td>4</td>
<td>Control II**</td>
<td>21.2</td>
<td>32.6</td>
<td>11.4</td>
<td>1.66</td>
<td>0.83</td>
</tr>
</tbody>
</table>

* Control: All control animals received 0.85% saline.
E: Compound E acetate (cortisone).
F: Compound F acetate (hydrocortisone).

† S.D.: Standard deviation
S.E.: Standard error
S.E.D.: Standard error of difference between experimental and control means.

# Significance equal to †/– 3 S.E.D.
**Control II animals received neither X-ray nor injection.
**TABLE 2**

Eruption Rates of the First Maxillary Molar
in Rats Injected Between Days 11 and 16

<table>
<thead>
<tr>
<th># of Rats</th>
<th>Treatment</th>
<th>Eruption (mm) Day 11</th>
<th>Eruption (mm) Day 16</th>
<th>Eruption (mm) Total</th>
<th>% of Day Cont.</th>
<th>S.D.</th>
<th>S.E.</th>
<th>S.E.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Control*</td>
<td>.799</td>
<td>1.709</td>
<td>1.015</td>
<td>.182</td>
<td>100</td>
<td>.0303</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.1 mg E*</td>
<td>.622</td>
<td>1.889</td>
<td>1.267</td>
<td>.253</td>
<td>139</td>
<td>.0643</td>
<td>.0816#</td>
</tr>
<tr>
<td>6</td>
<td>0.1 mg F*</td>
<td>.706</td>
<td>1.911</td>
<td>1.204</td>
<td>.241</td>
<td>132</td>
<td>.0299</td>
<td>.0586#</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>.581</td>
<td>1.598</td>
<td>1.017</td>
<td>.203</td>
<td>100</td>
<td>.0504</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.5 mg E</td>
<td>.564</td>
<td>1.828</td>
<td>1.264</td>
<td>.253</td>
<td>125</td>
<td>.0328</td>
<td>.0743#</td>
</tr>
<tr>
<td>6</td>
<td>0.5 mg F</td>
<td>.524</td>
<td>1.743</td>
<td>1.219</td>
<td>.244</td>
<td>120</td>
<td>.0260</td>
<td>.0715#</td>
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<tr>
<td>5</td>
<td>Control</td>
<td>.632</td>
<td>1.673</td>
<td>1.041</td>
<td>.208</td>
<td>100</td>
<td>.0590</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.75 mg E</td>
<td>.522</td>
<td>1.830</td>
<td>1.308</td>
<td>.262</td>
<td>126</td>
<td>.1774</td>
<td>.0973</td>
</tr>
<tr>
<td>2</td>
<td>0.75 mg F</td>
<td>.550</td>
<td>1.481</td>
<td>0.921</td>
<td>.184</td>
<td>88</td>
<td>.005</td>
<td>.0591</td>
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<tr>
<td>6</td>
<td>Control</td>
<td>.599</td>
<td>1.580</td>
<td>0.981</td>
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<td>100</td>
<td>.0361</td>
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<tr>
<td>6</td>
<td>1.0 mg E</td>
<td>.671</td>
<td>1.635</td>
<td>0.964</td>
<td>.193</td>
<td>98</td>
<td>.0729</td>
<td>.0813</td>
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<tr>
<td>6</td>
<td>1.0 mg F</td>
<td>.464</td>
<td>1.492</td>
<td>1.028</td>
<td>.206</td>
<td>105</td>
<td>.0378</td>
<td>.0523</td>
</tr>
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<td>4</td>
<td>Control</td>
<td>.569</td>
<td>1.538</td>
<td>0.969</td>
<td>.194</td>
<td>100</td>
<td>.0361</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.5 mg E</td>
<td>.831</td>
<td>1.558</td>
<td>0.727</td>
<td>.145</td>
<td>75</td>
<td>.0175</td>
<td>.0637#</td>
</tr>
<tr>
<td>3</td>
<td>1.5 mg F</td>
<td>.519</td>
<td>1.597</td>
<td>1.078</td>
<td>.216</td>
<td>113</td>
<td>.0297</td>
<td>.0681</td>
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<tr>
<td>6</td>
<td>Control</td>
<td>.572</td>
<td>1.598</td>
<td>1.026</td>
<td>.205</td>
<td>100</td>
<td>.0293</td>
<td></td>
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<td>7</td>
<td>2.0 mg E</td>
<td>.503</td>
<td>1.492</td>
<td>1.989</td>
<td>.198</td>
<td>97</td>
<td>.0401</td>
<td>.0496</td>
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<td>7</td>
<td>2.0 mg F</td>
<td>.466</td>
<td>1.347</td>
<td>0.881</td>
<td>.176</td>
<td>86</td>
<td>.0890</td>
<td>.0937</td>
</tr>
</tbody>
</table>

* Control: All controls received 0.85% saline.
  E: Compound E acetate (cortisone).
  F: Compound F acetate (hydrocortisone).

† S.D.: Standard deviation
S.E.: Standard error
S.E.D.: Standard error of difference between experimental and control means.

# Indicates significance equal to +/− 3 S.E.D.
**TABLE 3**

Eruption Rates of the First Mandibular Molar

in Rats Injected Between Days 11 and 16

<table>
<thead>
<tr>
<th>No. Rats</th>
<th>Treatment</th>
<th>Eruption (mm)</th>
<th>Day 11</th>
<th>Day 16</th>
<th>Total</th>
<th>Day Cont.</th>
<th>S.D.</th>
<th>S.E.</th>
<th>S.E.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Control*</td>
<td>.520</td>
<td>1.269</td>
<td>.749</td>
<td>.150</td>
<td>100</td>
<td>.135</td>
<td>.045</td>
<td>----</td>
</tr>
<tr>
<td>9</td>
<td>0.1 mg E*</td>
<td>.491</td>
<td>1.354</td>
<td>.863</td>
<td>.171</td>
<td>114</td>
<td>.174</td>
<td>.058</td>
<td>.022#</td>
</tr>
<tr>
<td>6</td>
<td>0.1 mg F*</td>
<td>.520</td>
<td>1.478</td>
<td>.958</td>
<td>.195</td>
<td>130</td>
<td>.164</td>
<td>.067</td>
<td>.024#</td>
</tr>
</tbody>
</table>

* Same footnotes as TABLE 2.

# Indicates significance equal to +/- 3 S.E.D.
# Table 4

## Histological Measurements

of the First Maxillary Molar Region*

<table>
<thead>
<tr>
<th>No.</th>
<th>Obs.</th>
<th>Treatment</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>Control</td>
<td>34.9  9.9  38.6  9.9  11.7  25.8</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>0.1 mg E**</td>
<td>46.9  5.9  31.4  9.4  9.3  22.9</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Control</td>
<td>47.8  10.2  48.8  9.5  16.2  27.9</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>1.0 mg E</td>
<td>50.3  6.5  40.6  11.9  11.9  32.5</td>
</tr>
</tbody>
</table>

* All measurements were made with an ocular micrometer and are expressed in low power units with the exception of blood vessel measurements which are in high power units.

- **H.S.**: Haversian space of alveolar bone.
- **T.**: Trabecular envelope surrounding H.S.
- **A.h.**: Height of alveolar bone, i.e., Superior aspect of alveolar bone to the dentino-enamel junction.
- **D.**: Thickness of dentin at the dentino-enamel junction.
- **C.T.**: Thickness of connective tissue of the gingivae.
- **b.v.**: Diameter of major pulpal vessels.

**E** refers to Kendall's compound E or cortone acetate. Treated rats were injected daily from 11 and 16 days.
TABLE 5

Measurements Showing Growth of Alveolar Bone* in Rats Injected Between Days 11 and 16

<table>
<thead>
<tr>
<th>0.1 mg Cortisone (mm)</th>
<th>1.0 mg Cortisone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.556</td>
<td></td>
</tr>
<tr>
<td>6.605</td>
<td></td>
</tr>
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<td>6.359</td>
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<tr>
<td>6.369</td>
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<tr>
<td>6.234</td>
<td></td>
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<tr>
<td>6.535</td>
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<tr>
<td>6.881</td>
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<td>6.114</td>
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<tr>
<td>7.133</td>
<td></td>
</tr>
<tr>
<td>mean 6.709</td>
<td>mean 6.443</td>
</tr>
<tr>
<td>+ .644**</td>
<td>+ .120</td>
</tr>
<tr>
<td>+ .372**</td>
<td>+ .049</td>
</tr>
<tr>
<td>+ .394**</td>
<td>+ .123</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>0.1 mg Hydrocortisone (mm)</th>
<th>1.0 mg Hydrocortisone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.073</td>
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<tr>
<td>6.013</td>
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<td>5.971</td>
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<td>6.788</td>
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<td>6.707</td>
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<tr>
<td>6.853</td>
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</tr>
<tr>
<td>6.602</td>
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</tr>
<tr>
<td>mean 6.738</td>
<td>mean 6.146</td>
</tr>
<tr>
<td>+ .109</td>
<td>+ .151</td>
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<tr>
<td>+ .055</td>
<td>+ .062</td>
</tr>
<tr>
<td>+ .140</td>
<td>+ .129</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control (mm)</th>
<th>Control (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.932</td>
<td>6.977</td>
</tr>
<tr>
<td>6.977</td>
<td>7.469</td>
</tr>
<tr>
<td>6.811</td>
<td>6.805</td>
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<tr>
<td>6.683</td>
<td>6.946</td>
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<tr>
<td>6.797</td>
<td>7.061</td>
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<tr>
<td>6.441</td>
<td>6.441</td>
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<tr>
<td>mean 6.748</td>
<td>mean 6.946</td>
</tr>
<tr>
<td>+ .257</td>
<td>+ .276</td>
</tr>
<tr>
<td>+ .129</td>
<td>+ .113</td>
</tr>
</tbody>
</table>

* Measurements according to method outlined on p. 15.
**S.D. Standard deviation; S.E. Standard error; S.E.D. Standard error of difference between experimental & control means.
TABLE 6

X-ray as an Experimental Procedure

<table>
<thead>
<tr>
<th>Test</th>
<th>D*</th>
<th>S.E.D.*</th>
<th>Significance*</th>
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<tbody>
<tr>
<td>1:2</td>
<td>0.30 gm</td>
<td>0.924 gm</td>
<td>No</td>
</tr>
<tr>
<td>1:3</td>
<td>0.94 gm</td>
<td>1.012 gm</td>
<td>No</td>
</tr>
<tr>
<td>2:3</td>
<td>0.54 gm</td>
<td>0.706 gm</td>
<td>No</td>
</tr>
<tr>
<td>4:5</td>
<td>0.38 gm</td>
<td>2.797 gm</td>
<td>No</td>
</tr>
<tr>
<td>6:7</td>
<td>0.06 gm</td>
<td>0.995 gm</td>
<td>No</td>
</tr>
</tbody>
</table>

1: Non-injected, non-X-rayed control:
N*= 4  mean= 11.4 gm
S.D.*= ± 1.66 gm  S.E.*= ± 0.83 gm

2: Saline-injected, X-rayed control:
N 36  mean=11.0 gm
S.D.= ± 2.43 gm  S.E. = ± 0.41 gm

3: Saline-injected, non-X-rayed control:
N=17  mean=10.46 gm
S.D. = ± 2.39  S.E. = ± 0.58 gm

4: 0.5 mg cortisone-injected, X-rayed experimental:
N= 6  mean= 6.95 gm
S.D.= ± 2.98  S.E. = ± 1.21 gm

5: 0.5 mg cortisone-injected, non-X-rayed experimental:
N=3  mean= 6.57 gm
S.D. = ± 4.37 gm  S.E. = ± 2.52 gm

6: 1.0 mg cortisone-injected, X-rayed experimental:
N=6  mean= 3.32 gm
S.D. = ± 2.17 gm  S.E. = ± 0.89 gm

7: 1.0 mg cortisone-injected, non-X-rayed experimental:
N=4  mean= 3.38 gm
S.D. = ± 0.90 gm  S.E. = ± 0.45 gm

* D: Difference between means of compared groups.
S.E.D.: Standard error of difference between compared means.
N: Number of animals in group.
Mean: Average, or mean weight gain.
A photograph showing the cephalostat and industrial X-ray unit used in the experiments on the first maxillary molar of the rat.
PLATE 2

EXPLANATION OF FIGURE

2 A photograph showing precocious eruption in a 0.5 mg cortisone-treated rat and normal eruption in a control (ages 16 days). Eruption of experimental rat: 1.323 mm; control rat: 0.760 mm. Arrow points to first maxillary molar.
PLATE 3
EXPLANATION OF FIGURES

3  A photomicrograph of the first maxillary molar of a control rat showing periapical and dental tissues X 100.

4  A photomicrograph of the first maxillary molar of a 0.1 mg cortisone-treated rat showing periapical and dental tissues X 100. A: ameloblasts; CT: connective tissue; D: dentin; ES: enamel space; HS: Haversian space; O: odontoblasts; P: pulp cavity; T: trabecular wall of alveolar bone.
PLATE 4

EXPLANATION OF FIGURE

A graph showing the effect of different dosages of cortisone on the total eruption during a five day period of the first maxillary molar of newborn rats. The brackets indicate the standard error. E: cortone acetate; F: hydrocortone acetate; C: control.
PLATE 5

EXPLANATION OF FIGURE

6 A graph showing the effect of different dosages of cortisone on body weight gain in newborn rats during a five day period. The brackets indicate the standard error. E: cortone acetate; F: hydrocortone acetate; C: control.
PLATE 6
EXPLANATION OF FIGURE

7 A roentgenogram of a (16 day old) rat head showing the lines superimposed to illustrate the method of bone growth and molar eruption rate measurement X 4. C: most rostral cusp of first maxillary molar; M: molar alveolar crest; I: incisor lingual alveolar crest; N: superior aspect of nasal bone; Line N-IM is indicative of bone growth.
PLATE 7
EXPLANATION OF FIGURES

8  A photomicrograph of the first maxillary molar of a control rat showing periapical and dental tissues X 100.

9  A photomicrograph of the first maxillary molar of a 1.0 mg cortisone-treated rat showing periapical and dental tissues X 100. A: ameloblasts; CT: connective tissue; D: dentin; ES: enamel space; HS: Haversian space; O: odontoblasts; P: pulp cavity; T: trabecular wall of alveolar bone.
PLATE 8

EXPLANATION OF FIGURES

10  A photomicrograph of the first maxillary molar of a control rat showing normal eruption (age 16 days) X 100.

11  A photomicrograph of the first maxillary molar of a 1.0 mg cortisone-treated rat showing apparent precocious eruption. (age 16 days) X 100.
APPROVAL SHEET

The thesis submitted by Stephanie Jean Zayachek has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis, and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

May 26, 1969
Date

[Signature of Advisor]