

## THE EFFECT OF THE ADMINISTRATION OF ŒSTROGENIC HORMONES ON THE NASAL MUCOSA OF THE MONKEY (MACACA MULATTA)

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THE problem of the naso-genital relationship in man, upon which some of the earliest observations had been made by Hippocrates and Celsus, received much attention from both rhinologists and gynæcologists towards the end of last century and at the beginning of this, until about 1912.

Starting with the publication of clinical observations by Mackenzie<sup>1</sup> (1884) and Fliess<sup>2</sup> (1897) a considerable literature<sup>3</sup> dealing with this subject has accumulated, dealt with in three main aspects: first, its bearing upon dysmenorrhœa; second, the relationship of epistaxis to menstruation and pregnancy; and, third, and to a less degree, the essential nature of the naso-genital relationship as a normal physiological process, which last was chiefly speculative. By far the greater part of it is confined to evidence derived from human clinical material, there being considerable disagreement amongst authors as to the condition in the nose at different physiological times, and there would seem to be a lack of controlled experimentation designed to throw light on the nature and conditions of the relationship.

Koblanck and Roeder<sup>4</sup> (1912) reported sexual hypoplasia in dogs and rabbits following the removal of the nasal conchæ in young animals. Owing to the high mortality from post-operative obstruction to breathing, suppuration and bronchitis, there survived for an adequate length of time only three rabbits and three dogs upon whom the operation had been done. That the animal's general condition had suffered was indicated by body weights less than those of the controls, but the sex-gland weights were markedly less and there was evident hypoplasia. The authors were not very satisfied with their results.

Karpow<sup>5</sup> (1929) performed double turbinectomy on male rabbits at 5 to 6 weeks. At 39 weeks the testicles were undescended and weighed 50 per cent less than those of the controls. Here again there was evidence of a general effect in the less weight of the treated animals. Recently, Nemours<sup>6</sup> (1935) has repeated this work, operating first on one side at the 4th to 5th week, and on the other ten days later. Of 7 animals that survived to 6 months of age, 5 grew normally and the testicles were normal in their descent and weight at death. In 2, however, the testes failed to descend, weighed markedly less than those of the controls, and histologically showed no spermatogenesis beyond the spermatocyte stage, at which time degenerative changes took place. The pituitary gland in these animals did

not show castration cells.<sup>7</sup> Such evidence of a specific effect is not convincing.

Borghesan<sup>8</sup> (1931), also in the rabbit, reported degenerative changes in the nose after castration, which observation Pighini and Porta<sup>9</sup> (1934) confirmed, finding definite re-integration of the affected nasal areas after treatment with liquor folliculi, with a histologically more marked improvement after treatment with crude extract of anterior lobe pituitary. In discussing these results, they admit that, if the amelioration produced by liquor folliculi be due to a hormone replacing the sex gland, the greater improvement which follows anterior pituitary treatment cannot be explained by gonadotropic action, since the animals are castrates. They consider this effect to be produced by direct action, and suggest the possibility of its being due to the growth hormone or the thyreotropic fraction, both of which such extracts undoubtedly contain.

Wiraboff<sup>10</sup> (1934) examined the nasal mucosa of rats and mice during the sex cycle, to ascertain whether the cycle coincided histologically with changes in the nasal mucosa. In a detailed study of 9 rats and 5 mice he found no histological variation with the cycle, the mucosa remaining normal throughout. In certain rats he observed a hyperæmia and leucocytic infiltration, from which he drew no conclusions, but did not believe this to be provoked by the cycle. "Menformon" injections neither prolonged the cycle nor affected the nose histologically.

In regard to histological studies made upon the nasal mucosa of the white rat in this connection it is as well to keep in mind the recent work of Smith and Craigie,<sup>11</sup> who describe a condition which occurs spontaneously in a high percentage of the supposedly normal rats which they have examined. From a "pathological process in the nasal cavity" there is a destruction of the olfactory cells, leading to an atrophy of the centrally directed processes that make up the olfactory nerves. In healing changes in the mucosa result, chiefly characterized by replacement of the sensory type of olfactory mucous membrane by ciliated columnar epithelium. "The vomeronasal organ is only rarely involved, thus accounting for the relative freedom of this centre from stunting."

We became interested in this problem from observation of a French-Canadian family of nine children all of whom suffered from atrophic rhinitis, as did both parents. In the cranial skiagrams of all of them definite evidence of dyspituitarism was found. Four of them, females ranging from 11 to 20 years, showed marked evidence of an early hypopituitarism, and, of these, 2 suffered from amenorrhœa. In a moderately large series of cases of atrophic rhinitis studied we have found this feature present in 80 per cent, and have come to the conclusion that the constitutional factor in this disease may be

related to pituitary function, both in the family and in the individual. Since the possibility arises that the anterior pituitary may exercise an effect on the nose, either directly in its control of facial differentiation, or possibly through the gonad by its gonadotropic action, it was decided first to investigate the effect of crystalline oestrogenic hormones on the monkey's nasal mucosa, since its physiological relationship is closer to man than to either the rabbit or the dog. In addition the monkey presents the advantage of possessing a nose which, anatomically, is very similar to man's.

ANATOMY OF THE NASAL CAVITY IN THE MONKEY  
(*Macaca Mulatta*)

(Fig. 1.) The nasal chamber is almost an isosceles triangle in shape, the base of which along the nasal floor, in a young adult, is about 40 mm. long; its vertical height is about 25 mm. The cranial wall is short and narrow and is formed by the cribriform plate of the ethmoid bone. The dorsal cranial wall is covered by the mucous membrane on the ventral aspect of the sphenoid bone. There are no sphenoidal ostia, since the sphenoidal sinus, like the frontal sinus, is absent in the monkey. The cavity, while in a sense bearing a close resemblance to the human nasal cavity, differs from it

in certain important features. It is relatively longer antero-posteriorly and shorter vertically than in man. Owing to prognathism and the poor development of nasal bones and cartilages its vestibular part is relatively much smaller than in man. The nostrils have their long axes in an obliquely transverse plane. In older animals they are relatively roomy but in the immature they are apt to be narrow and slit-like. The lateral nasal

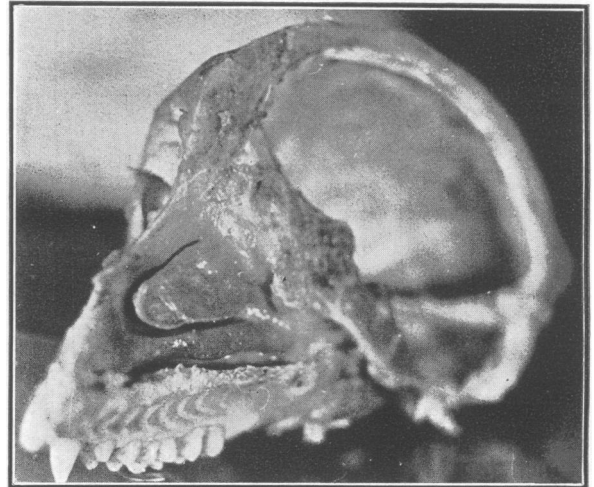


Fig. 1.—The right nasal cavity of *Macaca mulatta*.

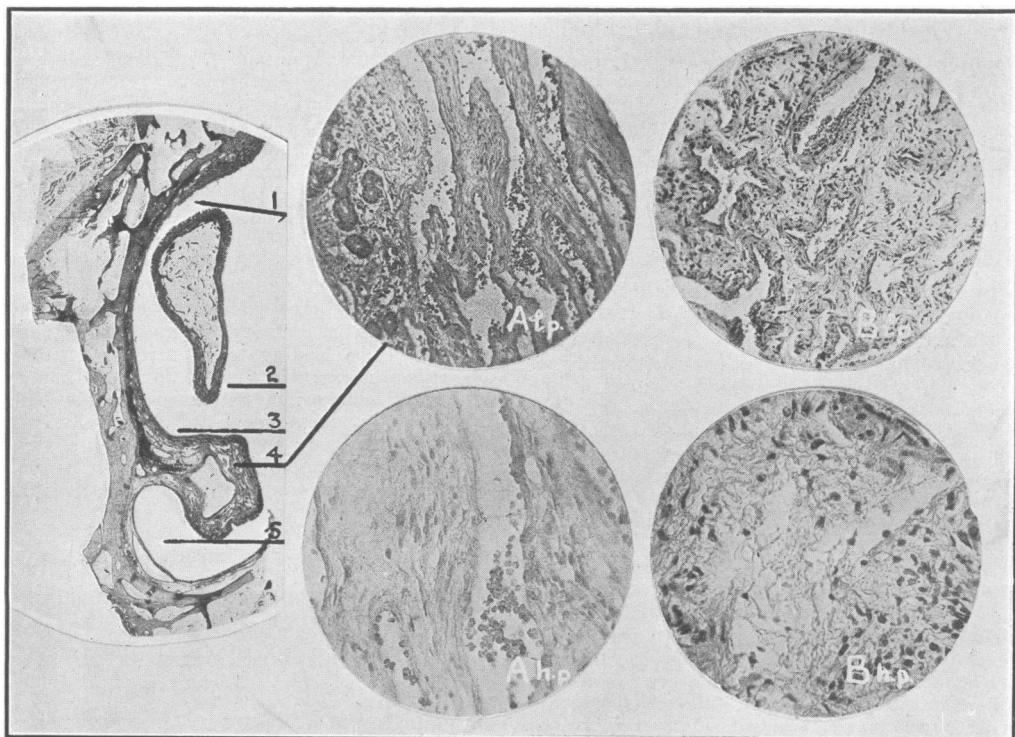


Fig. 2

Right nasal cavity (normal) — coronal section. 1. Superior meatus. 2. Middle concha. 3. Middle meatus. 4. Inferior concha—site of section A. 5. Inferior meatus.

Sections "A".—Normal structure of the cavernous tissue, in the inferior concha of an adult female monkey. (Low and high powers.) Sections "B".—After hypodermic injection of 1,000 gamma of crystalline keto-hydroxy-oestrin, in oil, daily for 14 days: the conchæ are swollen with a marked perivascular oedema and great opening out of the connective tissue, in the cavernous tissue septa. (Low and high powers.)

wall bears the nasal conchæ, which are homologous to the middle and inferior conchæ in man and occupy relatively similar positions. There is nothing corresponding to the so-called "concha superior" or to the "concha suprema" of the human nose, in which area the nasal wall is smooth and largely olfactory in function.

The *upper concha*, which we will call, for its human homologue, the "middle" concha, is a tongue-shaped process directed downwards and forwards from its attachment on the dorsal part of the lateral nasal wall. Its mesial surface is smooth and flattened, and its superior, anterior, and inferior borders are clean cut, somewhat sharpened and free. On directing the speculum upwards and outwards towards the lateral nasal wall the rounded, though thin, free border as it forms the tip of the concha can be clearly seen, together with the adjacent, most anterior parts of the lateral and mesial surfaces of the concha. Beyond, on the lateral side, are the confluent airways of the superior and middle meatuses and, mesially, the also relatively narrow airway between the flat mesial surface of the concha and the septum. The lateral surface of the concha is more convex and scroll-like than the mesial.

The mucous membrane covering the "*middle*" concha varies in colour with the age of the monkey, its physical condition, and whether or not it is mature; also, as we shall show, with the stage of the sexual cycle. In immature females it is paler in colour; especially if the monkey is somewhat anæmic, as it is apt to be if recently imported. In a sick animal it may be grey, even with a slightly faint cyanotic tint, which appearance is shared by other visible mucous surfaces. In healthy untreated adults there is considerable individual colour variation from a pale pink to a bright, healthy red. This zone of variation in normal animals we delimit on the charts by the zone R.-O.-P.

The *inferior concha* begins in front as an expansion from the dorsal part of the vestibule, from the lateral wall of which it gradually rises, somewhat ventral to the prominent tip of the middle concha, and under cover of which it gradually assumes the form of a rounded shelf, some 4 or 5 mm. in width and almost as much in thickness. This shelf extends dorsward to a little beyond the level of the posterior margin of the hard palate, where it merges into the lateral wall of the naso-pharynx. Its free or septal border is rounded and thick (about 4 or 5 mm.), and covered with mucous membrane, which ordinarily lies in rugæ. If the point of the speculum be depressed towards the floor of the nose and then directed outwards the light strikes this border tangentially, throwing the rugæ into relief; in an adult nose, almost the anterior half of the concha is thus made visible. When the cavernous tissue is distended with blood the rugæ are smoothed out and it becomes a deeper red or almost purple colour. At the anterior extremity, where the cavernous tissue is less well marked, the colour will then have a somewhat more fiery tint.

Caudal to the concha is the inferior meatus into the ventral part of which opens the naso-lacrymal duct. Above the concha, on the lateral nasal wall, when the middle concha is removed, can be seen the hiatus semilunaris into which the antrum opens. Between the conchæ and the septum the airway is relatively much narrower than in man, and deviation of the septum we have found relatively frequently. Changes in appearance in the inferior concha are not only less evident to the eye, but are more difficult to observe; they follow very closely, however, those occurring in the "middle" concha. In order to simplify our graphs we have charted only the variations found in the middle conchæ. It happens not infrequently that the degree of change in the two sides is not equal, in which case the mean of the two sides is taken.

#### THE MICROSCOPIC ANATOMY OF THE CONCHÆ

*The middle concha.*—Fig. 2 shows a coronal section through the anterior part of the middle concha and of the part of the inferior concha vertically caudal to it. The core of the concha is composed of a scroll-like process supported by thin bony trabeculæ, the interspaces of which are marrow-filled. The mucosa rests upon a thin plate of compact bone, the periosteum of which is intimately attached to the tunica propria of the mucous membrane. This is composed of an abundant connective tissue network, which contains many elastic fibres and is abundantly vascularized. It contains many mixed glands of the tubo-alveolar type, which branch freely in the deeper part and are lined with both mucous and serous cells. Deep to the glands, in this layer, are very numerous vascular spaces, and these are also to be found between the gland groups, where they are of smaller size. Separating this layer from the epithelium is a well-defined basement membrane upon which rests a stratiform, ciliated, columnar epithelium, the slender ends of whose cells rest on the basement membrane, their more bulky ends forming the free surface. Between these, and resting on the basement membrane, are other columnar cells which do not form part of the surface; also, there are many mucus-containing goblet cells. The epithelium is similar to the respiratory epithelium in man.

*The inferior concha.*—Here the bony lamina upon which the mucosa rests is roughly quadrangular, and between its supporting trabeculæ the spaces are marrow-filled. The mucous membrane has the same epithelial covering as in the middle concha. The tunica propria is intimately connected to the periosteum, but the mucous membrane in this situation is at least 3 or 4 times thicker than in the middle concha, owing to its containing in its submucous zone vascular cavernous tissue, which for the most part lies deep to the mixed glands, and is composed of large branching and communicating endothelial-lined venous spaces forming an "erectile" structure very similar to the inferior concha in man. In the latter, Schaeffer<sup>12</sup> (1920) describes the occurrence of circular and longitudinal bundles of smooth muscle imbedded in the walls of the venous channels of the plexus cavernosi concarum. Maximow<sup>13</sup> (1930) is of the opinion that this does not form an erectile tissue, owing to "the absence of septa containing smooth muscle". In *Macaca mulatta* we have found that the septa in the cavernous tissue of the inferior conchæ contain smooth muscle fibres.

#### THE ENVIRONMENTAL AND ATMOSPHERIC CONDITIONS

In an investigation such as the present one, in which the areas under observation are, in man, known to be highly liable to changes induced by atmospheric and other conditions, it is essential to describe the general environment of the animals as well as the temperature variations met with during the experimental period.

The monkeys are kept in six large indoor cages, 12 ft. by 9 ft. in area, and 8 ft. high, in a building heated in winter. Three of the cages communicate with three outside, wired-in enclosures on the roof, 15 ft. by 14 ft. and 6 ft. high, the doors of which the animals can open at will. Thus, half the colony has access to the outside on alternate weeks throughout the winter. Free use of it is made even in zero weather. Throughout the experimental period a record was kept of

indoor and outdoor temperatures and whether or not each monkey had had access to the outside. Artificial heating is from October onwards, but the indoor temperature is somewhat influenced by outdoor changes, so that temperature variations of from 58 to 72° F. were registered in the indoor cages. The maximum difference between outdoors and indoors was 60° F., in January. Throughout December, January and February the average difference (between outdoor and indoor temperatures) was from 40 to 50° F. It was at first thought that an animal having access to the outside would show considerable change in the vascular condition of the nasal mucosa in consequence, and it was feared that this might prove a complicating factor in observation. This fear, however, proved unfounded, and it can be said in passing that allowing the animals free access to the open air at all seasons has been very beneficial to their general health. Since its adoption no death from tuberculosis has occurred, while previously, when the animals were confined to small cages indoors, death from tuberculosis was not infrequent.

We have not found catarrhal affections of the respiratory passages in general to be common. Of the 29 monkeys used in the total series 62 per cent showed no trace of any catarrh throughout the entire period. Of the 38 per cent which did at one time or another show catarrh 19 per cent (5 animals) showed only a very slight, mucous discharge on one occasion, during the colder months. Two animals showed slight rhinitis at 2 examinations; 1 animal showed it 3 times, while 3 other animals had the condition intermittently; 1 animal showed discharge on 5 weekly examinations, another on 7 occasions, and 1 had a chronic condition with rhinitis on 11 occasions. Evidence of rhinitis is the presence of a thin mucous, or muco-purulent discharge, the mucosa being whitish, somewhat bleached looking and boggy. The animal does not seem to have any definite constitutional symptoms. The condition is not contagious, is confined to the nasal passages, and usually clears up in the course of 1 or 2 weeks. As the discharge diminishes there is a slight tendency to crusting. Such rhinitis as we have observed has at no time produced reddening of the mucosa, nor does the presence of a mild degree of rhinitis render invisible the physiological colour changes produced by oestrogenic hormone injections.

As the study proceeded, we became impressed with the well-marked, even striking, stability of

the monkey's nasal mucous membrane. Throughout the cold months, when the temperature is frequently in the region of zero, on chasing half the number from the outdoors into an atmosphere of 65 to 70° F. for examination, it was impossible to distinguish the animals which had been outdoors from those that had been indoors all week by any difference in the nasal mucosa. The variations in colour and activity of the mucosa which we report, when graphed with observations of outdoor and indoor temperatures on the days of observation and with the record of whether any given animal has had access to the outside on those dates or not, demonstrate a complete lack of any correlation between those facts.

It is well recognized that in man the degree of vascular and glandular activity of the mucosa is extremely labile, that even during a single nasal examination the condition of the mucosa may quite alter, from nervous or other causes. We have at no time observed this in any of our monkeys, although usually during the examination there is excitement due to being held. We have, however, noted temporary engorgement of the mucosa, especially of the middle concha, due to pressure upon the vessels of the neck by an attendant, when the animal is improperly held or when it struggles with the glottis closed. Associated flushing of the face gives indication that this factor is at work.

#### THE APPEARANCE OF THE MUCOSA ON SPECULUM EXAMINATION

The animal is held down with its lumbar region resting upon the back of the right hand of one attendant, which hand grasps the monkey's crossed elbows, while the left steadies the head, with the palm on the vertex, the face being turned slightly towards the examiner; a second attendant holds the legs. A Cameron nasal speculum is used, which carries its own light, projecting a brilliant beam between the jaws of the instrument. It is almost impossible to use a frontal mirror. The point must be directed first well outwards and upwards, to bring the middle concha into view, then downwards towards the floor of the nose as well as strongly outwards, to bring into view the inferior concha. The tendency at first is to have the point directed too far inwards, so that the septum fills the field; this is fairly often deflected, and if touched (it is very close to the point of the speculum) it bleeds very readily.

In cyclic animals varying states of appearance can be seen in the mucous membrane, and, over

a course of months, we have observed in the same animals three differing vascular patterns in the mucosa, irrespective of colour. (a) The mucosa may seem "homogeneous", that is to say, there are no visible capillaries or mottling of its surface. (b) It may be "mottled", showing a surface apparently composed of contiguous small circular zones, whose centre is somewhat lighter in colour, almost yellowish, without, however, the occurrence of any visible capillaries. (c) There may be visible surface capillaries, especially along the lateral aspect of the sharp-cut border of the tip of the middle concha. These three patterns seem to be to a great extent independent of the colour of the concha, which is usually most marked at the tip. The three patterns may pass from one to the other in the course of several weeks, but we have been unable to correlate them in any significant way with the cyclic change in vascularity which we are to report.

Normally, the mucosa has a moist appearance, which changes, when secretion is marked, to a clear glistening look, and this may exist apart from actual swelling. When this happens, there is a tendency for the vascular pattern, if it has previously been (b) or (c), to become (a). We have come to accept such glistening as evidence of glandular activity, describing it simply as an active mucosa. We have found it occurs in the prodromal stage of swelling of a physiological and not a pathological nature. It does not lead to the presence of discharge in the nasal cavity.

#### THE EXAMINATION PROCEDURE AND CONTROL OF THE OBSERVERS

During the first four months nasal examinations were made by three observers, thereafter by two. They were made weekly, at the same hour, in a room lit by a diffuse north light which proved ideal for colour judgment when using a speculum light. Under the same conditions two other trained observers independently recorded, every second day throughout the whole period, the colour and/or swelling of the sex-skin, face, nipples and back, and the presence or absence of vaginal bleeding. They did not disclose their findings to the nasal observers. Every three months the nasal findings and the record as to whether the animal had been out or not were charted on one-year graph-paper, after which the graphs of face, nipple, back and sex-skin activity (reddening and swelling) were charted. On similar paper a graph was made of the indoor and

outdoor temperatures in such a way that it could be accurately placed upon the nasal graph. Throughout the whole period the following facts were undisclosed to the observers—the identity of the monkey, its sex, whether it was sexually mature or not, whether it was intact or castrate, whether it menstruated regularly, the condition of its sex-skin, if it was then under treatment, and whether it had ever received treatment. The only facts supplied were the monkey's series' number and whether it had been allowed outdoors during the previous week, including the day of examination. It was felt that thus the vitiation of objectivity, clearly apparent in human clinical investigations of this nature in the past, might be avoided.

The most rigorous effort was made to confine the attention to the nasal appearance in a manner sincerely objective. Although it is to be regretted that owing to the nature and position of the examination site true objectivity of judgment is impossible, as no standard could be introduced for comparison, still it was felt that a truer estimate of reality would be possible if no observation were recorded unless it expressed unanimous opinion. In case of doubt the tendency to "mark down" the appearance was encouraged; thus if one observer was less impressed by a given degree of redness or swelling than the other two his marking was accepted. When there was a difference in colour of the two sides of the nose, in charting the observation the mean was taken, except in cases in which, after turbinotomy for histological examination, the visible parts of the conchæ had been replaced by scar tissue, and thus rendered useless as an index of colour change. Oedematous swelling, however, might be evident in it as the result of other changes in the non-visible part of the conchæ.

Abnormal redness and/or swelling was measured in three degrees. R.3 is an intense brilliant red of a vividness almost that of red typewriter-ribbon. R.2 and R.1 have also this characteristic quality, but are of less intensity. S.3 is applied to a middle concha when it is so swollen and oedematous that it seems to block the airway almost completely. In a lower concha the colour has not got the "brilliant" quality or vividness, but in R.3 it becomes a dusky, fiery red; or it may have a purple tint. Swelling is harder to see, apart from redness, in the lower concha. Both cause disappearance of the rugæ, but the glandular activity, the amount of mucous secretion, seem chiefly noticeable in the middle concha.



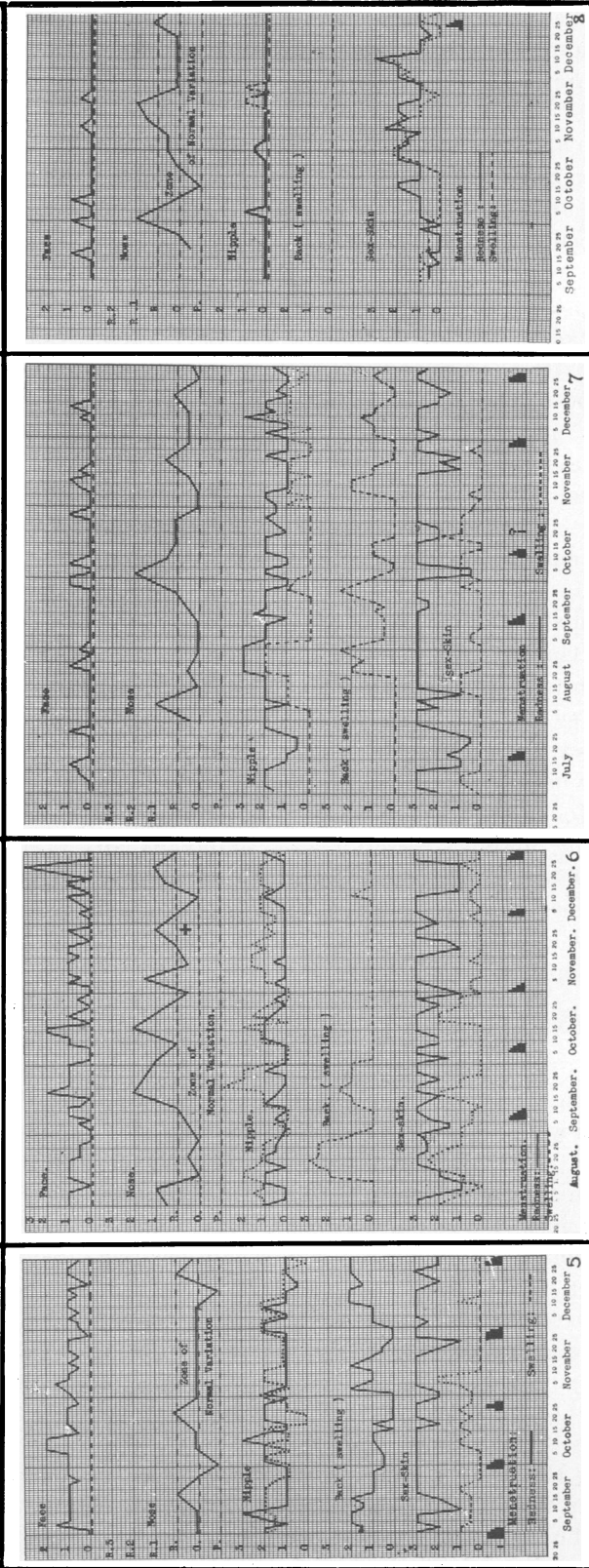
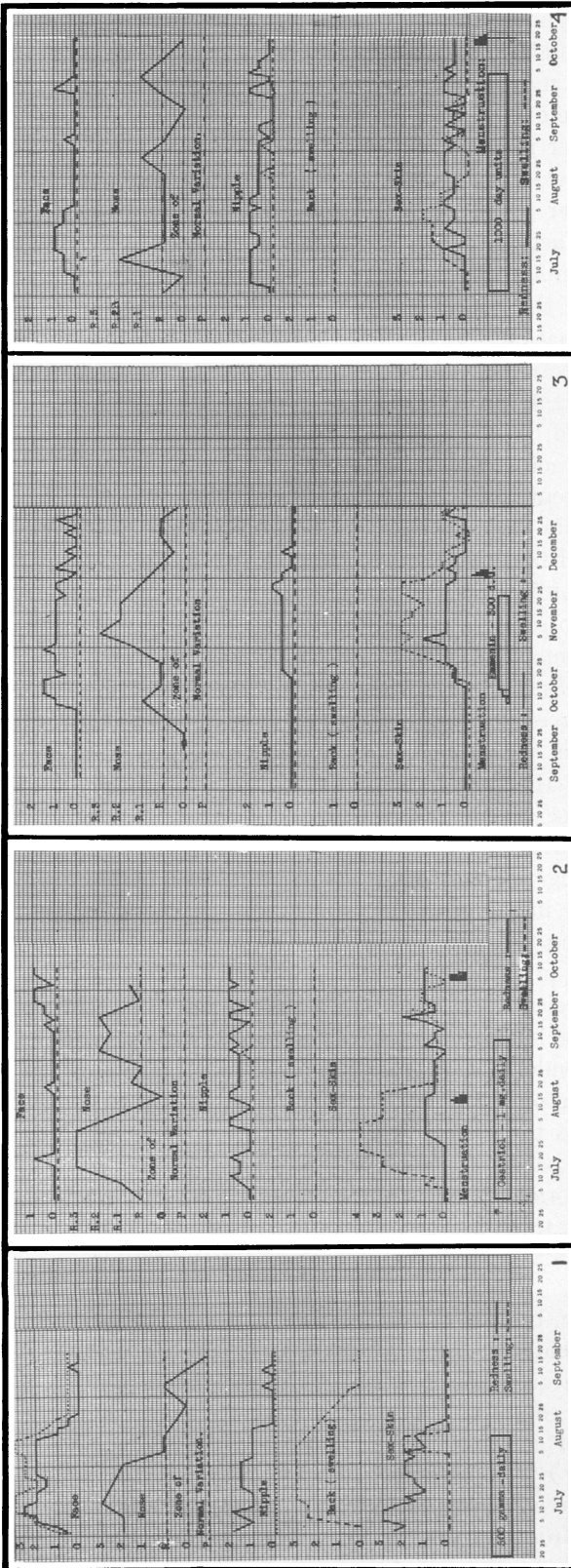


Chart 1.—Monkey No. 885—adult female castrate, treated with keto-hydroxy-estrin. Chart 2.—Monkey No. 2633—young adult female, oestriol-treated, 1 mg. in 10 per cent alcohol by mouth daily. Chart 3.—Monkey No. 2905—immature female, emmenin-treated, hypo. (300 d.u. b.i.d.). Chart 4.—Monkey No. 2632—young adult female, emmenin-treated by mouth. Chart 5.—Monkey No. 1295—adult female, untreated. Chart 6.—Monkey No. 1296—adult female, untreated. Chart 7.—Monkey No. 1299—cyclic adult female, untreated. Chart 8.—Monkey No. 2898—young adult female, untreated.

*Material examined.*—The colony consists of 28 animals. Three are males, 2 immature, and 1 adolescent; 5 are immature females, 4 are adolescent females (one a castrate), and 16 are mature females, of which 3 are castrates.

THE EFFECTS OF ADMINISTRATION OF OESTROGENIC HORMONES

*Oestrone.*—When nasal examinations commenced there were already 14 animals in course of treatment with oestrogenic hormones; these were studied, using the untreated remainder of the colony as controls. Two adult female castrates and one adolescent female castrate, treated with daily hypodermic injection of 500 gamma oestrone for 50 to 75 days, were examined during the last three to ten weeks of the period. Typical changes<sup>14</sup> were produced in the nipple, sex-skin, face and back. They were examined nasally twelve days after injection had begun, when the conchæ showed maximal redness (R.3) in one, and marked redness (R.2) in the other, this becoming maximal in about a week (Graph 1). The peaks were sustained for about two weeks, when, despite continued treatment, the colour steadily faded until about the time treatment was stopped it had reached normal level, preceding the fading of colour in the other sex-skin areas by about a week.

The adolescent castrate was seen only during the last two weeks of treatment when the nose showed marked reddening, as did also the nipple, face and sex-skin. Two intact adolescent females, treated similarly, were examined nasally only during the last two weeks of treatment. One showed abnormal redness on the three occasions examined, while the other did not; this does not mean, of course, that the nose may not have responded earlier in treatment.

*Theelol (Oestriol).*—A young adult female (Graph 2) and an immature male were treated daily with 1 mg. of Theelol in 10 per cent alcohol by mouth for seventy days, with weekly nasal examination throughout. Nasal reddening was maximal (R.3) in both animals after fourteen days' treatment, and in the female remained so for about two weeks, when it began to decline despite treatment, touching normal just before a menstrual period, which occurred on the forty-fourth day of treatment and lasted four days. After this the colour rose again during the last two weeks of treatment, and did not fall to normal till ten days before the next period. In the male animal the nasal response declined more rapidly, but marked variations in colour were seen for two months after treatment ceased.

*Emmenin.*—Three immature females (Graph 3) were treated with hypodermic injection of 300 day units twice daily, one for eighteen and two for forty-five days, with nasal examination throughout. The most mature animal showed maximal reddening, the two less mature, submaximal degrees. In all three increase in nasal colour was apparent a week after injections were begun. One adolescent female, given 1,000 units daily intramuscularly for one hundred days, whose nose was examined during the last forty days of the treatment, showed two maximal peaks of reddening, separated by an interval of twenty-four days.

Three animals (Graph 4) were given 1,000 units daily by mouth for periods of from fifty to ninety-five days. One, an immature male, showed a submaximal response at the thirtieth day of treatment which rapidly disappeared. This was synchronous with changes in the nipple and sex-skin. A young adult female (No. 2632) treated similarly for ninety-five days showed three submaximal peaks on the fifteenth, fifty-eighth and ninety-third days of treatment. An adult female castrate (No. 2265), treated for fifty days, showed a maximal nasal peak on the eighth day, and a submaximal one just before treatment ceased; castration was proved subsequently to have been incomplete.

One characteristic of the nasal response is worthy of note; it is most clearly evident and constant in the emmenin-treated animals, and much less frequently seen in those treated with the other hormones, namely periodic peaks in the colour response. With emmenin such peaks were found to occur, on the average, at intervals of about twenty-seven days (Table I). Em-

TABLE I.  
THE INTER-PEAK INTERVAL IN NASAL ACTIVITY IN MONKEYS TREATED WITH EMMENIN

Monkey	Peak intervals days	Average peak interval
No. 2901	27	28 days
	30	
No. 2903	23	24 days
	25	
No. 2905	28	25 days
	24	
	24	
No. 2263	23	23 days
No. 2635	30	29 days
	23	
	35	
	31	
No. 2632	44	30 days
	35	
	23	
	22	
	33	
	28	
No. 2265 (incomplete castrate)	28	26½ days
	40	

menin is relatively inert in the castrate, the presence of the ovary probably being necessary for its most effective action.<sup>15</sup> Its effect upon the nasal mucosa may be regarded as enhancing the normal premenstrual peak (Graph 5), making more clearly evident the nasal evidence of sexual cyclicality (Graphs 3 and 4).

*Untreated females.*—As a control group 12 females were examined under exactly similar conditions as the injected animals. Four were observed for eight months, 3 for seven months, 1 for six months, 2 for five and a half months, 1 for four months, and 1 for three months, those observed for the shorter periods being added to the series as time went on. At the beginning 7 were cyclic adults, while 5 were described as "immature". Of these last 3 were adolescent and developed the full cycle, with menstruation, during the latter half of the period of observation. One animal, examined for four months, remained immature and acyclic throughout. Two of the cyclic group menstruated irregularly. Only when the weekly state of the nose was charted on one-year paper, together with graphs of redness and/or swelling in the nipple, sex-skin, face and back, and also with the indoor and outdoor temperatures and observations as to whether or not the animal had been allowed out, did the following facts become apparent:—

1. The nasal variations are not significantly related to temperature variations.

2. There is a seasonal increase in the degree of colour of the nasal mucosa as well as in the degree of activity manifest in the several sex-skin areas, with which the former is coincident (Graphs 6 and 7). In the adults this occurs in September and October, at which season it is known that conception is most likely to take

place. In the "immature" group, however, this condition was best seen in November. This may be due to the fact that the series is very small (5 animals), or it may be that the adolescent animal responds somewhat more slowly to a seasonal stimulus (Table II).

TABLE II.

MONTHS IN WHICH THERE IS GREATEST ACTIVITY  
IN SEX AREAS

(Redness and/or Swelling, July, 1935 - February, 1936)

Cyclic Adult Females (Untreated)	Aug.	Sept.	Oct.	Nov.	Dec.
	No. 1294.....		x	x	x
No. 1295.....	x	x	x		
No. 1296.....	x	x	x	sl.	
No. 1297.....		x	x	x	
No. 1298.....	sl.	(regular and well marked activity)			sl.
No. 1299.....		x	sl.	sl.	
No. 1300.....		x	x		
"Immature" Females (Untreated)	2 (1 sl.)	6	5 (1 sl.)	2 (2 sl.)	1 sl.
	Aug.	Sept.	Oct.	Nov.	Dec.
No. 2898.....		x		x	x
No. 2899.....		x	x	x	
No. 2900.....			x	x	
No. 2902.....				sl.	
No. 2904.....				x	x
		2	2	4 (1 sl.)	2

x Indicates outstanding activity in sex-skin, nipple, face and nose.

sl. Indicates activity above other months, but not so marked as in x.

3. In the untreated cyclic female monkey there are periodic peaks in nasal mucosa colour and activity, most marked in September and October and least in January and February (Graphs 5, 6, 7). The peak-intervals vary in length in different animals; in the group they averaged 28 days. In the animal in which they were shortest, the average was 21 days, and in the animal in which they were longest, 33 days. In the group there was a total of 52 "monkey-months" nasal observation and 45 "nasal peaks" were seen (Table III). In 89 per

TABLE III.

IN 45 NASAL PEAKS OBSERVED IN CYCLIC MONKEYS  
THERE WAS CO-EXISTENT ACTIVITY IN THE OTHER  
SEX-SKIN AREAS AS UNDER:

	Cases	Percentage
Face.....	31	68.8
Nipple.....	40	88.8
Back (swelling).....	13	28.8
Sex-skin.....	38	84.4

cent of these there was coincident nipple activity, in 84 per cent sex-skin activity, in 69 per cent activity in the face, and in 28 per cent activity in the back (swelling). Finally, such nasal peaks were 8 per cent more regular as a manifestation of sexual cyclicality than uterine bleeding (Table IV). They were found to occur "pre-

TABLE IV.

CYCLIC ADULT FEMALES (UNTREATED)

		Percentage
Total "monkey-months" observation....	52	
Total menstrual periods.....	41	79
Missed menstrual periods.....	11	21
Total nasal peaks.....	45	87
Missed nasal peaks.....	7	13

Nasal activity in adult cyclic monkeys is 8 per cent more constant than menstruation in indicating the sex cycle.

menstrually" in 60 per cent, coincident with the bleeding in 16 per cent and "post-menstrual" in the remainder (Table V). We also observed in a number of instances

TABLE V.

RELATIONSHIP OF THE NASAL-ACTIVITY-PEAK  
TO MENSTRUATION — "ADULT MONKEYS"

	Cases	Percentage
"Premenstrual".....	23	60.5
Coincident with flow.....	6	15.7
Postmenstrual.....	9	23.8

that the nasal redness peak in a given month might be double, with a remission, synchronous with a double peak in sex-skin coloration, in an intermenstrual interval (see November, Graph 6).

In the immature untreated group of 19 "monkey-months" observation 13 nasal peaks were observed (Table VI). Their intervals were more irregular and

TABLE VI.

"IMMATURE" FEMALES (UNTREATED)

		Percentage
Total "monkey-months" observation....	19	
Total menstrual periods.....	10	52.6
Missed menstrual periods.....	9	47.4
Total nasal peaks.....	13	68.4
Missed nasal peaks.....	6	31.6

Nasal activity in "immature" female monkeys is 16 per cent (15.8) more constant than menstruation in indicating the sex cycle.

somewhat longer than in the adult group, averaging over 30 days; with 77 per cent of them there was coincident nipple activity, in 69 per cent also sex-skin and face activity, and in 1 case was there also swelling of the back (Table VII). Irregular menstruation or amenor-

TABLE VII.

IN 13 NASAL PEAKS OBSERVED IN "IMMATURE" FEMALE  
MONKEYS THERE WAS CO-EXISTENT ACTIVITY IN THE  
OTHER SEX-SKIN AREAS AS UNDER:

	Cases	Percentage
Face.....	9	69.2
Nipple.....	10	76.9
Back.....	1	7.7
Sex-skin.....	9	69.2

rhea was more frequent than in the adult, which accounts for the fact that in this group the nasal peaks were 16 per cent more regular as a manifestation of the



sexual cycle than uterine bleeding. As regards their position in the cycle (Table VIII), more than half of

TABLE VIII.  
RELATIONSHIP OF THE NASAL-ACTIVITY-PEAK TO  
MENSTRUATION—"IMMATURE" MONKEYS

	Cases	Percentage
"Premenstrual".....	4	57
Menstrual.....	3	43
Post-menstrual.....	0	

Only 7 nasal peaks out of 13 (53.8 per cent) can be related to a menstrual bleeding.

them were clearly "pre-menstrual", while the remainder continued or occurred while bleeding was taking place; none were "post-menstrual". Owing to amenorrhœa only half the peaks could be related to menstrual bleeding.

### DISCUSSION

We should like to make it quite clear that in a cyclic untreated animal, even after months of practice in nasal examination, none of the observers are of opinion that a single nasal examination would enable him to state with certainty the time in the cycle at which the inspection is made, due to the great variation in colour from animal to animal; but they are convinced that observations made over a period of several months will reveal variations that will assume significance when charted with graphs of activity in the other sex-skin areas. It is in this respect that the female monkey is superior to the human female as an object of observation in this problem. In woman, where the cycle, if amenorrhœa is present, is not clearly defined, use could be made of sex hormone excretion assay in the urine.

*The œstrin dosage necessary to elicit the nasal response.*—Since in the preliminary investigation large doses of the three oestrogenic hormones had been given for relatively long periods it was desirable to ascertain the smallest dose of œstrin capable of producing the characteristic effect on the nasal mucosa. For this purpose 10 of the females that had been examined throughout as controls for a period of about 5 to 6 months were first used in December and January. They presented the advantage of a very complete past record of both nasal and sex-skin activity, and the seasonal variation was then at its ebb. This might be considered as a disadvantage if the hypothesis were entertained that at that time the animals' responsiveness to œstrin might be less, but, on the other hand, it was felt that the observation of sudden activity at a time when the mucosa was apparently less active might gain in significance, especially if the dosage were small.

The doses given were 12.5, 25, 50, 100, 150 and 200 gamma. At the usual weekly examination of the colony these animals were mixed in with the others, so that the examiners could not identify and give them special scrutiny. After seven days' treatment all, except those given 12.5 gamma, whose colour was a high normal, showed a positive response, which increased with a further week's treatment, the reaction with the 12.5 gamma dose still being in doubt, since by the system of rigorous marking, the identity of the monkey not being known, a high normal or doubtful positive was deprived of any benefit of doubt and marked "normal".

Treatment was stopped, and when the nasal appearances had been steadily normal for some time, after about a month the treatment was repeated, when it became evident that with the same dose the animals did not react so well and it had to be increased 50 to 100 per cent to produce an equivalent effect. With the smaller dosage, smaller when compared with the early massive doses given, it was observed that the nasal reaction was somewhat changed in character; the redness was not so vivid and there was evidence of greater glandular activity, the mucosa appearing highly glistening and there being some clear discharge, which, however, never had any purulent quality.

Seven females, which had been treated with massive doses of œstriol or emmenin in the first survey, and had been allowed to recover and remain without treatment for three months or more, being under observation the whole time, were similarly treated. With a dosage of 25 gamma and upwards all gave the typical reaction.

In general, it must be said that there are a number of factors that complicate the question of the amount of crystalline œstrin necessary to effect nasal response, when injected hypodermically dissolved in corn oil. First, the sex of the monkey (females respond more readily); second, the age of the animal, whether it is sexually mature; and, third, whether or not it has recently been treated with an oestrogenic substance. It would appear that the previously untreated adolescent animal that is approaching maturity is the best test object. Very immature animals do not respond so well in any of the sex-skin areas, including the nose, and animals that have been mature for some considerable time and have shown a spontaneously rich coloration may possibly have small degrees of change, which are masked by the spontaneous coloration; or

they may have reached the maximal response beyond which change towards increase is very hard to evaluate. Fourth, the season of year at which the experiment is conducted; in autumn the animals are apparently more sensitive, but change is harder to see, since they are already highly coloured; whereas in January and February they would appear to be less sensitive, although changes are more easily seen. Finally, complications arise from the nature of the nasal site that it is difficult to see, and an artificial light has to be used of a certain intensity, so that one cannot be certain of slight degrees of change that might readily be seen on an external genital sex-skin. Besides this there is the attitude of the observer, who, with the animal's identity concealed from him, is most unwilling to compromise his judgment by recording as possibly abnormal anything which is not very clearly so. These facts must all be born in mind when the minimal effective dose is shown as being in the region of 25 gamma daily, for a week. At this time we treated the castrate monkeys for two weeks with very large doses of potent anterior pituitary gonadotropic hormone. We were able to record no significant change in the nasal mucosa.

*Histological examination after turbinotomy.*—Turbinotomy was performed on 16 animals showing varying degrees of change at different stages of treatment. The operation, from the histological standpoint, is unsatisfactory, since a nasal punch, even though it is the smallest available for human use, can only be introduced into the monkey's nose with difficulty, and it has to be used blindly; thus it is hard to be sure of getting a fair sample of tissue. At first, an attempt was made to get samples from both conchæ; later the middle alone was used. Fixation was in formalin and decalcification in 5 per cent hydrochloric acid.

Since the operation was done, in the main, for such clinical appearances as hyperæmia and swelling it is obvious that such treatment of small pieces of tissue, by the marked shrinking it causes, is apt to obliterate, or at least seriously to diminish, the histological confirmation of the clinical state. The histologist therefore has to base his opinion largely on the presence or absence of definite perivascular œdema in the submucosa. This he was able to find in about two-thirds of those which had shown abnormal redness and swelling. The histological appearance in a well marked case is shown in Plate 2 "A", from an animal treated for two weeks with 1,000 gamma

of cestrone. The clinical appearance was "R.3". The chief change occurs in the connective tissue of the cavernous septa, where the œdema is severe, spreading out the fibres, a change very similar to that found in the corium of the sex-skin, with œstriol treatment;<sup>14</sup> young fibroblasts can also be seen, especially adjacent to the basement membrane.

#### SUMMARY

1. The nasal mucosa of the intact monkey (*Macaca mulatta*) responds to the administration of œstrogenic hormones, crystalline Cestrone and Cœstriol, and Emmenin in the manner of, and synchronously with, the changes known to occur in the nipple, sex-skin, face and back.

2. The response is most clearly seen in the "middle" and inferior conchæ which show reddening and/or swelling. It is a specific response.

3. The substances mentioned are effective in males as well as females, in both immature and adult animals.

4. The effect can be produced in female castrates by Cestrone and Cœstriol.

5. It has not been obtained in castrate females with pituitary gonadotropic hormone.

6. The histological changes are similar to those known to be produced in the sex-skin of this animal by Cœstriol.

7. In the untreated immature and adult female animal peaks of nasal activity periodically occur normally at intervals of about 28 days; the peaks are chiefly "pre-menstrual" in time.

8. Such naturally occurring cyclical activity in the nose is most marked in the autumn months, when conception is most likely to occur.

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## STUDIES IN MINERAL METABOLISM\*

### II. CALCIUM AND THE KIDNEY: EXPERIMENTAL I.

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IN previous papers<sup>1, 2</sup> some of the lesions in the human kidney resulting from excessive calcium excretion were described. Further, it was suggested that the kidney lesions in some cases of renal rickets and of chronic pyelonephritis are primarily the result of injury by calcium. To study the natural history of mineral injury of the kidney we have undertaken experimental studies on animals. In the present series of papers will be recorded certain lesions produced in the kidneys of white and hooded rats after the injection of Collip's parathyroid extract. This work diverges from that of earlier observers.<sup>3, 4, 5</sup> For convenience of presentation the lesions will be described in the following order: (1) Intratubular and interstitial deposit of calcium and its removal; (2) parenchymal and interstitial reactions; (3) end-results; chronic kidney disease. The present paper deals with the details of the experimental procedures and with the first of the above group of lesions.

*The rats.*—We have used two strains. The first, albinos, the original stock of which was kindly supplied us by Prof. A. T. Cameron, has some tendency to a simple hydronephrosis and hydroureter. Marked hydronephrosis has occurred in about 1 in 200 rats, and mild in about 1 in 25. We have observed no natural tubular dilatation except in association with extreme hydronephrosis. The second strain, of Norwegian hooded rats, was kindly supplied us by Dr. Roy McCullagh, of the Cleveland Clinic. We have seen no congenital anomalies in these.

They are more resistant to the bad effects of parathyroid extract than are the albinos. The lesions produced in them by injection of the extract are the same in type as in the first strain but less in degree.

*Plan of injection.*—With two exceptions injections of all litters were begun when the rats were five days old. In the exceptions they were started on the third day. In early series all injections were given once daily subcutaneously. In more recent series intraperitoneal injection has been found simpler. Under treatment the rats nursed badly or not at all. The mortality was so high that a plan of interrupted injection was followed, thus:  $n$  units a day for 5 days, followed by 5 days to recuperate,  $n+1$  units a day for 5 days, 5 days' rest to recuperate, and so on up to a maximum of six injection periods. Then longer rest periods were given, two to four weeks, a week of injection, again a rest of four weeks, and so on. In this way the animals had time to recover from the ill effects of the extract. We think the time lapse probably important in the production of the lesions to be described. The minimum initial dose at 5 days was  $\frac{1}{2}$  unit, the maximum initial dose 5 units, the most satisfactory for our animals, 2 units. Animals were killed each 24 hours, and after each various dose in the early periods; after each period of injection, in the later one. Litter mate control was not possible. If only half a litter was injected the normal mates were so much more vigorous than the experimental animals that the latter had little chance of survival. We have obtained controls by killing whole litters, or individuals from litters, at various ages.

*Diet.*—Injections were started while the rats were nursing. They were weaned at 21 to 25

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