INTERACTIONS BETWEEN DEVELOPMENT AND BEHAVIOUR IN THE GUSTATORY SYSTEM

FUNGIFORM PAPILLAE IN THE DOMESTIC NORWAY RAT: THE PATTERN OF GROWTH, AND SOME BEHAVIOURAL MODIFICATIONS OF GROWTH

By

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ABSTRACT

The purpose of the work reported in this thesis is to delineate the growth pattern of the fungiform papillae during development of the domestic Norway rat, and to investigate some behavioural interactions with that development. It was found that growth occurs in two distinct spurts; the first begins during labour and is at least in part under uterine influences; the second begins with the sampling of solid foods and can be profoundly influenced by the weaning process and by some factor in the nursing experience. The total population of fungiform papillae following the second growth period is greater than that in adults, and remains at this level throughout adolescence.

ACKNOWLEDGEMENTS

I would like to thank Dr. G.K. Smith for his generous support and advice throughout this research and the preparation of this manuscript.

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I am grateful to my family for their forbearance and unstinting support..

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INTRODUCTION

Schwalbe (1868) first identified taste buds as the organs of taste in mammals and demonstrated the presence of these structures in a number of mammalian tongues: guinea pig, rat, rabbit, hare, deer, sheep, pig, horse, calf, ox, and man. The extensive body of literature concerned with gustation which has been produced since then is full of contradictions and controversies, many of which centre on the taste bud. There are disparate views on the number of cell types which form the taste bud (Graziadei, 1969), on the presence of synaptic structures between the taste cells and the nerve endings, and on the role of the receptor cell itself (Beidler, 1970). All of these problems are presently undergoing intensive investigation.

We considered another area of research to be potentially rewarding, that of the reported differences between adult and neonatal taste bud populations (von Ebner, 1902; Hermann, 1885). The distribution, the morphology, the function, and the innervation of neonatal taste buds are in need of clarification. In adult mammals, taste buds have a relatively limited distribution, most being associated with the fungiform, circumvallate, and foliate papillae on the surface of the tongue (figure 1). In young and foetal animals they may be more widespread, appearing, for example, on the tops of the papillae and in various parts of the pharynx where they are not associated with papillae but are embedded singly or in groups in the epithelium (Baradi and Bourne, 1953).

There is limited, and sometimes conflicting, evidence of a wider distribution of taste buds in neonates. Early studies (von Ebner, 1902; Hermann, 1885) reported greater numbers of taste buds in vallate papillae in embryonic and neonatal rabbits. These taste buds were said to be morphologically different to those found in adults, smaller and more globular in shape.

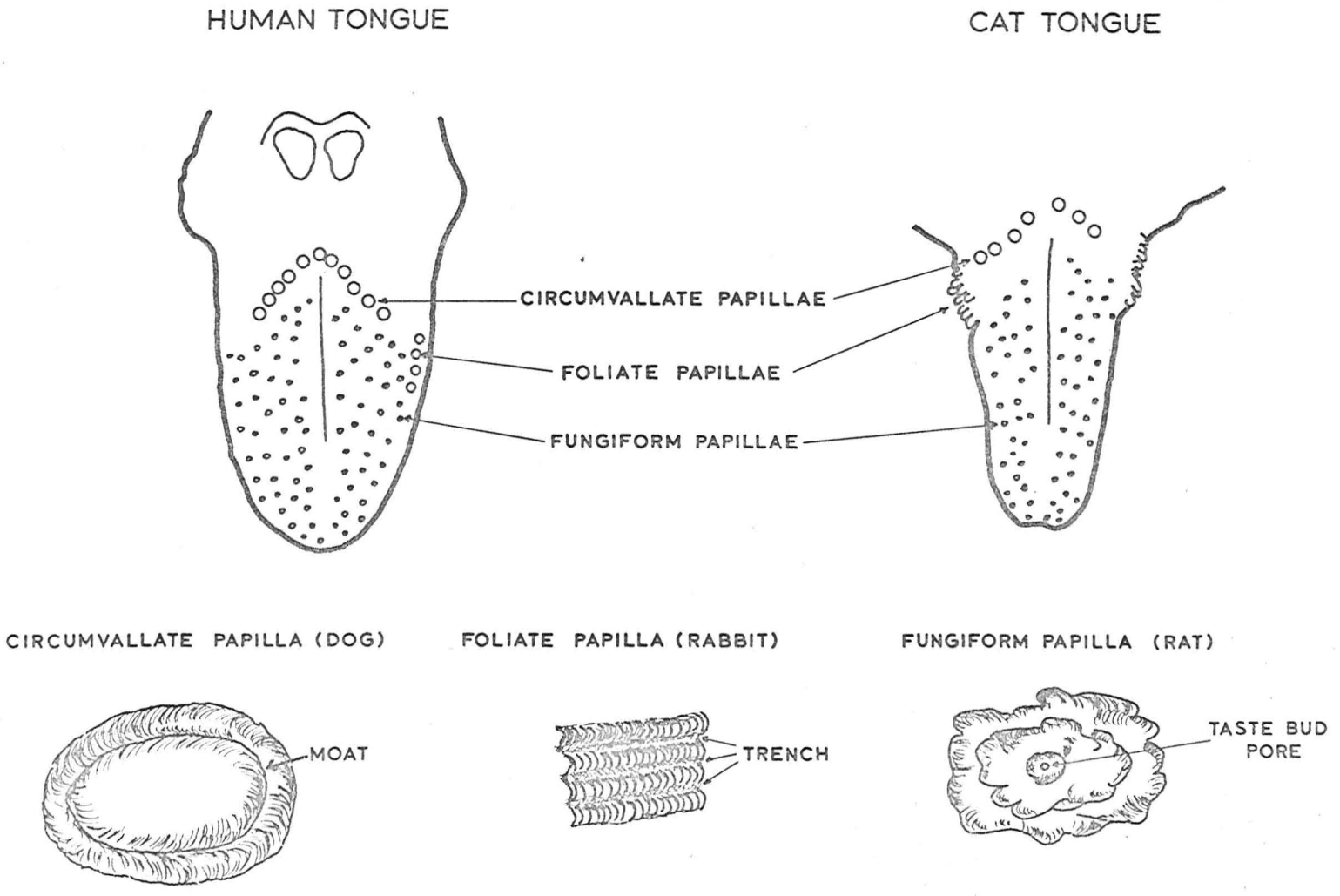


Figure 1. Distribution and types of papillae in adult mammalian tongues, from Crouch, 1969; Beidler, 1969.

Taste buds found in embryonic sheep appear to be morphologically the same as those of adults and are also apparently functional, as the chorda tympani responds to a variety of stimuli applied to the tongue, including its own amniotic fluid (Bradley and Mistretta, 1971).

Opinions on the presence of extra taste buds in the human neonate differ. Geldard (1953) reported that the vallate papillae are not fully populated until later in childhood, while Moncrieff (1951) reported that taste buds appear in the human embryo at three months after conception and are more widespread than in the child.

In a lengthy study of the various papillae in the rat, Fish, Malone and Richter (1944) concluded that the average population in fungiform papillae is essentially the same at all ages from 8 hours to 466 days.

Three hypotheses can be presented for a decrease in population from the neonate to the adult. Overproduction and subsequent reduction of cell populations during development is normal in the central nervous system and in a number of other organs and tissues (Levi-Montalcini, 1964). Some of these cells disappear during genetic and developmental programs (e.g. degeneration of the tail of the human foetus), and others later in ontogeny after failing to form functionally appropriate connections (Jacobson, 1970). The differentiation of sensory cells is always subsequent to the arrival of the sensory neuron, and their integrity is dependent on its continued presence (Morgan and Stellar, 1950). There is a comparable dependency of the neuron; contacts between terminals of the axon and the peripheral organs in development seem to be essential for the maturation and maintenance of the neuron (Jacobson, 1970). If overproduction of the taste buds is induced by the proximity of the developing sensory nerve, and the nerve fibers fail to establish connections with some of these

buds, this mutual dependency may bring about a reduction in both the taste bud and the nerve fiber populations.

Hermann (1885) suggested that the reduction in number of the taste buds over time might be attributed to loss through usage, with those areas being reoccupied by an overgrowth of the surrounding simple epithelium. The surface of the tongue is being subjected to almost constant erosive contact; ingestion of food--sometimes of extreme temperatures, grooming behaviour, and abrasion by the teeth and hard palate may all be deleterious to sensitive tissue, yet functional taste buds always remain (Beidler, 1965).

It has been demonstrated that the organs of taste have the power of regeneration. If the nerves innervating the taste buds are damaged and degenerate, the taste buds disappear; if the nerve regenerates, the taste buds reappear (Guth, 1957, 1958; Zalewski, 1970); further, the life span of a cell within the bud is about 250 hours (Beidler, 1965). These findings indicate that the taste bud is a dynamic rather than a static system, and would argue against usage being the factor responsible for a population decrement by adulthood. This explanation of loss suggests that taste buds which have disappeared were in essence like those that remained. The preceding explanation of developmental overproduction of cells implies that those buds present in the neonate but absent in the adult did not achieve a functional state.

A third hypothesis, that the additional taste buds in the neonate might serve some function peculiar to development, might be proposed. For the rat, the sense of taste is an important faculty. Adult rats can select the constituents of a complete diet for normal growth and health

(Richter, 1941) and can learn aversions to both poisoning and deficient diets (Rozin, 1968, 1969). While the initial learning is based on post-ingestional states, subsequent intake is determined by taste. The flavor of a diet has been shown to function as a learned cue in food selection (Harper, 1967). Moreover, initial diet selection by weanling rats is mediated in part by taste (Galef and Henderson, 1971).

For the present however the paucity of information available on the developing system of taste precludes acceptance of any hypothesis. There are certain basic questions that must first be answered. Some of these questions are presented here.

1) Is there a real difference in the populations of taste buds between the neonate and the adult?
2) If there is a difference, what are its dimensions? Where does it occur, at what age do changes from one population level to another take place, and how great is the difference?
3) If there is a difference, are environmental or experiential factors involved in the difference?

These questions outline the area of investigation for this thesis.

EXPERIMENT 1

The number of taste buds in the neonate has been reported as being greater than that in adults (Hermann, 1885; von Ebner, 1902), and as being the same (Fish, Malone and Richter, 1944). Are there more taste buds in the neonate? If there are, how great is the difference, and when does the change take place? The purpose of this experiment was to answer these questions, by determining the populations of taste buds as a function of time.

It has previously been noted that taste buds occur mainly in connection with three types of lingual papillae - fungiform, foliate, and circumvallate - and also singly or in groups in epithelium. The areas of the oral cavity where taste buds may be found are many. To count every taste bud in an animal would not only be tedious but also very nearly impossible; with the exception of the tongue, excision of the areas for examination would be almost certain to destroy bud-bearing tissue. In view of these problems, it was decided to restrict the area of investigation to the tongue itself, and to examine only the population of fungiform papillae. There are several reasons for selecting this method. The area is easily accessible. Each fungiform papillae, in the rat, is the site of a single taste bud (Fish, Malone and Richter, 1944). These structures are easily discerned, even with the unaided eye.

SUBJECTS

A breeding program was set up to supply one or two litters of hooded rats each day. Seventy females and six males of the Long Evans strain, obtained from Blue Spruce Farms and McMaster Health Sciences, were

used for this program. Conception times were determined by vaginal lavage, and the information noted for the records of each pup. Weaning of pups was allowed to occur naturally. Each mother was housed with her litter in a standard plastic laboratory cage, with food and water always available. The average litter size was twelve. Individual histories for every pup were kept for conception time, day and hour of birth, number of litter mates, and sex. This breeding program was the source of subjects for this and all subsequent experiments.

METHOD

To obtain the count of fungiform papillae, the animal was injected with a lethal dose of Nembutal and, immediately vital signs ceased, the entire tongue was excised. The tongue was then rinsed in cool tap water, food particles and other debris were removed with a fine brush, and the dorsal surface was painted with a solution of 0.1% methylene blue. The tongue was then aligned on a slender wedge of cork and clamped between two glass slides. This method of staining turns the simple epithelium blue, the filiform papillae - burr-like projections not involved in taste - a very deep blue, and leaves the fungiform papillae white (Tateda and Beidler, 1964). The pore of the taste bud can frequently be seen as an intense blue spot in the center of the papilla. By using a graticule in the eyepiece of a Zeiss binocular operating microscope a large-scale reproduction of the tongue, giving the exact location of every fungiform papilla on the dorsal surface, could be made (e.g. Figure 7).

PART A

Random sampling at various ages was done first as an aid in determining the most useful sampling pattern to follow. From this it was decided to map tongues at 5-day intervals from birth to maturity to identify any time periods where changes were occurring, and to then obtain more frequent samples at these periods. The number of subjects contributing to each point, in this and all other experiments, was four, each animal in the sample coming from a different litter.

RESULTS

Sampling at 5-day intervals showed that large changes are taking place within the first 25 days of life (Figure 2). There is a sharp increase in the number of papillae between birth and day 5, and a second, less precipitous rise begins after day 10. From 25 to 75 days the population size remains virtually at the same level, and then decreases over the next 15 days to a point midway between the day 5 and day 25 levels. There were no sex differences in the counts.

PART B

Closer examination was indicated for two time periods, prenatal to 5 days post partum, and from day 10 to day 25. Data for the first time period was collected for each day from day 1 to day 5. This revealed that the growth was completed within the first 24 hours of extra-uterine life. Samples were then taken at day 20 post conception, during labour, at birth, 15 minutes after birth, and hourly for the first 24 hours post partum.

Data for the second period were obtained for each day from day 10 to day 30.

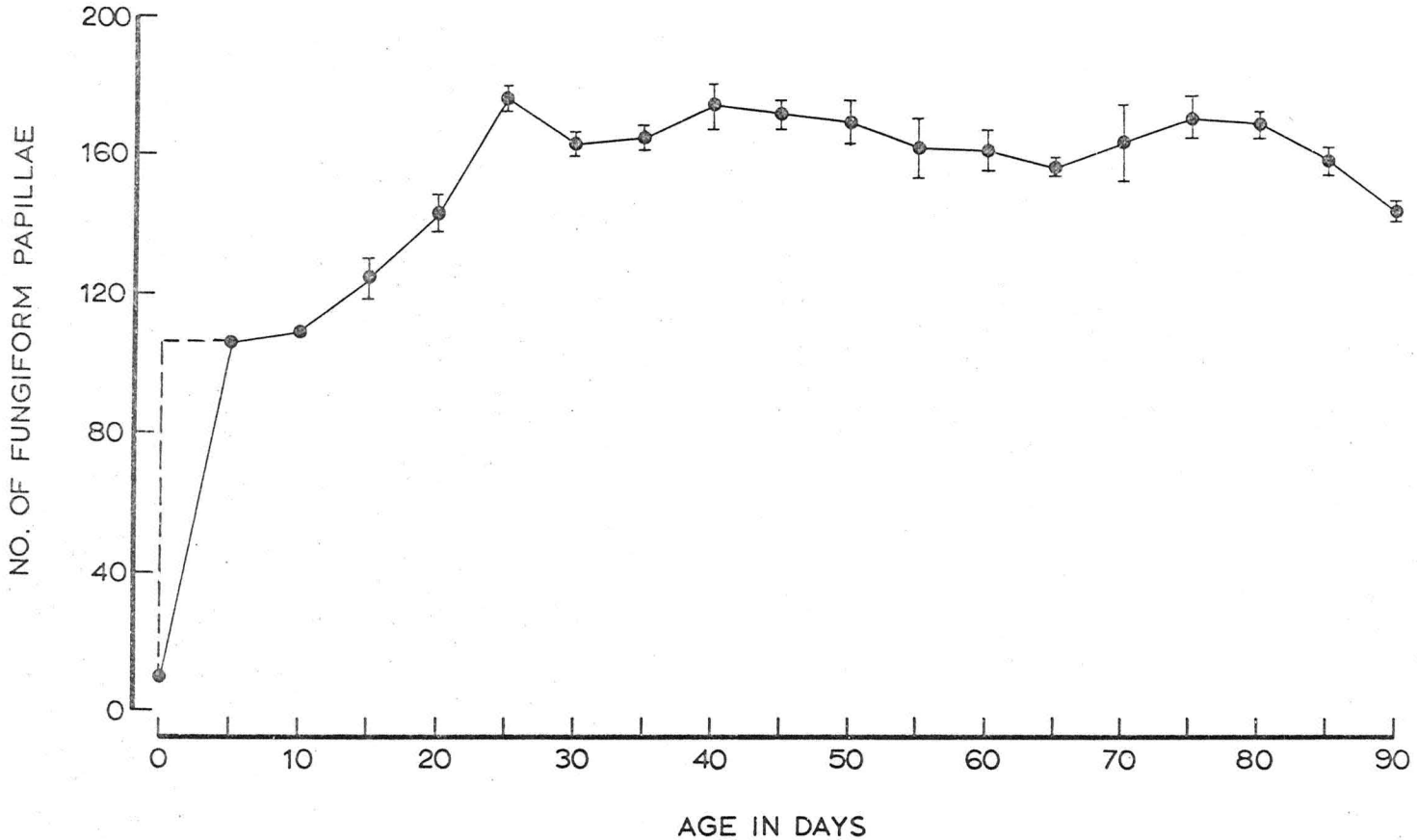


Figure 2. Mean populations of fungiform papillae at 5-day intervals from birth to maturity in the domestic rat. Figure 3 data (dotted line) is included to illustrate the presence of the two distinct growth periods.

RESULTS

The first rapid growth period, or 'initial' population appearance, is shown in Figure 3. Prior to the onset of labour there are no papillae discernible on the foetal tongue. During the course of labour, 5 pairs of papillae appear on either side of the median sulcus (Figure 4a) and this condition still exists 15 minutes after birth. A period of extremely rapid growth follows (Figures 4b, 4c, 4d) which ends at about 8 hours post partum. The population at this time is approximately 110, and there is no further change until the second growth period begins.

In this second period of change the boundaries of the increase are day 15 and day 24 (Figure 5). Up to day 14 the population counts vary only slightly from that of 8 hours. On day 15, two of the sample were still close to this level (113, 119) and two showed the beginnings of the second growth (126, 141). By day 16, all of the sample were into the second growth period, the lowest count being 126 papillae.

From day 24 to day 75, the populations of papillae remain fairly constant, with the majority falling between 170 and 180. The highest count observed over this period was 203 (Figure 6), a day 24 count, contributing to the peak on this day. There is a slow decrease beginning around day 75 to the adult level after day 90 (Figure 7).

DISCUSSION

The total number of fungiform papillae has been reported elsewhere as varying from 114 to 221, with an average of 178.8 (Fish, Malone and Richter, 1944). That study examined 103 rats ranging in age from 8 hours to 466 days. The data which Figure 2 depicts, however, have an overall

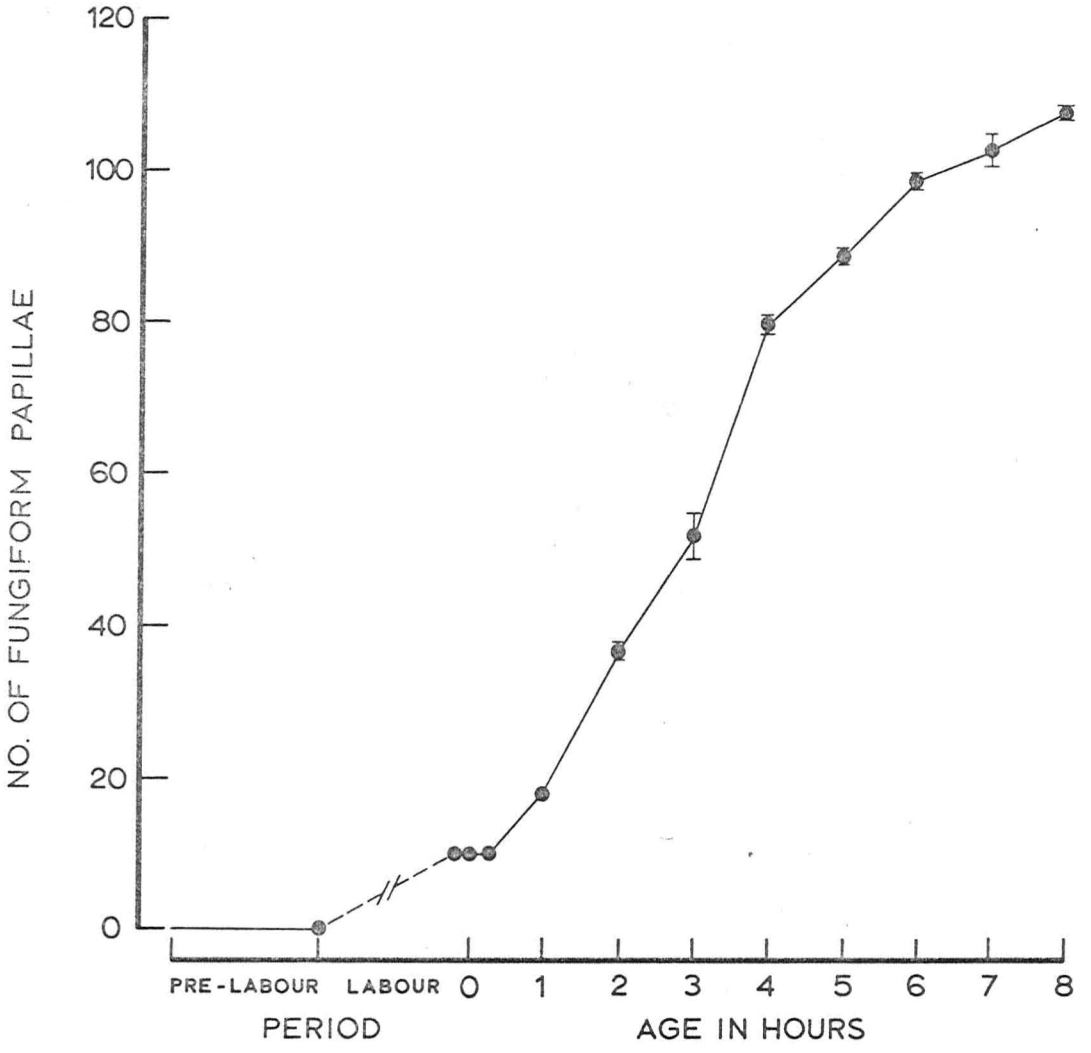
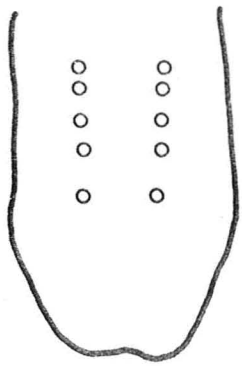
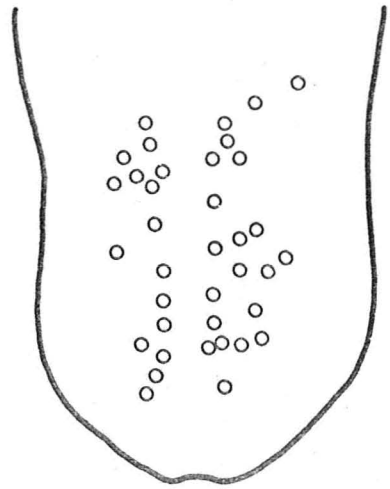


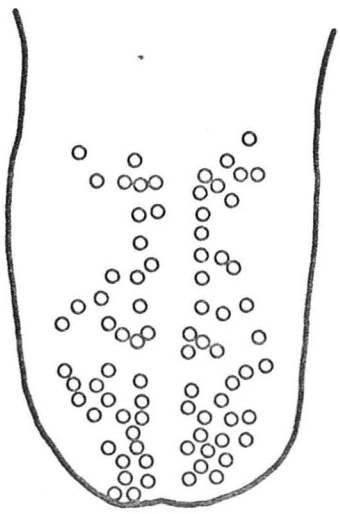
Figure 3. Mean populations of fungifrom papillae from 20 days post-conception to 8 hours post partum.



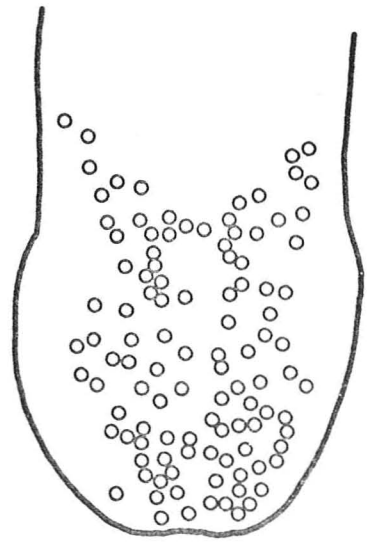
4a - BEFORE BIRTH



4b - 2hrs. - 37 PAPILLAE



4c - 4hrs. - 80 PAPILLAE



4d - 8hrs. - 108 PAPILLAE

Figure 4. Distribution of papillae in neonatal rat tongues, magnification 312.5 x.

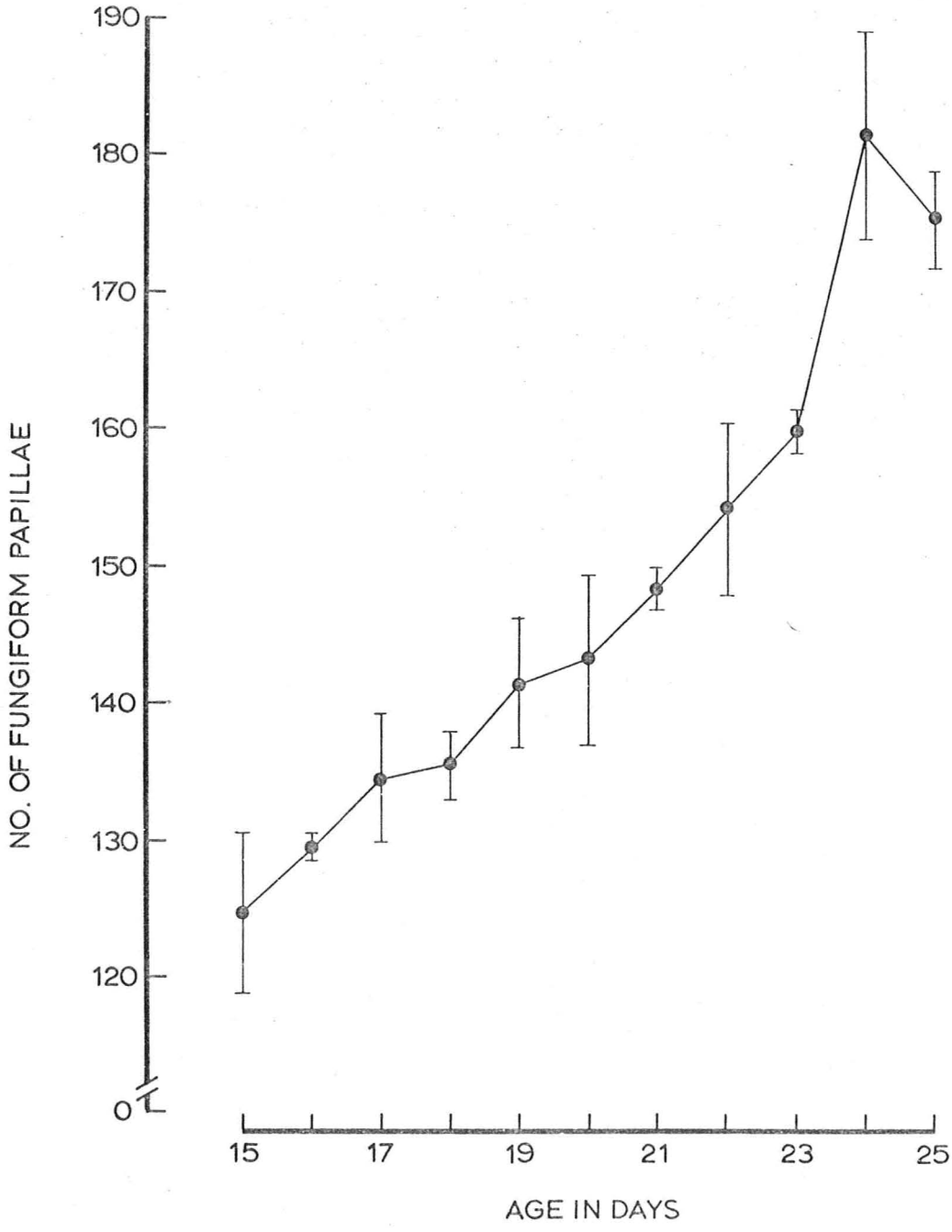


Figure 5. Mean populations of fungiform papillae at 24-hour intervals from day 15 to day 25 post partum.

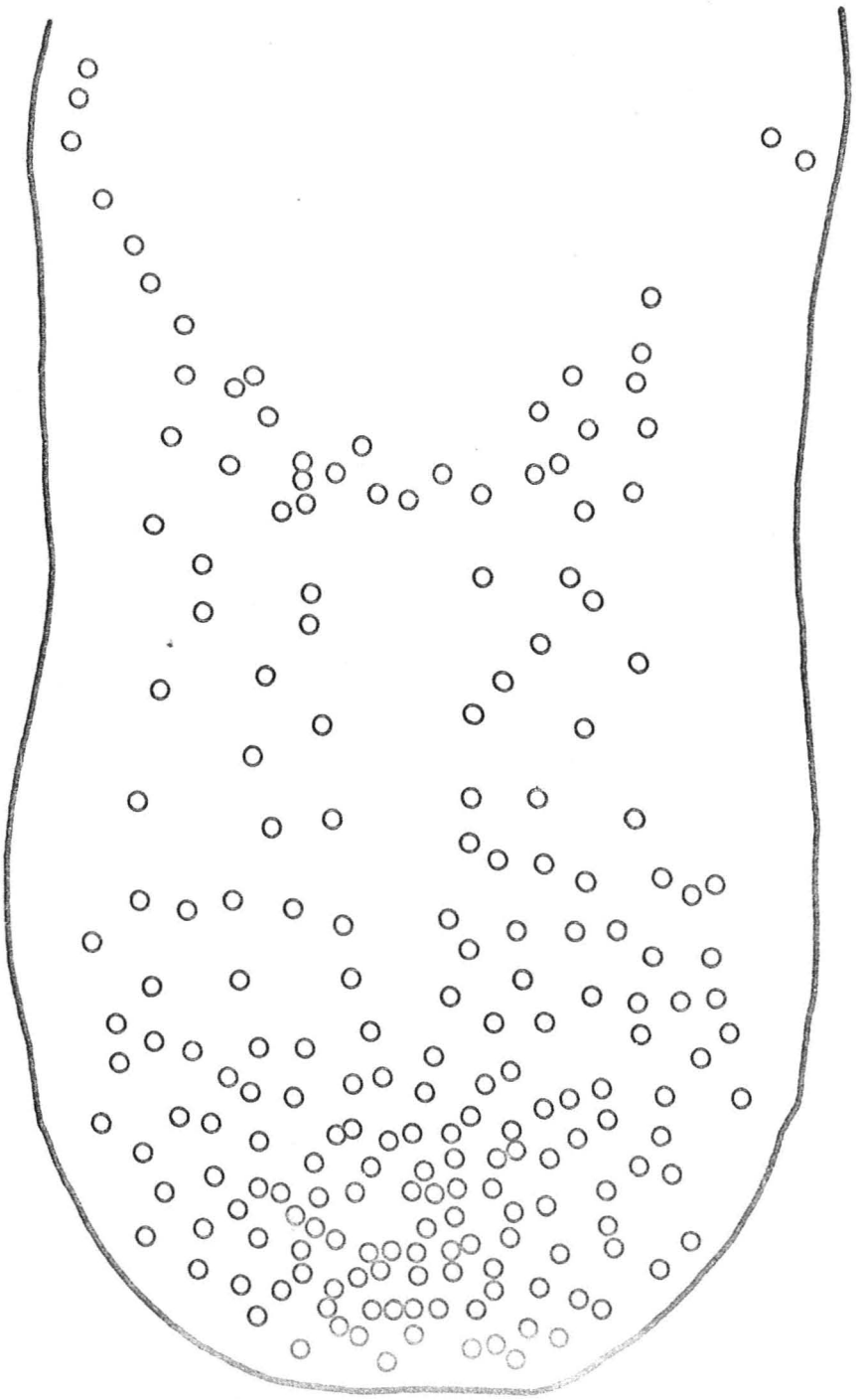


Figure 6. Distribution of papillae in weanling rat tongue at maximum growth level, magnification 312.5 x.

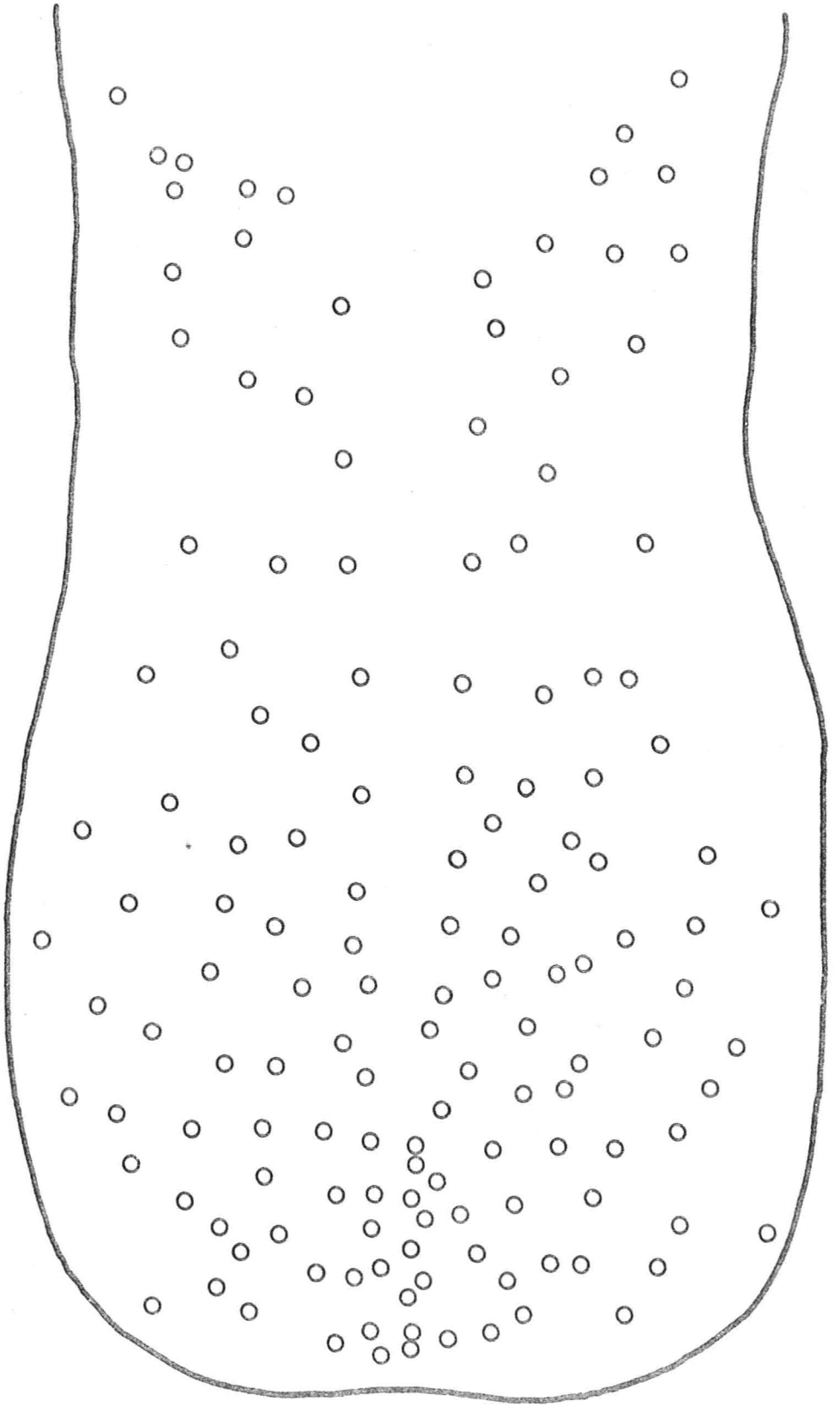


Figure 7. Distribution of papillae in the adult rat tongue at 90 days of age, magnified 312.5 x.

mean of 147.7, and range from 10 to 203. This difference is attributable in part to the addition of birth-time data in the present study, which reduces the mean by 7.7. Further, the 1944 study included the ventral surface of the tongue which has a small population of fungiform papillae. Unsystematic observation of this area has yielded counts of 20 to 30 papillae.

There is no real discrepancy then between the mean numbers of papillae found in the two studies. There is, however, disagreement in the interpretation of the data. Fish et al. state that the average number of papillae "remained essentially the same at all ages from 8 hours up to 466 days". In the light of the present study it would appear that 103 counts spread over such a wide range would fail to yield sufficient information about the nature of the curve, leading to this erroneous conclusion.

This experiment answers the first two questions that have been posed about the populations of papillae in neonate and adult. During development the infant rat has more papillae than the adult. Growth of the papillae occurs in two spurts which coincide with changes in feeding behaviour. This larger population persists throughout adolescence and decreases shortly before maturity.

EXPERIMENT 2

The initial population of papillae begins to appear during labour and its development is complete within the first 8 hours post partum. The appearance of this population may be a fixed developmental stage. It may also be under the influence of one or more of the physiological events coincident with parturition. There is a complex shift in maternal hormone levels that initiates labour and enables parturition to occur. The birth process itself or the change from uterine sustenance to the oral ingestion of milk may be influential in the completion of the growth period. The complex actions of maternal and foetal hormones in parturition are as yet poorly understood (Fuchs and Poblete, 1970) and are beyond the scope of the present work. We examined the possible influence of some of the other factors on the development of the initial growth of papillae. The following studies are preliminary in nature.

METHOD

In any investigation involving parturition it is important to know the expected time of birth with reasonable accuracy. Vaginal lavage is a reliable method of determining conception. In rats and mice the time of ovulation is quite constant within each of the various strains (Nalbandov, 1958). Timed from the morning (9:00 a.m.) when the lavage shows the presence of sperm cells, birth can be expected to occur in these Long Evans rats 21 days and 7 hours (± 3) later. In this breeding program 76 out of 83 gravid rats delivered within this time range and those that did not delivered in the following light cycle. Rats favour daylight hours (Liggins, 1972). Six conditions were examined in this experiment.

A. Post partum separation

As soon as the entire litter was born and cleaned, the pups were taken from the mother and maintained in a cotton wool nest kept warm by an incandescent light bulb. Brief nursing experienced before the removal is evidenced by a clearly visible white bar across the abdomen. This manipulation was done to see if the mechanical stimulation and nutrient intake are necessary to maintain the rate of growth of the first population of papillae.

RESULTS

Pups from this condition had initial populations of papillae which conformed closely with those of the normal curve (Figure 8). Withholding of maternal care and suckling after parturition was complete had no influence on the rate of growth of the papillae. It did result in some dehydration of the tongue.

B. Prevention of suckling

The mamillae of these mothers had been destroyed by cauterization under brief ether anesthesia several days earlier. The litters were left with the mothers and received all normal maternal care except suckling. This was done to see if development of the papillae population will occur in the absence of all suckling experience.

RESULTS

Pups barred from any suckling experience whatsoever were also normal in this development. Neither the growth nor the rate of growth were affected by this deprivation (Figure 8).

	0 hr.	2 hr.	4 hr.	8 hr.
Normal	10	37.5	80.5	108.5
Post parturition separation	10	42.5	79.75	107.0
Prevention of suckling	10	41.0	83.25	110.0
Delayed delivery (progesterone)	-	-	-	110.25
Natural late delivery	67.25	101.75	109.25	-

Figure 8. Mean populations of newborn rats under 3 birth conditions and 2 feeding conditions.

C. By-passing the birth process

Jost and Picon (1970) reported that a number of changes in metabolic processes in the neonate were determined by the actual passage from the intra-uterine to the extrauterine conditions. A method of avoiding this event is by surgical delivery. Pairs of mothers were matched for expected delivery dates. When one of a pair gave birth the other was delivered under Nembutal sedation by Caesarean section. The natural birth mother was then given the surgically delivered pups to nurse, to see if the population of papillae would develop normally without the stimulation of the birth process.

RESULTS

The attempt to foster surgically delivered pups to a naturally delivered mother failed. Pups died, either in utero or very shortly after, probably as a result of the anesthetic crossing the placental barrier. Use of a cervical fracture in place of Nembutal as anesthesia will avoid this effect.

D. Early induced parturition

The induction of labour by oxytocin has been observed in a variety of mammals (von Tienhoven, 1968). The number of hours by which the gestation period can be successfully shortened varies across species. A 2-day reduction is readily obtained in guinea pigs (Schofield, 1964), and 12 to 24 hours in rabbits (Fuchs and Fuchs, 1958), but only 4 to 6 hours in rats (Fuchs and Poblete, 1970). These studies were examining myometrial activity and all involved slow infusion of oxytocin over 2 to 4 hour periods (dose in rats - 360 mU).

In this experiment pregnant rats were injected subcutaneously with doses of 500 mU or 1.0 U oxytocin 5 hours before expected delivery time.

RESULTS

Early parturition induced by oxytocin was not achieved. In one case violent contractions were produced but delivery was not accomplished before expected term.

E. Delayed delivery

Administration of progesterone prevents parturition from occurring in some species. Short (1960) did not consider progesterone to be the controlling hormonal factor in parturition. It is ineffective with pregnant guinea pigs (Schofield, 1964). When successful in rabbits, a single 10 mg. injection delays parturition for about 3 days (Fuchs and Fuchs, 1958). Moudgal (1969) obtained a definite postponement of parturition in rats by a single injection of 8 mg. progesterone on the 20th day of pregnancy. Smaller doses were ineffectual.

In this experiment, pregnant rats were injected subcutaneously with 8 mg. progesterone. The time of the injection was varied in an attempt to control the length of the effect. Injections were given on the 20th day of pregnancy, and at 8 hours and 3 hours before expected term.

RESULTS

Postponement of delivery by progesterone was accomplished in all cases. Day 20 injections resulted in prolongation of pregnancy to the 25th day. All pups were born dead. Counts done on two of these tongues showed a full initial population (108, 112). Day 21 injections delayed parturition 24 to 48 hours. In these litters, only the first 2 or 3 pups delivered were dead, and the remainder (8 or 9) were living. Again the full compliment of papillae were present at birth. Litters removed surgically 8 hours after expected term also had completed growth of papillae (110, 111). (Figure 8).

F. Natural late delivery

Data for this condition was collected over the duration of all experiments, whenever a pregnant rat failed to deliver at expected term. Delivery occurred in all cases (4) the following day, 20 to 22 hours past expected term.

Tongues were mapped, when appropriate, at 0, 2, 4, and 8 hours.

RESULTS

Pups born under these conditions have very different populations. Counts at birth were 30, 88, 56, and 95 (mean 67.25). The rate of growth, in each litter, was normal. In that litter, for example, where the population at birth was 30, which is the 2-hour level in normal term neonates, the full population was present at 6 hours post partum. Similar growth rates were evident in the other three litters tested (Figure 8). It would appear that every pup in a given litter born late has the same number of papillae at birth.

DISCUSSION

The results show that the oral ingestion of food is not a necessary stimulus to the development of the initial population of papillae as, in its absence, development takes place in the normal fashion.

In those pups delivered late by progesterone treated mothers the population of papillae is at the same level as if birth had occurred at the expected time. This was true whether birth took place or the pups were removed surgically. These results would suggest that the birth process does not affect the development of this population, and indeed suggest that the process is a fixed developmental one. If this is so, then the period of interest is not measured from birth but from conception.

The data from both of the suckling conditions, from progesterone delayed birth, and from the normal curve places the development of the first population of papillae as beginning 21 days and 7 hours post conception. The data from

naturally delayed birth is very different. It does not conform to either post conception or post partum age. This discrepancy may be due to delayed implantation, or to a slowing or change in the pattern of shifting hormonal balances occurring during labour. However, if the onset of growth of the papillae is rigidly programmed and delayed implantation, rather than uterine conditions, is responsible for the delayed birth, the advanced growth of the papillae population would imply that delay of implantation also results in a lengthened period of gestation.

EXPERIMENT 3

The most interesting information supplied by the first experiment is that two distinct periods of growth occur, and that they are coincident in time with changes in the mode of nutrient intake. The first growth of papillae takes place as the young animal is making the transition from intrauterine sustenance to ingesting mother's milk; the second growth begins with the first sampling of solid foods.

This close coupling of development with changing behaviour suggests that one may be dependent on the other. The following experiment was designed to examine the behavioural aspects of food intake that occur in conjunction with the development of the second population of papillae. Under normal conditions, weanling rat pups begin sampling solid foods at about day 16, while still suckling (Galef and Henderson, 1971). Daily consumption of solids gradually increases over the next two or three weeks until this is the sole supply of nutrients, the mother having ceased by then to supply milk. This experiment was designed to limit ingestion, over the period when the second population normally develops, to either solid foods or mother's milk.

METHOD

Early Weaning Condition

Food intake in this condition was limited to solid foods. Four litters of pups were removed from their mothers on day 15 post partum and each litter was placed in a standard plastic laboratory cage, where they were introduced to a diet initially consisting of a warm, very thin mixture of Pablum and Esbilac. Standard rat chow and water were also available.

Removal of the mother at this stage in development is believed to affect subsequent adult learning in the pups, an effect which can be prevented by supplying a fat-rich diet (Novakova, 1966). Esbilac, which is a substitute for mother's milk, was included in the diet for this reason.

Weanling rats are slow to initiate feeding away from the nest if adults are not present at the feeding site (Galef and Clark, 1971). To prevent a possible initial period of food deprivation for this reason in these Early Weaning pups, each one was hand fed until eager consumption from the feeding dish was observed. Five minutes handling per pup was sufficient generally to effect the transition in diet.

Late Weaning Condition

Food intake in this condition was limited to mother's milk. Four mothers with their litters were moved at 5 days post partum to modified plastic laboratory rat cages. In each cage the floor was covered by an elevated grid of half-inch hardware cloth, so that all faeces would drop through out of the reach of the young. The regulation top designed to hold food and water was replaced by a flat top constructed of half-inch hardware cloth. The water bottle was suspended at a height which made it available only to the mother. A small ($8\frac{1}{2}$ x 5 x $\frac{1}{2}$ in.) nesting box was provided at one end of the cage. The mother was removed 3 times each day for 1-hour feeding periods in a separate cage. This schedule was maintained for the duration of the experiment.

RESULTS

All litters in both conditions compared by observation with non-experimental pups appeared to develop normally in size and activity. Pups from each of the eight litters were taken at 17, 20, 24, and 28 days of age, and their tongues were mapped by the same

method as in Experiment 1. The results are shown in Figure 9, against the normal curve obtained in Experiment 1. Pups in the Early Weaning condition had an accelerated growth pattern, attaining the full second population four days earlier than under normal weaning conditions. Those in the Late Weaning condition, however, experienced a very strong retardation, the full population not yet attained at 28 days of age.

DISCUSSION

It is evident that these weaning conditions had a powerful effect on the second growth pattern of papillae without noticeably affecting general development. Pups from both conditions seemed normal and healthy in every respect. Those in the Late Weaning condition began eating solids immediately when the experiment was terminated.

There are three factors involved in these manipulations that could be affecting the development of the second population. First, if some quality of the nursing experience is responsible for the changes in development, it must be inhibitory in nature. When suckling is the only source of nutrients, that quality would be present in a greater amount than in normal weaning where part of the nutrient intake is in the form of solid foods. This would suggest that normal development is under some repression as well. Total removal of suckling would remove all inhibition allowing rapid development.

Secondly, solid food intake may stimulate growth. The proportion of total intake that is in the form of solid foods would determine the rate at which the papillae would develop. These factors could both be involved as the direction of influence is the same.

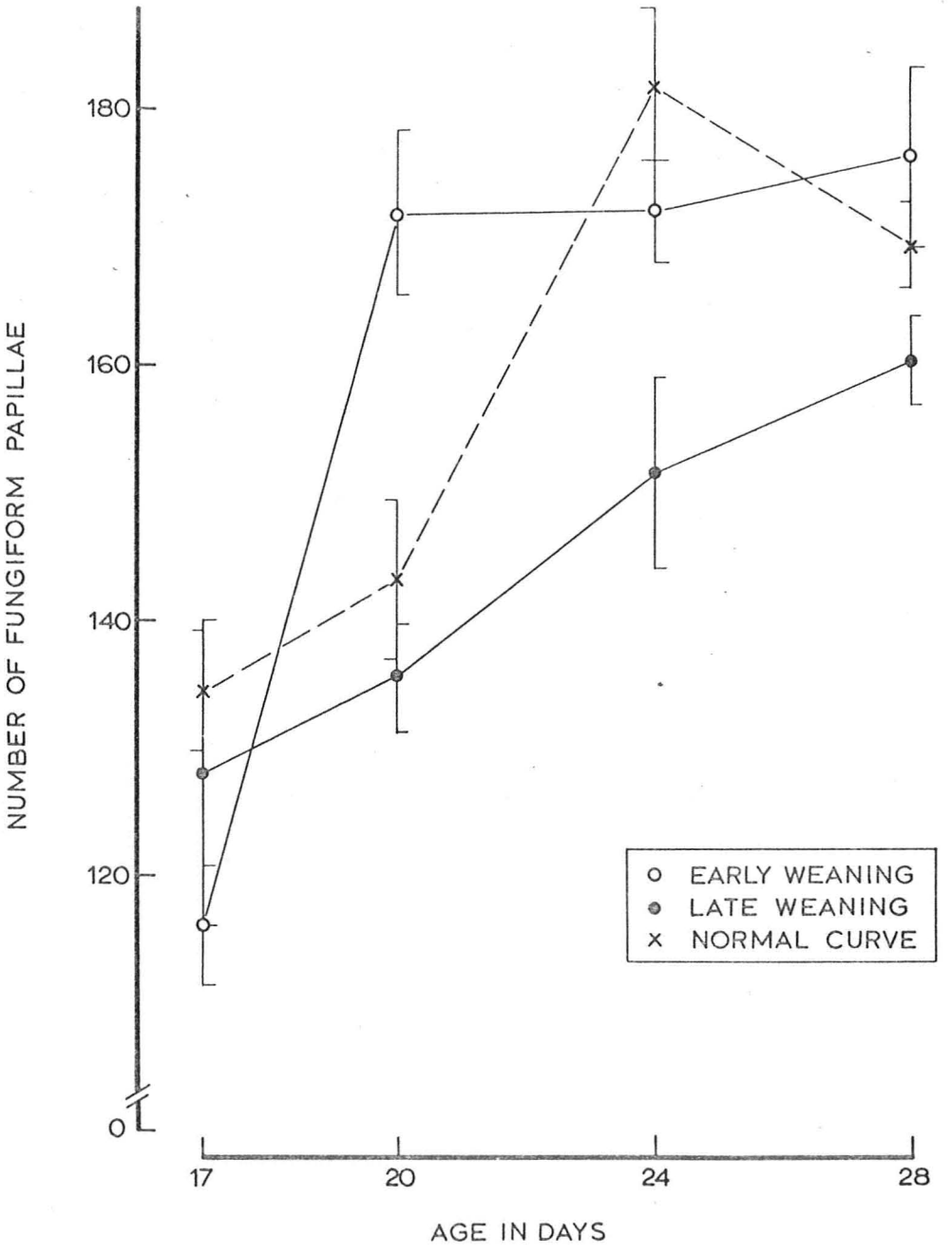


Figure 9. Effects of Early and Late Weaning conditions on development rate of fungiform papillae in weanling rats.

Thirdly, maternal deprivation occurred in both conditions. In Early Weaning, deprivation was total, in Late Weaning there were three hourly periods daily. As the expected result of deprivation is retardation, the obtained results would argue against maternal deprivation being a strong factor. If there is an effect, controlling for it should tend to move the Late Weaning curve closer to normal but should also tend to advance the Early Weaning condition even more.

Acquisition of the second population of papillae has been separated by more than a week by the two conditions. Identification of the factor responsible for this powerful effect can only be made by further experiments.

EXPERIMENT 4

In the preceding experiment the development of the second population of papillae was greatly altered by the imposed weaning conditions. Whether the effect is due to only one of the factors involved or to some combination of factors is still to be demonstrated. This experiment examines the nursing experience.

If there is some quality of the nursing experience that acts to delay the growth of the second population of papillae, it may be temporal in nature. That is, if an inhibiting agent for the second population is present in the nursing experience, its effect may attenuate over time and varying the age of the milk supply in respect to the age of the pups should affect the development of the second population. This was the design of the following experiment.

METHOD

Four groups, each consisting of three female rats, were bred so that the second litter to be born in a group arrived 10 days after the first, and the third was 5 days after the second. Ten of the twelve mothers gave birth to 12 pups, one to 11, and one to 13. It was possible therefore to have all mothers nursing 12 pups for most of the experiment. Mothers were housed separately with their litters in standard laboratory cages, with food and water always available.

Within every set, on the day following birth of the second litter (B) a cross-fostering procedure was begun that gave the first mother (A) and the B mother 6 11-day-old pups and 6 1-day-old pups each. This held until the third litter (C) was one day old, when further cross-fostering was done. C mothers got half of each of the groups from the A and the B

mothers, resulting in a litter of 4 groups of 3 each. A and B mothers gave up the rest of their natural pups to each other and got 6 C pups each. Now all mothers again had litters of 12 to nurse. Pups remained in these positions for the duration of the experiment. The details of this two-tiered cross-fostering are shown in Figure 10.

Pups originating with A mothers were moved back to 1-day-old milk on either their 11th or 16th day of life, or on both. Pups originating with C mothers were moved ahead to 16-day-old milk or 6-day-old milk when they were one day of age. Pups originating with B mothers went in both directions to keep litter sizes equal, one group (bAC) being moved first ahead and then back.

The method used for cross-fostering these pups was intended to reduce as much as possible the likelihood of rejection by the mothers. Mothers were removed to holding cages with food and water for two hours. Pup exchange was made immediately, and watch kept for the two hours to ensure that they remained mixed together in the nest site. If they separated, they were put together again. This was to encourage the acquisition by the fostered pups of nest odours familiar to the fostering mother.

RESULTS

The results for three of these groups are shown in Figure 11. Representing a move to older milk is the C group (cB) who were moved ahead to milk 5 days older than themselves. For movement backward, the A group (aBB) who were returned to 1-day-old-milk on their 11th day of life are shown, and the A group (aBC) who moved back twice to a new milk supply. The normal curve is represented by the dotted line. The significant differences between these individual groups is maintained if all groups moving in each direction are summated (Figure 12).

	First Cross-foster	Second Cross-foster
A mother (12 pups)	6 a pups (aA) 6 b pups (bA)	6 c pups (cA) 3 bA pups (bAA) 3 bB pups (bBA)
B mother (12 pups)	6 a pups (aB) 6 b pups (bB)	6 c pups (cB) 3 aA pups (aAB) 3 aB pups (aBB)
C mother (12 pups)		3 aA pups (aAC) 3 bA pups (bAC) 3 bB pups (bBC) 3 aB pups (aBC)

Coding: first letter - origin
second letter - first foster mother
third letter - second foster mother

Figure 10. Design for cross-fostering pups to mothers with older or younger milk supply.

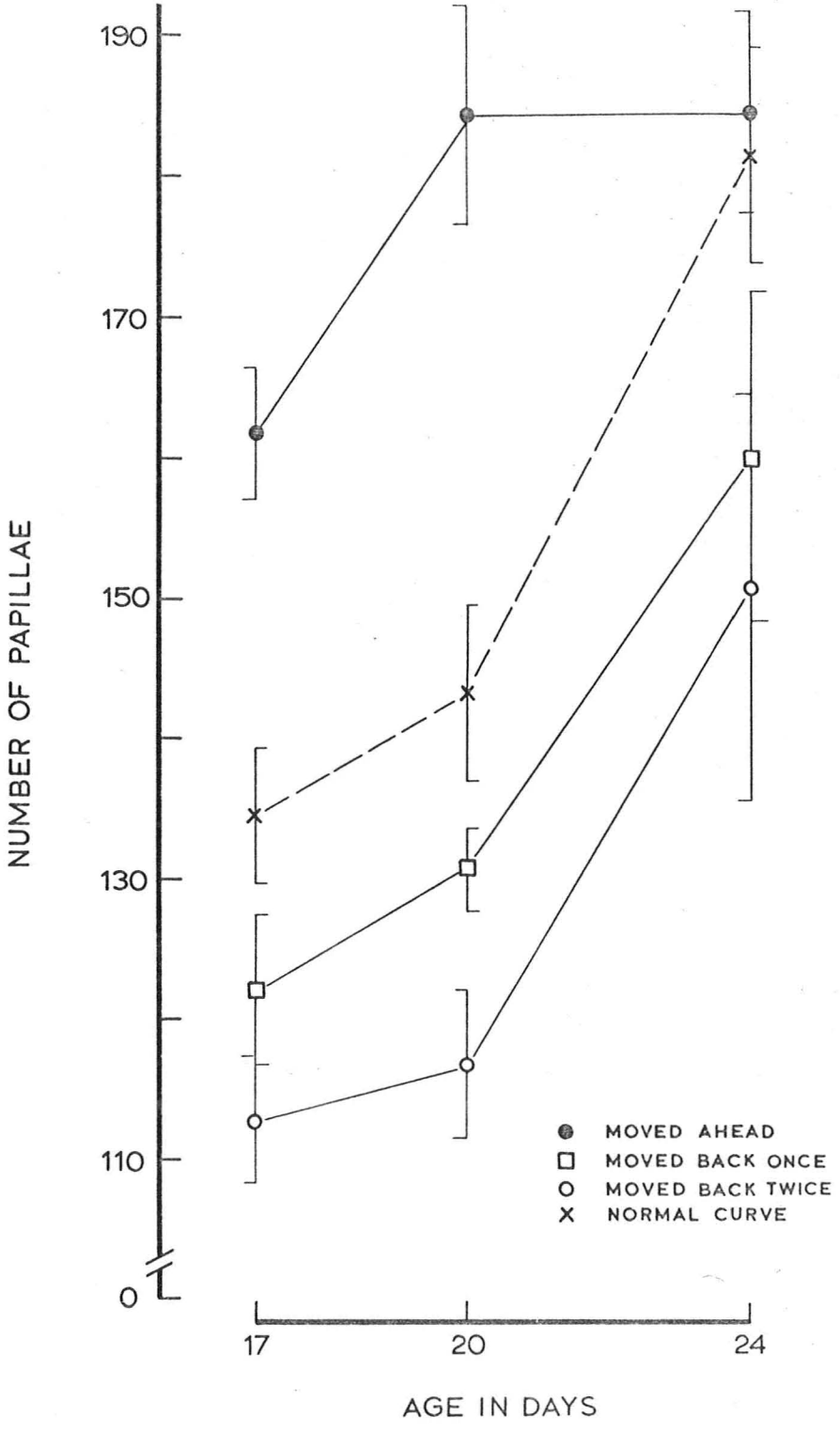


Figure 11. Effects of moving to a foster mother of earlier or later parturition date on developmental rate of fungiform papillae in weanling rats.

Figure 12. Results of shift in age of milk supply.

	Day 17		Day 20		Day 24	
	Ahead	Back	Ahead	Back	Ahead	Back
Mean	150.9	122.4	164.4	129.6	177.5	158.3
SE_M	3.88	3.86	4.7	4.28	3.59	4.98
n	18	16	18	16	17	15
t	5.797		5.422		3.186	
Significance	.001		.001		.01	

Those pups moved to an older milk supply develop the second population early, and those moved back to new milk develop this population late, as compared to normal development. The separation between the two experimental curves is approximately one week.

DISCUSSION

The limited number of sampling points (3) imposed by this cross-fostering design has resulted in some loss of information. Earlier sampling than day 17 of those moved to older milk, and later sampling than day 24 of those moved to younger milk would have yielded the end points of these curves. That information is not necessary, however, for the present purposes. It is clear that some part of the nursing experience has a profound effect on the development of the second population of papillae.

Those conditions in which pups were moved shortly after birth to an older milk supply have an inherent limitation. The pups, for example, moved at 1 day of age to a mother already 16 days post partum were probably weaned before the experiment was over, as the mother would be 39 days post partum when the pups were tested for day 24.

There are some interesting observations to be made of maternal behaviour in a cross-fostering plan such as this. There is no uniformity of acceptance. Some mothers on finding, for example, half of their newborn litter turned into 11-day-old pups resume nursing immediately. Others will crouch on the far side of the cage for an hour or more before accepting the litter. Still others will sort them out into separate groups for a day or two and, although both groups are nursed, will favour one over the other. This preference could not be predicted by size of pups, or own versus alien pups. All mothers, however, gave maternal care appropriate to the age of the particular pup (e.g. grooming).

This variance in maternal acceptance might have caused the differences seen in this study because favoured pups grew vigorously while non-favoured pups were, in a number of cases, seriously retarded in general development. This retardation stunted overall growth, delayed acquisition of the startle response, and delayed eye-opening for days. However, the papillae population of these seriously deprived rat pups developed according to the experimental manipulation and not to their general development. In a group of three 20-day-old pups, for example, the body weights and population counts were 16 grams - 171, 37 grams - 188, and 41 grams - 166.

Since this maternal behaviour was not predicted nor was it uniform, these pups were not concentrated in any one group. They appeared randomly across conditions, underlining the strength of the phenomenon. The development of the second population of papillae in these retarded rat pups in accordance with experimental manipulations would further suggest that this development is distinct from general development and is determined by nutrient intake.

DISCUSSION

We have assumed that each fungiform papilla in the rat is the site of a single taste bud (Fish, Malone and Richter, 1944). Therefore the preceding experiments report on changes taking place in populations of fungiform papillae, and probably of the taste buds themselves. Observations were limited to the fungiform papillae on the dorsal surface of the tongue and no attempt was made to see if similar differences exist in the circumvallate or foliate papillae, or in other known sites of taste bud population.

Three questions were posed about the development of taste bud populations in the growing rat. The first two questions dealing with the parameters of development were investigated in the initial experiment. There is a development of papillae in the neonatal period, achieved in two separate and distinct growth spurts, which total to a greater population than in adults. These two periods of neonatal growth occur at times when the manner of nutrient intake is also changing.

The transition from intrauterine to extrauterine conditions is accomplished fairly rapidly, and the initial growth spurt is of comparably short duration. However, investigation of this period did not support the idea that changing the manner of nutrient intake was involved in the development of this population. There is some evidence that it is not a fixed developmental process but is under uterine influences.

The second growth spurt is realized at the time young rats are beginning to eat solid foods, and is much slower in developing, taking days to be completed while the first growth takes hours. This second growth

period, however, appears to be strongly influenced by the transition to solid foods. Forcing the weanling rat into an abrupt transition to solid foods in the absence of the mother precipitates the development of the second population; withholding solid foods entirely serves to delay it. Of the three factors involved in this experiment, inhibitory effect of nursing, stimulating effect of solid food intake, and maternal deprivation, the nursing situation seems the most promising place to look for the responsible factor.

It has been shown that the milk of a mother rat provides not only nutrition but also information on what solid foods to eat at weaning (Galef and Henderson, 1971). Moving the rat pup to a mother giving older milk serves to hasten the development of the second population of papillae, and to a mother giving younger milk serves to retard it.

These two effects may be adaptive. If the function of the second population of papillae is to aid in diet selection, acceleration of its growth following loss of the mother would be adaptive. At the same time, if the presence of this second population is necessary before the young rat will try ingesting solids, it would be equally adaptive to retard its onset until general development had reached an appropriate level (e.g. eye opening) for the weanling to safely go out in search of foods. There are a number of experiments to be done to identify the variable responsible for this control.

There is a further area to be investigated. It is not known if taste buds in neonates are functional, and it will be necessary first to determine when innervation takes place. Following that, the response patterns to stimuli in the two populations must be investigated. While

any discussion at this time of the possible significance of these two distinct growth periods can only be speculative, they may serve different functions.

The results of the experiments reported here suggest behavioural control of considerable strength over development. A question which needs answering, and is currently under investigation, is whether development can also control behaviour. Manipulations of milk age alter the time of the second change. When the change is advanced in time, does the transition to solid foods begin at an earlier age also?

A further investigation just beginning is to determine how long the onset of the change can be delayed by repeatedly switching pups back to a newly parturate mother. This will aid in investigation of the initial population if the two growth periods can be separated by a large interval of time.

SUMMARY

1. The presence of a larger population of fungiform papillae in neonates than in adults was confirmed. There are about 30% more papillae in neonates.
2. The development of the neonatal population of papillae takes place in two distinct spurts. The first growth period begins shortly before birth and is completed by 8 hours post partum. The second growth period begins about day 15 and is completed by day 24.
3. The initial growth produces about 60% of the total neonatal population. This growth is not affected by withholding suckling, or by the birth process, or by delaying birth experimentally. It would appear to be a fixed developmental process, except that naturally lengthened gestation does alter the time of its onset, suggesting that it is in part under uterine influences.
4. The second growth occurs at the time when the weanling rat is learning to ingest solid foods and takes 10 days for completion. The combined populations persist throughout adolescence and begin to decline around day 75, reaching the adult level about day 90.
5. Removing the mother, at day 15 and thus limiting the weanling's diet to solid foods accelerates acquisition of the second population of papillae by four days. Preventing access to any food other than mother's milk retards acquisition by more than four days.
6. Moving pups forward or backward on their milk supply has equally powerful effects on the development of the second population of papillae. Pups who are moved back to "new" milk are about four days later than normal in beginning to develop the second population, while pups who are moved ahead to "older" milk are advanced by about four days.

7. Development of this second population appears to be independent of development in general, and to be determined by nutrient intake.

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