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Hormonal control of lactation

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THE HORMONAL CONTROL

OF LACTATION

by

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Medicine. 1935

To my dear friend Dr. Fred Fouts,
who has been my inspiration from
childhood on, and by whose help
I have been able to continue my
studies in the profession he has
practiced so nobly, this paper is
respectfully dedicated.

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INTRODUCTION

The mammary gland is an anatomical structure common to all mammals, and is the medium for the nourishment of the young for a varying period following intrauterine life. To synchronize the development and function of the mammary gland with the development and birth of the young requires a high degree of coordination beteween ovaries, uterus, pituitary gland and mammary gland, which will be the subject of this paper.

Each mammary gland in a woman is composed of from 15 to 25 individual lobes radiating from the mammary papilla or nipple and separated from each other by layers of connective tissue and adipose tissue. Each lobe is an independent, compound, branched alveolar gland, having a separate opening on the surface of the nipple by means of its excretory or lactiferous duct.

The secreting portions of the gland, the alveoli, consist of a basement membrane, a layer of myo-epithelial cells, (which serve to associate the mammary gland morphogenetically with the sweat glands) and a layer of low col-

umnar epithelial cells. These latter elements
secrete the complex product, milk, by diffusion
of the constituents from within the cell into the
lumen of the alveolus, and, possibly, during strong
sucking the portion of the cell which projects
into the lumen may be broken off with its contain-
ed secretions; hence the gland is of the apocrine
type.

The secreting alveoli pass over into
excretory ducts lined by cuboidal or low columnar
epithelium, which unite with other ducts to form
larger ducts in which the epithelium is taller
and cylindrical, finally uniting to form the main
excretory or lactiferous duct, which is lined by
stratified epithelium and which in turn is re-
placed by stratified squamous epithelium at some
distance from its opening on the nipple. Each
lactiferous duct is provided with a dilitation,
the sinus lactiferous, in the area beneath the
areola or pigmented circular area of skin surr-
ounding the nipple.

The primordium of the mammary gland
appears in the human embryo of 8mm. as a paired
thickening of the epidermis, the milk line, ex-

tending from the upper limb bud to the inguinal
fold. Only a portion of each milk line in the
costal region continues to thicken to form a
pair of lens-shaped plates, which later become
hemispherical or club shaped thickenings pro-
jecting into the underlying dermis. These are
called mammary buds, and in other mammals a
a number of such buds may form, or they may
develop at different points along the milk line.
The mammary buds give rise to a number of cell
columns from their lower surface, which project
into the underlying connective tissue, and later
become lactiferous ducts. In man there are 15 to
25 of these primary sprouts. These give rise to
secondary sprouts or cell columns which are the
primordia of the excretory ducts. By enlongation
and branchings the complex duct system of the
gland is formed.

At birth the mammary gland is 3.5 to 9
mm. in diameter, and the lactiferous ducts have
formed, with a few excretory duct branches. In
males there is a regression of the gland, and only
the rudimentary nipple remains, with its surround-
ing arebla. In females there is a slow evolution

of the duct system throughout childhood to
puberty, when the whole process is speeded up,
the gland increases in size due to the deposit-
ion of fat, the nipple increases in size, and
the duct system becomes complete.

There is no development of secretory
portions until the advent of pregnancy. Then
there is a rapid multiplication of the epithel-
ium at the ends of the excretory ducts, and the
secretory alveoli or lobules are formed. This
is especially rapid diring the first half of
pregnancy, and is accompanied by a loss of fat
from the gland to make room for the secreting
elements. During the last half of pregnancy
multiplication of the epithelial cells slows
down, and a secretion is formed in the alveoli,
which is colostrum. In the first few days after
delivery the colostrum is replaced by milk,
which continues to be secreted for the period
of suckling of the child. (42, 2)

In other animals the development of the
mammary gland at birth, before and at puberty
varies somewhat from species to species. For

example, in the ferret there is no duct development even after puberty, while there may be even full lactation in some marsupitals and possibly the dog after oestrus. A full discussion of these differences is given by Turner (2) and may well account for a number of discrepancies in the findings of the various investigators to be discussed later.

THE HORMONAL CONTROL OF LACTATION

In the past half century a great many investigators have directed their efforts toward finding the explanation for the growth, development and functioning of the mammary gland. Much has been accomplished, but there is still much left to be explained. Many discrepancies have arisen beteween the findings of the various laboratories, all of which cannot be explained on the basis of specie differences, and there is much to be desired in the way of standardization of methods and materials in the various centers of experimentation. Practically all the experimental work has of necessity been done on laboratory animals, and the field of practical applications is still unlimited.

Prior to 1895 physiologists believed that the coordination beteween the developing embryo and the mammary glands was due to direct nervous connectin beteween the uterus and mammary glands. As evidence of this was cited the commonly observed contraction of the uterus following stimulation of the nipples. (62, 2) In 1894 Mirinoff (43) observed that following the complete

severance of these nerves in pregnant animals the
gland would continue to develop and would secrete
milk after parturition. This was confirmed in 1896
by Goltz and Ewald (23) who completely removed
the lumbar cord from a bitch. She subsequently
conceived, and gave birth to a litter of puppies
which she was able to suckle normally. Routh (57)
(1893) observed that complete paraplegia below
the level of the sixth dorsal vertebra did not
inhibit lactation in man.

The nervous control was also disproven
in another way. Ribbert (53) (1893) was able to
transplant the mammary gland of a guinea pig
into the skin of the ear. During a subsequent
pregnancy the gland enlarged as usual, and
lactated following parturition. Pfister (52)
(1901) repeated the experiment on a rabbit.

On the other hand, it had been shown
by Knauer (34) and Halban (24) (1900) that
oophorectomy in young animals would cause regress-
ion of the mammary glands, and they would not attain
normal pubertal size. That this was not due to
the severance of nervous connections they demon-
strated by grafting the ovaries to the peritoneum

or intramuscularly. When this was done success-
fully the mammary glands attained their normal
pubertal growth.

Therefore it has been generally accept-
ed as true that the source of stimulation to the
mammary gland is hormonal, due to a "chemical
messenger" (62) rather than nervous. This is
true of the growth stimulus; as will be seen
later the lactation stimulus is held be some to
have a nervous factor in its control. Subsequent
investigations have been directed toward finding
the nature and origin of the hormones responsible,
for mammary hypertrophy and lactation.

Lane-Claypon and Starling (36) (1906)
were the first to attempt by experimental means
to find the hormones responsible for mammary
growth and lactation. They injected filtered aqu-
eous extracts of placenta, fetuses, uterine tissue
and ovaries, as well as combinations of these into
female rabbits. They did not castrate their
animals, so the results they obtained were prob-
ably due to the rabbits own ovaries, and not the
weak extracts they used, especially since their
figures show only a duct development, similar

to that observed after reaching puberty. (18)

Frank and Unger (18) (1911) repeated the
experiments of Lane-Claypon and Starling, also
with negative results. Other investigators in
this period who used aqueous extracts failed to
get conclusive results, at best only a slight
duct growth. It has since been shown that the
tissues they extracted do contain the oestrus
producing hormone, but the amounts extracted in
aqueous solutions are very small. This fact,
together with the fact that they failed to recog-
nize the necessity of removing the ovaries,
made what slight results they achieved value-
less, since it might easily have been a normal
oestrus growth. (2).

Following the report in 1912 by
Iscovesco (32) that lipoid extracts of the ovary,
corpus luteum and placenta cause distinct.
changes in the female genital tract and mammary
glands, this line of attack was taken up by
several investigators. Fellner (21) (1913)
obtained duct system growth in normal and
castrate male and female guinea pigs and rabbits.
Herrmann (29) (1913) (30) (1915) observed

growth of the mammary gland in castrate and
normal female rabbits; Frank and Rosenbloom (19)
(1915) slight growth in castrate rabbits and rats.
It has since been shown that the success of these
men was due to their successful extraction of
the oestrus producing hormone. (2)

Since the effect of the ovaries on the
mammary glands had already been observed by
Knauer (34) and Halban (24) it is only natural
that these organs should be subjected to con-
siderable study as to a possible relationship
to pregnancy development. The experiments just
mentioned, with lipoid extracts of the ovaries
was a start, but the real impetus to this line
of research was given by Allen and Doisy (1) (1923)
by the introduction of the rat test unit for the
oestrus stimulating hormone of the ovaries, and
the determination of the tissues in which this
hormone is found in the greatest concentration.
This hormone has been named by various investig-
ators "oestrin" "theelin" and "menoformon".

Early in the investigations of the
effects of theelin it was observed that one of
its effects is on the mammary glands. From that

time to the present this effect has been studied
by a great many men, only a few of whom will be
mentioned.

Hartman, et al (23) (1926) produced
duct system growth in the oppossum by injections
of follicular and placental hormones (theelin).

DeJongh and Dingemanse (14) (1931)
injecting 200 units of menoformon per day into
male guinea pigs produced marked growth of the
mammary glands, and if the dose were suddenly
reduced to 2 units a day the glands secreted
milk.

Turner, et al (66, 67, 68, 1930 & 1931)
using castrated immature male and female rabbits
and rats were able to produce only duct system
growth (pubertal development) by the injection of
the oestrogenic hormone, whether obtained from
the ovary, placenta, amnionic fluid or urine
of pregnancy. In 1932 they used crystalline
theelin and theelol (69) and produced signific-
ant duct growth but no lobule formation in the
mammary glands of male rabbits, castrated female
rats and male mice. No difference was observed
beteween the effects of theelin and theelol.

Bradbury (7) (1932) made similar find-
ings in the mouse. He found that the sexually
mature gland consisted only of galactophores
(primary duct system) and that injections of
theelin had no effect on this gland. A similar
duct growth could be brought about in castrate
immature males and females, however, by the
injection of theelin.

From the above experiments it seems
clear that the initial development of the mammary
gland is under the influence of theelin. This
hormone, produced by the ovary, stimulates the
pubertal development of the duct system, which
may continue to develop during each oestrus
cycle. Theelin secreted by the placenta may
cause the initial increase in the duct system
during pregnancy. This hormone seems to be
ineffective in stimulating lobule formation in
most species, however. In the guinea pig apparent-
ly lobules may be formed by administering large
doses of theelin, for secretion is obtained
when the dosage is reduced. (14) In other animals
a slight lobule may be noted at times. (2, 13)
But this slight lobule formation does not begin to

compare with the rapid hyperplasia which occurs
during the first half of pregnancy, and therefore
an additional hormone has been sought which
would stimulate pregnancy hyperplasia.

It is only natural that so prominent a
structure as the corpus luteum should be subjected
to some study. Starling (62,36) and Frank and
Unger (18) used aqueous extracts of this gland,
but as has been previously stated their extracts
were too weak to have any effect. Iscovesco (32)
and Fellner (21) were more successful with lipoid
extracts, but we have already mentioned that they
extracted theelin from the gland, and not the
corpus luteum hormone.

The discovery in 1911 by Ancel and
Bouin (3) of the condition of pseudopregnancy in
the rabbit gave the corpus luteum angle a fresh
impetus. The rabbit does not normally ovulate
until copulation occurs, hence corpora lutea are
not found except in the pregnant condition.
By mating does with vasectomized bucks they were
able to induce ovulation and corpus luteum format-
ion in the non-pregnant animal They found that
the corpus luteum persisted for about 15 days,

about half the duration of a normal pregnancy.
During this time the mammary glands underwent
rapid lobule hyperplasia, so they were convinced
that the corpus luteum is responsible for the
development of the mammary glands during the
first half of pregnancy. They ascribed the
development of the mammary glands during the
latter half of pregneney to the so-called "myo-
metrial gland".(4) However, Hammond (26) (1917)
showed that this structure is not constant, being
found in only an occasional rabbit, and not at
all in other species. He also pointed out that
the corpus luteum persists throughout normal
pregnancy, and concluded that the development of
the mammary gland during the latter half of
pregnancy is due to the same factor which causes
its development in the first half, namely the
corpus luteum.

In 1930 Corner (13) by using a highly
potent extract of corpus luteum, (progestin)
was able to carry pregnant does to full term which
had been deprived of their ovaries 18 hours
after conception, a proceedure which otherwise
results in abortion. In these rabbits mammary

growth and lactation occurred normally. He
reasoned that if the corpus luteum of pregnancy
were responsible for mammary gland development, he
should be able to produce similar development
in spayed non-pregnant does by injections of
progestin. He made this test, but could produce
no changes in the gland.

However, these rabbits were deprived
of oestrin, since they were spayed, and it had
been pointed out by Hissau (31) (1929) that the
corpus luteum does not exhibit its effects on
the symphysis pubis and endometrium without
the immediately previous action of oestrin. So
Corner used the method of Jares (33)(1930) to
subject the rabbit to the continued action of
both oestrin and progestin, namely, inducing
ovulation and new crops of corpora lutea at
of a few days by intravenous injections of 10
c.c. of filtered unine of pregnant women. He
found that continued action of progestin even
when preceeded by the action of oestrin does not
induce proliferation or lactation in the mammary
gland.

Turner and Frank (67) (1931) found that

injections of progestin in immature male and
female castrate rabbits produce no changes in the
mammary glands, even when preceeded by inject-
ions of theelin. Realizing that during pregnancy
a large amount of theelin is being secreted by the
placenta, they attempted to duplicate this by
injecting large doses of theelin in their rabbits
simultaneously with their progestin injections.
By this method they obtained a full development
equal to that during pregnancy.

Bradbury (7) (1932) made similar find-
ings in the mouse. He found that lutinization
of the ovaries by means of injections of pregnant
women's urine causes mammary hyperplasia, but
not if the ovaries are absent.

Selye, et al (58) (1933) confirmed the
above findings in the rat. They found that
intense luteinization of the ovaries produced
by injections of pregnancy urine causes mammary
gland growth.

Nelson and Pfiffner, (45, 46) (1930,
1931) found marked hyperplasia in the glands of
immature male and female guinea pigs and young
male rabbits which were injected with only a

corpus luteum extract. Turner (2) has pointed
out, however, that their extracts probably contain-
ed oestrin as well as progestin.

At this point it would seem that mammary
gland growth in most animals is caused by the
action of two hormones, oestrin initiating duct
system growth, and oestrin plus progestin causing
lobule formation. As we shall see later the
corpus luteum problem is not nearly so easily
settled as that.

So far we have purposely avoided the
problem of secretory activity , for it is in
connection with this function that the most im-
portant discoveries with regard to the hormonal
control of lactation were made. Lane-Claypon and
Starling (36) concluded that the substance
which gives the growth stimulus to the mammary
glands inhibits their secretory activity by
direct action upon the secreting cells, for the
reason that a cell cannot be both growing and
secreting at the same time. This view was held
by many of the subsequent investigators.

Evidence supporting this idea, aside
from the clinical evidence that these early

investigators based their ideas upon has been put
forth by DeJongh and Dingemanse (14) in their work
with guinea pigs previously mentioned. Lactation
was noted when the amount of oestrin injected
was suddenly reduced, just as occurs at partur-
ition following expulsion of the placenta.
Also, Selye et al (58) noted lactation in rats
following the removal of the ovaries which had
undergone intense luteinization under the influence
of pregnancy urine, and had been accompanied by
mammary gland growth. However, lactation did
not occur if the pituitary gland were absent,
and that could mean only one thing, that the
pituitary has some role in the hormonal control
of lactation.

In 1924 Evans (15) had shown that inject-
ions of an alkaline extract of the anterior lobe
of the hypophysis would cause persistence of pre-
existing corpora lutea as well as causing intense
luteinization of Graafian follicles without
ovulation. Using the method of Evans, Parkes
(51) in 1929 injected such an extract in pseudo-
pregnant rabbits and was able to continue the
luteal phase beyond the usual 15 day period.

In these animals he obtained a growth of the mamm-
ary glands equal to full term pregnancy, and
therefore decided that the corpus luteum was
responsible. But Corner(13) using the method of
Jares (33),that is, pregnancy urine injections,
could note no change in the mammary glands alth-
ough luteinization of the ovaries was produced
equally as well as by the anterior hypophysis
extracts. He assumed that some other factor must
be present in the anterior hypophysis extracts
which was responsible for the mammary gland
growth obtained by Parkes. He then injected spay-
ed virgin rabbits with alkaline extracts of the
anterior pituitary gland, and obtained both hyper-
plasia and secretion in the mammary glands. His
rabbits were mature, but virginal, the mammary
glands having reached the pubertal state before
the injections.

Stricker and Grueter (64, 65) (1928 &
1929) had also been able to produce mammary
hyperplasia and lactation in rabbits by the
injection of an aqueous extract of the anterior
hypophysis. They obtained their results first
by injections in the latter part of pseudopreg-

nancy. Later they removed the ovaries on the tenth day of pseudopregnancy and were still successful, indicating that the ovaries were not responsible for their results. Still later they learned that it was not even necessary that the animal be pseudopregnant, but it was only necessary that the mammary gland be developed by previous pseudopregnancy or pregnancy. They were successful under these circumstances in producing lactation in rabbits, dogs, hogs, and cattle by their anterior pituitary extracts. They could not induce lactation in virgin rabbits.

Shortly after Corner (13) had published his work, Riddle (54) (1931) and his associates who were studying the physiology of reproduction in birds, found that some of the extracts of the anterior pituitary which they were injecting to determine their effect on the reproductive system of pigeons, also caused an enlargement of the crop glands. These are two dorso-lateral areas in the crop of pigeons and doves of both sexes which normally cannot be differentiated from the rest of the crop mucosa, but which undergo remarkable hypertrophy at the end of the brooding period,

and produce by secretion and desquamation of the
mucosa cells a substance called crop milk.
This is mixed with partially digested food in
the crop and regurgitated to feed the young.
This process is analogous to lactation in that
it represents a phase of reproduction consequent
to ovulation, occuring at a considerable time
afterward, and at the time of a new phase of
alimentation in the young.

Riddle (54) determined that this
growth occurred after previous section of the
nerve supply, so it could not be conditioned by
nervous control. He was able to produce crop
gland growth by injections of anterior pituitary
extracts, but not by pregnant urine. He was un-
able to determine whether it was either of the two
known hormones of the anterior pituitary (growth,
sex maturity) which was responsible, or a third,
unknown hormone. He suggests that the crop gland
response might form a convenient means of standard-
ization of the hormone responsible.

A year later Riddle (55) was able to
state that the hormone responsible for the crop
gland response is a separate hormone, which would

still produce this response when freed of the
growth and sex maturity fractions. He gives the
method of making such a separation, and proposes
the name"prolactin" for the hormone. He found
that male and female mature guinea pigs and mature
female rabbits would also respond to this hormone
by lactation; the males after previous treatment
with theelin and progestin. Lactation began 2 to 3
days after beginning the treatment in rabbits,
3 to 5 days in guinea pigs. The term and quantity
of secretion were highly variable. In all cases
(pigeons, guinea pigs, rabbits) the gonad-stimulat-
ing principle and growth principle, when freed of
prolactin, failed to give any lactation or crop
gland response.

In a subsequent publication (56) (1933)
Riddle gives very complete and extensive experiment-
al data which shows that prolactin is a separate
hormone; that it is capable of producing the crop
gland response in doves and pigeons and the lact-
ation response in guinea pigs, rabbits, rats,
oppossum and monkeys; that the growth or gonad-
stimulating hormones are incapable of doing this;
that prolactin is effective in castrate and hypo-

physectomized animals. He also gives detailed
directions for the preparation of prolactin and
its assay, using the crop gland response. In a
recent article (6) (1934) he has shown that the
hormone prolactin is a protein substance, digest-
ed by trypsin.

All the investigators who have used
prolactin or similar preparations of the anterior
pituitary are agreed that it does stimulate
lactation under the proper conditions. Here
the agreement stops. Some men have held that it
not only stimulates lactation, but it also promotes
gland growth (lobule formation). There is also
a difference of opinion as to whether oestrin only
is sufficient to prepare the gland for the action
of prolactin, or whether progestin also is need-
ed. We will first consider the problem of
prolactin and gland growth.

This difference of opinion came up in
the earliest envestigations. It will be remember-
ed that Corner (13) found both hyperplasia and
secretion to result from prolactin injections in
his rabbits, while Stricker and Grueter (64, 65)
were unable to produce lactation in virgin animals,

but only if the mammary glands had been previously
developed by pregnancy or pseudopregnancy.

Nelson (46) (1931) suggests that the
ovarian factors (oestrin and progestin) are respon-
sible for the early growth of the mammary glands,
and that the profound growth during the latter
partoof pregnancy is controlled by the anterior
pituitary.

Asdell (5) (1932) found that potent
lactogenic extracts are without effect in immature
rabbits. He produced full mammary development
in ovariectomized rabbits which were just mature.

Catchpole et al (38) (1933) found that
the mammary glands of rabbits respond to the
lactation hormone by both duct and alveolar
growth, and lactation.

Weichert (70) (1934) found that the
ovariectomized rat does not respond to the lactogen-
ic hormone, but when the ovaries are present,
respond by both growth and lactation.

On the other hand, Riddle (56) (1933)
states that "we have become fully convinced that
prolactin has not in the least favored the
growth and development of mammary tissue in the

individuals with which we have worked". (Guinea pigs and rabbits.)

The latter view is held by Turner and his associates. Garner and Turner (22) (1933) could produce no growth of the mammary glands by prolactin injections in young ovariectomized rabbits. Turner (2) cites unpublished data by Garner, in which they not only failed to get duct growth in immature glands, but also they failed to get lobule formation where ducts only were present. His explanation of the apparently positive results of others is a logical one. He thinks that in all cases where lactation is produced by injections of prolactin, lobules were already present, and the apparent hyperplasia is only a distention of the lobules by secretion. It has been shown that in some mature animals a few lobules may be present. This would explain the onset of lactation in such animals. In immature animals and males lobules would not be found, and in these no one has been able to produce lactation without previous treatment with ovarian hormones. Lyons, et al (37, 9,39) Nelson et al (45,46,47,48,49,50) Bradbury (7) Asdell (5) Evans (17) .

Some such explanation as given by
Turner is necessary, for certainly in the normal
pregnant animal secretion and growth do not
occur simultaneously, but rather in sequence.
Nelson (50) has suggested that perhaps the anter-
ior lobe hormone acting together with the ovarian
hormones promotes growth, but lowering the
oestrin level (removal of the placenta) allows
the anterior lobe hormone to stimulate secretion.
This would apparently be refuted by the normal
development(but failure to lactate) of mammary
glands in hypyphysectomized pregnant animals.
Selye et al (59, 71)

In considering the preparation needed
before the mammary gland can be stimulated to
lactate by the action of prolactin, we find a
considerable controversy over the role played by
the corpus luteum.

Corner (13) thought that the corpus
luteum is unnecessary, since he used spayed virgin
rabbits in which he thinks it highly unlikely that
corpora lutea ever existed. Stricker and Grueter
(64, 65) thought previous sensitization by the
corpus luteum is necessary, since they could not

produce lactation in virginal rabbits. De Jongh
and Dingemanse (14) produced lactation by injections
of oestrin in male guinea pigs, so evidently
progestin is unnecessary in that animal. Nelson
(49) did the same, except that he followed the
oestrin injections with prolactin in order to
obtain lactation, instead of reducing the dosage
of oestrin. It would therefore seem that the
corpus luteum is not necessary in the guinea pig.
Catchpole and Lyons (8) found that no previous
corpora lutea are necessary in rabbits, but
that lutein sensitization makes them more
reactive to prolactin. They suggest that the
ovaries which do not show evidences of corpus
luteum formation do contain theca lutein cells
which normally go to make up the corpus luteum,
and think that these cells may be a factor in
preparing the gland. Asdell (5) found that the
corpus luteum is not necessary for the lactation
response in the rabbit and state that a goat which
had never been in heat was made to lactate by
prolactin injections.

On the other hand, Bradbury (7) finds that
in the mouse the lactation hormone is not effective
unless alveoli formation has been produced by

luteinization of the ovaries by means of pregnancy
urine. Also, Evans and Simpson (16) find that in
spayed mature virgin rats it is impossible to
produce mammary gland growth and secretion by means
of prolactin injections, even if progestin also is
given. Weichert et al (70) confirmed these find-
ings, but were able to produce gland growth in
the absence of the ovaries by properly propor-
tioned injections of ovarian hormones (oestrin
and progestin) . They point out that this is a
distinct species difference. That there is a
distinct species difference in the necessity
for the previous sensitization by progestin is
also pointed out by Nelson (50) and Selye et al (71)

It would seem therefore, that prolactin
is the hormone secreted by the anterior hypophysis
which initiates lactation. It probably does not
promote lobule formation. It is capable of act-
ing on a gland only if the gland has had lobules
previously formed. There is a species difference
in the matter of lobule formation. Some animals
may form lobules under the influence of theelin
alone, others require the action of progestin also.
Probably both hormones play a part in normal pregnancy.

We have not as yet considered why
lactation occurs only at the termination of
pregnancy. It seems to be quite generally
accepted that it is the antagonism of theelin
that prevents lactation. Nelson (50) as well
as Smith and Smith (61) were able to inhibit
lactation by injections of theelin. Nelson(50)
has shown also that it is the theelin secreted
by the placents which inhibits lactation. He
did this by castrating pregnant guinea pigs and
having them go to full term, only lactating after
parturition. Since mammary gland growth occurred
normally there must have been a source of theelin.
Removal of the pregnant uterus did not cause
lactation, it the ovaries were left, but removal
of both resulted in lactation. Removal of the
pregnant horn of the uterus and the ovaries leaving
the sterile horn resulted in lactation, therefore
some factor aside from the uterus was responsible.
Removal of the foetuses and ovaries, leaving the
placenta did not result in lactation as long as
the placenta was retained. Therefore the placenta
must elaborate the theelin which inhibits lactation.

That retention of the placenta will in-

hibit lactation has been observed a number of
times. Halban (24) Smith and Smith (61) Stimson (63)
Transplanted placental tissue will do the same,
as long as the grafts are active. Frankl (20)

So then, lactation occurs at the termin-
ation of pregnancy because the oestrin or theelin
content of the blood falls, due to the loss of
a source of this hormone, the placenta. But how
does the presence of oestrin inhibit mammary
activity? Nelson (50) believes it is by an action
on the anterior pituitary, preventing the release
of prolactin. When thes inhibitory factor is
removed, the anterior pituitary secretes prolactin.
This is shown by the fact that simultaneous inject-
ions of oestrin and prolactin result in lactation
in a properly prepared animal. On the other hand,
he thinks that large amounts of oestrin may act
directly on the mammary gland itself, for if
a large amount of oestrin is injected together
eith a corresponding dose of prolactin, no lact-
ation results. We suggest that this problem
could be clarified considerably, as well as the
problem of the role of prolactin on mammary
development during pregnancy, by some means of

determining the amount of prolactin in the blood of a pregnant animal. The fact that mammary development may continue in an hypophysectomized pregnant animal does not necessarily preclude the possibility of the pituitary being responsible in part, for there are fetal hypophyses which might secrete prolactin.

Another puzzling finding is that of Selye et al (12, 71) who state that pregnant, hypophysectomized rats and mice secrete milk for a few hours after parturition. We would be inclined to attribute this to fetal hypophyseal hormones circulating in the blood of the mother, but they also find (71) that distention of the uterus with paraffin prevents this secretion. They postulate a nervous influence on the hypophysis by the pregnant (or distended) uterus, inhibiting the release of prolactin. They ascribe the secretion of milk for a few hours after parturition in their hypophysectomized animals to a functional stimulus to the mammary gland by the uterus.

Another controversial matter is that of a possible nervous influence on the pituitary by the act of suckling. It would seem that the exper-

iments of Mirinoff (43), Ewald (23), Routh (57)
Ribbert (53) and Pfister (52) previously mentioned
should be enough to disprove any possibility
of a nervous control.

Although Hammond (27) found that when the
teats of certain mammary glands of a rabbit
were occluded to prevent the young from suckling
the corresponding glands would undergo involution,
even when adjoining glands were in an active
state of lactation; and Nelson (50) found exactly
the same to be true, Selye et al (60, 71) found
exactly the opposit. They tied the galactophores
of a gland, and it remained filled under the
stimulus of suckling. Excising the nipple of
one gland and allowing the opposit gland to be
sucklid, they found that the gland which was not
suckled due to the absence of a nipple remained
in active lactation. They take this to mean
that the act of suckling by means of a nervous
stimulus to the hypophysis causes the release of
prolactin, which continues to stimulate lactation
in the mammary gland that is not being suckled.
Evidently more work needs to be done to clarify
this point. It seems that it would be easy to

settle this question by seeing how long a gland
could be kept secreting under the influence of
prolactin injections but without being drained,
for the whole matter hinges about the question as
to whether mere distention of the gland by re-
tained secretions will result in its involution,
or whether the absence of prolactin is necessary.

But that experiment cannot be satis-
factorally carried out because of another puzzl-
ing finding, namely that continued injections
of prolactin are ineffective in continuing
lactation, even when increasingly large amounts
are injected. Riddle (56) states "it seems,
though it is not proved, that initial light dosage
with prolactin develops in castrate female guinea
pigs a mammary state in which the lactation
response is unusually difficult to obtain later
eith increased and adequate amounts of prolactin."
Nelson (50) "We have never been able to maintain
lactation induced by pituitary extracts indefin-
ately even when increasing amounts were administer-
ed." Asdell (5) was able to prevent the normal
decline in milk production in goats for only a
short time by means of prolactin injections.

Evans (17) made similar observations with goats, but found that after a lapse of 40 days was again able to increase the yield.

Evidently prolactin injections induce in the mammary gland a refractory state, which is extremely difficult to explain, inasmuch as the hypophysis has been shown to be necessary not only for the initiation of lactation but also for its continuation. Collip et al (12) Selye et al (59, 71)

A phase of the lactation problem which has hardly been touched upon is the relationship of the pancreas to the mammary glands. Markowitz et al (40, 41) report three cases in which depancreatized bitches failed to show mammary growth in pregnancy or lactation following parturition, and one case in which a depancreatized bitch suckled two pups for a month following parturition. Chaikoff et al (10, 11) report that five out of six depancreatized bitches kept alive by special diet and insulin failed to lactate when given prolactin in much larger amounts than necessary to produce lactation in normal animals. Also one case in which a depancreatized bitch showed neither

growth nor secretory activity in the mammary glands
when she became pregnant 3 months after pancreatect-
omy. These experiments seem to point to the
necessity of the pancreas for lactation. but
no further work has been done to prove or disprove
this finding.

So far, only a few practical applications
have been made with the lactation stimulating
hormone. Catchpole et al (9) has produced lact-
ation in virgin heifers, Evans (17) has done the
same with virgin goats. Asdell (5) was able to
prevent the normal decline of milk production
in goats for a short time.

The only work that has been done on
human subjects was by Kurzook et al (35) A
series of 37 maternity cases, most of which
showed an inadequate milk supply on the 5th.
or 6th. day after parturition, and in the clinical
opinion of the obstetrical staff would not im-
prove in their supply, were given 50 to 200 units
of prolactin made as described by Riddle (56) in
single or repeated doses. Most of the cases
showed a gain of from 50 to 400 gm. of milk per
day. The failures were easily accounted for on the

basis of insufficient breast tissue; injections given too soon following delivery; or subjects which were already producing the maximum amount. The provisional dosage as shown by their findings is 150 units followed by 100 units in from 12 to 24 hours.

CONCLUSIONS

The mammary gland develops its duct system under the influence of the female sex hormone, theelin or oestrin. A part of this development may be accomplished before or during puberty. In some animals slight lobule formation may occur also as the result of oestrin stimulation at or following puberty. The completion of the duct system, and the lobule formation during pregnancy is due to increased amounts of oestrin secreted by the placenta, and also in some animals supplemented by the secretion of progestin from the corpus luteum. Prolactin, the hormone secreted by the anterior pituitary which is necessary for the initiation and continuation of lactation, is prevented from forming or prevented from acting by the oestrin, but on removing the placenta the oestrin level of the blood falls, and lactation occurs, to continue for a variable length of time if the breast is emptied, but stops if it is not, either because the distention causes involution or because the lack of stimulation of the nipples fails to stimulate reflexly the release of prolactin.

Injections of prolactin cause a refractory state to be created in the gland against the action of prolactin.

The pancreas may be necessary for the development and functioning of the mammary gland.

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