Resumen por el autor, Naohide Yatsu.

Sobre los cambios de los órganos reproductores en la parabiosis heterosexual de ratas albinas.

En las parabiosis de macho y hembra (individuos unidos mediante una operación quirúrgica), algunos de los folículos de Graaf se desarrollan normalmente y forman cuerpos amarillos, mientras que la mayoría experimenta cambios regresivos. En estas parabiosis el útero no se modifica de modo marcado, aun cuando puede presentarse una hiperplasia de la subserosa. Los folículos ováricos de las parabiosis de macho y hembra castrado no se desarrollan normalmente, ni tampoco se forman cuerpos amarillos. Los quistes folículos y los cuerpos atrécticos son abundantes. Hay un aumento aparente de las células intersticiales, entre las cuales existen unas cuantas células luteínicas. El útero es el órgano más afectado mediante la unión con un macho castrado. Especialmente clara es la producción de hidrometrias. El testículo y la próstata no son afectados por la unión con hembras normales o estériles.

Translation by José F. Nonides
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ON THE CHANGES IN THE REPRODUCTIVE ORGANS
IN HETEROSEXUAL PARABIOSIS OF
ALBINO RATS

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SEVEN FIGURES

The actions of products from various endocrine organs have
been studied of late so extensively that one can hardly keep in
touch with the literature relating to even a single organ. Of
the methods employed for such investigations parabiosis has not
so far been used as it ought to be. And, I think, much new light
will be thrown in the future upon endocrinology by making use
of this method.

In the fall of 1916, several series of experiments in joining
together two albino rats of different sexes were begun at the
Zoological Institute of the Imperial University, with the help of
Mr. Masanosuke Takesita. The object of these experiments
was to obtain evidence regarding the effects of male and female
hormones in mixed condition upon united individuals. The
present investigation was more immediately suggested by Lillie's
paper on the theory of the free-martin which appeared in "Science"
('16). In this paper he expresses the view that in the hetero-
sexual twins of cattle the reproductive organs of the female
fetus are impaired by the influence of sexual hormones from the
male fetus, provided a blood circulation has been established

1 In carrying out the experiments described in this paper, I am indebted to the
Tokugawa Memorial Fund.

2 Mr. Takesita's death took place rather suddenly in November of 1919, just
after the operative part of the present investigation came to an end. He gradu-
ated from the Imperial University in 1916. At the time of his death he was
thirty years old. I owe a great deal to his skill and intelligence in carrying out
this study.
between the two. I thought it would be interesting to see how
the male hormones act upon the female and vice versa in adult
heterosexual parabiosis.

As to the method of parabiotic operation I am indebted not
a little to Dr. Rikurô Matsuyama. His kindly advice was in-
valuable. When I began this work he was engaged in a study
of uraemia produced by extirpating the kidneys from one compo-
nent of rat parabioses. Later, however, he directed his atten-
tion to the line which I was following. He obtained results
almost the same as mine, and published an excellent paper in
Japanese in August, 1919. Yet, since his paper is written in a
language only slightly intelligible to our confrères outside this
country and because of the existence of discrepancies in our
work and of additional observations, the publication of the present
paper may not be regarded as superfluous.

Most of the rats used for the experiments were bred in the
laboratory. Their ages, body length and body weight were
noted at the time of operation (parabiosis and gonadectomy)
and when killed. All the parabiotic pairs from which the material
was taken had been healthy. It is, therefore, almost certain
that any alteration found in the preparations is due to the
action of mixed hormones produced by united individuals.

MALE-FEMALE PARABIOSES

I have twenty-four successful cases of male-female parabioses.
The period of union ranges from 11 to 179 days. It need hardly
be mentioned that the actual parabiotic period does not cor-
respond with that of union for the blood circulation becomes
established between two animals only after at least ten days.
The youngest animal operated on was twenty-nine days old and
the oldest ninety days old. The individuals used for parabiosis
were in some cases from the same litter and in others from differ-
ent litters, but in all cases they were of the same age.
1. Changes in the ovary

The ovaries of parabiotic pairs were cut into sections and compared with those of unoperated females at corresponding ages, which had been kept separated from the males.

At the outset it might be mentioned that the time of appearance of the first corpus luteum, that is, the first ovulation comes at about the same time in the ordinary females and in the parabiotic females. Ovulation may continue for a considerable length of time, as is indicated in a case (161-day-old female, 111 days in parabiotic union) in which several apparently normal eggs at the metaphase of the second maturation mitosis were found in the oviduct. And this is also substantiated by the fact that both Morpurgo ('08) and Matsuyama '19) obtained normally developed fetuses from parabiotic females.

While the formation of the corpora lutea is taking place on the one hand, a great many graafian follicles are found undergoing regressive changes on the other. In fact, many more follicles degenerate in the ovaries of parabiotic females than in ordinary females. This I do not hesitate in attributing to the influence of the male component. Predominance of degenerating follicles makes itself evident after thirty days' union with the male. The longer the parabiotic period, the larger the number of degenerating follicles. It may also be added that the regressive changes set in more in younger follicles than older ones.

There are several modes of degeneration of the follicles. But it is important to note that none of the modes is peculiar to parabiosis. The only difference found between those in the normal ovary and in that of parabioses is in degree of change.

The first indication of degeneration of the follicle is the dissolution of the cells composing the cumulus oophorus. First the egg is set free in the follicular cavity. Then it is divided or rather fragmented into five or six cells of different sizes in the manner described by Hennuguy ('94) in the rat and by Spuler ('01) in the guinea-pig. Sometimes there are two nuclei in one cell. These 'blastomeres' disintegrate in the follicular fluid so completely that no trace of them can be found. The above changes also take place in very young follicles.
Pari passu with the dissolution of the egg goes the disintegration of the stratum granulosum. This is accomplished in two ways. The first mode is the gradual dissolution of the component cells from the inside, reducing the layer to a very thin sheet of cells, often separated from the theca interna by a narrow space. In an advanced stage, this layer is fenestrated, appearing in sections as an interrupted ring. The other means of destruction is by phagocytes that have entered the follicle from without. Whence they come I am not able to ascertain. At any rate, the phagocytes are much larger than the granulosum cells and are readily detected by ingested débris. They migrate toward the follicular cavity until they occupy a position in the fluid, as is shown in figure 1. As to the fate of these cells, I am unable to offer any suggestion. It is very probable that they also disintegrate in the follicular fluid.

As the granulosum layer disappears, the theca interna increases in thickness. Sometimes this process goes on only in a restricted area, sometimes along the entire layer. In either case the cells constituting the theca interna increase in size. These cells however, are quite different from the lutein cells. The nucleus and the cell body of the former are much smaller, more granular, and stain more deeply with haematoxylin than those of the latter.

Fig. 1 Portion of a degenerating follicle (forty-seven days of parabiotic union). P., phagocytes; G.-st., granulosum; I., theca interna; E., theca externa. × 560.
The cytoplasm of the lutein cells is more eosinophilic. In cases where the central cavity is obliterated, a corpus atreticum composed of modified theca interna cells is formed.

Side by side with the above-described atretic follicles are found those with the theca cells modified into the lutein cells. Such follicles have often a large central cavity. Matsuyama thinks that this type is peculiar to the ovaries of parabioses, but in my sections of the ovaries of old females this kind of follicle is not uncommon.

A feature that may rightly be called characteristic of the ovaries of parabiosis is the enormous growth of the interstitial cells.

Matsuyama has noticed this peculiar change also. This is met with especially in the female, which is operated upon when young; that is, at a stage in which the follicles have not yet attained their full size.

It cannot be doubted that the interstitial cells are modified theca cells. Consequently, the follicular cavity is found, in most cases, inside the mass of these cells and in some cases degenerating ova and débris of the granulosum layer, as is shown in figure 2.

The lutein cells are sometimes found among the interstitial cells. They can readily be distinguished, since the former have large nuclei and a single nucleolus in each, while the latter
have small nuclei, with scattered chromatin granules. Is the formation of lutein cells due to immigration or transformation? I think both processes may go on, judging from the fact that few mitotic figures are met with.

In conclusion it may be said that in parabiosis the male exerts a certain deteriorating effect upon a good many graafian follicles of various stages, though some of the follicles remain apparently normal and are able to discharge fecundable eggs. The injurious effect of the male hormones does not produce anything peculiar to parabiosis, but accelerates degeneration processes that would take place in normal ovaries.

2. Changes in the uterus

The changes in the uterus of the parabiotic female are usually not so marked as those in the ovary. As a matter of fact, changes, if any, are so slight that they do not at all affect the normal development of the fetuses, as was shown by the cases of Morpurgo and Matsuyama.

In some females, however, changes are noticeable after a month of parabiotic union. The hyperplasia of the stratum subserosum is the main feature. The uterine glands decrease in number. The muscular layers become thinner. The eosinophile leucocytes seem to be more abundant than in the normal uterus.

It is interesting to note that in two cases the uteri have been modified exactly like those of female + castrated male parabioses, which will be described in the next section. In the two cases the testes were smaller than in other males of parabiotic union, though spermatogenesis was going on normally.³

³ In one of the two males (139 days old when killed, 93 days of parabiotic union, body weight 160 grams, body length 182 mm.) the right testis was 0.977 gram and the left 0.967. In the other male (159 days old when killed, 106 days of parabiotic union, body weight 195 grams, body length 170 mm.) the right testis was 0.338 gram and the left 0.310 gram. In comparison it may be stated that in the males of similar age and of similar length of parabiotic period the testes weigh between 1.100 and 1.200 grams.
FEMALE + CASTRATED MALE PARABIOSES

Males were castrated, and after various intervals they were united with females. I have fourteen successful cases of this kind of parabioses. The period of union ranges from 18 to 179 days.

Contrary to what one might expect, the changes in the ovary and the uterus are more marked in these cases than in the above-described female + uncastrated male parabioses.

1. Changes in the ovary

The ovary is so affected by the influence of the castrated male that it ceases to discharge the eggs, judging from the fact that no normal corpora lutea are formed and what look like them are nothing more than the corpora atretica. All the follicles undergo regressive changes of one kind or another. The processes of degeneration are different in follicles of different stages of development. They are the same as those described by Böshagen ('04), Benthin ('11), Cohn ('09), and others. It may here be mentioned that none of the regressive changes is peculiar to this kind of parabiosis, all being met with in the normal ovary, as is the case with male-female parabioses. But all the modifications come in intensified form.

Of several modes of change of the follicles, I may mention first of all a most striking one, which I would not hesitate to regard as characteristic of this type of parabiosis. This is cyst formation due to enormous growth of the stratum granulosum. One example is shown in figure 3. Here one sees uneven growth of this layer. As the follicular fluid accumulates the follicle is distended, reducing the wall to extreme thinness. Sometimes the blood-vessels make their way into the cumulus oophorus. The egg in the follicular cysts usually undergoes degeneration without fragmenting. It may occasionally divide, but as far as I know it does not act as in younger follicles.

Harms ('11) has described a case of this kind of parabiosis in Rana temporaria, but I am not able to learn from his paper how the female organs were affected by the castrated male.
This remarkable growth of the glanulosum layer takes place not long after parabiotic operation. Indeed, I have a case in which the ovary showed this change only eighteen days after union.

At one portion of the follicular cyst one often sees an accumulation of the lutein cells formed from the theca interna. I have no strong evidence to oppose the view that this represents a stage on the way to formation of a corpus atreticum. But I am rather inclined to believe that any follicle once distended on the way to become cystic, will remain as such and never transform into the solid corpus atreticum. As a matter of fact lutein cells are found in the wall of half-grown follicles. This is represented in figure 4. Here the lutein cells are formed not only from the theca interna directly, but also from the interstitial cells. After the disintegration of the granulosum cells of such follicles they turn into solid corpora atreticae though sometimes a small cavity appears in them.

Other changes, such as unusual growth of the interstitial cells, are exactly the same as in male-female parabioses.

2. Changes in the uterus

The uterus, of all the female organs I have examined, is the most affected by the union with a castrated male. The striking change makes itself evident as early as eighteen days after parabiotic operation. The normal structure of the uterus will not here be described, since that has been so fully studied by Po-
wierza ('12) in the mouse and by Beiling ('06) in the rat. The first indication of the change is the rapid growth of the mucous layer. This is soon followed by the accumulation of turbid fluid in the uterine cavity. The subserosa or vascular layer, with not a few eosinophils, becomes thinner. The uterine glands may remain for some time compressed within the now narrow subserosa. Finally they disappear completely. The longitudinal muscles are no longer found. The diameter of this distended uterus is 6 mm., while that of the normal one is not more than 1½ mm. The wall is reduced to one-tenth the normal thickness. This hydrometral condition is not accompanied by hydrosalpinx, which Fischel obtained experimentally ('14). No noticeable changes take place in the tubal part.

Fig. 4 Part of a follicle showing invasion of lutein cells into st. granulosum (G.) from interstitial cells (In.) (107 days of parabiotic union). I., theca interna; E., theca externa. × 186.
The testes of male-female parabioses (twenty-four cases from 11 to 179 days) and of male+spayed female parabioses (eleven cases from 6 to 148 days) were weighed and cut into sections.

It is interesting to note that the histologic structure of the testes is not affected at all by the union with either unoperated

Fig. 5 Section of normal (A) and distended uterus (B). $\times 4.5$.
Fig. 6 Portion of distended uterus (eighteen days of parabiotic union). Notice the remnant of longitudinal muscle fibers. $\times 420$.
Fig. 7 Portion of half-distended uterus (twenty-three days of parabiotic union). Here one notices a uterine gland to the right. $\times 97$. 
or spayed female. In the latter combination, however, the sperm formation is somewhat delayed. In one case the spermatozoa could not be seen in a ninety-eight day rat joined for thirty-two days with a spayed female.

RESULTS

That the spermatozoa are functional in male-female parabioses is shown by the fact that a female which had been kept with a male-female parabioses gave birth to a litter. Copulation took place in this case 134 to 135 days after parabiotic operation.

In passing it may be mentioned that the prostate is not affected at all by the union with either normal or spayed female. In castrated male-female parabioses the prostate atrophies as in the solitary castrated male.

1. In male-female parabioses some graafian follicles undergo the normal course of growth and the corpora lutea are formed, while a large majority of follicles undergo regressive changes. None of the changes is peculiar to this kind of parabiosis.

2. In male-female parabioses the uterus is not modified very markedly. Sometimes hyperplasia of the subserosa is noticed.

3. In the ovary of castrated male-female parabioses none of the follicles develops normally. No corpora lutea are formed. Follicular cysts and corpora atretica are abundantly produced. Noticeable growth of the interstitial cells takes place and sometimes the lutein cells are met with in the interstitial cell groups.

4. The uterus is most affected by the union with a castrated male. Hydrometra of various grades is the remarkable feature. The uterine tubes are normal.

5. The testis is not affected at all by the union with either normal or spayed females. The same is true of the prostate.

COMMENT

From the above-described parabiotic experiments rather unexpected results were obtained. One would naturally suppose that the female organs would be more affected by the male with the testes. But as a matter of fact they are more influenced
by the castrated male. To account for this phenomenon I think it is very probable that the endocrine organs of the male are affected by castration, and that the ovary and uterus of united individuals are in turn influenced by the hormone or hormones produced from these organs. But what organ partakes in this process and how I do not know.

It is also interesting to note that the testis is not impaired in the least by the ovary of the female to which it is united.

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