A Theoretical Approach to the Regulatory Mechanism in the Brain-Hypophyseal-Gonadal System with Reference to its Mathematical Modeling and Computer Simulation*

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Synopsis

A theory to understand the total performance of complicated reproductive functions in mammals and to explain its integrative mechanism is presented with special reference to its mathematical formulation and digital computer simulation. Functional connections of components and subcomponents in the brain-hypophyseal-gonadal system are described through evaluation of experimentally established facts and our own hypotheses. Input-output relations of informations carried by neural spikes, 3 kinds of neurohormones and 3-5 kinds of hypophyseal and gonadal hormones are expressed in graphical, literal and mathematical terms. Sexual and species differences in both function and structure are also dealt with. The brain of both sexes is assumed to contain 3 different functional subcomponents such as neural, neurosecretory and store mechanisms. The neural mechanism is composed of detectors for photoperiodicity, copulatory stimulation and blood levels of hormones, and neurons which send either stimulatory or inhibitory signals to regulate the production and release of neurohormones. It is also assumed that each hormone can exert a positive feedback effect on the brain at first for a short period activating the stimulatory neural mechanism to be concerned, and then a negative effect for a longer period through the inhibitory mechanism. If the threshold, time constant and output level of each detector and neuron are adequately determined in response to neural or hormonal inputs, it is suggested that the secretory pattern of gonadotropins and sex hormones may be cyclic as in most female species and superficially non-cyclic as in the male. On the basis of such assumptions, a general principle for the regulatory mechanism is theoretically synthesized. This is exemplified by chronological changes in the dynamic state of the 4-day estrous cycle in the rat. This theory seems to be well evidenced by a result of numerical analyses by computer, showing a considerably good accordance with the performance of the real system.

Causal analyses of hormone actions or secretory functions as a local phenomenon (unit processes) have been extensively carried out by means of modern experimental techniques. However, at the present time, it appears considerably difficult to know how an endo-

crine activity is regulated in the organism as a whole. In current endocrinology, there seems to exist a tendency that much attention is paid to quantify unit processes exquisitely. Most workers in this field are relatively indifferent to draw a total figure of hormonal integration. Since hormones are secreted under mutual relationships among a number of components via multiple feedback loops and transmit informations essential for the harmonic maintenance of the body, an emphasis should be laid upon the necessity of endocrinology of regulatory mechanisms.

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Fig. 1. Schema of procedures in our systems approach to the regulatory mechanism.

However, too little thing is known to synthesize the total figure of integration although we have plenty of accumulated analytical data on unit processes obtained by endocrinological and physiological methods. Consequently it becomes necessary to supplement the lack of knowledge theoretically. In this respect a systems control approach might be most useful at present. Figure 1 diagrammatically shows our procedures in both theoretical and experimental researches. As to the validity and limitation of such an attempt, Caspari and Horvath (1970) state as follows: "If a system has as many as three or more control loops, it is literally impossible to predict its performance by trying to isolate each component and

separately determine its effect on the system. Some form of modeling which can simulate all known or hypothesized interactions is necessary in order to describe total systems performance. Because of the incomplete state of knowledge of this aspect of biology, all models derived in this manner should be regarded as highly tentative and subject to further testing and verification."

In this article we deal with a systems control approach to the mammalian brain-hypophyseal-gonadal system which is one of the most complex systems in the living organism. This system regulates the reproductive function through three different types of information carriers, such as nerve impulse signals, neuro-

hormones and hormones. The structure and function of the system is much more complicated than similar regulatory systems like the brain-hypophyseal-thyroidal system and the brain-hypophyseal-adrenocortical system. The gonad is different in its structure and secretion between the male and the female. The brain itself also undergoes sexual differentiation at an early stage of life (Harris, 1964). Hence the brain-hypophyseal-gonadal system behaves in quite a different way between the two sexes at maturity, i.e. cyclic activity in the female exhibiting estrous or menstrual cycles, and superficially non-cyclic activity in the male. In addition, activity patterns of the system change in the course of life, i.e. sexual differentiation, puberty, sexual maturation and senescence. Various kinds of externally expressed sexual behavior (estrus, copulation, pregnancy, parturition, lactation, etc.) are modified by internal physiological states of the system. Moreover, components and information carriers associated with the reproductive function are so many as shown in Figure 2 and Table 1. The systems control approach is now the most effective tool to understand such a complicated regulatory system dynamically.

Although in the present study our attention is mainly focused on the dynamics of 4-day estrous cycles in the female rat, we try to find

a general principle for the integration of reproduction in both male and female mammals, which can explain all kinds of known phenomena, on the basis of revisions of our previous papers (Inoué *et al.*, 1969a, b; Nakamura *et al.*, 1969; Sekiguchi *et al.*, 1969; Inoué, 1970). Similar but theoretically different attempts were also reported by several investigators (Thompson, 1966; Thompson *et al.*, 1969; Schwartz and Hoffmann, 1967; Schwartz, 1969a, b; Horrobin, 1969).

The Total System

The total system concerned with the regulation of reproductive functions is composed of the following three types of components:

- (1) Information-processing components such as the brain, the hypophysis, the gonad and the reproductive organs.
- (2) Information-transmitting components such as the hypophyseal portal system, the general circulation system and some sensory pathways.
- (3) Information-eliminating components such as the submandibular gland, the liver and the kidney.

Besides these, there are several associated components such as the thyroid, the adrenal and the posterior hypophysis, which may exert

Table 1. The production site, name and abbreviation of neurohormones and hormones concerned with the brain-hypophyseal-gonadal system*

Type	Production site	Abbreviation	Name
Neurohormone	Hypothalamus	FSH-RF	FSH-releasing factor
		LH-RF	LH-releasing factor
		PIF	Prolactin inhibiting factor
Hormone	Hypophysis	FSH	Follicle-stimulating hormone
		LH	Luteinizing hormone
		LTH	Luteotropic hormone or prolactin
	Gonad		
	Ovary	EH	Estrogen
	Testis	PH	Progesterin
		AH	Androgen

* Gonadotropin(s)

Sex hormones

* Placenta is excluded here.

minor effects on the regulatory function. The placenta plays an important role in case of pregnancy. They are, however, not dealt with in this simplified research.

The functional connections of the above components are shown in Figure 2. The direction of information flows is indicated by arrows. Broader lines mean that not only information flows but also transfer of substances *per se* is relatively important. Carriers of humoral informations related to this system are shown in Table 1. As to disturbances from the environment, we assume that all external conditions are kept constant. Consequently, only photoperiodicities and copulatory stimuli which may affect the gonadotropin secretion are dealt with here. State values chosen from the total system to describe mathematically, are summarized in Table 2.

Brain

The brain is the most complicated component in this regulatory system. The hypothalamus plays an essential role in the control of reproductive functions. Especially the medial basal hypothalamus is required and named as the hypophysiotropic area (Halász, 1969). The other brain regions such as the epiphysis and the limbic system including the hippocampus, the habenular nuclei and the amygdalar complex are known to participate in the regulation of the hypothalamus. Since

anatomical connections in the brain are not yet fully understood, we divided the brain into the three different subcomponents merely according to its function, i.e. the neural, the neurosecretory and the store mechanisms. The neural mechanism detects and processes both hormonal and neural signals and sends messages to the other two intracerebral mechanisms by means of nerve spikes. The neurosecretory mechanism receives neural inputs from the neural mechanism and produces neurohormones, which are transported to the store mechanism. Functional connections in detail are assumed as exhibited in Figure 3.

The neural mechanism contains detectors for all hormones shown in Table 1, and for sensory inputs coming from peripheries. Photoperiodicities and copulatory stimuli have a great importance especially in the adult female. In the case of sexually mature female rats, if the light-darkness rhythms are kept constant, a kind of biological clock concerned with LH-RF release (BC in Figure 3) is triggered to work 9 hr after the initiation of illumination (Everett *et al.*, 1949; Kobayashi *et al.*, 1968). Recent studies by Kawakami *et al.* (1970) demonstrated that considerably wide regions of the basal hypothalamus and the limbic brain may be related to the clock mechanism. Thus light exerts a trigger effect on the brain-hypophyseal axis, but changes in

Fig. 2. Functional connections of components concerned with the brain-hypophyseal-gonadal system. Arrows indicate the direction of information flows. Broader lines mean that the transfer of information-carrying substances is important.

Table 2. The symbol and definition of state values extracted from the brain-hypophyseal-gonadal system

Symbol	Definition* (Unit)
F, L, H, A, E, P, F _N , L _N and H _N	Amount of secreted FSH, LH, LTH, AH, EH, PH, FSH-RF, LH-RF and PIF, respectively, in the production site ($\mu\text{g}/\text{unit time}$)
N _F , N _L and N _H	Intensity of neural stimuli from the neural mechanism in the brain to cause the synthesis of FSH-RF, LH-RF and PIF, respectively, in the neurosecretory mechanism (1/unit time)
N _f , N _l and N _h	Intensity of neural stimuli from the neural mechanism in the brain to cause the release of FSH-RF, LH-RF and PIF, respectively, from the store mechanism (1/unit time)
N _b	Intensity of neural stimuli from the intracerebral biological clock to cause the transient release of LH-RF from the store mechanism (1/unit time)
f _M , l _M and h _M	Concentration of FSH-RF, LH-RF and PIF, respectively, in the store mechanism ($\mu\text{g}/\text{ml plasma}$)
G _F , G _L and G _H	Conductance of the boundary between the store mechanism and the descending hypophyseal portal system related to the release of FSH-RF, LH-RF and PIF, respectively (1/unit time)
f _R , l _R and h _R	Concentration of FSH-RF, LH-RF and PIF, respectively, in the descending hypophyseal portal system ($\mu\text{g}/\text{ml plasma}$)
f _P , l _P and h _P	Concentration of FSH, LH and LTH, respectively, in the ascending hypophyseal portal system ($\mu\text{g}/\text{ml plasma}$)
α	Ratio, in which hypophyseal outputs enter into the general circulation system
V _{HD} , V _{HA} , V _G and V _M	Volume of the descending and ascending hypophyseal portal system, the general circulation system and the store mechanism, respectively (ml or cm ³)
f _r , l _r , h _r , f, l, h, a, e and p	Concentration of FSH-RF, LH-RF, PIF, FSH, LH, LTH, AH, EH and PH, respectively, in the general circulation system ($\mu\text{g}/\text{ml plasma}$)
U, V and W	Grade exhibiting the state of follicular growth, ovulation and luteinization, respectively
Y and Z	Epithelial thickness (μ) and smear pattern, respectively, in the vagina

* Abbreviations, see Table 1.

photoperiodicity do not immediately alter the state of the total system. Therefore photic inputs are, although necessary, not a leading factor to control the system. Contrarily, copulation causes a definite change in most female animals at a certain stage of sex cycles, usually bringing about the state of pregnancy and then lactation in which the performance of the total system is entirely different from the previous state.

We propose that there are both stimulatory and inhibitory neurons (designated as N^s and Nⁱ, respectively, in Figure 3), which control the production and release of each neuro-

hormone. Another important assumption is that the sexual difference in the regulatory function of the brain may not be derived from structural differences, the same neural circuits existing in both sexes. We assume that the sensitivity, working capacity and/or time constant of the neural mechanism may differentially develop between the male and the female, and also between the spontaneous ovulator with or without functionally maintained corpora lutea and the reflex ovulator in mammalian species.

In our mathematical model, neural activities in each route in Figure 3 are not con-

Fig. 3. Functional connections in the brain. Informations are transmitted from the left to the right. White and black spots indicate excitatory and inhibitory "synaptic" junctions, respectively. Abbreviations are as follows.

BC: Intracerebral biological clock

C: Detector for copulatory stimulation

D with suffix: Detector for each hormone written in the left

N with suffix: Neurons regulating the production and release of neurohormones. s and i indicate stimulatory and inhibitory, respectively. F, L and H show relations to FSH-RF, LH-RF and PIF, respectively.

NS with suffix: Neurosecretory cells. Each suffix indicates the same relation as N.

S with suffix: Store mechanism. Each suffix shows the same relation as N.

sidered, and both stimulatory and inhibitory inputs to NS_F , for example, are concentrated as N_F . Since activities defined as N_F are modified by feedback effects of blood levels of androgen, estrogen, progesterin, FSH and FSH-RF, N_F can be expressed as a function of these state values. Here is a possibility that N_F is affected by the summation of effects of each hormone, hence:

$$N_F = K_{Fa}U_{-1}(T_{Fa}-a) \cdot (N_{Fa}-a) \\ + K_{Fe}U_{-1}(T_{Fe}-e) \cdot (N_{Fe}-e) \\ + K_{Fp}U_{-1}(T_{Fp}-p) \cdot (N_{Fp}-p)$$

$$+ K_{Ff}U_{-1}(T_{Ff}-f) \cdot (N_{Ff}-f) \\ + K_{Ffp}U_{-1}(T_{Ffp}-fp) \cdot (N_{Ffp}-fp) \\ \dots\dots\dots(1)$$

where U_{-1} means the symbol of unit step function, T and N with suffix located in parentheses the threshold level to each hormone (for example, T_{Fa} is the threshold level to androgen) and the spontaneous activity of neurons (for example, N_{Fa} is the spontaneous activity of androgen-sensitive neurons), respectively, and K with suffix constants. All values are assumed non-negative. T, N and

K with suffix may be functions of sexuality, physiological state, age and species.

On the other hand, there are also possibilities that N_F is affected by the product of all terms in equation (1) or the summation of products of some terms. The latter possibility is most likely, so far as results of our computer analyses are concerned.

N_F , inputs to the store mechanism S_F , is formulated in the same way. N_L and N_I , to which photic and copulatory stimuli are added, as well as N_H and N_h can be treated similarly but omitted here.

The neurosecretory mechanism, composed of neurosecretory cells in the hypothalamus, is mathematically described as follows.

$$\frac{d}{dt} \begin{pmatrix} F_N \\ L_N \\ H_N \end{pmatrix} = - \begin{pmatrix} \tau_{FN} & 0 & 0 \\ 0 & \tau_{LN} & 0 \\ 0 & 0 & \tau_{HN} \end{pmatrix} \begin{pmatrix} F_N \\ L_N \\ H_N \end{pmatrix} + \begin{pmatrix} K_{FN} & 0 & 0 \\ 0 & K_{LN} & 0 \\ 0 & 0 & K_{HN} \end{pmatrix} \begin{pmatrix} N_F \\ N_L \\ N_H \end{pmatrix} \dots\dots(2)$$

where τ and K with suffix mean the time constant and the constant, respectively, related to each neurohormone.

The location of the store mechanism lies in the median eminence of the basal hypothalamus, to which neurohormones are transported through the axons of neurosecretory neurons. On the other hand, Kobayashi (1970) has recently suggested that there is another possible route for neurohormones to reach the descending hypophyseal portal system. According to him, neurohormones are liberated into the liquor of the third ventricle, absorbed by ependymal cells located in the periventricular regions, and transported through their axons to the portal vessel. A number of synapses attached to the soma and axon of the ependymal cells may account for the neural regulation to the release of neurohormones. Consequently, both the liquor and the ependymal cells are also regarded as a member of the store mechanism.

The quantity and movability of neurohormones in the store mechanism located in the hypothalamic median eminence are formulated as in equations (3) and (4), respectively.

$$V_M \frac{d}{dt} \begin{pmatrix} f_M \\ l_M \\ h_M \end{pmatrix} = - \begin{pmatrix} G_F & 0 & 0 \\ 0 & G_L & 0 \\ 0 & 0 & G_H \end{pmatrix} \begin{pmatrix} f_M \\ l_M \\ h_M \end{pmatrix} + \begin{pmatrix} F_N \\ L_N \\ H_N \end{pmatrix} \dots\dots\dots(3),$$

$$\frac{d}{dt} \begin{pmatrix} G_F \\ G_L \\ G_H \end{pmatrix} = - \begin{pmatrix} \tau_{GF} & 0 & 0 \\ 0 & \tau_{GL} & 0 \\ 0 & 0 & \tau_{GH} \end{pmatrix} \begin{pmatrix} G_F \\ G_L \\ G_H \end{pmatrix} + \begin{pmatrix} K_{GF} & 0 & 0 & 0 \\ 0 & K_{GL} & 0 & K_{Gb} \\ 0 & 0 & K_{GH} & 0 \end{pmatrix} \begin{pmatrix} N_f \\ N_l \\ N_h \\ N_b \end{pmatrix} \dots\dots\dots(4)$$

where τ and K with suffix mean the time constant and the constant, respectively, related to each neurohormone.

As seen in state equations (2)–(4), characteristics in all responses of the brain-hypophyseal-gonadal system are assumed to be approximated tentatively by a first-order lag system.

Hypophyseal portal system

This localized vascular system combines the brain and the hypophysis, transmitting humoral informations from one to the other. The descending blood flow conveys neurohormones from the hypothalamus to the anterior hypophysis, while the ascending flow carries a part of hypophyseal hormones exerting the so-called short feedback effects on the brain (Motta *et al.*, 1969). This system has a significance to send informations in concentrated condition directly and rapidly to the neighboring target organ. Therefore the efficiency is very high. The direct effect of estrogen on the hypophysis is brought about through the descending portal stream via the general circulation system.

On the basis of the above consideration, we formulate the state of the descending and the

ascending hypophyseal portal system as equations (5) and (6), respectively.

$$V_{HD} \frac{d}{dt} \begin{pmatrix} f_R \\ l_R \\ h_R \end{pmatrix} = - \begin{pmatrix} \tau_{fR} & 0 & 0 \\ 0 & \tau_{lR} & 0 \\ 0 & 0 & \tau_{hR} \end{pmatrix} \begin{pmatrix} f_R \\ l_R \\ h_R \end{pmatrix} + \begin{pmatrix} G_F & 0 & 0 \\ 0 & G_L & 0 \\ 0 & 0 & G_H \end{pmatrix} \begin{pmatrix} f_M \\ l_M \\ h_M \end{pmatrix} \dots\dots(5),$$

$$V_{HA} \frac{d}{dt} \begin{pmatrix} f_P \\ l_P \\ h_P \end{pmatrix} = - \begin{pmatrix} \tau_{fP} & 0 & 0 \\ 0 & \tau_{lP} & 0 \\ 0 & 0 & \tau_{hP} \end{pmatrix} \begin{pmatrix} f_P \\ l_P \\ h_P \end{pmatrix} + (1-\alpha) \begin{pmatrix} F \\ L \\ H \end{pmatrix} \dots\dots\dots(6)$$

where τ with suffix means the time constant related to each neurohormone or hormone.

Hypophysis

This organ secretes three kinds of gonadotropins as shown in Table 1. We assume that not only the release but also the production of gonadotropins is regulated by the neurohormones and estrogen. FSH and LH are synthesized and released by the presence of FSH-RF and LH-RF, respectively, while the production and release of LTH are inhibited by PIF. Estrogen is known to exert dual effects on the gonadotropin secretion, acting either stimulatory or inhibitory (van Tienhoven, 1968). However, we hypothesize that in normal physiological conditions it acts only as an inhibitor so long as the direct effect on the hypophysis is concerned. Gonadotropins are transported by blood streams of the general circulation system as well as of the ascending hypophyseal portal system.

Thus, our mathematical model for the gonadotropin secretion is as follows.

$$\frac{d}{dt} \begin{pmatrix} F \\ L \\ H \end{pmatrix} = - \begin{pmatrix} \tau_F & 0 & 0 \\ 0 & \tau_L & 0 \\ 0 & 0 & \tau_H \end{pmatrix} \begin{pmatrix} F \\ L \\ H \end{pmatrix}$$

$$+ \begin{pmatrix} K_{FF} & 0 & 0 & -K_{Fe} \\ 0 & K_{LI} & 0 & -K_{Le} \\ 0 & 0 & K_{Hh} & -K_{He} \end{pmatrix} \begin{pmatrix} f_R \\ l_R \\ h_R \\ e \end{pmatrix} \dots\dots\dots(7)$$

where τ and K with suffix mean the time constant and the constant, respectively, related to each hormone and neurohormone.

General circulation system

This vascular system collects hormones from the glands and transport them to separately locating target organs. As blood vessels belonging to this system are distributed throughout the body, hormones can reach all bodily places equally as well as almost simultaneously. Thus, circuits for humoral informations are connected in parallel, forming a striking contrast to those for neural informations in series connection. As to concentrations of gonadal and hypophyseal hormones as well as hypothalamic neurohormones, the following equations (8), (9) and (10), respectively, are formulated.

$$V_G \frac{d}{dt} \begin{pmatrix} a \\ e \\ p \end{pmatrix} = - \begin{pmatrix} \tau_a & 0 & 0 \\ 0 & \tau_e & 0 \\ 0 & 0 & \tau_p \end{pmatrix} \begin{pmatrix} a \\ e \\ p \end{pmatrix} + \begin{pmatrix} A \\ E \\ P \end{pmatrix} \dots\dots\dots(8),$$

$$V_G \frac{d}{dt} \begin{pmatrix} f \\ l \\ h \end{pmatrix} = - \begin{pmatrix} \tau_f & 0 & 0 \\ 0 & \tau_l & 0 \\ 0 & 0 & \tau_h \end{pmatrix} \begin{pmatrix} f \\ l \\ h \end{pmatrix} + \alpha \begin{pmatrix} F \\ L \\ H \end{pmatrix} + \begin{pmatrix} \tau_{fP} & 0 & 0 \\ 0 & \tau_{lP} & 0 \\ 0 & 0 & \tau_{hP} \end{pmatrix} \begin{pmatrix} f_P \\ l_P \\ h_P \end{pmatrix} \dots\dots\dots(9),$$

$$V_G \frac{d}{dt} \begin{pmatrix} f_r \\ l_r \\ h_r \end{pmatrix} = - \begin{pmatrix} \tau_{fr} & 0 & 0 \\ 0 & \tau_{lr} & 0 \\ 0 & 0 & \tau_{hr} \end{pmatrix} \begin{pmatrix} f_r \\ l_r \\ h_r \end{pmatrix} + \begin{pmatrix} \tau_{fR} & 0 & 0 \\ 0 & \tau_{lR} & 0 \\ 0 & 0 & \tau_{hR} \end{pmatrix} \begin{pmatrix} f_R \\ l_R \\ h_R \end{pmatrix} \dots\dots\dots(10)$$

where τ with suffix means the time constant related to each hormone or neurohormone.

Gonad

As already mentioned, there are sexual differences in both structure and secretion of the gonad. Sex hormones secreted by the gonad are shown in Table 1.

Patterns of the production and release of ovarian hormones differ largely according to stages of the life history and of the sex cycle, being closely related to the ovarian structure. We suppose here that during the course of follicular growth the ovary secretes mainly estrogen under the stimulatory influence of mainly FSH, that at the time of follicular maturation both FSH and LH provoke ovulation, and that LH not only induces the successive luteinization of follicles but also alters the secretory state of the ovary from the estrogen phase to the progestin phase (*vide infra*). If relatively large amounts of LTH are involved in the luteinization process, as occurring spontaneously in some species such as women and guinea-pigs or induced by the copulatory stimulation, corpora lutea are activated for a certain period, secreting progestin in a high amount. Otherwise, as in case of the rat, relatively small amounts of LTH can hardly activate corpora lutea. Taking such facts and assumptions into consideration, the interrelationship between the function and the structure of the ovary is expressed as the following non-linear differential equations (11) and (12).

$$\begin{aligned} \frac{d}{dt} \begin{pmatrix} E \\ P \end{pmatrix} = - \begin{pmatrix} \tau_E & 0 \\ 0 & \tau_P \end{pmatrix} \begin{pmatrix} E \\ P \end{pmatrix} + \begin{pmatrix} 0 & K_{E'1} & 0 \\ 0 & K_{E1} & 0 \end{pmatrix} \begin{pmatrix} f \\ 1 \\ h \end{pmatrix} \\ + \begin{pmatrix} K_{E'f}U & K_{E1}U - K_{E1}'e & 0 \\ 0 & K_{P1}W & K_{Ph}W \end{pmatrix} \begin{pmatrix} f \\ 1 \\ h \end{pmatrix} \end{aligned} \quad \dots\dots\dots(11),$$

$$\frac{d}{dt} \begin{pmatrix} U \\ V \\ W \end{pmatrix} = - \begin{pmatrix} \tau_U & 0 & 0 \\ 0 & \tau_V & 0 \\ 0 & 0 & \tau_W \end{pmatrix} \begin{pmatrix} U \\ V \\ W \end{pmatrix}$$

$$\begin{aligned} + \begin{pmatrix} K_{Uf} & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & K_{Wh} \end{pmatrix} \begin{pmatrix} f \\ 1 \\ h \end{pmatrix} \\ + \begin{pmatrix} 0 & -K_{U1}V & -K_{Uh}W \\ 0 & K_{V1}U & -K_{Vh}W \\ -K_{Wf}U & -K_{W1}V & 0 \end{pmatrix} \begin{pmatrix} f \\ 1 \\ h \end{pmatrix} \end{aligned} \quad \dots\dots\dots(12)$$

where $0 \leq U \leq 1$, $0 \leq V \leq 1$, $0 \leq W \leq 1$,
 $0 \leq U + V + W \leq \text{Constant}$,

and τ and K with suffix mean the time constant and the constant, respectively, related to each hormone and/or ovarian structure.

The male gonad, testis, secretes androgen under stimulatory influences of FSH and LH. Differential effects of these hormones are not so clear as in the ovary, but LH is believed to stimulate the interstitial cells of the testis. The role of LTH in the testicular regulation is uncertain. Structural changes in the testis are not so dynamic and striking as in the ovary, so far as the induced effect by gonadotropins is concerned. Hence, we formulate the androgen secretion simply as equation (13).

$$\frac{d}{dt} A = -\tau_A A + K_{Af}f + K_{A1}1 \dots\dots\dots(13)$$

where τ and K with suffix mean the time constant and the constant, respectively, related to each hormone.

Reproductive organs

Important reproductive organs are the genital tract composed of the uterus and the vagina in the female, and the prostate and the seminal vesicle in the male. They react sensitively to sex hormones, and change their structure and function. Most of the reproductive organs are merely the target of gonadal hormones and hence the indicator of a state of the total system. Luminal contents of the vagina change with a close relation to blood level of ovarian hormones, although there is a considerably long time lag. Consequently, patterns of vaginal smears, es-

pecially in small laboratory rodents, are an excellent indicator to know the internal activities of the regulatory system, without giving disturbances or injuries. In addition, the utero-vaginal junction (vaginal cervix) is important as a sensory receptor for copulatory stimuli, which are transmitted to the brain via nervous pathways and alter the performance of the system. In the state of pregnancy, the placenta develops in the uterus after nidation. This acts also as an endocrine organ which participates in the regulation of the system.

Here we try to describe the vagina in mathematical term, assuming that the thickness of the epithelium (Y) is a linear function of concentrations of estrogen and progesterin, and that smear patterns (Z) based on the classical study of Long and Evans (1922) are expressed in quantized values.

$$\frac{d}{dt} \begin{pmatrix} Y \\ Z \end{pmatrix} = - \begin{pmatrix} \tau_Y & \tau_{YZ} \\ \tau_{ZY} & \tau_Z \end{pmatrix} \begin{pmatrix} Y \\ Z \end{pmatrix} + \begin{pmatrix} K_{Ye} & K_{Yp} \\ K_{Ze} & K_{Zp} \end{pmatrix} \begin{pmatrix} e \\ p \end{pmatrix} \quad \dots\dots\dots(14)$$

where τ and K with suffix mean the time constant and the constant, respectively, related to each structure and hormone.

Experimental analysis of dynamic characteristics of the uterus and its mathematical description together with computer simulations are reported elsewhere (Inoue and Sekiguchi, 1970a, b).

Submandibular gland, liver and kidney

Little is known as to the fate of hormones. Since each component in the regulatory system is dynamically maintained through quantitative information changes in circulating hormones, knowledge on effective amounts of hormones in the blood and on clearance rate is urgently necessary.

At present, gonadotropins are known to be inactivated in the submandibular gland (Tojo *et al.*, 1969) and gonadal hormones are inactivated in the liver. All hormones and neurohormones are excreted into the urine through the kidney. The half-life of exogenously ad-

ministered hormones seems to be approximately 30 min in LH, 150 min in FSH (Bogdanove and Gay, 1969) and 10 min in androgen (testosterone) and estrogen (estradiol) (Ulrich and Kent, 1968). In our mathematical model, amounts to be inactivated in the tissue or excreted from the body are assumed to be proportional to the concentrations in the general circulation system as described in equations (8)–(10).

The equations (1)–(14) except for (13) are summarized in Figure 5 in the form of a block diagram. This indicates the total regulatory system for the female. Here the neural mechanism is treated as a black box. Vectors and matrices are expressed as vector values which are represented by a symbol of the first element, for example;

$$\mathbf{f} = \begin{pmatrix} f \\ l \\ h \end{pmatrix}, \quad \boldsymbol{\tau}_e = \begin{pmatrix} \tau_e & 0 \\ 0 & \tau_p \end{pmatrix}.$$

The principle of regulation

The regulatory mechanism involved in the brain-hypophyseal-gonadal system is theoretically synthesized here. We exemplify the case of the normal female rat with 4-day estrous cycles under constant environmental conditions, and refer briefly to sexual and species differences as well as other physiological states, such as pregnancy, lactation and gonadectomy. Changes in the developmental course will not be dealt with here.

As shown in Figure 5, an estrous cycle consists of 2 days' diestrus (D_1 and D_2), 1 day's proestrus (P) and 1 day's estrus (E), although each duration does not strictly accord with 24 hours. A diurnal photoperiodicity is supposed to be an alternation of 12-hr illumination (8:00–20:00) with 12-hr darkness (shown black in Figure 5).

Recent studies by Miyake and his associates demonstrated that estrogen (Hori *et al.*, 1968) and progesterone (Uchida *et al.*, 1969) are secreted alternately. Hence we call the predomi-

Fig. 4. Block diagram of the total system in the female. The definition of symbols, see Table 2 and text. s means the Laplace operator. Vectors or matrices of state values or constants in the text are shown as vector values represented by the first element.

Fig. 5. Chronological changes in information quantity during the 4-day estrous cycle of the rat. Explanations, see text.

nant stage of estrogen the EH phase and that of progesterone the PH phase (Figure 5). In the ovary, the follicular growth takes place during the former, while during the latter the follicular maturation, ovulation and luteinization occur successively.

Then what can bring about such secretory activities in the ovary? As shown in Figure 2, multiple feedback loops determine the state of the system. Consequently, there is no leading actor playing an absolutely decisive role in the regulatory performance of the system. Therefore, any state of an estrous cycle may be destined by the preceding state and accompany the next state. Now let us begin with a state of the neural mechanism in the brain at the beginning of the EH phase, looking at Figures 3 and 5.

The neural mechanism activating LH-RF production (N_L^s) is closely related to the intracerebral biological clock (BC). This

clock sends daily a stimulatory information of less than 20 min's duration to N_L^s regularly 9 hr after the initiation of illumination. In other words, it has a 9-hr latency to the onset of photic stimulation and generates a pulse-like output at a 24-hr interval. This pulse becomes an input indirectly to the neurosecretory mechanism concerned with LH-RF (NS_L) via N_L^s , and directly to the LH-RF store mechanism (S_L). Hence the production and release of LH-RF increase in proportion to the input. We assume that there is also the same clock in the male, for cyclic gonadotropin secretion is suggested in male rats (Inoue, 1964, 1965a; Kihlström, 1966). However, its output is supposed not so effective as in the female because of much lower level.

On the other hand, estrogen secreted by the ovary (EH) reaches the detector in the neural mechanism (D_e) and modulates activities of both N_L^s and N_L^i (the neural mechanism inhibiting LH-RF production). If we define the threshold and the time constant for EH of N_L^s and N_L^i as T_{Le}^s and T_{Le}^i , and τ_{Le}^s and τ_{Le}^i , respectively, the following inequalities may be written.

$$T_{Le}^s < T_{Le}^i, \tau_{Le}^s < \tau_{Le}^i \quad \dots\dots\dots (15).$$

Consequently, only a positive feedback effect of estrogen appears at first, causing more elevated production and release of LH-RF. As a result, released LH-RF is transported to the hypophysis through the descending hypophyseal portal system, and induces LH secretion. LH can also exert a stimulatory effect at first on the neural mechanism and then an inhibitory effect later, known as the short feedback action. Hence the following inequalities may be formulated similarly.

$$T_{Li}^s < T_{Li}^i, \tau_{Li}^s < \tau_{Li}^i \quad \dots\dots\dots (16).$$

Combined effects of BC, EH and LH on the neural mechanism increase inputs to NS_L and S_L gradually up to the onset of the PH phase as illustrated in Figure 5. When BC is triggered on the afternoon of proestrus (P), which

corresponds with the so-called critical period, the excitability of N_L^s is sharply raised to the maximum, exceeding the threshold level (T_L^s). to cause the LH surge from the hypophysis. Then a large amount of LH-RF is transferred to the hypophysis.

Meanwhile, a rise in EH level during the EH phase similarly provokes a positive feedback effect on the brain, activating the $D_e \rightarrow N_F^s \rightarrow NS_F - S_F$ route at first. Consequently, FSH-RF and then FSH are released more and more, which in turn induce more EH secretion. FSH is also assumed to activate FSH-RF production and release through the short feedback pathway prior to a negative influence. As a result, when increased EH exceeds a certain level, the $D_e \rightarrow N_F^i \rightarrow NS_F - S_F$ route is activated, bringing about decrease in FSH-RF and then FSH as well as EH (negative feedback effects). Hence relations in the threshold and the time constant between the stimulatory and the inhibitory routes may be expressed as inequalities (17).

$$T_{Fe}^s < T_{Fe}^i, \tau_{Fe}^s < \tau_{Fe}^i \quad \dots\dots\dots(17).$$

The same relations may be applicable to each effect of FSH and PH, although the latter is almost negligible at the EH phase. Combined effects change chronologically, as shown in Figure 5.

As to the neural mechanism concerned with PIF, activities of the $D_e \rightarrow N_H^s$ or N_H^i route may be very low, manifesting the change as illustrated in Figure 5. Here the same relations, as in the case of inequalities (15)–(17), may exist:

$$T_{He}^s < T_{He}^i, \tau_{He}^s < \tau_{He}^i \quad \dots\dots\dots(18).$$

The effect of LTH and PH at the EH phase seems negligible. We hypothesize that the effects of estrogen on the neural mechanism as expressed in inequalities (15), (17) and (18) may be mutually related in the following manner.

$$\left. \begin{array}{l} T_{Fe}^s < T_{Le}^s < T_{He}^s; T_{Fe}^i < T_{He}^i \leq T_{Le}^i \\ \tau_{Fe}^s < \tau_{He}^s \leq \tau_{Le}^s; \tau_{Fe}^i < \tau_{He}^i \leq \tau_{Le}^i \end{array} \right\} \dots\dots\dots(19).$$

The relation of $T_{Fe}^i < T_{Le}^i$ is evidenced by experiments of chronic estrogen administration (Inoué, 1965b).

Thus, the gonadotropin secretion, under the above-described regulatory influences of the brain and the ovary (the direct effect of estrogen on the hypophysis, *vide ante*), may be like the schematic graphs in Figure 5 (below). Precise data on LH secretion were already obtained by Kobayashi *et al.* (1968). Although there is no detailed survey, some suggestions are available as to circulating quantities of FSH (McClintock and Schwartz, 1968) and to LTH (Kwa and Verfohstad, 1967) during an estrous cycle of the rat.

In case of the male, relations explained as the effects of EH may be replaced by those of androgen (AH). Consequently, AH instead of EH plays an important role in the regulation of the brain-hypophysis complex. Normally no secretion of PH occurs in the testis. Therefore, activities through the $D_p \rightarrow N_L^s$ route (Figure 3) which is characteristic in the estrous cycle do not appear, without provoking a sudden rise in LH release. This and the lower output of BC may account for the superficially non-cyclic reproductive function in the male. We assume, however, that the pathway $D_p \rightarrow N_L^s$ may exist also in the male rat, being able to activate LH release from the hypophysis, if exogenous PH reaches the brain (Inoué, 1969). Similarly, the pathways from D_e to the neurosecretory and/or the store mechanism may exist in the male too, exhibiting no functions in normal conditions just like those from D_a in the female.

Now let us consider the regulatory mechanism which governs the PH phase. A steep rise of LH level at proestrus, caused by the sudden increase in LH-RF as described before, then induces not only PH secretion from the ovary, but also suppresses the EH secretion (Uchida *et al.*, 1969). Now the EH phase is replaced by the PH phase. Morphologically, matured follicles shed ova and change into corpora lutea. PH in turn acts on the brain, being detected by D_p , and activates at first

N_F^s , N_L^s and N_H^s (positive feedback) and then N_F^i , N_L^i and N_H^i (negative feedback). Hence, similarly the following inequalities may be written.

$$\left. \begin{array}{l} T_{Fp}^s < T_{Fp}^i; \tau_{Fp}^s < \tau_{Fp}^i \\ T_{Lp}^s < T_{Lp}^i; \tau_{Lp}^s \ll \tau_{Lp}^i \\ T_{Hp}^s < T_{Hp}^i; \tau_{Hp}^s \ll \tau_{Hp}^i \end{array} \right\} \dots\dots\dots(20).$$

In comparison with the male, D_p of the female rat is so sensitive (Inoué, 1969) that N_L^s and N_L^i are greatly modified by PH levels. The results are then informed to the hypophysis by means of neurohormones, to cause changes in secretory patterns of gonadotropins (Figure 5). In these processes too, the short feedback actions of each gonadotropin may participate in the regulation of the neural activities, just as described in the inequalities (16).

PH secretion continues even after the cessation of LH release, exhibiting the second peak in diestrus (D_1). This lower peak is explained as the result of autonomous secretory activities of newly formed corpora lutea (Uchida *et al.*, 1969). In fact, this stage accords with the period, in which the volume of fresh corpora lutea grows to the maximum. If copulatory stimuli reach the brain during a rather long period (from the onset of the PH phase to D_1 , unpublished data of Inoué), the neural mechanism concerned with LTH secretion (the routes connected with C in Figure 3) is triggered to suppress N_F^s , N_L^s and N_H^s and activates N_F^i , N_L^i and N_H^i . Consequently, the production and release of neurohormones are inhibited and only LTH becomes freely and abundantly liberated to bring about successive secretion of PH in elevated levels. This state characterizes pregnancy or pseudo-pregnancy. However, our discussion should be back to the PH phase of normal estrous cycles.

The suppressive effect of PH on the brain is especially important to the chronological sequences of performance at the PH phase.

As expressed in the inequalities (20), after a short period of the positive feedback action, the high level of PH exerts a considerably strong negative effect on the neural mechanism. The second peak is assumed to enhance the effect further and elongate the duration. Hence, the neural activities concerned with the production and release of each neuro-hormone rise very slowly (Figure 5).

If such negative effect of PH is entirely removed, the diestrous stage might disappear. Contrarily, if such effect continues longer, the duration of diestrus might be longer. The non-cyclicity in the male reproductive function and the persistent-estrous state, spontaneously occurring or experimentally induced, in the female may be the former case. Reflex ovulators showing constant estrus (for example, the rabbit) may also belong to the same category. Meanwhile, normal rats exhibiting 5-day estrous cycles may be the latter case. Furthermore, the other spontaneous ovulators with longer estrous or menstrual cycles, such as the guinea-pig or the woman, may belong to the latter category. The length of a cycle is determined by the length of PH-secreting activity of the ovary, i.e. the life span of the corpus luteum.

Associated problems arising from various experimentally obtained facts may be explained by our theory. These are modified states of the total system caused by the effects of exogenous hormones, anesthetics or stressful agents and by the lack of some informations in case of brain lesions, hypophysectomy or gonadectomy. Especially, chronological secretory patterns of gonadotropins in gonadectomized condition, which is believed acyclic in both male and female rats (Inoué, 1961), may be analysed by means of this theory and equations. In addition, developmental changes throughout the life history as characterized by the sexual differentiation of the brain, puberty and senescence, could be pursued on the extension of our hypothetical basis.

Fig. 6. Flow chart with some numerical values used for computer programming (from Nakamura *et al.*, 1969).

Computer simulation of the rat estrous cycle

We have tried to analyse dynamic changes in the performance of our theoretically synthesized model of the brain-hypophyseal-gonadal system by computer. The above-described differential equations were partially simplified and transformed into difference equations. Our attention was focused only on a normal 4-day estrous cycle of the rat. One cycle was divided into 288 sampling intervals (sampling was done every 20 min). Since almost all numerical values for variables and constants are unknown, we gave appropriate values in the trial-and-error manner as shown in a feed back loop of Figure 1. Initial values of EH,

PH and LH were adopted from Miyake(1968). Calculations started in the midnight between D₁ and D₂. The flow chart for computer programming and some numerical values used in a calculation is shown in Figure 6. We used digital computers NEAC 2101 and 2230 (Nippon Electric Co., Tokyo). Detailed procedures of programming and numerical analyses were described elsewhere (Nakamura *et al.*, 1969).

Figures 7 and 8 exhibit a set of calculated results. Structural changes in the ovary (follicular growth, ovulation and luteinization in sequence) are considerably well simulated, although follicular maturation and ovulation occur a little earlier in this calculation than those in the real system. We can notice that

Fig. 7. Computer simulation of changes in the ovarian structure during the estrous cycle. The definition of symbols, see Table 2 and text.

Fig. 8. Computer simulation of changes in the plasma concentration of hormones during the estrous cycle. The definition of symbols, see Table 2 and text.

FSH and EH are secreted in accordance with the follicular growth and that ovulation takes place at the time of the maximal LH secretion. Thus numerical connections between these phenomena proved to be too strong in this

case. Corpus luteum formation synchronizing with the second rise of PH level fits the performance of the real system. It is clearly demonstrated that corpora lutea are not maintained further. Calculations to obtain

more exquisite simulations, which may be available for dynamic analyses of the total system, are now in progress.

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