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Receptor ontogeny and hormonal imprinting

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Continuous interaction with the environment is a basic feature of cell life. Precise recognition of the environment is a prerequisite for cell function and survival. The environment of the unicellular organism is the external world. Transition from unicellular to multicellular forms of life involves dramatic alterations in the recognition system since, while the environment of the multicellular organism is still the external world, that of the single cell may be, depending on its localization, either the external or the internal milieu[15,16]. The at least partial 'internalization' of the environment at the level of multicellularity results in specialization of the recognition system (for the needs of the community). While the developing nervous system acquires the ability to receive scores of different signals and to control the entire (multicellular) organism, the primordial – chemical – system of signal recognition acquires different functions which become integrated into the complexity of the functions of the organism.

Differentiation of 'self' from 'non-self' (foreign) seems to exist at all levels of phylogenesis, because even unicellular organisms combine and form colonies exclusively with their own kind, and either escape or devour the organisms recognized as 'foreign'. From this basic mechanism develops at the multicellular level the marker – receptor recognition system, whose functions are to furnish morphogenesis and to maintain homeostasis of the immune system. Thus the immune system of multicellular organisms screens both the external and the internal environment for chemical (molecular) signals emitted by 'self' and 'foreign' cells. The second complex of the chemical recognition systems is represented by the pheromone system, which controls the relations between individuals, and is oriented exclusively towards the external environment. Last but not least, the endocrine system of multicellular organisms evolves from the primitive chemical recognition system of the unicellular organism. The endocrine system coordinates cellular functions initially in the absence of, but later in collaboration with, the nervous system. Since the endocrine system operates inside the multicellular organism, the recognitive function of the cells controlled by it is oriented towards the internal environment.

Analysis of the recognition system of multicellular organisms from the point of view of environmental relations has revealed that essentially four sub-systems operate in that system, of which one interacts exclusively with the external environment (pheromone system), two can process both external and internal signals (nervous system and immune system), and one responds exclusively to internal signals (recognition part of endocrine system). The operation of the sub-systems is genetically encoded, within the limits set by the needs and potentialities of the species. However, it appears that only the sub-system which responds exclusively to external signals (pheromone system) is encoded in every detail. In each condition in which recognition of the internal environment, i.e. recognition of the individually varying 'self' is decisive,

normal regulation presupposes the operation of the controlling and controlled factors in a complementary code system, and adjustment (adaptation) of the two factors to one another. In all probability, since individual variation tends to increase with the progression of evolution, adaptation, too, obtains an increasing importance. This is reflected by the immune differentiation of 'self' from 'foreign'. The immune system recognizes as 'self' all structures which were present in the course of its development, and as foreign all those with which it has interacted in its fully developed form, regardless of whether the latter structures are useful or noxious for the organism. The same applies to behavioral imprinting, which plays a decisive role in the fundamental adaptation of affective responses. In this light it appears logical that the endocrine system, too, has to adapt itself for self (individual milieu), because its entire function is oriented on the internal environment.

The endocrine system can be subdivided into two main functional entities, the hormones and the hormone receptors. Supposing that these structures are not stable from the very beginning, but adaptable to one another, the conclusion lies close at hand that either is capable of adapting to the other[12]. From the phylogenetic point of view the hormones appear to be more dynamic structures than the receptors[56], for they may vary considerably by mutation. For example, only about 38% of the amino acid components of the vertebrate hormone insulin have persisted in an unchanged form throughout the course of about 500 million years' evolution, whereas the receptors of the hagfish, which took a different evolutionary path about 500 million years ago, are still able to bind vertebrate insulin[68, 69]. On the other hand, ontogenetic investigations[12] suggest that the hormones have been stable structures ever since they appeared, and adaptability seems to be primarily the property of the receptors.

Receptor alterations during ontogenetic development

The hormone-binding capacity of receptor-bearing cells changes greatly in the perinatal period. The glucagon sensitivity of embryonic rat liver cells is only 1% of the adult value after 15 days of prenatal life, and 23% of it after 21 days. Prenatal hepatocellular insulin binding capacity is 11% and 45% relative to the adult value at 15 and 21 days of prenatal life, respectively[9]. The luteinizing hormone (LH) receptors of the rat testicle can be detected as early as at 15.5 days of prenatal life, show a considerable increase in binding capacity at 18.5 days, and become fully active by the 5th postnatal day[84]. The receptors of the follicle stimulating hormone (FSH) appear in the rat embryo at 17.5 days of intrauterine life. Their binding capacity remains low until 19.5 days, but tends to increase markedly from 20.5 days on. The hepatic insulin-binding capacity of the human fetus tends to increase gradually, whereas the number of somatomedine receptors shows practically no change[77]. At the same time, the number of insulin receptors has been found to decrease in adulthood, from the neonatal value of 44,600/cell to 7100/cell[82], and this quantitative decrease has also been substantiated by other experimental observations[4]. The somatotropic receptors of the female rat increase 5 to 6 times in number from the 8th to the 28th postnatal day[65]. The hepatic glucagon receptors of the guinea pig fetus are 3 times more numerous at 58 than at 65 days of age, and relatively less numerous in adulthood[55]. Prenatally the glucagon receptors do not differ in amount between 58 and 65 days of embryonic life, but their binding affinity is relatively greater at 65 days, although their number is about 50% less than in adulthood. The beta adrenergic receptors of chickens are less numerous at 9 days of age than between 4.5 and 7.5 days, and still fewer at 12–16 days[1]. While adrenalin is able to act on neonatal rat hepatocytes in culture, insulin has hardly any influence on them[17]. The triiodothyronine (T_3) binding capacity of the rat is considerably greater in early prenatal life than at the newborn age[37], and is lower in adulthood than neonatally[36].

The above experimental observations suggest that the number of receptors, and to a lesser degree their affinity for the hormone, does change during the perinatal period. It cannot be stated that the number, or binding capacity, of the receptors tends to increase from neonatal to adult age, nor is there firm evidence to the contrary. Nor it be stated that an increase or decrease in binding capacity with increasing age would apply to any given hormone or receptor. It appears that receptor numbers vary with the degree of maturity at birth, probably also with the sex, and almost certainly with the type of the hormone, too, because certain hormones which play a morphogenetic role, prenatally, are known to switch over to another function post-natally.

In view of this it seems logical that the receptors of morphogenetic hormones already appear in fetal life, and are probably more numerous and display a greater binding affinity in that period than after birth[57]. However, the number of receptors is in itself not conclusive evidence of the intensity of hormone action for, although the receptor structure may itself be present prenatally, its postreception mechanism may be out of operation to protect the developing cell against an undesirably strong hormonal influence[54]. Perinatal changes in the number and affinity of the receptors seem to be associated with the gradual stabilization of the originally unstable (plastic) binding site (e.g. hormone receptor stabilization takes about one month in the rat). In principle, stabilization could be the result of a spontaneous – genetically encoded – differentiation associated with that of the receptor-bearing cell membrane or cytosol, but it may also be an adaptation phenomenon which is facilitated by the initial perinatal plasticity of the receptor structure. Perinatal adaptation is in all probability required for the establishment of the final receptor number and structure.

Why is receptor adaptation necessary? An approach based on information theory

The main components of an information system are the signal emitter, the signal receiver, and the channel through which the signal (information) passes from emitter to receiver. Information transfer presupposes that either the emitter and receiver operate in the same code system, or the receiver is dynamic enough to receive and process practically all signals, regardless of their code system[12]. The receiver structures of unicellular organism

are probably of the latter type, inasmuch as their dynamic membrane seems to be able, under the influence of the signal molecule, to present a complementary interacting pattern to a great variety of signal molecules. Receptor formation by such a mechanism is, however, definitely not characteristic of multicellular (endocrine-system-possessing) organisms, in which exchange of information with a closed internal environment presupposes the encoding of the signal emitter and signal receiver by the same genetic coding system. Thus it appears that at the level of the organisms possessing an endocrine system, the signal emitter and signal receiver necessarily operate within the same (known) complementary code system.

Nevertheless, taking into consideration that although the signal emitter (endocrine cell) and signal receiver (target cell) carry the same genetic information, different details of them may operate in the different cell types; it is by no means certain that the interrelationships of the gene details responsible for coding the emitter and receiver are heritable. In other words, although the internal environment is heritable, its hereditary transmission may not a priori be coordinated with the heritage of the cells it controls. There is reason to postulate that the endocrine changes associated with certain pathological or nearly pathological phenomena represent only a marginal sector of the variations which fall into the normal range, and account for the great variability of the receptor–hormone relationship. In this light the signal emitter and signal receiver, and the code of their operation are deterministic, but adequate operation of information transfer requires tuning of the emitter and receiver to one another, to ensure the functioning of a living system. Perinatal adaptation, which ultimately results in the establishment of the final, reproducible, receptor number and, probably, receptor affinity, seems to be the underlying mechanism of mutual adjustment[11, 13].

The third main component of the information system is, apart from the code, the channel, which in higher organisms is represented by the circulation or in certain cases, e.g. in the case of paracrine secretion, by the intracellular space. Through this channel pass various signal molecules, along with molecules with other (non-signal) functions, which may be capable or incapable of binding to receptor structures. Thus the target cell (i.e. its receptor) is obliged to select from among a mass of diverse molecules those carrying the appropriate information, and to differentiate them from the general 'noise'. Selection is difficult, because the hormones form hormone families[6, 7] (partly because it is easier to 'use' a modified version of an established signal molecule, partly because the hormone synthesizing cells are in many cases similar) and the more similar the signals, the more difficult it is to distinguish one from the other.

Exactly for this reason it is imperative that the target cell adapts to the specific hormone while the receptor is still plastic, in order that certain factors which avert the confusion of similar signals can take effect. One such factor is probably the circumstance that the specific hormone and the related molecules do not simultaneously appear in the perinatal period at the concentrations required for receptor adaptation[48, 50, 51, 63, 70]. Furthermore, the binding (transport) proteins of the circulating blood may also play a role in the control of the hormone concentration. On the other hand, as the availability of the receptor for the hormone is limited, the delay of receptor maturation relative to hormone availability also facilitates precise adaptation. At all events, this concept of mutual adaptation of receptor and hormone presupposes a precise programming of the critical events (timing of specific hormone peak and receptor maturation).

The chemical code system of the living organisms is, despite the existing similarities between hormones, fairly variable. In the case of polypeptide hormones, the amino acid sequences appear to be fairly simple, but their association into secondary, tertiary and quaternary structures increases the intricacy of the molecular structure. Since the closest relationship (in terms of amino acid sequence) has been demonstrated exactly between polypeptide hormones, these sequences seem to account for the specificity of their code. The amino acid-type, steroid-type and fatty acid-type hormones arise by different codes, and their specificity is associated with certain determinant groups. The lower the phylogenetic level of the organism, the less specific appear to be its receptors, and the greater are the overlaps of signal molecules (e.g. of polypeptide hormones) on one another's binding sites[22, 52]. Probably the phylogenetic process of receptor formation is being reproduced during the ontogenetic development of the receptor, and adaptation is required to impress the 'image' of the specific hormone on the 'memory' of the cellular receiver structure.

Experimental evidence for hormonal imprinting

An experimental approach to the substantiation of neonatal adaptation (imprinting) calls for the creation of either hormone deficiency or hormone excess during the critical period. The effect of both treatments is assessed in adulthood.

Polypeptide hormones

The thyrotropic hormone (TSH) is a polypeptide hormone. Treatment of newborn rats with thyroxine (T_4) for several days depresses hypophyseal TSH-production, thus giving rise to so-called neonatal hyperthyroidism[49]. Animals so treated while newborn show no decrease in the basic thyroid hormone level in adulthood, but no increase either in T_3- or T_4-production in response to TSH[30]. It appears that normal receptor development requires the presence of TSH, because absence of TSH in the critical neonatal period accounts for depression of adult response to TSH. On the other hand, neonatal exposure to a massive dose of TSH also results in depression of adult thyroidic response, not only in rats[29], but also in chickens[43]. The period required for the normal course of imprinting can be fairly well assessed in chickens; these are more mature than the rats at birth, and, accordingly, the development of their hypothalamic-pituitary-thyroid axis also is more advanced at hatching[67]. While the chick embryo is irresponsive to TSH at 8 days of prenatal life, it shows a lifelong alteration in receptor responsiveness when exposed to TSH at 12

days[71], after the above axis has developed. For the rat, the optimal hormone dose is 20–50 IU; doses above or below that range are depressive or indifferent rather than stimulatory[72].

Testing follicle stimulating hormone (FSH) on the gonads of day-old chicks has shown that neonatal exposure to FSH (of chicks) amplified the FSH-receptor[21]. Certain organs of the chickens so treated developed more intensively also without reexposure, owing in all probability to an increased responsiveness of the target cells to the endogenous hormone. Gonadal response proved to be still greater after reexposure to FSH in adulthood.

A single neonatal treatment with vasopressin unequivocally accounted for an increase in adult response to vasopressin[33].

Mice exposed to met-encephalin neonatally on a single occasion showed a considerable increase in sensitivity to opioids in adulthood[76].

The effect of a single neonatal exposure to insulin can be assessed from alterations in the hepatic hormone binding and hepatocellular responsiveness of the adult[59]. There is, however, a sex variation, for neonatal insulin exposure enhances adult insulin binding in females, but depresses it in males, and has an adequate effect on the blood glucose level, too.

Imprinting by related hormones; the disturbing effect of 'noise'

TSH and the gonadotropins are related, in that they have a common alpha subunit and their beta subunits differ only slightly, just to a degree which furnishes specificity of action[83]. TSH-gonadotropin binding overlaps can occur in adulthood without any appreciable consequence in function[2, 3, 75], but they have far-reaching consequences in the critical perinatal period.

Assessment of the effects of exposure to FSH or TSH on different parameters of chicken gonads has shown that the two hormones develop qualitatively the same action when applied to day-old chickens[20]. Although FSH has a greater influence on the germinal elements than TSH and, vice versa, the latter has a more powerful action than the former on the interstitium, each has an appreciable effect on the target cells of the other. Quantitative measurements have revealed that the gonadal action of TSH was also superior to that of FSH at lower dose levels. This cannot be ascribed to a contamination, on the one hand because the preparations used were very pure, on the other because the effect of contamination cannot supersede hormone action. The functional overlaps could be substantiated by light and electron microscopic studies which showed that the two hormones stimulated the function of the same organelles[35, 78, 79].

Evidence of TSH-FSH functional overlap at the neonatal age has strongly suggested that these hormones bind to the same receptors in that period. This has prompted us to investigate the influence of structurally similar (related), but functionally different hormones on receptor development.

Rats[29] and chickens[43] neonatally exposed to a single dose of gonadotropin had a decreased T_4-level in adulthood. Thyroidic response to TSH was weak, to judge from a negligible rise in the T_4-level.

Treatment of newly-hatched chicks with TSH amplified the gonadotropin receptors to a similar degree to FSH itself, to judge from morphological and functional (hormone level) evidence of a greater response to FSH on reexposure[43]. TSH proved to be in several respects more active than FSH, whereas it failed to amplify the receptors for itself, to judge from the experimental observation that response to TSH of adult chickens neonatally preexposed to TSH did not differ from that of the not-preexposed control. The gonadal response of neonatally TSH-treated rats was qualitatively similar to that of the chickens, but quantitatively less conspicuous.

Neonatal influence of exogenous TSH or FSH on the receptors also alters adult sexual behavior[24], although the functional overlap of TSH is of a lesser degree than in the case of morphological or hormonal indexes.

The polypeptide hormones vasopressin and oxytoxin are also related molecules, differing from each other only in two amino acid components. No functional overlap between them is demonstrable in adulthood, but neonatal exposure of rats to oxytocin amplifies the receptors for vasopressin as well as for oxytocin itself and this effect is still demonstrable in adulthood[33].

Overlapping imprinting by steroid hormones

Neonatal treatment with sexual steroids influences the hypothalamic receptors and through them the adjustment of sexual constitution[5, 8, 10, 44, 47]. However, target cells other than hypothalamic may respond to steroids at receptor level, and imprinting by overlapping steroid hormones can also occur.

Rats treated neonatally with a single dose of diethylstilbestrol (DES) showed at 6 weeks of age a decrease of about 50% in uterine estradiol receptor numbers without, however, any change in receptor affinity[28]. A single neonatal exposure to allylestrenol instead of DES accounted for a decrease of about 30% in the number of estradiol binding sites, also without causing any alteration in their affinity. Neonatal exposure to DES or allylestrenol also gave rise to a considerable alteration (reduction) of adult uterine DES-binding capacity.

Primary exposure to DES in early pregnancy (at days 9 and 14 of gestation) instead of the newborn age resulted in death or stillbirth of all fetuses. The offspring of the allylestrenol-treated females were born alive, but no change in the uterine binding capacity was demonstrable relative to the control in that case. At the same time, a decrease of about 50% was observed in the number of the thymic glucocorticoid receptors[62].

Rats neonatally treated with glucocorticoid (dexamethasone) showed a decrease of about 33% in thymic glucocorticoid receptor numbers in adulthood[61].

The steroid structure is very widely distributed in the living world; it is demonstrable in both plant and animal organisms. Thus the steroid structure is characteristic not only of steroid hormones, but also of a variety of materials which occur in the natural environment of man, and act on him either advantageously as drugs, or noxiously

as carcinogens. The basic structure of the digitalis derivatives and of benzpyrene is also steroid-like.
Rats treated neonatally on a single occasion with digoxin or ouabain[26] responded to reexposure in adulthood with an increase in the blood digoxin level (after digoxin treatment), i.e. by decreased elimination of digoxin. A single neonatal treatment with benzpyrene[25] accounted for a 33% decrease in adult thymic glucocorticoid reception. Neonatal exposure to ouabain had no influence on adult thymic glucocorticoid binding. Conversely, however, neonatal triamcinolone treatment[60] depressed adult myocardial ouabain binding and ouabain sensitive ATP-ase activity by about 33%.

Imprinting with amino acid type hormones

A single neonatal treatment with catecholamines (epinephrine, isoproterenol, dopamine) alters considerably the adrenergic vascular response of the adult rat. Isoproterenol alters adult response to norepinephrine and to vasopressin as well[34].
A single treatment with melatonin at the newborn age accounted for about a 25% increase in the adult T_4-level without any further exposure to the hormone. Since melatonin is a thyroid inhibitor, neonatal treatment with it presumably gives rise to desensitization of the thyroidic melatonin receptors[31]. AT the same time melatonin inhibits the action of TSH on the thyroid gland.

The importance and sensitivity of imprinting

The foregoing considerations permit the following conclusions:
1. Presence of the hormone is an essential prerequisite of the amplification (imprinting) of the receptor.
2. Related hormone molecules, whose chemical structure differs from that of the specific hormone but not to such an extent which would prevent their binding to its receptor, induce either an adequate imprinting characteristic of the hormone proper, or a different (faulty) imprinting.
3. By interfering with the normal course of imprinting, the related molecule biases response to the specific hormone without, however, adapting the receptor for itself; thus no nonspecific response occurs in adulthood. The exception is the case in which the cell membrane is artificially damaged during imprinting with the related hormone; in such case the related molecule involved in imprinting acquires a lasting advantage over the adequate hormone.
4. In principle, adaptation of the hormone and receptor to one another is optimal if it takes place without 'foreign' interference, although faulty imprinting could presumably be corrected by 'artificial' interference.
5. The specific hormone itself can damage the receptor, if it appears at an inappropriate time and concentration.
Thus hormonal imprinting is a species–dependent, time–dependent (and probably also sex–dependent) biological phenomenon, whose effects may be different exactly for the reasons outlined above. Its effect is portrayed above all by the numbers of (maximal) evokable receptors, for no appreciable affinity changes have been demonstrable by receptor kinetic analysis. *Imprinting is in all probability not the exclusive property of hormone receptors,* as non-hormone molecules acting at receptor level can also induce it (in their own receptors or in related binding structures). This has been demonstrated in the case of glucose-like molecules, such as glucosamine and mannose[19], and of drugs, chemicals, etc.
Once induced and established, imprinting persists for the lifetime of the organism, as has been demonstrated in the FSH-TSH, and vasopressin-oxytocin systems. In the unicellular *Tetrahymena,* imprinting has been shown to persist by transmission from one cell generation to the other[32]. This is, in all probability, the case also in higher organisms, for in these, too, cells treated in the neonatal age represent distant ancestors of those which are present in adulthood. When treatment is performed at the newborn age (when imprinting occurs), and its effect is measured in adulthood, many new generations arise in the time interval between, which obviously 'remember' the event of imprinting. The 'heredity' of imprinting seems to persist still longer. The progeny generation of rats treated with insulin neonatally, and mated in the adult age, showed the same pattern of imprinting after maturity as the parent generation, in that insulin binding was decreased in the males, and increased in the females[27]. Since the F_1 rats had not been treated with insulin neonatally, their changed insulin binding was clearly acquired by hereditary transmission. The effect of parental imprinting was still greater in those F_1-rats which had themselves been exposed to insulin at the newborn age. A similar effect was also observed if only one parent of the two had been treated, but the binding capacity (affinity) of the receptor was weaker in such cases. Parental imprinting was no longer demonstrable in the F_2 generation.

Is Haeckel's principle valid in ontogenetic development of hormone receptors?

The receptors of the adult rat are highly selective, and can therefore (functionally) differentiate the closely related hormones FSH and TSH from one another. This selectivity is not yet present at the newborn age, when the rat gonads respond equally to FSH and TSH. At a lower phylogenetic level, in the frog, FSH-TSH overlap can also occur in adulthood[52]. Treatment of adult male frogs with chorionic gonadotropin characteristically elicits ejaculation (this was formerly utilized for pregnancy testing). TSH has the same effect on the adult male frog, although at a higher dose than FSH[22]. While this effect of TSH is inferior to that of chorionic gonadotropin, it is certainly superior to that of the luteinizing hormone. Moreover, TSH potentiates the action of the gonadotropic hormone in the frog[23]. This does not, of course, mean that hormonal imprinting can also be induced at the adult age at the lower levels of phylogenesis; it signifies rather that the receptors of phylogenetically lower organisms are as incapable of differentiating related hormones from one another in adulthood as those of higher organisms are in the perinatal period, but the effects of hormone overlap are dissimilar at the two phylogenetic levels.

Hormonal imprinting in cell lines

Cell lines have generally been developed from mammalian cells which had been subject to hormonal imprinting in vivo. Nevertheless, long-term maintenance by serial passages in all probability results in loss of the 'memory' of the event, because imprinting usually takes place in mammalian cell lines exposed to a hormone in vitro, to judge from an increase in hormone binding after a few days[14]. The effects and the overlap of TSH and FSH can be reliably studied in Chinese hamster ovary (CHO) cell cultures. TSH enhances CHO cell division to the same extent as FSH[39]; pretreatment with FSH amplifies the CHO-cell receptor not only for itself, but also for TSH; and vice versa, TSH amplifies it for both itself and FSH[41]. By analogy with perinatal imprinting, TSH is superior in action to FSH, and amplifies the receptor to a greater degree for FSH than for itself, also in vitro.

Likewise, insulin, too, can induce imprinting in cell culture[40], at a very low concentration (10^{-13} M) and in a relatively short time (1 h). However, the insulin imprinting induced in cultured cells is not equivalent to that induced in the living (animal) organism, because it expires, or becomes disturbed, after a certain time. This can probably be explained by the circumstance that cultured mammalian cells have necessarily been subject to hormonal imprinting in vivo, and their primary exposure in culture brings about a revival rather than a primary induction of imprinting. It should also be taken into consideration that while the effect of hormonal imprinting is maintained by the endogenous hormone in the living organism, this is not the case in culture.

Hormonal imprinting at enzyme level

Each hormone represents simultaneously the ligand of the specific receptor, and the substrate of the enzyme responsible for its degradation or synthesis. Since, like receptors, the enzymes are molecules of a well defined steric structure and have a ligand (the substrate), there is reason to postulate that imprinting also takes place at enzyme level. Six-week-old rats neonatally treated with DES or allylestrenol showed no change in basic hepatic mirosomal enzyme activity, but did show a change in response to testosterone, which failed to cause an activity increase relative to the control within 48 h. Allylestrenol proved to be more active than DES under the given conditions of the experiment[38].

These experimental observations suggest that *hormonal imprinting,* which had formerly been thought to be an exclusive property of molecules acting at receptor level, *can be regarded as a more general phenomenon, appearing and taking effect presumably in all binding site – ligand interrelationships, irrespective of the nature of the latter's function.*

The mechanism of imprinting

In the initial stage of evolution, as portrayed by present-day unicellular organisms, imprinting occurs in a dynamic open system, in which the continuously changing membrane patterns are screening the environment for specific signal molecules. In the presence of the specific signal molecule, the interacting membrane pattern becomes amplified and is transformed into a receptor. This persists of it acquires a selection advantage, through which the receptor structure, the cell carrying it, and the organism of which the cell forms a part, survive and become integrated into the mechanism of evolution[12, 15, 16]. In higher organisms the receptors are genetically encoded (pre-programed) structures, since at the level of multicellularity the cell has not the chance to form any kind of receptors for itself. However, the encoded receptor structure requires amplification, which is accomplished by imprinting.

The hormones act primarily on the membrane receptors or the cytosolic receptors, both in the critical perinatal period and later in life. The hormone molecules bound by membrane receptors become internalized into the cytoplasm, and if they do not become degraded therein, they presumably also develop action intracellularly[58, 73, 74, 80, 81]. Since the effect of hormonal imprinting is demonstrable in the progeny generation, there is reason to postulate that the hormone enters the cellular nucleus, too, and develops intranuclearly a gene level action. This presumption is supported by the experimental observation that the effect of hormonal imprinting was demonstrable in the F_1 offspring generation of rats neonatally exposed to the hormone, although the circumstance that it did not reappear in the F_2-generation does not support suggestions of a strong genetic effect.

Radioisotope studies have shown that the labeled hormone content of lymphocytes was several orders greater in the perinatal period, and even at one week of age, than in adulthood[42]. Since the internalized hormones, at least the amino acid-type hormones, are also accumulated in the nucleus, their nuclear presence may contribute to the establishment of imprinting. Polypeptide hormones, too, have, apart from membrane receptors, intracellular and nuclear membrane associated receptors, which probably serve as mediators of imprinting[73, 74, 80, 81]. Since membrane DNA is being transposed to the nucleus, its involvement in the mechanism of imprinting cannot be excluded either.

Inhibitors of endocytosis and cellular protein synthesis inhibit hormone binding to cells in culture, and on simultaneous treatment with TSH they inhibit imprinting by TSH to different degrees, depending on TSH-binding[14]. Of the protein synthesis inhibitors, cycloheximide and cytochalasin (which acts on the microfilamentary system) inhibit imprinting to a still greater degree, from which it follows that hormonal imprinting presupposes the normal operation of the intracellular cxycloheximide-sensitive and cytochalasin-sensitive systems, irrespective of the binding relations. Although these findings suggest the involvement of receptor internalization and resysthesis in imprinting, the underlying mechanism is still obscure. Experiments in unicellular model systems are expected to throw more light on this problem.

Chemically the hormone receptors are glycoproteins, composed of a protein part and an oligosaccharide chain. Since the sugars also serve as determinant groups, they may play a certain role in hormonal imprinting. It ap-

pears that those lectins which bind to simple sugars, like for example pea lectin, are not able to induce imprinting either for themselves, or for hormones interacting with receptor structures which contain a like sugar molecule[18]. Against this Helix and Datura lectins, which bind to amino sugars, are able to induce imprinting for insulin, thus pretreatment with these lectins enhances insulin binding in culture. Datura lectin deserves special mention, for it imprints the receptor also for itself, to judge from its increased binding on reexposure. Presumably the hexosamine oligomeres (to which the Datura lectin binds) are also involved in the induction of imprinting.

The four stages of the development of encoded adaptation-requiring dynamic systems in mammals

It appears that, in any living being, adaptation (imprinting) can take place only at a given stage of ontogenetic development. This applies especially to mammals, for the chances of imprinting are limited by the particularities of the maternal-fetal relationship.

Experiments on steroid imprinting have shown that allylestrenol, which had a firm imprinting effect when applied in the perinatal or neonatal period, had no influence on the target organ during gestation, but developed a strong lifelong effect on the receptors of another organ[62]. Analysis of this phenomenon helps in an understanding of the nature of the factors averting or promoting imprinting, if the non-hormonal adaptation-requiring systems are also taken into consideration. Inhibition of prenatal steroid imprinting may have been associated partly with the protective barrier separating maternal from fetal circulation, and partly with the immaturity of the fetal steroid receptors. The time-dependent differences in action should therefore receive special consideration, and to facilitate this approach, we divided the ontogenetic development of the recognition system theoretically into four stages.

The first stage, which essentially coincides with the morphogenetic period, is characterized by full protection of the receptors against interaction with the hormone. At this stage presumably not so much the (as yet inaccessible) hormone receptors, as the receptors involved in cell-cell recognition (by marker recognition) are predominant, and contribute to the normal course of morphogenesis. Since little is known about the vulnerability of these receptors, it is poorly understood why destructive processes involving morphological changes also at organ level arise in that period. The marker-receptor system cannot be fully incriminated for such changes. At all events, its seems unlikely that either normal or abnormal imprinting could take place in the first stage of the recognition system's development.

The second stage is characterized by a relative protection of the developing hormone receptors, which have not yet come into their final shape (and quantity), because the factor(s) responsible for their amplification are lacking. Protection is justified, for maternal hormones are present in considerable amounts in the fetal circulation. Were adaptation (imprinting) to take place at that stage, it *would necessarily be oriented towards the quality and level of maternal hormones,* which would be deleterious, particularly in respect of sex differences. Relative protection, furnished by the immaturity, irresponsiveness, coating, etc. of the developing receptor seems to prevent at this stage binding of the fetal hormone, or the like maternal hormone, to the receptors of the target organ. However, the factors furnishing relative protection of the target organ apparently cannot protect 'foreign' receptors against the influence of the hormone. Perhaps this mechanism was involved in the experiment mentioned earlier, in which perinatal allylestrenol treatment damaged severely the thymic glucocorticoid receptors of rats, but had no influence whatever on its own specific (uterine) receptors[62].

After the stage of relative protection follows the third stage, in which the system is open to lifelong hormonal imprinting, behavioral imprinting, and adjustment of the individual sexual constitution. This critical stage falls into the perinatal period. It cannot be precisely determined whether this stage commences before or after birth. Its beginning varies in all probability with the type of the developmental mechanism and also with the species, and may also depend on maturity at birth and on sex. Immunological adaptation (the capability of differentiating 'self' from 'non-self') begins somewhat earlier than hormonal and behavioral imprinting (although it is still in progress during their course). Behavioral imprinting can, naturally, begin only postnatally, since behavioral adaptation presupposes interaction with an external environment. The length of the open stage varies with the species, but it ends in all species in the early postnatal period. For example, the hormone receptors of the rat mature by the end of the third to fourth postnatal week.

The fourth stage can be defined as a state of relative openness, in which the learning mechanisms of the nervous system evolve, to persist for a lifetime. At this stage, probably, imprinting-like phenomena also occur in those cells which have preserved their fetal characteristics (hemopoietic and gonadal cells), and are interacting with certain (foreign) molecules for the first time. It has been shown experimentally that erythrocyte neogenesis involves the presentation of new receptors in adult patients treated for long periods with digoxin[66].

Thus the dynamic recognition systems necessarily take their final shape by adaptation to the given (internal and/or external) environmental conditions. Adaptation may be qualitative or quantitative; for example, immune adaptation is almost exclusively qualitative, taking place in the very period in which 'self' and 'non-self' are differentiated for a lifetime. In the case of hormone receptors, adaptation has mainly quantitative aspects, since the future (final) receptor numbers also depend on the perinatally available hormone concentration. In other words, while the main function of hormonal imprinting is to increase the quantity of adequate receptors, immune adaptation furnishes a qualitative rather than a quantitative alteration of immune cells (receptors), probably because the immune system has to cope with the reception of both internal and external signals, whereas the hormone receptor is concerned with that of internal signals only. In this respect the immune system resembles the nervous system, being capable of 'learning' for a lifetime, despite the fact that it continues to refuse 'foreign' substances after the stage of self-recognition has terminated, unlike hormone

receptors, whose 'learning' potential expires after acquiring the capacity of self-recognition.

Reference to immune-biological phenomena in the context of hormonal imprinting and ontogenesis seems to be justified, since many details of the two mechanisms seem to be alike. In the period of 'learning' to differentiate 'self' from 'non-self', the immune system suppresses the appropriate cell clones, whereas the hormone, on the contrary, activates the receptor system. Nevertheless, both mechanisms promote self-recognition. No 'self' input can take place in either system after termination of the critical adaptation period. Like immune receptors, the hormone receptors keep the difference between 'self' and 'foreign' in a lifelong 'memory'. A deeper insight into the mechanism of the immunological memory may probably throw more light on the mechanism of hormonal imprinting, and vice versa; better knowledge of the latter could perhaps promote the understanding of the immunological memory since, according to present knowledge, the genetic determinism of the immunological memory is also loose[53, 64], and perhaps requires the presence of the antigen for stabilization, exactly as receptor 'memory' requires the presence of the hormone in order to develop.

Medicinal aspects of hormonal imprinting

The quality of the hormone and of its target cell (receptor) is genetically determined. The time of appearance, and the quantitative relations of hormone and receptor are also genetically encoded. Disturbances can nevertheless occur in their interaction and mutual adaptation, e.g. if membrane differentiation occurs earlier, or the hormone appears later, than at the optimal time. Such circumstances may disturb receptor adaptation, and lead to an abnormal increase or decrease in cellular hormone binding capacity. If a structurally related (foreign) molecule appears in the critical period of primary receptor-hormone interaction, it may give rise to faulty imprinting by diminishing either the number or the affinity of receptors. The foreign molecules capable of affecting receptor development may not only be members of the same hormone family, but also certain products of the chemical and drug industry, which are structurally related to the specific hormone. Careful studies on steroid-like molecules, such as digoxin and benzpyrene, have attracted attention on the hazards of exposure to such molecules in the perinatal period. In the present era of 'chemicalization' the possible presence of such molecules in the air and water and/or in various drugs, may seriously affect the future health of the human infant if exposure occurs in the critical perinatal period.

Clarification of the still obscure details of the mechanism of hormonal imprinting may provide useful medical tools for the diagnostic identification, prevention, and/or correction of endocrine diseases, latent endocrinopathies, and non-physiological interventions hazardous to human health.

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The special case of hormonal imprinting, the neonatal influence of sex

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Key words. Alpha-fetoproteins; androgens; brain; estrogens; estrogen antagonists; estrogen receptors; genitalia; gonads; hypothalamus; preoptic area; receptor imprinting; reproductive tract; sexual differentiation; sexually dimorphic nucleus.

The chain of events leading to reproductive success is based on the participation of a variety of organs and tissues with different structures and functions. The *brain* controls behavioral orientation and sexual identification as well as gonadotropic hormone (GTH) release from the pituitary gland. A sex specific pattern of gonadotropic hormone release will stimulate maturation and release of male or female germ cells respectively. A properly developed *internal duct system* will then transport the germ cells to the outside. Species with internal fertilization need appropriately developed *external genitalia* for the transfer of germ cells from the male to the female individual.

Historical perspectives

The question about which factors may determine the fate of a developing fetus, causing it to become either male or female, has occupied many previous cultures and scientists. Ancient Greek ideas about sexual differentiation centered mainly around two hypotheses. The 'hypothesis of laterality', established by Anaxagoras of Clazomenae (about 440 BC), claimed that semen from the right testis would produce male offspring, semen from the left testis would produce females. This hypothesis further claimed that male fetuses are carried in the right horn of the uterus, females in the left[1]. The 'thermal hypothesis' of Empedokles of Akras (about 460 BC) claimed that temperature was an important factor in sex determination[85]. Conception in a hot uterus would produce a male, in a cold uterus a female. Aristotle of Stagirus (384 to 322 BC) favored the thermal hypothesis. He observed in sheep and goats that they would produce male offspring when warm winds were blowing from the south during copulation, but female offspring when cold winds were blowing from the north[4]. Plato postulated that the first human generation consisted only of men. Those men of the first generation, who had been cowardly or had spent most of their lives in wrong-doing, were reborn in the second generation as women[86].

Environmental influences on sexual differentiation

The 'thermal hypothesis' of Empedokles may actually not be that far off after all. It has been shown that frog larvae develop a male phenotype when raised at an elevated water temperature; at a low temperature they develop into females[83]. In some species of lizards breeding of the eggs at temperatures below 26 °C will prime the embryos for female development, whereas at temperatures above 26 °C the embryos will develop into males[95]. In two species of turtles, *Emys orbicularis* and *Testudo graeca* the temperture effect on sexual differentiation is reversed. Male development is induced during breeding at temperatures below 28 °C and female development is induced during breeding at above 32 °C[82].

Another environmental influence which may effect sexual differentiation is the concentration of potassium and calcium ions in the water. Three- to four-fold elevation of calcium ions in the water will stimulate the larvae of *Discoglossus pictus* to develop into females. Five- to