The Inductor Theory of Sex Differentiation[1)]

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(With 2 Text-figures)

Introduction

About the end of the last century the search for the causes of embryonic differentiation led to the discovery of the phenomenon of morphogenic induction. Obviously the fundamental causes of differentiation rest always with the constitution of the embryonic cells and blastemas. But the classical experiments by Herbst, Sachs, and Klebs furnished evidence of trigger mechanisms, that initiate processes of differentiation and often impart a particular direction to them. The morphogenic "impulses", "substances", or "means" of these investigators were of very heterogeneous nature; but they had in common that directly or indirectly each could induce a characteristic process of differentiation.

When the present writer started his studies on the many faceted process of sex differentiation in *Rana temporaria*, he soon perceived that all embryos and primordial germ cells are bipotential, and that the factors which decide the alternative of male or female differentiation may be placed in three groups : genetic, environmental, and localized internal agents (Witschi 1914 a, b).

a In regard to *genic constitution*, breeding experiments (including R. Hertwig's *Rana esculenta* series) prove that individuals differ not by presence or absence of male (M) or female (F) determining genes but merely by their quantitative balance. Goldschmidt's formula for *Lymantria* was adapted for the case of sex races of frogs, mainly by the addition of a minor female determining factor (f), carried in the Y chromosome. For instance, if the sum of all female gene quanta exceeds that of the male genes, the individual may be called a *genetic female*; more exactly one may say that its female constitution is *epistatic*, the male *hypostatic*. Under optimal developmental conditions sex differentiation is led by the epistatic sex; the hypostatic sex sometimes can manifest itself temporarily but as a rule it remains entirely subdued (Witschi 1914b, 1929 a, b).

b *Environmental conditions*, such as extreme temperatures or delayed fertilization, can partly or completely reverse the genetic determination.

c *Localized internal factors* enter most directly in evidence in cases of her-

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maphrodite development of sex glands. Simultaneously, gonia start differentiating in the male or the female direction according to their location in the medulla or in the cortex of the gonad. Germ cells not included in either part, do not differentiate at all, but eventually degenerate. This leads to the conclusion that germ cells assume the male or the female sex character, not on the basis of their genic constitution, but in response to inductive stimuli received from without. The *cortex* is an inductor of female differentiation, the *medulla* an inductor of male differentiation. These facts and considerations served as the fundaments of the *inductor theory of sex differentiation* of 1914. Supported by experimental evidence, it has found wide-spread acceptance.

In subsequent years the theory was further developed. It is the purpose of this contribution to the anniversary volume honoring Professor Tohru Uchida, to summarize the progress that has been made through work from laboratories in many parts of the world, including particularly also the Zoology Department of Hokkaido University. It is hoped that the review may assist in the planning of further investigations which should be directed toward critical analysis and resolution of unsolved problems rather than confirmation of already established principles.

Histology of the inductors

Cortex and *Medulla* are topographic terms that apply mainly to gonads of selachians, amphibians, and all higher vertebrates, but not to teleosts (D' Ancona 1956), cyclostomes, and invertebrates. Moreover, the exceptional development of ovocytes within seminal tubules (e. g. Ichikawa 1937, Uchida and Hanaoka 1949) indicates that not the cortex as an anatomical unit should be considered as the feminizing inductor. Rather a more detailed histologic analysis of the events of sex differentiation leads to the conclusion that *follicle cells* are the inductive element of the cortex, and that *interstitial cells* are the inductive element of the medulla (Witschi 1921, 1929). Since also medullary gonia usually are enclosed in follicle cells, one must assume that the interstitial cells exert control over both, follicle and germ cells (Fig. 1). Evidently not only sexual differentiation, but also other basic life processes of the germ cells, growth, respiration, multiplication, and maturation, are under control of what basically seem to be *nurse cells*. In conclusion, the analysis of available facts indicates that in amphibians and higher vertebrates *feminizing induction* emanates from follicle cells usually located in the cortex, and *masculinizing induction* issues from interstitial cells of the medulla. Follicle cells in the medulla are embryologically derived from the cortex but only exceptionally maintain or assume feminizing induction. Considering all facts, it seems justified to adhere to the terms *cortical* and *medullary induction*, and hence to *corticin* and *medullarin* (Witschi 1931) as names for the inductive substances which presumably they release.

Interrelationships between sex determining factors

Returning now to the interrelationship of the three groups of sex determining factors, it is evident that *the genes F and M must control the cortical and medullary inductors*, and that possibly this is their sole function. At a certain developmental stage an interaction becomes established between chromosomal genes and cytoplasm of follicle cells, as a result of which the cortex becomes endowed with its

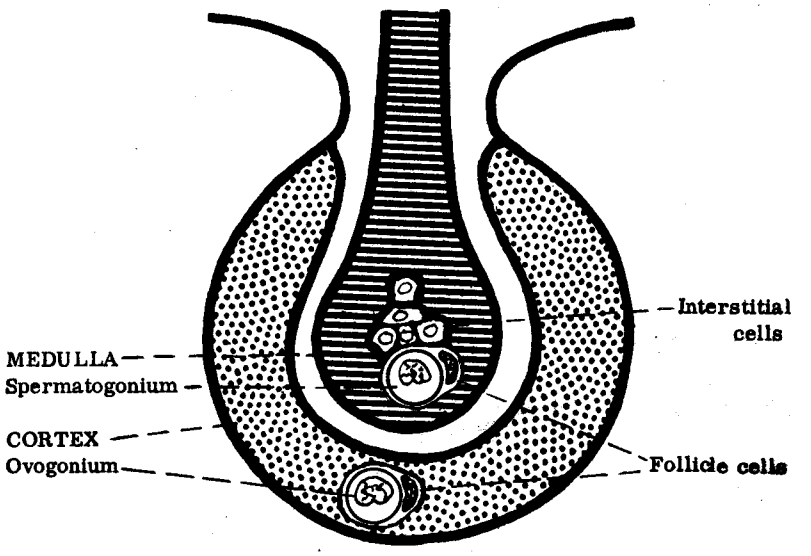

Fig. 1. Diagram of cortical and medullary inductors of sex (Chang and Witschi 1956).

inductive quality (Fig. 2). Simultaneously or somewhat later, medullary induction develops in the interstitial cells (Fig. 2). Its mechanism is more complex. It evokes not only male differentiation but controls also the follicle cells around medullary gonia (Fig. 1), inhibiting their innate feminizing capacity. Thus the germ cells are temporarily maintained in an undifferentiated condition (protogonia; Beccari 1933).

The *unimportance of the genic constitution of the germ cells for the direction of sexual differentiation* was demonstrated experimentally by Humphrey (1933) who had genetically male gonia migrate into genetically female somatic gonad primordia and vice versa. Sexual differentiation always followed the genic type of the somatic (i.e. inductor) cells.

External factors have never been shown to interfere by changing the genic constitution. In all sufficiently analyzed cases it was found that they *affect*

adversely one or the other of the inductor systems (Fig. 2). This is most clearly illustrated in the instances of sex reversal by means of simple surgical operations, such as they were performed in toads (Harms 1921) and in chickens (Benoit 1923). In the former, the male gonads usually are capped by purely cortical lobes (Witschi 1933), which after ablation of the testicular parts (i.e. the entire medulla) develop to maturity and produce fertile eggs. Conversely in hatching female chicks the right gonad rudiment consists almost or entirey of medulla only (Domm 1929),

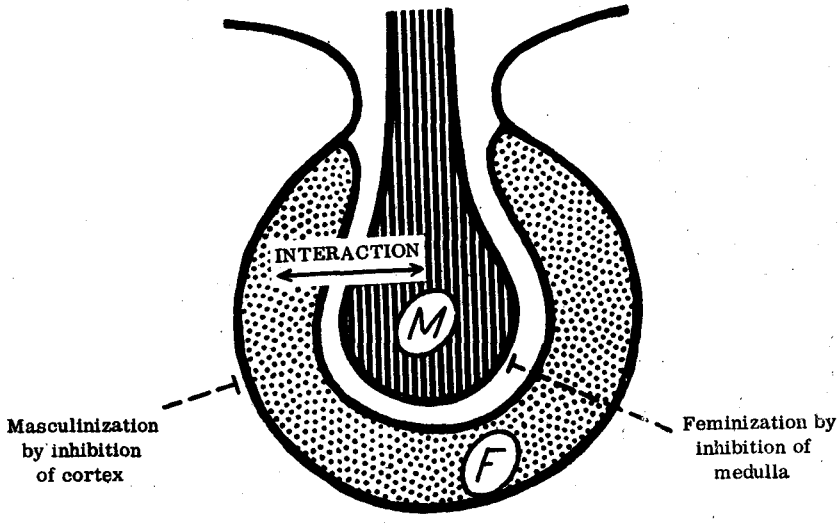

Fig. 2. Relationship between sex determining factors.
M and *F* represent gene reactions which in specific cells establish medullary and cortical inductors.
Interactions between inductors decide medullary or cortical prevalence.
External agents *feminize* if they suppress medullary function; they *masculinize* if they suppress cortical function.

which after sinistral ovariotomy develops into a testis. Each operation produces sex reversal, because the genetically epistatic sex can no longer manifest itself, after its adequate inductor system had become removed. On the other hand the hypostatic sex can now express itself. That ovogenesis in the sex reversed toad proceeds normally, in spite of male genetic constitution, is unequivocally proven by breeding experiments (Ponse 1950). Similarly it was shown that the spermatogenesis in the right testicle of sex reversed hens proceeds with female Z O chromosome complements (Miller 1938, Witschi 1957). The surgical method of elimination of one or the other inductor system is very effective but can only be practiced in a few favorable objects. Broader use may be made of temperature treatments

which in principle duplicate the surgical method. In amphibians extreme high temperature causes destruction of the cortex, while low temperature delays medullary development. The former treatment leads to masculinization of the females, the latter feminizes at least temporarily the males (Uchida 1937, Witschi 1942). Other agents (steroid hormones, overripeness) producing similar effects, will be referred to later.

Inductive antagonism

The described experiments illustrate also two other fundamental qualities of the inductors : *mutual inhibition* and *compensatory hypertrophy*. The development of the cortical lobes of the toad, and of the right medullary rudiment of the chick, following removal of the gonadal parts of opposite sex, proves that before the operation such growth and differentiation had been suppressed. In the unoperated hen the right rudiment usually becomes a sterile vestige within a few months following hatching. The cortico-medullary antagonism is particularly witnessed in parabiotic pairs of salamanders (Witschi 1936) and in heterosexual cattle twins with anastomosed blood vessels (Witschi 1939). In most instances blood-born antagonism results in the suppression of cortical development in the female partner. However, the adversity is mutual and in the combination of a large female tiger salamander with a small male jeffersonian, testicular differentiation in the latter is greatly delayed (Witschi 1937).

The comparative study of cortico-medullary antagonism reveals a highly varied picture. In some species of mammals as well as of amphibians the cortical and medullary antagonists are not primarily carried by the blood but diffuse through the tissues with falling gradients of concentration (Witschi 1932). In the toad the effects are locally narrowly limited, and of course in hermaphrodites eggs and sperms mature side by side, apparently without antagonistic interference. Such comparative studies show that antagonists are not essential to primary sexual differentiation, but to *gonochoristic sex distribution*. Indeed the establishment of antagonistic reactions must have provided the basis for the evolution of gonochorism in the vertebrate line. Interference is on a lower level in races or species tending toward hermaphrodism, than in their strictly gonochoristic relatives (Uchida 1943, Uchida and Hanaoka 1941, Witschi 1936). Observations of such nature lend support to an evolutionary interpretation.

In many species the antagonism appears slowly and only late in embryonic development. It is characteristic for birds that the left testis for some time is covered by a fairly thick cortex while the center of the ovary is ocupied by a massive medulla of testicular type (Witschi 1935). In *Bufo* larvae one observes a struggle for either cortical or medullary predominance that sometimes is prolonged into the adult stage (Witschi 1933). Such observations are of interest because they suggest that the antagonisms develop in response to the presence of the respective inductor substance. Hence, corticin and medullarin each appear in the

role of an antigen while their antagonists resemble antibodies. Originally it had been proposed simply that each inductor releases a stimulating as well as an inhibitive principle (corticin$^+$ and corticin$^-$ or medullarin$^+$ and medullarin$^-$; Witschi 1937). But now, with an approach to some general serologic principles, this interpretation may be modified by ascribing each stimulator the character of an *antigen*, that causes the other inductor system to produce an *antibody* (Chang and Witschi 1956).

Thus the cortex is now presumed to produce *corticin and antimedullarin* and the medulla *medullarin and anticorticin*. That the antagonists issue from the gonads and not from some somatic sources is evidenced by a simple experiment with parabiotic salamanders. If in a heterosexual pair the predominant member is castrated, the reduced gonads of the partner at once begin to grow (Humphrey 1931, Witschi 1943). The reaction cannot be ascribed to hormones from the castrate's hypophysis for, at the larval stage, the cortico-medullary antagonism remains unaffected by ablation of the hypophyseal placode at the neurula stage (Chang 1955).

Further experiments will have to decide whether the inhibitive agents are produced independently or in response to the appearance of inductor substance of opposite denominator. They might shed some light also on the etiology of *human male pseudohermaphrodism*, which possibly results from an interaction between a male fetus and its mother (Witschi, Nelson, and Segal 1957). The fact that even in mildest degrees this malformation shows hypospadias, places its teratogenic origin into the beginning of the third fetal month, i.e. shortly following testicular differentiation. At that time an exchange of antigens and antibodies across the chorionic vesicle seems not too improbable. On the other hand this early loss, by the testes, of control over the course of secondary sex differentiation practically rules out the pregnancy estrogens as possible causative agents of male pseudohermaphrodism in man.

Chemical nature of inductive substances

Since the days of Herbst and Sachs countless morphogenic substances have been described. However the chemistry of actual induction in embryogenesis is still a field of research full of exciting problems but lacking final solutions. In the intensive, worldwide search for the inductor of axial organization (Spemann's organizer) Needham (1942) and Waddington found that some steroids are powerful inductors; but effective are also many other materials and at the present the attention of investigators has turned to an entirely different group of substances, tissue proteins and their fractional derivatives (Yamada and Takata 1956).

The analysis of the inductors of sex differentiation meets with particular difficulty because it must deal not with one single factor but with a pair of interacting systems, each with stimulating as well as inhibiting functions. Clearly, in *Bufo*, feminization after surgical ablation of the testicular gonad region is brought

about by removal of an inhibitor. But how about the mechanism of feminization of genetic males in the administration of estrogenic hormones (Chang 1955)? Is estradiol likewise offsetting the medullary inhibitor or is it directly stimulating the cortex? The fact that in the treated larvae the cortical lobes are reduced in size (Takahashi 1956) speaks against the second alternative. Direct observation shows that medulla and cortex both are retarded, apparently the former more than the latter.

Since steroid hormones often or always produce such inhibitory effects (Witschi 1950), the question arises whether or not the natural inhibitors, *antimedullarin* and *anticorticin*, might be of their class. Even a cursory examination of the review of steroid hormone effects presented in Table 1 suggests a negative answer. But before entering upon the discussion of this table, it is important to remember that in heterogenous parabiotic twin combinations the interactions often differ markedly from those in homogenous pairs (Witschi and McCurdy 1943). Such taxonomic specificity indicates that in all probability *the antagonists are proteins*, certainly not steroids.

Turning to the consideration of *corticin* and *medullarin*, the primary, stimulating inductors of sex differentiation, no direct indication of their chemical nature seems available. If it were assured that the inhibitors are their antibodies, a protein-like constitution could be postulated because, as stimulators, they would appear in the role of antigens. However at the present this concept should be valued only as a working hypothesis. This seems the more appropriate as some investigators are inclined to assume direct induction by steroids. Vannini (1950) in a thorough discussion of this problem conceded that the specific inhibitors may well be proteins, but held that testosterone and estradiol, or some closely related substances, can actually be recognized as the primary medullary and cortical inductors. His conclusions however were mainly based on *experiments with frogs*, which, as examination of Table 1 shows, hold a quite exceptional position. At the larval stage masculinization by androgens and feminization by gynogens are obtained with great ease. However one must not overlook the fact that induced feminizations according to Gallien's (1944) experiments are only transitory effects. Since high dosages of estradiol are masculinizing (Padoa 1938) it appears that both hormone types have only one and the same *lasting effect*. In experiments spreading over many years and involving thousands of tadpoles it was also found that many other steroids, but particularly progesterone, pregnenolone, dehydroandrosterone, desoxycorticosterone, cortisone, hydrocortisone, and adrenosterone produce masculinizing effects, each at characteristic levels of concentration. Equilenine feminizes larvae, like estradiol, but it is not known whether the reversal of the males might last beyond metamorphosis and until maturity. Stilbestrol, benzestrol, and pregnandiol were without effect over a wide scale of concentrations (unpublished assays, Iowa laboratory).

Quite contrary are the results obtained with *lower anurans and salamanders*.

Table 1. Effects of steroid hormones on gonad differentiation in vertebrates with urogenital connection in the male sex.
fem. feminizing; *masc.* masculinizing; *inc.* incompletely; *temp.* temporarily.

	Androgens	Gynogens	Authority
Selachians	Feminizing	Feminizing	Chieffi
Rana (XY)	Masculinizing	Temp. feminizing high doses : masc.	Dantchakoff, Foote, Gallien, Mintz, Padoa, Vannini, Witschi
Pseudacris (XY?)	Masculinizing	Feminizing (temp. ?) high doses : masc.	Chang, Foote, Witschi (unpublished)
Bufo (ZW)	—	Temp. feminizing	Chang
Alytes	—	Feminizing	Witschi
Discoglossus	—	Feminizing	Gallien
Xenopus (ZW)	—	Feminizing*	Allison, Chang, Gallien, Segal, Witschi
Hynobius	Feminizing	Feminizing	Hanaoka
Ambystoma (ZW)	Feminizing	Feminizing	Bruner, Burns, Foote, Mintz, Witschi
Pleurodeles (ZW)	Feminizing	Feminizing*	Gallien, Mintz
Triturus	—	Feminizing	Uchida, Hanaoka
Lizards	Inc. feminizing	Partly fem.	Dantchakoff
Birds (ZO)	Inc. feminizing (partly masc.)	Partly fem.	Boss, Dantchakoff, Gallagher, Kozelka, Willier, Witschi, Wolff
Opossum	—	Partly fem.	Burns
Rodents (XY)	—	—	Bruner, Green, Ortiz, Price, Raynaud, Witschi

* Sex reversed animals were raised to reproductive age and bred.

Here, all types of steroid hormones are feminizing, if they affect sex differentiation (Table 1). The lasting character of feminizations has been proven by breeding experiments with sex reversed genetic males of *Pleurodeles* (Gallien 1954) and *Xenopus* (Chang and Witschi 1956). Treating *Pleurodeles* larvae with testosterone, Gallien and Mintz obtained a total of 100♀ + 3⚥ + 2♂. Similar work with *Ambystoma* by Foote, Bruner and Witschi produced 138 ♀ + 74 ⚥ + 5♂. Together these 238♀ + 77⚥ + 7♂ illustrate beyond possible doubt the feminizing effect of androgens on sex differentiation in salamanders. The work of Hanaoka on *Hynobius* points in the same direction. Moreover Asayama (1951) has shown that also desoxycorticosterone has a feminizing effect on gonad differentiation of *Hynobius*. In our own laboratory we used all-female genetic stock of *Ambystoma* (received from Dr. Humphrey) to test the possibility of masculinizing influences of testosterone (Mintz 1947), methyltestosterone, ethinyltestosterone, pregnenolone and cortisone. No sign of masculinization was observed at any dosage level. At the present time we know of no hormone that shifts sex differentiation of salamanders or *Xenopus* in the testicular direction.

In conclusion, two groups of amphibians can be distinguished which respond in opposite directions to the administration of steroid hormones. The characteristic results are *masculinization in frogs and evidently also treefrogs*, but *feminization*

in other anurans and urodeles. It is noteworthy that so far the separation line coincides with that between the XY and ZW chromosomal types.

In *salamanders the results of hormone treatments are in sharp contrast with those of heterosexual parabiosis and of gonad transplantation.* In well known experiments Humphrey (1945) even succeeded breeding normal with sex reversed female *Ambystomas* (ZW×ZW). The gonads of the latter contained several testicular segments which had developed *under the influence of implanted testes.* Complete spermatogenesis was also observed in the gonads of a female (ZW) *Ambystoma* for four years in parabiosis with a male (ZZ); (Witschi 1937). In view of the fact that in the same species administration of so-called androgens feminizes testes, but does not alter ovarian development, the conclusion seems unavoidable that the natural inductor substances are not of steroid nature. Statements to the contrary by Burns (1939, 1955) evidently are based on unfortunate errors in the evaluation of rudimentary gonads from young animals. Also, his conclusions are not supported statistically by the inadequate material (11 animals in the testosterone experiment).

Pertinent and even of a particular interest are some experiments by Ichikawa (1937) with newts. Testes implanted into castrated females produce enough androgen to masculinize the nuptial sex characters. But this does not suppress the progress of testicular ovogenesis in large parts of the transplants. Whatever the exact chemical nature of newt androgen may be, the case proves that at physiologic levels it is not an inhibitor of cortical induction.

In *birds* and *mammals* (Table 1), only instances of partial and temporary sex modification have been obtained by the application of steroid hormones. Experiments with chicks offer a remarkable parallel to those with salamanders. Most hormone treatments produce degrees of feminization, though the systematic search of 16 androgenic substances by Wolff (1949) revealed also masculinizing effects by testosterone and androstanediol at certain dosage levels.

Numerous attempts to gain control of sex differentiation in mammals by administration of steroid hormones to pregnant mothers, or directly to embryos and fetuses, were without result. Yet, some side effects, inhibitions and minor abnormalities, give evidence that the hormones pass the chorion without difficulty. These experiments provide no support for a contention that the agent responsible for the free-martin effect in cattle twins might be a steroid hormone.

Blastophthoria and masculinization

Since first reported by Pflüger in 1882, it has been amply confirmed that delayed fertilization of frog eggs may become the cause of a shift in the sex ratio in favor of the male. Later work revealed that overripeness leads to degradations of the egg and the germ, falling under the general class of blastophthoria. Not all parts of a germ need be affected at the same rate; but among the many ensuing deficiencies (Witschi 1952) degeneration of primordial germ cells is one of the more

conspicuous. In cases of heavy damage the gonia entering the gonad primordia are few and small. If less affected, the original number may still be considerable but they are slow to multiply and often even diminish in number. Analogous observations in *Hynobius* are reported by Mikamo (1956).

As a consequence of gonial deficiency the gonad primordia of affected animals, whether genetically male or female, have only a poorly developed cortex with few follicular cells. Since an obvious correlation exists between the number of gonia and that of follicle cells, the inductive capacity of the cortex is lowered. Consequently the medulla, being not similarly affected, sooner or later may start a compensatory development that leads to testicular differentiation, also in the genetic females.

While thus the analysis of masculinization by blastophthoria corroborates the inductor theory, it also contributes toward an understanding of the *cause of sex reversal in man.* Advances in nuclear cytology have led to the recognition that men exhibiting the Klinefelter syndrome, particularly characterized by small testes, often are chromosomally of the female sex. Their infertility, and also the finding of intermediate stages of sex reversal, indicate that a paucity of germ cells at the time of embryonic sex differentiation was an essential cause of testis formation. The individual's female chromosomal constitution in no way opposes this course of development (Witschi 1957; Witschi, Nelson and Segal 1957).

Even though the analysis of the process of sex differentiation is foremost a subject of theoretical biology, the possibility of application and verification of discovered principles in studies of clinical cases and of human reproduction adds a good measure of importance and satisfaction to the more idealistic rewards gained from scientific work.

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