Olfactory Stimuli in Mammalian Reproduction

Odor excites neurohumoral responses affecting oestrus, pseudopregnancy, and pregnancy in the mouse.

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Almost all vertebrates are equipped with visual, olfactory, and auditory perception, but there is wide variation in the extent to which animals of different groups rely on the different senses for contact with their environment, and in the extent to which their lives are influenced by specific sensory stimuli. Among the higher vertebrates an instructive comparison can be made between birds and mammals. In most species of birds the sense of smell is poorly developed, and vision plays a major part in social and other responses. There are notable exceptions to this generalization, as was recently emphasized by Bang (*1*), but generally it is true that vision is far more significant in the life of birds than olfaction; in particular, visual stimuli are potent exteroceptive factors in the reproductive processes of birds, whereas olfactory stimuli are not known to have such a role. In mammals the situation is reversed. Many mammals have indifferent vision, especially color vision, and most depend more on smell than on sight to assess their environment; light and dark appear to be the only purely visual stimuli known normally to act as exteroceptive factors in mammalian reproduction. By contrast, smell is a potent factor in mammalian social life, and the odorous substances produced by mammals are implicated, as shown by well-authenticated observations, not merely in defense, territory marking, and identification but also in sex attraction and the evocation of sexual and aggressive behavior. Thus, Kelley (*2*) observed that the pregnant ewe, which is unattractive to the ram, could be made attractive by smearing her vulva and perineum with a swab taken from the vagina of an oestrous ewe. Interest in this field is age-old, as witness the traditional use of musk, civet, and castoreum (from the beaver) in high-quality perfumes. Whether there are interspecific effects of sex attractants, other than the conditioning of reflexes, may be doubted, but the intraspecific effects are well established.

Direct neural effects, however, are not the only ones evoked in mammals by odor. In the last few years evidence has accumulated that odor can constitute an exteroceptive factor affecting the oestrous cycle and pregnancy, and that in doing so it acts through neurohumoral mechanisms analogous to those evoked by visual stimuli in birds and by light and dark in certain mammals. The recognition of odor as an exteroceptive factor of this kind in mammals is comparatively new and is likely to open up a wide field of investigation. In this article we review the relevant evidence and discuss its wider biological implications.

Social Effects on Reproduction in Mice

Interaction between female mice (Lee-Boot effect). It is often assumed that the oestrous cycle in the mouse is spontaneous and regular until senescence, only being interrupted from time to time by pregnancy (or pseudopregnancy) and lactation. Recent observations, however, have shown that this traditional concept is far from complete, for it is now recognized that the oestrous cycle of the female is profoundly influenced by her social environment. These effects are produced by means of neurohumoral mechanisms for which the primary—perhaps the sole—stimulus is olfactory.

The earliest report of such a reaction came from workers in Holland, who found that when female mice were housed together in groups of four there was an increase in the number of spontaneous pseudopregnancies, which occurred in up to 25 percent of all cycles (*3*). That true pseudopregnancies rather than short periods of anoestrus were involved was shown by the fact that the formation of deciduomata was possible during the longer cycles induced by females in each other. The effect could be prevented by excision of the olfactory bulbs, or by housing the females individually (*4*). The prolonged cycles were apparently not dependent on physical contact between the females (*5*), although Dewar (*6*) observed male mating activities (chasing and mounting) between oestrous females in his colony and suggested that such behavior might have been a contributory factor.

Another type of interaction was observed when female mice were housed in larger groups of 30 per box; the oestrous cycles became highly irregular, and the majority of the mice became anoestrous for long periods. The reaction, however, was readily reversible, and over 90 percent of such females mated within 5 days of being paired with a stud male (*7*). Thus, it appears that the grouping together of female mice leads to mutual disturbance of oestrous cycles, by the intervention of pseudopregnancies if the groups are small or of anoestrus if the groups are large.

Effect of the male on the oestrous cycle (Whitten effect). At about the same time that these observations were made, Whitten (*8*) drew attention to the unexepected pattern of mating behavior among females housed in groups before being paired. He found that mating did not take place with approximately equal frequency on the first four nights after pairing, as it does in females which have been housed individually before the introduction of the male (*9*). Fewer females than had been expected mated on the first, second, and fourth nights, but mating on the third night greatly exceeded expectation, amounting, in the experiments reported, to 46 percent of the matings for all females paired, or to over 50 percent of the matings for females that mated within 4 days (Fig. 1). This reaction, also, was independent of contact between individuals. When the

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Table 1. Interactions between two strains in pregnancy block (23 or more females per group).

Stud ♂	Stud ♀	Strange or alien ♂	Blocked pregnancies (%) ○ ● P/CBA	Blocked pregnancies (%) ○ ● P/Dutch
♂ (○)	♀ (○)	♂ (○)	25	25
		♂ (●)	81	78
♂ (●)	♀ (○)	♂ (●)	16	30
		♂ (○)	88	87
♂ (○)	♀ (●)	♂ (○)	31	32
		♂ (●)	78	84
♂ (●)	♀ (●)	♂ (●)	*	48
		♂ (○)	52	88

*Not tested.

male was confined in a wire basket in the box containing the female for 2 days before being released, the peak mating occurred on the first night after his release—that is, as before, on the third night after exposure. Among such females, therefore, a new cycle had been initiated by the introduction of the caged male, so that oestrus was synchronized in the same way as by contact with the male.

It was also found that if a male was confined within a stock box containing 30 females, the oestrous cycle was shorter and more regular than it was in his absence and the incidence of abnormal cycles was reduced (*9, 10*). From his experiments Whitten concluded that the effects on the oestrous cycle and on mating behavior were brought about by olfactory stimuli from the males.

Effect of males on pregnancy (Bruce effect). If the newly mated female is removed from the stud male and exposed to other males of the same strain (strange males) and, more particularly, to males of a strain different from that of the stud male (alien males), pregnancy and pseudopregnancy (the expected result of an infertile mating) are both blocked, to a large extent. The female returns to oestrus within 3 to 4 days of exposure, as if coitus had not occurred, and if the strange (or alien) male has access to the female, fertile mating follows. Experiments in which genetically marked male mice were used show that all offspring born after such successive matings were sired by the second male and that, therefore, the original pregnancy from the stud mating was blocked. As in both the Lee-Boot and the Whitten effects, contact between the sexes is not necessary. Pregnancy-block occurs if the newly mated female is caged inside a stock box containing males (*11, 12*).

The female is vulnerable to the influence of males for the first 4 days of pregnancy, day 0 being the day on which the vaginal plug from the stud mating is found. Exposure of the female on day 5 *post coitum,* by which time discrete swellings are apparent in the uterus, is much less effective, and the reaction is virtually abolished by day 6. The response is slow to develop, exposure for at least 2 days being necessary to produce a maximum number of blocked pregnancies, although some females return to oestrus after an exposure of only 12 hours (*13*). Apparently successive pregnancies in a female can be blocked without impairing her subsequent fertility.

Strain Differences

Since alien males have much greater pregnancy-blocking capacity than strange males, strain differences are of particular interest in the block to pregnancy. As the reaction concerns three individuals (stud male, stud female, and strange or alien male), there are eight combinations in which the influence of two strains can be shown. The effect on the incidence of pregnancy block is given in Table 1. The experiment was performed twice, once with a randomly mated ablino strain P and inbred strain CBA mice, and again with the same albino strain in combination with randomly mated Dutch mice, offspring of some purchased from a dealer in 1959.

Irrespective of the strain to which the female belonged, pregnancy was blocked in about 30 percent of the females when the stud male and the second male belonged to the same strain; when they belonged to different strains it was blocked in about 80 percent of the females. The introduction of a third strain into the reaction failed to raise the incidence of blocked pregnancies; in a few tests involving five strains combined in various ways, 52 out of 65 females returned to oestrus within 7 days. Thus it appears that the induction of a blocked pregnancy is determined by the relationship between the males and the differences between them perceived by the female.

Exposure of the female to alien males before mating appeared to blunt somewhat her powers of discrimination. Reexposure to the same alien males after mating resulted in blocked pregnancies in only about 60 percent of the females 22 out of 36), while exposure to strange males of females exposed to alien males before mating resulted in blocked pregnancies in about 40 percent of the females (14 out of 33).

Furthermore, the presence of the stud male with the female largely eliminated the reaction of the female to alien males surrounding the pair. In such circumstances only eight out of 40 females returned to oestrus within 7 days. When the females were returned to the stud males after separation from them

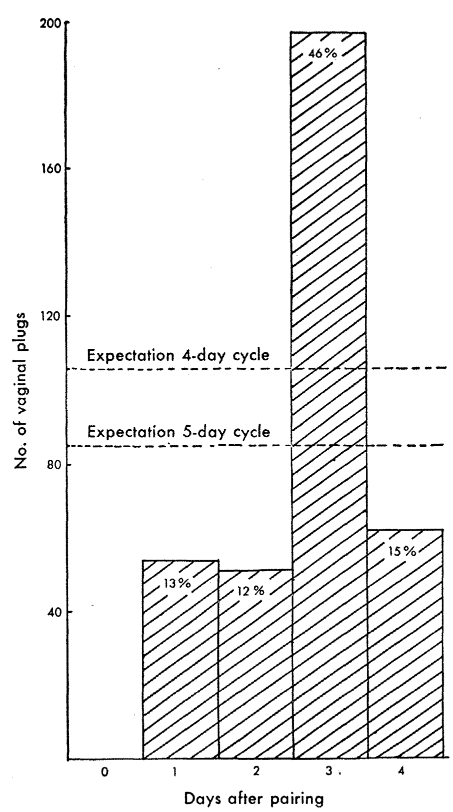

Fig. 1. Incidence of mating over the first 4 days after pairing: 425 females were paired, of which 364 mated within 4 days. [From W. K. Whitten (*8*)]

for 24 hours, none of 46 females returned to oestrus (*12*), 42 females carried the litter to term, and four became pseudopregnant. Thus, not only can the female "recognize" the stud male after separation from him for 24 hours but she can distinguish between males of his strain and males of a different strain; she can also, even without contact, distinguish between strange males and strange females. In the block to pregnancy, the key to this discrimination lies with the stud male.

The fact that pregnancy block is brought about by the proximity of males, and that physical contact with the newly mated females is unnecessary, shows that the effect is mediated by the appearance, sound, or smell of the males. It seemed unlikely for various reasons that sight was implicated, and as the effect is exerted in total darkness, it was felt that the conclusive experiment of blinding female mice was not justified. An investigation of the possible effects of sound was started by making a tape recording of the noises made by the male mice in the test situation, with the idea of subjecting newly mated female mice to repeated playbacks during the 3-day test period. Squeaking and chatterings of possible interest were picked up by the microphone placed among the males, but the main noise recorded was the general and obviously nonspecific pattering of tiny feet. Before the formidable technical difficulties in this experiment could be overcome, another line of work had made it unnecessary.

Whitten (*8*) had shown that the effect of the presence of the male on the oestrous cycle could be reproduced to some extent by placing the females in soiled cages recently vacated by males. Early attempts to bring about pregnancy block by the same method were unsuccessful, but finally the effect was produced in 28 of 33 newly mated female mice by placing them, under conditions of restricted ventilation, in 2-liter glass jars previously occupied by five alien males, on bedding known to retain mouse smell (laboratory coat cloth), and by replacing the jars with other soiled jars twice daily for 3 days (*14*).

Transference of this type of experiment from tall glass jars to ordinary flat mouse boxes showed clearly that the important variable was the twice-daily replacement of the container with a container vacated by males immediately before, rather than the restricted ventilation or the nature of the bedding. Thus, pregnancy was blocked in newly mated females placed individually in boxes vacated by five alien males to the extent of 83 percent and 74 percent, respectively, when the soiled boxes were renewed twice daily over 3-day and 2-day test periods (*15*). These figures are remarkably similar to those for the incidence of pregnancy block in females exposed for 3 or 2 days to the proximity of alien males (*13*). Results almost equally impressive were obtained when the males were allowed to soil sawdust bedding. Renewal of the soiled boxes once daily was much less effective.

The experiments with soiled boxes show that pregnancy block is caused by some substance coming from the males and left behind by them, and the results throw an interesting light on the volatility or instability of the substance. There is a remote possibility that ingestion rather than olfaction is involved. However, the fact that neither pregnancy block (*16*) nor the Lee-Boot and the Whitten effects occur in females rendered anosmic by destruction of the olfactory bulbs seems to establish beyond reasonable doubt that olfactory stimuli are involved.

Consideration of individual and strain differences raises some interesting questions. It is most unlikely that every male mouse produces a different odorous substance. Probably "spectra" of odors are involved, which differ slightly between individuals of the same strain and markedly between certain strains. Even human beings can differentiate between the smells of some strains, as is well recognized by those who work with strains isolated in separate rooms. The differences are less obvious when, as is more often the case, several strains

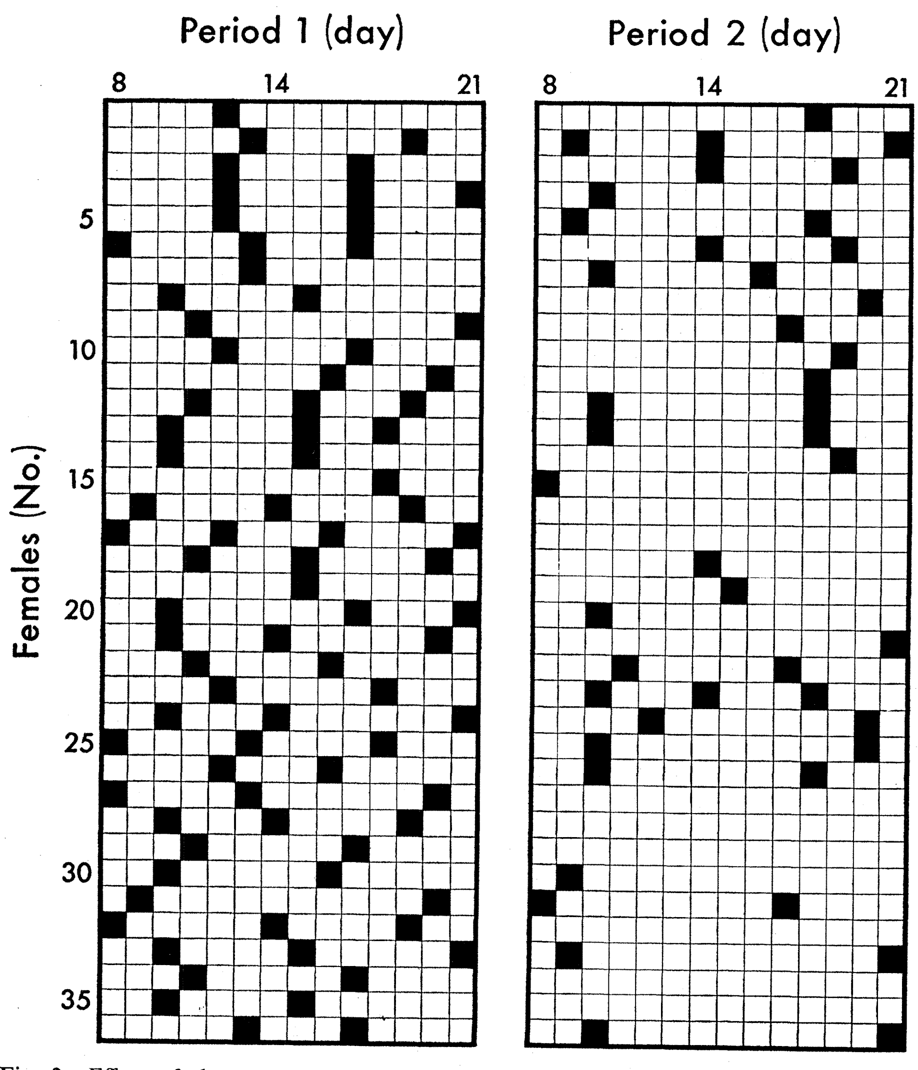

Fig. 2. Effect of the presence of a castrated male on the incidence of oestrus (solid squares). Each horizontal row represents one female. In period 1 (left), females were housed in small groups (two to five) with one castrated male; in period 2 (right), males were removed and all females were housed as a single group in a stock box. One week was allowed for adaptation at the beginning of each period; vaginal smears were examined daily during the second and third weeks.

are kept in one room. There is almost certainly some overlapping of the "spectra" of odoriferous substances contributing to the final effect; it is possible to discriminate more easily between certain strains than between others. In a recent experiment, expert perfumers found that five strains of mice kept at the National Institute for Medical Research fell broadly into two odor groups. In the light of the experiments on strain differences, it is not so much the olfactory acuity as the olfactory memory of the females which is remarkable.

Source of the Male Odor

So far, no information has been obtained as to the source or nature of the operative odor of the male mouse. It is not even certain that all components of the odor spectrum come from the same source. Sexual vigor plays little or no part in pregnancy-blocking capacity. Infertile males completely lacking in sex drive are as effective in inducing the block to pregnancy as are their fertile and more vigorous companions (*12*). Removal of the preputial glands from young adult male mice failed to impair their pregnancy-blocking capacity, 60 out of 81 pregnancies (74 percent) being blocked as compared with 58 out of 76 (76 percent) in concurrent experiments with intact males. Males castrated as young adults retain this capacity to some extent, and experiments now in progress indicate that even when castration is carried out before puberty, the capacity to block pregnancy still develops. Castrated males also produce the Whitten effect (Fig. 2).

The males do not appear to react to the presence of females as regards their output of odorous substances. No difference in pregnancy-blocking capacity of individual groups of males relative to the length of the interval between tests was apparent. Removal of the olfactory lobes does not impair the pregnancy-blocking capacity in the male [50 out of 65 pregnancies (77 percent) were blocked by exposure to anosmic males].

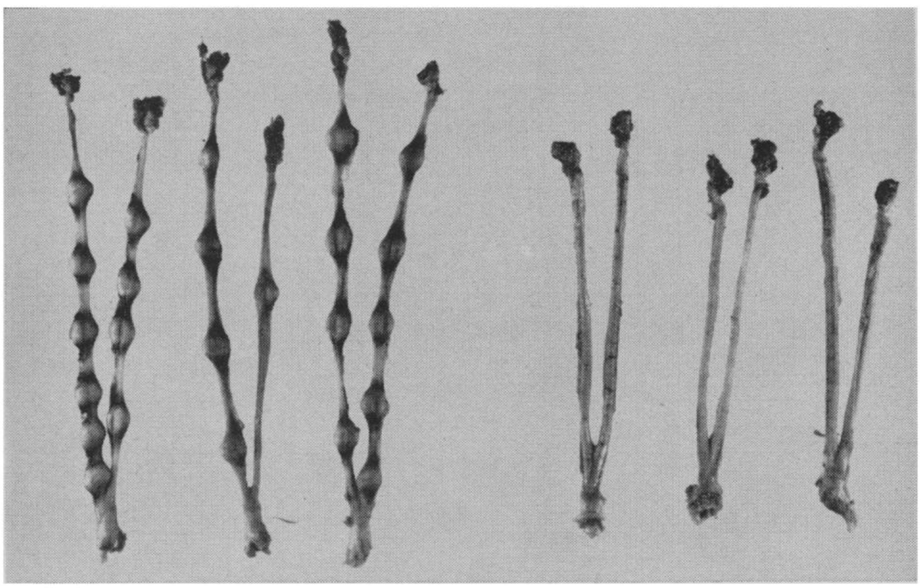

Fig. 3. Effect on implantation of exposure to strange male, as seen in mouse uteri 7 days after mating with stud male. (Group at left) Normal pregnancy; (group at right) pregnancy blocked by the presence of strange male 24 hours after stud mating. Second mating 4 to 5 days after first.

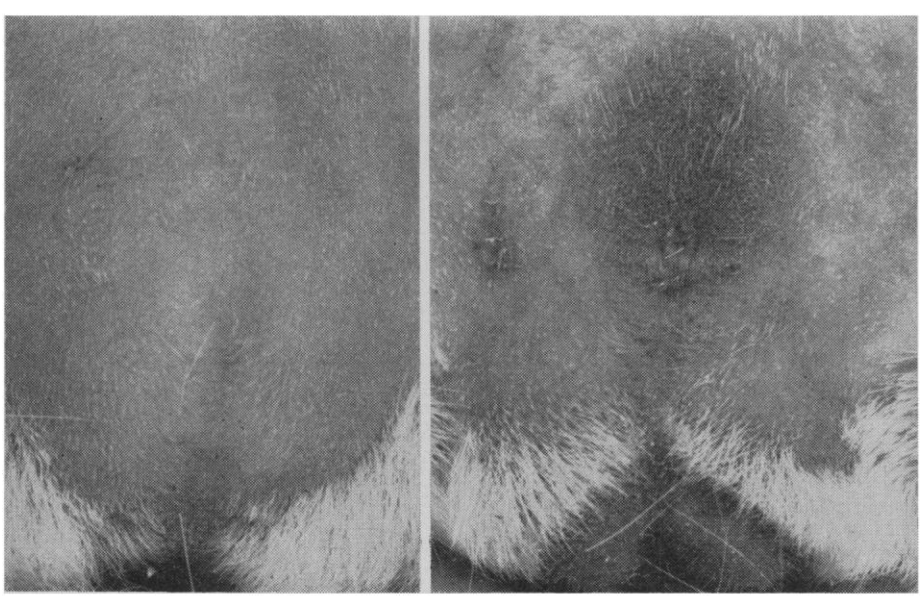

Fig. 4. Supracaudal gland of guinea pig (hair removed). (Left) Adult female; (right) adult male.

Endocrine Factors in Pregnancy Block

The experiments discussed above raise a host of questions about the chain of events by which olfactory stimuli lead to failure of pregnancy and especially about the hormone factors involved. The external symptom of pregnancy block—continuation of the 5-day oestrous cycle as though mating had not occurred—is highly suggestive of failure of the corpora lutea of ovulation to undergo the functional development that would normally occur after mating, with consequent failure of progestational changes in the endometrium and inhibition of implantation. This interpretation accords with the fact that implantation does not take place in a blocked pregnancy (Fig. 3), that the segmented eggs cannot be recovered after they leave the fallopian tube, and that histological examination of the ovaries shows that in many cases the corpora lutea have not developed as they usually do after mating (*12*). The question arises, therefore, as to why development of the corpora lutea does not occur. The effect of the presence of males in interrupting anoestrus of social origin in mice presumably depends on the induction of a flush of follicle-stimulating hormone from the adenohypophysis. Similarly, the effect of the presence of males in interrupting socially induced pseudopregnancy might be due either to a flush of this hormone, terminating pseudopregnancy and actively causing the return of oestrus, or to a breakdown in the supply of prolactin, which would terminate pseudopregnancy at an early stage and allow the continuation of the normal 5-day cycle.

The possibility that the test situation is sufficiently stressful to excite adrenal complications has also to be considered. In the light of these considerations, experiments were carried out to determine whether pregnancy block could be prevented by the administration of Luteotrophin (prolactin) and whether olfaction-induced pregnancy block could be simulated by the injection of follicle-stimulating hormone or ACTH.

The results were clear-cut. Ten international units of prolactin given daily over the 3-day period during which the females were in the presence of alien males prevented the block to implantation, while block occurred, as usual, in controls (*17*); injection of substantial doses of follicle-stimulating hormone or of ACTH, once, or daily over the usual test period, to newly mated females isolated from males did not produce pregnancy block. It seems, therefore, that the immediate endocrine cause of the block is failure of prolactin secretion by the adenohypophysis, with consequent failure of corpus luteum development and return to oestrus in the ordinary way. This interpretation is confirmed by the fact that pregnancy block on exposure to the presence of males does not occur in lactating mice mated at post-partum oestrus, presumably because of the increased amount of circulating prolactin (*18*). How the failure of prolactin secretion is initiated by the olfactory stimuli is not known, but no doubt the hypothalamus is involved.

The Odoriferous Glands of Mammals

The dramatic effects produced in female mice by odorous substances emanating from the male but of unknown nature and origin raises acute problems about animal odors. The odoriferous glands of mammals differ remarkably in origin and position and in the nature of their products. As Champy (*19*) remarks, they have nothing in common except their odoriferous nature. Anatomically they may be occipital (Arabian camel), suborbital (antelope), on the throat (Californian mastiff bat), scapular (fruit bat), sternal (opossum), lateral (shrew), dorsal (golden hamster), axillary (man), supracaudal (guinea pig) (Fig. 4), interdigital (ruminants), or even scrotal (potto). In mice they have been described on the soles of the feet (*20*) and elsewhere. Probably the best known odoriferous glands in mammals are the anal or perineal glands of the stoat, civet, and other Mustelidae and the preputial glands of the musk deer, the muskrat, and the beaver. A vast amount of information on the location and morphology of the odoriferous glands of mammals is to be found in *Die Hautdrusenorgane der Saugetiere*, by Schaeffer (*21*). Such glands are not, of course, restricted to mammals. The fetid smell of the crocodile, for instance, arises from so-called musk glands in the cloaca. Moreover, specific odorous substances are not only produced by special glands but may appear in the urine or feces from hepatic or other sources.

Interpretation of the significance of the odoriferous glands is complicated by the fact that they may or may not be present in both sexes, and that they may or may not be affected by gonadectomy and gonadal hormones. The anal glands of the rabbit are very similar in buck and doe, are reduced in both sexes by gonadectomy, and are restored, though somewhat differently, by both estrogens and androgens. The supracaudal gland of the guinea pig is developed only in the adult male, is reduced by castration, and is restored by androgen. The flank glands of the golden hamster differ superficially in male and female only in size. In rats and mice, preputial glands are present in both sexes and in both are affected by gonadectomy and gonadal hormones. The sternal gland of the opossum is much better developed in the male, is reduced by castration, and is restored by testosterone. The malodorous flank gland of the male goat is also reduced by castration. On the other hand, gonadectomy does not affect the lateral gland of the short-tailed shrew, which is well developed in males and non-oestrous females but is reduced at oestrus and reduced still more during pregnancy (*22*). Other examples could be given showing the difficulty of making generalizations in this field.

In spite of the wealth of anatomical information available, little is known

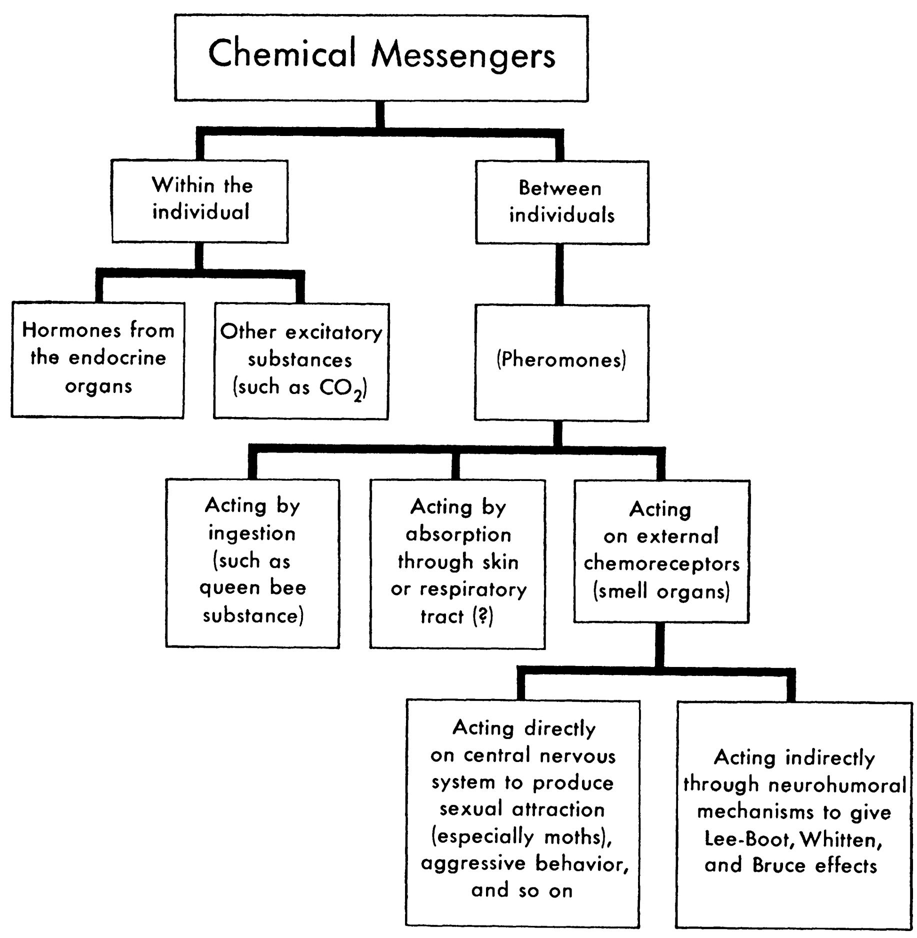

Fig. 5. Types of chemical messengers, especially types of those that integrate populations of individuals (pheromones).

about the nature or functions of the substances produced. Because of the interest of the perfume manufacturers, a great deal of chemical work has been done on the preputial glands of the musk deer and muskrat and on the perineal glands of the civet cat; muskone, civetone, dihydrocivetone, and a large number of other macrocyclic compounds have been isolated from these sources. Muskone has also been isolated from the preputial glands of the boar (*23*). Civetone is of especial interest because it has a chemical resemblance to Δ^{16}-androstenol, a steroid with a pleasant musky smell, related to the male hormones and present in pig testes and human urine. In other ways, also, the musk odor is far from specific, and a large number of artificial musks (many bearing, to the layman, little olfactory resemblance to natural musk) have been prepared; some of them (for example, musk xylene) are manufactured in enormous quantities for inclusion in cheap toilet preparations. From the point of view of the biologist, however, chemical work on animal odors has hardly begun.

Exocrinology

The odorous substances produced by male mice and effective under certain conditions in blocking ovoimplantation in females obviously belong to a group of substances, now coming into prominence, which offer an interesting contrast with hormones. According to Starling's classic definition, hormones are substances produced in one part of the body and carried by the circulating blood to another part where they evoke a response. This definition could include excitatory substances such as carbon dioxide, but in the course of time the term *hormones* has become restricted to the products of internally secreting glands, the endocrine organs. In any case, hormones are involved essentially in the integration of the individual. But Starling also used the term *chemical messengers* in this connection, and this term can now be given a much wider connotation, to include not only internal secretions involved in the integration of the individual but external secretions involved in the integration of populations of individuals (Fig. 5).

A notable example of this group of substances has recently been the subject of outstanding work by Butler, Callow, and Johnston (*24*)—that is, the queen substance of honey bees, 9-oxodec-2-enoic acid (*25*)—which is secreted by the mandibular glands of the queen bee and is ingested by the worker bees (undeveloped females), who are thereby retained in sexless subjection. If the supply of this substance is cut off by the death of the queen, the ovaries of some of the workers may develop, but the most obvious response is that the workers start to build queen cells in an attempt to requeen the hive.

The effect of the first-hatched mosquito larvae on the remaining unhatched eggs of the batch (*26*) may also be due to an externally secreted chemical messenger. Other interesting examples of these external chemical messengers, for which the name "pheromones" (*27*) has recently been suggested (*28*), are the sexual attractants of moths, which, at relatively enormous distances and in fantastic dilutions, stimulate the chemoreceptors of the antennae of the opposite sex. The sexual attractants of moths seem to be strictly analogous to the odorous substances of mammals which serve to mark sex and oestrous condition and evoke sexual behavior. Such effects, however, must be clearly distinguished from those that lead to pregnancy block in mice. Sex attraction and sexual and aggressive behavior induced by odors is an instantaneous, or at least very rapid, response, evoked directly through the central nervous system and needing only a brief stimulus. In strong contrast, pregnancy block in mice depends on a neurohumoral chain of events, initiated by a continuous olfactory stimulus which must last 2 to 3 days for maximal effect.

The effects of the presence of the male on the oestrous cycle in mice (*8*) and possibly in sheep (*29*) and goats (*30*) and on implantation in mice (*11*) appear to be the first effects of their kind to be described in mammals. The extension of this line of work to other species, perhaps even to man, and the identification and location of the substances involved are likely to be of absorbing interest. Endocrinology has flowered magnificently in the last 40 years; exocrinology is now about to blossom (*31*).

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31. We thank Dr. Dorothy Price for calling our attention to the supracaudal glands of the guinea pig, and Messrs. Ernie, Ferguson, and Kingston of Boake A. Roberts and Co. Ltd., for their comments, as expert perfumers, on the mouse odors characteristic of our various strains. We are also indebted to Dr. Delphine Parrott for the removal of the preputial glands and for the anosmic male mice, and to Miss M. V. Mussett for the tests of statistical significance. We acknowledge, with thanks, the generous supply of strain CBA mice made available by Dr. Mary Lyon of the Medical Research Council (Radiobiological Research Unit), Harwell.