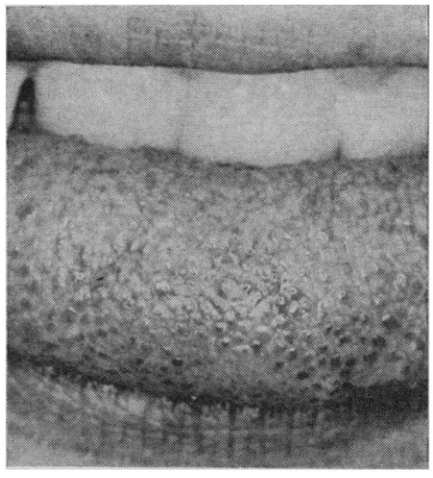

Fig. 1. Tip of the tongue of a healthy child. The dark spots represent blood vessels seen through the clear epithelium of the fungiform papillae.

This observation may also advance our understanding of the disease. The absence of taste buds provides an anatomical basis for deficiency in taste discrimination. It also raises the question of how much of the symptomatology might be attributed to some general defect in peripheral receptors. The mechanism for the absent axon flare is primarily peripheral (*6*). Many other features of the disease—the subnormal response to oxygen and carbon dioxide (*7*), postural hypotension, and areflexia—may also have their origin in defects of peripheral receptors.

In test animals, severing the chorda tympani is followed by disappearance of the taste buds (*8*). Examination of the tongue in four human subjects with a history of destruction of the chorda tympani incidental to middle ear surgery revealed, in two of them, a very striking reduction in fungiform papillae. Although we cannot say now whether the papillae are present but inapparent because of some transformation, or whether they have disappeared, the finding does demonstrate a strong interdependence of the papillae and their neural connections and suggests that a neural deficiency during the development of the fetus might result in lack of formation of papillae and the associated taste buds. A recent finding favors this possibility. In the embryonic chick that is functionally denervated with botulinum toxin, the effector organ, in this instance skeletal muscle, fails to develop normally (*9*).

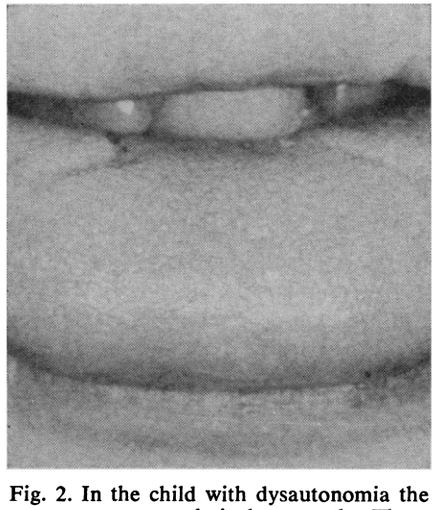

Fig. 2. In the child with dysautonomia the tongue appears relatively smooth. There are no fungiform papillae.

A study with the electron microscope (*10*) of the sequence of development of the fungiform papillae and taste buds in rats revealed that the papillae develop in the absence of any demonstrable neural elements in the immediate vicinity. Nerves then grow inward, and taste buds appear. Thus the papillae appear to arise in response to some non-nerve stimulus. Hence the basic mechanism for dysautonomia may not be neurogenic. A deficiency in some non-neural, controlling factor may be primary, such as the salivary gland factor described recently by Levi-Montalcini (*11*).

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X Chromosome DNA Replication: Developmental Shift from Synchrony to Asynchrony

Abstract. *The X chromosome in* Melanoplus differentialis *is negatively heteropycnotic in the early spermatogonial cell generations but positively heteropycnotic in the final premeiotic interphase. Autoradiography after administration of tritiated thymidine reveals that this condensation change is paralleled by a change in the time of DNA replication in the X chromosome relative to that in the autosomes.*

Sex chromosomes which show precocious condensation during interphase (positive heteropycnosis) are asynchronous in DNA replication; DNA synthesis continues in such chromosomes after it ceases in most other chromosomes (*1*). Developmental changes in the pattern of chromosome condensation are well known and provide an opportunity for testing the possible correlation of replication sequence and heteropycnosis. We have studied DNA replication in testicular cells of the grasshopper *Melanoplus differentialis* in which a striking change in the pattern of X chromosome condensation occur. Thus the X is positively heteropycnotic and forms a typical sex chromatin body in the final premeiotic interphase and meiotic prophase (Fig. 1*b*), but the X is negatively heteropycnotic in the earlier spermatogonial cell generations where no sex chromatin body is visible at interphase (Fig. 2*b*) and where the X is slightly undercondensed at metaphase (Fig. 3, *b* and *d*) (*2*, *3*). We have observed a developmental change in the relative time of X-chromosome replication which parallels this change in chromosome condensation.

Melanoplus differentialis males (*4*) under light CO_2 narcosis were injected with tritiated thymidine (*5*) and either killed after 25 minutes or given at that time a second injection of nonradioactive thymidine (*6*) and then killed at 2- to 4-hour intervals from 4 to 46 hours after injection. The testes were fixed, stained in bulk by the Feulgen reaction, and squashed, and autoradiographs were prepared (*7*).

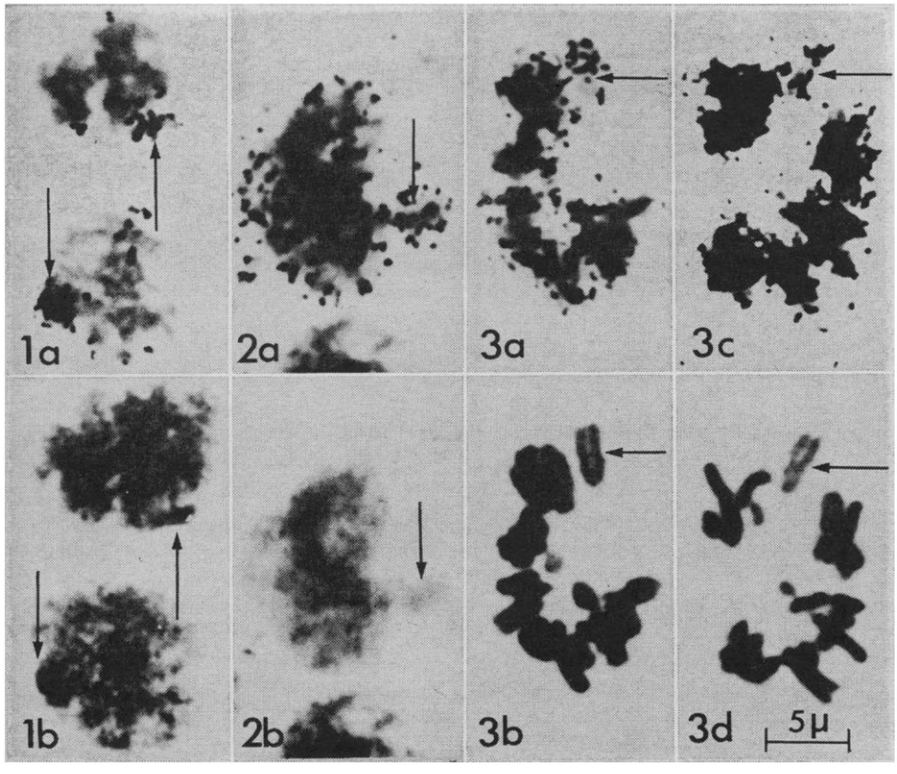
We have confirmed earlier reports (*2*, *3*) that the X chromosome in younger spermatogonia can be identified during interphase by its characteristic position (Fig. 2*b*); the X can be positively identified at this site in late

anaphase and early prophase, when it is negatively heteropycnotic. If asynchronous replication occurs the autoradiographs prepared from material fixed 25 minutes after application of precursor should show some interphase nuclei with the silver grains largely restricted to the X chromosome. However, no examples of this were seen in 1031 nuclei scored; a typical example of the usual X plus autosomal pattern found is shown in Fig. 2. But in this same material the earlier reports of incorporation only into the X in the immediately premeiotic nuclei were confirmed, 13 percent of 967 nuclei showing this pattern (Fig. 1). The complementary class, labeled only over the autosomal region, occurs both in younger spermatogonia (4 percent of the total) and in immediately premeiotic spermatogonia (26 percent of the total). Thus there may be a very short time period in the younger spermatogonia when some autosomes, but not the X, are in DNA synthesis. However, this is not certain since at the relatively low grain densities employed some labeling which is limited to the autosomes will be due to the randomness of radioactive decay. Thus no asynchrony of X and autosomes is detectable in younger spermatogonia; but the question arises whether the parameters of DNA synthesis are such that asynchrony is present but not resolved. For example, temporal resolution would be reduced if the duration of DNA synthesis were shortened, if the duration of the tritiated thymidine pulse were unaltered. This question was examined by counting silver grains over younger and older spermatogonia to obtain an estimate of the relative rate of incorporation. Incorporation into younger spermatogonia is more rapid; they have an average of 2.8 times as many grains as older (positively heteropycnotic X) spermatogonia (100 nuclei of each type were scored). But asynchrony as great as in the positive-X spermatogonia would still be detectable if present in the earlier spermatogonial generations. Thus at a threefold reduction in temporal resolution, 4.3 percent (⅓ of 13 percent) of the younger spermatogonia should show labeling of the X only. Actually, of the 967 nuclei scored, not one showed this pattern.

Table 1. Labeling of mitotic chromosomes in 698 spermatogonial cells fixed at various intervals after injection of tritiated thymidine.

Injection-fixation interval (hours)	Labeling pattern (No. of cells)	
	X plus autosomes	Autosomes only
4	8	8
8	67	7
10	63	8
12	59	5
14	28	1
16	59	1
18	50	6
20	80	7
22	61	0
24	16	0
32	108	10
40	41	5

This result has been checked by a second, more sensitive, method based on the labeling pattern in prophase or metaphase cells of testes fixed at intervals after injection of tritiated thymidine (*8*). As successively greater intervals elapse between injection and fixation, labeled prophases and, later, metaphases are seen; if the cell population is reasonably regular in progression to division, most such nuclei will have been at the very end of DNA synthesis when labeled precursor was available. Late replication is readily detected by this method even if the labeled precursor is continuously available and if progression to division is irregular (*9*). Metaphases in materials fixed at still greater intervals after injection provide information concerning the incorporation pattern at correspondingly earlier portions of the period of DNA synthesis. One experimental run will be described in detail; less systematic but confirmatory results were obtained in several other experiments. Under our conditions (*4*) these general observations apply. In material fixed 4 hours after injection, about 20 percent of the spermatogonial prophases are labeled; at 8 hours, about half the metaphases are lightly labeled; at 10 hours, 95 percent of the metaphases are labeled; at longer intervals up to 40 hours, all metaphases are labeled. Thus the interphase period after synthesis averages about 3 hours, and the period of DNA synthesis about 35 hours. The DNA replication pattern in prophases and metaphases where the negatively heteropycnotic X



Figs. 1–3. Tritiated thymidine autoradiographs of *Melanoplus* spermatogonial nuclei before (above) and after (below) removal of the silver grains. The arrows indicate the X chromosome. Fig. 1. Last premeiotic interphases showing labeling largely limited to the positively heteropycnotic X. Figs. 2 and 3. Earlier spermatogonial nuclei showing the usual X plus autosome labeling pattern. Fig. 2. Interphase. Fig. 3. Two examples of metaphases with slight negative heteropycnosis of the X. Fixed 10 hours after injection of tritiated thymidine.

can be certainly recognized is given in Table 1. Undercondensation alone permits recognition of the X in prophase, but in metaphase the undercondensation is often marginal, and the separation of chromosomes is generally incomplete (Fig. 3). However, characteristically the X lies away from the autosomes, and then even slight heteropycnosis can be diagnosed. Furthermore the daughter chromatids of the X are well separated distally at metaphase, and this is not seen in the autosomes until late metaphase or early anaphase (Fig. 3) (*3*). Labeling of the X alone was never seen, even in the labeled cells fixed at 4, 8, and 10 hours. Only the autosomes are labeled in 8 percent of the cells; this is a high estimate and at no time is there a large fraction of such cells. Hence it appears that the negatively heteropycnotic X chromosome of young spermatogonia undergoes DNA synthesis throughout the period of DNA synthesis, although at any time synthesis may be low or absent in the X of some cells. Certainly the typical pattern (92 percent of the cells) is as shown in Fig. 3. The density of silver grains in this series of slides is not consistently different from that in the materials fixed 25 minutes after injection of tritiated thymidine, an indication that the pulse duration is about half an hour as suggested by Lima de Faria (*1*). Thus the pulse duration is about 1/70 of the period of DNA synthesis; in other words—temporal resolving power is very high.

We conclude that in cell generations in which the X chromosome is negatively heteropycnotic, its replication is synchronous with autosomal replication, but in the last premeiotic interphase the X is both positively heteropycnotic and asynchronous in replication. Similar developmental alterations in the temporal sequence of replication are implicit in recent studies on mammalian cells (*10*); ours is the first direct demonstration. This study provides additional support for the view that late replication and positive heteropycnosis are intimately, perhaps causally, related and hence provides some basis in fact for hypotheses relating late replication to the genetic repression associated with heteropycnosis (*11*).

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Tylotrich (Hair) Follicle: Association with a Slowly Adapting Tactile Receptor in the Cat

Abstract. *The small raised tactile areas on the skin surface of the cat which evoke action potentials that convey both temporal and spatial information to the central nervous system are* Haarscheiben *or tylotrich pads. Each pad is an integral part of a tylotrich follicle, a skin appendage that is highly specialized for sensory function.*

Slowly adapting cutaneous mechanoreceptors with afferent nerves have been described in the cat, dog, and primates (*1–4*). Iggo (*2*) and Tapper (*4*) have shown that in the cat the receptors are contained within specialized structures called touch spots, which are raised hemispherical domes on the skin surface. Touch spots are richly vascularized and contain specialized cells in the epidermal layer (*3*). Thus the touch spot seems strikingly similar in morphology to the well-known *Haarscheibe* (*5, 6*) or tylotrich pad (*7*).

We describe here the tylotrich pad in the skin of the cat and suggest that the pads are the structures seen by Winkelmann (*8*), as well as the structures also called "touch spots" or "touch corpuscles" (*1–4, 9*).

Three adult female cats were anesthetized and the hair was clipped from the right dorsa, venters, and hind limbs; the remaining hair stubble was removed with barium sulfide. Erected tylotrich pads were visible to the eye immediately after depilation, and this erectile response lasted 5 to 10 minutes. The pads ranged from 0.16 to 0.42 mm in diameter, and they were larger on the caudal venter than on the caudal dorsum (Table 1). Similar results are reported for the mouse and sheep (*10*).

About 60 tylotrich pads with some surrounding tissue were excised from the various skin areas. For study of nerve endings, some specimens were impregnated with gold chloride or were fixed in chloral hydrate and impregnated with silver nitrate (*11*). The remaining tissues were appropriately processed, then stained with alum hematoxylin and eosin, Weigert's hematoxylin and eosin, or van Gieson's picric acid–fuchsin stain and alum hematoxylin.

Each tylotrich pad is composed of a thickened and distinctive epidermis underlaid by a convex area of fine connective tissue that is highly vascularized and well innervated (Fig 1, *a* and *b*). Along the base of the pad epidermis,

Table 1. Diameter of tylotrich pads in the cat.

Caudal area	Pads		t*	P†
	Observed (No.)	Diam. M ± S.E. (mm)		
Dorsum	23	.256± .007	3.16	<0.01
Venter	21	.300± .012		

* Based on Fisher's small sample t-test.
† Probability that the difference between the means is due to chance alone.