women beyond middle life by vertebral and femoral fractures. The fractures appear to result from an age-related loss of bone mass, judged from the well-known reductions in cortical and trabecular thickness and from decreasing femoral density (total weight divided by total volume) (*3*). Reductions in mass of 25 to 50 percent have been estimated for osteoporotic spines of women in whom rates of calcium accretion have been found normal (*4*). To explain this discrepancy, increased resorption rates have been proposed (*4*), but seemingly relevant is our evidence in "averaged" aging femurs that remodeling occurs without apparent net loss of compact bone and that theoretical surface areas substantially increase. From the youngest to oldest groups, vertebral osteoporosis of significant degree increased from 19 to 90 percent (*5*). Thus, from mean data only, femurs of largest diameter and surface areas were found in the group with highest incidence of significant vertebral atrophy.

Our measurements were made at the section where the transverse diameter, although minimal, is less than the anteroposterior diameter which is enhanced by the prominent linea aspera. Dimensional changes of similar type and magnitude would not be expected in other sections of the femur with different stress-structural relationships. Indeed, as shown in Table 2, the increases in diameter with age were less at sections above midshaft, 1.8 mm at the section (circular) just below the lesser trochanter [section 24 of Koch (*2*)] and only 0.9 mm at the femoral neck section of minimal diameter.

This suggests that flexural stress with bowing, which is maximal at about midshaft, activates the periosteal accretion of bone. Meanwhile the trabeculated, nontubular and, thus, more rigid femoral neck undergoes a proportionately smaller increase with age. For older femurs at midshaft, gains in section moduli mean increased resistance to flexural forces. This has more significance for subjects in whom skeletal ingredients are diminished, since the resistance of the shaft to flexure can be maintained even with less bone provided it has been remodeled into a cortex of larger diameter.

However, this entire osteoporotic femur will bow less and store less elastic energy. With resistance to flexural forces being decreased in the neck relative to the shaft, the femoral neck becomes the most vulnerable site for fracture. Similarly, trabeculated, nontubular, rigid vertebrae become prone in later life to compression fractures from minimal flexural forces since there is no compensatory increase in vertebral diameter with age (*5*).

We have not shown that all femurs participate similarly in structural remodeling which, since mean data only are presented, could represent either a progressively differing population sampled for successive age groups or an increasing rate of "dropping out" of women with smaller femoral diameters. However, these two explanations seem untenable since the diameters of both the metacarpal and lumbar vertebrae remained constant despite significant cortical thinning (*5*). Whereas the mean diameters of adult radii are unchanged with age (*6*), rib diameters increase (*7*). If changes in the tibia and fibula are also found to parallel those of the femur, they may reflect a progressive adaptation to the erect state in which flexural and longitudinal compression forces on leg bones from lifelong weight-bearing decline proportionately less than do predominantly flexural forces on the arms and predominantly compression forces on the spine.

Table 2. Periosteal diameter of femurs at three sites. The subjects were selected at random from the oldest and the youngest groups.

Age group	No. of subjects	Periosteal diameter (mm)*		
		Midshaft	Subtrochanter†	Femoral neck‡
45–49	30	31.03 ±0.50	34.20 ±0.52	35.91 ±0.42
75–90	30	34.63 ±0.39	35.95 ±0.35	36.85 ±0.39
Increase		3.60	1.75	0.94
P		<.001	<.01	<.10

* Mean ± standard error. † Lesser; section 24 of Koch (*2*). ‡ At section of smallest diameter.

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1. From the point of measurement to the lowest point on the superior surface of the femoral neck, the mean distance was 19.0 ± 1.5 cm.
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9. The study was supported by funds from the USPHS, Division of Accident Prevention, under contract SAph. 77270.

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Identification of Acetylcholine in Sympathetic Ganglia by Chemical and Physical Methods

Abstract. *Extracts of sympathetic ganglionic chains contain a substance which behaves like acetylcholine biologically, chromatographically, and electrophoretically. The melting point of the tetrachloroaurate salt of this substance is identical to that of acetylcholine tetrachloroaurate. No other choline esters have been detected in these extracts. Perfusion of sympathetic ganglia with C^{14}-choline indicates that C^{14}-acetylcholine is released.*

Acetylcholine was the first choline ester isolated from natural sources (*1*). More recently, a number of choline esters have been found in animal tissues (*2*). The choline ester in sympathetic paravertebral ganglionic chains is thought to be acetylcholine (*3*). This hypothesis is based on results obtained with nonspecific chemical tests, bioassay procedures, and classical pharmacological tests. In the investigation described here, more definitive techniques

Table 1. The chromatographic behavior of the acetylcholine-like substance as compared with C^{14}-acetylcholine. Descending paper chromatography was conducted for 17 hours in each system.

Solvent (vol : vol)	R_F*	
	Extract †	C^{14}-ACh ‡
n-Butanol-water (9 : 1)	0.09	0.08
n-Propanol-water (9 : 1)	0.32	0.32
n-Propanol-water-formic acid (8 : 1 : 1)	0.65	0.65
n-Butanol-ethanol-acetic acid-water (8 : 2 : 1 : 3)	0.51	0.50

* Each set of R_F values for a solvent represents one experiment. † Purified acetylcholine-like substance obtained from beef sympathetic ganglia. ‡ C^{14}-acetylcholine.

were used to test the above hypothesis. Paper chromatographic and paper electrophoretic methods were used to characterize the acetylcholine-like substance derived from sympathetic ganglia. In addition, the tetrachloroaurate salt of the purified acetylcholine-like substance obtained from beef sympathetic ganglia was prepared and its melting point determined (*4*).

The data obtained by paper chromatography and paper electrophoresis (Tables 1 and 2) indicate that the choline ester present in sympathetic ganglia is acetylcholine. The melting point of the tetrachloroaurate salt of the acetylcholine-like substance is 164° to 167°C. Acetylcholine tetrachloroaurate melts at 164° to 166.5°C and a mixture of the two salts melts at 165° to 167°C. Perfusion of the cat superior cervical ganglion with C^{14}-choline resulted in the release of a C^{14}-labeled substance which has been tentatively identified as acetylcholine.

A 10-percent solution of trichloroacetic acid (*5*) was used to extract the sympathetic ganglionic chains excised from beef and cats. These extracts were purified (*6*) and subjected to paper chromatography and to paper electrophoresis (*7*). To serve as a reference standard, C^{14}-acetylcholine was mixed into the purified extract. A radioactive (strip) counter was used to ascertain how far the C^{14}-acetylcholine had moved. The location of the acetylcholine-like substance was detected after elution of suitable zones with 0.9-percent NaCl solution and bioassay of the eluates.

Table 2. The relative electrophoretic mobility (Rm) of the acetylcholine-like substance in beef extracts. In each case a 0.5*M* ammonium acetate buffer was used. The electrical gradient was 17.55 v/cm except at *p*H 6.0 where it was 21.74 v/cm.

*p*H of buffer	Time * (hr)	Rm †
3.0	3¼	0.98
4.0	3	1.00
5.0	4	0.99
6.0	2	1.02

* The time the compounds were subjected to the electrical gradients. † Each Rm value represents one experiment; Rm values were computed with reference to the mobility of C^{14}-acetylcholine and are expressed as a ratio.

The chromatographic behavior of the acetylcholine-like substance obtained from beef ganglionic chains is illustrated in Table 1. The electrophoretic mobility of the same substance is depicted in Table 2. In each system the acetylcholine-like substance moves essentially the same distance from the origin as does C^{14}-acetylcholine. Almost identical results were obtained when extracts from the sympathetic ganglionic chains of cats were examined. No other choline esters were detected in these extracts.

The contraction of the isolated rectus abdominis muscle of the frog and the vasodepressor response in cats were the tests used for the bioassays. The sensitivity of the two biological test objects to acetylcholine and to the acetylcholine-like substance was comparable. Physostigmine salicylate (50 μg/kg, given intravenously) potentiated the vasodepressor activity of both acetylcholine and the acetylcholine-like substance. The prior administration of atropine sulfate (0.2 mg/kg, given intravenously) blocked the effects of both substances on cat blood pressure. Furthermore, neostigmine bromide ($1 \times 10^{-6} M$) potentiated the effects of both acetylcholine and the acetylcholine-like substance on the isolated frog muscle. Treatment of the frog muscle with *d*-tubocurarine chloride (10 μg/ml) prevented acetylcholine and the acetylcholine-like substance from inducing a contractile response.

The biological activity of both acetylcholine and the acetylcholine-like substance was destroyed after incubation in cat blood for 4 minutes at 25°C. Similarly, both compounds were inactivated when they were incubated in a saline solution of *p*H 10 for 1 minute at 100°C.

The above biological and pharmacological tests are commonly used by investigators to identify acetylcholine in tissue extracts (*5*). However, these tests cannot be relied on for the definitive characterization of acetylcholine, especially when they are applied to crude extracts (*2*). Our tests were performed on the purified acetylcholine-like substance.

The chemical substance concerned with the transmission of nerve impulses in sympathetic ganglia has been shown to possess acetylcholine-like properties (*8*). We have attempted to identify this chemical mediator with the use of C^{14}-choline. The superior cervical ganglion of the cat was perfused with Locke's solution buffered at *p*H 7.4 (*9*). The Locke's solution also contained 20 μg of physostigmine salicylate and 5 μg of C^{14}-choline chloride per milliliter. Preliminary electrophoretic (*10*) and chromatographic (*11*) data indicate that all of the radioactivity in the effluent of a ganglion whose preganglionic fibers are electrically stimulated can be accounted for as C^{14}-choline and C^{14}-acetylcholine. The relative electrophoretic mobility (Rm) and R_F values of one radioactive compound were the same as the corresponding values for acetylcholine. The other radioactive compound possessed the same electrophoretic and chromatographic properties as choline.

The data provide strong evidence that acetylcholine is present in sympathetic paravertebral ganglionic chains and add support to the view that it functions as a chemical mediator of preganglionic sympathetic nerve activity. Choline esters other than acetylcholine do not appear to participate in the above process.

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11. Paper chromatography with *n*-butanol-water (9 : 1 by volume) was used to characterize the radioactive compounds. Descending chromatography was conducted for 18 hours. Nonradioactive choline and acetylchoine were used as reference standards. The R_F of choline was 0.07 and the R_F of acetylcholine was 0.10.
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"Cytoplasmic" Sterility in Drosophila paulistorum Which Is Ultimately Dependent on Nuclear Genes

Abstract. *A case of hybrid sterility in* Drosophila paulistorum *is due to an incompatibility of the Y chromosome of certain strains with the cytoplasm of other strains. The constitution of the cytoplasm responsible for the sterility is not, however, independent of the chromosomal genes. After seven backcrosses of the hybrid females to males of the same strain, fertile male progenies are finally obtained.*

At least three different kinds of hybrid sterility occur within the superspecies *Drosophila paulistorum*. This superspecies consists of six races or incipient species; hybrids between the races are fertile as females but sterile as males (*1*). The hybrid females can be backcrossed to males of the parental races, and the backcross progenies consist again of fertile daughters and sterile sons. The sterility of the backcross males depends upon the genetic constitution of their mothers; all the sons of a female carrying any mixture of the chromosomes of the parental races are sterile, even if some of these sons themselves carry only the chromosomes of a single race (*2*). This is, then, an instance of a genic sterility operating through a maternal effect, the genes responsible being distributed in all three pairs of the chromosomes which the species possesses. Evidently, the sterility of the F_1 males is due to a different mechanism, since F_1 hybrids are descendants of pure rather than hybrid mothers.

A third kind of sterility has been reported (*3*), so far in only a single cross, between strains from Mesitas and those from Santa Marta, Colombia. Both the Mesitas and Santa Marta strains belong to the Transitional race of *D. paulistorum*. The cross Mesitas female × Santa Marta male gives fertile hybrids of both sexes, but the male progeny of the reciprocal cross is sterile. The hybrid females can be backcrossed to males of either parental strain; the male progenies of these backcrosses are sterile if they carry the Y chromosome of Mesitas in the Santa Marta cytoplasm, or the Y chromosome of Santa Marta in Mesitas cytoplasm. Genetic analysis consequently suggests that the sterility is caused by an interaction of the Y chromosome of the Santa Marta strain with the cytoplasm of Mesitas. This inference was based on a study of four backcross generations to both parental strains. Males with cytoplasm of Mesitas origin and a Santa Marta Y chromosome were sterile even when about 97 percent of their genome (other than the Y chromosome) were of Santa Marta origin. Since the earlier report (*3*) was published, additional backcrosses have been made to test the possibility that the cytoplasmic difference may eventually be overcome by the nuclear genes (*4*).

Fifteen new strains of *D. paulistorum* were obtained (*5*) from Mesitas, Columbia. These strains behaved like the old Mesitas strain (*6*), and were pooled into three stocks. All the experiments crossing Mesitas with Santa Marta were, therefore, made in triplicate; the results were uniform and can be described jointly. Mesitas females were crossed to Santa Marta males and the hybrid females were backcrossed repeatedly to Santa Marta males. The male progenies of the first four backcross generations were, as before, sterile. By the fifth or sixth backcross generation some motile spermatozoa were seen in the sperm-storing organs of the females with which the hybrid males were tested, but still none of the eggs deposited by these females hatched. The male progeny of the seventh backcross generation is, however, entirely fertile.

A similar situation was observed in the crosses with the Santa Marta cytoplasm. Six backcrosses to Mesitas males yielded sterile male hybrids, but the seventh backcross generation gave at least some fertile males. The progeny of the seventh backcross is expected to have more than 99 percent of the genes of the recurrent parent. Thus, by repeated backcrosses the origin of the cytoplasm is finally overcome by the chromosomal genes. If the properties of the cytoplasm were transmitted independently of the nucelar genes, the sterility of the backcross males would have to be retained irrespective of the number of the backcrosses made, as it was after some 60 generations in the experiments of Laven (*7*) on a cytoplasmic sterility in crosses between certain mosquitoes. This is, however, not the case in *Drosophila paulistorum*. Finally, the male progeny of the seventh backcross generation of (Santa Marta female × Mesitas male) × Mesitas male was crossed to pure Santa Marta females. All the hybrid males thus obtained were sterile, just as the initial Santa Marta female × Mesitas male F_1 males.

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Lateral Geniculate Nucleus and Cerebral Cortex: Evidence for a Crossed Pathway

Abstract. *Lesions were placed in the lateral geniculate nucleus of cats, and degeneration was traced in the brains after survival times ranging from 5 to 21 days. Degenerated fibers could be seen in the corpus callosum and in the lateral and suprasylvian gyri of the opposite hemisphere. Results suggest the presence of a crossed geniculocortical pathway.*

A number of earlier authors (*1*) suggested the possibility of a tract connecting each lateral geniculate nucleus with the contralateral cortex by way of the corpus callosum. Such a pathway was proposed to account for the paradoxical sparing of the macular portion of the visual fields in man after massive