containing large numbers of mitochondria and vesicles typical of osteoclasts and other giant cells. For electron microscopy the tissues were fixed in paraformaldehyde buffered with collidine and osmic acid, and embedded in epon (*5*).

In one osteoclast was a centrosphere containing seven pairs of centrioles (Fig. 1). The number of pairs corresponded to the number of nuclei in the cell. We examined serial sections of giant cells from rat bone, a human aneurysmal bone cyst, a human osteoclastoma, and several human granulomas to determine if this property of a common centrosphere was characteristic of all giant cells. The granulomas were selected because we felt they would offer a technical advantage since they have a greater number of giant cells per unit of tissue. Since they are not mineralized, difficulties in serial sectioning were reduced. Eleven osteoclasts were sectioned for electron microscopy. Each of the 11 cells had a single centrosphere containing centriole pairs corresponding to the number of nuclei. The largest centrosphere contained nine centriole pairs (Fig. 2). In this cell, as in others, the centrosphere was so large that not all of the centrioles could be identified in any one ultrathin section. We reconstructed the centrosphere by comparing the position and number of centrioles seen in successive sections. To better determine the number of nuclei and the position of the centrosphere, we examined stained epon thin sections (1 μ) with the light microscope before making ultrathin sections (80 mμ) of the same cells for study with the electron microscope. With this technique, giant cells could be readily located. Serial sections of cells identified in this manner either contained a centrosphere, the ratio of nuclei to centrioles being 1 : 2, or contained no centrosphere or centrioles. In the latter case, we presumed that the centrosphere was lost in the original sectioning process. Serial sectioning of the smaller giant cells made determination of the ratio of nuclei to centrioles much simpler.

Examination of the osteoclastoma revealed several "osteoclasts." However, serial sections of these cells revealed centrioles scattered in the cytoplasm. No giant centrosphere could be detected. Similarly, the giant granuloma cell also did not have any special arrangement of centrioles. In all granulomas examined centrioles were found (Fig. 3), but no special centrosphere was present. Apparently, the special arrangement of the centrosphere is a unique feature of the normal osteoclast and is not a feature common to all multinucleated cells.

The organization of centrioles within one centrosphere in the osteoclast could be the result of incorporation of centriole pairs from mononucleated cells, after they fuse, and subsequent inclusion into a common centrosphere. The single centrosphere may be related to the need for regulating cell cleavage in a cell with a large ratio of cell volume to cell surface. However, granuloma cells with a comparable ratio did not have this arrangement. It is possible, but unlikely, that this arrangement represents a mechanism for the production of multiple nuclei from one mitotic configuration. Autoradiographic studies of bone after the administration of tritiated thymidine (*6*) have indicated that some labeled nuclei occur in osteoclasts. This finding was interpreted by the author as evidence that multinucleated cells are formed by fusion. Studies of giant cells with electron microscopy (*3*) have provided supportive evidence for the fusion mechanism although mitotic figures within forming giant cells have also been observed (*3*), indicating that both mitosis without cell cleavage and fusion of preexistent cells occur. The difference between osteoclasts and other giant cells, with respect to the arrangement of the centrosphere, may be related to the ultimate fate of the cell. Giant cells often are subject to disintegration and to removal by other phagocytes. In the case of the osteoclast, there is some suggestion that this cell dissociates into uninucleated cells and reenters an osteo-progenitor pool (*6*, *7*). If this idea is proved correct, the arrangement of the centrioles into a common body would facilitate the orderly cleavage of this giant cell into subunits with a full complement of cell parts.

Centrioles have long been recognized as structures that have a special role in the process of cell division. They are related to the formation of the division spindle and to formation of continuous tubules of the midbody, the site of cell cleavage. The observation of a peculiar centriole configuration in osteoclasts, which is not generally characteristic of other giant cells, suggests that a special mechanism for formation and cleavage of these cells may be involved.

JAMES L. MATTHEWS
JAMES H. MARTIN
GEORGE J. RACE
Department of Anatomy,
Baylor University College of Dentistry,
Baylor Medical Center, Dallas, Texas
ELLIOTT J. COLLINS
Schering Corporation,
Bloomfield, New Jersey

References and Notes

1. N. Hancox, in *The Biochemistry and Physiology of Bone*, G. H. Bourne, Ed. (Academic Press, New York, 1956), p. 213.
2. P. D. Toto and J. J. Magon, *J. Dental Res.* **45**, 225 (1966).
3. J. S. Sutton and L. Weiss, *J. Cell Biol.* **28**, 303 (1966).
4. H. E. Jordan, *Anat. Rec.* **30**, 107 (1925).
5. J. A. Lynn, J. H. Martin, G. J. Race, *Amer. J. Clin. Pathol.* **45**, 704 (1966).
6. R. W. Young, *J. Cell Biol.* **14**, 357 (1962).
7. M. Heller, F. C. McLean, W. Bloom, *Amer. J. Anat.* **87**, 315 (1950); E. A. Tonna, *Lab. Invest.* **15**, 435 (1966).
8. Work supported by grants from Medical Research Foundation of Texas and from the Schering Corporation. We thank James Roan for his technical assistance with the electron microscope.

7 November 1966

Muscle-Spindle Histochemistry

Abstract. *Reduced nicotinamide adenine dinucleotide tatrazolium reductase is abundant in cat intrafusal muscle fibers, whereas in the toad its activity is equal to that in extrafusal fibers. Spindles of both species contain little fat. In sections stained for adenosine triphosphatase bound to myofibrils, two types of intrafusal muscle fibers appear in spindles of both the cat and toad.*

In recent years great interest has been focused on a possible correlation between muscle histochemistry and physiology. Much physiological (*1*) and morphological (*2*) information has been presented favoring the existence of two types of intrafusal muscle fibers. However, only a few histochemical investigations on muscle spindles have been published, and only one of them deals with those generally used in spindle physiology. As demonstrated by the histochemical tests for various oxidative enzymes (*3*), phosphorylase (*4*) and myoglobin (*5*), there are three types of intrafusal muscle

fibers in the mouse (*3, 4*) and dog (*5*). Histochemical tests for mitochondrion-bound adenosine triphosphatase have shown two types in the cat (*6*). Regardless of fiber types, chicken muscle spindles are rich in oxidative enzymes (*7*).

Histochemical studies of extrafusal muscle fibers of mammals are more numerous. "White" muscle (low in myoglobin and in oxidative enzymes) consists of three types of fibers in the rat (*8*) and cat (*6, 9, 10*). Some controversy exists about "red" muscle (rich in myoglobin and oxidative enzymes). In the soleus muscle, one (*6, 10*) as well as two (*8, 9*) types of fibers have been described.

Physiologically, limb muscles of mammals can be classified as fast- or slow-contracting muscles (*11*), corresponding to the morphological "white" and "red" types. Wuerker, McPhedran, and Henneman (*12*) stated that this classification was also valid for motor units of cat muscles, and they also discussed the possibility of a third type of unit being present in the "white," fast-contracting gastrocnemius muscle. The soleus muscle was concluded to contain only one type of unit (*6*). Both muscles consist of fibers corresponding to the twitch type of frog muscle (*13*).

Although, in the frog, there is no separation into "white" and "red" muscles, physiological differences are present between individual muscle fibers, which are classified as twitch and slow fibers (*14*). Physiological experiments on single fibers of the iliofibularis muscle have disclosed at least three types of fibers, as have histochemical tests for fat and oxidative enzymes (*15*). Three types of muscle fibers have been identified histochemically in frog gastrocnemius sartorius, and rectus abdominis muscles as well (*16*).

Thus, as far as both frog and cat extrafusal muscle fibers are concerned, fairly good agreement is present between histochemical and physiological results. Since there is strong support for the existence of two types of intrafusal muscle fibers (*1, 2*), I analyzed histochemically spindles from species commonly used in spindle physiology. I used cat gastrocnemius and toad (*Xenopus laevis*) iliofibularis muscles. Sections (16 μ) were cut in a cryostat at −23°C and were stained for various enzymes, as well as for glycogen and fat. Only those results obtained with staining for reduced nicotinamide adenine dinucleotide ($NADH_2$) tetrazolium reductase, fat, and myofibrillar-bound adenosine triphosphatase will be presented. The methods used for visualization of $NADH_2$-tetrazolium reductase and myofibrillar-bound adenosine triphosphatase were those described by Scarpelli, Hess, and Pearse (*17*) and Padykula and Herman (*18*), respectively, with some modifications. Sections were incubated for 40 and 45 minutes, respectively, at 37°C (cat) and 20°C (toad). Sudan black B was used to demonstrate fat (*19*).

Muscle spindles of cat gastrocnemius muscle had extremely high activity of $NADH_2$-tetrazolium reductase (Fig. 1). In all spindles examined, only one type of intrafusal muscle fiber was demonstrated by this technique. To exclude the possibility that the equal intensity of staining in the intrafusal muscle fibers was caused by overincubation, I incubated preparations for as little as 5 to 10 minutes, but there was still no difference between the fibers. The high activity of this enzyme makes the method suitable for identification of cat muscle spindles.

The toad muscle spindles did not have the extremely high activity of $NADH_2$-tetrazolium reductase noted in the cat. The intensity in the intrafusal muscle fibers was equal to that in the extrafusal ones. This implies that, with respect to activity of this enzyme in the intrafusal fiber, a clear species difference is present between toad and cat. Furthermore, in toad spindles, two types of fibers could often be seen. One was small and darkly stained, and one was large and sparsely stained (Fig. 2). Spindles with only one type of intrafusal muscle fiber were also observed.

Extrafusal muscle fibers can store fat in proportion to their content of oxidative enzymes (*20*). When, however, the spindle in Fig. 1 was stained for fat, the intrafusal muscle

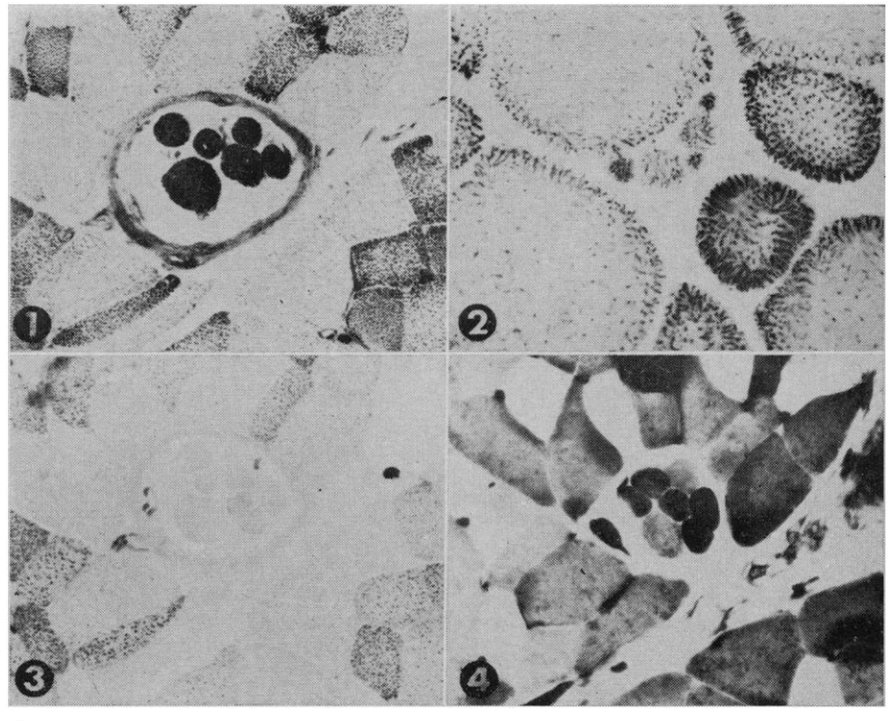

Fig. 1. Muscle spindle from cat gastrocnemius muscle stained for $NADH_2$-tetrazolium reductase. Note the high activity of this enzyme in the intrafusal muscle fibers compared with that in the extrafusal ones. Only one type of fiber is visible inside the spindle (× 250). Fig. 2. Muscle spindle from toad iliofibularis muscle stained for $NADH_2$-tetrazolium reductase. The high activity of this enzyme seen in cat gastrocnemius muscle spindles (Fig. 1) does not occur in toad spindles. Spindles with two types of fibers are often seen, as in the figure. The smaller fibers have a higher content of the enzyme (× 250). Fig. 3. The same muscle spindle as that in Fig. 1, stained for fat. The intrafusal muscle fibers lack fat or have an extremely low content. Only one type of intrafusal muscle fiber is present, whereas there are three distinct types of extrafusal fibers (× 250). Fig. 4. The same spindle as that in Figs. 1 and 3 stained for myofibrillar-bound adenosine triphosphatase. Two types of intrafusal muscle fibers and three types of extrafusal muscle fibers are seen (× 250).

fibers were almost unstained (Fig. 3). This means that, despite a high activity of oxidative enzymes, lipid droplets are scanty or absent in intrafusal gastrocnemius fibers of the cat. On the other hand, when the cat muscle spindle in Figs. 1 and 3 was stained for myofibrillar-bound adenosine triphosphatase, two types of fibers were observed (Fig. 4). The smaller fibers had a higher content of this enzyme.

Toad muscle spindles stained for fat always appeared almost unstained, just as cat spindles did, whereas in sections stained for myofibrillar-bound adenosine triphosphatase, spindles with one or two types of intrafusal muscle fibers were identified. In physiological investigations, two types have usually been reported (*1*).

It can be inferred that all intrafusal muscle fibers of cat spindles showed equal staining intensity when stained for $NADH_2$-tetrazolium reductase or fat, whereas morphological and physiological studies generally disclose two types of fibers. When the same spindles were stained for myofibrillar-bound adenosine triphosphatase, two types of fibers appeared. However, other staining reactions may reveal even more types (*4, 5*). Thus, it seems premature—at any rate with respect to intrafusal muscle fibers—to correlate physiological and histochemical results until those chemical processes specifically associated with the physiological properties are better known.

BO NYSTRÖM

Department of Anatomy and Histology, Karolinska Institutet, Stockholm 60, Sweden

References and Notes

1. B. Katz, *J. Exp. Biol.* **26**, 201 (1949); S. W. Kuffler, C. C. Hunt, J. P. Quilliam, *J. Neurophysiol.* **14**, 29 (1951); C. Eyzaguirre, *ibid.* **20**, 523 (1957); I. A. Boyd, *J. Physiol. London* **144**, 11 P (1958); C. Eyzaguirre, *ibid.* **150**, 169 (1960); A. Crowe and P. B. C. Matthews, *ibid.* **174**, 132 (1964); R. S. Smith, *Acta Physiol. Scand.* **60**, 223 (1964); ———, *ibid.* **62**, 195 (1964); ———, in *Nobel Symposium 1, Muscular Afferents and Motor Control*, R. Granit, Ed. (Almqvist and Wiksell, Stockholm, 1965), p. 69; K. Diete-Spiff, *J. Physiol. London* **183**, 65 P (1966).
2. S. Cooper, in *Structure and Function of Muscle*, G. H. Bourne, Ed. (Academic Press, New York, 1960), vol. 1, p. 381; I. A. Boyd, *Phil. Trans. Roy. Soc. London, Ser. B* **245**, 81 (1962); D. Barker, in *Symposium on Muscle Receptors*, D. Barker, Ed. (Hong Kong Univ. Press, Hong Kong, 1962), p. 227; S. Cooper and P. M. Daniel, *Brain* **86**, 563 (1963).
3. T. Ogata and M. Mori, *Acta Med. Okayama* **16**, 347 (1962).
4. C. Wirsén and K. S. Larsson, *J. Embryol. Exp. Morphol.* **12**, 759 (1964).
5. C. Wirsén, *J. Histochem. Cytochem.* **12**, 308 (1964).
6. E. Henneman and C. B. Olson, *J. Neurophysiol.* **28**, 581 (1965).
7. N. I. Germino and H. D'Albora, *Experientia* **21**, 45 (1965).
8. J. M. Stein and H. A. Padykula, *Amer. J. Anat.* **110**, 103 (1962).
9. T. Ogata and M. Mori, *J. Histochem. Cytochem.* **12**, 171 (1964).
10. B. Nyström, *Nature* **212**, 954 (1966).
11. D. Denny-Brown, *Proc. Roy. Soc. London, Ser. B* **104**, 371 (1929); A. J. Buller, J. C. Eccles, R. M. Eccles, *J. Physiol. London* **150**, 399 (1960); R. Close, *ibid.* **173**, 74 (1964).
12. R. B. Wuerker, A. M. McPhedran, E. Henneman, *J. Neurophysiol.* **28**, 85 (1965).
13. A. J. Buller, D. M. Lewis, R. M. A. P. Ridge, *J. Physiol. London* **180**, 29 P (1965).
14. S. W. Kuffler and E. M. Vaughan Williams, *ibid.* **121**, 289 (1953); *ibid.*, p. 318.
15. J. Länneregren and R. S. Smith, *Acta Physiol.* **68**, 263 (1966).
16. V. Dubowitz and A. G. E. Pearse, *Histochemie* **2**, 105 (1960).
17. D. G. Scarpelli, R. Hess, A. G. E. Pearse, *J. Biophys. Biochem. Cytol.* **4**, 747 (1958).
18. H. A. Padykula and E. Herman, *J. Histochem. Cytochem.* **3**, 161 (1955).
19. H. M. Carleton and R. A. B. Drury, *Histological Technique* (Oxford Univ. Press, London, 1957).
20. L. A. Carlsson, S. O. Liljedahl, C. Wirsén, *Acta Med. Scand.* **178**, 81 (1965).
21. I thank Miss I.-L. Andersson for technical assistance. Supported by a grant from Svenska Sällskapet för Medicinsk Forskning: Ernst and Martha Janson Foundation.

27 January 1967

Lysine Transport in Human Kidney: Evidence for Two Systems

Abstract. *Experiments with slices of human kidney cortex from two control subjects demonstrated two distinct transport systems for lysine (α and β) which differ greatly in affinity and capacity. Both systems were found in kidney from two patients with cystinuria. Studies with rat kidney confirmed these findings. These experiments defined only a single transport system for cystine in kidney from both control and cystinuric subjects.*

Intestinal absorption and renal tubular reabsorption of dibasic amino acids in man and other mammals is accomplished by specific, energy-dependent, active transport processes. Renal clearance (*1*) and stop-flow (*2*) analyses, oral-absorption studies (*3*), and uptake experiments with gut mucosa in vitro (*4*) indicate that the dibasic, monocarboxylic amino acids—lysine, arginine, and ornithine—are transported by a common system in the gut and kidney and that this system may be shared by cystine, a dibasic dicarboxylic amino acid. This transport system is defective in individuals with cystinuria, an inherited disorder leading to impaired intestinal absorption and marked urinary hyperexcretion of these dibasic amino acids. However, several important findings from experiments in vivo and in vitro have not been explained satisfactorily by the prevailing concept of a single renal and intestinal transport mechanism for this group of structurally related compounds. (i) Cystine clearance has equaled or even exceeded inulin clearance in all homozygous cystinuric subjects studied, but simultaneous clearances of lysine, arginine, and ornithine have been significantly less than the clearance of inulin (*5*). (ii) Lysine uptake was reduced by only 50 percent in kidney from homozygous cystinuric subjects in vitro and was further inhibited by arginine (*6*). (iii) Cystine uptake by kidney from cystinuric subjects was not significantly impaired, nor could cystine be shown to share a common transport mechanism with lysine, arginine, or ornithine (*6, 7*). (iv) In subjects homozygous for type I cystinuria (*8*), mediated intestinal transport of cystine, lysine, and arginine was completely abolished, in contrast to the partial transport defect in kidney tissue (*6*). The first two observations are consistent with the hypothesis that the mutations responsible for homozygous cystinuria alter the configuration, quantity, or activity of a single carrier protein or enzyme with different affinities for the several dibasic amino acids. The other two observations, however, strongly suggest an alternate hypothesis, namely, the presence of two transport systems in human kidney for dibasic amino acids, only one shared by the gut and mutant in cystinuria.

There is ample evidence for this alternative hypothesis in nonmammalian systems. Ames (*9*) reported that histidine enters *Salmonella typhimurium* by two distinct processes, one shared by the other aromatic amino acids and one transporting only histidine. This conclusion was based on the demonstration of two apparent affinity constants (K_m) for histidine uptake. Similar results were reported subsequently for galactose uptake in *Escherichia coli* (*10*) and for β-alanine and diaminobutyric acid transport in ascites tumor cells (*11*). Our studies tested the hypothesis that dibasic amino acid transport in human kidney also depends on more than a single mechanism.