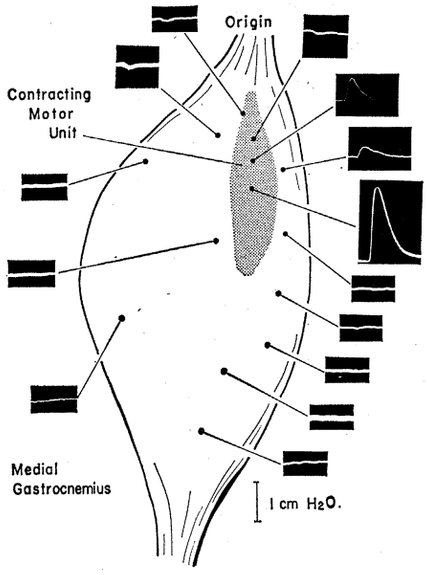

Fig. 2. Pressure changes in the cat's medial gastrocnemius occurring with twitch contractions of a motor unit producing dimpling of the shaded area. Unit was caused to contract by stimulation of a ventral root filament. Sharp increases in pressure were recorded within the motor unit territory; slight decreases in pressure appeared at some neighboring sites; and more distant sites showed no effect.

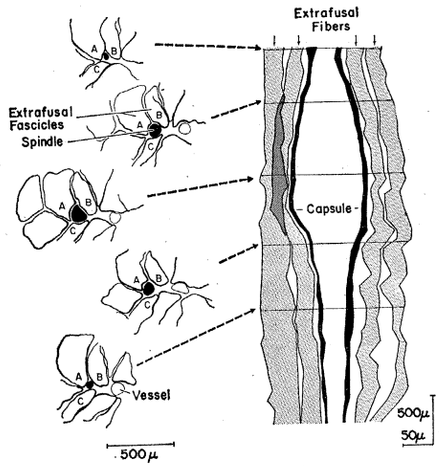

Fig. 3. The five projections at the left represent selected views of cross-sections of a tenuissimus spindle and adjacent muscle fascicles. Where the section passes through the spindle equator, distortion of fascicles *A* and *B* by the impinging capsule is shown. At the right is a longitudinal sectional view of the same spindle as reconstructed from the serial cross sections. The horizontal scale of the representation is expanded six times the vertical scale. Two extrafusal fibers of fascicle *A*, and two from *B* are shown. Their course is seen to bow around the capsular bulge.

stimulation of a ventral root filament, abrupt rises in pressure were obtained in a restricted area at the center of the dimple marking the contraction; at some marginal sites slight drops in pressure were detected; and through most of the muscle no changes were detected (Fig. 2).

2) Reconstruction from serial sections of capsules of various shapes, and relation of the capsules to adjacent muscle, strongly suggest that the equatorial region of the spindle is subjected to pressure during contraction of neighboring extrafusal fibers. Spindles lie in interfascicular clefts where they appear, at least after histological fixation, to be freely suspended in extracellular space. However, the swollen equatorial region presses outward against adjacent fascicles to a degree sufficient to cause deformation of the fascicles (Fig. 3, fascicles *A* and *B*). The course of individual extrafusal fibers is warped from a direct course by the equatorial swelling. Pressure upon the equator thus may derive not only from thickening of extrafusal fibers during contraction, but also from that force vector exerted normal to the line of tension along the shortening extrafusal fiber.

3) Teasing of spindles following maceration and reconstructions from serial sections show that the motor poles of many intrafusal fibers insert into the capsule. Systematic examination of 128 intrafusal fibers from the proximal ends of 25 extensor digitorum brevis and tenuissimus spindles of the cat shows that 60 percent of the intrafusal fibers are clearly "intracapsular," and only 32 percent extend distinctly beyond the capsule as "percapsular fibers." The latter fibers usually have their equatorial nuclei in a bag arrangement, while "intracapsular fiber" nuclei are in single file.

The "intracapsular fibers" in this pressure-sensing mechanism would function, presumably, by causing sustained stretching of the sensory endings, thus altering their sensitivity to changes in pressure (Fig. 4). Since separate motor systems for innervation of small and large intrafusal fibers probably exist (*2*), the length sensing mechanism associated with the large "percapsular fibers" could be independently adjustable. Each system would be expected to have distinct reflex connections centrally. The pressure sensing mechanism might serve to monitor contractile activity in different parts of the muscle

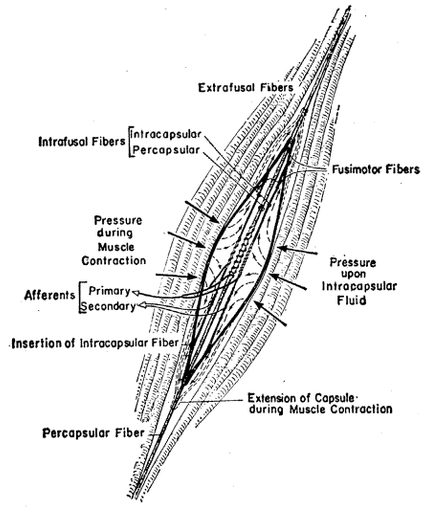

Fig. 4. Schematic diagram of a mechanism in the muscle spindle for converting alterations in intramuscular pressure into changes in sensory discharge.

and play a part in balancing participation by the surrounding motor units. The state of the muscle would thus be gauged by three sensory modalities: (i) tendon organs for total tension developed; (ii) annulospiral endings, for length of the muscle; and (iii) "flower-spray" terminals, for pressure gradients.

CHARLES F. BRIDGMAN
EARL ELDRED

Department of Anatomy, University of California Medical Center, Los Angeles 24

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3 October 1963

Cystinuria: In vitro Demonstration of an Intestinal Transport Defect

Abstract. *A defect in the transport of* L-*cystine and* L-*lysine has been found in the intestinal mucosa of patients with cystinuria. Transport studies in normal intestinal mucosa, in contrast to similar studies in the kidney, show that cystine and lysine are mutually inhibitory.*

Cystinuria, a hereditary disease of man, is characterized by formation of cystine renal calculi and by excessive excretion in urine of cystine and the dibasic amino acids, lysine, arginine,

and ornithine (*1*). The characteristic urinary excretion pattern led Dent and Rose to suggest that the four amino acids share a common renal transport mechanism which is defective in cystinuric patients (*2*). Recent studies with kidney slices from normal and cystinuric subjects have failed to support this suggestion (*3*). Although lysine and arginine transport were defective in kidney from cystinuric patients, cystine transport was normal. Furthermore, no competition could be demonstrated between cystine and the dibasic amino acids in renal tissue from either normal or cystinuric subjects.

Milne, Asatoor, and co-workers suggested that an intestinal as well as a renal transport defect exists in cystinuria (*4*). Their hypothesis was based on indirect evidence obtained by measuring urinary and fecal excretion of diamines after lysine, arginine, and ornithine, but not cystine, had been fed to normal and cystinuric subjects. This hypothesis has now been investigated directly with material obtained by intestinal biopsy. The results, in contrast to those obtained with kidney slices, indicate a defect in the intestinal transport of cystine as well as lysine in cystinuria and suggest competition between these substances.

Intestinal mucosa was obtained with a Rubin tube which was placed at the ligament of Treitz under fluoroscopic control. Jejeunal mucosa thus obtained accumulates amino acids against a concentration gradient (*5*). The uptake of L-cystine and L-lysine in normal and cystinuric subjects was most rapid during the first 30 minutes of incubation and then approached a plateau. Subsequent measurements of concentration were therefore carried out at 45 minutes, except in the studies of inhibition where the measurements were made during the period of rapid accumulation.

After 45 minutes of incubation, intestinal mucosa from four normal volunteers concentrated lysine 13-fold and cystine 4.5-fold, while similar tissue from four cystinuric subjects failed to concentrate either of these amino acids significantly (Table 1). Mucosa from normal and from cystinuric subjects concentrated glycine five-fold; hence the transport defect in cystinuria is not a generalized one.

The finding of Hagihira *et al.* (*6*) that cystine inhibited lysine transport in segments of hamster gut was difficult to reconcile with previous reports (*3, 7*) that similar inhibition could not be demonstrated in renal tissue from several species, including man, and their work prompted a search for inhibition in normal human intestinal mucosa. These investigations demonstrated that lysine accumulation was inhibited by arginine or cystine and that cystine uptake was inhibited by lysine. However, neither the transport of cystine nor the transport of lysine was affected by the presence of glycine (Table 2).

Thus it has been demonstrated directly that a defect in the transport of cystine as well as lysine exists in the intestinal mucosa of patients with cystinuria. Of perhaps greater interest is the demonstration that cystine accumulation can be inhibited by lysine and that lysine accumulation can be inhibited by cystine. In these important respects, intestinal transport of cystine and lysine differs from renal transport.

These results and the observations of Frimpter (*8*) of a markedly elevated renal arteriovenous difference for cysteine and an apparently normal renal arteriovenous difference for cystine in

Table 1. Uptake of L-lysine-C^{14} and L-cystine-S^{35} by gut mucosa from normal subjects and patients with cystinuria. Biopsy specimens, weighing 1 to 7 mg, were placed in 25-ml erlenmeyer flasks containing 2.0 ml of Krebs-Ringer bicarbonate buffer, *p*H 7.4, plus the labeled amino acids. The flasks were gassed for 30 seconds with 95 percent O_2 and 5 percent CO_2, sealed, and incubated for 45 minutes at 37°C in a Dubnoff metabolic shaker. After termination of the incubation, the tissue was removed from the flask, blotted, weighed, and placed in 1.0 ml of distilled water. The tissue amino acid pool was equilibrated with the distilled water by boiling for 6 minutes. Aliquots of the aqueous tissue supernatant and remaining medium were counted in a liquid scintillation spectrometer. Paper chromatography of the tissue supernatant indicated that greater than 90 percent of the recovered radioactivity was found with the appropriate R_F for the amino acid studied. The tissue radioactivity was converted to counts per minute per milliliter of intracellular fluid by correcting for the measured tissue water and inulin spaces. The distribution ratio is defined as the ratio of the counts per minute per milliliter of intracellular fluid to the counts per minute per milliliter of medium, and values greater than 1.0 indicate concentration against a chemical gradient. The *p* values were obtained from Student's *t* test.

Patient	Age	Sex	Distribution ratio	
			L-Lysine (Medium concn., 0.065 m*M*)	L-Cystine (Medium concn., 0.03 m*M*)
			Normal subjects	
A.K.	23	M	12.6	3.2
J.W.	36	M	15.8	5.8
C.M.	33	M	13.8	5.1
P.R.	26	M	11.3	3.8
			Mean ± SD = 13.4 ± 1.91	Mean ± SD = 4.5 ± 1.26
			Cystinuric subjects	
L.C.	19	F	1.2	0.9
E.B.	25	M	1.8	1.5
P.C.	21	F	1.3	1.0
J.G.	48	M	1.3	1.3
			Mean ± SD = 1.4 ± 0.26 ($p < .001$)	Mean ± SD = 1.2 ± 0.28 ($p < .001$)

Table 2. Inhibition of normal gut mucosal uptake of L-lysine-C^{14} and L-cystine-S^{35} by a second amino acid. Except where indicated, the conditions of the experiments were the same as those described in Table 1.

Substrate	Inhibitor *	No. of observations	Distribution ratio †	Significance of difference from control (*p*)
L-Lysine ‡	None	12	4.57 ± 1.84	
L-Lysine ‡	L-Arginine	4	1.13 ± 0.22	< 0.001
L-Lysine ‡	L-Cystine	4	1.77 ± 0.26	< 0.001
L-Lysine ‡	Glycine	3	4.81 ± 0.40	> 0.6
L-Cystine §	None	10	3.61 ± 0.85	
L-Cystine §	L-Lysine	4	2.31 ± 0.35	< 0.01
L-Cystine §	Glycine	3	3.63 ± 0.96	> 0.9

* All inhibitor concentrations are 2.4 m*M*. † Average distribution ratio ± S.D. ‡ Concentration 0.065 m*M*; 15-minute incubation. § Concentration 0.03 m*M*; 30-minute incubation.

a cystinuric patient indicate that further investigations into the pathogenesis of cystinuria must consider sulfhydryl-disulfide interaction between cysteine and cystine and must explain the observed differences in renal and intestinal transport.

SAMUEL THIER
MAURICE FOX
STANTON SEGAL
National Institute of Arthritis and Metabolic Diseases, Bethesda 14, Maryland
LEON E. ROSENBERG
National Cancer Institute, Bethesda 14, Maryland

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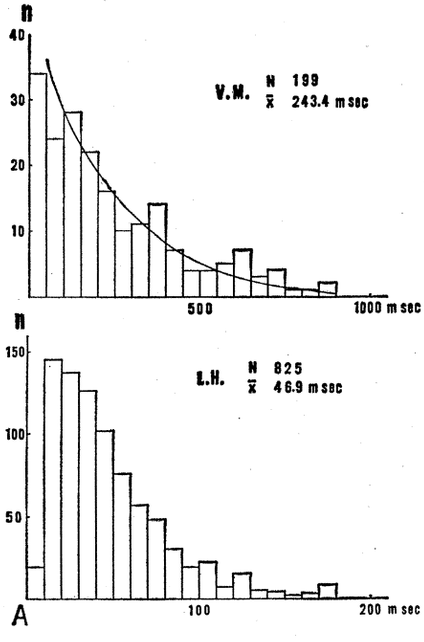
2 January 1964

Reciprocal Activities of the Ventromedial and Lateral Hypothalamic Areas of Cats

Abstract. *Statistical treatment of recordings of spontaneous unit discharges from the ventromedial nucleus and the lateral area of the hypothalamus (the activities in one area being recorded while the other was stimulated) revealed significant reciprocal relations. The concept that glucose-sensitive neurons are present in the ventromedial nucleus was supported by the effects on the spontaneous unit discharges of injecting glucose and other solutions intravenously.*

The important role of the hypothalamus in the nervous regulation of food intake has already been emphasized. Bilateral lesions in the lateral hypothalamic area (LH) of mammals cause aphagia and those in the ventromedial hypothalamic nucleus (VM) induce polyphagia (*1*). Electrical stimulations of the former through chronically implanted electrodes elicit feeding behavior (*2*), while stimulations of the latter suppress it (*3*). The results of several electroencephalographic studies of these regions supported the behavioral experiments (*4*). In this report we describe analytical studies of the activities of single neuronal units in the lateral hypothalamic area and in the ventromedial hypothalamic nucleus, yielding information on the reciprocal activities of these areas (*5*).



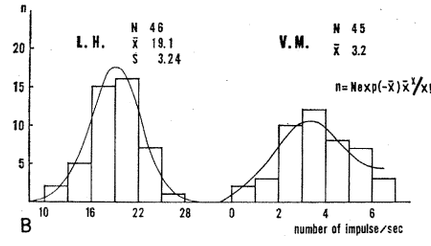


Fig. 1. Spontaneous unit discharge patterns in cats under ether anesthesia. *A*, Distribution of intervals between successive discharges recorded simultaneously in the ventromedial hypothalamic nucleus (VM, top, in a series of 199 potentials) and in the lateral hypothalamic (LH, bottom, 825). Ordinate: n is the number of intervals between $x + \Delta x$. The mean interval $\bar{x}$ is shown. The solid curve was plotted according to $n = N \left(\frac{\Delta x}{\bar{x}}\right) \exp\left(\frac{-x}{\bar{x}}\right)$. *B*, Distribution of number of spontaneous unit discharges per second in the VM (right) and the LH (left). The discharge pattern of the VM closely corresponds to the Poisson distribution and that of the LH to the Gaussian distribution.

Cats were anesthetized with ether and made to respire artificially through tracheal cannulae. Glass pipette recording microelectrodes and concentric, bipolar, metal-stimulating electrodes were inserted in the ventromedial hypothalamic nucleus (F 11.5, S 1.2, H-5, according to Jasper's atlas) and the lateral hypothalamic area (F 11.5, S 3.5, H-4) at the level of the VM. Electrode locations were later verified histologically. (Insertions were successful in 18 out of 29 cats.) When a stable series of spontaneous unit discharges was detected, tetanic shocks of 20 to 50 cy/sec for a few seconds (pulse duration 0.05 msec; current strength 10^{-6} to 10^{-5} A) were applied through one of the stimulating electrodes. This was followed by intracarotid injections of test solutions (0.3 ml of 10 percent glucose; 10 percent NaCl, Tyrode solution or water). Tactile, auditory, or visual stimuli did not affect the hypothalamic neurons under investigation.

Simultaneous recordings of spontaneous unit discharges in the LH and VM of more than 1 minute duration were attained in seven cats. The frequency of the discharges was usually low in the VM (2 to 7 cy/sec) and high in the LH (8 to 20 cy/sec). The distributions of the time intervals of the spontaneous unit discharges (discharge intervals) and the number of discharges per second (discharge frequency) were compared with simple theoretical distributions, and the significance of the differences was estimated by the Chi-square test.

In all VM recordings, the discharge interval closely resembled the exponential distribution and the discharge frequency the Poisson distribution ($p < .05$ to 0.01) (Fig. 1), like the spontaneous miniature end-plate potential (*6*). On the other hand, in all LH recordings, the discharge interval did not fit the exponential distribution but the Gaussian curve described the discharge frequency ($p < .05$ to 0.01) (Fig. 1). It was confirmed theoretically that even when different class intervals were used (different scales on the abscissa), the patterns of discharge intervals did not correspond to the exponential distribution. Thus, completely