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Choline Esterase Activity of Skeletal Muscle in Various Conditions.

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Very little information is available as to possible changes in acetylcholine (Ach) metabolism under pathologic conditions. The concentration of Ach as determined in tissue extracts is not a measure of its activity. Determination of the activity of choline esterase (ChE) in tissue however may be expected to yield information, though indirect, with regard to changes in the activity of the hypothetical transmitter substance.

The present study, which we intend to continue, deals with the activity of ChE in striated muscle in a number of pathologic conditions.

So far ChE activity in muscle has generally been determined per hour and per 100 mg of tissue (QChE). It has been shown by Marnay and Nachmansohn[1] that at the neuromuscular junctions of striated muscle, there is a concentration of ChE 30,000-50,000 times as high as in the surrounding muscle tissue. The distribution of the motor endplates throughout the muscle is not uniform. It is therefore obvious that a comparison based on QChE values is not valid unless the entire muscle is extracted for the determination and the weight of the whole muscle is taken into consideration. Assuming that for any given species, the same muscle, within certain variations, will contain a fixed number of motor endplates, the total choline esterase (TChE = QChE $\times$ muscle weight) will represent a comparable measure of ChE activity. If muscles of different weight are compared it must also be considered that their ChE content as shown below is not directly proportional to muscle weight.

The effect of nerve degeneration on choline esterase activity was first studied by Martini and Torda.[2] In the denervated gastrocnemius muscle of rats, they found a progressive decrease of the QChE varying from 12 to 75% from the second to the twelfth day after denervation.

Feng and Ting[3] reported a decrease of the ChE activity of 20-30% in the nerve-containing tibial portion of the toad-sartorius between the twelfth and the thirty-fourth day after section of the sciatic nerve. No changes were found in the nerve-free pelvic portion of the sartorius.

Recently it has been stated by Couteaux and Nachmansohn[4] and by Nachmansohn and Hoff[4a] that in the denervated gastrocnemius of guinea pigs there is no significant fall in the QChE during the first 2 weeks after denervation. From the third to the fourth week on, coinciding with morphological changes in the endplates, a 20-30% decrease in the QChE was said to occur at the endplates. In view of these discrepancies in the available data the ChE activity of denervated voluntary muscle has been reinvestigated. It also appeared of interest to study the effect of nutritional muscular dystrophy on the ChE activity of the muscle.[5]

[1] Marnay, A., and Nachmansohn, D., *C. R. Soc. de biol., Paris*, 1937, **124**, 942.

[2] Martini, E., and Torda, K., *K. Wschr.*, 1937, **23**, 824.

[3] Feng, T. P., and Ting, Y. C., *Chin. J. Phys.*, 1938, **13**, 141.

[4] Couteaux, R., and Nachmansohn, D., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 177.

[4a] Nachmansohn, D., and Hoff, E. C., *J. Neurophys.*, 1944, **7**, 27.

[5] Nerve endings in guinea pigs with nutritional muscular dystrophy have been described as normal by Rogers, Pappenheimer, and Goettsch (*J. Exp. Med.*, 1931, **54**, 167) and in rats by Pappenheimer (*Am. J. Path.*, 1939, **15**, 179). Telford (*Anat. Rec.*, 1941, **81**, 171), however, found them reduced in number in the degenerated muscles. Re-examination with a modified Ranvier gold chloride method (Pappenheimer and Stoerk, unpublished observations) has shown complete disappearance of the hypolemmal nerve endings together with the entire motor end plate in the dystrophic fibers, although epilemmal neurites are well-preserved.

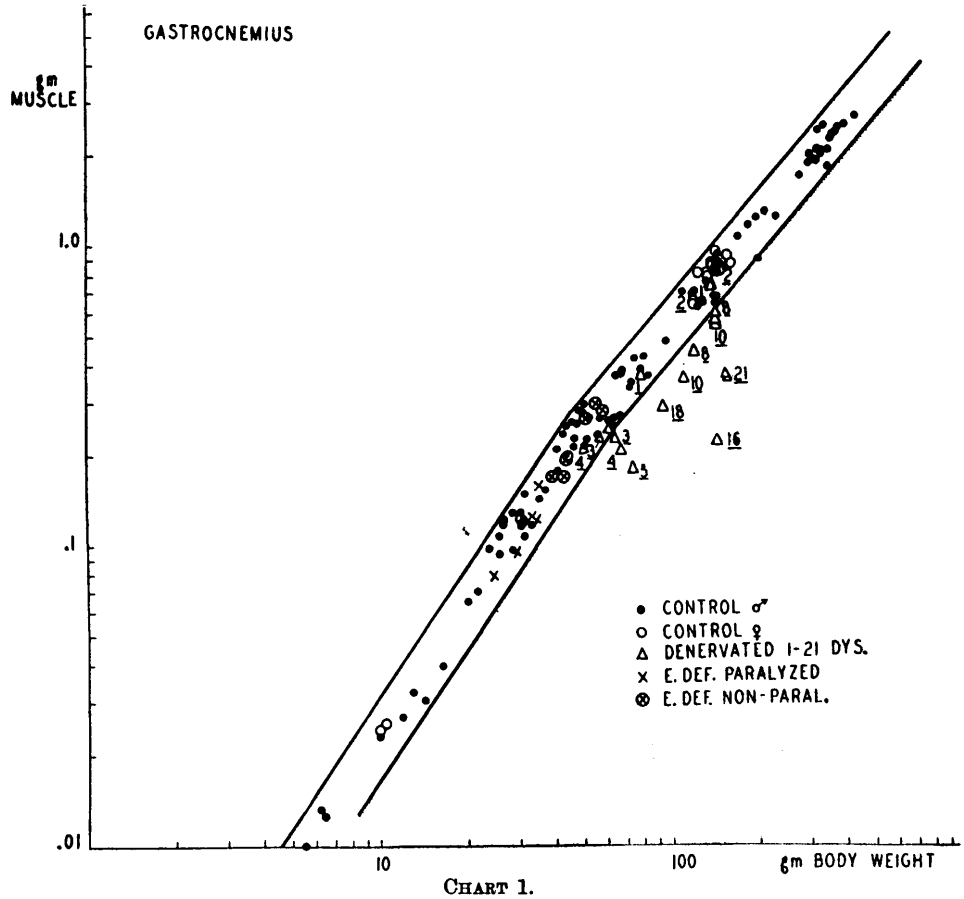

CHART 1.
Log. log. plotting of gastrocnemius weight against body weight.

Seventy apparently healthy albino rats of the Sherman strain maintained on Rockland rat pellets were used as normal controls. They varied in age from one day to 6 months and in body weight from 5.5 to 469 g. In 18 male rats the right sciatic nerve was cut and a portion of the nerve approximately 1 cm in length was removed. Muscular dystrophy was induced by a diet deficient in vitamin E in 7 young nursing rats according to the technic described by Goettsch and Ritzmann.[6] The animals were killed 2 days after the onset of paralysis. Six animals raised under identical conditions but showing no clinical or histological evidence of muscular dystrophy served as controls. All animals were killed with ether. The right gastrocnemic muscle was dissected, and weighed. The entire muscle was then extracted with Na-Mg buffer by grinding with silica. The ChE activity of the suspension was determined with the manometric method (Ammon[7]). The contralateral muscles of the animals in which the sciatic nerve was sectioned were used as controls. In all E-deficient animals the contralateral muscle was examined histologically.

A graphic presentation of the data is given in Charts I, II, and III. In Chart I gastrocnemius weight is plotted against body weight on a log., log. grid. The usual straight line relationship with a single "break" is observed

Two days after section of the sciatic nerve, the hypolemmal axons in the gastrocnemius were also seen to have disappeared. The epilemmal axons and the sole plates showed no appreciable changes. No Wallerian degeneration had as yet occurred.

[6] Goettsch, M., and Ritzmann, J., *J. Nutr.*, 1939, **17**, 371.

[7] Ammon, R., *Arch. ges. Physiol.*, 1933, **233**, 486.

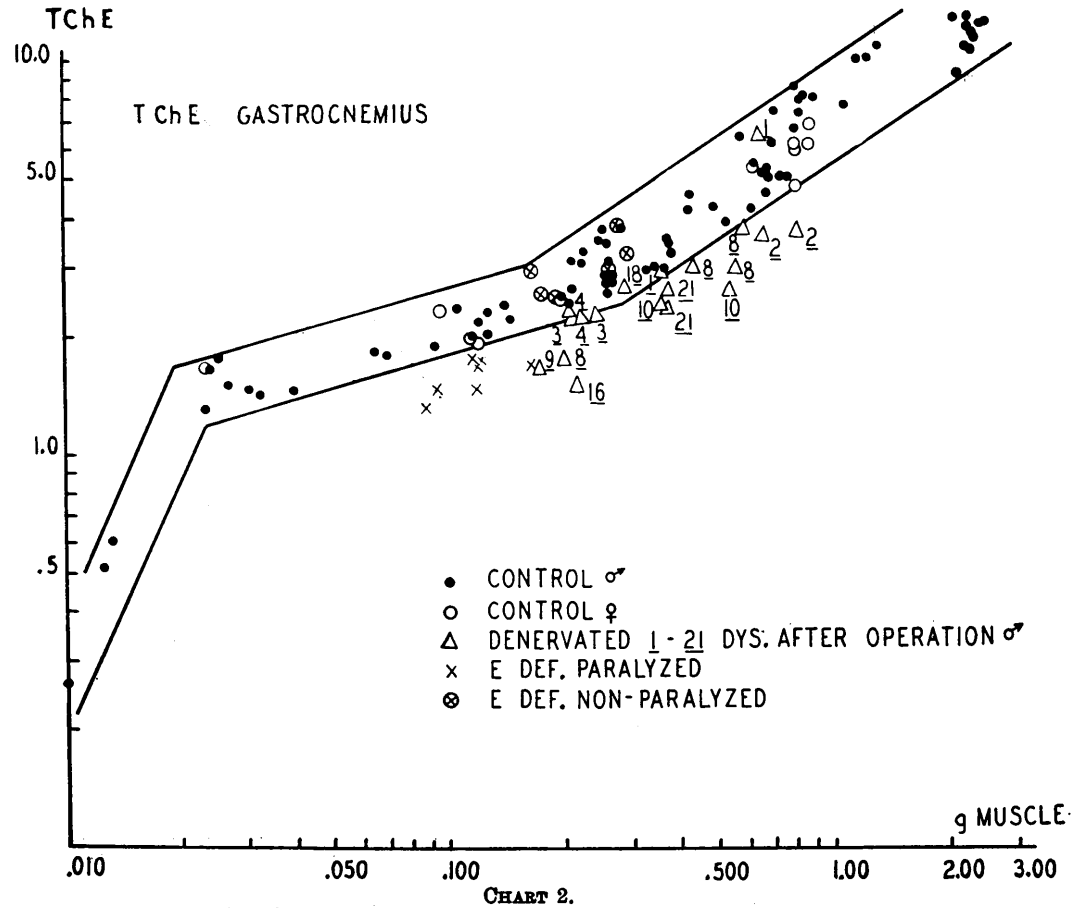

CHART 2.

Log. log. plotting TChe of gastrocnemius against gastrocnemius weight. The band includes all the controls.

as in many other organs or body constituents (Needham,[8] Zucker *et al.*[9]). The denervated gastrocnemii rather soon after section of the nerve are seen to be reduced in size. The muscular atrophy however is not progressive and it appears that in the later stages both failure of growth of the muscle and atrophic changes account for the deviation from the normal range of relative weight.

In Chart II the ChE activity of the whole muscle (TChE) is compared with the weight of the muscle on a log. scale. The width of the band includes all the data obtained from the 88 controls, expressing both biological variations and experimental error. Muscle weight and not the usual body weight has been chosen as ordinate because of the growth retardation of the muscle combined with atrophic changes occurring after denervation. the log.-log. relation obtained presents 2 "breaks" indicating changes in the rate of relative increase of the enzyme concentration. The first occurs after about 1 week of age of the animals, the second close to the onset of sexual maturity. The bulk of the TChE values in muscles denervated for more than one day, and in animals with muscular dystrophy, fall below the range of the established norm. On an average, the TChE of these two groups is reduced by approximately one-third. This reduction in the TChE of the

[8] Needham, J., *Biochemistry and Morphogenesis*, Cambridge Univ. Press., 1942.

[9] Zucker, T. F., Zucker, L., and Sperry, W. M., *Abstracts 108th A. C. S. Meeting*, Sept., 1944, p. 2B.

TABLE I.
TChE of M. Obliqu. Inf. Oculi.

Autopsy No.	Diagnosis	Sex	Age	Muscle wt.	TChE
14,414	Meningitis	♂	18	.236	9.2
415	Lymphosa	♀	58	.138	8.2
418	Arterioscl.	♀	84	.188	14.7
419	Lymphosa	♀	73	.155	11.3
421	Ca of lung	♂	51	.325	13.9
422	Paget Dis. of bone, Pneumonia	♂	69	.201	11.3
423	Diab. mellit., Glomerulo nephr.	♀	30	.205	9.6
424	Mult. myeloma	♂	67	.211	13.3
425	Cardiac insuff.	♀	74	.177	11.9
427	Pneumonia	♂	60	.247	11.3
432	Myel. leucem.	♀	37	.151	8.8
462	Myasth. gr.	♀	30	R.235	4.9
				L.231	4.6
476	Hyperthyroid	♀	42	.186	9.2

TABLE II.

No.		Cc Prostigm. (1:10,000)	Min after Ist inj.	G gastrocn. wt	TChE
Control	1	—	—	.847	5.95
,,	2	—	—	.795	4.65
,,	3	—	—	.855	6.75
,,	4	—	—	.612	5.27
,,	5	—	—	.790	6.02
,,	6	—	—	.793	5.80
Exp.	7	1 × .2	30	.797	5.47
	8	1 × .4	30	.724	5.47
	9	1 × .8	died after 15	.616	4.45
	10	1 × 1.0	30	.870	5.12
	11	2 × .2	150	.905	5.78
	12	4 × .2	270	.930	4.65
	13	4 × .2	270	.850	4.25
	14	4 × .4	270	.807	3.67

All injected animals showed marked symptoms of prostigmine poisoning beginning about 10 minutes after the injection. The maximal response appeared after *ca.* 30 minutes. The effect lasted throughout the intervals in the animals which received repeated injections.

denervated muscle obviously is not a function of the time elapsed after the section of the nerve, for it is essentially the same after 2 days as after 21 days. The question as to the specificity of the fraction of the enzyme which remains active after denervation and in the dystrophic muscle may only be answered by further studies.

It seems probable that the transmission of the nerve impulses is disturbed in patients with myasthenia gravis. In 2 instances (Jones and Stadie,[10] Goodman *et al.*,[11]) the activity of ChE has been examined in muscle tissue obtained by biopsy from patients with myasthenia gravis and has been compared with that of controls. No differences were found. The comparison of small portions of muscle with regard to their ChE activity, however, as pointed out above, is open to criticism. ChE is known as a very stable enzyme. It therefore appeared safe to use tissue from autopsy material. The inferior oblique muscles of the eye were dissected from a patient who died of myasthenia gravis and in whom a large thymoma (12 x 6 x 4 cm) was present. The ChE activity of the whole muscle was determined as described above and was compared with that of muscles from patients dying from various other diseases. The findings are summarized in Table I where it is seen that the enzyme activity in the patient with myasthenia gravis was only about half as high as in the lowest of the controls. From the data on hand it cannot be decided whether or not all

[10] Jones, M. S., and Stadie, W. C., *Quart. J. Exp. Physiol.*, 1939, **29**, 63.

[11] Goodman, L., Carlson, R. I., and Gilman, A., *J. Pharm.*, 1939, **66**, 15.

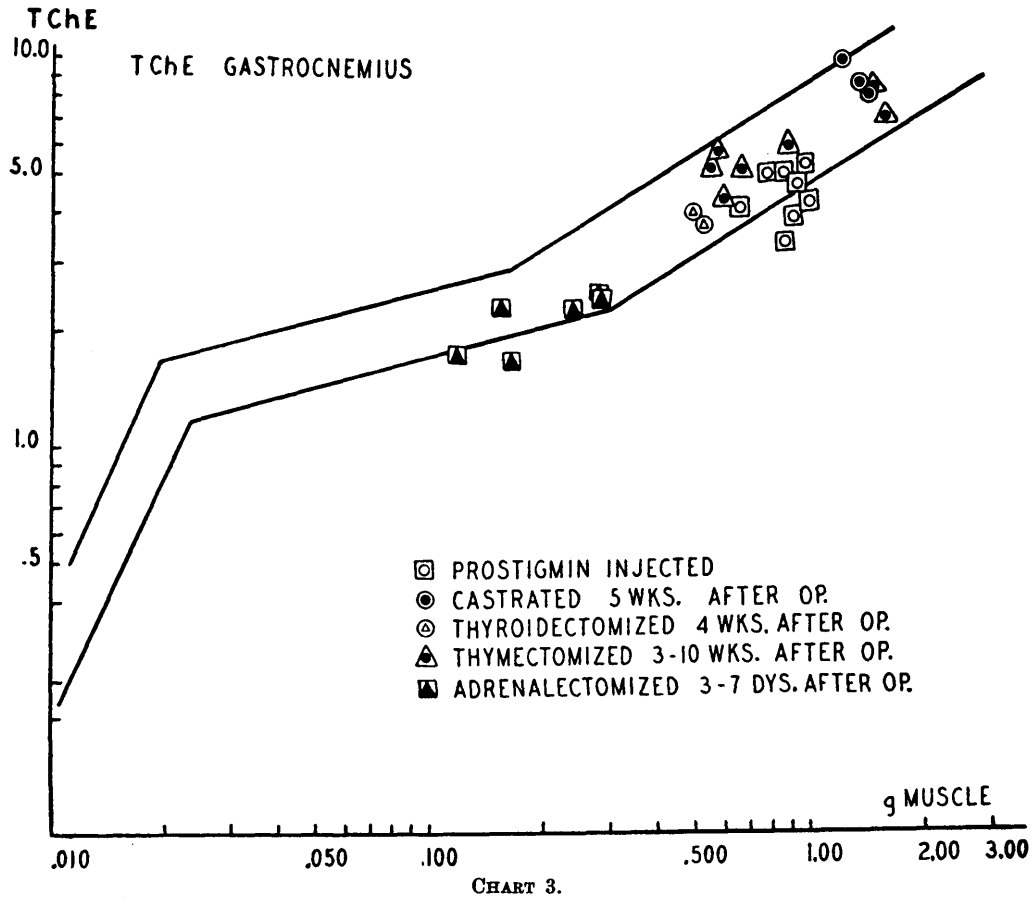

CHART 3.
Comparison of range of data (controls) from Chart 2 with experimental data.

of this reduction of the ChE activity was due to the prostigmine treatment (.5 mg almost every hour) the patient received before death. It is seen from Table II and Chart III that in rats, even the repeated injection of extremely large amounts of the drug causes only a relatively small reduction of the enzyme activity. Thymectomy in rats did not influence the activity of ChE as seen from Chart III.

In hyperthroidism an increased activity of ChE was found in the serum by Antopol *et al.*[12] The muscle ChE in a patient who died from Grave's disease (Table I) and in 2 thyroidectomized rats (Chart III) was unchanged.

Other experimental findings are summarized in Chart III and Table II. It has been shown by Glick[13] that the decrease in the ChE activity observed in the blood of prostigmine injected animals is not reflected in tissue (gastric mucosa). This is also brought out from part of our data. It was found that most marked effects of prostigmine poisoning may be observed without measurable reduction in the activity of the enzyme. Repeated large doses of prostigmine, however, did significantly lower the activity of ChE in muscle. In 3 castrated and 2 parathyroidectomized animals the enzyme activity was unchanged. That in 2 of 6 animals after adrenalectomy the TChE fell below the range of the controls appears of interest. Additional data are needed to confirm or refute the significance of this observation.

Summary. The choline esterase activity

[12] Antopol, W., Tuchman, L., and Schifrin, A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 46.

[13] Glick, D., *J. Gen. Physiol.*, 1938, **21**, 297.

of the whole gastrocnemic muscle (TChE) in rats was found to be reduced by approximately one-third after denervation and in nutritional muscular dystrophy. It was slightly lowered after repeated injections of high doses of prostigmine and after adrenalectomy. Single injections of high doses of prostigmine had no such effect.

Thymectomy, thyroidectomy, parathyroidectomy, and castration had no effect on the TChE of the rat gastrocnemius.

The TChE of the musc. obliqu. inf. oculi in a patient who died from myasthenia gravis was found to be only half as high as that of the lowest in a series of 12 controls. It was not possible to decide whether or not all of this reduction of the enzyme activity was an effect of prostigmine treatment. The ChE activity in the muscle of a patient dying from hyperthyroidism was within the range of the controls.

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Effect of Soybean Phosphatides on Utilization of the Fat-soluble Vitamins.

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We reported[1] that the utilization of vitamin A and carotene in the rat is influenced by an as yet unidentified factor present in soy bean lecithin. Patrick and Morgan[2] confirmed our findings in regard to the chick and found that the same factor influences vitamin E utilization as well as vitamin A metabolism. They also showed that yeast, extracted with certain fat solvents, was lacking in this specific factor. Earlier, Polskin[3] reported that lecithin plays an important role in the conservation of the hepatic vitamin A reserves of the chick.

Jensen, Hickman, and Harris[4] have questioned our findings on the grounds that soy bean lecithin contains a certain amount of vitamin E and they express the belief that the observed effects were due to the increased vitamin E intake provided by soybean lecithin in our diet. They could obtain no marked difference from addition of soybean lecithin to the diet of the rats in their experiments. Apparently they overlooked our suggestion that the unknown factor is contained in yeast, which was proved by Patrick and Morgan. Inasmuch as the basic diet of Jensen, Hickman, and Harris contained 8% yeast, their observations do not bear upon the problem under consideration.

To exclude the possibility that the effects of the unknown factor may be attributed to vitamin E, the following experiments were carried out in which the amount of vitamin E contained in soy bean lecithin was taken into consideration. The vitamin E content of soy bean lecithin* was determined according to the method of Emmerie and Engel, and Scudi and Buhs, as 1.3 mg per g.

Experimental. Female albino rats, Sherman strain from our laboratory colony, 28 to 30 days old, weighing approximately 52 to 60 g were kept in individual metal cages with wire mesh floors. The basal diet fed had the following percentage composition: casein, Labco, 20; cerelose, 72; salts, Sure's No. 1, 4; cottonseed oil, 4. The diet was supplemented with thiamine, 2 mg; riboflavin, 4 mg; pyri-

[1] Slanetz, C. A., and Scharf, A., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 17.

[2] Patrick, H., and Morgan, C. L., *Science*, 1943, **98**, 434.

[3] Polskin, L. J., A thesis submitted to Rutgers University, New Brunswick, N.J., May, 1940.

[4] Jensen, J. L., Hickman, K. C. D., and Harris, P. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 294.

* This assay was made by Food Research Laboratories, Inc., Long Island City.