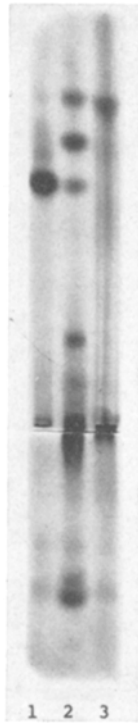
The Genetics of a Soluble Acidic Protein in Skeletal Muscle[1]

The microtubule protein must be one of the older proteins in an evolutional sense, for microtubules are the components of bacterial flagella as well as the mitotic spindle of all eukaryotes. In vertebrates, aside from mitotic spindles, the cilium of certain epithelial cells, the tail of spermatozoa, neurofilaments of the nerve axon and sarcotubules of the skeletal muscle utilize this protein. It has been shown that the microtubule protein is an acidic protein of globular shape having a molecular weight of 120,000.

It was of extreme interest to us to find out whether the microtubule protein used in different organelles of vertebrates is specified by a single gene locus or by a group of duplicated gene loci. The present paper describes the autosomal inheritance of a soluble acidic protein found in skeletal muscle of the toad and the quail. In the quail it will be shown that this protein is a dimer having the molecular weight of 120,000, and the possibility that it is a constituent of sarcotubules will be considered.

When the skeletal muscle extracts of the toad (*Bufo americanus*) and the Japanese quail (*Coturnix coturnix japonica*) were subjected to starch gel electrophoresis at ph 8.6, an intensely stained protein banding pattern was identified on the gel plate at a position far toward the anode (Figure). Allelic polymorphism of this protein was observed. The homozygotes showed a single band, while the heterozygotes showed 3 bands. The progeny test done on the quail confirmed the single autosomal locus inheritance of this protein. The mating between heterozygotes (1/2) such as shown in column 2 of the Figure, produced 3 types of progeny (1/1, 1/2 and 2/2) in the expected 1:2:1 ratio.



Vertical starch gel electrophoresis of quail skeletal muscle extracts. Extracts were prepared by mincing and sonicating 5 mg of pectoralis muscle in 2 volumes of pH 7 adjusted distilled water at 4 °C. A supernatant was obtained by centrifugation in the cold at 40,000 *g* for 30 min. Protein concentrations were determined using the Biuret method and were occasionally estimated by the 280/260 mμ absorbance ratio. Vertical starch gel electrophoresis was carried out, as previously described[9] at pH 8.6 in borate buffer. Gels were fixed and protein bands stained with Amido Schwartz. Column 1: 1/1 homozygotes with a single intensely stained band. Column 2: 1/2 heterozygotes with 3 bands. Column 3: A single band of 2/2 homozygotes.

Binding specificity of H^3-colchicine

Extracting solution	Counts/min H^3-colchicine	Boiled extract + H^3-colchicine	Protein (cpm/mg)
Quail pectoralis muscle			
dist. H_2O pH 6.5	4,872	953	2,528
P-Mg (pH 6.5)	6,350	1,383	3,443
P-Mg + 0.1 m*M* GTP	7,809	1,067	4,420
P-Mg + 0.1 m*M* GTP + 0.1 m*M* UTP	10,245	1,340	5,517
P-Mg + 0.1 m*M* GTP + 0.1 m*M* UTP + $\sim 1 \times 10^{-5} M$ Unlabelled colchicine	1,445	1,470	–
Rabbit serum	1,045		–
Rat brain			
P-Mg + 0.1 m*M* GTP	52,312	1,504	30,827

The sarcoplasmic extracts obtained by homogenizing muscle tissue under conditions of low ionic strength generally contain all of the soluble enzymes, the sarcotubule protein, and a number of unidentified proteins present in small amounts[2]. Since this particular protein represented 30% of the total protein, it seemed most reasonable to consider that it might be the muscle microtubule subunit. The fact that this protein can be extracted from skeletal muscle but not from cardiac muscle indicates that this protein is neither actin nor myosin. Furthermore, the procedure of EISENBERG and EISENBERG[3,4], which is supposed to specifically extract sarcotubules did extract this protein.

To further establish the identity of this protein, molecular weight measurements were made using zone-sedimentation procedures in a preparative ultracentrifuge and by Sephadex G-200 gel filtration[5]. Exponential sucrose gradients were designed to obtain constant velocity sedimentation. I^{125}, labelled IgG, was used as an independent marker and an *S* value of 5.7 was obtained in this way. Using the equation[7] $\frac{S1}{S2} = \left(\frac{MW\,1}{MW\,2}\right)^{2/3}$, the molecular weight was estimated as 119,000. Filtration of the sarcoplasmic extract through Sephadex G-200 gel columns was accomplished in the manner of ANDREWS[6] and gave a molecular weight estimate of 120,000 in good agreement with the value obtained in the ultracentrifuge. Location of the protein in gradient and Sephadex fractions was made by collecting and electrophoresing

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appropriate fractions in starch gels. Those quail which typed as 1/2 heterozygotes were used since the resultant banding pattern was more distinctive.

It has been shown that one dimeric molecule of microtubule protein specifically binds with 1 mol of colchicine[6]. To test colchicine binding, the minced muscle was extracted in distilled water, P-Mg[7] buffer or P-Mg buffer containing nucleoside triphosphates (Table). Supernatant solutions and appropriate controls were then incubated with $2.5 \times 10^{-7} M$ H^3-colchicine at 37°C for 1 h. Colchicine binding was then assayed using the Whatman DE81 filter method described by WEISENBERG et al.[8].

Cold colchicine was added to a final concentration of $1 \times 10^{-5} M$, incubation mixtures were diluted with 9 ml of P-Mg buffer and gravity filtered through Whatman DE81 paper. Collected protein was then washed 5 times with P-Mg buffer and counted in a Beckman LS233 liquid scintillation spectrometer to 2% standard error. The binding of H^3-colchicine is specifically blocked by $1 \times 10^{-4} M$ unlabelled colchicine and sources lacking the microtubule do not bind the tritiated alkaloid at all.

Investigations are currently underway to study the kinetics of colchicine binding and to extend the microtubule assay to other tissues of the quail. Since 2 allelic forms are maintained in the stocks, many questions regarding gene redundancy for such a conservative protein may be answerable.

Zusammenfassung. Ein Hauptbestandteil von wässerigem Skelettmuskelextrakt ist ein dimeres, saures Protein mit einem Molekulargewicht von 120 000. In der Wachtel *Coturnix coturnix japonica* und in der Kröte *Bufo americanus* wird dieses Protein von einem einzelnen autosomalen Locus kontrolliert.

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Phosphoglucomutase Polymorphism in the Rainbow Trout, *Salmo gairdneri*[1]

Zone electrophoresis of phosphoglucomutase (PGM) using muscle extract from rainbow trout, *Salmo gairdneri*, was carried out by ROBERTS and TSUYUKI[2], who reported 2 distinct isozymes at pH 8. At higher pH's less clear zones of activity up to a maximum of 5 were seen. They did not report interindividual polymorphism. WOHNUS (unpublished) has found PGM isozyme polymorphism in the Amphibian genera *Bufo* and *Rana*.

Other electrophoretic studies of PGM have largely involved human tissue extracts. SPENCER et al.[3] found polymorphism for phosphoglucomutase in man with electrophoresis of erythrocyte lysates. They reported 2 groups of bands, involving a total of 8, with variation restricted to the slower migrating group. They postulated 2 loci which are now labelled PGM_1 for the slower bands and PGM_2 for the faster bands. PGM_2 polymorphism has been reported (HOPKINSON and HARRIS[4]), and recently, a faster additional variable group of isozymes in man from the placenta, liver and kidney has led to the postulation of a third locus, PGM_3. The PGM_3 banding is the most prominent banding in cultured fibroblasts, while in muscle they are 'extremely weak and barely detectable' (HOPKINSON and HARRIS[5]). PARRINGTON et al.[6] found no genetic linkage between PGM_1, PGM_2 and PGM_3 in a familial study. Considerable differences in PGM_1 allelic frequency in human populations indicate the occurrence of genetic drift or natural selection (MOURANT and TILLS[7]).

In the present study, skeletal muscle extracts of rainbow trout, *Salmo gairdneri*, were run in vertical starch gel electrophoresis at pH 8.6 using the technique of WOHNUS. Gels were run for 16 h at 450 volts in an Otto Hiller V-type electrophoresis apparatus. The staining mixture consisted of 0.1 g $MgCl_2$, 0.035 g TPN, 0.075 g MTT tetrazolium, 0.001 g phenazine methosulfate, and 0.30 g glucose 1 phosphate dissolved in 50 ml 0.03 *M Tris* buffer (pH 8.0) to which was added 25 λ of glucose-6-phosphate-dehydrogenase (G-6-PD) just before use.

A total of 72 rainbow trout from a commercial fish farm (Happy Jack's Fish Farm, Azusa, California) were studied. A band that migrated slightly to the cathode was seen in the same position in all the zymograms, and was designated (a). A variable, intense banding pattern appeared on the anodal side of the origin, and consisted of 2 distinct bands or single bands at 1 of the 2 corresponding positions. The band closest to the origin was designated (b), while that which migrated farthest was called (c). Thus some specimens showed (b) and (c), while others showed only (b) or (c). The (a), (b) and (c) bands can be seen in the Figure. Of the 72 specimens, 17 were (bc), 2 were (b) and 53 were (c). If one interprets the 2-band (bc) combination as the heterozygous combination of 2 alleles that are respectively homozygous in the single (b) and (c) phenotypes, the fit to Hardy-Weinberg expectations is as follows:

No. tested: 72. $p(b) = 0.15$, $q(c) = 0.85$

	Phenotypes		
	(b)	(bc)	(c)
Observed	2	17	53
Expected	1	18	52

The results are consistent with an assumption of a Hardy-Weinberg equilibrium.

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