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 WOHNUS (unpublished) has found PGM isozyme polymorphism in the Amphibian genera *Bufo* and *Rana*.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.

[1] Supported in part by a grant No. CA-05138 from the National Cancer Institute, U.S. Public Health Service and in part by Animal Resources grant No. FR00433.
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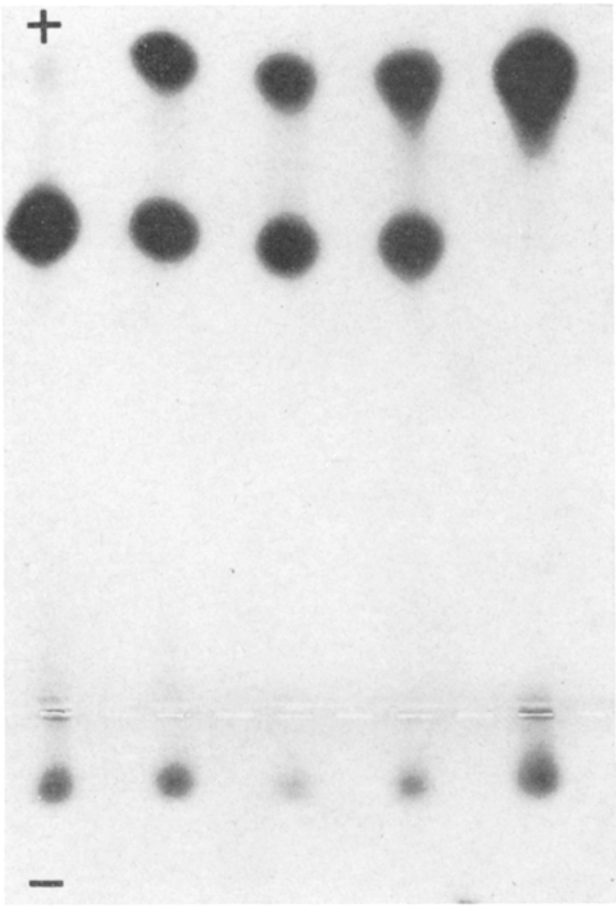

Zymogram for phosphoglucomutase from skeletal muscle homogenates of 5 rainbow trout showing the (bb) homozygote, (bc) heterozygote (3), and (cc) homozygote.

Finally, a very faint non-variable band, designated (d) migrated still farther to the anode. Thus all specimens were identical for (a) and (d). Because of the wide separation of (a) and (d) with the insertion of (bc) bands it is assumed that (a) and (d) represent products of 2 different homozygous loci. Consequently, a total of 3 loci are postulated for phosphoglucomutase in rainbow trout.

The possibility exists that these 3 loci are homologous to the PGM_1, PGM_2 and PGM_3 loci in man, an idea supported by the similar nature of the fastest band. HOPKINSON and HARRIS'[5] description of the PGM_3 banding as barely detectable in human muscle extracts would apply to the trout muscle zymograms. In the present study, this band has not been studied in extracts of other tissues which might show it more clearly. Despite the apparent similarity of trout and human phosphoglucomutase in being under the control of 3 loci, the total number of isozymes in man is about twice the number in trout.

Zusammenfassung. Es wird aufgrund von Stärke-Gel-Zymogrammanalysen der Phosphoglukomutase in 72 Forellen (*Salmo gairdneri*) die Existenz eines polymorphen und zweier nicht variierender Gene ermittelt. Der Polymorphismus beruht auf 2 Allelen mit übereinstimmenden Frequenzen im Hardy-Weinberg-Gleichgewicht.

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Purothionins in *Aegilops-Triticum* spp.

Purothionin was first obtained from the endosperm of hexaploid wheat (*Triticum aestivum* L.) and crystallized by BALLS et al.[1]. This high sulphur protein moiety of a proteolipid has bactericidal and fungicidal activity[2]. Recent work[3–6] has established that the crystallized material is a mixture of approximately equal amounts of 2 forms: purothionins α and β. Molecular weight determinations, aminoacid composition and other properties indicate that the 2 forms are very closely related[5]. We have found that both the allohexaploid *T. aestivum* L. (genomes *ABD*) and the allotetraploid *T. durum* Desf. (genomes *AB*) synthetize the α and β forms[6]. This note is to report some phylogenetic implications of purothionins.

The diploid species *T. monococcum* (*A*) synthetizes only the β form, suggesting that the *A* genome of *T. durum* is responsible for the genetic control of β form synthesis and the *B* genome for that of the α form. Analysis of the potential *B* genome donor, namely, the diploid species *Aegilops speltoides* ($S = B$), which does synthetize the α form, substantiates the hypothesis. This indicates that α and β purothionins are the result of divergent evolution at the diploid level and have come to coexist by the convergent process of alloploid formation.

We have further investigated the occurrence of α and β forms in the remaining species of the *Aegilops-Triticum* group. A micromethod was used because only small amounts of material were available. The samples, 200 to 400 mg of ground kernels were macerated for 2 h with twice the amount (v/w) of petroleum ether (b.p. 35–60°C). The supernatant was transferred with the aid of a capillary tube to a piece of paper (Whatman No. 3, 2×8 mm) and evaporated in the process. Lipid was dissociated from purothionin by treating the paper with 1 *N* HCl in ethanol: petroleum ether (3:1) with the aid of a capillary and then was extracted by immersion in petroleum ether for 1 h. The dried paper was wet with buffer and the purothionins fractionated by starch-gel electrophoresis.

The results are summarized in the Figure. The occurrence of the previously described[7] linoleate (L) and palmitate-

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