coeliac patients by using Leu-Pro, Phe-Pro and Glu-Pro, the zymograms were almost identical. These present results and the findings of others (Lindberg, Norden & Joseffson, 1968; Douglas & Booth, 1969) strongly suggest that the decreased activities of some peptidases in untreated coeliac intestine may be due to secondary phenomena attributable to the abnormal morphology of the mucosa.

To obtain more specific information about the intestinal hydrolysis of gluten, ^{125}I -labelled gliadin peptides were used to study intestinal peptidases. Results will be presented on the suitability of these peptides as substrates for intestinal peptidases.

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Multiple Forms of Pig Intestinal β -Galactosidase and Related Activities

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Studies on humans (Hsia, Makler, Semenza & Prader, 1966; Gray & Santiago, 1969), rats (Asp & Dahlqvist, 1968) and a number of other species indicate that intestinal β -galactosidases fall into two main groups. 'Acid' β -galactosidases have low pH optima, are readily solubilized, work best on hetero galactosides and are inhibited by *p*-chloromercuribenzoate. 'Neutral' β -galactosidases are essentially disaccharidases with neutral pH optimum that are associated with brush-border particles. They are not readily inhibited by *p*-chloromercuribenzoate. Multiple forms may occur within these groups and the substrate specificity may include glycosides with a β -glucosidic configuration.

We have examined pig intestinal mucosa and compared its enzymes with those described in pig kidney by Abrahams & Robinson (1969). Four enzymes with activity towards 4-methylumbelliferyl β -D-galactoside can be separated on DEAE-cellulose at pH 6.5 in 10mm-phosphate buffer by using a NaCl gradient. Form I is unretained by the columns; forms II, III and IV are eluted successively with increasing salt concentration. Form I has narrow specificity in requiring the galactose configuration at C-4, but hydrolyses hetero glyco-

sides (β -galactosides and α -arabinosides) as well as lactose. Forms II and III have identical specificities, hydrolysing β -glucosides and β -galactosides and the corresponding pentosides, but have no effect on lactose. Form IV has hexosidase but no pentosidase activity, and it is most effective on lactose. Unlike forms I, II and III it is not readily inhibited by *p*-chloromercuribenzoate and closely resembles the 'neutral' lactases previously reported. On starch gel electrophoresis at pH 6.8, form I remained at the origin, forms III and IV migrated rapidly towards the anode at the same rate, and form II showed intermediate mobility. Forms III and IV could be resolved electrophoretically on thin agarose gels. At pH 5.0 form I is dissociated into two bands migrating in opposite directions but retaining β -galactosidase activity. The two forms of soluble β -glucosidase reported by Abrahams & Robinson (1969) in pig kidney have the same substrate specificities and electrophoretic mobility as forms II and III reported here.

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Isoenzymes in the Study of Wild Populations

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The superior resolution of starch-gel electrophoresis in the separation of proteins has added a new dimension to studies in genetics. This technique has enabled us to examine variation at the molecular level. It has been shown that normal body proteins are polymorphic and that the actual molecular variants present are genetically determined. Thus with the aid of this biochemical technique it is now possible to detect and analyse the action of both directed and stabilizing selection in natural populations at the molecular level.

In the present study samples from a wild population of the house mouse, *Mus musculus*, living on the small island Skokholm off the coast of Pembrokeshire in South Wales (Berry, 1968) were surveyed at six loci. Gene frequencies were computed at the beginning and end of the breeding

season in two successive years. For comparison wild mice from the neighbouring mainland were sampled at the same time.

Blood was obtained from the suborbital canthal sinus of trapped mice and the unharmed animals were released again into the population. The serum and erythrocytes were separated and frozen for later analysis. The proteins were separated by horizontal starch-gel electrophoresis. In the erythrocytes the systems studied were haemoglobin *Hbb* (Gluecksohn-Waelsch, Ranney & Siskin, 1957) and the erythrocyte peptidases *PepC* and *PepB* (Lewis & Truslove, 1969). In the serum two esterase loci were examined, the pre-albumin esterase designated *Es2* (Petras, 1963) and a post-albumin esterase *Es5* (Petras & Biddle, 1967). In the β -globulin fraction of mouse serum the transferrins *Trf* were studied.

Both the Skokholm and mainland mice were polymorphic at all six loci. In fact of the 15 alleles found in the mainland samples all but one, that of *Es2*^c, occurred on the island. Of the mainland mice 17.8% are heterozygous at these polymorphic loci compared with 25.3% of the island mice. Of interest is the fact that on Skokholm natural selection was seen to be acting on the variants at three loci. Heterosis or an excess of heterozygotes over that expected on the Hardy-Weinberg equilibrium was found to exist at the beginning of the breeding season (March) at the *Es2* locus and at the end of the breeding season (September) at the *Hbb* locus. The gene frequencies at these loci returned to the expected value in September at the *Es2* locus and in March at the *Hbb* locus. Thus powerful selection pressures are shown to operate at both ends of the breeding cycle. There was selection against mice carrying the *PepC*² allele during the summer. These trends were repeated in both years of our study. Marked numerical fluctuation in population size occurs during the breeding season from about 200 animals at the beginning to about 2000 at the end. Neither genetical nor physiological investigation (Berry, Jakobson & Moore, 1969) has uncovered any factor that can be seen to contribute to this.

These studies support the assumption that polymorphism is a widespread and important phenomenon and that it is generally associated with and maintained by heterozygous advantage. Electrophoretic separation of proteins enables us to witness selection in operation and to observe and analyse its immediate effects. Investigation by this method at the gene level by using a sampling technique that does not harm the individual or interfere with his contribution to subsequent populations has become a powerful tool in ecological and population genetics. Gene frequencies have been determined in man (Harris, 1969) and in *Drosophila pseudoobscura*

(Lewontin & Hubby, 1966), but this is the first time that a population has been studied through several generations where the forces contributing to the gene frequencies found can be better assessed.

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The Role of Thiol Groups in the Multiple Forms of an Invertebrate Creatine Kinase

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The creatine kinase (EC 2.7.3.2) of the marine polychaete *Murphysa sanguinea* has been purified and found to consist of five electrophoretically distinct forms, none of which appears to be associated with particulate matter of the cell or to show organ-specific distribution (Fitzsimmons & Doherty 1970). Carbohydrate was found to be associated with the enzyme protein, but was removed by gel filtration in 0.1 M-KCl without affecting the enzyme activity or multiplicity. Only partial separation of the multiple forms has been achieved to date despite the wide variety of fractionation procedures tested. It has been found that rechromatography of a mixture of forms 3, 4 and 5 on DEAE-cellulose at pH 8.3 resulted in the recovery of 16% of the total protein as forms 1 and 2. Refractionation of forms 3, 4 and 5 by precipitation with 65% (v/v) ethanol in the presence of magnesium acetate failed to produce detectable amounts of forms 1 and 2. Forms 1 and 2 had a considerably lower specific activity than a mixture of forms 3, 4 and 5, but were activated by incubation with 0.25 M-dithiothreitol to the extent of 350%. Under the same conditions forms 3, 4 and 5 show only a 26% increase.

Immunological studies indicated a close relationship of all five forms of the enzyme. An apparent interconversion of the molecular forms detected by gel electrophoresis was achieved by incubation with dithiothreitol and with the oxidized form of this compound. Titration of the thiol groups of the different enzyme forms with 5,5'-dithiobis-(2-nitrobenzoic acid) showed that both the easily titratable thiol groups and the total thiol groups