Amino acid derivatives such as acetylleucine and leucylhydroxamic acid do not promote the exchange. The simple dipeptides leucylglycine and glycylleucine are inactive. Chloramphenicol at a concentration of 100 μg/ml did not affect the reaction. The amino acid analogues, norleucine, tryptozan and O-F-phenylalanine, did not inhibit the exchange promoted by their corresponding amino acids, leucine, tryptophan and phenylalanine. The analogues could not substitute for the amino acids in promoting the exchange.

The rapid rate, as well as the widespread distribution of the exchange reaction, suggests that it may reflect a system of major metabolic importance. The relationship of this reaction to protein synthesis must await the demonstration of its requirement in a system where amino acids are being actively incorporated into protein. The significance of the fact that the reaction is apparently specific for only 8 amino acids is not readily apparent. It is possible that these 8 AA represent a primary activation mechanism and the apparently inactive amino acids could be secondarily activated through some transfer reaction with one of the 8 active amino acids. Work now in progress is directed toward the purification of this enzyme system and to the determination of the active form of the amino acid.

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The preparation of a water soluble dinitrophenyl-gliadin

The detailed examination of the chemistry and structure of the gliadin of wheat is made especially difficult by the lack of methods for fractionating it. Consequently, although the protein has been shown by physico-chemical methods to comprise several components it has not been possible to prove whether these are distinct individuals or the products of association from a single monomer. It is the purpose of this communication to draw attention to the fact that by treatment with fluorodinitrobenzene gliadin may be divided into two fractions, one of which is water soluble and is a more suitable subject for certain studies than the native protein.

Gliadin was prepared from Manitoba No. 2 flour as previously described (MILLS[1]). A solution of the dried gliadin in 30 ml 50% ethanol-water was passed through a column of Amberlite IRA 400 resin which had been equilibrated against 50% ethanol. To this solution were added 50 mg $NaHCO_3$ and 0.2 ml fluorodinitrobenzene. The mixture was shaken for 30 min and then allowed to stand for 18 hours. After removing the precipitate, the supernatant liquid was poured into 100 ml of deionised water. The soluble DNP-gliadin was precipitated by the addition of 15 ml of saturated NaCl solution, was washed rapidly with a small amount of water and dried over P_2O_5 *in vacuo*. The final yield was 27%.

A typical sample of this product, referred to as S-gliadin, contained 16.2% total N, 0.56% S and amide N equal to 28.1% of the total N, all on an ash and moisture free basis. It was readily soluble in water or alkaline buffers of low ionic strength but was insoluble below pH 6. The precipitate formed by acidifying aqueous solutions could only be dissolved in alkaline buffers, *e.g.* 0.005 *M* $NaHCO_3$. In alcoholic solutions (50–70%) it was soluble at all pH values.

In contrast to the findings of KOROC[2] and DEUTSCH[3] a complex mixture of dinitrophenyl derivatives was liberated on hydrolysis (18 h in equal parts of 5 *N* HCl and formic acid). Of the fifteen chromatographically distinct derivatives isolated only three have so far been identified, namely: DNP-aspartic acid, DNP-serine and DPN-valine. In each case only traces were present.

Characterisation in the ultracentrifuge indicated that the results were dependent on the nature of the solvent. In aqueous glycine buffer of pH 9.4 the behaviour was that of a system of molecules capable of forming a number of association complexes; while in 0.005 *M* $NaHCO_3$ the S-gliadin was present mainly as a single component of very high sedimentation rate. In alcoholic solutions a single well defined, though usually asymmetrical peak was always observed. Some of the results obtained in these solvents are summarized in Table I. $S_{20,w}$ fell in the range 2.15–2.35 Svedbergs except when the solution was buffered with acetate or citric acid-phosphate

mixtures, when values of 1.8 and 2.51 Svedbergs respectively were found. It was also found that the value of $S_{20,w}$ in solutions containing glycine buffer or sodium chloride was dependent on the concentration of S gliadin down to the least observable levels. In other solvents however, $S_{20,w}$ was independent of protein concentration below 1 %.

TABLE I

VALUES OF $S_{20,w}$ AT ZERO CONCENTRATION FOR S-GLIADIN IN SOLVENTS CONTAINING ALCOHOL

Solvent	*Buffer salts*	*pH*	$S_{20,w}$
70 % ethanol-water	0.03 *M* HCl	2.05	2.3
70 % ethanol-water	0.03 *M* acetic acid	4.5	2.35
70 % ethanol-water	0.033 *M* NaCl	5.6	2.2
70 % ethanol-water	0.01 *M* acetic acid and 0.02 *M* sodium acetate	6.1	1.8
70 % ethanol-water	0.01 *M* Na_2HPO_4 and 0.005 *M* citric acid	6.35	2.51
70 % ethanol-water	0.015 *M* glycine and 0.015 *M* NaCl and 0.018 *M* NaOH	9.15	2.25
70 % ethanol-water	0.005 *M* $NaHCO_3$	9.75	2.33
70 % ethanol-water	0.01 *M* $NaHCO_3$	9.8	2.26
70 % ethanol-water	0.02 *M* $NaHCO_3$	9.9	2.35
70 % ethanol-water	None	7.20	2.35
60 % ethanol-water	None	7.15	2.21
50 % ethanol-water	None	6.95	2.15

Although it is not clear from these results whether S-gliadin is a homogeneous protein derivative it would appear that a fractionation of the native protein has been achieved and further studies are being pursued.

Specially milled samples of flour were provided for these investigations by Dr. T. MORAN and Dr. D. W. KENT-JONES, and many of the ultracentrifugal experiments were made by Miss ANNE DOUGHTY.

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The exchange of $H^{14}COOH$ with the carboxyl group of pyruvate by *Clostridium butylicum* and *Micrococcus lactilyticus*

Escherichia coli decomposes pyruvate through a phosphoroclastic reaction leading to acetylphosphate and formic acid according to equation (1)[1].

$$CH_3COCOOH + H_3PO_4 \rightarrow CH_3COPO_4H_2 + HCOOH \quad (1)$$

In addition, anaerobically grown cells possess the enzyme system, formic hydrogenlyase, which catalyzes the breakdown of formate to H_2 and CO_2. Certain strict anaerobes, like *Cl. butylicum* and *M. lactilyticus* ferment pyruvate to acetyl phosphate, CO_2 and H_2, according to equation (2)[2,3].

$$CH_3COCOOH + H_3PO_4 \rightarrow CH_3COPO_4H_2 + CO_2 + H_2 \quad (2)$$

The similarity in the end products of pyruvate breakdown in these two groups of microorganisms suggested that the CO_2 and H_2 in the Clostridial fermentation may arise from formate. However, KOEPSELL AND JOHNSON[4] using cell-free extracts of *Cl. butylicum* which fermented pyruvate according to equation (2), demonstrated the inability of such extracts to ferment formate. This observation was confirmed by WILSON *et al.*[5] who also demonstrated a complete and rapid equilibration of $^{13}CO_2$ with the carboxyl group of pyruvate in this system. Under identical conditions $H^{13}COOH$ failed to exhibit any measurable exchange with pyruvate. Because of these observations, it has been assumed[6] that pyruvate is fermented by *Cl. butylicum* by direct decarboxylation to an acetaldehyde complex and CO_2. The hydrogen gas is then assumed to arise from the oxidation of the hypothetical aldehyde complex to acetyl phosphate.

During a study of the exchange of $H^{14}COOH$ with pyruvate carboxyl by extracts of *E. coli*[7,8,9], it was observed that the exchange reaction required the participation of at least two