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

enzymes[10]. One of these enzymes could be obtained from another source, namely, certain strict anaerobes such as *Cl. butylicum*, *Cl. pasteranium* and *M. lactilyticus*. Studies on the latter preparations unexpectedly disclosed that extracts of *Cl. butylicum* and *M. lactilyticus* prepared by grinding with *alumina*, were *alone* capable of rapidly exchanging formate with the carboxyl group of pyruvate (Table I).

TABLE I*

FORMATE-PYRUVATE EXCHANGE BY DIFFERENT EXTRACTS OF *Cl. butylicum*

Extract from	*Pyruvate (c.p.m./μM)*
Glass ground cells	2.3
Alumina ground cells	1518

Conditions: 100 μM PO_4 buffer pH 8.0; 50 μM K pyruvate, 50 μM K formate ^{14}C (4110 c.p.m./μM) 20 units CoA, 12.5 μg cocarboxylase, 20 μM dilithium acetylphosphate, 0.05 μM $MnCl_2$, 5 μM β-mercaptoethanol and 0.1 ml of each extract in a final volume of 1.0 ml. Incubated for 20′ at 37°.

* See ref.[9] for methods of analysis.

TABLE II

EFFECT OF pH ON THE EXCHANGE OF $^{14}CO_2$ AND $H^{14}COOH$ WITH PYRUVATE CARBOXYL

M. lactilyticus additions	*pH*	*Pyruvate (c.p.m./μM)*
$H^{14}COOH$	8.0	1420
$^{14}CO_2$	8.0	1239
$H^{14}COOH$	6.5	706
$^{14}CO_2$	6.5	2618
Cl. butylicum		
$H^{14}COOH$	8.0	579
$^{14}CO_2$	8.0	2455
$H^{14}COOH$	6.5	247
$^{14}CO_2$	6.5	2271

Conditions: 100 μM PO_4 buffer; 50 μM K pyruvate, 50 μM K formate (2950 c.p.m./μM) or 50 μM $H^{14}CO_2K$ (7560 c.p.m./μM) 0.3 ml extract in a final volume of 1.0 ml. Incubated for 30′ at 37°.

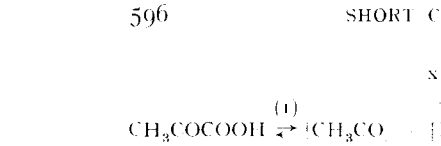
Since previous investigators[5] could not find evidence for this reaction in *Cl. butylicum* extracts, the characteristics of the exchange in the present preparations were carefully examined.

The effect of pH on the exchange of both $H^{14}COOH$ and $^{14}CO_2$ with pyruvate carboxyl by extracts of *Cl. butylicum* and *M. lactilyticus* revealed pronounced effects, particularly on the formate exchange reaction.

It can be seen (Table II) that with extracts of *M. lactilyticus* the incorporation of $H^{14}COOH$ into pyruvate is about 2 times more effective at pH 8.0 than at pH 6.5, while the reverse picture is seen with $^{14}CO_2$. In general, the data with *Cl. butylicum* extract are similar except that the effect of pH on the incorporation of $^{14}CO_2$ is not evident. This may well be due to a quantitative rather than a qualitative difference between the extracts, since *Cl. butylicum* carries out a more vigorous exchange of $^{14}CO_2$, than does *M. lactilyticus*. Since the previous investigators had utilized glass-ground extracts and had worked at pH 6.0 to 6.5 (where pyruvate fermentation is optimal), it is now evident why the formate exchange in these systems is not detected.

The enzyme system responsible for the formate exchange reaction in extracts of *Cl. butylicum* and *M. lactilyticus* shares a number of properties in common with the formate exchange system in extracts of *E. coli*. The most significant of these is its extreme lability. Like the *E. coli* system, the present extracts exhibit pronounced dilution effects[8,11] and are inactivated by such procedures as ammonium sulfate fractionation, dialysis, isoelectric precipitation and treatment with Dowex-1. The formate exchange system in these extracts, as in *E. coli*[8], appears to require the presence of phosphate, since the reaction is strongly inhibited when Tris buffer is substituted for phosphate and the inhibition is gradually overcome by the addition of increasing concentrations of phosphate. The reaction is strongly inhibited by oxygen, by 2 μm/ml each of diacetyl, versene and sodium hypophosphite, all of which likewise inhibit the *E. coli* exchange system[12,13]. In contrast with the *E. coli* system which is strongly inhibited by 10 μM/ml of triphenyltetrazolium, the present preparations are only inhibited about 10% by this compound. The rate of the formate exchange appears to be independent of the net breakdown of pyruvate, that is, formate exchange occurs rapidly in extracts which are no longer capable of carrying out the forward reaction of equation 2. This has been observed with the formate exchange system in the *E. coli* system by CHANTRENNE AND LIPMANN[9] and for the CO_2 exchange system in *Cl. butylicum* by WOLFE AND O'KANE[14].

The finding that *Cl. butylicum* and *M. lactilyticus* are able to exchange formate, as well as carbon dioxide, with the carboxyl group of pyruvate, raises the question of the state of oxidation of the two-carbon moiety which participates in the exchange. This finding also raises the question of the mechanism of fermentation of pyruvate by these extracts and the relationship of the formate exchange system to the fermentation reaction. It would appear that in order to exchange



Scheme 1

formate, the two-carbon moiety would have to be on the same oxidation level as acetate. If the exchange reaction can be assumed to reflect an initial step in the fermentation of pyruvate, then these organisms may be capable of cleaving pyruvate by a reaction similar to the phosphoroclastic reaction in *E. coli*. If this were the case, CO_2 would presumably be reduced to the formate level of oxidation prior to exchange with pyruvate carboxyl. These ideas are diagramatically presented in Scheme 1.

We visualize in step 1 a cleavage of pyruvate to a 2-carbon fragment at the oxidation level of acetate and a one carbon fragment at the oxidation level of formate. The brackets are intended to signify that these are not free compounds, but possibly exist in some bound form. The exchange of CO_2 with pyruvate carboxyl would require conversion to the formyl moiety through an oxidation reduction reaction with some unknown carrier (x) (step 2). In support of this suggestion are the following observations: (1) The exchange of CO_2 and pyruvate in these systems, which possess strong reducing power in the form of a powerful hydrogenase system, is extremely rapid and goes to completion; whereas similar CO_2 exchanges with animal[15] and microbial[16,17] preparations, lacking a potentially powerful reducing system, are slow and proceed to the extent of only a few per cent. It would appear then that the rapidity and completeness of the reaction can be correlated with reducing ability and the reducing ability may control the rate of step 2 in Scheme 1. (2) Certain dyes which can act as electron acceptors have been reported to inhibit CO_2 exchange in *Cl. butylicum*[14]. This observation could be interpreted to indicate that the dyes inhibit the availability of electrons for the reduction of CO_2. (3) It is well known that hydrogen production by these anaerobes decreases with increasing pH[18] and that hydrogenase functions very poorly at an alkaline reaction[19]. Thus the decreased ability of *M. lactilyticus* to exchange CO_2 at an alkaline reaction would also be consistent with Scheme 1. Conversely, if the ability to utilize the formyl compound through the hydrogen evolving system (step 2) decreases as the pH increases, one would expect an increasing formation of formate (step 3) as well as increased exchange with free formate as the pH becomes more alkaline. The fact (Table II) that formate exchange is considerably better at the alkaline pH is consistent with this idea. In this connection, it is interesting to note that OSBURN *et al.*[20] reported a gradually increasing accumulation of formate during glucose fermentation by *Cl. butylicum* as the pH was shifted from pH 4.5 to 7.5 with bicarbonate.

These considerations suggest that a bound form a formate may be an intermediate in pyruvate fermentation by *Cl. butylicum* and *M. lactilyticus* and that its fate, *i.e.*, conversion to CO_2 and H_2 or production of free formate, is regulated among other things by pH. The bound formate, rather than an acetaldehyde complex, could then be the immediate source of hydrogen in the fermentation. Work is now in progress to attempt to identify both the C_2 and C_1 moieties involved in this exchange.

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