folic acid requirement by thymine or thymidine[2]. The incorporation was confirmed by use of a portion of the thymine-2-^{14}C obtained from the previous experiment. Organisms were grown in 100 ml of medium A[3] without Tween, with 0.12 mg of labeled thymine added. After 4 days growth, pyrimidines and purines were isolated from the combined nucleic acids as described above. Of these, thymine was the only compound with detectable activity, 100 counts/min/μmole, as compared with a starting activity of 59,200. The small degree of incorporation of thymine is in agreement with the results of FRIEDKIN AND WOOD[7] in this organism and others. They have found that thymidine incorporation is 27 times that of thymine in *Tetrahymena*.

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Quantitative aspects of CO_2 fixation during protein synthesis from ammonium acetate

Micro-organisms, growing on synthetic media containing ammonium acetate as sole carbon source, rapidly incorporate isotope from added $^{14}CH_3COONH_4$ into protein[1,2]. In the presence of unlabelled $NaHCO_3$, this synthesised protein is less radioactive than in its absence. Short-term experiments[2] indicated that the protein initially synthesised under such conditions may derive as much as 70% of its carbon from the unlabelled bicarbonate.

These results could be explained either by the occurrence of a CO_2-fixation reaction of hitherto unsuspected magnitude, or by an increase in the steady-state concentration, due to the addition of bicarbonate, of a compound in ready equilibrium with CO_2 lying on one of the earliest stages of the pathway from acetate to protein. The following experiments support the latter explanation.

Growing cultures of acetate-grown *Pseudomonas* KB1[3] were suspended at pH 7.0 in 15 ml of medium containing 25 m*M* ammonium acetate (Flask A) or 25 m*M* ammonium acetate + 10 m*M* $NaHCO_3$ (Flask B) as carbon source. The 500 ml flask A was gassed with 100% O_2 and stoppered tightly, whilst 5% CO_2/95% O_2 was continuously bubbled through the medium in the similar flask B. At zero time, 0.75 ml of a solution of $^{14}CH_3COONa$, containing 30 μmoles of acetate and giving $1 \cdot 10^7$ counts/min under the conditions of radioassay used, was added to each flask. Samples (2 ml) were removed every 30 min and the protein, precipitated with trichloroacetic acid, was hydrolysed in sealed tubes at 110° for 18 hours. The amino acid nitrogen concentration was estimated colorimetrically[4] and the radioactivity of the amino acids was determined by direct assay on paper after autoradiography.

At both 22° and 30° (Table I), the specific activities of the protein synthesised in the presence of $^{12}CO_2$ were 83–85% of that synthesised in its absence. This shows that CO_2-fixation accounts for only 15–17% of the carbon incorporation in the synthesis of protein from ammonium acetate, and is of an order consistent with the known $C_3 + C_1$ condensations[5,6].

Recent experiments with acetate-grown *Pseudomonas*[7,8] have shown that acetate enters the tricarboxylic acid cycle at two sites, to form citrate at one and malate at the other. The present results and those obtained previously[2] are consistent with these findings. Cell-free extracts of *Pseudomonas* KB1 contain malic enzyme[10] and can form malate from pyruvate and CO_2. It is therefore likely that the addition of $NaH^{12}CO_3$ temporarily raises the steady-state concentration of malate or oxaloacetate (Fig. 1), causing the protein synthesised in the earliest stages of the short-term experiments to be derived from a "pool" of C_4-compounds of low radioactivity.

TABLE I

THE EFFECT OF $^{12}CO_2$ ON THE INCORPORATION OF ^{14}C FROM $^{14}CH_3COONH_4$ AT 22° AND 30°
(Flask A: Growth on $^{14}CH_3COONH_4$; Flask B: growth on $^{14}CH_3COONH_4$ + $^{12}CO_2$)

Temp. (°C)	Time (h)	Protein concentration (μg N/sample)		Total ^{14}C incorporated (counts × 10^{-3} min/sample)		Specific activity of synthesised protein (counts × 10^{-3} min/μg N synthesised)	
		A	B	A	B	A	B
22	0	14	14	–	–		
	0.5	16	16	26.6	22.0	13.3	11.0
	1.0	19	18	60.8	42.6	11.4	10.3
	1.5	21	21	85.6	72.6	12.4	10.0
	2.0	26	26	144	121	11.7	9.7
	2.5	28	28	169	141	12.2	10.0
					Average	12.2	10.2
30°	0	14	13	–	–		–
	0.5	21	20	88.9	74.2	12.7	10.6
	1.0	31	30	213	179	12.4	10.5
	1.5	45	44	371	319	11.3	10.0
	2.0	65	64	611	527	12.0	10.4
	2.5	69	70	662	591	12.7	10.7
					Average	12.0	10.4

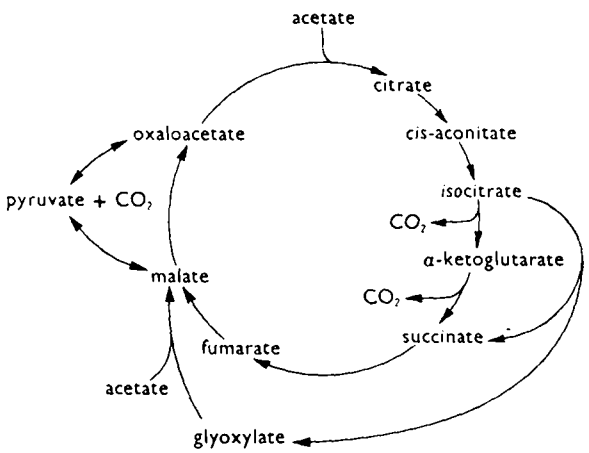

Fig. 1. Suggested interrelationships of CO_2 with the tricarboxylic acid and glyoxylate[9] cycles.

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Cross linking of collagen by S- and N-mustards

When di-(2-chloroethyl) sulphide (mustard gas, H) is applied to the skin, about 12% of the amount which penetrates remains bound to skin tissue. The extent of the resultant vesication has been shown to be approximately proportional to the amount of H fixed[1].

The present work was therefore carried out to examine the reaction between mustard gas and related S-compounds (S-mustards) and N-compounds (N-mustards) and collagen, the major protein component of skin. PIRIE[2] investigated the reaction between H and ox-corneal collagen and found the reaction product differed in certain aspects from untreated collagen. Swelling in acid and alkaline solution was much reduced and solubility in boiling water and digestibility by proteolytic enzymes were negligible. These results suggest that H might be acting as a cross-linking agent, stabilising the structure of the collagen molecule. On the other hand, ALEXANDER, FOX, STACEY AND SMITH[3] found that neither H nor the N-mustard, di-(2-chloroethyl) methylamine (HN2), brought about any obvious cross-linking of the fibrous protein of wool. In the present study an attempt was made to obtain further evidence for the cross-linking of collagen by some S- and N-mustards and other cross-linking agents. A study was therefore made of the effect of these various substances on the shrinkage temperature, T_s, of collagen. THEIS[4] has defined T_s as "the specific point at which the increasing disruptive tendencies exceed the diminishing cohesive forces, thus making the shrinkage temperature actually a measure of the structural stability of collagen expressed in temperature units". Thus, if cross-linking has occurred and if measurements of T_s are made under similar conditions of pH and ionic strength[4], an increase in T_s would be expected.

Standard hide powder (B.D.H.) was used in these experiments and treatment with the various reagents was carried out as follows:

(a) With the N-mustards, 2,4-dinitrofluorobenzene (FDNB), 1,3-difluoro-4,6-dinitrobenzene (DFDNB) and tri-acryl formal, 150 mg of hide powder were suspended in 10 ml of 0.25 *M* phosphate buffer, pH 7.4, containing the reagent (10 mmoles) and the suspension agitated for 16 h at 37°. The fall in pH during the incubation was never greater than 0.5 of a unit.

(b) In the case of the S-mustard, however, the phosphate buffer employed in (a) was too weak to maintain the pH during treatment and a much higher concentration of buffer ion was obviously necessary. Since S-mustards react readily with anions[5] it is undesirable to use high concentrations of buffer, and another method of treatment was adopted. The hide powder was suspended in 10 ml distilled water, 0.1 *N* NaOH was added as required to maintain pH between 6.8 and 8.2 (phenol red) and the reaction allowed to continue till the pH stopped decreasing (about 1.5 h). Tests using HN2 under these conditions gave similar results to those obtained with assay method (a), and consequently the two procedures (a) and (b) are strictly comparable.

In order to keep conditions of pH and ionic strength the same when determining T_s, the fibres were spun down hard after treatment with the agent and the supernatant discarded. The fibres were resuspended in 0.025 *M* phosphate buffer, pH 7.4, and spun down again. This process was repeated 6 times and finally the fibres were suspended in a small volume of buffer. Measurements of T_s were carried out by the micro-technique described by BORASKY AND NUTTING[6]. The temperature at which shrinkage occurred was read to the nearest 0.5°. It was observed, as reported by these authors, that different samples of the same group of fibres gave values of T_s varying by as much as 3°. Observations were therefore made on at least 10 groups of fibres from each sample and the mean and standard deviation calculated.

The effects of the various treatments on the T_s of hide powder are shown in Table I. The main finding was the fact that treatment with a multifunctional S- or N-mustard brought about a considerable rise in T_s, whereas treatment with the corresponding mono-functional compounds either brought about a much smaller increase in T_s or did not raise it significantly. This result is most easily explained on the assumption that the multifunctional mustards have brought