lyophilized, and the protein dried at 60° for 2.5 hours as described above. Then 25 μl H_2O were added and after 2 hours back-reaction at 38° the solutions were cryodistilled and the density of the water determined. Exchange values of 60 and 62 respectively were found, in good agreement with curve II.

Our wish to correct our previous errors as quickly as possible had led us to omit a discussion of the behavior of SANGER's A-chain on which experiments are in progress. It should, however, be noted that renewed experiments with the tetrapeptide leucyltriglycine, using the improved technique, have shown that all 6 labile hydrogen atoms exchange instantaneously if the peptide is lyophilized prior to dissolution, so that it is brought into rapid contact with the solvent. The exchange with H_2O during the drying over P_2O_5 is considerably slower than in the case of insulin, which may be due to the fact that the lyophilized peptide is crystalline. A similar assumption may explain why the A-chain does not seem to exchange with P_2O_5[1].

The method and results will be described more fully elsewhere.

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Influence of methionine on protein and nucleic acid synthesis in *Pseudomonas hydrophila* *

The recent findings of SCHMIDT *et al.* showed an increase in the biosynthesis of acid-insoluble and acid-soluble adenine and guanine fractions in yeast cells grown in a synthetic medium supplemented with methionine. The present investigation deals with the influence of DL-methionine on nucleic acid and protein synthesis in *Pseudomonas hydrophila*.

Pseudomonas hydrophila NRC 492 was grown at three concentrations of DL-methionine in a synthetic medium[2] for 24 hours with constant shaking. The cells, harvested in a centrifuge, were washed four times with ice cold water, frozen and dried.

The following estimations were made on the freeze-dried material.

a. *Protein:* 20 mg of the freeze-dried material was incubated with 2 ml of 5% trichloroacetic acid (TCA) at 0° C for 2 hours and centrifuged at 4° C. The residue, washed twice at 4° C with 5% TCA, was taken up in 3% NaOH and the protein in a 4 ml aliquot was estimated by the colorimetric biuret method of HILLER *et al.*[3] Intensity of color was measured in a Beckman spectrophotometer at 550 mμ.

b. *Total nucleic acid phosphorus* (TNA-P): 30 mg of the freeze-dried material was incubated with 4 ml of 5% TCA at 0° C for 2 hours and centrifuged at 4° C. The residue was washed four times at 4° C with 5% TCA, once with ethanol, three times with a mixture of ethanol: ether (3:1) at room temperature. The washed residue was extracted three times with 2 ml aliquots of 5% TCA at 90° C for 10 min. The TCA extracts were made up to 10 ml and phosphorus was estimated in an aliquot according to the method of KING[4].

c. *Desoxyribonucleic acid* (DNA-P): 80 mg of the freeze-dried material was incubated with 5 ml of 5% TCA at 0° C for 2 hours and centrifuged at 4° C. The residue was washed with TCA and lipid solvents as described above. The washed residue was taken up in 2 ml *N* KOH and the mixture was kept for 18 hours at 37° C. It was then chilled in an ice bath and DNA and protein were precipitated with 0.4 ml 6*N* HCl and 2 ml 5% TCA. The mixture was held at 0° C for 15 min and centrifuged at 4° C. The residue was washed at 4° C with 5% TCA. The washed residue was extracted three times with 1 ml aliquots of 5% TCA at 90° C for 10 min and the phosphorus in the extracts estimated according to the procedure of KING[4].

d. *Ribonucleic acid phosphorus* (RNA-P): RNA-P was obtained by substracting DNA-P from TNA-P.

The turnover of ribonucleic acid phosphorus as effected by DL-methionine was studied by using ^{32}P. *P. hydrophila* was grown in a synthetic medium[2] with or without added 0.02 *M* DL-methionine. Labeled phosphorus (1.5 μc/ml of medium) was supplied as $H_3{}^{32}PO_4$. Twenty-four hour cultures were centrifuged and the cells were washed six times with ice cold water. The washed cells were suspended in 7% TCA (2.5 ml TCA per g of wet cells) and held at 0° C for two hours. The suspension was then centrifuged at 4° C and the residue was washed six times with 5% TCA at 4° C and with

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lipid solvents as described above. The residue was extracted three times for 1 hour each at 100° C with 10% NaCl. To the pooled extracts two volumes of ethanol were added. The precipitate of Na-nucleate was washed with ethanol and ether and dried. The Na-nucleate was hydrolysed with 0.3 *N* KOH at 37° C for 18 hours and the RNA nucleotides were separated by paper ionophoresis and their activities determined according to procedure of DAVIDSON AND SMELLIE[5].

TABLE I

INFLUENCE OF DL-METHIONINE ON THE NUCLEIC ACID AND PROTEIN SYNTHESIS IN *Pseudomonas hydrophila*

DL-*methionine concentration in the medium*	*Dry weight of cells, mg (400 ml culture)*	*In 100 mg dry cells*		
		mg protein	*µg DNA-P*	*µg RNA-P*
1.	475	53.23	37.8	707.5
2. 0.01 *M*	540	66.10	36.1	745.2
3. 0.02 *M*	546	71.43	36.2	782.0
4. 1.05 *M*	570	73.32	37.4	843.1

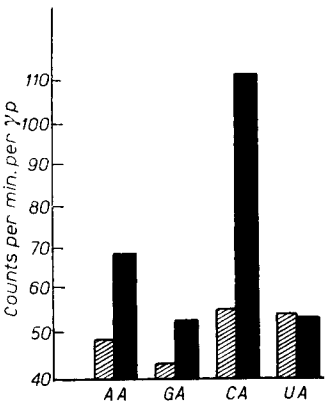

Fig. 1. Effect of DL-methionine on incorporation of ^{32}P into ribonucleotides of *P. hydrophila*. AA = adenylic acid; GA = guanylic acid; CA = cytidylic acid; UA = uridylic acid. Black: with 0.02 *M* DL-methionine. Shaded: control.

In *P. hydrophila*, DL-methionine caused an increase in growth and in the synthesis of protein and ribonucleic acid, but had no apparent effect on DNA content of the cells (Table I). In addition, presence of methionine in growing cultures of *P. hydrophila* brought about an increase in the turnover of RNA phosphorus (Fig. 1). CALDWELL AND HINSHELWOOD[6] reported that the amount of DNA per cell of *Bacterium lactis aerogenes* remained approximately constant even if the organisms were grown under a wide range of conditions. The ribosenucleic acid content on the other hand showed considerable variation. SPIEGELMAN AND KAMEN[7] showed that turnover of RNA phosphorus in yeast cells was several times higher when the cell was producing protein and that several factors inhibiting the protein synthesis also inhibited the replacement of RNA phosphorus. In *P. hydrophila*, methionine brought about higher production of protein and higher turnover of RNA phosphorus. Thus there was a positive correlation between protein and RNA synthesis. This evidence lends further support to the accumulated data showing that RNA is involved in protein synthesis[9–11].

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