From the specific activities of different RNA fractions the relative amounts of pRNA and rRNA could be calculated, and they proved to agree very well with the values obtained by direct determination. The composition of RNA was characteristic of the tissues: the content of rRNA was about 30 % in lymphatic cells and about 15 % in the liver; the rest was pRNA. The nucleotide compositions of pRNA and rRNA differ quite markedly, the content of guanylic acid being higher in the former than in the latter.

It appears that the phenol method of KIRBY[1] roughly fractionates two classes of RNA differing in function in the cell. Like DNA, the bulk of pRNA which is insoluble in 1 *M* NaCl may be duplicated only during the cell division cycle (*cf.* HOTTA AND OSAWA[3]), and is metabolically rather inert. On the other hand, rRNA is metabolically very active, turning over at a very high rate. The mode of linkage of RNA to protein must be quite different for the two fractions of RNA, since only pRNA can be released by aqueous phenol. Distribution of the two forms of RNA among different subcellular fractions is now being explored.

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β-Alanine-α-alanine transaminase of *Pseudomonas*

Several papers have appeared in the literature which indicated transamination of β-alanine[1-2], but the exact nature of the reaction as well as the products have not been completely elucidated. A partially purified enzyme preparation from *Pseudomonas* sp. (No. 23) is now shown to catalyze the transamination of β-alanine with pyruvic acid, resulting in the formation of stoichiometric quantities of malonic semialdehyde and α-alanine.

Cells were grown and extracts were made as described previously[3] except that β-alanine was used as a major carbon and nitrogen source in the growth media. The enzyme activity was assayed by the appearance of malonic semialdehyde using a colorimetric reaction with diazotized *p*-nitroaniline[4]. The enzyme was purified about 20 fold by heat treatment (10 min at 60°), followed by fractionation with $(NH_4)_2SO_4$ and dialysis against 0.02 *M* potassium phosphate, pH 8.0, for 3 h at 0°.

TABLE I

THE STOICHIOMETRY OF THE REACTION

The complete incubation mixture contained 90 μmoles β-alanine, 80 μmoles sodium pyruvate, 600 μmoles potassium phosphate, pH 8.0, and 1.2 mg enzyme in a final vol. of 6.0 ml. After the incubation was carried out for 40 min at 35°, the reaction was stopped with 0.6 ml 10% $HClO_4$. Pyruvic acid was determined spectrophotometrically with reduced diphosphopyridine nucleotide and crystalline lactic dehydrogenase[5]. Malonic semialdehyde was determined manometrically with aniline citrate[6], and colorimetrically with diazotized *p*-nitroaniline. Malonic semialdehyde was converted to acetaldehyde by steam distillation of the acidified reaction mixture and was determined colorimetrically with *p*-hydroxydiphenyl[7] or as the 2,4-dinitrophenylhydrazone, and also spectrophotometrically with reduced diphosphopyridine nucleotide and crystalline yeast alcohol dehydrogenase[8]. α-Alanine and β-alanine were determined colorimetrically with ninhydrin after separation by ion-exchange chromatography with a Dowex-50 column[9]. α-Alanine was also determined microbiologically using *Leuconostoc citrovorum* 8081[10] and the Henderson-Snell medium[11] which contained leucovorin. (We are indebted to Miss S. ISHIDA and Dr. T. SUZUKI for this assay.)

System	*Δ β-Alanine*	*Δ Pyruvic acid*	*Δ α-Alanine*		*Δ Malonic semialdehyde*				
							As acetaldehyde		
			Ninhydrin assay	*Bioassay*	p-*nitroaniline assay***	*As CO_2 Aniline citrate assay*	p-*Hydroxy-diphenyl assay*	*As 2,4-dinitro-phenylhydrazone*	*Enzymic assay*
Complete	— 15.0	— 16.0	+ 16.5	+ 16.3	+ 17.0	+ 15.5	+ 13.8	+ 16.1	+ 16.6
Complete with boiled enzyme*	— 0.8	— 0.3	0.0	0.0	+ 0.5	0.0	+ 0.2	0.0	0.0
Complete minus β-alanine	—	— 0.2	0.0	0.0	+ 0.2	— 0.4	0.0	0.0	0.0
Complete minus pyruvate	+ 0.3	—	0.0	0.0	+ 0.2	+ 0.1	0.0	0.0	0.0

All the numbers are expressed in μmoles.
* Heated in boiling water for 10 min.
** Calculated from oxaloacetate as standard.

The stoichiometry of the reaction is shown in Table I. α-Alanine was also identified by paper chromatography using five different solvent systems*. Malonic semialdehyde was isolated from a large-scale incubation mixture by ether extraction after acidification of the solution, and was identified as 2,4-dinitrophenylhydrazone by paper chromatography using three different solvent systems by comparison with an authentic sample**. Further evidence for the identity was provided by the quantitative decarboxylation with aniline citrate and by the production of equivalent amount of acetaldehyde upon steam distillation from the acidified reaction mixture. Acetaldehyde was also identified as the 2,4-dinitrophenylhydrazone.

The reaction appears to be freely reversible, since the enzyme preparation catalyzes the stoichiometric formation of pyruvic acid and β-alanine from α-alanine and malonic semialdehyde. Pyruvic acid acts as specific amino acceptor in the forward reaction. Neither α-ketoglutarate, β-ketoglutarate, β-ketoadipate nor oxaloacetate could replace pyruvate as amino acceptor. However, γ-aminobutyrate acts as amino donor to some extent. Preliminary evidence indicates that pyridoxal phosphate stimulates the activity of the dialyzed enzyme preparation. Studies are now in progress in order to elucidate the further metabolism of malonic semialdehyde.

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* Paper chromatography was carried out on Whatman No. 1 paper. R_F values for α-alanine and β-alanine with isopropanol–water (8:2) were 0.33 and 0.25; with ethanol–ammonia–water (18:1:1) 0.23 and 0.11; with *n*-butanol–pyridine–water (1:1:1) 0.43 and 0.34; with phenol–water (8:2) 0.52 and 0.62; with *n*-butanol–acetic acid–water (4:2:3) 0.27 and 0.33, respectively.

** The authentic sample was prepared from malonic semialdehyde which was synthesized chemically[12]. We are indebted to Mr. H. KONDO, Drs. K. HAYASHI and T. SUZUKI for the chemical synthesis of malonic semialdehyde. Paper chromatography was carried out on Whatman No. 1 paper and sprayed with dilute alkali. R_F values for the 2,4-dinitrophenylhydrazones of malonic semialdehyde and pyruvic acid with isopropanol–isoamylalcohol–pyridine–water (20:4:1:5) were 0.56 and 0.45; with isoamylalcohol–ethanol–0.1 *M* carbonate, pH 10.7 (5:1:2, upper layer) 0.31 and 0.13; with *n*-butanol–ethanol–0.1 *M* carbonate, pH 10.7 (6:1:1, lower layer) 0.65 and 0.56, respectively.