Amino acid transfer from sRNA to microsome
II. Isolation of a heat-labile factor from liver supernatant

Although the experiments of the previous paper[1] indicate that the supernatant effect is partly due to an SH requirement, in many experiments the liver supernatant caused an additional stimulation that could not be fully substituted by SH compounds or a GSH-generating system. A requirement for a further supernatant fraction, probably protein, will be more clearly brought out in the present paper. In order to demonstrate this requirement, a means was needed of further depleting the microsomes of stubbornly adhering material; DOC in moderate concentration had been used to purify microsomes without destroying activity[2,3]. It will be shown that washing with 0.5 % DOC yielded a preparation that needed supplementation by a heat-labile fraction of liver supernatant.

Rat-liver microsomes were prepared in LITTLEFIELD AND KELLER'S medium A (see ref. 4), using 24-h fasted animals in order to deplete liver glycogen, which appears to be important. The rinsed microsomes were homogenized in 0.5 % DOC-medium A (10 ml per liver) and recentrifuged at 105,000 × *g* for 2 h. The pellets were rinsed several times with medium A, and finally homogenized in the same medium without DOC. The homogenate of such particles withstood lyophilization and storage at —20° for as long as 3 weeks with little or no loss of activity. Soluble RNA was isolated by the phenol method from briefly heated *Escherichia coli* paste. Since *E. coli* sRNA had been found to be as active as rat liver sRNA in the transfer of leucine to liver microsomal particles, the more easily available bacterial sRNA was used exclusively in these experiments. Amino acid loading was carried out with an alumina-ground *E. coli* extract as the enzyme source.

TABLE I

EFFECT OF SUPERNATANT ON DOC-EXTRACTED PARTICLES

The medium contained in a volume of 1 ml: DOC-extracted particles (2.0 mg protein); 0.24 mg sRNA loaded with amino acids, including 1500 counts/min [^{14}C]leucine (10 μC/μmole); 0.003 *M* ATP; 0.01 *M* PEP; 30 μg PEP-kinase; 0.0003 *M* GTP; 0.100 *M* Tris–HCl, pH 7.0; 0.05 *M* KCl; 0.006 *M* $MgCl_2$. After a 5-min incubation at 35°, 5 % trichloroacetic acid was added and the precipitate was extracted with 5 % trichloroacetic acid at 90° for 15 min. The precipitated protein was washed with 5 % trichloroacetic acid and with ethanol–ether (3:1), plated, and counted in a Nuclear Chicago windowless gas-flow counter.

Additions	*[^{14}C]leucine transferred counts/min*
None	5
GSH, 0.010 *M*	19
105,000 × *g* supernatant	277
GSH, 0.010 *M* + 105,000 × *g* supernatant	249

Table I presents experiments with DOC-washed particles where even in the presence of an SH compound practically no activity developed except on addition of 105,000 × *g* supernatant. The DOC wash after dialysis also stimulated, indicating that the active factor had, indeed, been eluted by this procedure. Similar supernatant

Abbreviations: SH, sulfhydryl; DOC, desoxycholate; sRNA, soluble ribonucleic acid; GSH, glutathione; ATP, adenosine triphosphate, GTP, guanosine triphosphate; PEP, phosphoenolpyruvate; Tris, tris(hydroxymethyl)aminomethane.

stimulation could be obtained with microsomes from a mouse plasma cell tumor[5], but here washing with the usual salt medium was sufficient.

Fig. 1 shows the effect of increasing amounts of supernatant on leucine transfer. An almost complete dependence on supplement made this preparation a convenient assay system. The active component was found to be completely destroyed by heating at 60° for 5 min, and was non-dialyzable. Ammonium sulfate and acetone fractiona-

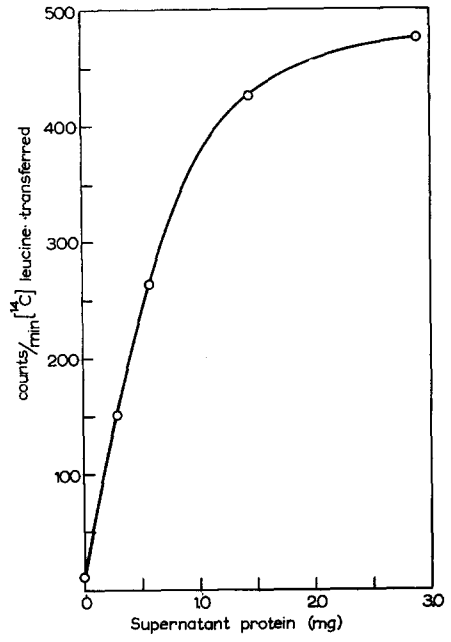

Fig. 1

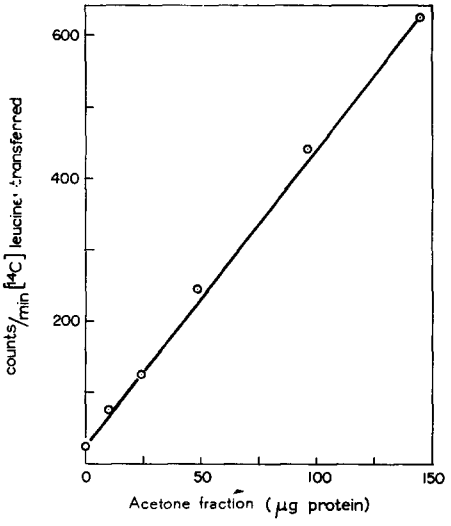

Fig. 2

Fig. 1. Dependence of leucine transfer on added 105,000 × *g* supernatant. Incubation mixture and conditions were the same as in Table I except as follows: DOC particles, 2.5 mg protein; 0.29 mg sRNA; 0.010 *M* GSH.

Fig. 2. Dependence of leucine transfer on a purified supernatant factor. Incubation mixture and conditions were the same as in Table I except as follows: DOC particles, 2.5 mg protein; 0.10 mg sRNA; 0.010 *M* GSH.

Fig. 3. Time curves of leucine transfer with different amounts of a purified supernatant factor (lower curve, 15 μg protein; upper curve, 75 μg). Incubation mixture and conditions were the same as in Table I except as follows: DOC particles, 2.5 mg protein; 0.14 mg sRNA; 0.010 *M* GSH. The solid square indicates transfer in the absence of GSH, and is paired with the upper 5-min value.

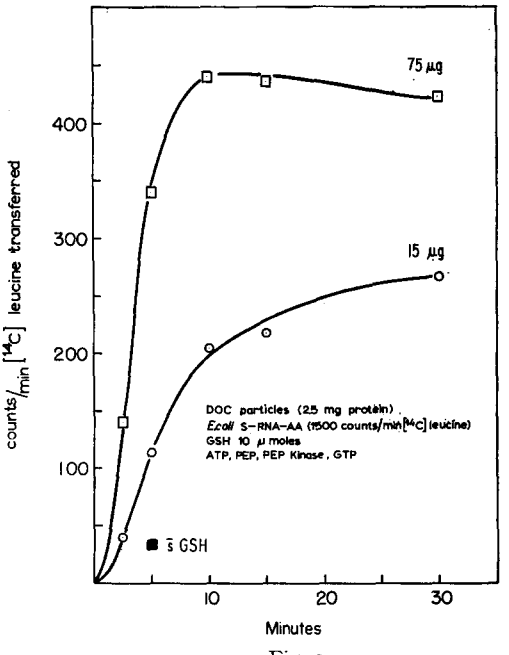

Fig. 3

tion yielded a 30-fold purification with 30% over-all recovery (Table II). Unfortunately, the purified preparation was rather labile.

Fig. 2 shows an assay of purified factor, and Fig. 3 time curves for leucine transfer at two different concentrations. In each case, 0.010 *M* GSH was present. It may be noted in Fig. 3 that the SH effect, not apparent with unfractionated supernatant (Table I), was brought out clearly with the purified fraction. Cysteine and GSH were equally effective.

TABLE II

PURIFICATION OF THE SUPERNATANT FACTOR

One unit equals counts/min [^{14}C]leucine transferred in 5 min per mg of original 105,000 × *g* supernatant protein, adjusting to values in the straight part of the assay curve. All samples were tested with the same microsome preparation. In other runs the 26–32% acetone fraction had the highest specific activity.

Fraction	*Total units*	*Specific activity units/mg*	*Recovery %*
105,000 × *g* supernatant (10 rat livers)	4370	1	
39–50% (w/v) ammonium sulfate (pH 7.0)	2650	3.3	60
20–26% acetone (pH 6.4, –6°)	1340	30	30

We conclude that the analysis of a reaction requiring the integrity of a particulate component, such as microsomal protein synthesis, presents experimental difficulties that remain to be further resolved. The progress reported here is modest. At present we can distinguish reasonably clearly three factors that participate in the compounding of the active amino acids carried by sRNA into a protein. These are: (a), GTP, long ago found by KELLER AND ZAMECNIK[6]; (b), an oxidizable group, possibly sulfhydryl; and (c) a heat-labile fraction which, so far, is poorly identified. Obviously further work is needed before an even preliminary discussion can be attempted.

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