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† Present address: Biology Department, Brown University, Providence 12, Rhode Island.

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*AMINO ACID INCORPORATION AND THE REVERSION OF ITS INITIAL PHASE WITH CELL-FREE TETRAHYMENA PREPARATIONS**

BY J. MAGER† AND FRITZ LIPMANN

ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK CITY

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Cell-free preparations of various biological systems present a rather uniform pattern of amino acid incorporation. However, often the different systems seem to be more or less appropriate for studying a particular step in the sequence of reactions from initial activation[1] to final incorporation into protein. In the present study, *Tetrahymena pyriformis* was chosen as a test organism. This protozoön yields subcellular fractions analogous to those obtained with mammalian tissue homogenates. The *Tetrahymena* system was found to be particularly suitable for the demonstration of a reversibility of transfer of activated amino acid to the soluble ribonucleic acid fraction.

FRACTIONATION OF HOMOGENATES

Tetrahymena pyriformis "W" was kindly supplied to us by Dr. G. W. Kidder. It was grown in a shallow layer of 2 per cent Difco proteose at 26° for 40 hours. The yield was approximately 0.7 gm. of dry weight per liter. The organisms were collected by decantation and centrifugation at 400 *g* for 5 minutes, suspended in one-fourth of the original volume of distilled water, and washed twice in the centrifuge. The washed residue was suspended in 50 ml. of 0.4 *M* sucrose solution, containing 0.1 *M* Tris[2] buffer, pH 7.5, and 0.005 *M* potassium chloride, and the suspension was left on ice for 10 minutes to lyse partially. This preparation separated, on centrifugation for 15 minutes at 18,000 *g*, into a well-packed bottom layer, a fluffy viscose intermediate layer, with a clear supernatant on top. The supernate and the intermediate layer were decanted and discarded, the pellet was re-homogenized with a Teflon homogenizer in 10 ml. of 0.25 sucrose to complete cellular disintegration, making the preparation suitable for differential centrifugation. Cell debris and nuclei were spun off at 1,500 *g* and discarded. For further fractionation, the sucrose concentration was raised to 0.35 *M*. On centrifugation at 12,000 *g* for 15 minutes a mitochondrial fraction was sedimented. The turbid supernate of this was further separated by centrifugation at 105,000 *g* for 90 minutes, into a translucent microsomal pellet and a supernatant soluble fraction.

ASSAY FOR INCORPORATION

For assaying amino acid incorporation, an incubation mixture was used, containing, in a final volume of 1 ml., the following ingredients: 100 μmoles Tris buffer, pH 7.6; 6 μmoles magnesium chloride; 0.3 μmoles DL-leucine-1-C^{14}; and an ATP-generating system consisting of 1 μmole ATP, 5 μmoles lithium carbamyl phosphate with 0.01 ml. of a dialyzed sonic extract of *Streptococcus faecalis*, which served as carbamyl phosphokinase.[3]

TABLE 1*

INCORPORATION OF LEUCINE† INTO SUBCELLULAR FRACTIONS OF *Tetrahymena*

Fraction	C.p.m. per Mg. Protein
Mitochondria	187
Microsomes	258
Soluble fraction (S.F.)	8
Microsome + S.F.	684
Mitochondria + S.F.	135

* Standard reaction mixture as described in assay for incorporation; in addition: mitochondria twice washed with 0.25 *M* sucrose, 5.6 mg. protein; microsomes, 2 mg. protein; S.F., 1.5 mg. protein; incubated for 60 minutes at 25°.

† Radioactive valine, alanine, phenylalanine, and serine gave similar but only about half as much incorporation.

The radioactive leucine was kindly prepared for our use by Dr. Loftfield. It contained 5 μC per μm, which in our Nuclear counter is equivalent to 8×10^6 counts per μm. With only the L-component reactive, this corresponds to the addition in 0.3 μmoles of DL-leucine of roughly 1×10^6 counts per test. The counting technique used was the same as described by Weiss *et al.*[1]

To determine protein-bound amino acids, the TCA precipitate was washed with

hot TCA according to Schneider[4] and with ether-alcohol to remove a lipid material. The non-protein (nucleotide-bound) amino acids were determined as the difference between cold and hot TCA-washed residue.

Incorporation into Microsome-Protein.—The activities of various fractions and combinations are shown in Table 1. It appears that, as with liver and other systems, the highest rate of incorporation was obtained with a mixture of microsomal and supernatant fractions. The mitochondrial fraction gave a significant amino acid incorporation, which, however, seemed not to be increased by the addition of the soluble fraction. The soluble fraction alone showed no uptake of amino acids into *protein.*

As shown in Table 2, the stimulatory effect of the homologous supernate on the incorporation into *Tetrahymena* microsomes could be duplicated to a large extent by a supernate derived from a liver homogenate. Likewise, liver microsomes were found to respond strongly to the addition of *Tetrahymena* supernate, demonstrating the interchangeability between protozoan and mammalian preparations.

TABLE 2*

INCORPORATION OF RADIOACTIVE LEUCINE INTO VARIOUS COMBINATIONS

	Fractions	Total c.p.m. "Protein-bound"†	Total c.p.m. "Nucleotide-bound"†
1a	Tetrahymena microsomes	650	...
	Tetrahymena microsomes + *Tetrahymena* supernate	1,780	...
	Tetrahymena microsomes + liver supernate	1,235	...
1b	Liver microsomes	794	...
	Liver microsomes + *Tetrahymena* supernate	3,115	...
	Liver microsomes + liver supernate	4,276	...
2	Supernate *Tetrahymena*	4	946

* Incubation analogous to that of Table 1.
† "Protein-bound" refers to *hot* TCA-treated; "nucleotide-bound" refers to *cold* TCA-washed residue.

TABLE 3*

REVERSAL OF LEUCINE INCORPORATION INTO NUCLEOTIDE-BOUND FRACTION OF SUPERNATE

No.	Added after Initial 10 Min. Incubation	Total c.p.m. after 1st 10 Min.	Total c.p.m. after 2d 10 Min.
1	None	1,120	1,138
	KF + AMP	..	982
	KF + PP	..	294
	KF + PP + AMP	..	68
2	None	1,240	1,175
	AMP	..	1,260
	PP	..	815
	PP + AMP	..	328

* Incubation analogous to that described in Table 1, but only supernate was used. The compounds added after 10-min. initial incubation were present at the following levels: KF, 10 μmoles; AMP, 5 μmoles; PP, 5 μmoles. All counts refer to residue precipitated and washed with *cold* TCA and ether-ethanol ATP, 2 μmoles instead of regeneration system.

Attachment to Soluble Polynucleotide.—In the second experiment presented in Table 2, where the sediment was extracted with cold TCA only, an incorporation was obtained with the non-particulate supernate alone. The amino acid incorporated under these conditions, however, in contrast to the microsomal, protein-bound acids, was released by treatment with hot TCA, with RNA-ase, or with dilute alkali. This is interpreted to indicate formation of polynucleotide-bound amino acid in the supernate, in a similar manner to that described by Hoagland

et al.[5] and by Weiss *et al.*[1] for liver and pancreas, respectively. The order of magnitude of this incorporation was similar to that found in the microsomal protein.

Reversion.—This incorporation into the RNA fraction was found, as shown in Table 3, to be reversed by the addition of PP or, more effectively, by a mixture of PP and AMP. Fluoride increased the effect, presumably by inhibition of the rather abundant pyrophosphatase present in the preparation. Without fluoride (cf. No. 2 in Table 3), AMP alone was inactive, while with fluoride it probably showed a slight effect. In contrast to PP, inorganic P was entirely inactive.

COMMENTS

It appears that the protozoön *Tetrahymena* contains an amino acid incorporation system rather similar to that of higher animals. The non-particulate fraction here likewise incorporates amino acids in a linkage which is hot-TCA-, RNA-ase-, and alkali-unstable and is therefore presumably due to linkage with soluble polynucleotide. This incorporation is of a magnitude similar to that in the microsomal protein.

The *Tetrahymena* preparation proved to be rather suitable for showing a reversibility of the initial step, namely, the amino acid attachment to soluble polynucleotide. Such a reversion is obtained by the addition of the PP and is considerably enhanced by PP + AMP. This indicates that the transfer of the activated amino acid, presumably amino acyl adenylate, to the polynucleotide may be reversed on addition of both reaction products. The pronounced effect of AMP to reverse incorporation gives welcome support to a two-step reaction (cf. also Holley[6]) to be formulated as follows:

$$\text{ATP} + \text{amino acid} \rightleftharpoons \text{amino acyl-AMP} + \text{PP}$$
$$\text{Amino acyl-AMP} + \text{polynucleotide} \rightleftharpoons \text{amino acyl-polynucleotide} + \text{AMP}$$

The inability of AMP alone to reverse appreciably may mean that in this sequence the amino acyl adenylate is not a free intermediary. It may also, however simply indicate that the reversal of the second reaction is energetically unfeasible without a simultaneous reversal of the first step, which we know to be quite exergonic backwards. A non-reversal by AMP in this interpretation may indicate the second step to be rather irreversible in *forward* direction and balancing the endergonic initial reaction. We prefer, at present, not to discuss this somewhat foggy situation further, but feel that the present formulation is probably an over-simplification.

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† This work was carried out during the tenure of a Warburg-Magnes fellowship of the Hebrew University, Hadassah Medical School. Present address: Department of Biochemistry, Hebrew University, Hadassah Medical School, Jerusalem.

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[2] The following abbreviations have been used: AMP, adenosine monophosphate; ATP, adenosine triphosphate; P, phosphate; PP, pyrophosphate; TCA, trichloracetic acid; RNA, ribonucleic acid; Tris, tris (hydroxymethyl) amino methane.

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*SOLUTION OF A PAIR OF INTEGRAL EQUATIONS FROM ELASTOSTATICS**

BY R. W. FREDRICKS

INSTITUTE OF GEOPHYSICS, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA

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Introduction.—In the theory of elastostatics, certain mixed boundary-value problems lead to dual integral equations. For instance, one may consider the elastostatic boundary-value problem presented by specifying zero shear stress over the entire plane boundary of an elastic half-space, while the normal component of stress vanishes on this surface exterior to a finite strip and the normal component of displacement is specified within this strip. This two-dimensional problem is formulated conveniently in terms of the type of dual integral equations considered in this paper. For the details of the formulation the reader is referred to the work of Sneddon.[1]

If the normal component of displacement is decomposed into its even part, $g(x)$, and its odd part, $h(x)$, the resulting integral equations are

$$\begin{aligned} &\int_0^\infty \xi^{-1}P(\xi)\cos(\xi x)d\xi = g(x), \qquad 0 \le x < a, \quad (a) \\ &\int_0^\infty P(\xi)\cos(\xi x)d\xi = 0, \qquad a < x \le \infty, \quad (b) \end{aligned} \tag{1}$$

and

$$\begin{aligned} &\int_0^\infty \xi^{-1}Q(\xi)\sin(\xi x)d\xi = h(x), \qquad 0 \le x < a, \quad (a) \\ &\int_0^\infty Q(\xi)\sin(\xi x)d\xi = 0, \qquad a < x \le \infty. \quad (b) \end{aligned} \tag{2}$$

The systems of equations (1) and (2) have been considered by Sneddon,[2] who transformed the kernel functions into Bessel functions of order one-half. The resulting dual integral equations bear a formal resemblance to the equations solved by Busbridge.[3] However, the restrictions on the solution of Busbridge are violated by the equations (1) and (2), and Sneddon's use of this solution is not justified.

Solution of the Dual Integral Equations.—Since the superposition theorem holds for elastostatics, it is possible to decompose the normal displacement prescribed on the surface $-a < x < a$, $z = 0$, into an even and an odd function of x, as above. It is possible to decompose further both the even and odd functions $g(x)$ and $h(x)$ and solve a number of separate sets of dual integral equations. To the term-by-term decomposition of $g(x)$ and $h(x)$, there will correspond a term-by-term decomposition of the unknown functions $P(\xi)$ and $Q(\xi)$. After solving the separate problems, one may recombine the solutions to obtain the solution of the composite problem.