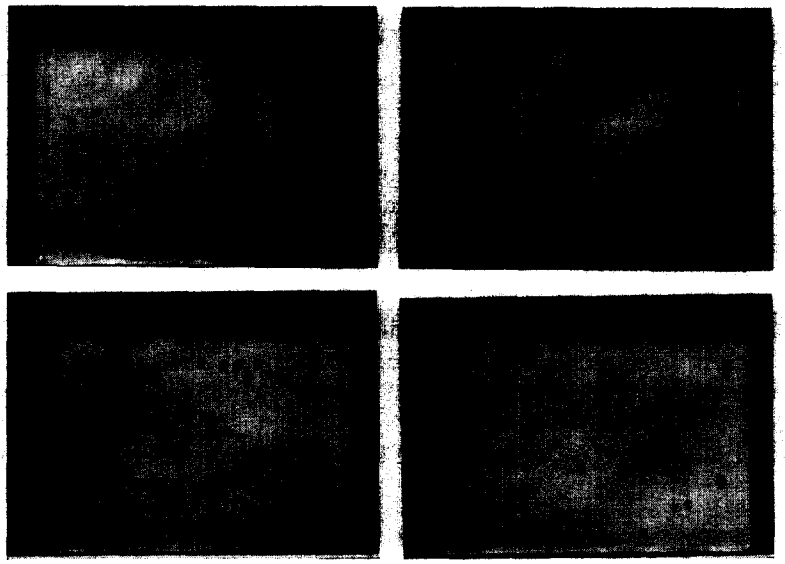

Fig. 1. Photographic reproduction of descending paper chromatograms of the compounds No. 1–50, listed in Table I, on Whatman paper No. 1 in *n*-butanol–acetic acid–water (12 : 3 : 5). Upper row: ninhydrin-treated papers after impregnation with copper salt. Lower row: Same compounds treated with ninhydrin without copper impregnation.

has proved useful also in spot-test technique on filter paper. Photographic reproductions of ninhydrin-treated paper chromatograms of all compounds listed in Table I on copper-impregnated as well as untreated paper are presented in Fig. 1.

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Received June 2nd, 1959

Biochim. Biophys. Acta, 38 (1960) 148–150

Chloramphenicol and chlortetracycline inhibition of amino acid incorporation into proteins in a cell-free system from *Tetrahymena pyriformis*

Growth of *Tetrahymena pyriformis* "W" in 2.5% proteose peptone (Difco) was considerably delayed or completely arrested by chloramphenicol at the relatively high levels ranging from 25 to 150 μg/ml medium. Similar concentrations of the

Abbreviations: ATP, GTP, CTP, UTP, the triphosphates of adenosine, guanosine, cytidine and uridine respectively; PEP, phosphoenol pyruvate; RNA-ase, ribonuclease; Tris, tris(hydroxymethyl)aminomethane.

antibiotic produced a 30 to 60 % inhibition of amino acid incorporation into protein in both intact cells and in crude cell-free homogenates. Thus, the action pattern of chloramphenicol in *T. pyriformis*, notwithstanding the low order of susceptibility of this protozoon, conformed essentially with its mode of action in bacteria as revealed by the study of GALE AND FOLKES[1].

It appeared puzzling, therefore, that the previously described microsomal system for amino acid incorporation into protein, which has been isolated by differential centrifugation from crude homogenates of *T. pyriformis*[2], proved to be refractory to chloramphenicol added in amounts as high as 250 μg/ml reaction mixture. This finding, agreeing with results obtained in similar experiments with microsomal preparations from rat and guinea-pig liver (prepared according to KELLER AND ZAMECNIK[3]) suggested the possibility that some protein-synthesizing system, other than the microsomal system, may be responsible for the inhibitory effect of chloramphenicol observed *in vivo* and in the whole homogenates. In fact, the existence of such a system could be demonstrated in the heavy subcellular fraction left after separation of the microsomal particles. This fraction which was mainly composed of mitochondria will be referred to as the "mitochondrial system" although it is not at all certain that the amino acid incorporation activity adheres to the mitochondrial structure.

The capacity of the mitochondrial system to incorporate amino acids into its proteins was found to be ATP-dependent and was significantly enhanced by addition of a mixture of GTP, CTP and UTP. Its specific activity in terms of amounts of [^{14}C]leucine incorporated per mg protein was 3- to 4-fold lower in the mitochondrial than in the microsomal preparation. The saturating level of [^{14}C]leucine required for optimal incorporation was about $5 \cdot 10^{-4}$ *M i.e.* nearly 5 times higher than the corresponding value for the microsomal system. Again in contrast to the microsomes, the presence of cell sap (supernatant resulting from centrifugation of the homogenate at 105,000 $\times$ *g* for 1 h) did not appear essential for the activity of the mitochondria, under the conditions employed, since a twice-washed preparation showed only slight stimulation on addition of cell sap. The two distinctive characteristics, however, sharply differentiating the mitochondrial system from its microsomal counterpart were: resistance to treatment with ribonuclease and susceptibility to strong inhibition by chloramphenicol. A comparative summary of the salient features of the two systems is presented in Table I.

Omission of the extraction with hot trichloroacetic acid from the standard procedure used for assaying the incorporation of the radioactive amino acid into protein[2,3] revealed the appearance of labeled non-protein material in the chloramphenicol-containing reaction mixtures. In the control experiments on the other hand, all the radioactivity incorporated appeared to be protein-bound, as evidenced by the practically identical values obtained by either the standard assay or the modified procedure (see Table II). In the light of the latter observation it appears reasonable to assume that chloramphenicol causes the accumulation of an amino acid compound which is precipitable by cold trichloroacetic acid and destroyed by heating in trichloroacetic acid at 90° for 10 min. The labile nature of the amino acid linkage in the above compound was further indicated by the finding that, following brief exposure to 1 *N* NaOH or to neutralized 0.5 *M* NH_2OH, all the radioactivity was split off, as evident from the fact that the labeled amino acid was no more precipitable by cold trichloroacetic acid but remained in the trichloroacetic acid supernatant.

TABLE I

FACTORS INFLUENCING AMINO ACID INCORPORATION INTO PROTEINS OF THE MITOCHONDRIAL AND MICROSOMAL SYSTEM

The complete system contained in 1 ml total volume the following ingredients: 100 μmoles Tris buffer pH 7.4, 120–150 μmoles sucrose, 25 μmoles KCl, 5 μmoles $MgCl_2$, 1 μmole ATP, 5 μmoles PEP, 25 μg crystalline pyruvate kinase, 0.4 μmole L-[1-^{14}C]leucine (about $1.2 \cdot 10^6$ counts/min), cell sap (2–3 mg protein) and either mitochondria twice-washed in 0.25 *M* sucrose (8–10 mg protein) or microsomes (4–6 mg protein) prepared as previously described[2]. Where indicated the following compounds were added: nucleoside triphosphates: GTP, CTP and UTP, 0.25 μmole each; crystalline ribonuclease (Worthington), 50 μg; chloramphenicol, 100 μg; chlortetracycline, 100 μg. Incubation, 30 min at 30°. Assay and procedure as described[2].

Reaction mixture	*Incorporation (counts/min/mg protein)*	
	Mitochondria	*Microsomes*
Complete system	168	656
Minus ATP-regenerating system	15	80
Minus cell sap	144	110
Plus mixture of nucleoside triphosphates	288	640
Plus RNA-ase	165	20
Plus chloramphenicol	39	649
Plus chlortetracycline	22	624

TABLE II

INHIBITION BY CHLORAMPHENICOL AND CHLORTETRACYCLINE OF AMINO ACID UPTAKE BY PROTEINS OF THE MITOCHONDRIAL SYSTEM

Complete system and conditions essentially as in Table I. All experiments run in duplicate and zero-time controls included for each combination.

Addition to complete system	*Incorporation (counts/min/mg protein)*	
	Standard assay	*Hot extraction omitted*
None	212	208
Chloramphenicol	38	148
Chlortetracycline	25	106

Experiments done with chlortetracycline, under conditions similar to those reported for chloramphenicol, yielded strictly analogous results.

Further characterization of the compound accumulating in the presence of chloramphenicol and chlortetracycline may aid in locating the exact site of action of these antibiotics and may also contribute towards the elucidation of the mechanism involved in the amino acid uptake by the mitochondrial system.

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Received June 25th, 1959