carbon analogue. A set of four 2-halogenoethylamines was similarly tested; inhibition increased with hydrophobic character of the side chain. The inhibition was irreversible for the 2-halogenoethylamines in contrast to the reversible inhibition by the N^1-alkylnicotinamides. For both sets of inhibitors, concentrations were found that inhibited uptake of NMN with little or no inhibition of PAH uptake, demonstrating a specificity for the NMN system.

Inhibitors of protein synthesis, puromycin and cycloheximide, were used to find whether the turnover of one or more components of the NMN transport system was sufficiently rapid to be detected. Incubation of slices with either inhibitor resulted in time-dependent inhibition of uptake of NMN. PAH uptake was not inhibited by these agents.

Presence and properties of dihydrofolate reductases within the genus *Trypanosoma*

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Dihydrofolate reductase activity was detected in extracts of seven species representing all four major subgroups of the protozoan genus *Trypanosoma*. The enzyme assay was based on the decrease in absorbency at 340 mμ in the presence of extract, dihydrofolic acid and NADPH (Jaffe & McCormack, 1967). The species studied were rat-adapted bloodstream forms of *T. brucei*, *T. rhodesiense*, and *T. equiperdum* (*brucei* subgroup); *T. congolense* (*congolense* subgroup); *T. vivax* (*vivax* subgroup); *T. lewisi* and *T. cruzi* (*lewisi* subgroup). Dihydrofolate reductase activity was also detected in culture forms of *T. rhodesiense* and *T. cruzi*.

The amount of dihydrofolate reductase per mg of protein appeared to be greater in extracts of trypanosomal populations in the log phase of growth and less in extracts of relatively stationary populations.

When an equimolar amount of folic acid was substituted for dihydrofolic acid in the standard assay system, or when NADH replaced NADPH, very little decrease in absorbency at 340 mμ was observed, indicating the superiority of dihydrofolic acid as substrate and NADPH as hydrogen donor in the reaction mediated by trypanosomal dihydrofolate reductase. Such properties are characteristic of this enzyme from whatever source. The Michaelis constants (K_M) for dihydrofolic acid ranged from 3.3×10^{-6} M (*T. brucei*) to 2.3×10^{-5} M (*T. cruzi*); the Michaelis constants for NADPH with respect to those trypanosomal reductases so studied were quite similar, around 1×10^{-5} M.

The trypanosomal reductases, like those from other genera, were strongly inhibited by 4-amino analogues of folic acid, such as aminopterin and methotrexate. For example, the concentration of methotrexate required for 50% inhibition (ID50) ranged from 2×10^{-10} M (*T. equiperdum*) to 1×10^{-5} M (*T. cruzi*).

Burchall & Hitchings (1965) found that dihydrofolate reductases from mammalian sources on the one hand and from bacterial sources on the other exhibited two markedly dissimilar patterns of sensitivity to the inhibitory action of various 2,4-diaminopyrimidines and related heterocyclic compounds. We found that the analogous reductases from the bloodstream form of trypanosomes were also inhibited by this class of compounds and

that they, as a group, showed yet a third distinctive pattern of sensitivity to these agents. At the same time it was observed that the reductases from trypanosomes of African origin (*T. brucei*, *T. rhodesiense*, *T. equiperdum*, *T. congolense*, and *T. vivax*) had closely similar drug sensitivity profiles, clearly distinguishable in certain respects from those of the reductases from two species of non-African origin (*T. lewisi* and *T. cruzi*). Thus, for example, the ID50 of 2,4-diamino-5-(3',4',5'-trimethoxybenzyl) pyrimidine (trimethoprin) for the reductases of the seven species was: *T. brucei*, 5×10^{-7} M, *T. rhodesiense*, 2.5×10^{-7} M; *T. equiperdum*, 1×10^{-6} M; *T. congolense*, 5×10^{-7} M; *T. vivax*, 7×10^{-7} M; *T. cruzi*, 1×10^{-5} M and *T. lewisi*, 1.5×10^{-5} M.

No significant differences have yet been found in the properties of dihydrofolate reductases from bloodstream and culture forms of either *T. cruzi* or *T. rhodesiense*.

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Evidence for the storage of oxytocin with neurophysin-I and of vasopressin with neurophysin-II in separate neurosecretory granules

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Two of the soluble, high molecular weight constituents of neurosecretory granules isolated from bovine pituitary posterior lobes (Dean & Hope, 1967) are known as neurophysin-I and neurophysin-II: each of these proteins can bind oxytocin and

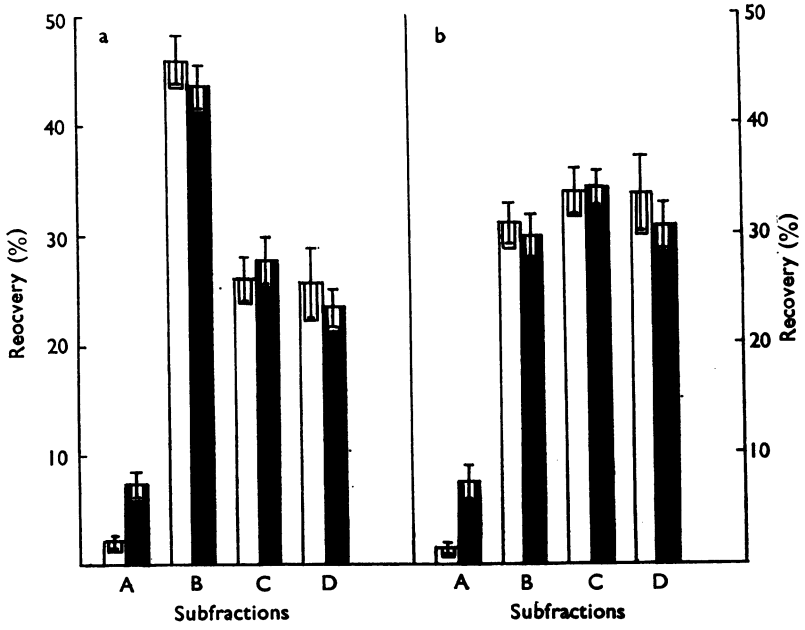

FIG. 1. Distribution of oxytocic and pressor activities and of neurophysin-I and -II in subfractions of neurosecretory granules taken from a density gradient. Column height represents the percentage of material recovered (mean±S.E.M., $n=7$). (a) □, Oxytocin; ■, neurophysin-I; (b) □, vasopressin; ■, neurophysin-II.