participate in the virus assembly. Furthermore, if after incubation in guanidine, infected cells are maintained in media containing FPA or Py11, neither virus particles nor infectious virus are produced, in spite of the remarkable synthesis of virus RNA.

These data lead to the conclusion that guanidine impairs the ability of capsid precursors to participate in the assembly of infectious virus particles[8].

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Riassunto. La guanidina altera irreversibilmente la capacità delle proteine capsidiche del poliovirus di partecipare alla formazione di particelle infettanti.

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Chromatographic Evidence for the Synthesis of Possible Sleep-Mediators in *Trypanosoma brucei gambiense*

Trypanosoma brucei gambiense produces a chronic disease in man characterized by prolonged sleep states, mental depression, and physical inertia. The onset of abnormal sleep periods corresponds to the penetration of trypanosomes into the cerebrospinal fluid[1]. One member of the protozoan family Trypanosomatidae, *Crithidia fasciculata*, as well as the ciliate, *Tetrahymena pyriformis*, have been reported to contain the indolamine, serotonin (5-hydroxytryptamine)[2]. This compound has been suggested to be a mediator of slow-wave sleep in mammals[3]. We have investigated the metabolism of tryptophan and 5-hydroxytryptophan, two immediate precursors of serotonin, in *T. b. gambiense* in an attempt to demonstrate the synthesis of serotonin or of other sleep-mediators.

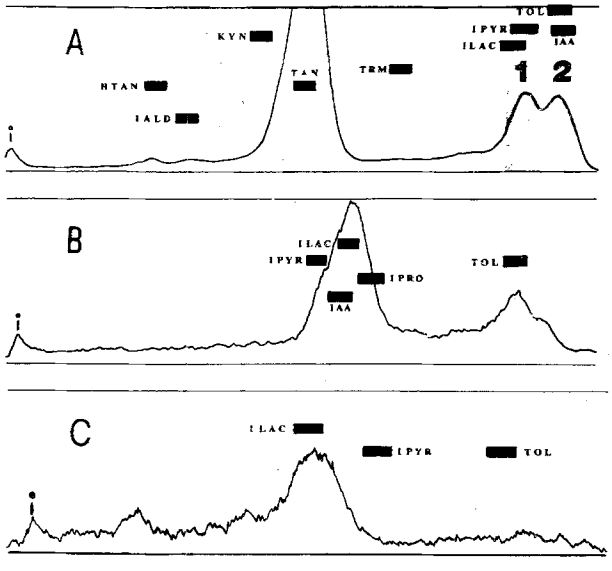

Fig. 1. (A) Radioactivity scan (10,000 cpm range) of chromatogram of supernatant from incubation of trypanosomes for 90 min with tryptophan-3-C^{14}, developed in butanol: acetic acid: water (4:1:1). Shaded areas 1 and 2 were eluted from the chromatogram and re-chromatographed in other solvent systems. (B) Scan (3,000 cpm range) of chromatogram of eluate from area 1 in A, developed in ethanol (95%): ammonia: water (16:1:3). (C) Scan (300 cpm range) of chromatogram of eluate from peak in B, developed in benzene: acetic acid: water (125:72:3). Abbreviations: HTAN, 5-hydroxytryptophan; IAA, indole acetic acid; IALD, indole acetaldehyde; ILAC, indole lactic acid; IPRO, indole propionic acid; IPYR, indole pyruvic acid; KYN, kynurenine; TAN, tryptophan; TOL, tryptophol: TRM, tryptamine.

Materials and methods. Bloodstream forms of this parasite were harvested from infected rats and separated from blood cells by column chromatography through diethyl amino ethyl (DEAE) cellulose (Sigma Chemical Co.)[4]. The trypanosomes were suspended in 5.0 ml of 0.1 *M* potassium phosphate buffer, pH 7.4, containing NaCl (0.1 *M*), KCl (0.006 *M*), $MgSO_4$ (0.002 *M*), 1% D-glucose, vitamin mix (100× concentrated, 1.0 ml/100 ml buffer, designed for minimum essential Eagle medium, Microbiological Associates, Bethesda, Maryland), and enzyme cofactors (6,7-dimethyl-5, 6, 7, 8-tetrahydropterine, 1 m*M*; pyridoxal phosphate, 5×10^{-5} *M*; α-ketoglutarate, 8 m*M*; NADH, 1.4 m*M*; NAD^+, 0.6 m*M*; and NADPH, 6.7×10^{-4} *M*) and incubated for 90 min at 37 °C with 5.0 μCi of either L-tryptophan-3-C^{14} (3.82 mg/mCi) or DL-5-hydroxytryptophan-3-C^{14} (2.1 mg/0.05 mCi) (New England Nuclear). The concentration of trypanosomes was approximately 100 million/ml. Sonicated trypanosome preparations were also incubated with these C^{14}-substrates; sonication was carried out using a Branson S-75 sonifier at setting 5 for 45 sec.

Three volumes of absolute methanol were added to terminate the incubations, and after centrifugation and removal of precipitated protein, the supernatant extract was concentrated to 3.0 ml by evaporation. 100 μl aliquots of this were chromatographed in one dimension along with known, purified standards on 57 cm strips of Whatman 3MM chromatography paper using butanol: acetic acid: water (4:1:1). The developed chromatograms were scanned for radioactivity using a Packard radiochromatogram scanner, and radioactive peaks not identical with the substrate were eluted from the chromatogram and re-chromatographed in as many as 4 other solvent systems. Aliquots of the incubation extract were also subjected to two-dimensional paper chromatography using butanol: acetic acid: water (4:1:1) and ethanol (95%): ammonia: water (16:1:3). Control incubations were run simultaneously with the experimentals: these included a boiled preparation, a zero time incubation, an incubation of only buffer and substrate, and incubations of centrifuge-purified, sonicated platelets and erythrocytes.

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Results. Both living and sonicated trypanosome preparations converted C^{14}-tryptophan to 2 radioactive metabolites, which were identified as indole lactic acid and tryptophol (indole-3-ethanol) (Figures 1 and 2). Tryptophol and indole lactic acid were also identified as metabolites of tryptophan in two-dimensional paper chromatography. Only 1 metabolite was formed from C^{14}-5-hydroxytryptophan; this was identified as 5-hydroxytryptophol (Figure 3). No synthesis of serotonin or tryptamine was detected, nor was tryptophan hydroxylated to 5-hydroxytryptophan. There was no metabolism of tryptophan or 5-hydroxytryptophan in any of the controls.

Preliminary results indicate that the pathway from tryptophan to tryptophol involves a transamination with α-ketoglutarate as amino-acceptor, followed by decarboxylation of the C-1 carbon of the side chain. The pathway does not appear to proceed through tryptamine.

Discussion. 5-Hydroxytryptophol is a naturally occurring metabolite of serotonin in the brain and pineal of mammals[5,6]. The 'tryptophols' have been suggested to be the substances which trigger the onset of paradoxical, or rapid-eye-movement, sleep[7,8]. These compounds have been reported to cause sleep, convulsions, and death by respiratory depression when injected in high doses into mice, rats, cats, and chicks[7–9].

Because the African trypanosomes invade the cerebrospinal fluid and possibly brain tissue of the host during the late stages of this disease, they may metabolize brain stores of tryptophan to tryptophol, which could directly affect nervous tissue, causing lethargy, prolonged sleep states, and even death. However, tryptophol synthesized outside the central nervous system may have little effect on the host, since it does not readily cross the blood-brain barrier unless present in high concentrations[10]. High levels are not likely to be attained in the blood due

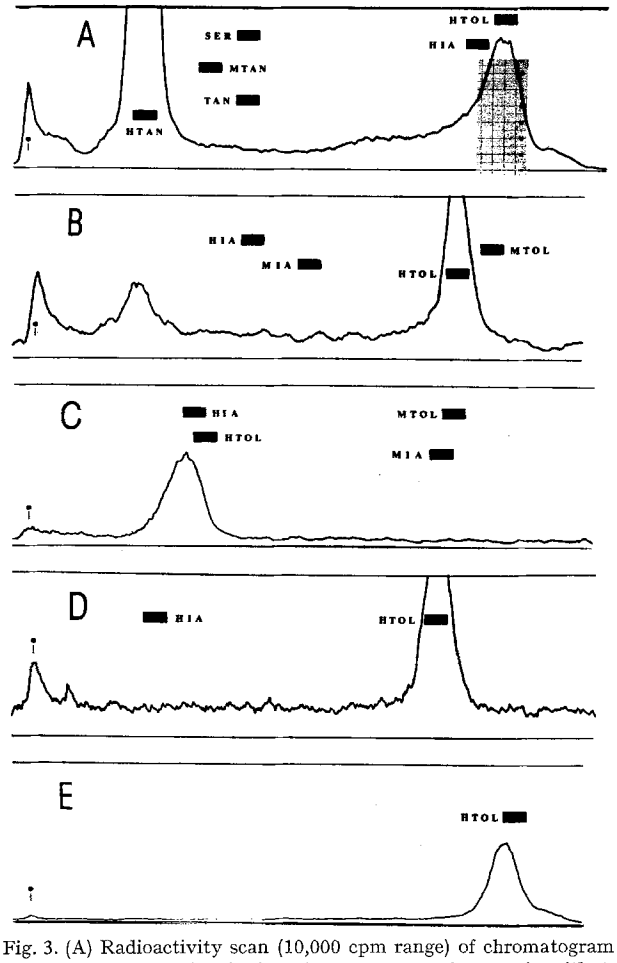

Fig. 3. (A) Radioactivity scan (10,000 cpm range) of chromatogram of supernatant from incubation of trypanosomes for 90 min with 5-hydroxytryptophan-3-C^{14}, developed in butanol: acetic acid: water (4:1:1). Shaded area was eluted and rechromatographed in 4 other solvent systems (B–E). (B) Scan (1,000 cpm range) of chromatogram of eluate from shaded area in A, developed in ethanol (95%): ammonia: water (16:1:3). (C) Scan (1,000 cpm range) of chromatogram of eluate from peak in B, developed in benzene: acetic acid: water (125:72:3). (D) Scan (300 cpm range) of chromatogram of eluate from peak in C, developed in isopropanol: ammonia: water (100:5:10). (E) Scan (1,000 cpm range) of chromatogram of eluate from peak in B, developed in methyl ethyl ketone: propionic acid: water (75:25:30). Abbreviations: HIA, 5-hydroxyindole acetic acid; HTAN, 5-hydroxytryptophan; HTOL, 5-hydroxytryptophol; MIA, 5-methoxyindole acetic acid; MTOL, 5-methoxytryptophol; SER, serotonin; TAN, tryptophan.

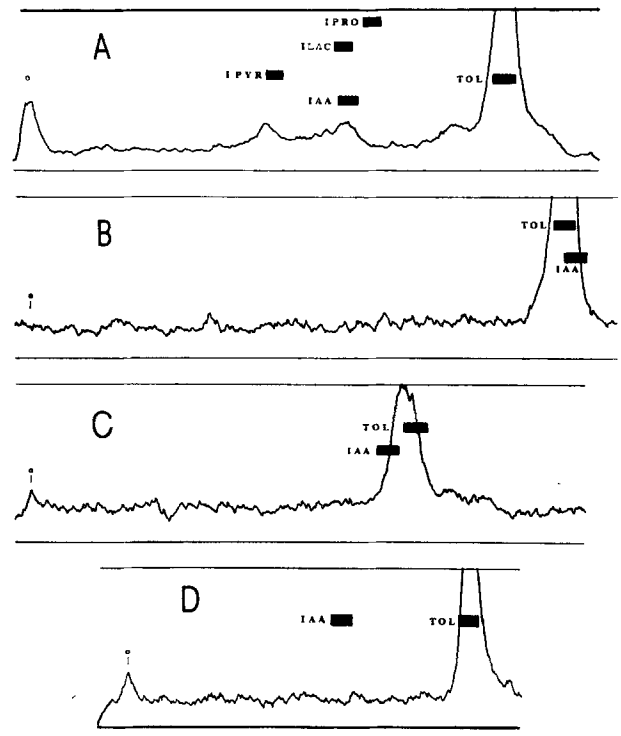

Fig. 2. (A) Radioactivity scan (1,000 cpm range) of chromatogram of eluate from area 2 in Figure 1A, developed in ethanol (95%): ammonia: water (16:1:3). (B) Scan (300 cpm range) of chromatogram of eluate from peak in A, developed in methyl ethyl ketone: propionic acid: water (75:25:30). (C) Scan (300 cpm range) of chromatogram of eluate from peak in A, developed in benzene: acetic acid: water (125:72:3). (D) Scan (300 cpm range) of chromatogram of eluate from peak in C, developed in isopropanol: ammonia: water (100:5:10). Abbreviations as in Figure 1.

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to rapid metabolism of this compound in the liver[10,11] and excretion in the kidney[12].

The significance of this pathway in the overall metabolism of this trypanosome is presently obscure. The initial transamination of tryptophan could serve to increase the intracellular concentration of glutamate, thereby stimulating conversion of pyruvate to alanine by alanine aminotransferase, an enzyme present in high levels in this parasite (unpublished data). The result, in addition to detoxification of intracellular pyruvate, might be an increased rate of glycolysis due to removal of the end-product[13].

Résumé. Les trypanosomes ont converti les substrats L-tryptophan et DL-5-hydroxytryptophan en métabolites tryptophol (indole-3-éthanol) et 5-hydroxytryptophol, deux composés qui produisent le sommeil chez la souris et le poussin. Les effets possibles de ces composés soporifiques chez un homme infecté par ce parasite et leur rôle dans le métabolisme du parasite, sont discutés.

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12 May 1973.

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COGITATIONES

The Mechanism of Specific Precipitation: Another Look

The 'lattice' (= framework = alternation) theory of specific precipitation, proposed by Marrack[1], was later adopted by Heidelberger and Kendall[2,3], by Pauling[4], and by various other workers in the field. Briefly, it supposed that (assumed) multivalent antibody united with multivalent antigen, each such union involving only a single combining group of each reagent, that the bimolecular compound thus formed then united with another molecule of antigen or antibody (or with an antibody-antigen compound already formed), and that this process continued until the resulting aggregates were so large that they perforce separated out of the solution as a precipitate. The idea had a refreshing mechanistic simplicity, and to workers trained mainly in chemistry, and not over-familiar with the earlier literature of immunology, the theory seemed almost self-evident, as indeed some of its proponents claimed it was. This lattice theory, in spite of some opposition, has become the generally accepted theory.

Nevertheless, it was clear from the beginning that the lattice theory was not an adequate explanation of all the known facts. Rather surprisingly for a theory suggested by chemists, it took no account of solubility. Heidelberger and Kendall[5] explicitly stated '...aggregation would occur regardless of the affinity of the groupings for water.'

But, contrary to this idea, it was already known that affinity of the groupings for water, i.e., solubility, did play a role. For example, it had been found that precipitates made with protein and horse anti-protein antibodies were soluble in excess antibody, whereas such precipitates made with rabbit antibody were not. Clearly a solubility effect. Boyd and Purnell[6] studied the precipitating behavior of the different kinds of antibody in detail.

The lattice theory demanded that antigen be multivalent, which was never in much doubt, and which subsequent investigation has amply confirmed (cf. [7]), and assumed antibody also to be multivalent, a more doubtful point. Later work has in fact not confirmed it, but it does seem that most antibody is divalent (cf. [7]), and divalent antibody can be visualized as forming 'lattices' with multivalent antigens or haptens.

The lowest valence that can be called multivalent in this sense is three, and in fact some trivalent haptens have been observed to precipitate with appropriate (divalent) antibody. But with divalent haptens and divalent antibodies lattice formation is hard to visualize. Rather awkwardly, some divalent haptens have been observed to precipitate. However, some of these haptens are probably aggregated in solution[8], and thus become effectively multivalent, which may be an explanation.

It was suggested that divalent haptens, even when they did not precipitate, should form long ...hapten-antibody-hapten-antibody... chains. This should confer on the mixture a pronounced birefringence of flow, but a careful examination of such mixtures by Hooker and Boyd[9] did not reveal any such birefringence. In fact, later studies by Epstein, Doty and Boyd[10], using the light scattering technique, indicated that the chains formed in their mixtures were short. They did not precipitate.

It was obvious that the behaviour of di- and multivalent haptens when mixed with antibody would be a critical test of the lattice theory. Boyd[11] studied the reactivity of 34 different haptens containing from 1 to 6 specifically reactive groups, and found that 6 divalent haptens, 4 trivalent haptens, and 1 hexavalent hapten failed to precipitate. He was able to connect these observations plausibly with the solubility of the haptens. He concluded that the possibility of lattice formation is by no means sufficient to ensure that a hapten will precipitate.

Boyd[11] stated, 'As an explanation of precipitation, it would seem that neither the alternation nor the Bordet theory is adequate. The alternation theory seems to be simply incorrect, and the Bordet theory too vague to

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