TENTATIVE IDENTIFICATION OF A LIPID MOBILIZING SUBSTANCE FROM THE CALF MID-BRAIN

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An earlier report described the isolation and some properties of a lipid mobilizing factor (LMF) from the calf mid-brain, (1968). This factor has been tentatively identified as a hypoxanthine-like compound. LMF was indistinguishable from the purine base, hypoxanthine by paper electrophoretic and paper chromatographic techniques, and in u.v. absorption spectra.

MATERIALS AND METHODS

The method of preparation of the mid-brain extract and the procedure developed for its purification has been reported (1968). The LMF preparation used in the following experiments was obtained by column chromatography on carboxymethyl cellulose (1968), it is the most highly purified preparation.

Ultraviolet Absorption Spectra were determined in a Beckman Model DB-G Spectrophotometer with an automatic recording attachment. Spectral curves were made under acid, neutral and alkaline conditions. The buffers employed were 0.1N HCl, 0.02M sodium phosphate, and 0.02N NaOH for pH 1.0, 2.0 and 11.2, respectively (1967).

Paper Chromatography was conducted in four different solvent systems (1967): Solvent No. I: Isobutyric acid/conc NH_4OH/water, 66/1/33; pH 3.7. Solvent No. II: Ethanol/1.0N ammonium acetate, 7/8;

pH 7.5. Solvent No. III: 0.2M sodium phosphate, pH 5.8/ammonium sulfate/n-propanol, 100/60/2. Solvent No. IV: Isobutyric acid/conc NH_4OH/water, 57/4/39; pH 4.3.

Chromatograms were developed at 27C for 13 hours (Solvent No. II), and 16 hours with Solvents I, III and IV. The chromatograms were air dried and viewed under ultraviolet light; the u.v. absorbing areas were outlined and their R_f values determined.

Paper Electrophoresis was carried out employing a water-cooled E and C electrophoresis apparatus with a custom made high voltage power supply. The buffer system was 0.20M Na_2HPO_4 + 0.1M citric acid, used in the appropriate proportions to give pH 5.0 and pH 7.0 (1959). Electrophoretic mobilities were measured at 1,500 volts for 1 1/2 hours (pH 5.0 and 7.0), and at 3,000 volts for 2 hours (pH 7.0), and 1,500 volts for 4 hours, pH 7.0.

RESULTS AND DISCUSSION

Ultraviolet Absorption Spectra. Solutions of LMF absorb in the ultraviolet region with maximal absorption at 248 mμ. The absorption spectra of several purines and pyrimideines were compared with LMF; two, only, had absorption maxima identical to LMF; these were, the nucleoside, inosine (hyoxanthine riboside), and the purine base, hypoxanthine. The area of maximal absorption for the catecholamines, in this system, was 278 mμ.

The absorption spectra for LMF, inosine and hypoxanthine at pH 1.0, 7.0 and 11.2 are shown in Figs. 1, 2 and 3, respectively. Under acid and neutral conditions, maximal absorption was at 248 mμ for LMF, inosine and hypoxanthine. At pH 11.2, inosine absorbed maxi-

mally at 252 mμ, while LMF and hypoxanthine absorbed maximally in the areas of 256-258 mμ, repectively.

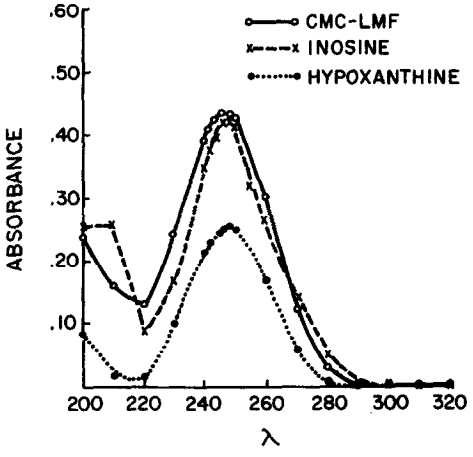

FIG. 1. Ultraviolet absorption spectra at pH 1.0.

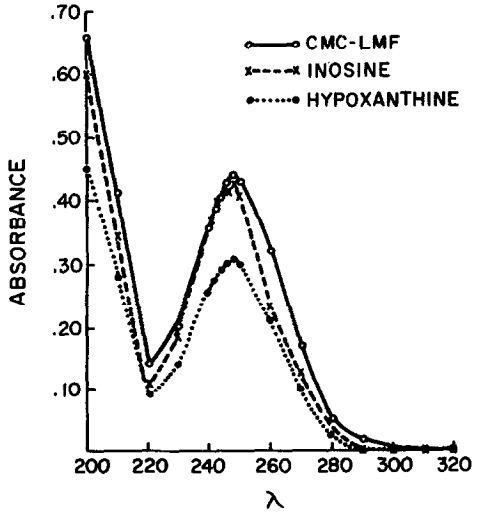

FIG. 2. Ultraviolet absorption spectra at pH 7.0.

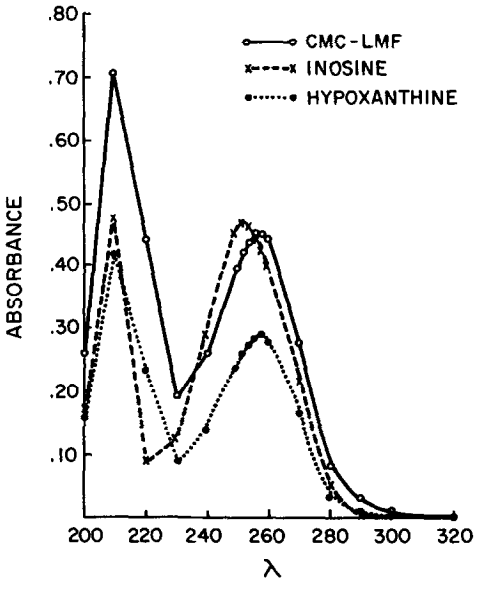

FIG. 3. Ultraviolet absorption spectra at pH 11.2.

Paper Chromatography. Descending chromatography was carried out in buffer systems at pH 3.7 to pH 7.5; the results are shown in Table 1. Of the four solvent systems employed, three (I,III, and IV) differentiated between LMF and inosine, and when LMF and inosine were co chromatogrammed they were resolved into two separate components. No distinction between LMF and hypoxanthine was evident in any of the solvent systems. In two solvents, I and IV, inosine migrated more slowly than LMF and hypoxanthine; whereas in solvent III inosine preceded LMF and hypoxanthine. LMF had R_f values identical to hypoxanthine in all four solvents and chromatogrammed together, LMF and hypoxanthine moved as a single entity.

Electrophoretic Mobilities. The pH of the buffer systems chosen for electrophoresis was based on pK_a values for inosine and hypoxanthine; pH 7.0 provided conditions whereby hypoxanthine should have

TABLE 1

PAPER CHROMATOGRAPHIC STUDIES

Compounds	R_f Values			
Solvents:	I	II	III	IV
Inosine	0.56	0.68	0.23	0.63
LMF	0.68	0.67	0.18	0.70
Inosine*	0.56		0.23	0.62
+		0.67		
LMF	0.68		0.18	0.70
Hypoxanthine	0.69	0.66	0.17	0.68
LMF	0.68	0.65	0.17	0.68
Hypoxanthine*				
+	0.68	0.64	0.17	0.68
LMF				

*Denotes co-chromatography.
The amounts of inosine and hypoxanthine applied to the paper were 4.26 µg and 2.48 µg, respectively; 50 µl of CMC-LMF were used, this is equivalent to 7.15 µg of hypoxanthine.

its greatest mobility; and pH 5.0 should give optimal conditions for inosine.

The application of 1,500 volts for 1 1/2 hours, at either pH 5.0 or pH 7.0, did not separate LMF from inosine or hypoxanthine. However, at pH 7.0 there was an indication that LMF and inosine were pulling apart; when a current of 3,000 volts was applied for 2 hours, LMF and inosine were resolved into two separate components, with

TABLE 2

ELECTROPHORETIC MOBILITIES

Compounds	v/cm^2/hour
Inosine	- 0.072
LMF	- 0.062
Inosine*	- 0.076
+	
LMF	- 0.062
Hypoxanthine	- 0.066
LMF	- 0.067
Hypoxanthine +	
+	- 0.071
LMF	

*Denotes co-electrophoresis.
The amounts of inosine and hypoxanthine applied to the paper were 4.26 µg and 2.48 µg, respectively; 50 µl of CMC-LMF were used, this is equivalent to 7.15 µg of hypoxanthine.

inosine in advance of LMF (Table 2). LMF and inosine also were separated with a current of 1,500 volts for 4 hours. Hypoxanthine and LMF were inseparable and moved as a single component under all conditions tested. Epinephrine, at pH 7.0, migrated towards the anode and during electrophoresis at 1,500 volts for 4 hours, it moved completely off the paper.

Our incubation system consisted of 0.06M phosphate buffer, pH 7.4 (1968), the only addition to the medium was the substance being tested for its lipid mobilizing effect on adipose tissue; thus, LMF was the sole factor contributing to the stimulation of lipid mobilization. It was of interest, therefore, to test the action of commercial hypoxanthine in our system. Two preparations (Sigma and P-L Biochemicals, Inc.) were tested, over a wide range of concentrations--no lipolytic activity was observed. While the evidence indicates that LMF and hypoxanthine are identical, it is possible that LMF has some grouping not present in commercial hypoxanthine, which confers lipid mobilizing ability but does not contribute to u.v. absorption or electrophoretic and chromatographic mobilities. Presumably this grouping is not a sugar, or at least not ribose, since LMF and inosine could be separated under certain conditions.

In summary, LMF and hypoxanthine were indistinguishable by electrophoretic and chromatographic techniques, and by their u.v. absorption spectra. LMF appears to be a hypoxanthine-like substance.

Additional purification studies are in progress and a full length publication is in preparation.

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