albumin, hydrocortisone migrated approximately 3 times as far as progesterone. This observation demonstrated that the considerable transport of progesterone by albumin (Fig. 1, bottom) and the lack of transport of hydrocortisone (Fig. 1, top) could not be the result of different intensities of adsorption on the paper.

The studies show that approximately 2 to 10 percent of hydrocortisone and more than 90 percent of progesterone were transported by and, therefore, were bound to human serum albumin. The hormone concentrations employed were only a fraction of the maximal binding capacities of serum albumin solutions for these compounds. Even at this level, a substantial proportion of the steroids was in free form.

References and Notes

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Continuous Ascending Chromatography—New Technique

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In recent years an impressive list of papers has been published in which the technique of ascending paper chromatography (*1*) has been the primary means of separation or identification. Where the Rf values for the desired components are small, the choice has been continuous descending chromatography or longer sheets of paper for the ascending method with necessarily larger and more elaborate chromatographic chambers. Significant diffusion occurs when the resolving solvent remains in contact with the paper after the solvent front has reached the top of the conventional ascending chromatogram. Lengthening the chromatogram does not adequately circumvent this effect, since the rate of solvent movement is markedly retarded by gravity. The new technique alleviates this shortcoming.

The procedure described here depends on the rapid removal of the solvent as it reaches the top of the chromatogram by capillary ascent (wick effect). Any suitable container is fitted with a glass, cork, wood, polyethylene, aluminum, or stainless steel cap that has one or more slits to permit the top of the ascending paper chromatogram or chromatograms to protrude. Generally, it is sufficient to seal the cap to the jar with Scotch tape. The container, preferably glass, is kept to a minimum height commensurate with the distance that the desired components will travel during the required development. The volume of the container is also kept to a minimum in order to decrease, or essentially eliminate, the time usually allotted for liquid-vapor equilibration (*2*). Ideally, the chamber should approximate the dimensions of the chromatogram. This arrangement may be approached by cutting prescription bottles or other flattened flasks to the desired height. However, spoutless beakers, test tubes, and jars of varying heights are also used. The wick that extends above the cap may be sandwiched between two short wedges of paper in order to seal the opening and to increase the evaporation area.

For most of the solvent systems used in this laboratory, there is sufficient evaporation from the wick under ordinary room conditions to remove the solvent front. For higher boiling solvents systems, the positive draught from a conventional hood or fan is adequate. If evaporation is not sufficient to remove the solvents rapidly, the wick can be bent downward and the solvent front can run off into a beaker. In practice, we have had little occasion to resort to this latter arrangement.

We have found that the new technique is more effective than the continuous descending procedure when there is need for a continuous system. Although our applications have been mainly in the fields of antibiotics and alkaloids, the dye FD&C Blue No. 1 is used simply for illustrative purposes. A commercially available descending chromatographic chamber was equilibrated overnight for the descending technique, and a 250-ml beaker with stainless steel cap was used for the ascending procedure. The FD&C Blue No. 1 was spotted from aqueous solution on Whatman No. 1 paper, air-dried for 5 min, and resolved with methyl cellosolve acetate and water (4 : 1) in the respective chambers. The results are illustrated in Fig. 1.

Typical of other findings is the appearance of three well-defined components in FD&C Blue No. 1 after 18 hr of resolution by the ascending method as against one diffused zone by the descending technique. One

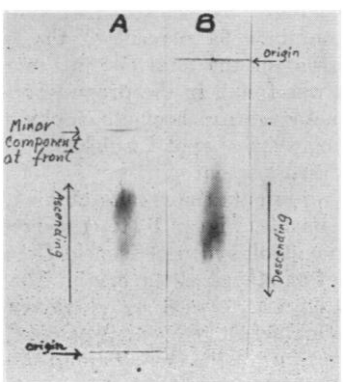

Fig. 1. (*A*) Continuous ascending chromatogram after 18 hr. (*B*) Continuous descending chromatogram after 18 hr.

component, a minor constituent, travels relatively fast and concentrates at the front, as is shown in Fig. 1. During the first 6 hr of resolution, the descending chromatogram exhibits this minor component, but it is lost through diffusion on further development. After 48 hr, the descending chromatogram did exhibit two components but in a decidedly diffused and streaked condition.

The simplicity and rapidity of the new technique affords the laboratory a continuous system for ascending chromatography at a minimum cost.

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Detoxication of Drugs and Other Foreign Compounds by Liver Microsomes

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Recent studies demonstrating that diethylaminoethyl diphenylpropylacetate HCl (SKF 525-A) inhibits the rate of biotransformation of drugs metabolized by a diversity of metabolic pathways (*1, 2*) suggested that the tissue catalysts responsible for their metabolism possess certain factors in common. This report describes experiments which show that common denominators in the metabolism of a variety of foreign compounds (Table 1) are most unusual and include localization of the enzyme systems in liver microsomes and requirements for both reduced triphosphopyridine nucleotide (TPNH) and oxygen.

Table 2. Requirements for the demethylation of monomethyl-4-aminoantipyrine by dialyzed rabbit liver homogenate.

Additions	Amount of 4-aminoantipyrine formed (μM)
Complete system*	1.53
Minus Mg^{++}	0.99
Minus glucose-6-phosphate	0.87
Minus nicotinamide	0.15
Minus TPN	0.14
Diphosphopyridine nucleotide in place of TPN	0.20

* To 2 ml of liver homogenate (1 : 2 in 0.2*M* phosphate buffer, *p*H 7.4) were added 100 μM of nicotinamide, 75 μM of $MgCl_2$, 0.2 μM of TPN, and 5 μM of monomethyl-4-aminoantipyrine to a final volume of 5 ml. Incubation was for 1 hr at 37°C in air.

From the results in Table 2, which show the requirements for the demethylation of monomethyl-4-aminoantipyrine in homogenates of dialyzed rabbit liver, it is seen that the reaction is enhanced by nicotinamide, triphosphopyridine nucleotide (TPN), Mg^{++}, and glucose-6-phosphate. Similar requirements were also found for the metabolism of the other drugs listed in Table 1.

The cellular localization of the biochemical reactions was determined by measuring the activity of cellular fractions separated by differential centrifugation of liver homogenates (*11*). Table 3 shows that both microsomal and soluble fractions of liver are required for drug metabolism. Nuclei and mitochondria, on the other hand, show virtually no activity.

The role of the soluble fraction in the reactions was suggested by the afore-mentioned stimulation by glucose-6-phosphate together with the presence of considerable glucose-6-phosphate dehydrogenase activity in the soluble fraction. These observations indicate that TPN might be required in the homogenate in the reduced form, TPNH, and that glucose-6-phosphate

Table 1. Reactions catalyzed by enzyme systems in liver microsomes. Analytic methods are described in the references cited.

Type of reaction	Substrate	Products	Reference
Barbiturate side-chain oxidation	Evipal (hexobarbital)	Keto-evipal	(*3*)
	Nembutal (pentobarbital)	Nembutal alcohol and nembutal carboxylic acid	(*4*)
Dealkylation	Pyramidon (dimethyl-4-aminoantipyrine)	4-Aminoantipyrine + formaldehyde	(*5*)
	Monomethyl-4-aminoantipyrine	4-Aminoantipyrine + formaldehyde	(*5*)
	Monoethylaniline	Aniline + acetaldehyde	(*6*)
	Ephedrine	Norephedrine + formaldehyde	(*7*)
Deamination	Benzedrine (amphetamine)	Phenylacetone + ammonia	(*8*)
Ether cleavage	Codeine	Morphine + formaldehyde	(*9*)
	Phenacetin (*p*-ethoxyacetanilide)	*p*-Hydroxyacetanilide + acetaldehyde	(*9*)
Hydroxylation	Aniline	*p*-Hydroxyaniline	(*10*)
	Acetanilide	*p*-Hydroxyacetanilide	(*10*)