pyrine transformation at 12 days of age with that of a 6 months of age brother. Figures 1 and 2 show the ability of the brain, at every day of age, to take up the administered drugs, both oxazepam and aminopyrine.

In some experiments it was possible to test the metabolizing power of the isolated brain *in situ* of both male and female newborns from the same litter and at the same day of age: at the eighth day (glucuronoconjugation in two male and female newborns) and at the twelfth (glucuronoconjugation, demethylation, and acetylation in four male and female newborns). In these experimental arrangements no significant differences were noted between male and female newborn animals from the same litter.

CONCLUSIONS

Related to the days of age, the brain isolated *in situ* of newborn dogs from the same litter showed a drug metabolism which was evaluated by studying the glucuronoconjugation of oxazepam and the demethylation and acetylation of aminopyrine. No metabolizing activity was present before 4 days of age; at 8–12 days the drug metabolism was evident, but only after 12–24 days of age did it begin to become quantitatively similar to that of the adult.

These great differences in cerebral drugs metabolism induced by the age of the newborns, seem to be interesting particularly in view of both the ability of the brain to take up the administered drugs and the detoxication mechanisms of the brain itself. In the authors' experimental conditions no significant differences were evident between the metabolizing activity of male and female newborns from the same litter and of the same age.

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Detection and Separation of Aliphatic Polycarboxylic Acids by Reversed-Phase Partition Thin-Layer Chromatography

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Abstract □ The application of reversed-phase partition TLC, employing Silica Gel HF chromatoplates impregnated with silicone and developed in glacial acetic acid–dioxane–water–formic acid (4:1:1:6), proved suitable for the separation of agaricic acid (α-hexadecylcitric acid), norcaperatic acid (α-tetradecylcitric acid), and citric acid.

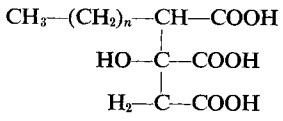
Keyphrases □ Polycarboxylic acids, aliphatic—detection, separation □ Phosphomolybdic acid solution—spot visualization □ Potassium permanganate solution—spot visualization □ TLC, reversed phase partition—detection, separation

Volatile compounds of a homologous series can be fractionated usually by adsorption TLC but compounds of longer chain lengths are resolved, in most cases, by reversed-phase partition chromatography. Kaufmann and Makus (1) employed this technique for the separation of homologs of fatty acids, alcohols, and triglycerides. Malins and Mangold (2) successfully separated saturated and unsaturated fatty acids by using siliconized chromatoplates. Other investigators have utilized reversed-phase partition procedures for the separation of fatty acids and their methyl esters (1–3), cholesteryl esters of higher fatty acids (4), keto acids, lactones, and hydroxy fatty acids (5).

A number of hydrophobic agents have been employed as the stationary phase in reversed-phase partition chromatography. Silicone appears to be the preferred hydrophobic agent but undecane, tetradecane, squalane, polyethylene, and paraffin have also been used. Numerous adsorbants have been utilized in this procedure but Silica Gel G and kieselguhr G are usually employed.

The recent isolation and identification of norcaperatic acid (α-tetradecylcitric acid) from the mushrooms *Gomphus floccosus* (Schw.) Singer [syn: *Cantharellus floccosus* Schw.] (6) and *Polyporus fibrillosus* Karst. (7) have accentuated the need for a convenient method for the differentiation and detection of members of this aliphatic polycarboxylic acid group. Agaricic acid

(α-hexadecylcitric acid) had been previously isolated from *Fomes officinales* (Fr). Faull. in 1907 (8). The structural formulas of agaricic acid (I) and norcaperatic acid (II) may be represented as:



I, $n = 15$
II, $n = 13$

EXPERIMENTAL

Preparation of Chromatoplates—All traces of any protective film were removed from the surface of 24 noncorrosive, precleaned, 7.62 × 2.54-cm. (3 × 1-in.) microscopic slides. The cleaned slides were arranged end-to-end on a smooth surface in groups of four and both sides of each group taped to the surface by overlapping 0.635 cm. (0.25 in.) of the slide with 2.54 cm. (1 in.) wide masking tape according to the method of Connors and Eriksen (9). A glass stirring rod was firmly drawn over the overlapping tape in order to obtain a uniform distance between the top of the tape and the surface of the slide. The adsorbant was prepared by adding 24 ml. of distilled water to 10 g. in a 125-ml. conical flask and vigorously agitated for 45 sec. A portion of the slurry was poured on the head slide of each group and spread uniformly over the slides by drawing a glass stirring rod over the surface of the tape. An adsorbant thickness of approximately 150 μ was obtained in this manner. The coated slides were allowed to air dry for 4 hr. and then placed in a preheated oven at 120° for 1 hr. After heating, the plates were cooled to room temperature in a desiccator containing calcium chloride. The adsorbant layer was rendered hydrophobic by impregnating with a thin film of silicone by the method of Mangold (10). Seventy milliliters of a 5% solution of silicone[1] in diethyl ether was placed in a Petri dish and the slides dipped into the solution for 5 sec., removed, placed in a horizontal position, and allowed to dry. Upon evaporation of the solvent the prepared chromatoplates were ready for use.

Preparation of Reference Solutions—Solutions of agaricic acid (0.1%), norcaperatic acid (0.1%), and citric acid (1%) were prepared by dissolving the respective compounds in 95% ethanol.

Spray Reagents—A 10% solution of phosphomolybdic acid in ethanol and a 3% solution of potassium permanganate in concentrated sulfuric acid were prepared.

Chromatographic Procedure—A syringe[2] was employed for spotting the reference solutions. One microgram of agaricic and norcaperatic acid and 10 mcg. of citric acid were spotted 5 mm. from the bottom of the chromatoplate. The desired developing solvent was placed in a Coplin staining jar and allowed to equilibrate for 10 min. prior to developing. The chromatoplates were allowed to develop for a distance of 6 cm. (approximately 20 min. developing time), removed from the chamber, air-dried, sprayed with the desired reagent, and placed in a 160° oven for 15 min. Both agaricic and norcaperatic acid appeared as bluish-green spots on a greenish-yellow background when sprayed with the phosphomolybdic reagent. Since citric acid did not react with this spray reagent it was necessary to use potassium permanganate in concentrated sulfuric acid in order to detect this compound.

[1] Dow Corning 200 fluid, viscosity 350 at 25°.
[2] York Termo Micro, MS-10.

RESULTS AND DISCUSSION

Chromatographic procedures for the separation of aliphatic polycarboxylic acids have not been previously reported. Graf and Liu (11) have reported the successful separation, by TLC, of a number of chemical intermediates, which they obtained in their attempts to synthesize agaricic acid. Their method included kieselguhr G as the adsorbant and eight different solvent systems. All of these systems were tested but were found totally unsuitable for the separation of agaricic and norcaperatic acid. In the course of the authors' investigation, over 40 different solvent systems were tried with Silica Gel G, kieselguhr G, aluminum oxide, mannitol, and lactose as adsorbants. Migration of agaricic and norcaperatic acid occurred with several of these systems but separation of the two acids was not accomplished.

Various procedures for the separation of mixtures of long-chain compounds of a homologous series by partition TLC in reversed phase have been reported in the literature (1–5). Employing this technique, 25 different solvent systems were tried with siliconized Silica Gel G and Silica Gel HF as adsorbants. Best results were obtained with chromatoplates prepared with Silica Gel HF, impregnated with silicone, and developed with a solvent system composed of glacial acetic acid–dioxane–water–formic acid (4:1:1:6). The two aliphatic polycarboxylic acids were rendered visible by spraying with a 0.1% phosphomolybdic acid solution. As little as 0.5 mcg. of either acid could be detected with this spray reagent. Agaricic and norcaperatic acid appeared as discrete bluish-green spots at R_f 0.39 and 0.53, respectively. Citric acid was detected by spraying with a 3% potassium permanganate in concentrated sulfuric acid and appeared at R_f 0.87. Both spray reagents were necessary since agaricic and norcaperatic acid were insensitive to the potassium permanganate reagent; as was citric acid to phosphomolybdic acid.

This reversed-phase partition thin-layer chromatographic method should prove especially useful in screening fungal extracts for micro amounts of aliphatic polycarboxylic acids and also of value in the interpretation of results in both the chemical synthesis and biosynthesis of these acids.

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