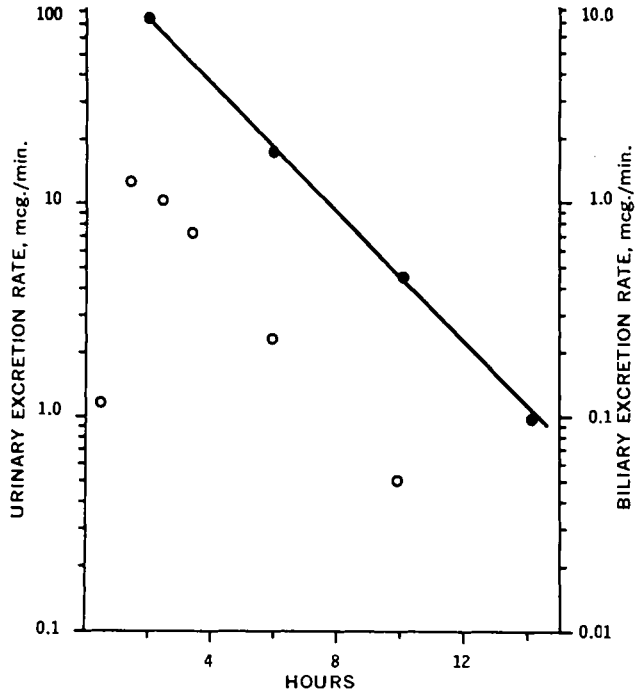

Figure 1—*Rates of urinary (●) and biliary (○) excretion of riboflavin by an 11-year-old girl after intramuscular injection of 17.5 mg./m.² riboflavin as the phosphate.*

We recently sampled the bile of a 3.5-month-old boy with refractory diarrhea who received only parenteral alimentation; the diarrhea was under control at the time of the study. Duodenal fluid was obtained by a continuous siphon through a Levin tube placed in the duodenum. Hourly collections started 1 hr. before and extended to 4 hr. after intramuscular injection of 10 mg. riboflavin as the phosphate. Gallbladder contraction was stimulated by the hourly administration of corn oil. Analysis of these samples (6) yielded a total of only 0.042 mg. riboflavin, while 9.54 mg. was recovered in the urine over 36 hr. Since the intestinal intubation procedure does not assure complete collection of bile and since the patient was quite young, we could not conclude with certainty that biliary excretion of riboflavin is negligible.

However, a subsequent study was carried out in an 11-year-old girl with complete obstruction of the common bile duct who had external biliary drainage through a temporary common bile duct cannulation. This patient was also receiving only parenteral alimentation at the time of the study. She was given 17.5 mg. riboflavin as the phosphate per m.² body surface area intramuscularly, urine and bile being collected completely at intervals from 2 hr. before to 24 hr. after injection. Preinjection excretion rates of apparent riboflavin by fluorometric assay (6) were 0.54 and 0.049 mcg./min. for urine and bile, respectively. Total recovery of injected riboflavin was 88.7% of the dose from the urine and only 0.97% from the bile. Known amounts of riboflavin added to bile obtained before injection of the vitamin were recovered quantitatively.

The time courses of urinary and biliary excretion of riboflavin by the girl are shown in Fig. 1. The kinetics of urinary excretion are similar to those of normal children given the same dose of the vitamin by the same route (data to be published). Urinary and biliary excretion rates of riboflavin were essentially parallel.

It appears that biliary excretion of riboflavin is negligible in man and that the secondary excretion rate maxima observed in man after large oral doses of the vitamin are due to further absorption of riboflavin. This is probably related to the discharge of bile into the intestine, but the mechanism of the apparent absorption-enhancing effect of bile is still uncertain (3). Future studies on patients whose bile is completely diverted from the intestine, and who are given riboflavin orally with and without bile, may establish more definitively the role of bile in riboflavin absorption.

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Biological and Chemical Evaluation of a 43-Year-Old Sample of *Cannabis* Fluidextract

Keyphrases □ *Cannabis* fluidextract—stability of 43-year-old sample □ Cannabinoids—presence, activity in 43-year-old *Cannabis* fluidextract

Sir:

The instability of *Cannabis sativa* L. preparations has been generally accepted as fact because of numerous observations of loss in biological activity with time.

We now report the preliminary results of studies on a 43-year-old sample of *Cannabis* fluidextract. We found that this preparation, in spite of the long shelf storage, still contained enough biological activity to conform with the requirements of USP X (1). Furthermore, TLC and GLC showed the presence of cannabidiol, Δ^1-tetrahydrocannabinol, and cannabinol in significant amounts.

To the best of our knowledge, the only long-term study on the stability of *Cannabis* preparations ever published is that of Eckler and Miller (2). They reported that both fluidextract and granulated drug moistened with alcohol retained their biological activity (in the dog ataxia assay) after 5 years of storage, while the same drug lost all its activity after dry storage for the same period of time. This observation and its practical significance have apparently gone unnoticed until now.

The fluidextract tested by us (in 1970) was a 4-oz., corked, amber glass bottle of "Fluid Extract/Cannabis, USP"[1]. The bottle had been stored at ambient temperature which at times exceeded 38°. It was almost full, and the total solids content was found to be 12.6%. The fluidextract was clear and dark green.

The alcohol was removed from aliquots of the original fluidextract, which were then reconstituted in propylene glycol and tested for biological activity by two methods. The characteristic ataxia in dogs, according to USP X, was produced by oral administration of the extract. A new assay was an operant conditioning lever-pressing procedure, based on alternative responses of food approach and shock avoidance (3). Rats were trained to make one response after injection of a synthetic Δ^1-tetrahydrocannabinol (4 mg./kg.) and the alternative response after injection of the vehicle alone (propylene glycol, saline–polysorbate 80). The fluidextract, at a dose of 25 mg./kg., elicited the drug response in 10 of the 11 animals tested (4). A series of tests with various drugs conducted in the same animals showed that this bioassay was highly specific to Δ^1-tetrahydrocannabinol and *Cannabis* extracts containing this substance.

The presence of Δ^1-tetrahydrocannabinol in the fluidextract was confirmed by TLC, using a method described previously (5) with minor modifications. Small aliquots of the fluidextract were applied to 0.25-mm. plates of silica gel G (Merck), along with samples of authentic cannabidiol, cannabinol, and Δ^1-tetrahydrocannabinol standards. The plates were developed with benzene–diethylamine (99:0.6) and air dried. Spraying with fast blue B salt reagent revealed the cannabinoids by their characteristic colors as well as by their $R_{\text{cannabidiol}}$ values, as previously reported (5). This procedure clearly showed the presence of significant quantities in the fluidextract not only of Δ^1-tetrahydrocannabinol but also of cannabidiol, cannabinol, and, in smaller amounts, at least five other components which gave pink to purple colors with fast blue B.

The actual concentrations of cannabidiol, cannabinol, and Δ^1-tetrahydrocannabinol in the fluidextract were determined by GLC, using authentic reference standards. The GLC analysis was performed on the original fluidextract and cannabinoid standards as well as on their trimethylsilyl derivatives by procedures similar to those of Davis *et al.* (6), with no significant differences between the results. We found that the fluidextract contained 0.1% cannabidiol, 0.4% Δ^1-tetrahydrocannabinol, and only 0.04% cannabinol. In addition, the GLC showed at least 11 other components. Three of these may be hydrocarbons, which do not form trimethylsilyl derivatives.

The foregoing biological and chemical assays indicate that this commercial *Cannabis* preparation lost little, if any, of its activity and that its cannabinoids survived the storage of 43 years. The obvious implication is that long-term storage of *Cannabis* may be practical in the form of an alcoholic extract to prevent deterioration of the pharmacologically active constituents.

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[1] Manufactured by Parke-Davis & Co., Detroit, Mich. The label bore a product number (598), lot number (2804334), and date (Manufactured Feb.-23-27).