graph making one revolution an hour whereas the lower was made on a much slower drum running overnight. Figure 2 is an ichthyogram produced by a goldfish placed in a 1:10,000 solution of mercurochrome (H., W. & D.) and surviving for 24 hours. Figure 3 shows the effect of a dilute solution of mercuric chloride (1:100,000), which caused death in a few hours. Figure 4 reveals the results obtained with a solution containing a mixture of mercurochrome, 1:10,000, and bichloride of mercury, 1:100,000.

SUMMARY

1. A simple method for graphically recording on the kymograph the neuromuscular activity and general behavior of small fish has been described.

2. This method has been employed by the authors for pharmacological study of the comparative effects of various drugs on goldfish.

3. A striking difference was noted in the ichthyograms produced by goldfish placed, respectively, in solutions of certain organic mercurials and of inorganic mercury salts, the former being much less toxic than the latter.

4. The ichthyometric method has proved useful not only in differentiating between organic and inorganic mercury compounds but also in discovering the presence of inorganic mercury contaminants in mercurochrome.

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"To be guided by reason is to obey the laws of nature."—Ernest Wood

Non-Toxic Character of Ursolic Acid.*

Preliminary Study

By J. A. Lubitz and C. R. Fellers†

INTRODUCTION AND REVIEW OF LITERATURE

Ursolic acid is a monohydroxytriterpene acid (1) of the formula $C_{30}H_{48}O_3$ (2). It is widely distributed in nature, having been found in *uva ursi* leaves (3), mistletoe (4) and the skins of apples (5), pears (6) and cherries (7). Cranberry pomace obtained in the commercial canning of cranberry sauce was also found to contain ursolic acid (8). According to Winterstein and Stein (9), ursolic acid in the form of a saturated solution of its sodium salt is toxic to fish. This reported toxicity to fish might be due to the use of a saturated solution or possibly to still other factors. As ursolic acid is being introduced as an emulsifying agent in pharmaceutical and food preparations, it was thought advisable to determine whether or not it possessed toxic properties.

EXPERIMENTAL

Description of Sample.—The ursolic acid used in this study was prepared from cranberry skins.[1] This ursolic acid was a fairly pure sample although it had not been crystallized from alcohol. The yield of ursolic acid manufactured from dried cranberry skins is about 10 per cent.

Toxicity.—Rats, guinea pigs, chickens and rabbits were fed ursolic acid orally at levels of from 1000 to 5000 mg. per Kg. of body weight. These animals were placed in individual cages with abundant drinking water, but were left without food for 24 hours. The ursolic acid was then fed mixed with dried bread crumbs in the case of the rats and guinea pigs, laying mash in the case of the chickens and Purina Fox Chow (a prepared dry feed) in the case of the rabbits. The animals were observed for 12 days during which time no toxic manifestations were noted. Autopsies were then performed on a representative number of animals. No abnormalities were evident; therefore, it would seem that ursolic acid is not injurious to these animals when fed orally in large quantity. The liver, kidneys, heart, lungs, adrenals, stomach, intestinal tract and ovaries (in the case of females) were carefully examined

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* Contribution No. 409, Massachusetts Agricultural Experiment Station, Amherst.

† Nutrition Laboratory, Massachusetts State College.

[1] Ursolic acid obtained through the courtesy of Cranberry Canners, Inc., South Hanson, Mass.

microscopically and while a histological study was not made, gross microscopic examination showed no differences from normal tissue.

Each of three adult male subjects was given ursolic acid orally in doses of 0.5 Gm. daily (20 mg. per Kg. body weight) over a 3-day period. No symptoms or discomfort of any kind were noted. We may conclude, consequently, that ursolic acid in moderate amounts is innocuous to humans.

SUMMARY

1. According to the literature (9) ursolic acid in a saturated solution of its sodium salt is toxic to fish.

2. Ursolic acid was found to be non-toxic to rats, guinea pigs, chickens, rabbits and humans.

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A Colorimetric Method for the Assay of Diethylstilbestrol*

By Earl B. Dechene, B.Sc.

Various methods have been described in the literature (1, 2, 3) for the quantitative estimation of diethylstilbestrol. These methods have the disadvantage of a narrow range of applicability, or they require apparatus not generally available in control laboratories. An attempt was therefore made to devise a colorimetric method which would be applicable to the estimation of relatively small amounts of diethylstilbestrol present in pharmaceutical preparations.

* From The Research Laboratories, Charles E. Frosst & Co., Montreal, P. Q.

It was found that the xanthoproteic reaction was suitable for this purpose and in this paper is presented a method which is simple, rapid and accurate, for the estimation of diethylstilbestrol in tablet, ampul and suppository preparations.

It should be emphasized that the method to be described is applicable only to those preparations in which diethylstilbestrol is the only phenyl derivative present.

EXPERIMENTAL

The Color Test.—Transfer an aliquot of a methanol solution of pure diethylstilbestrol to a test-tube graduated at 16 cc. Remove the methanol in a boiling water bath with the aid of vacuum. To the residue add 0.75 cc. of concentrated nitric acid (sp. gr. 1.42) and place the tube in a boiling water bath for ten minutes. Cool the tube to room temperature and add 4 cc. of 10% ammonium hydroxide. Cool again to room temperature and dilute to 16 cc. with water.

Experiments showed that the color produced by the reaction was stable over long periods of time and was unaffected by various concentrations of reagents over a wide range. The 10-minute period of heating was found to ensure maximum color development.

Using this method on amounts from 0.25 mg. to 1.75 mg. of diethylstilbestrol the yellow colors produced were found to be linear functions of the concentrations of diethylstilbestrol present when measured in a B. & L. Duboscq Colorimeter.

Attention was next turned to the effect on the color reaction of those substances commonly used as tablet excipients, *e. g.*, starch, lactose, acacia, magnesium stearate, calcium phosphate. It was found that when a methanol extract of various known amounts of diethylstilbestrol was made in the presence of a proportionate amount of a mixture of these excipients and compared with a methanol solution of 0.5 mg. diethylstilbestrol as standard, practically quantitative results were obtained.

The following procedure was therefore applied to the assay of tablets.

Weigh a number of tablets equal to 5 mg. to 10 mg. of diethylstilbestrol and determine the average weight of one tablet. Powder the tablets and take a weight of the powder equal to approximately 5 mg. of diethylstilbestrol. Macerate the powder in 10 cc. of methanol for 15 minutes with the aid of a glass rod. Filter and transfer an aliquot equal to 0.5 mg. of diethylstilbestrol to a test-tube graduated at 16 cc. Treat as described under Color Test, and compare the color to that given by 0.5 mg. of a standard prepared in the same way.

Using this method in a considerable number of assays on carefully prepared tablets an average deviation of $\pm 1\%$ was obtained with a maximum deviation of $\pm 5\%$ in only two instances.