

be changed to the desirable straw yellow color of medicinal cod-liver oil. Also the offensive odor of cod oil may be more or less removed by different deodorizing processes but if it is removed by "blowing" the oil with air any vitamin content which the cod oil originally possessed is decreased as the result of the oxidizing action of the air.

Inasmuch as cod oil and non-edible cod-liver oils sell at a much lower price than medicinal cod-liver oil and the cost of chemically refining such oils is not at all prohibitive it is a profitable business to renovate non-edible oils and sell them as medicinal oil. Since such renovated oils are on the market it behooves the consumer who really desires an oil rich in the fat-soluble vitamins to demand information concerning the vitamin content of any oil in which he may be interested.

Studies of the vitamin content of oils produced by promptly rendering strictly fresh livers as compared with the vitamin content of oils produced by the rotting process have shown that the rotted oils contained relatively little vitamin A. Thus it is apparent that if one wishes a cod-liver oil rich in the fat-soluble vitamins he cannot be guided by physical appearance or chemical analysis of a given oil, for it is not at all difficult to produce a renovated oil which will meet all the specifications set for a medicinal oil and still such an oil may be lacking or nearly so in the desired fat-soluble vitamins.

From this it is evident that while it is possible by chemically refining to so improve a cod oil, that one obtains a renovated oil which is fairly satisfactory as regards color, odor and taste, chemical refining does not enhance the original vitamin content of cod oil. To be sure of obtaining a cod-liver oil with a high vitamin potency one must insist on an oil that has been biologically tested for its vitamin content.

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A PHYTO-PHARMACOLOGICAL METHOD OF ASSAYING DIGITALIS.*

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INTRODUCTION.

During the last few years one of the authors in collaboration with various associates has been developing a study concerning the effects of drugs and poisons on plant protoplasm. In comparing the results thus obtained with the same substances on animal tissues a scientific phyto-pharmacological method of testing toxicity has been developed in contradistinction to the zoöpharmacological methods. This method has already yielded very interesting results in the study of the relative toxicity of various chemical substances on animal and plant tissues respectively.

Thus it has been shown that, while cocaine was very poisonous for animals, on the other hand certain poisons of animal origin were found to be much more poisonous to plants than to animal test objects. In connection with these phyto-pharmacological studies Macht and Lubin made a brief examination of various digitalis principles and studied their toxicity on the seedlings of *Lupinus albus*.

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It was found that all of these principles were more or less deleterious to the growth of the plant; these experiences suggested the present investigation.

It was thought worth while to study the toxicity of the galenical preparations of digitalis and more particularly the tincture upon the growth of the plants with the hope of possibly finding a simple relationship between the concentration of the digitalis preparations on the one hand and their relative toxicity to plant protoplasm on the other. This investigation is still in progress but the results already in hand are sufficiently clear-cut and interesting to warrant this preliminary report.

METHOD OF STUDY.

The method of study was the same as that employed by the senior author and his associates on other occasions. The toxicity of solutions of tincture of digitalis from 0.1% to 1% upon the growth of the single straight and well-demarcated roots of the seedlings of *Lupinus albus* was noted and this toxicity was compared with the toxicity of various digitalis preparations on animals, as determined by the cat method.

In studying the growth of the seedlings the following procedure was followed:

The dry seeds were soaked over night in tap water at ordinary temperature. On the following day the swollen seeds were planted with the hilum downward in moist, finely ground sphagnum moss. The planted seeds were placed in a thermostat and left at a constant temperature of 20° C. On the third day after planting, the roots of the seedlings are of convenient length for measurement and are ready for study. After recording the exact length of the root it is placed in an upright test-tube of hard glass containing a nutrient solution, the seed resting upon the upper edge of the tube. The solution employed was the so-called Shive Solution which contains calcium nitrate, magnesium sulphate, and mono-potassium acid phosphate. Such a solution was prepared by mixing 10.4 cc. of 0.5 molar solution of calcium nitrate, 30 cc. of 0.5 molar solution of magnesium sulphate and 36 cc. of 0.5 molar phosphate, with distilled water to make one liter. The normal growth of the *Lupinus* rootlets was studied by immersing the seedlings in a mixture of equal volumes of Shive Solution and distilled water. The digitalis solutions were added to the mixture of distilled water and Shive Solution before employing the latter as a nutrient solution. After measuring accurately the length of each rootlet and placing the seedlings in the control and drug solutions, the whole was again placed in the incubator and left at a constant temperature of 20° C., and the effect of the drug solutions was determined after twenty-four hours. Ten seedlings were placed in the control solution, on one-half Shive Solution and one-half distilled water, and ten seedlings were employed to study each concentration of the tincture. The alcoholic content of various dilutions of the digitalis tincture was kept constant. The assay of the tincture upon the cats was carried out in the following manner:

Lightly anesthetize the animal with ether and cannulate the femoral vein. The tincture was diluted to ten times its volume with normal saline solution and allowed to run into the femoral vein, 10 cc. in the first 5 minutes and subsequently 1 cc. a minute until cessation of the heart occurs and the respiration continues for a short time thereafter. The heart-beat is counted throughout the experiment and

a typical digitalis death as described above marks the end-point of the successful experiment.

RESULTS.

An examination of the data so far in hand has shown that the toxicity of various solutions of tincture of digitalis for plants, the alcoholic content remaining constant, varied directly with the concentration of the drug solutions: this is well illustrated by the following table of experiments of June 26th and 27th.

EXPERIMENTS JUNE 26-27.

| Solution tested. | Growth. | Solution tested. | Growth. |
|------------------|----------|------------------|----------|
| Shive Control | 100% | Tinct. Digitalis | 0.5% 83% |
| Tinct. Digitalis | 0.1% 96% | Tinct. Digitalis | 0.6% 81% |
| Tinct. Digitalis | 0.2% 92% | Tinct. Digitalis | 0.7% 79% |
| Tinct. Digitalis | 0.3% 89% | Tinct. Digitalis | 0.8% 74% |
| Tinct. Digitalis | 0.4% 86% | Tinct. Digitalis | 0.9% 72% |
| | | Tinct. Digitalis | 1.0% 70% |

Having found that the growth of the seedlings is inversely proportional to the concentration of the tincture in the nutrient solution, some other specimens of tincture obtained from different leaves were tested in a similar way and it was found that the toxicity of the various preparations upon the same crop of seedlings, maintaining other conditions constant, was not the same. This difference in toxicity was found to run parallel with the relative toxicity of the same preparations for cats. Thus, the relative toxicity of two different tinctures of digitalis on the *Lupinus* seedlings was in the ratio of 107.6% to 100%. The relative toxicity upon the mammalian heart as determined by the cat method was 11.28 cc. in one case and 10.48 cc. in the other, a ratio closely corresponding to that obtained by the phyto-pharmacological method.

The present investigation is still in progress. The authors propose to examine a large number of digitalis tinctures by the above method and it is thought very likely that, after a sufficient number of experiments have been performed, a practical method will be developed for assaying digitalis, without recourse to animal experiments.

ABSTRACT OF DISCUSSION.

The foregoing paper was discussed by Messrs. Viehoever, Smith, Nitardy, Munch, Pittenger, and Schneider. A question was raised concerning the effect of alcohol upon the growth of the seedlings. The author made clear, that the alcoholic content of all tests was kept constant, and thus retardation of growth was a function of the activity of the Digitalis alone.

Reference to the time required for the experiment was made by the author in response to questions. He said that the time required for two measurements of the seedlings is about one hour; the time required for the growth of the seedlings in the Digitalis solutions is about twenty-four hours. The author stated that no bacteriological difficulties were experienced, arising from growths in the nutrient solutions.

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