initial absorption can be recovered. That the substance responsible for the recovered absorption is uracil is indicated by the absorption spectrum and by the characteristic shift in absorption (*2*) upon making the solution of the recovered material alkaline to pH 11.4.

Some increase in absorption takes place if the irradiated solution is allowed to stand at pH 7.0, but the rate of increase is extremely slow at room temperature. This rate may be accelerated by increase of temperature; immersion of the irradiated solution into boiling water for 15 min can bring an increase in absorption comparable to that produced by addition of acid. Recovery in acid solution, however, has given more reproducible results.

Irradiation of uridine has led to qualitatively similar results. Uridine appears to be some 16 times as labile to ultraviolet irradiation as uracil, when compared at pH 7.

Under these conditions of irradiation, thymine, cytosine, adenine, and guanine, and adenylic and guanylic acid are not decomposed. However, preliminary experiments have given evidence of a similar reversible phenomenon with cytidylic acid.

More complete details of this work will be published elsewhere.

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## A Polarographic Determination of Digitoxin[1]

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In conjunction with a polarographic investigation of a variety of organic compounds of pharmaceutical interest, a study of the polarographic properties of digitoxin was instituted. Fieser (*1*) reported that the cardiac glycosides gave polarographic half-wave potentials in the region between −1.9 and −2.0 volts. The present investigation confirms Fieser's work and elaborates on the qualitative and quantitative polarographic properties of digitoxin. The results of this study show that digitoxin may be determined in concentrations as low as 0.1 μg in 50% alcohol solution and may also be extracted by means of suitable solvents from complex mixtures and determined in similar low concentrations.

The method used to carry out these investigations was as follows: A stock solution of the pure drug[3] was prepared by dissolving 25 mg of digitoxin in 12.5 ml of absolute alcohol and diluting to 25 ml with distilled water. Varying amounts of the stock solution were added to 0.5 ml of 0.2 *N* tetraethyl ammonium hydroxide in a Heyrovsky reaction vessel and diluted to 5 ml total volume with 50% alcohol solution in order to study the half-wave potential and height of break at different concentrations. For extremely low concentrations, a stock solution of lower concentration was prepared and diluted in a similar manner. Nitrogen was bubbled through the prepared solutions for a period of 15–20 min and the polarogram recorded. This process was repeated until a satisfactory curve of height versus concentration had been determined for the concentrations under investigation and the average half-wave potential was calculated.

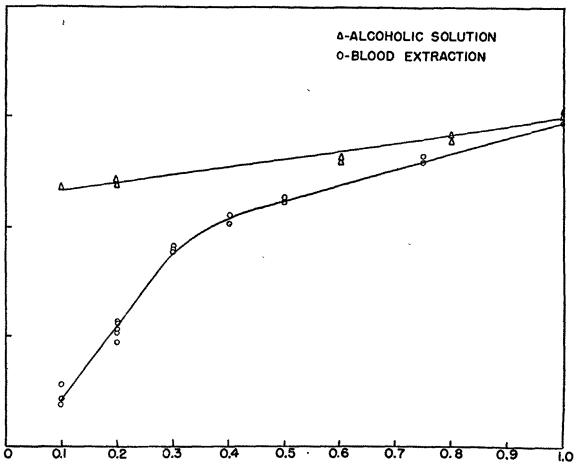


FIG. 1. Height versus concentration curves for digitoxin between 0.1 and 1.0 μg. Upper curve (△), alcoholic solution; lower curve (○), extraction from blood. Abscissas, concentration of digitoxin in μg; ordinates, height in in. (1 in. = 0.508 μa).

The study of digitoxin in blood was carried out by a combination of extraction and polarographic work. The most satisfactory extraction solvent was found to be petroleum ether. The procedure followed for the investigation in this portion of the experiments was as follows: Varying amounts of stock solution were added to 10 ml of mixed blood, and 2.5 times the total volume of petroleum ether used for extraction. The combined mixture and extraction solvent solution was placed in a separatory funnel and shaken. After thorough settling, the residual blood was drawn off. The remaining solution was shaken and allowed to settle until no blood residue appeared after shaking. The petroleum ether fraction was placed in an evaporating dish and evaporated to dryness. This residue was dissolved in 2.5 ml of absolute alcohol and decanted so that no alcohol-insoluble components would be in the final solution. The alcohol solution of the residue was diluted to 5 ml with distilled water and 2.5 ml of this solution was placed in a reaction vessel with 2.0 ml of 50% alcohol solution

[1] This investigation was supported in part by research grants from the Division of Research Grants and Fellowships of The National Institutes of Health, U. S. Public Health Service, and The American Cancer Society.

[2] The author wishes to thank Dr. C. L. Gemmill for suggesting this problem and for his guidance in this study.

[3] Grateful acknowledgment is made to Dr. K. K. Chen, Eli Lilly and Company, Indianapolis, Indiana, for the purified digitoxin used in this study.

and 0.5 ml of 0.2 *N* tetraethyl ammonium hydroxide. Nitrogen was bubbled through the solution for 15–20 min and the polarogram recorded. In this investigation, as in the study of the alcoholic solutions of digitoxin, multiple runs were made until a satisfactory height-versus-concentration curve was obtained and the average half-wave potential was calculated.

The results of these experiments show that digitoxin may be determined in concentrations as low as 0.1 μg in both alcoholic solutions and in blood. Multiple determinations run on concentrations between 0.1 and 0.4 μg of digitoxin in blood show the error in this method to be ±0.02 μg. Fig. 1 shows the height of polarographic break for various concentrations of digitoxin in alcoholic solutions and in blood extracts. This figure also shows the relationship between height of break for alcoholic and blood determinations. It may be seen that in concentrations down to approximately 0.6 μg the two curves coincide reasonably well; however, below this concentration the curve of digitoxin extracted from blood drops sharply and approaches zero. This drop from the alcoholic curve may be due to the distribution of digitoxin between the extraction solvent and blood at these low concentrations. The use of the arbitrary curve, although it varies from the curve of digitoxin in alcoholic solution, is based upon the results of multiple determinations which show a low error (± 0.02 μg) in the concentrations where the deviation is greatest. The average half-wave potentials were found to be −1.965 in alcoholic solution and −1.958 when extracted from blood.

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## Effect of Sulfadiazine on Survival of the Mammalian Embryo[1]

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It has been shown by Detwiler, Copenhaver, and Robinson (*2*) that sulfadiazine in solutions of 1% or higher is frequently toxic to *Amblystoma* embryos in early developmental stages. More recently, Copenhaver and Detwiler (*1*) showed that 2% concentration of sulfadiazine caused a failure to survive to the stage of yolk resorption. They also observed abnormalities of various structures in the organism. S. Y. P'an (*4*) has shown that sulfamethiazine when administered to normal male rats produces gross and microscopic atrophic changes in the testes, seminal vesicles, and anterior prostate. Recently, Figge *et al.* (*3*) reported on the influence of sulfonamide drugs on cancer susceptibility, and reproduction in mice. He has observed that there is a decrease in reproduction in mice maintained on constant medication with certain sulfonamides. Two of the authors (Yntema and Hammond) have found that sulfadiazine is more lethal to chick embryos during the first half of the second day of incubation than it is subsequently. The drug appeared to interfere with the development of the vascular system. These studies suggested that sulfonamide administration might interfere with early development of the mammalian embryo.

TABLE 1

| Expt. No. | Treatment* | Given on days | No. of mice | No. of deliveries (full term) | Percentage deliveries | $\chi^2$ | $P$ |
|---|---|---|---|---|---|---|---|
| 1 | Untreated | | 56 | 27 | 48.2 | 7.64 | 0.0057 |
| | Sulfadiazine† | 5–15 | 56 | 13 | 23.2 | | |
| 2 | Untreated (isocaloric) | | 49 | 16 | 32.7 | 1.6 | 0.109 |
| | Sulfadiazine | 5–15 | 47 | 10 | 21.3 | | |
| 3 | Sulfadiazine | 8–12 | 79 | 33 | 41.8 | 15.24 | 0.0001 |
| | " | 6–10 | 79 | 11 | 13.9 | 10.84 | 0.001 |
| | " | 10–14 | 79 | 29 | 36.7 | | |
| 4 | Sulfadiazine | 6 | 109 | 15 | 13.8 | 23.18‡ | |
| | " | 7 | 104 | 15 | 14.4 | 22.39‡ | |

* Administered only in the diet in Experiments 1–3. In Experiment 4, one injection of 12 mg sulfadiazine sodium was given intraperitoneally, in addition to administration of sulfadiazine in the diet for the 6th or 7th day.

† Eight-tenths percent of diet.

‡ Compared with untreated controls in Experiment 1.

To investigate this, we selected white mice as our experimental animal and used sulfadiazine as a representative of the sulfa group of drugs. A diet of Purina dog chow containing 0.8% by weight of sulfadiazine was employed. The breeding cages contained four females and one male. Females were examined each morning. Those having vaginal plugs were removed, numbered, and placed in individual cages. Only the females showing vaginal plugs were selected for the investigation.

Experiment 1 (Table 1) represents the results obtained when such animals, beginning on the 5th day following conception, were fed sulfadiazine in the diet for a period of 10 days. The percentage of deliveries in this treated group was 23.2%, in contrast with 48.2% in the untreated control group. When untreated animals were fed isocalorically there was a reduction of deliveries, as indicated by the results of Experiment 2. However, this reduction did not reach the level noted in the sulfadiazine-treated animals.

It was of interest to localize more accurately the time at which the sulfonamide was effective. Experiment 3 indicates the results. Here it was shown that the period from the 6th to the 8th day was the one at which the sulfonamide was effective.

To localize further the most effective interval, Experiment 4 was conducted, in which animals were given sulfonamide for 1 day only. Here sulfadiazine sodium was

[1] This investigation was supported by a research grant from the Hendricks Fund at Syracuse University.