placed in a chloroform-washed 10 x 50 mm Whatman extraction thimble caught and held in place in the sugar tube constriction. About 3.5 ml of chloroform were added and the cholesterol was extracted by refluxing for 30 min. After cooling, chloroform was added to the 4 ml mark, 2 ml of a fresh mixture of 1 part of conc. sulfuric acid and 20 parts of pure, dry acetic anhydride were added, the contents mixed well and the color read in the photoelectric colorimeter at 575 mμ after standing in the dark for exactly 10 min. The cholesterol content was translated from a standard curve. The results of the various determinations are summarized in Table I.

Results and discussion. Histamine shock, passive and active anaphylaxis: Neither ACTH nor cortisone had any effect on the fatal outcome of the reaction, regardless of dosage, number of injections and total dose, time relationship of the challenge dose to the pretreatment, etc. The details are published elsewhere(1).

Chemical and cellular studies: The figure listed as normal for most of the values was the average of a number of determinations taken from apparently healthy guinea pigs. Some values were also obtained from guinea pigs shocked passively and in histamine shock, neither group having had any previous ACTH or cortisone therapy. The statistical evaluation of a significant difference for the results obtained indicates that the difference between the normal and the experimental means varies within at least a 98% confidence limit.

Summary. Normal values for the following constituents of the blood of guinea pigs are presented: blood cell count, serum proteins, cholesterol, chloride, uric acid, creatine, creatinine and glutathione. Physiological alterations were produced by the administration of the adrenocorticotrophic hormone and cortisone as evidenced by an increase in the uric acid/creatinine and albumin/globulin ratios, a decrease in the relative mononuclear and an increase in the polymorphonuclear leucocyte counts.

The author gratefully acknowledges the technical assistance of Miss Betty J. Hargis and Miss Margaret D. Werle.

Received April 26, 1951. P.S.E.B.M., 1951, v77.

Rate of the Enzymatic Breakdown of Digitoxin.* (18771)

JAMES G. HILTON.† (Introduced by C. L. Gemmill.)

From the Department of Pharmacology, University of Virginia Medical School, Charlottesville, Va

The breakdown of digitoxin by strong acids to form digitoxigenin and digitose has been described by Stoll(1) and others. A similar effect by heart and skeletal muscle was described by Fischer(2). However, in neither of these cases was any attempt made to study the reaction rates of these changes. The development of a polarographic method for the analysis of digitoxin (Hilton)(3), in very small quantities has made it possible to study the breakdown of digitoxin and to determine the reaction rate constants for these reactions in dilute acid and in extracts of heart, liver, kidney, and yeast. In the case of the heart extract attempts were made to purify the essential enzymatic component which was responsible for the reaction.

Methods. The breakdown of digitoxin by 0.85% hydrochloric acid was carried out as follows: 9.0 ml of 0.85% hydrochloric acid were placed in each of six 50 ml Erlenmeyer flasks which were shaken in a constant tem-

* This work was aided in part by a grant from Eli Lilly Co., Indianapolis, Ind.

† Present address: Department of Pharmacology, University of Tennessee, Medical School, Memphis, Tenn.

1. Stoll, A., *The Cardiac Glycosides,* The Pharmaceutical Press, London, 1937.

2. Fischer, H., *Arch. f. exp. Path. u. Pharmakol.*, 1928, v130, 111.

3. Hilton, J. G., *J. Pharm. and Exp. Therap.*, 1950, v100, 258.

perature bath at 29.7 ± 0.05°C for 30 minutes. After this period of time, 0.100 mg of digitoxin in 50% ethyl alcohol was introduced into each flask. Periodically after the addition of digitoxin a vessel was removed, and the contents were extracted with 25 to 35 ml of petroleum ether. The extract was evaporated to dryness and the residue was analyzed polarographically for its digitoxin content. The breakdown of digitoxin by the extracts of various organs and yeast was carried out in the same manner as the hydrochloric acid reaction. In these studies the extracts used were prepared by mixing 10 g of dried, defatted organs‡ with 100 ml of phosphate buffer (0.20 molar, pH 7.31) and allowed to stand for 1½ hours. After this time, the mixture was filtered, diluted to 100 ml in a volumetric flask and allowed to stand at 5°C for 2 to 3 hours before using. The organs used in this study were: Heart, kidney, liver, and baker's yeast (Fleischman, dried for 24 hours at room temperature).

Results. Of the 5 substances studied, only acid, heart, and liver showed digitoxin splitting activity. A plot of the digitoxin remaining at given time intervals after the addition of the drug may be seen in Fig. 1. This figure is for acid only, however, the plots of digitoxin splitting activity for heart and liver extracts are very similar. Zero time extractions and controlled samples run on 0.85% saline and phosphate buffer show the complete extractability of the digitoxin added. In saline and phosphate buffer, no breakdown was observed in time intervals up to 240 minutes.

The shape of the time-concentration plot, and the fact that a log concentration-time plot is a straight line indicates that this reaction may be a first order reaction. Reaction rate constants at 29.7°C calculated by the equation $K = \frac{2.303}{t} \log \frac{c_0}{c_t}$ were as follows:

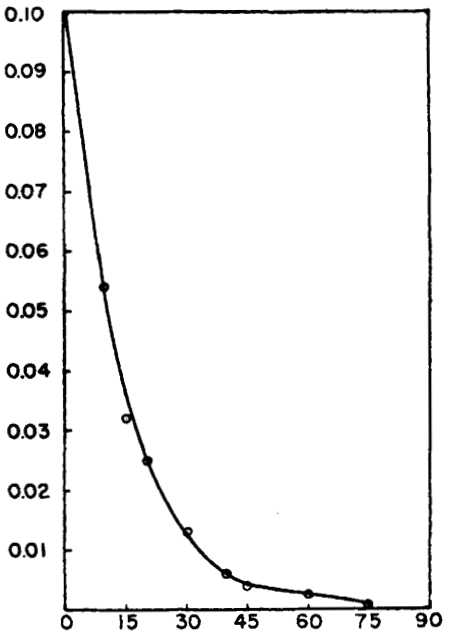

FIG. 1. Breakdown of digitoxin by .85% hydrochloric acid. Ordinates, digitoxin remaining in mg; abscissae, time in min.

0.0765 g hydrochloric acid 6.85×10^{-2}, standard deviation§ 0.48×10^{-2}; active principle in 0.9 g dried, defatted heart 5.15×10^{-2}, standard deviation 0.14×10^{-2}; active principle 0.9 g dried, defatted liver 5.80×10^{-2}, standard deviation 0.40×10^{-2}.

A purification of heart extract was attempted by dialysis of the filtered extract against cold running water for 24 hours. This produced a grayish brown precipitate, soluble in phosphate buffer and having approximately 5 times the activity of the residual solution. Further dialysis of the phosphate buffer failed to reprecipitate the active component, but addition of ethyl alcohol in the cold (5°C) to the extent of 50% by volume produced a reddish brown solid which showed a high digitoxin splitting activity. This solid was soluble in phosphate buffer (0.20 Molar pH 7.31) and showed typical protein reactions.

Discussion. The breakdown of digitoxin to digitoxigenin in acid medium has been shown to be a rapid reaction which may be of the first order. The breakdown of digitoxin by

‡ The dried defatted organs used in this study were obtained from VioBin Corp., Monticello, Ill. These tissues were dried and defatted at 37° C by azeotrophic process. The medium for both drying and defatting was ethylene dichloride.

§ Standard deviation of individual determinations from run average.

heart and liver extracts is similar in type and rapidity. The activity of these tissues appears to be due to some substance present in the tissues and not due to a pH effect. The presence of this substance in heart and liver tissues which splits digitoxin, and thus changes the solubility characteristics, may in some manner explain the action of digitoxin and its mode of excretion.

Summary. A study of the breakdown of digitoxin to form digitoxigenin by hydrochloric acid and extracts of dried heart, liver, kidney, and yeast has been carried out. The results of this study showed the hydrochloric acid and extracts of heart and liver have the ability to cause this breakdown, while extracts of kidney and yeast are inactive in this reaction. Reaction rate studies gave the following rate constants at 29.7°C: 0.85% hydrochloric acid 6.85×10^{-2}, heart extract (10 g dried, defatted powder per 100 ml phosphate buffer) 5.15×10^{-2}, liver extract 5.80×10^{-2}. Purification of the heart extract yielded a reddish brown solid which had a high enzymatic activity and gave typical protein reactions.

The author wishes to express his thanks to Dr. C. L. Gemmill for his advice and guidance in carrying out this work and to Dr. K. K. Chen, of Eli Lilly Co., and Dr. F. E. Kelsey, of the Department of Pharmacology, University of Chicago, who kindly supplied the drugs used in this study.

Received November 28, 1950. P.S.E.B.M., 1951, v77.

Effect of X-Radiation upon Succinoxidase of Rat Kidney. (18772)

M. LEMAY.* (Introduced by H. M. Patt.)

From the Division of Medicine, Argonne National Laboratory, Chicago

Hammett(1), irradiating a solution of glutathione with radium, found a decrease in sulfhydryl groups and an increase in disulfide groups and suggested that radium irradiation in "non-tissue destroying amounts" had its main effect on cell growth by decreasing the concentration of sulfhydryl. Barron and coworkers(2-4) reported both *in vitro* and *in vivo* experiments showing inhibition of –SH enzymes following small amounts of X-irradiation. In the latter experiments, inhibition was relatively marked in kidney slices from X-irradiated animals with succinate as a substrate, presumably as a result of inhibition of the –SH enzyme, succinic dehydrogenase.

Since succinic dehydrogenase is a widely distributed enzyme whose activity as affected by irradiation could be easily measured, further study of the effect of irradiation on the succinoxidase system seemed indicated.

Methods. Litter-mate Sprague-Dawley rats weighing 150 to 200 g were divided into control and irradiated groups. The irradiated animals received 400 r to 800 r total-body X-radiation (200 kv, 15 ma, and 1.5 mm Cu and 3 mm Bakelite filters). Irradiation was given at approximately 17 r per min, and the animals were killed by a blow on the head within 2 hr following irradiation. Five additional animals were given 800 r (250 kv) at the faster rate of 180 r per min and killed within 7 min after irradiation. The kidneys were removed immediately and 5% homogenates were made in a glass homogenizer with cold distilled water. Kidney homogenates were assayed for succinoxidase, succinic dehydrogenase, and cytochrome oxidase activity by the method of Schneider and Potter(5) as applied by Case and Dickens(6). Kidney

* Present address: Dept. Radiotherapeutics, Univ. of Cambridge, Cambridge, England.

1. Hammett, F., *Protoplasma,* 1932, p15, 422.

2. Barron, E. S. G., Dickman, S., Muntz, J. A., and Singer, T. S., *J. Gen. Physiol.,* 1949, v32, 537.

3. Barron, E. S. G., and Dickman, S., *J. Gen. Physiol.,* 1949, v32, 595.

4. Barron, E. S. G., 1946, Effects of X-rays on tissue metabolism, CH-3654.

5. Schneider, W. E., and Potter, V. R., *J. Biol. Chem.,* 1943, v149, 217.

6. Case, E. M., and Dickens, F., *Biochem. J.,* 1949, v43, 481.