added are given in Table I. Except for the sodium salts, which are considered separately, the sulfonamides are listed in order of increasing toxicity. Sulfadiazine was the most satisfactory of the group. Cultures grew well at all concentrations tested, even up to five times saturation in plasma. Planimeter measurements suggested that the drug had a stimulating action at higher concentrations. Sulfadiazine was the least soluble of the series (Table I) and its pH in plasma was somewhat below the optimum. Next in order came succinyl sulfathiazole and sulfapyrazine, the growths equaling or somewhat exceeding those of the controls at all concentrations tested. Then came sulfapyridine and sulfaguanidine: cultures containing these drugs grew well up to saturation and somewhat beyond; only at about double the saturation point was the growth less than in the controls. Sulfathiazole, next on the list, when at half saturation had no inhibiting influence on growth, but at saturation and beyond there was early and severe degeneration of nerve cells. Succinyl and nicotinyl sulfanilamide and sulfanilamide itself fall into much the same class inasmuch as they are toxic at approximately half saturation. Of these, succinyl sulfanilamide has a selective effect at concentrations in excess of saturation, producing dissolution of the initially migrating nerve cells and severe vacuolization of neuroglial and wandering cells. Growth of cultures to which sulfanilamide were added was satisfactory up to half saturation; above saturation there was no growth whatever.

The sodium salts, because of their high solubility, were tested over a limited range in the lower concentrations. Judging these compounds at like levels of percentage concentration of saturation, they fall into the following order of increasing toxicity: sodium sulfadiazine, sodium sulfathiazole and sodium sulfapyridine. Cultures containing sodium sulfadiazine and sodium sulfathiazole, respectively, grew as well as the controls up to concentration of approximated 2.0 mg/cc.

Conclusions. Brain of 8-day-old chick embryos cultivated *in vitro* in plasma to which sulfonamides in various concentrations were added, responded differently in accordance with the sulfonamide tested. Cultures containing sulfadiazine and succinyl sulfathiazole grew better than the controls in all concentrations tested. The growths in sulfapyrazine and sulfapyridine equalled those of the controls. The other sulfonamides showed varying degrees of toxicity. Preliminary experiments suggested strongly that the toxicity is to be ascribed to inherent properties of the drugs, not their pH.

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15070 P

Effect of Digitalization on the Coagulation Time in Man.

L. SOKOLOFF AND M. I. FERRER. (Introduced by A. C. DeGraff.)

From the Department of Therapeutics, New York University, College of Medicine.

Within recent years a number of communications concerning the influence of digitalis on the coagulability of the blood has appeared. Tanaka[1] found that parenterally administered strophanthin shortens the blood coagulation time of dogs and Werch[2] reported that digifolin exerts a similar effect in rabbits. Macht[3] observed that several of the glycosides shorten the coagulation time of cat plasma to the

1 Tanaka, H., *Okayama-Igakkai-Zasshi*, 1928, **40**, 1826.

2 Werch, S. C., *Quart. Bull. Northwestern U. M. Sch.*, 1943, **17**, 50.

3 Macht, D. I., *Ann. Int. Med.*, 1943, **18**, 772.

order of 1 10 to 1 /5 of its initial value *in vivo* and also act *in vitro*. Recently Massie *et al.*[4] stated that digitalis leaf in clinical doses shortens the coagulation time of the whole blood of man. Digitalis has also been reported to make heparin less effective in prolonging the blood coagulation time of man and dogs.[5] As a result, several clinical notes have appeared suggesting that digitalis therapy may favor the development of thrombotic disease.

Selection of Material. Ten patients exhibiting some degree of heart failure were studied. Because an observation period of several days was required before specific therapy was initiated, these patients were necessarily not too seriously ill. Because admixture of tissue juices exerts a thromboplastic influence on the coagulation process, patients who did not have a sufficient number of easily penetrable veins were not accepted as subjects. Other medications which have been considered to influence coagulability of the blood were withheld, including salicylates, aminophylline and mercurial diuretics. In one instance (S.W.) a single dose of mercupurin was administered after initiation of digitalis therapy. Since mercurials have been considered to increase coagulability of the blood[6] and since no shortening of the coagulation time occurred, we feel justified in including this case. The coagulation time was determined for at least 4 days before onset of digitalization and for a comparable period during initiation of this therapy. The rapid method of Eggleston was followed. In 9 cases the U.S.P. XII leaf preparation and in one case the purified glycoside digitoxin was used. Six patients were digitalized to the point of minor toxicity, 5 receiving the whole leaf, and one receiving digitoxin. Of the remaining 4 patients, all received digitalis leaf. Two received 15 and 22 U.S.P. XII digitalis units, respectively, in 5 days. The third patient received 21 units in 4 days, the fourth 13 units in 6 days. All 10 patients can be considered adequately digitalized since a full therapeutic effect was obtained in each instance and the average initial digitalizing dose with this preparation is 12 digitalis units.

Method. The method was essentially that of Lee and White. Particular attention was paid to performing the venepuncture expeditiously; unless this was accomplished directly, the specimen was rejected. An 18-gauge needle was used and the tourniquet removed as soon as the antecubital vein was entered. Three test tubes, 14 mm x 100 mm, were jacketed in thermos-jug water baths between 35° and 40°C. All tubes were cleaned daily with chromic acid cleaning solution. Just before use, they were rinsed with sterile, physiological saline solution, as were the needles and syringes. Three cc of blood were withdrawn into a 5 cc syringe and 1 cc transferred gently to each of the test tubes. The samples were allowed to stand for 4 minutes. The first tube was then tilted every half minute until the blood failed to flow down the side. The second and finally the third were similarly tilted. The time at which the blood ceased to flow in the third was considered the coagulation time.

Results. A considerable variation in the coagulation time was observed from day to day in several of the patients. This reflects in part the intrinsic coagulability of the blood, and in part the essentially crude procedure through which many extrinsic variables operate, particularly the agitation against the foreign surface of the container. Forty-seven determinations were made before and 42 following onset of digitalization. In 4 cases there was some shortening of the average coagulation time following initiation of the therapy; in 5 cases there was a slight prolongation; and in one case, no change at all. The average time for all determinations before digitalization was 9.45 minutes (S.D. = $\pm$1.55 for 46 degrees of freedom) and 9.50 minutes (S.D. = $\pm$1.41 for 41 degrees of freedom) during digitalization. The population standard deviation, which indicates the reproducibility of the method is $\pm$1.18 minutes with 69 degrees of freedom. An analysis of variance was computed: that due to digi-

[4] Massie, E., Stillerman, H. S., Wright, C-S, and Minnich, V., *Arch. Int. Med.*, 1944, **74**, 172.

[5] de Takats, G., Trump, R. A., and Gilbert, N. C., *J. A. M. A.*, 1944, **125**, 840.

[6] a. Wiebel, H., *Klin. Wchnschr.*, 1940, **19**, 1013; b. Link, K. P., *Harvey Lecture*, 1943-44, **39**, 162.

talis was 0.060; that between patients was 8.762; that reflecting the interaction (digitalis × patients) was 2.089; the error within patients was 1.386. It is evident that the clotting times of individual patients differed significantly among themselves regardless of therapy. However, the variance due to treatment with digitalis is considerably less than that due to experimental error. Consequently, these experiments fail to support the hypothesis that oral digitalization increases the coagulability of the blood as measured by this technic.

Summary. Whole blood coagulation times were determined before and during oral digitalis therapy of 10 patients. No significant alteration of coagulability as so measured was observed.

15071

The Anti-Histamine Effect of Imidazol.*

HAROLD C. MORRIS AND CARL A. DRAGSTEDT.

From the Department of Pharmacology, Northwestern University Medical School, Chicago, Ill.

From a study of several compounds (histidine, arginine, cysteine, etc.) which inhibit the action of histamine upon smooth muscle structures, Ackermann and Wasmuth[1] postulated that histamine owes its pharmacological activity to its =NH imine group, and to its NH_2 group, the former acting as an anchoring group with the chemical receptors of the smooth muscle, and the latter as the stimulating agent. The inhibiting compounds, having imine but no amine radicals, bind the chemical receptors, have no pharmacological effects themselves, but block the effects of histamine. Rocha e Silva[2] confirmed such a view by showing that various histamine compounds in which the amine radicals were masked, were inactive themselves but acted as blocking agents against histamine.

On the basis of this theory one could expect that imidazol (Glyoxalin) would possess antihistamine properties because it presents a free imine radical.

Imidazol was therefore tested as to its counteracting effect against the stimulating action of histamine upon the surviving guinea pig intestinal strip and its vaso-depressor action in anesthetized cats. Following Rocha e Silva's technic, histamine was added to the bath in a series of concentrations to determine the dose which produced a marked but submaximal contraction. Imidazol (Eastman), neutralized, was then added to the bath in increasing amounts, noting the effect of each increment upon the response to the test dose of histamine. In a series of experiments it was found that imidazol would block the effects of histamine when present in a ratio of 1500 to 1. That the imidazol did not act by damaging the intestinal strip was indicated by the fact that there was prompt recovery of histamine responsiveness on washing. In the cat, the intravenous injection of imidazol (1 to 128 mg per kilo) diminished, but did not obliterate the depressor effect of histamine. Doses of 1 mg per kilo reduced the effect of histamine about 25%, and doses of 128 mg per kilo about 60%. The latter dose approximates the maximally tolerated dose.

Summary. Strong concentrations of imidazol can block the effect of histamine upon the guinea pig intestine, and large doses can decrease the vasodepressor effect of histamine in the cat, but the amounts required are so great as to offer no promise of clinical or laboratory usefulness.

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[1] Ackermann, D., and Wasmuth, W., *Z. f. Physiol. Chem.*, 1939, **258**, 28.

[2] Rocha e Silva, M., *J. Pharmacol. and Exp. Therap.*, 1944, **80**, 399.