TOXICOLOGICAL STUDIES ON CONGO RED.*

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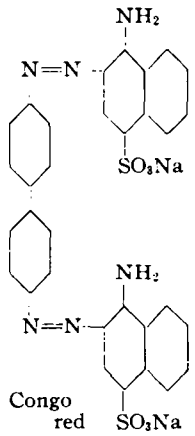
INTRODUCTION.

Congo red, long known as a laboratory reagent, has been used as an indicator for free hydrochloric acid, and as a test for amyloid and for the estimation of the functional state of the reticulo-endothelial system (1, 2). In recent years, however, its use has been extended to therapeutic procedures, and it has been employed empirically in the treatment of pernicious anemia (3), as a hæmostatic agent in cases of pulmonary tuberculosis (4, 5), uterine bleeding (6) and purpura hemorrhagica (7). Although, according to older authorities (8), the dye possesses no bactericidal action, congo red has more recently been advocated in the treatment of streptosepticemia, and in some quarters it is evidently regarded as a chemotherapeutic agent. Thus, for instance, Green (9) advocates injection of 20 cc. of a one per cent solution of congo red on each of three to seven successive days in the treatment of *Streptococcus viridans* septicemia. In connection with such intravenous employment of congo red solutions, further information concerning the potency or toxicity of various samples of the dye obtainable on the market was held by the writers to be very desirable. The present investigation was begun with this object in view and was further stimulated by a few clinical reports of severe collapse and even of sudden death following intravenous injection of congo red administered by physicians who purchased preparations of the dye on the open market. In one such fatal case death was reported to be due to cerebral embolism.



Congo red

EXPERIMENTS ON BLOOD COAGULATION.

Taliaferro and Haag (10) in an excellent monograph on the toxicity and effect of congo red on blood coagulation have reported that injection of small doses of a one per cent solution of the dye (1 to 5 cc. per kilo weight) diminished the coagulation time of rabbits' blood, as determined by a modified capillary glass tube method. When the dye was injected in larger doses, however, they found that the coagulation time of rabbits' blood was markedly delayed. The same authors found further that small doses have no effect on coagulation time of dogs' blood for a period of three hours after injection of the dye. On the other hand, these writers reported that doses of 10 to 15 cc. of a one per cent solution of congo red, administered to ten patients, definitely decreased the blood coagulation time of the majority. In the same paper, Taliaferro and Haag stated that experiments *in vitro* revealed that small amounts of congo red had no effect at all on coagulation time of rabbits' blood.

The present writers have made studies on coagulation time of cats' blood *in vitro* with results quite at variance with those obtained by the previous authors in experiments *in vitro* with rabbits' blood. Tests were made with oxalated blood plasma and blood serum of cats by the well-known Howell-McLean method (11, 12), a procedure which admits of accurate and simultaneous determination of coagulation time of a large number of samples. To a given quantity of the plasma, usually 0.5 cc., is added a little blood serum, usually 0.1 cc., and the onset

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of jellying or clotting is conveniently observed by gently tilting the small glass containers. When the mixture ceases to flow and the tubes can be completely inverted without spilling, the clotting-point has been reached.

Employing the method described above, the authors made numerous experiments in which mixtures of plasma and serum in different proportions were compared with mixtures of plasma with sera in which there had been dissolved various quantities of congo red. Controls were made with physiological saline. Thus it was found that when very dilute solutions of congo red were employed in this manner coagulation time was greatly *shortened*, while that of serum containing greater concentrations of congo red was *prolonged*, and in extreme cases clotting was prevented altogether. The subjoined table summarizes the results obtained in many such experiments. These findings corroborate and extend the conclusions reached by Taliaferro and Haag and emphasize the importance of the concentration of the dye in the blood in relation to coagulation phenomena. Dilute solutions of congo red hasten coagulation time while stronger concentrations delay it. Inasmuch as coagulation time may vary not only with the dose of a thromboplastic drug but also with different species of animals and different individuals of the same species, the question of a safe therapeutic dose must be seriously considered in the case of each patient.

TABLE I. EXPERIMENTS WITH CONGO RED ON COAGULATION OF CATS' BLOOD.

Substance Tested.	Coagulation Time.
0.5 cc. plasma + 0.1 cc. serum with saline (control)	15 minutes
0.5 cc. plasma + 0.1 cc. serum with congo red, 1:500	remained fluid over night
0.5 cc. plasma + 0.1 cc. serum with congo red, 1:5,000	9 minutes
0.5 cc. plasma + 0.1 cc. serum with congo red, 1:50,000	6 minutes

TOXICITY FOR CATS.

To ascertain whether or not different lots of congo red have approximately the same toxicity, the writers procured a number of samples of the dye from the open market and compared them with other samples taken from our own stock, and also with a congo red which had been repurified several times in these laboratories. The pharmacological method of testing toxicity may be described as follows: A one per cent solution of the dye was prepared in normal physiological sodium chloride. This was injected at regular intervals, 1 to 5 cc. at a time, into the femoral vein of cats under light ether anesthesia. Simultaneously a continuous record of the respiration and blood pressure was made on a kymograph. Table II shows the figures obtained with five different lots of the dye. Thus in Lot A the average lethal dose was 320 mg., a figure agreeing with that obtained by Taliaferro and Haag in experiments on rabbits. This particular preparation was a quantity of congo red which had been repurified chemically several times in these laboratories according to the method suggested by Kolthoff (13). Chemical analysis showed that its purity was approximately 95 per cent. The hydrogen-ion concentration of this solution was 7.1. The other samples of congo red which were tested gave readings varying all the way from 102 to 270 mg. per kilo weight of cats. The writers found that the toxicity of a given sample of the dye was but little affected by variations in the hydrogen-ion concentration, *provided* it was on the alkaline side or close to the neutral point. The most interesting finding, however, was the great difference in toxicity of the five different samples of congo red which were repeatedly examined.

TABLE II. TOXICITY OF CONGO RED FOR CATS ON INTRAVENOUS INJECTION.

Lot.	Number of Cats Used.	p_H	Average Lethal Dose Per Kilo.	Remarks.
A	6	7.1	320 mg.	Repurified several times
B	6	6.9	225 mg.	Not repurified
C	4	6.8	102 mg.	Impure sample
D	4	10.5	270 mg.	Large quantities of sodium chloride and 3% of sodium sulfate
E	4	9.5	120 mg.	Large quantities of sodium chloride and traces of sodium sulfate

In a number of the cats used for assay (not listed in Table II) death occurred suddenly after injection of but small quantities of congo red, *i. e.*, much less than the usual lethal dosage. Such

animals exhibited a phenomenon not unlike that encountered in blood pressure experiments with cats when a clot is formed in the cannulated artery. Post mortem examination in these cases, however, revealed no clot in the cannula itself and the reason for sudden death could not be definitely learned. Nevertheless such findings did not oppose the assumption that intravascular clotting had occurred in the cerebral circulation because the sudden arrest of respiration followed by fall in blood pressure was exactly like that noted in animals with embolism produced by intravenous injection of either oil or solid particles. Unfortunately the writers were not in a position to confirm this hypothesis by actual histological examination.

Some support was lent to this hypothesis by the findings made by the writers in other experiments in which the animals were injected with heparin (H. W. & D.) and the lethal dosage of congo red was determined by the method outlined above. In such tests it was generally found that the toxicity of the dye was definitely decreased when heparin had been previously administered. Figures 1 and 2 illustrate the effect produced by injection of a one per cent solution of the same sample of congo red in two cats, one of which received the saline solution of the dye alone while the other was previously given an effective dose of heparin.

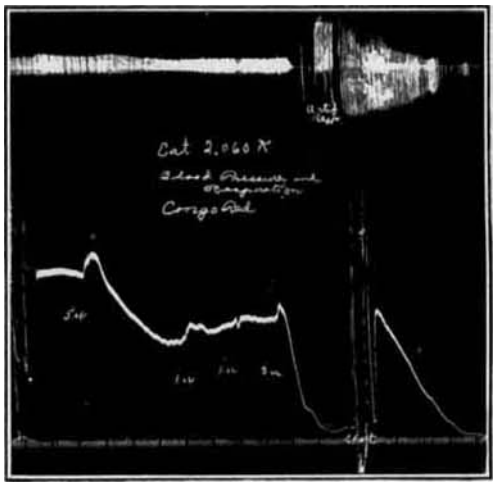

Fig. 1.—Sudden death after injection of 12 cc. of a solution of congo red, 1 per cent, with a picture of intravascular clotting.

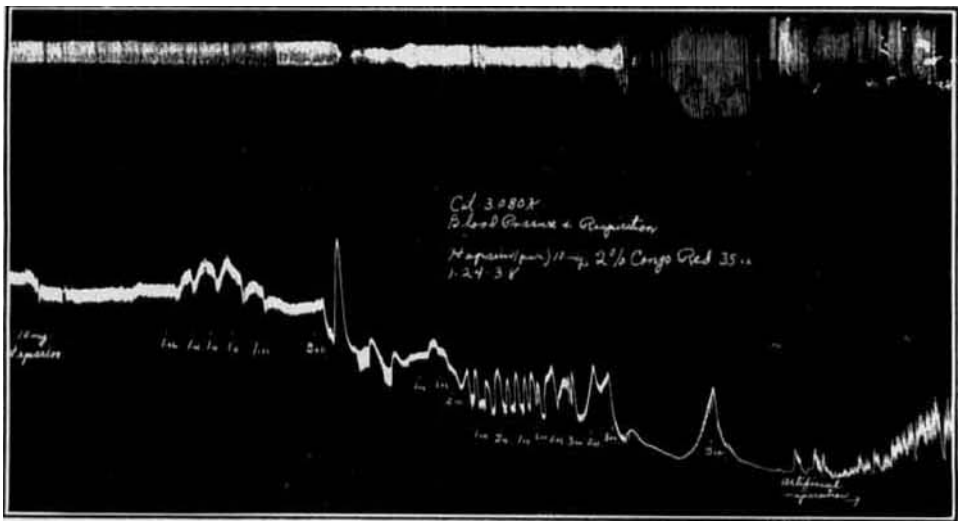

Fig. 2.—Injection of 12 cc. of a solution of the same lot of congo red, 2 per cent, after administration of 10 mg. of heparin.

TOXICITY FOR MICE.

The great difference in toxicity of various samples of congo red observed in over forty experiments on cats intravenously injected with respective solutions of the dye was also exhibited in the series of tests on white mice, injected intraperitoneally or subcutaneously or in the tail

vein. It was found that the lethal intraperitoneal dose for mice averaging 24 Gm. in weight varied with the different samples. Lethal doses per mouse ranged from 5 to 10 mg., depending on the lot of dye employed, but were virtually uniform for any given lot. In other series of experiments, small doses (1 to 3 mg. per mouse) of congo red were given intraperitoneally daily and it was found that with repeated injection even of small doses, chronic poisoning occurred, ending in death after several days. In such tests, heparin previously injected exerted no antidotal action.

DISCUSSION.

The present investigation yielded two principal findings. *First*, a striking difference in toxicity of the various lots of congo red for both mice and cats was noted. This toxicity is due partly to the intrinsic property of the congo red molecule itself, as the results obtained with repurified dye indicate, but it is greatly increased by the presence of impurities. Although some of the samples, such as Lot E, contained huge quantities of sodium chloride and sodium sulfate, the authors did not regard the presence of these salts *per se* as explanatory of the marked toxicity of the respective solutions.

Secondly, the finding made by Taliaferro and Haag in their studies on the effect of congo red on the coagulation of blood has been extended. The writers have found in experiments *in vitro* that congo red in great dilution hastens coagulation of cats' blood whereas the stronger concentrations of the dye may not only retard coagulation but even keep the blood permanently fluid. Whether these findings cast any light on the severe reactions occasionally encountered in patients injected with congo red cannot be positively asserted. Yet on the basis of the data already in hand, a word of caution with regard to the intravenous use of congo red may not be out of place. In the first place, in the case of such a complicated chemical as a dye, it is desirable to employ a preparation as nearly pure chemically as possible. In the second place, before administering the dye, the wise physician will recall the thromboplastic effects of congo red noted in animal experiments both *in vivo* and *in vitro*. The safest concentration to use must be determined by the physician in each individual case. The possibility of combining an anticoagulant such as heparin should also be considered in certain instances.

SUMMARY.

1. The effect of congo red on coagulation of cats' blood depends on the concentration of the dye employed, small doses of the drug hastening coagulation and large doses delaying it, according to experiments made *in vitro*.

2. The toxicity of various samples of congo red, obtained on the market, varies widely for both mice and cats.

3. In connection with clinical injections of congo red, only the purest brands of the dye should be employed and the possibility of a thromboplastic effect should be borne in mind in the case of each patient.

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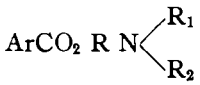
A SERIES OF CONTRIBUTIONS TO THE QUESTION OF THE RELATION BETWEEN CHEMICAL CONSTITUTION AND LOCAL ANESTHETIC ACTIVITY.

III. SUBSTITUTED CINNAMIC ACID ESTERS OF DIALKYLAMINO ALCOHOLS.

BY W. A. LOTT AND W. G. CHRISTIANSEN.

The first paper in this series dealt with the mechanism by which local anesthetics act and with hypotheses developed for use in orienting our efforts to produce new local anesthetics superior in one respect or another to existing ones. Particular emphasis was placed on the question of dual emulsions—systems which have water-in-oil and oil-in-water emulsions in equilibrium with each other and which can be altered or displaced in one direction or another by substances entering the system and having oil solubility as well as water solubility. Such substances would distribute themselves in accordance with their solubility coefficients and in so doing would alter the complex emulsion system. It is reasonable to expect that structural changes sufficient to significantly alter the oil solubility of a compound would modify the properties of the compound in so far as it is involved in complex emulsion systems.

Local anesthetics of the type studied in these researches may be represented by a general formula such as the following:



in which Ar represents an aromatic nucleus, R represents a polymethylene or substituted polymethylene group and R_1 and R_2 represent alkyl or substituted alkyl groups. These compounds may be described as esters obtained from aromatic acids and substituted amino alcohols. By appropriately altering the character of the acid or the alcohol one can change the oil-water solubility relationships.

The second paper was a report on anesthetics derived from alkoxy-benzoic acids. The character of the alkoxy group and other substituents in the benzene ring as well as the structure of the alkylaminoalkyl group were varied so as to produce substances having different distribution coefficients between oily and aqueous liquids and permit selection of the optimum combination of the acidic and alcoholic components.

It has long been known that the aromatic acid used for the preparation of a local anesthetic may be a cinnamic acid instead of a benzoic acid indicating that