Acridine orange as a pseudo-Schiff reagent[1] was first tried by KASTEN[2] and he obtained negative results. He therefore concluded that dyes without any primary amino group cannot be suitable for Feulgen type of staining. A more recent study with AO by KASTEN[3] indicated that different batches of this dye, manufactured by different firms, contain contaminants with primary amino group as judged by thin layer chromatography. Following this technique, the present author noted 4 distinct fluorescent zones in the chromatograms of the American product for which the solvent used consisted of *n*-butyl alcohol, ammonium hydroxide, ethyl alcohol and water in the proportion of 16:0.15:5:5. To obtain a dye that would show a single spot in the chromatogram, the dye solution was thoroughly washed with chloroform or amyl alcohol or *n*-butyl alcohol. The spectrophotometric analyses of the dye thus purified, as well as the unpurified dye solution (Figure 1), revealed 2 peaks in the UV-regions, one at 230 nm and the other at 270 nm and a 3rd peak in the visible region, viz. at 490 nm. It is important to mention here that in the visible region the curves for the unpurified dye and the butanol extracted dye differ from those of chloroform and amyl alcohol extracted dyes in not showing a secondary shoulder. Similar analyses of the fraction of the dye solution that went into solution in chloroform or amyl alcohol or *n*-butyl alcohol also revealed some difference (Figure 2). Results of staining with the unpurified dye, as well as the purified dye, indicated that perfect staining of DNA is possible with both. These results, therefore, do not corroborate KASTEN's[4] contention that dyes without any primary NH_2 group are not suitable for staining DNA aldehyde. Carbylamine reaction, to test the presence of primary NH_2 group as impurities in AO, also indicated that the samples of AO used in this investigation did not contain any contaminant with primary amino group, as judged by the absence of an offensive odour of carbylamine. It is pertinent to mention here that the present author has also obtained successful staining of acid hydrolyzed DNA with basic dyes, thionine blue and methylene blue[5] and rhodamine B[6], all of which are without any primary NH_2 group in their molecules.

Résumé. Après hydrolyse par l'acide hydrochlorique et teinture subséquente par une solution aqueuse d'acridine orange suivie de déshydratation régulière par l'alcool éthylique, des coupes de tissu, examinées au microscope fluorescent montrent des noyaux brillants fluorescents rouge-orange. On considère que cette teinte produite par la solution aqueuse d'un colorant qui ne contient aucun groupe aminé primaire dans sa molécule, est due à la réaction Feulgen modifiée.

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Standardized Method for the Production of Experimental Atrial Fibrillation

Several methods have been proposed for the experimental study of auricular fibrillation. Most of them are based on the original observations of BURN et al.[1]. These authors demonstrated in the dog's heart-lung preparation that during the infusion of acetylcholine, electrical stimulation of the right auricle caused the onset of atrial fibrillation, which continued as long as acetylcholine was infused. This method was applied with some modification by others. SCHERF and CHICK[2], NADEAU et al.[3], and NAHUM and HOFF[4] placed acetylcholine or some other cholinergic drug directly on the anterior surface of the right atria. HASHIMOTO et al.[5] and NAKAYAMA et al.[6] induced fibrillation by means of the administration of acetylcholine into the sinus node artery under constant pressure perfusion. Other cholinergic drugs have been used by CHIBA and HASHIMOTO[7] for the same purpose. Production of an hipokalemic state has been utilized by LEVEQUE[8] for the maintainance of the acetylcholine-induced fibrillation.

When the effects of antiarrhythmic drugs on auricular fibrillation are considered, the duration of the experimental arrhythmia is a critical parameter to be measured. In this communication, we attempt to present an experimental model based on the facts summarized above, in which the time of persistance of the arrhythmia is a highly predictable value and an appropriate criteria for the assessment of the antiarrhythmic activity of a drug.

Method. Experiments were carried out on 30 dogs of either sex, weighing 6 to 15 kg, anesthetized with morphine-chloralose-urethane (morphine 1 mg/kg s.c. followed 30 min later by chloralose 3%-urethane 30%, 2 ml/kg i.v.). The trachea was cannulated and artificial ventilation was initiated with a positive pressure pump, in order to allow a chest opening through the 4th right intercostal space. The pericardium was incised parallel and just anterior to the right phrenic nerve, exposing the right atria, and the heart was suspended loosely in a pericardial cradle. Two electrodes were implanted in the anterior surface of the right atria, near to the base of the appendage and about 5 mm apart. They were connected to a Grass S4 stimulator through nickel-steel alloy electrodes. A 3rd similar electrode was inserted 5 mm apart and connected to an ECG preamplifier related to a Sanborn two-channel recorder. Electrodes consisted of coil-springs conductors covered with silicone rubber. 2 mm of the tip were denudated from the cover and the coil-spring was stretched in order to form a pointed terminal. After insertion, a gently clockwise rotation allowed penetration of the tip into the atrial wall with a minimal trauma.

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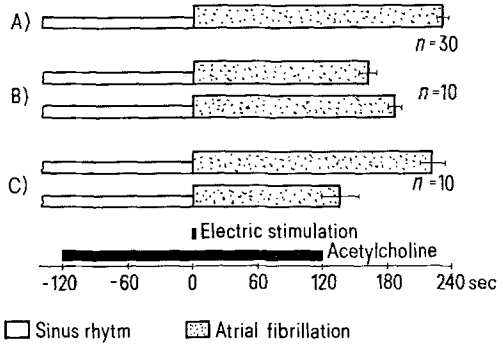
A polyethylene catheter in the femoral artery allowed blood pressure measurement on the Sanborn recorder. A thin polyethylene tubing was inserted into the saphenous vein and connected to a slow-injection syringe in order to inject acetylcholine at the very slow rates desired. Another polyethylene catheter was inserted into the controlateral femoral vein for injections of antiarrhythmic drugs or saline.

Results. Sustained auricular fibrillation was produced constantly when an appropriate dose of acetylcholine was perfused intravenously and a supraliminal stimulation was applied to the atria. Arrhythmia persisted as long as the acetylcholine infusion was maintained and for some time thereafter. Parameters of electrical stimulation were chosen after several controlled trials and consisted of repetitive square wave pulses of 3 msec duration, voltage output of 7 volts and a frequency of 900 cycles/min. The train of pulses lasted for 3 sec. The amount of acetylcholine (in mg per min/kg body wt.) had to be determined in each dog: after 2 min of infusion, the electrical stimulus was applied and the dose of acethylcholine was considered appropriate when the episode of fibrillation lasted for at least an additional period of 2 min. This dose ranged between 0.1 and 1.2 mg/kg/min.



Mean duration of auricular fibrillation. Acetylcholine infusion is installed 2 min before zero time. At zero time electrical stimulus was applied. Acetylcholine infusion lasted for 2 min after zero time. A) mean duration (± 1 standard error) of arrhythmia in 30 dogs. B) effects of saline, control and after saline, mean of 10 dogs. C) effects of quinidine, control and after the drug, mean of 10 dogs.

After 2 min of fibrillation, acetylcholine was stopped and the total time of arrhythmia measured.

When these standardized conditions were achieved, the duration of successive episodes of fibrillation was quite constant for each dog. Thus the average duration of 3 episodes, separated by intervals of 5 min of sinus rhythm, was taken as a control value. Results in 30 dogs showed an average of duration of 230 ± 8.4 sec (see A in the Figure).

In order to determine the validity of the method, 10 dogs were injected with 1 ml/kg saline, and another 10 with 8 mg/kg quinidine. Saline was unable to shorten the duration of arrhythmia (control value: 162.2 ± 19.3 sec; after saline: 187.8 ± 12.6 sec; $p > 0.2$, non-significant; B in the Figure). On the other hand, quinidine reduced significantly the time of fibrillation from a control value of 222.6 ± 22.7 to 136.1 ± 36.3 sec ($p < 0.01$, significant; C in the Figure). In fact, in 5 dogs, arrhythmia stopped even during the acetycholine infusion and in 2 dogs fibrillation did not appear at all after quinidine.

Conclusions. When an appropriate continuous infusion of acetylcholine is given to the dog and a supraliminal electrical stimulus is applied to the right atria, a sustained atrial fibrillation is obtained. Continuance of the infusion by a standardized lapse of 2 min before stimulation and maintainance of the arrhythmia for an additional standardized period of 2 min permit: 1. to assert the production of a persistent arrhythmia; 2. to obtain a remarkably constant duration of fibrillation for each animal.

This time of persistance was taken as a control value in order to test antiarrhythmic effects. Results obtained after injection of saline, compared with the injection of quinidine, a well recognized antiarrhythmic drug, seem to prove the validity of the method as a technique to measure antiarrhytmic activity.

Zusammenfassung. Methode zur Erzeugung von Vorhofflimmern bestimmter Dauer beim Hund.

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Selective Staining of Cell Nuclei with Haematoxylin Containing Transitional Metal Cations

It was shown by MELANDER and WINGSTRAND[1] that GOMORI's[2] haematoxylin gives excellent staining of resting cell nuclei, as well as chromosomes, of a wide variety of animals and plants. The incorporation of metal cations, particularly iron in oxazine dyes like celestine blue B (GRAY et al.[3]; DUTT[4]), gallocyanin (EINARSON[5]; SANDRITTER et al.[6]; DUTT[7]) and chromium, iron, nickel, cobalt and copper in gallamine blue (GRAY et al.[8]; DUTT, unpublished) does facilitate specific binding of these dyes with DNA and RNA, particularly with their phosphate groups. GOMORI's haematoxylin likewise contains chrom alum as one of the ingredients. This communication shows that chromium can be replaced by other transitional metal cations, and also presents evidence on the nature of specificity of this dye reagent towards nucleic acids.

Haematoxylin (C.I. No. 75290) used in this investigation was manufactured by the British Drug Houses Ltd., London. The dye reagent was prepared as follows: To a

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