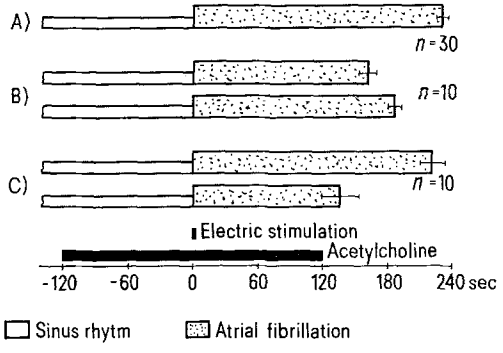
A polyethylene catheter in the femoral artery allowed blood pressure measurement on the Sanborn recorder. A thin polyethylene tubing was inserted into the saphenous vein and connected to a slow-injection syringe in order to inject acetylcholine at the very slow rates desired. Another polyethylene catheter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

[1] Y. MELANDER and K. G. WINGSTRAND, Stain Techn. *28*, 217 (1953).
[2] G. GOMORI, Am. J. Path. *17*, 395 (1941).
[3] P. GRAY, F. M. PICKLE, M. MASER and L. HEYWEISER, Stain Techn. *31*, 141 (1956).
[4] M. K. DUTT, Acta histochim. *44*, 1 (1972).
[5] L. EINARSON, Acta path. microbiol. scand. *28*, 82 (1951).
[6] W. SANDRITTER, G. KEIFER and W. RICK, *Introduction to Quantitative Cytochemistry* (Academic Press, New York 1966).
[7] M. K. DUTT, Acta histochim., in press.
[8] P. GRAY, M. W. DAY, L. HEYWEISER and C. NEVSIMAL, Stain Techn. *32*, 161 (1957).

1% aqueous solution of haematoxylin was added 100 ml of a 3% solution of ferrous sulphate, nickel chloride or cobalt chloride. To this were added 4 ml of a 5% solution of potassium dichromate and 0.2 ml of concentrated sulphuric acid. Then the solutions were filtered. These were usable immediately after preparation. All these dye reagents registered a pH of 1.4 when freshly made. This pH was adjusted to 2.0, 3.0, 4.0 and 5.0 with sodium hydroxide. After addition of the alkali, a lot of precipitate was produced when the pH was above 3.0.

The materials used were liver, kidney, testis, ovary, spleen and heart of a Holtzman strain of rat, and were fixed in 10% buffered neutral formalin. Paraffin sections (12 µm) were used throughout.

For staining, sections were hydrolyzed in 6 *N* HCl at room temperature for 10 min or longer, rinsed in water, stained for 3–5 min, washed thoroughly in water, dehydrated through a graduated series of ethanol, cleared in xylol and mounted.

Acid hydrolyzed sections following the above procedure of staining revealed extremely perfect deep blue-black nuclei at pH 1.4 to 3.0, without any staining of the cytoplasm. At pH 4.0 and 5.0 staining of nuclei was poor. When unhydrolyzed sections were stained, coloration occurred both in the nuclei and the cytoplasm. Sections treated with boiling 5% trichloracetic acid (TCA) and then stained, revealed rather pale staining of the nuclei. Sections after acid hydrolysis followed by treatment with phenylhydrazine at 60 °C for 2 h, washing in water and staining with the dye reagent containing either iron, nickel or cobalt, revealed perfect staining of the nuclei.

As to potency of the dye reagents during which they can be used with success, it can be said that they remain potent in localizing DNA easily upto 3 weeks, so that haematoxylin in combination with transitional metal cations can be considered to be quite stable even at room temperature. These dye reagents can, therefore, be used in routine studies dealing with detection of DNA in cell nuclei.

From the results presented above, it is logical to conclude that staining with haematoxylin containing transitional metal cations is due to nucleic acids. The role of acid hydrolysis is only to remove RNA (DEANE[9]; VENDRELY-RENDAVEL[10]). However, after acid hydrolysis, aldehyde molecules of DNA are also liberated and when these are blocked by phenylhydrazine, staining of nuclei still occurs. This suggests that staining of nuclei is not due to DNA-aldehyde but due to DNA-phosphate group. This is further substantiated by the fact that sections treated with 0.1 *N* sodium hydroxide at 60 °C for 10 min and stained revealed perfect staining of the nuclei. Alkali hydrolysis is known to remove RNA without affecting DNA (DAVIDSON[11]).

Since when sections are treated with boiling trichloracetic acid and then stained with haematoxylin, they reveal a rather pale staining of the nuclei without any staining of the cytoplasm, the conclusion is that nuclear basic protein (histone) is involved in the staining. Apparently the metal cations in the dye help in the binding of histone with the dye molecules.

Résumé. Les noyaux cellulaires des sections de tissus acido-hydrolysés montrent une teinte sélective quand ils sont colorés avec de l'hématoxyline contenant en solution aqueuse des cations métalliques transitionnels comme le fer, le nickel ou le cobalt à pH 1.4. Ces réactifs teints peuvent être utilisés pour les études routinières de la localisation du DNA.

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Department of Zoology, University of Delhi, Delhi 7 (India), 16 August 1973.

[9] H. W. DEANE, Am. J. Anat. *78*, 227 (1946).
[10] C. VENDRELY-RENDAVEL, Compt. r. Soc. Biol., Paris *143*, 294 (1949).
[11] J. N. DAVIDSON, *The Biochemistry of the Nucleic Acids*, 6th edn (Methuen and Co. Ltd., London 1969).

Simple Rheumatoid Agglutination Test with Non-Diluted Serum and Blood

Rapid slide agglutination tests using γ-globulin coated polystyrene latex particles (Hyland RA-test, Hyland Laboratories, and similar tests) are widely used for the detection of rheumatoid factor in the serum[1]. Bentonite[2], kaolin, charcoal[3] and raw latex can be used instead of the polystyrene latex in the RA-test. We found that ion-exchange resin particles could be used also as an adsorbent in the RA-test and made a new reagent (Resin RA-test) for testing whole blood as well as serum. We believe that Resin RA-test has some merits as compared with conventional RA-tests.

The Resin RA-test reagent consisted of 1.5 g of aggregated γ-globulin coated resin particles, 0.3 g of bovine serum albumin, 12 mg of acetyltryptophane, 0.5 g of

Table I. Relationship between Resin RA-test and Hyland RA-test[a]

Reagent		Hyland RA-test				Resin RA-test				Resin RA-test			
Dilution of Serum			1	*X*			1	*X*			20	*X*	
Intensity of agglutination		++	+	±	−	++	+	±	−	++	+	±	−
++[b]	(40)[c]	40				37	3			14	11	6	9
+	(22)	16	6				20	2			2	9	11
±	(21)	1	16	4				9	12				21
−	(322)	2	36	73	211				322				322

[a] Non-diluted serum and 20 times diluted serum were tested with Resin RA-test and Hyland RA-test. [b] Results of Hyland RA-test with 20 times diluted serum as standard. [c] Number of serum tested.