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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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.

Table II. Resin RA-test with non-diluted serum and whole blood[a]

Samples	Non-diluted serum		Whole blood		Hyland RA-test[b]
Volume (ml) used	0.01	0.04	0.01	0.04	
1[c]	++	++	++	++	++
2	++	++	++	++	++
3	++	++	++	++	++
4	−	−	−	−	−
5	−	−	−	−	−
6	±	±	−	−	±
7	±	−	±	±	±
8	++	++	++	++	++
9	−	−	−	±	±
10	+	+	+	+	+
11	++	++	++	++	++
12	++	++	++	++	++
13	+	+	++	++	+
14	+	+	+	+	+
15	++	++	++	++	+
16	++	++	+	+	+
17	++	+	+	+	+
18	−	−	−	±	−
19	±	±	−	±	±
20	+	++	±	+	+
21	++	++	++	++	++
22	−	−	−	−	−
23	−	−	−	−	−
24	−	−	−	±	±
25	++	++	++	++	++

[a] Each volume of serum or blood was mixed with 0.03 ml of Resin RA-test reagent. [b] Results of conventional Hyland RA-tets (20 × serum). [c] Number of serum.

saponin (a haemolytic agent), and 0.1 g of sodium azide in 100 ml of 1/10 *M* glycine buffered saline. The resin particles with a diameter of approximately 3 μm were obtained by crushing a weakly basic ion-exchange resin (Amberlite-IR 45, Rohm & Haas, Philadelphia) in a ball mill. Aggregated γ-globulin was prepared by heating 2% human γ-globulin (Cohn's Fr. II) in the glycine buffered saline for 10 min at 63 °C. The resin particles were added to aggregated γ-globulin solution. The suspension was shaken gently for 2 h at 4 °C. The γ-globulin coated resin particles obtained was washed 3 times with the buffer.

The stability of this reagent was similar to or rather superior to Hyland RA-test reagent. The ion-exchange resin used for preparing the reagent was widely available and inexpensive. Furthermore the resin particles could be easily prepared and treated. The procedure of testing was the same to Hyland RA-test. A drop of serum or blood was mixed with a drop of the reagent on a slide glass. The reaction was read as −, ±, +, ++ according to the intensity of agglutination.

Since conventional RA-tests use highly sensitive sera, for testing they have to be diluted approximately 20 times to avoid false positive reactions. When non-diluted sera were tested, many false positive reactions were observed as shown in Table I. On the other hand, Resin RA-test was specific, but not so sensitive that non-diluted sera could be used. As summarized in Table I, the reaction by Resin RA-test with non-diluted serum was well correlated with that by Hyland RA-test with 20 times diluted serum. However the former was slightly insensitive compared with the latter, and a few wrong negative results were observed in the former test. Thus this test required no serum dilution. Testing 20 times diluted serum, Resin RA-test gave weaker agglutinating reactions than Hyland RA-test (Table I). Since Resin RA-test reagent was capable of lysing red cells, we could also detect rheumatoid factor in a drop of whole blood. Though agglutinating reactions were slightly weaker and took place a little slowly, Resin RA-test with whole blood gave a similar result to that with non-diluted serum. Furthermore, when 0.01 to 0.04 ml of serum or whole blood were mixed with 0.03 ml of Resin reagent, similar results were obtained in all cases, as shown in Table II.

As described above, the rheumatoid agglutination test using this reagent can be performed easily with a drop of serum or whole blood. Since the test requires no serum dilution or even no separation of serum from blood, these screening tests can be employed in poorly equipped laboratories and in field work.

Zusammenfassung. Zerkleinerte Ionen-Austauscher-Partikel werden als Adsorbans von γ-Globulin im Rheumatoid-Agglutinationstest verwendet. Die Test-Substanz wirkt sehr spezifisch, ist jedoch im Gegensatz zu den konventionellen RA-Tests von geringerer Sensitivität, so dass der Agglutinationstest sowohl mit unverdünntem Serum als auch mit reinem Blut schnell und sicher durchgeführt werden kann.

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