serum was carried out at 0°C to prevent completion of the hemolytic reaction[10]. The results are given in the Table. There was a striking difference between LR_3 and FR_3 in that EA treated with LR_3 were not lysed by guinea-pig C′ in the presence of Versene, whereas EA treated with either guinea-pig serum or FR_3 were lysed. Thus LR_3, unlike FR_3, was either deficient in at least one component requiring divalent cations or treatment of guinea-pig serum with 'Liquoid' resulted in the formation of inhibitors.

It is also evident from the Table that LR_3 lysed partially complemented cells resulting from the reaction of EA with FR_3, whereas it did not lyse $EAC'_{1,4,2}$ prepared by the reaction of EA with whole guinea-pig serum. It seems, therefore, that the reaction of EA with FR_3 leads to the formation of complemented cells having the activities of C'_1, C'_2, C'_4 and that component of C'_3 present in FR_3, but absent from LR_3, and that lysis occurs on the addition of LR_3 because it has the C'_3 component missing in FR_3. This finding suggests that LR_3 and FR_3 react in a specific sequence with partially complemented cells and that FR_3 reacts first. It remains to be determined whether or not FR_3 reacts directly with $EAC'_{1,4,2}$. If it does, it is possible that $EAC'_{1,4,2}$ and LR_3 taken together might be useful reagents for the titration of the C'_3 component absent from LR_3 but present in FR_3. The results of this comparative study also suggest the possibility of utilizing partially complemented cells formed during the reaction of EA with FR_3 for the titration of the C'_3 component which is present in LR_3.

It is clear from these results that the three methods for preparing R_3 reagents are not equivalent and that it may be possible to utilize LR_3 and FR_3 for the titration of at least two of the components of the C'_3 complex.

Assay of formaldehyde and liquoid inactivated guinea-pig serum for components of complement that require Ca^{++} and Mg^{++} to react with sensitized sheep erythrocytes (EA)

Reaction[a] of EA with:	Source of C'_3	O.d.[b] (541 mμ)
Guinea-pig serum (0°C, 40 min)	Guinea-pig serum	0.680
FR_3 (23°C, 60 min)	Guinea-pig serum	0.680
LR_3 (23°C, 60 min)	Guinea-pig serum	0.030
FR_3 (23°C, 60 min)	LR_3	0.500
LR_3 (23°C, 60 min)	FR_3	0.030

[a] At the end of the time indicated for each reaction the mixtures were centrifuged to separate the cells which were washed three times with buffer and titrated for $EAC'_{1,4,2}$ in the presence of EDTA with the sources of C'_3 indicated. [b] Optical density is proportional to the fraction of cells lysed; the value 0.680 represents 100% lysis.

Riassunto. Sono stati esaminati comparativamente tre reagenti per la titolazione di C'_3, rispettivamente allestiti con siero di cavia inattivato con zymosan, formalina e «Liquoid». Le differenze esistenti tra essi sono state analizzate e discusse in base alle attuali conoscenze sulla natura del terzo componente complementare.

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Separation of Large Numbers of Lymphocytes from Human Blood

Preparation of large amounts of selected white blood cells from human blood are required for clinical chemistry investigations. We have recently described a method for obtaining human granulocytes with a high recovery in about 50 min[1].

This paper describes a method for obtaining large numbers of pure and well-preserved lymphocytes. Methods used to date proved to be unsatisfactory because they do not yield sufficient quantities of these cells, and do not eliminate a significant proportion of the contaminating erythrocytes and platelets[2–6].

Material and method. 250 ml of venous blood were collected in an Erlenmeyer flask containing 25 ml of 5% sodium EDTA. 1 Vol of blood was added to 4 Vol of 0.83% NH_4Cl and the mixture was poured into 100 ml tubes. After a few minutes the blood was centrifuged at 350 *g* for 10 min in a refrigerated centrifuge at 4°C. The sediment was washed a second time with NH_4Cl. The sediment was suspended in 5 ml of serum of the same donor and poured into a prewarmed small glass tube (20 · 1.2 cm) loosely packed with ordinary cotton. After 30 min incubation at 37° the tube was inverted and lymphocytes were eluted with warm 0.83% NH_4Cl.

The eluted material (7–10 ml) was centrifuged at 350 *g* for 10 min in 10 ml conical centrifuge tubes and the sediment washed twice with Tyrode's solution, saturated with a 95% O_2 and 5% CO_2 gas mixture.

Results and discussion. Our preparations contain over 90% of lymphocytes and virtually no erythrocytes and platelets. The presence of serum proteins was excluded by immunodiffusion. Over 50% of the lymphocytes were recovered. These cells were not clumped and could be counted easily. Electron microscopic examination showed perfect preservation of all intracellular structures. The viability of lymphocytes was demonstrated by the

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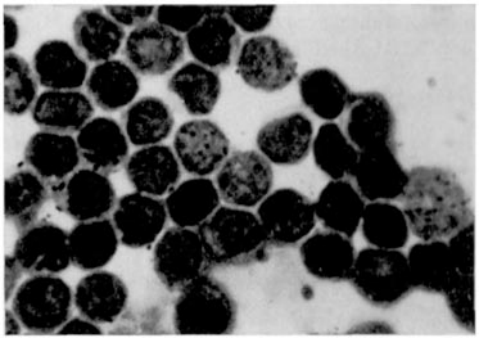
marked incorporation of tritiated uridine by lymphocyte slides prepared from suspensions obtained by the above-described method (Figure).



Very satisfactory results were obtained with the same method for isolating myelocytic cells from granulocytes in cases of chronic myeloid leukemia.

Riassunto. Viene descritto un metodo che consente di isolare una buona quantità di linfociti, completamente privi di eritrociti e di piastrine. Il metodo è basato sull'azione emolitica del NH_4Cl e sulla ritenzione dei granulociti e delle piastrine ad opera del cotone. Questo metodo è risultato molto soddisfacente anche per isolare le cellule di tipo mielocitario dai granulociti dal sangue di pazienti con leucemia mieloide cronica.

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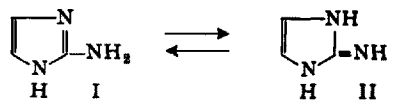
The Synthesis of Azomycin (2-Nitroimidazole)

Azomycin (2-nitroimidazole), an antibiotic active against *Trichomonas vaginalis*, has been isolated by several authors[1–4] from culture filtrates of different Streptomyces species. We have now carried out a synthesis of this compound through diazotization of 2-aminoimidazole followed by the Gattermann reaction with sodium nitrite (the nitration of imidazoles gives the 4 (or 5) derivatives only[5]). The aromatic character of 2-aminoimidazole (I) was discussed by Burtles and Pyman[6] who concluded that it could be better represented by the 2-imino-2,3-dihydroimidazole structure (II). Their opinion was based upon the fact that 2-aminoimidazole does not yield a benzylidene derivative with benzaldehyde[7] and does not couple with naphthols after treatment with nitrous acid[8]. Actually, in the reaction with nitrous acid in the presence of hydrochloric or acetic acid, a deep red colour was obtained by adding sodium hydroxide to the solution; this was interpreted[6] as a formation of a 4 (or 5) nitroso derivative. However, it is also possible to assume that 2-aminoimidazole reacts with nitrous acid as an aromatic amine (structure I), giving a diazo derivative which partially couples with itself yielding the red colour.



This assumption proved to be correct when 2-nitroimidazole was obtained, though in very poor yield, by treating 2-aminoimidazole dissolved in HCl with an excess of sodium nitrite, followed by addition of cuprous sulphite and sodium nitrocobaltate. Higher yields (about 30%) were obtained when 2-aminoimidazole hydrochloride (2.88 g) was diazotized in 40% fluoboric acid (13 ml) with sodium nitrite (1.67 g) and the resulting solution was treated with sodium nitrite (24.7 g in 50 ml of water) and copper powder (4.9 g). The 2-nitroimidazole (775 mg, m.p. 284°) was readily recovered from the acidified reaction mixture by extraction with ethyl acetate, evaporation of the solvent and recrystallization of the residue from ethanol. The product thus obtained and a sample of azomycin produced by fermentation showed identical melting points, UV- and IR-spectra and antimicrobial activity.

The demonstration that 2-aminoimidazole can be diazotized opens a way to the synthesis of a number of 2-substituted imidazoles. Thus, it was thought to be interesting to try the same reaction on an N-alkyl imidazole. Diazotization of 1-methyl-2-aminoimidazole[9] in fluoboric acid and treatment as described above with nitrite and copper powder gave 1-methyl-2-nitroimidazole (m.p. 100–102°, yield 30%) identical with the product obtained by methylation of azomycin[10].

Riassunto. La sintesi del 2-nitroimidazolo e del 1-metil-2-nitroimidazolo per diazotazione e reazione di Gattermann a partire dai corrispondenti 2-aminoimidazoli dimostra che, in determinate condizioni, questi ultimi si comportano come composti a carattere aromatico.

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