spermidine alone were revealed on 100 g fresh weight of Jerusalem artichoke dormant tubers.

Our data clearly show that spermine is a growth substance able to take the place of IAA to cause the cellular division. It is interesting to note that, in the literature, no substance having aliphatic structure was found to be able to act in a similar way. Our researches are continuing in order to decide at which metabolic level spermine acts.

Riassunto. È stato per la prima volta dimostrata che la spermina ($10^{-4}M$) ha un effetto di crescita simile a quello dell'acido indolacetico sugli espianti di *Helianthus tuberosus* in vitro.

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A Comparison of Methods for the Inactivation of Third Component of Guinea-Pig Complement[1]

For many years after the discovery of complement (C′) the consensus was that it consisted of four components, based on the fact that hemolytically active serum can be fractionated or inactivated to give reagents which are non-hemolytic when used alone, but which are fully active hemolytically when combined in pairs. The assumption was made that each reagent was deficient in one component, without regard to the possibility that more than one component might be absent from a given reagent and that the hemolytic system might therefore involve more than four components.

In recent years evidence has been obtained clearly establishing the complexity of C'_3 (third component of C′)[2-6]. It is possible, therefore, that an R_3 reagent, believed heretofore to lack C'_3, may in fact be deficient in more than one component. A study of various methods for preparing an R_3 reagent was therefore undertaken.

R_3 reagents were prepared as follows using guinea-pig serum: (1) by the inactivation of serum by 'Liquoid'[7] or formaldehyde, and (2) by the absorption of serum with Zymosan[8-9].

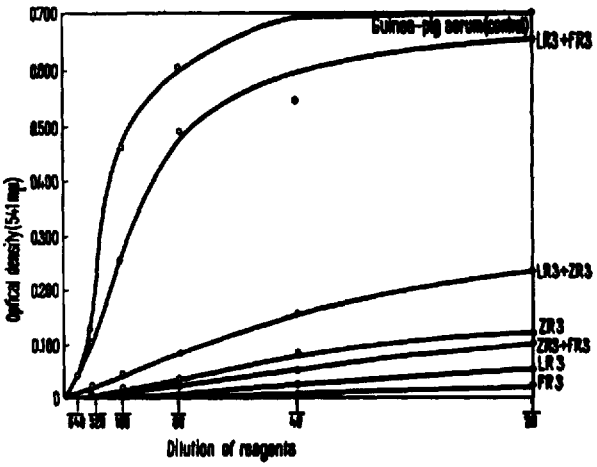
The R_3 reagents were assayed to ascertain whether or not they were deficient in more than one component and whether or not the missing components were the same.

Each of the R_3 reagents was non-hemolytic by itself and cross-titrated with reagents R_1, R_2 and R_4, which are deficient in C'_1, C'_2 and C'_4, respectively. The R_3 reagents were therefore lacking in one hemolytic component at least, presumably a component of the C'_3 complex, and contained C'_1, C'_2 and C'_4.

In order to learn whether the missing component in the three preparations of R_3 was the same or not, they were cross-titrated with each other in pairs over a wide range of concentrations. The results are shown in the Figure. Each R_3 preparation was essentially non-hemolytic, but LR_3 ('Liquoid' preparation) and FR_3 (formaldehyde preparation) taken together had virtually the same hemolytic activity as the untreated guinea-pig serum used as a control.

This finding suggests that the missing C'_3 factors in one preparation are different from those in the other, and although it gives no clue as to the exact number of components involved, the minimum number is certainly two. The Zymosan preparation (ZR_3) did not cross-titrate significantly with either LR_3 or FR_3, an indication that it must have lacked the components absent from both LR_3 and FR_3.

A further comparison of LR_3 and FR_3 was made with respect to those components of complement that require either Ca^{++} or Mg^{++} for immune hemolysis. For this purpose was used the reaction of these reagents with EA (sensitized sheep cells) to form $EAC'_{1,4,3}$, that is, complemented cells that are lysed by guinea-pig serum in the presence of Versene. Because neither reagent was hemolytic by itself, the reaction was carried out at room temperature, whereas the reaction with the untreated guinea-pig



Cross-titrations of R_3 reagents for hemolytic activity of complement. The reagents were tested either alone or in combinations taken two at a time; using guinea-pig serum as a control. Optical Density at 541 mμ is proportional to the fraction of cells lysed and is a measure of the hemolytic activity of complement.

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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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