show a reduction in the number of digits of both forefeet and hindfeet. The forefeet are generally tridactyl and the hindfeet tetradactyl.

Material and methods. We used matings between hypodactyl females (hd/hd) and normal heterozygous males (hd/+). In that type of mating, the ratio of normal to hypodactyl is not significantly different from 1:1[11].

Normal heterozygous males (hd/+) were introduced to the cage of females to mate, and 12 days later, the pregnant females were recognized by palpation.

In order to study the foetal blood, day 14 of gestation was chosen, since the nucleated red cells are still numerous at that stage. Five pregnant hd/hd rats were operated and the foetuses removed on day 14, blood smears were made and stained by the panoptic method. The microscopic image of the blood cells was projected on paper with a projection microscope. For each foetus, the outline of 200 nucleated red cells was drawn and the diameters directly measured. The frequency of each diameter was determined (36 foetuses from 5 mothers).

Results and discussion. Although it seems almost impossible to distinguish the abnormal foetuses macroscopically at that stage (the abnormalities become apparent on day 15), the study of the foetal blood cells permit one to observe two populations in the ratio 1:1 (Figure 1): in 17 foetuses, a very obvious macrocytosis is seen: about 80% of the cells have a diameter > 12 µm, while in 19 foetuses, only 36% are > 12 µm. The difference between the two percentages is statistically significant (method of χ^2: $p < 0.001$). Moreover, the aspect of the nucleated red blood cells is very different in the macrocytic cells to normals; their nucleus is generally large and irregular and paler than in normocytic cells (Figure 2).

The macrocytosis observed during the early foetal development of hd/hd rat resembles both that of br/br rabbit and the macrocytosis due to pyrimethamine. In the latter cases an impairment in the folate or vitamin B_{12} metabolism seems to be implicated. Moreover, in the hd/hd strain, foetal red cell macrocytosis is associated with sterility in the homozygous males, as has already been observed in man with such a metabolic failure[12].

The hd gene could be responsible for a disturbance of cell division which leads to macrocytosis. The abnormal nucleated red cells could then be destroyed too massively and could block up the smallest arteries of the extremities, which could give rise very soon to necrosis and amputation of the digits. Further work with vitamin treatment to the pregnant rat should determine the truth of this hypothesis.

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Rosette Formation by Human T and B Lymphocytes in the Presence of Adrenergic and Cholinergic Drugs[1]

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Summary. It was shown that adrenergic drugs, which increase the intracellular levels of cAMP, inhibit the rosette formation by T-lymphocytes, but stimulate the rosettes produced by B-lymphocytes. Cholinergic drugs, which increase the levels of cGMP, on the contrary, stimulate the formation of rosettes by T-lymphocytes but inhibit those produced by B-lymphocytes.

It was recently demonstrated that E-rosette formation by human T lymphocytes is either inhibited by drugs which increase the intracellular levels of cyclic adenosine monophosphate (cAMP)[2–4], or stimulated by drugs which raise the levels of cyclic guanosine monophosphate (cGMP)[5,6]. In this paper we present evidence suggesting that human T and B lymphocytes bear adrenergic and cholinergic receptors whose stimulation results in antagonistic effects on rosette formation.

Material and methods. Lymphocytes. Lymphocyte suspensions obtained from peripheral blood of normal subjects by Ficoll-Hypaque were adjusted to contain $2 \cdot 10^6$ cells/ml. *Red blood cells*: Unsensitized (E) sheep red blood cells (SRBC) were prepared weekly in Hank's solution. Sensitized (EAC) SRBC were prepared according to LAY and NUSSENZWEIG[7], using rabbit antiserum and human complement.

Drugs. The following drugs (Sigma Chemical Co.) were assayed: theophilline 10^{-3} *M*, papaverine 10^{-3} *M*, isoproterenol $2 \cdot 10^{-4}$ *M*, dibutyryl cAMP $2 \cdot 10^{-4}$ *M*, carbamylcholine 10^{-6} *M*, pilocarpine 10^{-3} *M*, dibutyryl cGMP $2 \cdot 10^{-4}$ *M*, atropine sulfate 10^{-6} *M*. Solutions of the drugs were prepared just before use in Hanks' solution with final pH adjusted to 7.4.

Rosette assays. Drug effects on rosette formation were assayed by incubation 0.45 ml of the lymphocyte suspension containing $2 \cdot 10^6$ viable cells/ml with 0.05 ml of the drug dilution, for 60 min at 37 °C. The mixture was separated in two tubes, each receiving, respectively, 0.5 ml of a 0.25% suspension of unsensitized (E) and sensitized (EAC) SRBC. Control tubes received no drugs. The tubes were then centrifuged at room temperature for 5 min at 200 *g* and incubated at 37 °C for 30 min (for B-lymphocyte rosettes) or at 4 °C for 60 min (for T-lymphocytes rosettes). The resulting pellets were gently resuspended and counted in a hemocytometer. The viability

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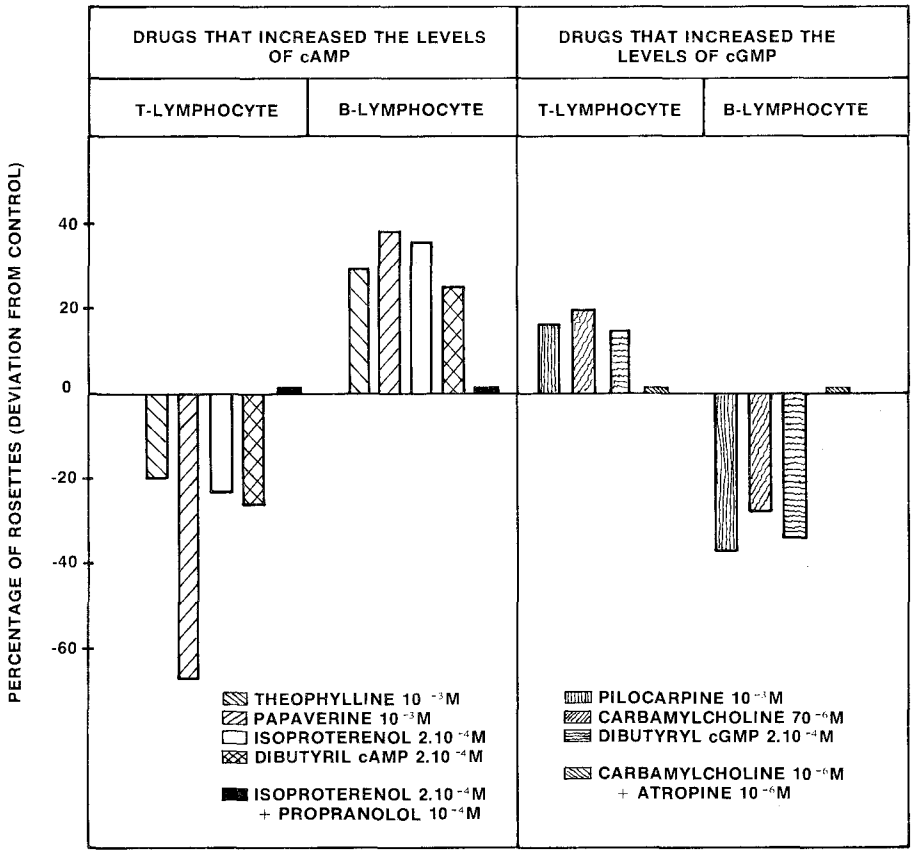
of the cells remained around 95%. Lymphocytes with 3 or more bound erythrocytes were counted as rosettes. Results were expressed as per cent of inhibition or stimulating relative to the per cent rosettes formed by control lymphocytes.

Results. As we can see in the accompanying table, theophylline (10^{-3} *M*) and papaverine (10^{-3} *M*), drugs recognized as capable of raising the intracellular levels of cAMP through a blockade of the phosphodiesterase activity, showed an inhibition of 20% ($p < 0.005$) and 68% ($p < 0.005$), respectively, in the capacity of T lymphocytes to form non-immunologic E-rosettes. The inhibition produced by isoproterenol ($2 \cdot 10^{-4}$ *M*) and dibutyryl cAMP ($2 \cdot 10^{-4}$ *M*) were, respectively, 21% ($p < 0.005$) and 24% ($p < 0.005$). The rosette formation by B-lymphocytes, on the contrary, were stimulated by the same drugs at identical concentrations. The stimulatory effects obtained with these substances were 28% ($p < 0.01$) for theophylline, 36% ($p < 0.005$) for papaverine, 34% ($p < 0.005$) for isoproterenol and 27% ($p < 0.005$) for dibutyryl cAMP. Propranolol (10^{-3} *M*) totally blocked the inhibitory effect of isoproterenol ($2 \cdot 10^{-4}$ *M*). Pilocarpine (10^{-3} *M*) and carbamylcholine (10^{-6} *M*), drugs capable of raising the intracellular levels of cGMP through stimulation of guanylcyclase, showed a stimulatory effect of 12% ($p < 0.005$) and 14% ($p < 0.005$), respectively, in the capacity of T-lymphocytes to form E-rosettes. Dibutyryl cGMP ($2 \cdot 10^{-4}$ *M*) produced a stimulatory effect of 11% ($p < 0.025$). The EAC-rosette formation by B-lymphocytes, on the contrary, were inhibited by the same substances at identical concentrations: pilocarpine 33% ($p < 0.005$), carbamylcholine 27% ($p < 0.010$) and dibutyryl cGMP 32% ($p < 0.005$). Atropine (10^{-6} *M*) totally blocked the inhibitory effect of carbamylcholine (10^{-6} *M*).

Discussion. We present in this paper new evidence that cyclic nucleotides cAMP and cGMP have the capacity of modulating the formation of rosettes by human T and B lymphocytes. The results obtained with drugs capable of modulating the intracellular levels of cyclic nucleotides were similar to those described by Chisari and Edgington[2], Galant and Remo[3], and Grieco et al.[4] using cAMP, and by Grieco et al.[5], and Lundak et al.[6] using cGMP. The rosette formation by human T lymphocytes with SRBC (E-rosettes) was either significantly inhibited by drugs which increase the intracellular levels of cAMP, or enhanced by drugs which raise the levels of cGMP. Unexpected results were obtained, however, with human B lymphocytes. Here the influence of the two nucleotides on rosette formation with sensitized erythrocytes (EAC-rosettes) were shown to be in opposite directions, since cAMP increased and cGMP decreased the percentage of rosette obtained. Explanations for this result are unclear at this moment.

Although the biochemical mechanisms through which cyclic nucleotides exert their effects are not yet understood, many regulatory events on cell membranes are known to be initiated by changes in cAMP and cGMP. It is known that the blood lymphocytes can be distinguished by cell function, membrane antigens and membrane receptors.

Human B lymphocytes have membrane receptors for activated complement (C3) which can be demonstrated by different methods. This complement receptor is de-



Stimulatory and inhibitory effects of drugs on rosette formation by human T and B lymphocytes. The percentage of rosette formation (increase and decrease) was calculated from controls taken as 100%.

Rosette formation by human T and B lymphocytes in the presence of drugs that raise the cellular levels of cAMP and cGMP. The percentage of inhibition or stimulation was calculated from controls taken as 100%

Drugs	Cases No.	T lymphocytes			B lymphocytes		
		Rosettes %	Inhibition %	Stimulation %	Rosettes %	Inhibition %	Stimulation %
None	65	53.3± 7.0	–	–	20.8±6.7	–	–
Theophylline $10^{-3}M$	29	41.0± 9.5[a]	20	–	25.0±8.6[b]	–	28
Papaverine $10^{-3}M$	23	17.0±11.3[a]	68	–	26.0±8.1[a]	–	36
Isoproterenol $2{\cdot}10^{-4}M$	13	48.3± 9.7[a]	21	–	31.3±7.3[a]	–	34
Dibutyryl cAMP $2{\cdot}10^{-4}M$	13	48.6± 9.0[a]	24	–	29.4±5.8[a]	–	27
Isoproterenol $2{\cdot}10^{-4}M$ + propranolol $10^{-3}M$	11	52.0± 8.0	NS	–	21.2±5.0	–	NS
None	24	47.8± 6.9	–	–	23.3±6.5	–	–
Pilocarpine $10^{-3}M$	9	55.4± 6.4[a]	–	12	16.0±2.7[a]	33	–
Carbamylcholine 10^{-6}	10	55.0± 6.7[a]	–	14	17.0±2.6[c]	27	–
Dibutyrol cGMP $2{\cdot}10^{-4}M$	10	53.0± 4.1[b]	–	11	15.5±3.5[a]	32	–
Carbamylcholine $10^{-6}M$ + atropine $10^{-6}M$	8	47.0± 6.8	–	NS	22.0±3.7	NS	–

[a] $p < 0.005$; [b] $p < 0.025$; [c] $p < 0.01$; NS = not significant.

stroyed by trypsin but is not blocked by antibody to Ig. Human thymocytes and T lymphocytes, on the other hand can be identified by surface membrane receptors which bind to SRBC to form non-immunological E-rosettes. They are dependent on the concentration of divalent cations[8], can be enhanced by treatment of lymphocytes by neuraminidase[9], and are inhibited by iodacetate[8], trypsin[8], azide[10], antilymphocyte serum[10], cytochalasin B[11]. The properties of human thymocytes and T lymphocytes of making spontaneous rosettes with SRBC appears to be conferred on T-cell precursors[12,13]. Curiously, it was found that theophylline and thymus extracts, known to increase the intracellular levels of cAMP, both have the capacity of stimulating E-rosette formation by human precursors lymphocytes and of restoring the immunological competence of neonatally thymectomized mouse. The results obtained in our experiments with rosette formation, suggest that cyclic nucleotides are modulator agents of lymphocyte membrane function. These nucleotides might be exerting their effects by modulating the synthesis of membrane receptors for SRBC and C3, by incorporating these receptors into plasma membranes or even by shedding this receptors from the lymphocyte membranes.

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Serum Immunoconglutinins and Complement in Systemic *Lupus erythematosus*[1]

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Summary. IK activity titrated by the sedimentation method in sera from patients affected with SLE was found to be negatively correlated with C4 and C3 complement factors levels. The significance of this data is discussed.

Generally the immunoconglutinins, (considered as autoantibodies with anti-complement specificity) formation reflects an in vivo complement fixation[2]. The IKs react specifically with fixed C3 and C4, in the KAF-unreacted form, i. e., C3b and C4b; once bound, IKs can trigger a new complement activation and so fix more C3 and C4[2]. These auto-antibodies are predominantly IgM, and the presence of free complement components, as well as EDTA, did not inhibit their reaction. On the contrary IK activity found in human saliva is referred to an unusual IgA class of antibody[3], and it is inhibited by free complement components or EDTA in solution. Thus, these two latter properties make the salivary IKs functionally similar to bovine conglutinins (non antibodies), which reacts in an EDTA reversible way, with the KAF-reacted form of C3(C3d)[2].

Serum raised IK values have been reported in many human pathological states: overall in infections[4,5], and in collagen-vascular diseases[6–8]; in all these conditions, a certain degree of complement consumption is usually found. Up to the present, the exact relation between IKs rizing and complement lowering, from the clinical standpoint, has not been investigated except for some cases of renal pathology[9–11].

We valued the IK titre and some complement functions in patients affected with Systemic *Lupus erythematosus*,