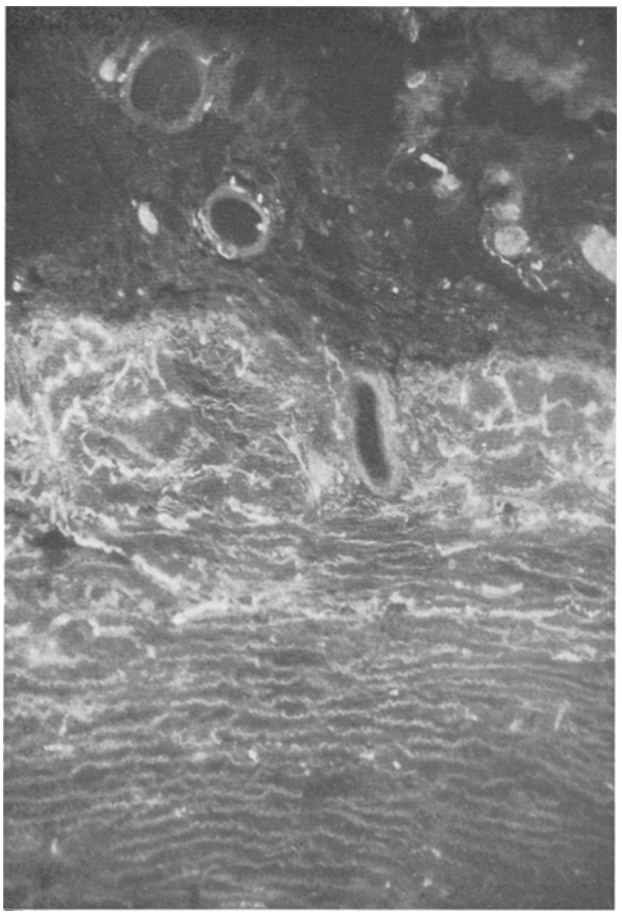
along the entire length of the ductus. However, chemical assay of catecholamines and uptake of ^{3}H-norepinephrine by the tissue suggested a nonuniform distribution of adrenergic nerves[3].

Although the quantity of smooth muscle in relation to the supporting elements varies among vessels from different locations, the muscular vessels of medium size generally have specific fluorescent fibers in the media while elastic vessels have none in the same area[4]. The ductus thus shares with other muscular vessels the feature of numerous fluorescent fibers associated with the media.



Cross-section of the ductus arteriosus of the fetal lamb. A large number of nerve fibers with intense specific fluorescence are seen around vasa vasorum in the adventitia (top) and among the autofluorescent elastic fibers and the nonfluorescent smooth muscle cells of the media (bottom). Fluorescence microphotograph. × 315.

Using the Cajal technique, BOYD[5] described a sensory innervation in the rabbit ductus similar to that found in the carotid sinus and aortic arch. In addition, he found fine fibers terminating in the musculature of the ductus which he presumed to be efferent but whose nature and origin were unclear. KENNEDY[6] also observed fine myelinated fibers in the ductus and suggested that the muscle fibers are innervated; but, the small number of fibers observed does not seem to justify this conclusion. He postulated that since closure of the ductus followed inflation of the lungs, a reflex might be involved. Subsequently, KENNEDY[7] attempted denervation of the ductus and stressed the importance of oxygen tension as a major determinant of closure.

Thus, the neural contribution to ductus closure remains obscure. However, the present demonstration of adrenergic innervation associated with the well developed musculature suggests that neural control may be a factor in the initiation and/or maintenance of contraction of the ductus[8–10].

Zusammenfassung. Nachweis adrenergischer Nervenfasern in der Media des Ductus arteriosus von Schafföten mit Falck'scher histochemischer Fluoreszenzmethode. Zusammen mit der gut entwickelten Muskulatur macht dies wahrscheinlich, dass Nerven am Kontraktionsgeschehen des Ductus arteriosus teilnehmen.

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[9] Supported by grants from USPHS No. HE-05157 and the American Medical Association Education and Research Foundation. I thank Dr. F. H. ADAMS and his colleagues for the fetal material; Drs. R. R. SONNENSCHEIN, F. N. WHITE, and D. MASUOKA for valuable suggestions and criticism; Mr. H. MCCAFFERY for efficient technical help.
[10] After this paper was submitted for publication, a report appeared (L. O. BORÉUS, T. MALMFORS, D. M. MCMURPHY and L. OLSON, Acta physiol. scand. *77*, 316 (1969)) on the presence of specific adrenergic nerve fibers in the media of the human fetal ductus arteriosus.

Rosette Formation in White Blood Cell Cultures from Patients with Acute Myeloblastic Leukemia

Rosette-formation of cells in vitro is a well-known immunological phenomenon observed under various experimental conditions[1–3]. In cultures of human leukocytes, for example, typical rosettes develop after addition of red blood cells which have been coated with immunoglobulin G. It was assumed that the mononuclear cells (macrophages, monocytes, large lymphocytes) participating in the formation of these rosettes, carry specific surface receptors for immunoglobulin G which, in vivo, may be involved in the apprehension and destruction of antibody-coated red blood cells or particles[4].

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This paper reports the observation of a rosette-formation in cultures of leukocytes from patients with acute myeloblastic leukemia (AML). In these spontaneously developed rosettes, leukemic cells were found to adhere to single monocytes which often contained phagocytozed material.

Materials and methods. Total and differential white blood cell counts were made from capillary blood from the following individuals: group A: 5 clinically healthy control persons; group B: 3 patients with chronic lymphocytic leukemia (CLL); group C: 2 patients with chronic myelocytic leukemia (CML), and group D: 8 patients with acute myeloblastic leukemia (AML). Simultaneously, 20–80 ml of venous blood from each individual were aspirated in 20 ml aliquots into heparin-rinsed 25 ml syringes containing 4 ml of dextran (Pharmacia Fine Chemicals, Upsala; mol. wt. 250,000; 3% in 0.9% NaCl). After careful mixing, the syringes were fitted with curved 20-gauge needles and incubated in a vertical position (needle upward) at 37 °C. 30 min later, the leukocyte-rich supernatant overlaying the erythrocytes was carefully expelled from the syringes and centrifuged at *200 g* for 10 min (4 °C). The resulting cell sediments from each individual were pooled and washed twice in Earle's balanced salt solution. The cells were then resuspended in 2 ml of the same medium. A small sample was withdrawn for a viability test with trypan blue and for determination of the total cell number. The cells were subsequently dispensed at a concentration of 2×10^6 viable cells per ml in minimum essential medium on Earle's base, supplemented with 15% fetal calf serum, penicillin (400 IU/ml) and streptomycin (400 γ/ml). This cell suspension was layered in 2 ml aliquots over Millipore filters (type HA, pore size 0.45 μ, diameter 23 mm), placed at the bottom of sterile liquid scintillation counter flasks (Packard). The flasks were tightly closed and then maintained at 37 °C. After predetermined incubation periods, the filters carrying the cells were removed from the flasks, stained by a methylene-blue parafuchsin procedure[5], dehydrated, embedded on slides and observed light microscopically.

At the time of the first withdrawal of blood for leukocyte culturing, the AML-patients were untreated. The CML- and CLL-patients were in relapse after previous treatment. Leukocyte cultures from all these patients were repeatedly performed also after initiation or resumption of therapy.

Results. Total and differential white blood cell counts. The Table summarizes the results of the total leukocyte counts in the controls as well as in the leukemic patients. From the data of the differential white blood cell counts, only the percentage of *monocytes* in the peripheral blood of the individuals is listed, since this cell type was found to be the only one of importance with respect to the findings reported in this study.

In the controls (group A), the percentage of monocytes was found to vary within normal limits. In smears from multiple blood samples taken at intervals from the patients with CLL (group B), no monocytes were observed. In contrast, a few typical monocytes were always present in the blood smears of both patients with CML (group C), although the percentage of this cell type was consistently low. 5 patients with AML (group D) also exhibited typical monocytes in their peripheral blood, while in 3 others, no such cells were observed before or during therapy.

White blood cell cultures on Millipore filters. After the first 4 h of incubation, the leukocyte cultures from the controls and from the leukemic patients showed a random distribution of the cells on the filters (Figure 1). In the cultures from the controls (group A) and from the patients with CLL (group B) and CML (group C), this pattern remained unchanged even after 22 h of incubation. In some of the specimens, a few irregularly arranged clusters consisting of various cell types (including neutrophilic granulocytes) were observed even at early stages. A rosette-like arrangement of cells was, however, never found in repeatedly performed Millipore filter-cultures from the blood samples of the groups A, B and C.

In contrast, in the leukocyte cultures from 5 patients with AML (Table, group D) who had monocytes in the peripheral blood (individuals No. 1–5), distinct rosettes

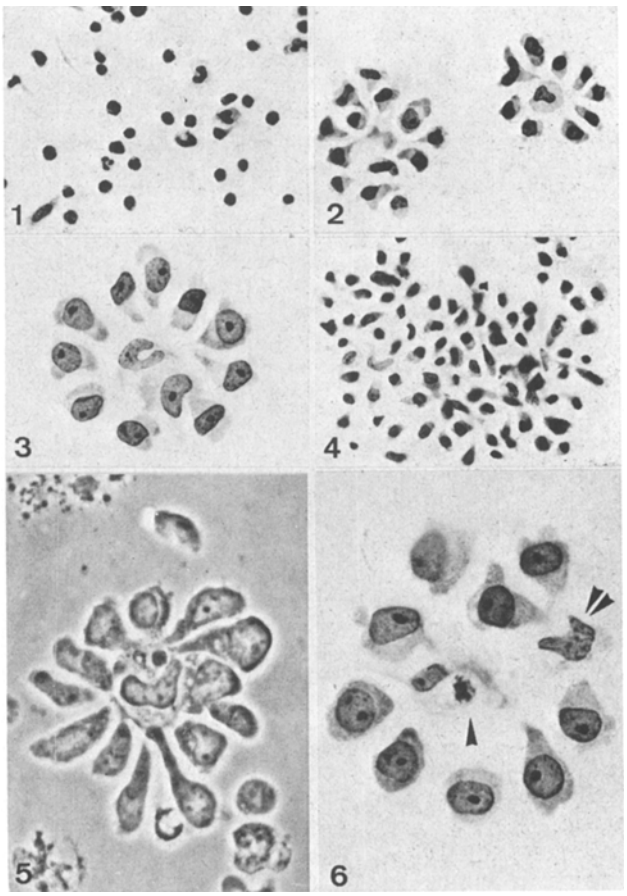

Fig. 1. Leukocyte culture from a patient with acute myeloblastic leukemia (AML), 4 h of incubation. × 390.

Fig. 2. Rosette formation in AML-leukocyte culture after 8 h of incubation. × 470.

Fig. 3. Single rosette with central monocyte surrounded by elongated leukemic myeloblasts, 21 h of incubation. × 780.

Fig. 4. Myeloblasts clustering around monocytes at higher cell concentration in the culture medium. × 390.

Fig. 5. Single rosette with elongated myeloblasts which are in direct contact with the monocyte cell surface. Semi-thin section from Epon-embedded leukocyte culture, 20 h of incubation. × 1000.

Fig. 6. Rosette with central monocyte (arrow) containing ingested cell revealing a pycnotic nucleus. A second monocyte (double arrow) is located among the peripherally distributed leukemic cells. The very pale monocyte cytoplasm is difficult to reproduce. × 1200.

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were visible after 8 h of incubation. All these rosettes consisted of a mononuclear cell surrounded by various numbers of myeloblasts (Figures 2, 3, 5 and 6). The central cells had usually an oval-, kidney- or horseshoe-shaped nucleus with one or several small nucleoli. The abundant, pale and poorly defined cytoplasm appeared homogenous. By cytological criteria, these cells were classified as monocytes. Frequently, they were found to contain a phagocytozed cell or cell remnants (Figures 5 and 6). Leukemic blast cells with a large, round or oval nucleus containing one or several prominent and deeply stained nucleoli were assembled around the monocyte. These blast cells were usually elongated and oriented towards the central cell, the cytoplasmic process often in direct contact with the surface of the monocyte (Figure 5).

Additional experiments indicated that the initial cell concentration in the culture medium was an important factor in obtaining well-defined rosettes. If the concentration exceeded 3×10^6 cells/ml, a loose monolayer developed and the myeloblasts tended to cluster irregularly around the monocytes (Figure 4).

No rosettes were evident in multiple white blood cell cultures from the 3 patients with AML, whose peripheral blood *lacked* monocytes (Table, group D; individuals Nos. 6–8).

Discussion. In this study, where leukocyte cultures from patients with AML, CML, CLL and cultures from healthy controls were checked for the presence of rosettes, such cellular arrangements were, without any exception, observed only in leukocyte cultures from AML-patients *having monocytes* in the peripheral blood. The presence of phagocytozed material in many of the centrally located cells in the rosettes indicates that at least part of these cells had, in the cultures, transformed into macrophages. Such a transformation of monocytes in vitro is known to occur within a few hours of incubation[6].

Although the significance of the spontaneous formation of rosettes in these AML-leukocyte cultures is, at the present time, far from being clear, one might speculate that mechanisms similar to those operative in other rosette-forming systems[1–3] may play a role. Thus, a rosette-like arrangement of erythrocytes around macrophages could be observed, when normal sheep red blood cells were added to lung macrophages from guinea-pigs which had been actively immunized with sheep erythrocytes[2]. The characteristic binding of these 2 cell types was thought to be due to the presence of *cytophilic antibodies*[7,8] elaborated in the animal, which conferred upon its macrophages the ability to adsorb the specific antigen.

Should a similar mechanism be responsible for the rosette-formation in the AML-leukocyte cultures, this would indicate, in the patients' serum, the presence of specific antibodies against the leukemic cells. The finding that gammaglobulin G or its Fc-fragment inhibits, in solution, the formation of rosettes[4], could thereby explain the absence of such cellular arrangements in vivo, i.e. in the AML-patients' peripheral blood[9].

Total white blood cell counts[a], percentage of monocytes in the conventional dry smears and occurrence of rosettes in the leukocyte cultures on Millipore filters

Group	Patient No.	Total cell count (cells/mm³)	% of monocytes (dry smears)	Rosettes in culture
A	1	5,200	5	—
Healthy	2	7,100	3.5	—
controls	3	3,500	4	—
	4	7,200	4	—
	5	4,550	8	—
B	1	106,600	0	—
Chronic	2	111,000	0	—
lymphocytic	3	298,000	0	—
leukemia CLL				
C	1	74,000	1.5	—
Chronic	2	49,000	3	—
myelocytic leukemia CML				
D	1	12,800	4	++
Acute	2	5,200	2	++
myeloblastic	3	186,000	1.5	++
leukemia	4	216,000	2	++
AML	5	23,000	22.5	++
	6	49,000	0	—
	7	168,000	0	—
	8	37,500	0	—

[a] Groups B and C: values before resumption of therapy. Group D: values before initiation of therapy.

Zusammenfassung. In Monozyten enthaltenden Leukozyten-Kulturen von Patienten mit akuter myeloischer Leukämie entstehen nach ungefähr 8 h Inkubationszeit Rosetten, die aus einem von Myeloblasten umgebenen Monozyten bestehen. In Analogie zu anderen in vitro-Systemen, in welchen sich eine Rosettenbildung beobachten lässt, könnte diese zelluläre Reaktion auf dem Vorhandensein von spezifischen Antikörpern im Patienten-Serum beruhen, welche gegen die leukämischen Myeloblasten gerichtet sind.

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Relation between Carcinogenicity and Metabolic Reduction of 4-Nitroquinoline 1-Oxide Derivatives

The reduction product of 4-nitroquinoline-1-oxide (4NQO), 4-hydroxylaminoquinoline-1-oxide (4HAQO) has been reported to be carcinogenic[1–3]. The metabolic pathway which converts 4NQO to 4HAQO was detected in animal tissues[4–6]. This report compares the susceptibility of a number of derivatives of 4NQO to be metabolized to the hydroxylamino compounds in relation to their carcinogenicity, as part of studies on their mechanism of action.

Materials and methods. All compounds were synthesized in this Institute. Compounds dissolved in a small amount