Zusammenfassung

Pflanzen, die mit Diazinon gespritzt wurden, enthalten biochemisch aktive Rückstände des Insektizids auch nach vergleichsweise langer Zeit; 50% der aktiven Stoffe waren vorhanden 25 Tage nach der Spritzung; 20% der ursprünglichen biochemischen Aktivität wurden noch nach 9 Wochen festgestellt.

Reines (99,9%) Diazinon enthielt ausser dem Diazinon eine andere cholinesterasehemmende Substanz, wahrscheinlich identisch mit dem Sauerstoffanalogon des Diazinons. In einem ein Jahr alten reinen Diazinonpräparat konnten 6 Komponenten papierchromatographisch nachgewiesen werden. 3 Bestandteile einer derartigen Mischung konnten identifiziert werden: Diazinon, sein Sauerstoffanalogon und sein S-ethylisomeres. Die beiden letzteren wurden wahrscheinlich bei der Behandlung von Diazinon mit Brom bzw. bei seiner Erhitzung auf 140°C als Hauptprodukte gebildet.

Demonstration of Specific and Non-specific Agglutinogens in the Normal Bone Marrow Erythroblasts

The presence in the bone marrow erythroblasts of agglutinogens (specific and non-specific) similar to those of the mature erythrocytes, though theoretically intuitive, has up to now been suggested only indirectly. For instance, BJÖRKMAN[1] reports the agglutinability of erythremic cells by influenza virus and by a serum containing cold agglutinins, and WAGNER[2] that of erythroblasts of the rat by an anti-rat-erythrocytes serum. More recently PISCIOTTA and HINZ[3], using circulating erythroblasts in a case of acquired haemolytic anaemia, have shown that they agglutinate with group-specific sera, and with sera containing autohaemoagglutinins.

The problem is especially interesting in relation to the suggested bone marrow involvement in course of immunohaemolytic syndromes: the bone marrow vulnerability in such pathological conditions, demonstrated by several different studies (GASSER[4], SACCHETTI, ROSSI, and DIENA[5]), would be supported by the finding of erythroblastic agglutinability.

The present work has been then directed to establish:

(1) whether group-specific antigens exist in the normal bone marrow erythroblasts, and the stage of their appearance in the cells; and

(2) whether the erythroblasts are agglutinated by non-specific agglutinating sera.

The erythroblasts of the bone marrow have been separated by a fractionated centrifugation technique (BRACCO, CURTI, and MASERA[6]), modified with the addition of Polyvinylpyrrolidone, which makes it possible to obtain in a short time (15 min from the bone marrow puncture) an erythroblastic suspension of 90–95%. For the agglutination tests with grouping sera, a drop of the agglutinating serum was added, in serological tubes, to a drop of an erythroblastic suspension at 40–50% in saline.

For sera containing auto-antibodies, the erythroblasts, at 2–4% in saline, were added to equal volumes of serum.

The readings of the agglutination, after incubation at the appropriate temperature for not longer than 30 min to avoid cellular damage, were taken macro- and microscopically.

In case of incomplete antibodies, compatible normal serum was added to the erythroblasts-serum-mixture, and the readings were taken as above.

All glassware used was siliconated.

Table I shows the results of some experiments of agglutination of normal erythroblasts by group-specific sera: the results obtained were in complete agreement with those obtained when mature erythrocytes of the same subjects were used instead of bone marrow erythroblasts.

Table I

Case No. 1		Case No. 2		Case No. 3	
Anti-A	–	Anti-A	+	Anti-A	–
Anti-B	–	Anti-B	–	Anti-B	+
Anti-M	+	Anti-M	–	Anti-M	+
Anti-N	–	Anti-N	+	Anti-N	+
Anti-D	+	Anti-D	–	Anti-D	+
Anti-Lewis Le^a	–	Anti-Lewis	+	Anti-Lewis	–
Anti-Lutheran Lu^a	+	Anti-Lutheran	–	Anti-Lutheran	+

+ denotes agglutination; – denotes no agglutination.

Inspection of the agglutinates, in freshly made preparations carefully observed with phase-contrast microscopy, or after staining of the slides, made it possible to recognize the presence of basophilic erythroblasts also in the earlier phases.

Table II

Agglutinating sera	Type of antibody	Optimal temperature	Agglutination
1	Incomplete	37	+
2	Incomplete	37	+ + +
3	Incomplete	20	+ +
4	Complete	4	+ + + +
5	Complete	20	+ + + +
6	Complete	4	+ + +

+, + +, + + +, + + + + denote varying degrees of agglutination.

Table II shows the results of erythroblastic agglutination by sera containing auto-antibodies, cold and warm type, complete and incomplete form.

Our observations indicate that the bone marrow erythroblasts possess group-specific antigens similar in all respects to those found on the mature erythrocytes. Such antigens are present also in the most immature basophilic erythroblasts: this fact demonstrates that the presence of antigens is unrelated to the appearance of haemoglobin in the cells. The demonstration that the erythroblasts are strongly agglutinated by sera containing auto-antibodies of different type seems to be significant: this suggests that, during the immunohae-

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[4] C. GASSER, Sang *26*, 6 (1955).

[5] C. SACCHETTI, V. ROSSI, and F. DIENA, Boll. Soc. ital. Ematol. (to be published).

[6] M. BRACCO, P. C. CURTI, and N. MASERA, Acta haematol. *6*, 91 (1951).

molytic process, the antibody may attach itself directly not only to the circulating red cells but also to the bone marrow normoblasts, thus causing the bone marrow impairment demonstrated by different studies (GASSER[4], SACCHETTI, ROSSI, and DIENA[5]).

This possibility refers to complete and incomplete antibodies, and also to antibodies acting optimally *in vitro* at temperatures below 37°C.

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Zusammenfassung

Es wurde untersucht, ob die Erythroblasten im menschlichen Knochenmark spezifische und unspezifische Antigene, entsprechend den Erythrozyten, besitzen. Diese Eigenschaft wurde auch bei den unreifsten basophilen Erythroblasten beobachtet.

Differences in the Desoxyribonucleoprotein Complex of Normal and Leukemic Human Lymphocytes

The biochemical findings of MIRSKY and RIS[1] indicate that histone occurs in constant quantitative ratio to DNA in cell nuclei of the same animal species.

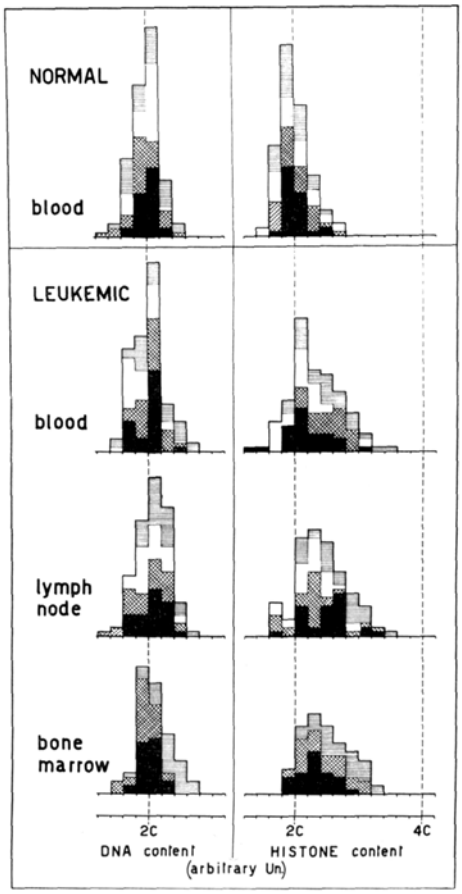
ALFERT and GESCHWIND[2], using an original staining method, observed that the nuclear content of the histone stained with Fast green at pH 8 is in constant ratio with corresponding nuclear content of Feulgen stained DNA.

These findings have been confirmed by us, in rat and normal human lymphocytes[3].

In proliferating tissues, such as young rat liver cells[4] and granuloblasts of normal human bone marrow[5], the Feulgen/Fast green ratio was found to be markedly lower, presumably[4] due to a higher Fast green stainability of the histone during nuclear synthesis of desoxyribonucleoprotein.

In order to check the behaviour of the nucleoprotein complex in leukemic nuclei, cytophotometric determinations of the Feulgen stained DNA and the Fast green stained histone were carried out in the lymphocytes of four cases of lymphatic leukemia.

The cytophotometric apparatus was that described by one of us, co-working with VIALLI[6]; the Feulgen reaction was performed as described by POLLISTER[7]; the Fast green staining according to ALFERT and GESCHWIND[2], as previously described[8].



Nuclear content of DNA and histone in lymphocytes of four normal subjects and of four cases of lymphatic leukemia. Values of each case are differently hachured and graphically exposed in function of the normal diploid (2 C) content. The data of bone marrow in one case are missing. In ordinate nuclear frequency; scales are linear.

The measurements were made in blood, bone marrow and lymphnode smears of leukemic subjects, and in normal blood smears, stained simultaneously (Figure).

Mean values, obtained in *blood smears* by thirty determinations in each case, are summarized in the Table.

The Table and the graphic show that the Fast green stained histone content of the leukemic cells is greater

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[5] S. PERUGINI, U. TORELLI, and M. SOLDATI, Riv. Istochim. norm. pat. *3*, 1 (1957).

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[7] A. W. POLLISTER, Rev. Hémat. *5*, 527 (1950).

[8] S. PERUGINI, U. TORELLI, and M. SOLDATI, Riv. Istochim. norm. pat. *2*, 449 (1956).

	DNA		Histone	
	Normal	Leukemic	Normal	Leukemic
Case 1	9·99 ± 0·12	9·85 ± 0·16	10·08 ± 0·15	10·92 ± 0·30
Case 2	9·84 ± 0·23	10·33 ± 0·21	9·99 ± 0·23	12·90 ± 0·32
Case 3	10·03 ± 0·13	9·42 ± 0·18	10·05 ± 0·30	10·62 ± 0·22
Case 4	9·90 ± 0·23	10·90 ± 0·36	9·88 ± 0·18	12·36 ± 0·31

(Mean values ± standard error)