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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Institute of Medical Pathology, University of Genoa (Italy), April 29, 1957.

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.

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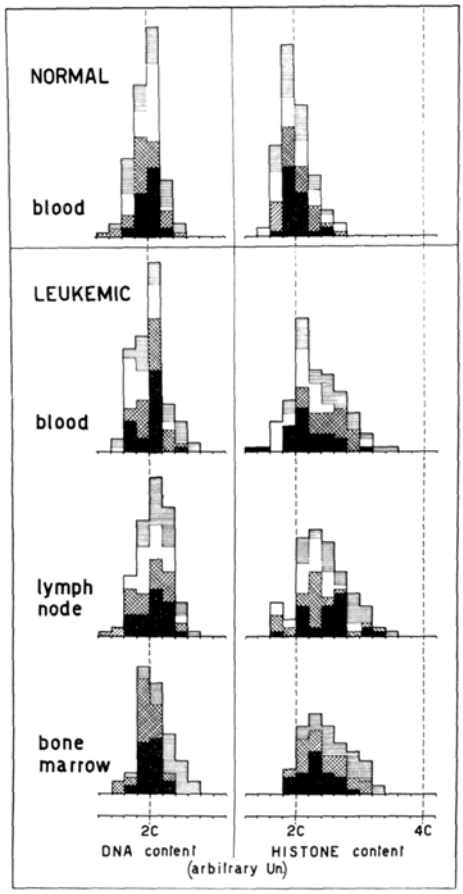
[3] S. PERUGINI, U. TORELLI, and M. SOLDATI, Riv. Istochim. norm. pat. *2*, 449 (1956); Riv. Istochim. norm. pat. *3*, 1 (1957).

[4] D. P. BLOCH and G. C. GODMAN, J. biophys. biochem. Cytol. *1*, 531 (1955). – S. PERUGINI, U. TORELLI, and M. SOLDATI, Riv. Istochim. norm. pat. *2*, 449 (1956).

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

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

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	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)

than that of normal lymphocytes, although the DNA values are similar in both cell categories, indicating the lack of a DNA movement in the pathological lymphocytes.

Our findings do not allow us to decide whether the decreased Feulgen/Fast green ratio in the leukemic lymphocytes is due to an absolute increase in histone, or to a modified Fast green stainability.

The biochemical observations of CRUFT *et al.*[9], indicating the abnormal properties of histones from malignant cells, seem to support the latter hypothesis.

In any case, it seems that in leukemic lymphocytes the properties of the desoxyribonucleoprotein complex are different from those of normal lymphocytes.

A detailed account of this work, with statistical analysis of the data, will be published elsewhere.

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S. PERUGINI, U. TORELLI, and M. SOLDATI

Institute of Medical Pathology, University of Modena (Italy), May 23, 1957.

Riassunto

Gli autori hanno determinato con metodo citofotometrico nel visibile il contenuto di acido desossiribonucleico (DNA) e di proteina istonica nei linfociti di 4 soggetti normali e di 4 pazienti affetti da leucosi linfatica.

I valori del DNA sono apparsi uguali nelle cellule normali e in quelle leucemiche, i valori dell'istone sono risultati in queste ultime superiori alla norma, onde il rapporto DNA/istone risulta essere nei linfociti leucemici inferiore a quello dei linfociti normali.

[9] H. J. CRUFT, C. M. MAURITZEN, and E. STEDMAN, Nature *174*, 580 (1954).

Muscle Adenylic Deaminase

After the discovery of adenylic deaminase by SCHMIDT[1], partially purified preparations of this enzyme have been described by a number of investigators[2]. Recently, the extensive purification and crystallization of this enzyme has been reported[3]. Most methods used for the preparation of the enzyme start with procedures which are ineffective in extracting the bulk of the enzyme[2]. The bulk of the adenylic deaminase is extractable only at high salt concentrations. The effect of increased concentration of salt upon adenylic deaminase extraction from acetone powders of tissues of several species is shown in the Table. As indicated in the Table, only after reaching molarities of 0·5 *M* and higher is the bulk of the enzyme extractable. Advantage has been taken of this, and of the denaturation and/or insolubilization of other enzymes by acetone treatment, to obtain with little effort an active preparation free of easily soluble enzymes and of myokinase. The procedure described here is intended to provide a simple reproducible preparation useful for analytical purposes. The data emphasizes the high level in skeletal muscle of this enzymatic activity.

Acetone powders of tissues are prepared by a standard procedure[4]. When kept at 0° in a dessicator they are stable for over 1 year. Some purification and concentration of the enzyme is accomplished as follows: Extract rabbit muscle acetone powder with 10 vol of water w/v for 10 min. All centrifugations were carried out at 5000 × *g* and at 0°C. The ammonium sulfate solutions were saturated and adjusted to pH 7·4 at 0°C. All the steps of the fraction procedure were carried out at 0°C.

The effect of salt concentration upon the extractibility of adenylic acid deaminase

Species	Extracting medium				
	Water	0·2 *M* KCl	0·25 *M* KCl	0·5 *M* KCl	1 *M* KCl
	Units/ml	Units/ml	Units/ml	Units/ml	Units/ml
Rabbit .	185	420	2200	4300	5500
Pigeon .	340	–	777	1200	1400
Chicken .	180	–	1000	1250	1400

Centrifuge and discard the supernatant fluid. Re-extract with 10 vol w/v of 0·2 *M* KCl and discard the supernatant fluid. Re-extract with 10 vol of 1 *M* KCl w/v twice and keep the supernatant fluids after centrifugation. The combined supernatant fluids have about 2000 units[5] and 7 mg protein per ml, thus yielding more than 20 times the enzyme activity, per gram muscle, of other procedures[5]. Add to the crude KCl extract ¼ vol of 0·6 *M* disodium phosphate and ⅓ vol saturated ammonium sulfate pH 7·4; discard the precipitate and precipitate the enzyme by adding ²/₃ vol of ammonium sulfate. (All vol refer to the initial volume of the crude KCl extract.) The recovery is from 90–95% and the specific activity is increased approximately 2 fold e.g. 600–700[5]. CONWAY[6] observed that saline was more effective than water for the extraction of the enzyme, however he did not explore further the effect of high concentrations of salts. He also showed that the adenylic deaminase appeared to have a very low affinity for the substrate and he discussed the possible existence of inhibitors. Whether the adenylic deaminase present in the preparation here described possesses tightly bound inhibitors is not known. The addition of crude acetone powder extracts with little adenylic acid deaminase activity, e.g. heart, is not inhibitory; however, when comparing activity at several dilutions, we have observed an apparent higher activity at higher dilutions of the enzyme. We have observed that when using initial velocities and less than 10–20% substrate utilization, there is essentially a doubling of rate by doubling the substrate concentration between 2×10^{-5} *M* and 2×10^{-3} *M*. This is in sharp contrast with the data of NIKIFORUK and COLOWICK[5] but in agreement with the data of CONWAY and COOK[6].

From the activity measurements shown in the Table it has been calculated that at 37°, pH 6·4[5] and 4×10^{-5} *M* 5-adenosine monophosphate, a gram of muscle can deaminate over 15 micromoles 5-adenosine monophosphate/min and this figure would be higher at higher concentrations of 5-adenosine monophosphate. It ap-

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