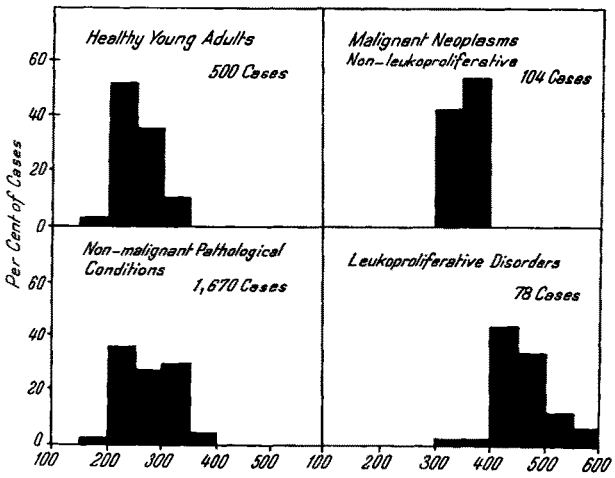

Fig. 1.–Distribution of the DPA indices in four groups of human sera.

tosus, polycythemia vera, and ADDISON's disease. The DPA index ranged from 160 to 376 units, with a mean value of 274 units, and a probable error of 4·78 units.

The third group was 104 persons, 5 to 88 years of age, with maignant neoplasms, not leukoproliferative, consisting of breast tumor, cancer of the prostate, cancer of the liver, ovarian tumor, skin cancer, brain tumor, osteogenic sarcoidosis, cancer of the esophagus, lung cancer, intestinal cancer, cancer of the tongue, and kidney tumor. The DPA index ranged from 303 to 406 units, with a mean value of 370 units, and a probable error of 5·02 units.

The fourth group, consisting of 78 leukoproliferative conditions or those involving the blood forming organs, 3 to 75 years of age, included acute and chronic leukemia, acute monocytic leukemia, and chronic granulocytic leukemia, reticulum cell sarcoma, lymphosarcoma, lymphoblastoma, multiple myeloma, and HODGKIN's disease. The DPA index ranged from 298 to 600 units, with a mean value of 448 units. The probable error was 5,38 units.

We were fortunate to be able to test several extremely early cases of lymphatic leukemia. These cases gave DPA indices of 298 to 304 units. All other cases of leukoproliferative disorders had a DPA index of 400 units or higher when we first tested them.

The mean values for the normal controls and the non-malignant pathological states differed by 26 units, but the mean value for the malignant neoplasms was 122 units higher than the normal. The mean value of the leukoproliferative disorders was 78 units higher than the malignant neoplasms and 200 units higher than the normals. This difference from malignant neoplasms is sufficiently high to be useful in the diagnoses of leukemia.

The figure shows the distribution of the DPA indices in the four groups of subjects. The highest DPA index values correspond to the leukoproliferative conditions. Multiple myeloma gave especially high values, 520 to 606 units. The index always becomes higher as the leukemia progresses. When drugs such as ACTH, Cortisone, Aminopterin (Lederle), triethylene melamine (Lederle), nitrogen mustards, or X-radiation were given to control the leukoproliferative disorders of patients, the steady increase in the DPA index nevertheless persisted. Even through the disease was in a state of remission or relapse, as revealed by the blood and bone-marrow studies, the DPA index continued to increase and always remained elevated. All other diseases showed a decrease to the normal index range when the patient was in the recovery stage.

The range, 303 to 406 DPA index units, for malignant neoplasms indicates that the test might also be useful as a screening test for malignancy. Leukemia may be easily distinguished from these processes during the advanced stages when the index is high. Border-line cases will require additional laboratory investigation.

Infectious mononucleosis gave no abnormal rise in the index reading, and therefore, it may be distinguished from leukemia even if the cytologic picture sometimes may be confusing.

Knowledge that the basic abnormality of a leukoproliferative disorder was present would lead to an earlier diagnosis and provide a basis upon which to begin the available therapeutic measures and to study new ones.

Further details of this work will be published elsewhere.

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Department of Research, Diagnostic Laboratoires, Miami Valley Hospital, Dayton, Ohio, June 28, 1954.

Zusammenfassung

Alle leukämischen Kranken hatten einen hohen Diphenylamin(DPA)-Index. Falls das mikroskopische Blutbild keine sichere Aufklärung gibt, kann der DPA-Index zur diagnostischen Unterscheidung zwischen Pseudoleukämie und echter Leukämie angewendet werden.

Alkali-Resistant Cooley's Anemia Hemoglobin is Different from Alkali-Resistant Fetal Hemoglobin

An alkali-resistant hemoglobin that has all the properties of normal fetal hemoglobin has been described in COOLEY's anemia[1] and related Mediterranean hemopathic syndromes[2].

This Hb and the fetal Hb react identically to the denaturation in aqueous alkaline solutions, move the same distance on paper electrophoresis, show the same properties in crystallization, solubility and ultraviolet absorption spectra.

Most authors are therefore inclined to believe that such hemopatic conditions are associated with a congenital inability to pass from fetal to adult mechanism of hemoglobin synthesis.

We all know the importance of the application of several independent methods to check the homogeneity of the protein specimens.

The following differences between fetal and COOLEY's anemia Hb already have been demonstrated in our Institute:

(1) While their correspondent oxyhemoglobins are known to be denaturated by alkali at the same rate, they are denaturated by acids at different rates[3];

(2) their carbon monoxide derivatives are denaturated both by alkali and by acids at different rates[1].

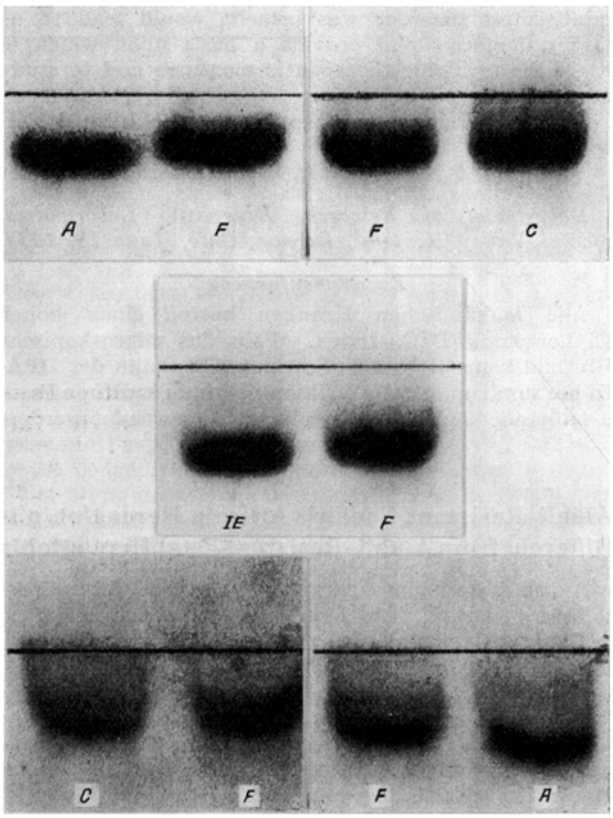
[1] F. VECCHIO, La Pediatria *54*, 529 (1946). – T. PUTIGNANO and L. FIORE-DONATI, Boll. Soc. ital. Biol. sper. *24*, 277 (1948). – K. SINGER, A. I. CHERNOFF, and L. SINGER, Blood *6*, 429 (1951). – A. M. LIQUORI, Nature *167*, 950 (1951). – A. RICH, Proc. Nat. Acad. Sci. U.S. *38*, 187 (1952). – G. SANSONE and F. CUSMANO, Boll. Soc. ital. Biol. sper. *26*, 1343 (1950).

[2] L. PEROSA, T. PUTIGNANO, and L. FIORE-DONATI, Boll. Soc. ital. Biol. sper. *25*, 1204 (1949). – S. AGNISETTA and S. MASSENTI, Minerva Med. *2*, 661 (1953). – S. CUTILLO, Personal communications.

[3] T. PUTIGNANO and S. COGNETTI, Boll. Soc. ital. Biol. sper. *28*, 1157 (1952).

Now we have been able to show that paper electrophoresis provides evidence of a different behaviour for fetal and COOLEY's anemia Hbs.

After repeated trials, we have used the following technique which is very similar to that described by REYNAUD[1]. The FLYNN and DE MAYO electrophoretic apparatus was used filled up with sodium veronal buffer (pH 9). Two Hb spots were applied on each the 6 cm strips of WHATMAN filter paper n.1, by means of PEROSA's gadget[2]. After 12 h electric current flow (120 W), immediate fixation was accomplished with an alcohol-ether mixture.



Examples of different electrophoresis runs. *A* = Adult normal Hb; *F* = Fetal Hb (umbilical cord blood); *C* = Hb of COOLEY's disease; *IE* = Congenital hemolytic spherocytic jaundice.

Finally the paper strips were dried at 80°C and the spots evidenced by naphtalene black. The Hb solutions were obtained from patients suffering from COOLEY's anemia, and from the umbilical cord and prepared by a method used also by SPAET[3].

The results obtained may be summarized as follows:

(1) In agreement with previous observations of REYNAUD, the migration speed of adult Hb is greater than that of the fetal, whereas the migration of COOLEY's anemia Hb has an intermediate rate.

(2) We have also noticed that the pattern of the spots, after evidencing with naphtalene black, was characteristically different for each kind of Hb considered (Figure).

(a) Adult Hb, besides its farther migration from the starting line, appears like a single and well defined band. (Identical behaviour has shown the Hb in one case of congenital hemolytic spherocytic jaundice.)

(b) Fetal Hb, besides its dislocation nearer to the starting line, shows a darker front band followed by a lighter one with distinct contours: the space between the second spot and starting line is colourless.

(c) The Hb of COOLEY's anemia, besides its intermediated speed between the fetal and adult Hb, shows a front band followed by a lighter one, wich unlike fetal Hb, does not show a sharp contour shading to the starting line.

These findings have been reproduced constantly. The pattern of adult Hb therefore substantiates ITANO's statement that the normal adult Hb is a form that occurs free of other components.

COOLEY's anemia and fetal Hbs, on the other hand, are resolved into two bands: the faster possibly corresponding to the adult Hb, the slower corresponding, in one case, to the fetal, and in the other, to COOLEY's Hb. These two bands, as shown above, have different patterns.

These results, which apparently have been obtained also in the DERRIEN's Laboratory[1], will be further discussed on another occasion.

The electrophoretic behaviour, the different resistance of Hb O_2 to denaturation by acids, and the different resistence of Hb CO to denaturation by alkali and acids, strongly suggest that the COOLEY's and fetal Hbs are different.

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Institute of Clinical Medicine, University of Bari, March 18, 1954.

Riassunto

Gli autori, basandosi sul fatto che l'Hb alcaliresistente del m. di COOLEY si comporta diversamente dall'Hb alcali-resistente del feto, sia per quanto riguarda la denaturazione dell'Hb O_2 con gli acidi, sia per quanto riguarda la denaturazione dell'Hb CO con gli acidi e con gli alcali, e sia, infine, per quanto riguarda il loro comportamento elettroforetico (su carta), prospettano la possibilità che le due Hb alcali-resistenti siano diverse una dall'altra.

[1] Y. DERRIEN, Personal communication.

[1] J. REYNAUD, C. r. Soc. Biol. *147*, 838 (1953).

[2] L. PEROSA and G. RACCUGLIA, Boll. Soc. ital. Biol. sper. *27*, 1590 (1951).

[3] T. H. SPAET, J. Lab. Clin. Med. *41*, 161 (1953).

In vitro Inhibition of Catalase by Ovomucoid

HARGREAVES and DEUTSCH[1] have discovered that the kochsaft of tumors is endowed with anticatalase activity.

We have found that hen's ovomucoid acquires, after boiling, a strong inhibitory power against catalase. The ovomucoid was prepared as follows: the egg white diluted 1:1 with saline was placed in a boiling water-bath for 15 min in tightly stoppered glass tubes. After filtration through paper, the clear filtrate containing the ovomucoid was used for the assays. In our experiments, 1 ml of filtrate was incubated for 1 h at 0°C with 0·5 ml of 0·02 per cent horse liver catalase (prepared according to BONNICHSEN[2]) and the catalase activity was then determined spectrophotometrically[3].

The anticatalase activity of ovomucoid has been found to be proportional to the time of boiling (Fig. 1 *a*),

[1] A. B. HARGREAVES and H. F. DEUTSCH, Cancer Res. *12*, 720 (1952).

[2] R. K. BONNICHSEN, Acta Chem. Scand. *2*, 561 (1948).

[3] F. ABRIGNANI and V. MUTOLO, Boll. Soc. ital. Patol. *3*, 96 (1953).