Electrophoretic, Nitrogen, Lipide and Enzyme Studies of the Plasma and Plasma Fractions in Cancer*

GEORGE H. L. DILLARD, H. ROWLAND PEARSALL, AND ALFRED CHANUTIN

(From the Biochemical Laboratory, Medical School, University of Virginia, Charlottesville, Virginia)

Electrophoretic and chemical studies of the plasma and plasma fractions of injured animals and diseased humans show marked variations from the respective controls (5, 6, 7, 13). The available information concerning the detailed changes of plasma proteins of patients with cancer is limited. This paper is concerned with the electrophoresis and chemistry of plasma and three plasma protein fractions together with data for three typical plasma enzymes in a group of patients with various types of cancer.

METHODS

The diagnosis for each patient is established. The diagnoses for the 36 cases studied are as follows: carcinoma of the bronchus, 3, pancreas, 3, prostate, 9, stomach, 5, colon, 2, breast, 3, ovary, 1, kidney, 1, larynx, 1, hepatoma, 1, myelogenous leukemia, 2, and lymphoblastoma, 5. These cases are free of detectable intercurrent infection or massive necrosis of tumor tissue. The control group consists of 17 healthy young men.

Fasting samples of blood (50 ml.) are drawn into heparin-moistened syringes and are centrifuged immediately in Lusteroid tubes. The fractionation of the separated plasma is begun within 30 to 60 minutes after the blood is collected.

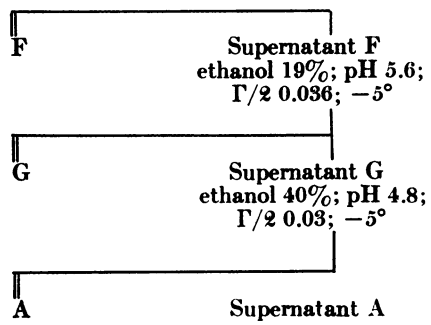
A relatively simple procedure for fractionating plasma is outlined in Diagram 1.

DIAGRAM 1

FRACTIONATION OF PLASMA[1]

Plasma

Ethanol 8%; pH 7.2; Γ/2 0.04; 0°



1. The plasma (18 ml.) is adjusted to pH 7.2 with 3 to 4 ml. of phosphate buffer (pH 6.4, Γ/2 0.15). Calculated amounts of water are added, the mixture cooled to 0 to 1.0°, and the ethanol concentration adjusted to 8 per cent with 95 per cent ethanol. The 5 × diluted plasma is stirred for 2 minutes and fraction F is removed by centrifuging at 0°.

2. Supernatant F is adjusted to pH 5.6 with an acetate-acetic acid buffer (pH 4.0; Γ/2 0.8) and sufficient 95 per cent ethanol is added to give a final concentration of 19 per cent. The final volume is 5.5 × the original plasma volume. Fraction G is removed by centrifuging at −5°.

3. Supernatant G is adjusted to pH 4.8 with about 0.8 ml. of acetate-acetic acid buffer (pH 4.0, Γ/2 0.4) and sufficient 95 per cent ethanol is added to yield a final concentration of 40 per cent. This represents a 7 × dilution.

The three fractions are dissolved in 0.85 per cent saline and brought to 10 ml. volumes shortly after precipitation.

Aliquots of each fraction are diluted with equal amounts of barbiturate-NaOH buffer (pH 8.6, Γ/2 0.1) and dialyzed against 2 liters of this buffer for 3 days in the cold. Electrophoresis is carried out in the Tiselius apparatus according to Longsworth's modification of the schlieren method (11) in a micro cell of 2 ml. capacity.[2] The ascending patterns are used for analysis and no attempt is made to calculate mobilities.

The plasma and fractions are analyzed for lipide carbon (18), cholesterol (16), and nitrogen.

* This investigation was supported by research grants from the National Cancer Institute, U.S. Public Health Service, and from the Office of Naval Research.

[1] The first step is similar to the procedure for obtaining Fraction I in Method 6 of Cohn et al. (3). Most of the fibrinogen is thus removed and avoids the clotting that is occasionally observed. The second step depends on the observation made by Cohn and associates (4) that practically all the globulins are precipitated by adjusting diluted plasma to pH 5.6 and 19 per cent ethanol at −5°. The conditions for obtaining the last fraction are similar to those used in Method 6 (3) for obtaining Fraction V. The nomenclature of plasma protein fractions is still confused and must await clarification by such workers as those at Harvard who are intensively studying this problem. Consequently, the 3 fractions obtained in this work are named F, G and A as a matter of convenience to indicate that fibrinogen, globulin and albumin are present in appreciable amounts in each of the respective fractions.

[2] Purchased from Pyrocell Mfg. Co., New York City.

Alkaline and acid phosphatases are estimated by the methods of Binkley, Shank, and Hoagland (2). Amylase is determined according to the procedure recommended by Andersch (1). The activity of these enzymes decreases particularly in the fractions, when stored at temperatures slightly above freezing. Quick-freezing of the samples prevents this deterioration and therefore all analyses are done immediately, or after storage in the frozen state. The nitrogen, lipide carbon, cholesterol content, and enzyme activities are expressed on the basis of 100 ml. of whole plasma.

ELECTROPHORETIC ANALYSES OF WHOLE PLASMA AND FRACTIONS

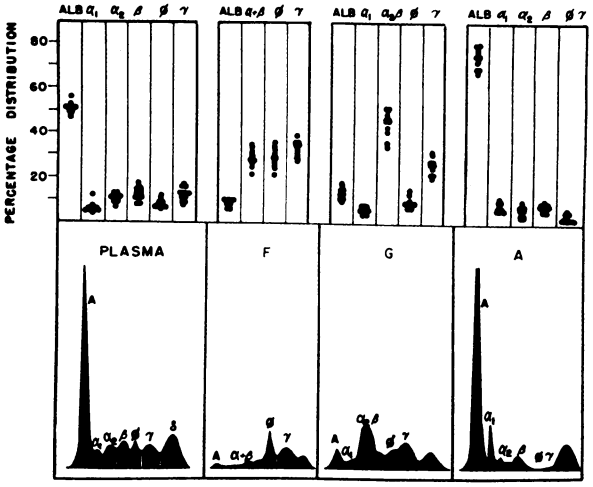


FIG. 1.—The distribution of the protein components of plasma and plasma fractions of healthy young men.

RESULTS

Typical electrophoretic patterns and the percentage distributions of the protein components of the plasma and 3 plasma fractions of healthy young men are shown in Figure 1. Fraction F contains most of the fibrinogen and small amounts of α, β and γ globulins. According to Morrison (12), most of these globulins are occluded by fibrinogen under the precipitation conditions. The greater portion of the β and γ globulins of the plasma are present in fraction G. The last fraction probably contains α_1 lipoprotein, α_2 glycoprotein, the β_1 metal combining protein as well as most of the plasma albumin.

The electrophoretic percentage distributions of the protein components of the plasma and plasma fractions of cancer patients are shown graphically in Figure 2. The most striking changes noted are the consistently low values for albumin in the plasma and in fraction A and the increases in the fibrinogen of fraction F.

About 75 per cent of the plasma nitrogen values of cancer patients are within the control range (Fig. 3). No correlation between the degree of cachexia and physical findings and the nitrogen concentration of the plasma is possible. About one-third of the values for fraction F are elevated. No consistently abnormal distribution for the fraction G values is seen. Approximately 80 per cent of the fraction A values are below the control range.

The distributions of lipide carbon and cholesterol in plasma and in fraction G are shown in Figure 4. Fractions F and A contain small amounts of cholesterol. No explanation can be offered for the lipide carbon values of the plasma which fall outside the control range. Most of the cholesterol values of the plasma and fraction G are in the lower portion or below the control range. The values for the per cent free cholesterol of the plasma are elevated in about 40 per cent of the patients but are not as marked in fraction G. The two highest values of 53 and 75 per cent are obtained in two cases of carcinoma of the pancreas with common duct obstruction. The remaining

ELECTROPHORETIC ANALYSES OF WHOLE PLASMA AND FRACTIONS

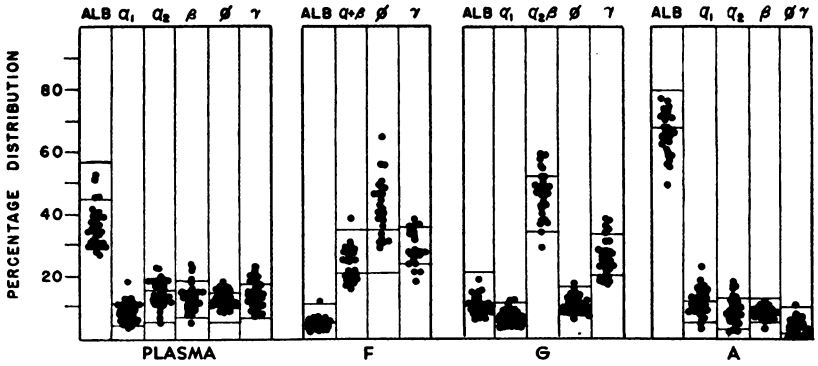


FIG. 2.—The distribution of the protein components of plasma and plasma fractions of cancer patients. The rectangles represent the respective control ranges.

elevated values cannot be related to any signs of liver damage.

Data for alkaline and acid phosphatase and for amylase in plasma and plasma fractions are shown in Figure 5. The alkaline phosphatase of the plasma and fraction A is elevated in about 50 per cent of the cases: a greater proportion of the fraction G values are above the control level. It is worthwhile pointing out that the activity of the alkaline phosphatase of fraction A is consistently greater than that of fraction G in each case.

The highest values for acid phosphatase are encountered in the plasmas of 5 patients with disseminated carcinoma of the prostate. When the plasma values are elevated, the enzyme is generally increased to about the same extent in fractions F and A.

Large variations from the control range are observed in the amylase content of the plasma, and fractions G and A. Attempts to account for these changes on the basis of clinical and pathologic observations are not successful.

DISCUSSION

It is generally concluded from electrophoretic studies of the plasmas of cancer patients that the changes observed are usually nonspecific (9, 10, 14, 15, 17). Pearsall and Chanutin (13) conclude that the degree of alteration in the plasma proteins appears to be determined by the severity of the disease. Generally, tissue destruction, infection, and cachexia are accompanied by a decrease in plasma albumin and usually by an increase in fibrinogen and possibly other globulins. The electrophoretic, nitrogen and lipide analyses of plasma fractions in these experiments do not aid in distinguishing cancer from other diseases.

Greenstein (8) has pointed out that alkaline phosphatase is elevated in metastases to bone and in lymphoid disease. In the present study increases in the activity of this enzyme are observed in all

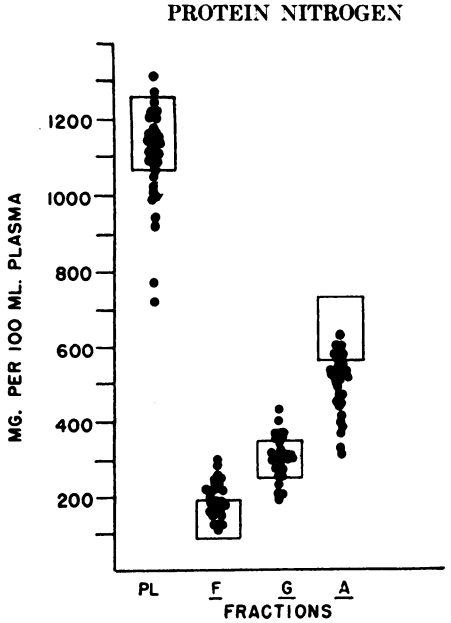


FIG. 3.—The distribution of protein nitrogen in the plasma and three plasma fractions of cancer patients. The rectangles represent the control ranges.

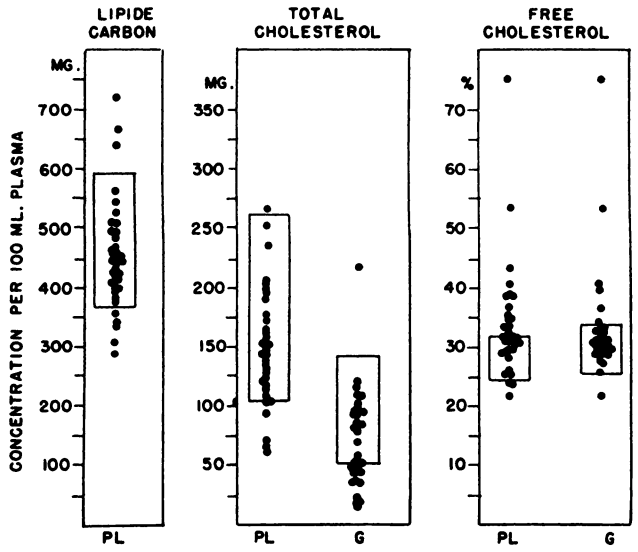


FIG. 4.—The distribution of lipide carbon in plasma and cholesterol in plasma and Fraction "Globulin" in cancer patients. The rectangles represent the control ranges.

cases with bone metastases and in 4 of the 7 cases with lymphoid malignancies. The marked changes in acid phosphatase are limited to disseminated carcinoma of the prostate. The amylase activity of the plasma and its fractions is too varied for interpretation. It appears that cachexia is not necessarily a factor in determining the changes in amylase concentration.

SUMMARY

Methods are outlined for fractionating small volumes of plasma into fibrinogen, globulin, and albumin-rich fractions by the low temperature-ethanol procedures.

Electrophoretic, nitrogen, lipide carbon, cholesterol and enzyme analyses of the plasma and three plasma fractions of 17 healthy young men and of 36 patients with carcinoma of the prostate, stomach, pancreas, bronchus, breast, colon, ovary, and larynx; lymphoblastoma, myelogenous leukemia, and hepatoma are presented. The electrophoretic and nitrogen analyses show: (a) a consistent decrease in albumin, (b) an increase in fibrinogen, and (c) few significant changes in the globulin components. The changes in the lipides of plasma and its fractions are due to cachexia or hepatic involvement. The distributions of the alkaline and acid phosphatases and amylase of plasma fractions are discussed.

The results presented for the plasma protein fractions do not yield information which is of value in the diagnosis of cancer.

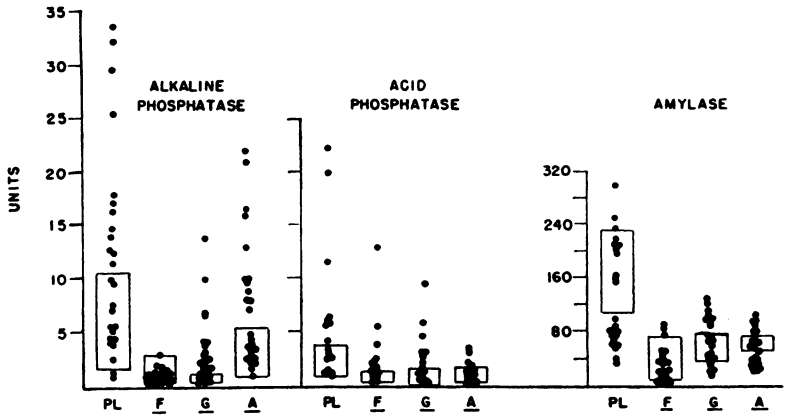


FIG. 5.—The distribution of three enzymes of plasma and three plasma fractions of cancer patients. The rectangles represent the control ranges.

REFERENCES

1. ANDERSCH, M. A. The Determination of Serum Amylase, with Particular Reference to the Use of β-Amylose as the Substrate. J. Biol. Chem., **166**:705–710, 1946.
2. BINKLEY, F., SHANK, R. E., and HOAGLAND, C. L. Modification of the King-Armstrong Method for the Determination of Phosphatase. J. Biol. Chem., **156**:253–256, 1944.
3. COHN, E. J., STRONG, L. E., HUGHES, W. L. JR., MULFORD, D. J., ASHWORTH, J. N., MELIN, M., and TAYLOR, H. L. Preparation and Properties of Serum and Plasma Proteins. IV. A System for the Separation into Fractions of the Protein and Lipoprotein Components of Biological Tissues and Fluids. J. Am. Chem. Soc., **68**:459–475, 1946.
4. COHN, E. J. Personal communication.
5. GJESSING, E. C., LUDEWIG, S., and CHANUTIN, A. Fractionation, Electrophoresis and Chemical Studies of Proteins in Sera of Control and Injured Dogs. J. Biol. Chem., **170**:551–569, 1947.
6. GJESSING, E. C., and CHANUTIN, A. An Electrophoretic Study of Plasma and Plasma Fractions of Normal and Injured Rats. J. Biol. Chem., **169**:657–665, 1947.
7. GJESSING, E. C., LUDEWIG, S., and CHANUTIN, A. Fractionation, Electrophoresis and Chemical Studies of Proteins in Sera of Control and Injured Goats. J. Biol. Chem., **174**:683–696, 1948.
8. GREENSTEIN, J. P. Biochemistry of Cancer, p. 343, New York, Academic Press, Inc., 1947.
9. LUETSCHER, J. A., JR. Biological and Medical Applications of Electrophoresis. Physiol. Rev., **27**:621–642, 1947.
10. LONGSWORTH, L. G., SHEDLOVSKY, T., and MACINNES, D. A. Electrophoretic Patterns of Normal and Pathological Human Blood Serum and Plasma. J. Exper. Med., **70**:399–413, 1939.
11. LONGSWORTH, L. G. The Observation of Electrophoretic Boundaries. Ann. N.Y. Acad. Sci., **39**:187–202, 1939.
12. MORRISON, P. R. Preparation and Properties of Serum and Plasma Proteins. XV. Some Factors Influencing the Quantitative Determination of Fibrinogen. J. Am. Chem. Soc., **69**:2723–2731, 1947.
13. PEARSALL, H. R., and CHANUTIN, A. Electrophoretic, Nitrogen, and Lipide Analyses of Plasma and Plasma Fractions in Disease. Am. J. Med. In press.
14. PETERMANN, M. L., and HOGNESS, K. R. Electrophoretic Studies on the Plasma Proteins of Patients with Neoplastic Disease. I. Gastric Cancer. Cancer, **1**:100–103, 1948.
15. PETERMANN, M. L., KARNOFSKY, D. A., and HOGNESS, K. R. Electrophoretic Studies on the Plasma Proteins of Patients with Neoplastic Disease. III. Lymphomas and Leukemia. Cancer, **1**:109–119, 1948.
16. SPERRY, W. M., and BRAND, F. C. The Colorimetric Determination of Cholesterol. J. Biol. Chem., **150**:315–324, 1943.
17. STERN, K. G., and REINER, M. Electrophoresis in Medicine. Yale J. Biol. and Med., **19**:67–97, 1946.
18. VAN SLYKE, D. D., and FOLCH, J. Manometric Carbon Determination. J. Biol. Chem., **136**:509–541, 1940.