Europ. J. Cancer, Vol. 16, pp. 679–685

0014-2964/80/0501-0679 $02.00/0

Fibrinogen Split Products, Antiproteases and Granulocytic Elastase in Patients with Lung Cancer

C. GROPP,* R. EGBRING and K. HAVEMANN

Medizinische Universitätsklinik, Marburg, Federal Republic of Germany

Abstract—*Fibrinogen degradation products (FDP) were found in the sera of 40% of patients with lung cancer before therapy. In patients with metastatic disease, 22 out of 26 had FDP in the serum. There was no sign of general intravascular coagulation and fibrinolysis. Plasminogen, antithrombin III and fibrinogen levels were normal or higher.*

The antiproteases α_1-antichymotrypsin, inter-α-trypsin inhibitor, and α_2-macroglobulin, were normal, but α_1-antitrypsin was found to be increased especially in patients with metastatic disease. In these patients a granulocytic elastase (ELP) could be demonstrated to be bound to α_1-antitrypsin. This ELP which may be released from granulocytes by circulating immune complexes, is able to digest fibrin and/or fibrinogen, leading to the formation of split products similar to those induced by plasmin. Furthermore, it is shown that the determination of FDP, ELP and α_1-antitrypsin ($\alpha_1 - AT$) may have a prognostic value in lung cancer patients.

INTRODUCTION

SEVERAL studies indicated an association between coagulation phenomena, tumor growth, and the dissemination of cancer cells. O'Meara [1] e.g., showed that extravascular deposition of fibrin occurred in and around the tumor mass. This precipitation was assumed to be caused by a coagulative factor released by the cancer cells. The formation of this tumor matrix was believed to be necessary for the growth and spreading of the tumor. It has been reported that tumor division would not take place before tumor cells stuck to the wall of the blood vessels causing the subsequent formation of a fibrin clot [2].

Fibrinogen degradation products (FDP) have been demonstrated in cancer patients by several authors [3, 4]. They occur predominantly in patients with metastases. The presence of FDP in the serum of cancer patients may be explained either by a breakdown of extra- and intravascular fibrinogen and fibrin, by general fibrinolysis or by intravascular coagulation with secondary fibrinolysis in cancer patients. The purpose of this study was to investigate the incidence of increased FDP in patients with lung cancer and to find the reason for the occurrence of these FDP.

Accepted, 24 October 1979.
*Address for correspondence: Dr. Claus Gropp, Med. Klinik, Mannkopffstraße 1, D-355 Marburg, Federal Republic of Germany.

MATERIALS AND METHODS

Serum and plasma of lung cancer patients with various histology were collected before therapy and stored at −40°C. For the determination of FDP in serum, 2 ml samples of blood were collected in glass tubes containing soy bean trypsin inhibitor (approximately 3600 NF units per tube) and bovine thrombin (20 NIH units per tube). In all patients the extent of disease was determined by broncho- and/or mediastinoscopy, by nuclear scans of bone and liver, by chest and bone X-rays, and by blood chemistries.

Determinations of FDP

FDP were determined in sera by the Thrombo-Wellcotest (Wellcome Reagents Limited, Beckenham, England). Antibodies used in this test were produced by means of highly purified preparations of human fibrinogen fragments D and E. After antibodies to all other serum proteins had been removed by solid-phase absorption, the specific antibody globulin was extracted and used to coat by

absorption a suspension of latex particles in glycine saline buffer. The sensitivity of the latex reagent is adjusted so that in the presence of FDP concentrations of at least 2 µg (fibrinogen equivalent) per ml, the latex particles clump together giving macroscopic agglutination. Degradation products were also determined by one-dimensional immunoelectrophyoresis according to Laurell, employing monospecific antisera against the human fibrinogen fragments D and E.

The plasma antiproteases α_1-antitrypsin (α_1-AT), α_2-macroglobulin (α_2-M), α_1-antichymotrypsin, and inter-α-trypsin inhibitor, were determined by a radial immunodiffusion technique with monospecific antisera (Behringwerke, Marburg, FRG).

An elastase-like protease (ELP) from human polymorphonuclear leukocytes was determined in the plasma by one-dimensional Laurell-electrophoresis [5]. Complexes between ELP and α_1-AT could be demonstrated by two-dimensional immunoelectrophoresis with monospecific antisera to α_1-AT and ELP as described previously [6].

Plasminogen, fibrinogen and antithrombin III (AT III) levels were determined by radial-immunodiffusion with monospecific antisera (Behringwerke, Marburg, FRG). AT III was also determined using chromogenic enzyme substrates (Coatest®/Antithrombin, Kabi Diagnostika, Stockholm, Sweden). In this test AT III activity in plasma is measured in the presence of an excess of heparin. Test plasma not defibrinated, is diluted in buffer containing EDTA and heparin. Thrombin is added in excess and the mixture left to incubate at 37°C for 1 min. An amount of thrombin is inhibited by the AT III-heparin complex in proportion to the amount to AT III. After neutralisation of the heparin with polybrene, the remaining amount of thrombin splits the *p*-nitroaniline (pNA) from the substrate. The release of pNA is allowed to continue for a certain period of time and then stopped with acetic acid. The absorbance of the free pNA measured at 405 nm, decreases linearly with increasing concentrations of AT III in the 25–125% range of normal plasma. The concentration of AT III is read from a standard curve prepared from human normal plasma dilutions.

In all patients platelets, partial prothrombin time, thromboplastin time and factor VIII activity and factor VIII associated antigen were determined. Circulating immune complexes were measured by the C_{1q}-deviation test as described previously [7]. All sera were also tested for the presence of endotoxin using the Limulus test [7]. In addition factor XIII activity was determined in the plasma of all patients by a radioactive method by incorporation of ^{14}C-labelled putrescin into casein [8].

RESULTS

Tests for FDP in 55 patients with lung cancer before therapy showed that no FDP could be found in the serum of the 29 patients without metastases. FDP could, however, be demonstrated in 22 out of 26 patients with metastases (Fig. 1). Immunologically in these patients fibrinogen fragments D and E could be shown by Laurell-electrophoresis. A comparison of the fibrinogen levels of patients without and with metastases showed a distinctly higher amount of fibrinogen in the plasma of the latter (Fig. 2). Plasminogen and AT III as determined by radial immunodif-

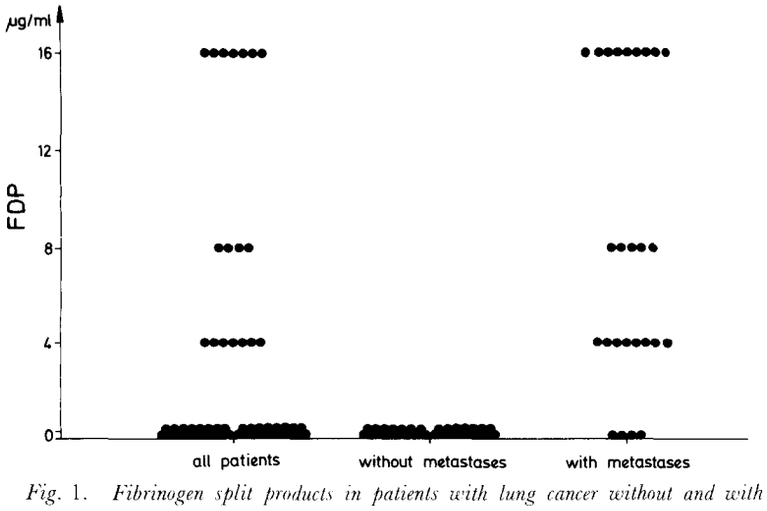

Fig. 1. Fibrinogen split products in patients with lung cancer without and with metastases.

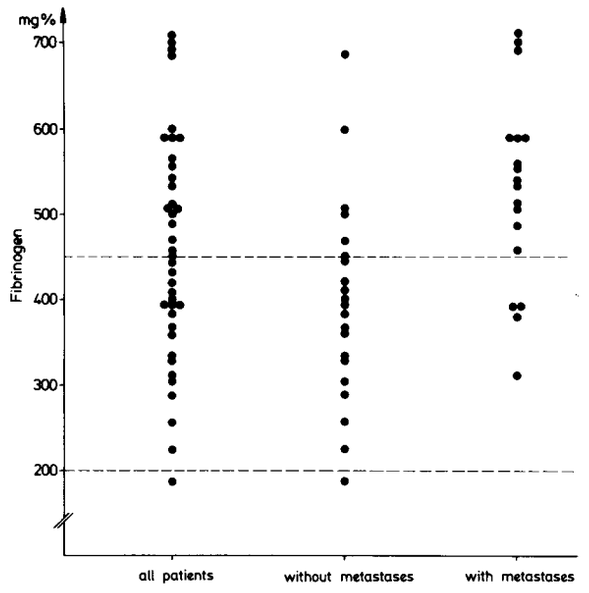

Fig. 2. Fibrinogen levels in patients with lung cancer without and with metastases.

fusion were normal in all patients with lung cancer. Similar results were obtained when chromogenic substrates were used for assaying AT III: three patients showed activities between 60 and 70%, five between 70 and 80%, while the majority had more than 80%. No abnormal levels of the plasma protease inhibitors α_1-antichymotrypsin, inter-α-trypsin inhibitor, and α_2-macroglobulin could be seen. α_1-AT, however, was increased in most patients with metastatic disease (Fig. 3).

Results obtained with two-dimensional Laurell-electrophoresis are shown in Fig. 4. When normal plasma was separated by two-dimensional electrophoresis using monospecific antisera to α_1-AT, α_1-AT appeared as a rapid migrating symmetrical peak (Fig. 4d). The same procedure was then followed with a number of sera of patients with metastatic carcinoma of the lung, with the result that an additional slowly migrating peak appeared (Fig. 4b). This additional peak could be identified using an antiserum against the granulocytic elastase-like protease (ELP) for the second dimension (Fig. 4a). The anti-ELP serum peak corresponded precisely with the slowly migrating peak which had been detected using an antiserum to α_1-AT. These α_1-AT–ELP complexes could not be shown in normal plasma (Fig. 4c), but have been described previously in patients with acute leucemia and septicemia [6, 7]. The amount of ELP in plasma of patients with metastatic disease could be measured routinely by one-dimensional electrophoresis using a standard curve obtained after addition of different amounts of ELP to normal plasma (Fig. 5). By this method, ELP could be detected in the plasma of 19 out of 40 patients with metastatic lung cancer, but in none of the patients with localized cancer. The amount of ELP read from standard curves in one-dimensional electrophoresis, ranged from 0.2 to 1.5 μg/ml. They showed a surprising correlation to FDP levels in the serum of these patients: patients (Fig. 6) with high ELP levels also had high amounts of FDP in their serum. All these patients had metastases. ELP and FDP were not demonstrable in patients without metas-

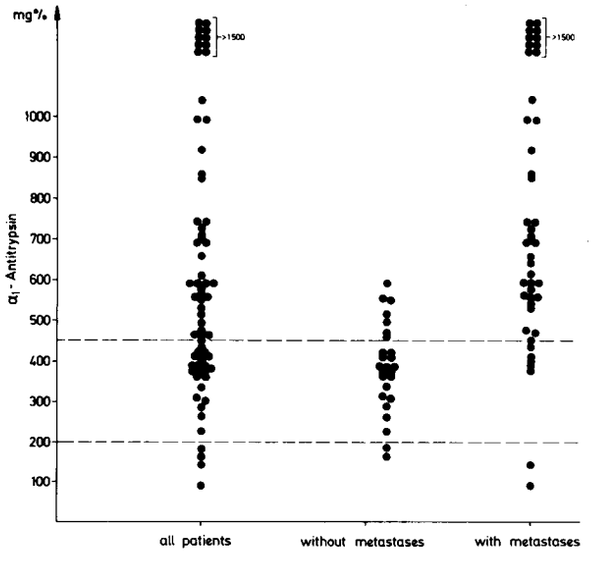

Fig. 3. α_1-Antitrypsin levels in patients with lung cancer without and with metastases.

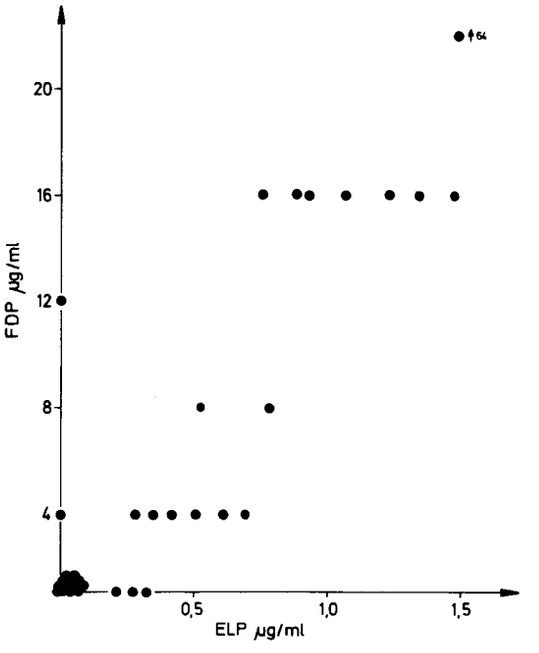

Fig. 6. Correlation between FDP and ELP levels in patients with metastatic lung cancer.

tases. When ELP levels and immune complexes were compared in the same patients a strong correlation existed between these two parameters (Fig. 7). Endotoxin could not be detected in any of the patients' sera. The factor XIII activity was lower than 50% in 15, between 50 and 100% in 18, and more than 100% in 19 out of 52 patients. The lowest factor XIII activity was found in patients with positive ELP in the plasma.

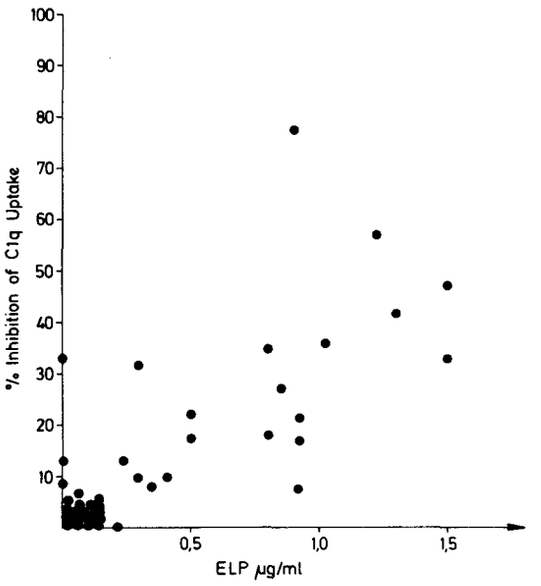

Fig. 7. Correlation between levels of ELP and circulating immune complexes (measured as % inhibition of C_{1q}-uptake) in patients with metastatic lung cancer.

DISCUSSION

FDP were found in the sera from 40% of patients with lung cancer at the time of diagnosis. It is noteworthy that in 22 out of 26 patients with metastases, FDP could be demonstrated. The occurrence of FDP in cancer patients may be due to disseminated intravascular coagulation which has been reported by Davis [7] in cancer patients after chemotherapy. In our patients, who were all investigated before therapy, no signs of general intravascular coagulation (low fibrinogen, low platelets, low plasminogen, low antithrombin III, low factor VIII) could be shown. These findings are in agreement with studies of Hedner *et al.* [3] and Carlson [4]. These authors had found FDP in the serum of patients with various malignancies but could not demonstrate any general intravascular coagulation disorder. They suggested that the FDP in those cases may derive from extravascular breakdown of fibrin by malignant cells. On the basis of our study we suggest another explanation for the occurrence of FDP in lung cancer patients. We could demonstrate in the plasma of these patients a granulocytic elastase-like protease (ELP) which is bound to α_1-AT. This ELP is known to be able to digest fibrin and fibrinogen, and to generate fibrinogen split products [8]. Furthermore, this ELP has been found in patients with acute leucemia and septicemia (but not in normal controls) who showed increased amounts of FDP without signs of disseminated intravascular coagulation [8]. Our data therefore suggest that FDP in the serum of lung cancer patients is caused by the digestion of fibrin and fibrinogen by ELP, a granulocytic protease which cannot be demonstrated in lung tumor extracts or lung tumor cell lines. Already in 1950, Tagnon *et al.* [9] demonstrated in mammalian tissues a factor, which could activate plasminogen to plasmin. Although in our patients normal plasminogen levels were measured at this time, it cannot completely be excluded that elevated FDP levels in lung cancer patients may also derive from digestion of fibrinogen or fibrin by plasmin. In our laboratory a radio-immunoassay is under investigation which specifically measures FDP which derive from ELP digestion. This radioimmunoassay will give additional information of the origin of the FDP in our patients. As FDP were present only in patients with metastatic lung cancer the determination of these split products seems to be of prognostic value.

Fibrinogen levels were normal or (especially in patients with metastases) elevated. The raised fibrinogen levels may be explained as a response to accelerated turnover. The higher α_1-AT levels in these patients may be explained in the same way, namely as a response to the accelerated clearance of the antiprotease by release of ELP. The very low activity of factor XIII in 15 out of 52 patients is also well explained by the destruction of factor XIII by ELP. This has been shown in a previous study [10].

The cause of ELP release from granulocytes in patients with lung cancer is unknown. In patients with acute leucemia or septicemia it has been shown that the release of granulocytic protease may be induced by endotoxin, severe fever, or by antigen–antibody complexes [11]. Because endotoxin could be excluded and severe fever was the exception in our patients at the time of diagnosis, the release of ELP by antigen–antibody complexes seems to be the most likely explanation. All sera of patients with lung cancer were neg-

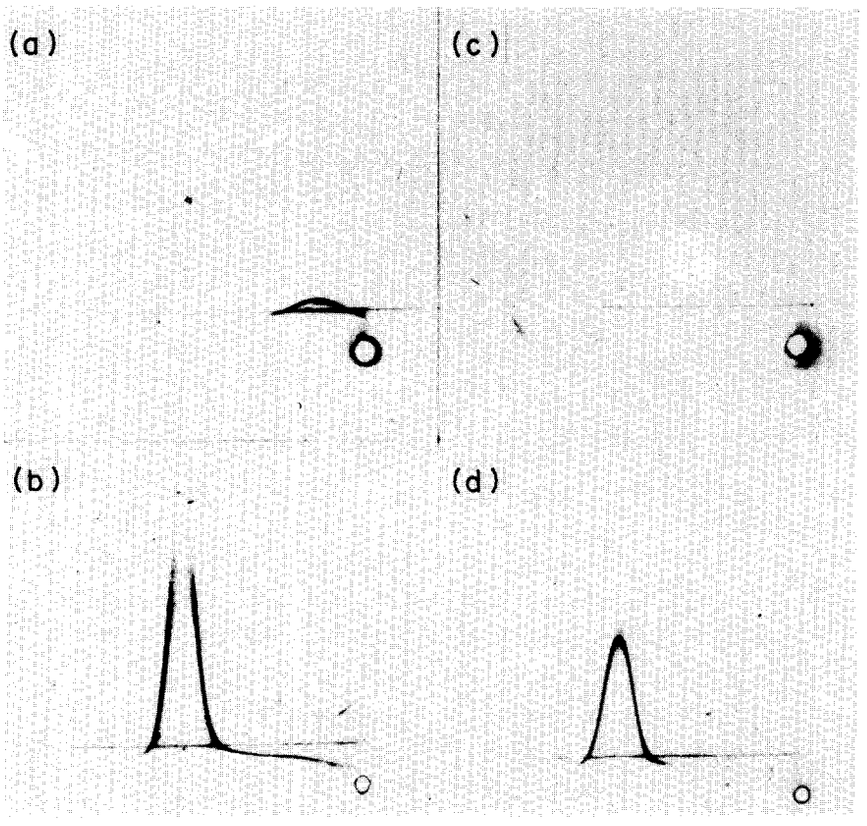

Fig. 4. *Two-dimensional Laurell-electrophoresis using monospecific antisera to* α_1*-antitrypsin.*
(b) plasma of a lung cancer patient; (d) plasma of a normal control and monospecific antisera to ELP; (a) plasma of a lung cancer patient; (c) plasma of a normal control.

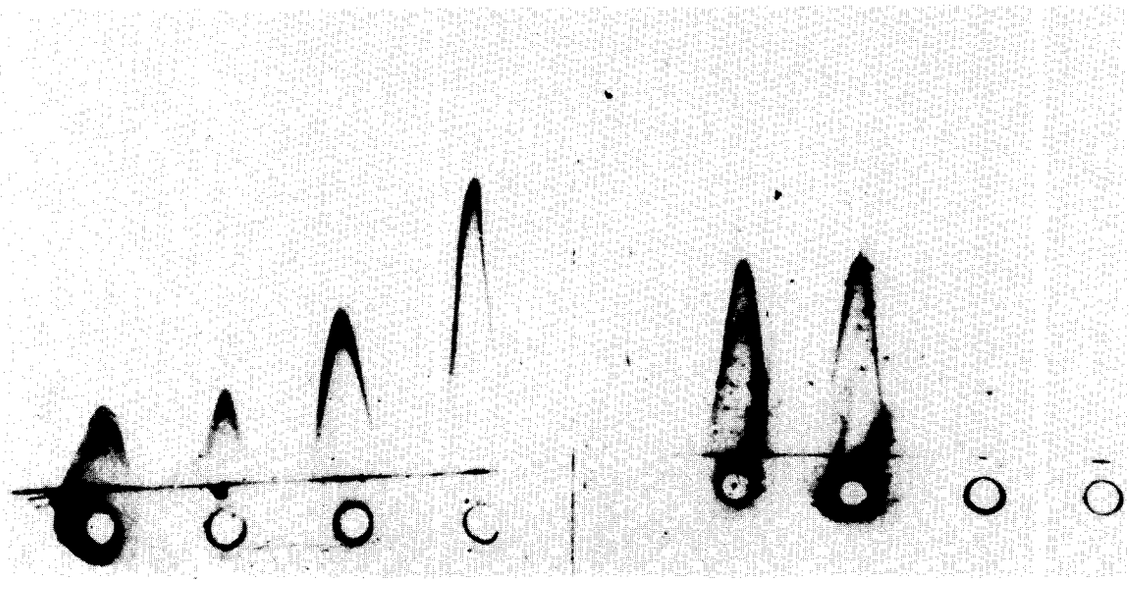

Fig. 5. *One-dimensional Laurell-electrophoresis of plasma of two patients with lung cancer (on the right) using antisera to ELP (standard curve on the left).*

ative for endotoxin but in previous studies as well as in this study we could show that circulating immune complexes are present in a high frequency especially in lung cancer patients with metastases [12].

As there exists a strong correlation between the amount of circulating immune complexes and the levels of ELP and fibronogen split products in our patients we believe that ELP is released by immune complexes. The release of ELP is then followed by the degradation of fibrinogen to FDP. Because fibrinogen split products can suppress immune reactions [13] and so may enhance tumor spread our results may contribute to the knowledge of tumor growth.

REFERENCE

1. R. A. Q. O'Meara, Coagulopative properties of cancers. *Irish J. med. Sci.* **394,** 474 (1958).
2. S. Wood, Jr., Pathogenesis of metastasis formation observed *in vivo* in the rabbit ear chamber. *AMA Arch. Pathol.* **66,** 550 (1958).
3. U. Hedner and J. M. Nilsson, Clinical experience with determination of fibrinogen degradation products. *Acta med. scand.* **189,** 471 (1971).
4. S. Carlsson, Fibrinogen degradation products in serum from patients with cancer. *Acta chir. scand.* **139,** 499 (1973).
5. C. B. Laurell, Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Ann. Biochem.* **15,** 45 (1966).
6. R. Egbring, W. Schmidt, G. Fuchs and K. Havemann, Demonstration of granulocytic proteases in plasma of patients with acute leukemia and septicemia with coagulation defects. *Blood* **49,** 219 (1977).
7. R. B. Davis, T. Athanasios and B. J. Kennedy, Comparative studies of blood coagulation and platelet aggregation in patients with cancer and non-malignant diseases. *Ann intern. Med.* **71,** 67 (1969).
8. R. Egbring, W. Schmidt and K. Havemann, Die vereinfachte radiologische Faktor XIII-Bestimmung und ihre klinische Anwendung bei kongenitalem Faktor XIII-Mangel. *Blut* **27,** 6 (1973).
9. H. J. Tagnon and G. E. Palade, Activation of proplasmin by a factor from mammalian tissue. *J. clin. Invest.* **29,** 317 (1950).
10. W. Schmidt, R. Egbring and K. Havemann, Effect of elastase-like and chymotrypsin-like neutral proteases from human granulocytes on isolated clotting factors. *Thromb. Res.* **6,** 315 (1975).
11. M. Gramse, C. Bingenheimer, W. Schmidt, R. Egbring and K. Havemann, Degradation products of fibrinogen by elastase-like neutral protease from human granulocytes. *J. clin. Invest.* **61,** 1027 (1978).
12. K. Havemann and W. Schmidt, Potentiating effect of adherent cell supernatants on lymphocyte proliferation. In *Proceedings of the Eighth Leukocyte Culture Conference.* (Edited by K. Lindahl-Kiessling) p. 181. Academic Press, New York (1974).
13. K. Havemann, C. Gropp, F.-G. Lehmann, P. Preisser, S.v. Kleist, Tumor-associated antigens in bronchogenic carcinoma. In *Immunodiagnosis and Immunotherapy of Malignant Tumors* (Edited by H.-D. Flad, Ch. Herforth and M. Betzler) p. 56. Springer, Berlin (1979).