ADVANCES IN CANCER RESEARCH

Edited by

GEORGE KLEIN SIDNEY WEINHOUSE

ADVANCES IN CANCER RESEARCH

Edited by

GEORGE KLEIN

Department of Tumor Biology
Karolinska Institutet
Stockholm, Sweden

SIDNEY WEINHOUSE

Fels Research Institute
Temple University Medical School
Philadelphia, Pennsylvania

Volume 30—1979

ACADEMIC PRESS New York San Francisco London
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 52–13360

ISBN 0–12–006630–0

PRINTED IN THE UNITED STATES OF AMERICA

79 80 81 82 9 8 7 6 5 4 3 2 1

CONTRIBUTORS TO VOLUME 30

Numbers in parentheses indicate the pages on which the authors' contributions begin.

FAYE C. AUSTIN, *Cell Biology Section, Laboratory of Viral Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014* (301)

JOSEPH BALÓ, *Department of Pathological Anatomy, Semmelweis Medical University, Budapest, Hungary* (151)

CHARLES W. BOONE, *Cell Biology Section, Laboratory of Viral Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014* (301)

E. H. COOPER, *The Unit for Cancer Research, University of Leeds, Leeds, England* (1)

NECHAMA HARAN-GHERA, *Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel* (45)

CHRISTOPHER R. KINTNER, *The McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706* (239)

VALERI F. KLIMASHEVSKI, *Laboratory of Experimental Tumors, N.N. Petrov Research Institute of Oncology, USSR Ministry of Public Health, Pesochny-2, Leningrad 188646, USSR* (165)

HIROSHI KOBAYASHI, *Laboratory of Pathology, Cancer Institute, Hokkaido University School of Medicine, Sapporo, Japan* (279)

ALEXEI J. LIKHACHEV, *Laboratory of Experimental Tumors, N.N. Petrov Research Institute of Oncology, USSR Ministry of Public Health, Pesochny-2, Leningrad 188646, USSR* (165)

WILLIE MARK, *The McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706* (239)

ALPHA PELED, *Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel* (45)

KAZYMIR M. POZHARISSKI, *Laboratory of Experimental Tumors, N.N. Petrov Research Institute of Oncology, USSR Ministry of Public Health, Pesochny-2, Leningrad 188646, USSR* (165)

JACOB D. SHAPOSHNIKOV, *Laboratory of Experimental Tumors, N.N. Petrov Research Institute of Oncology, USSR Ministry of Public Health, Pesochny-2, Leningrad 188646, USSR* (165)

V. S. SHAPOT, *Cancer Research Center, USSR Academy of Medical Sciences, Moscow, USSR* (89)

JOAN STONE, *Department of Mathematics, University of Bradford, Bradford, England* (1)

BILL SUGDEN, *The McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706* (239)

CONTENTS

Role of Hydrazine in Carcinogenesis

JOSEPH BALÓ

Experimental Intestinal Cancer Research with Special Reference to Human Pathology

KAZYMIR M. POZHARISSKI, ALEXEI J. LIKHACHEV,
VALERI F. KLIMASHEVSKI, AND JACOB D. SHAPOSHNIKOV

The Molecular Biology of Lymphotropic Herpesviruses

BIEL SUGDEN, CHRISTOPHER R. KINTNER, AND WILLIE MARK

Viral Xenogenization of Intact Tumor Cells

HIROSHI KOBAYASHI

Virus Augmentation of the Antigenicity of Tumor Cell Extracts

FAYE C. AUSTIN AND CHARLES W. BOONE

ADVANCES IN CANCER RESEARCH, VOL. 30

ACUTE PHASE REACTANT PROTEINS IN CANCER

E. H. Cooper and Joan Stone

The Unit for Cancer Research, University of Leeds, Leeds, England; and Department of Mathematics, University of Bradford, Bradford, England

I. Introduction

The acute phase reactant proteins (APRPs) are mainly glycoproteins that alter their plasma concentration in response to stimuli produced by many forms of tissue injury, acute and chronic inflammation, connective tissue disorders, and cancer. Clearly as they respond to such a wide variety of stimuli it is self-evident that any changes in these proteins must be regarded as nonspecific. (For general review see Owen, 1967; Koj, 1974; Fisher and Gill, 1975.) Nevertheless, the quantitative and temporal responses of individual members of the APRPs can differ according to the nature of the stimulus and therefore may have diagnostic implications (Laurell, 1974; Hiramatsu *et al.*, 1976; Fisher and Gill, 1975).

The interest of APRPs in cancer can be considered from both a fundamental and an applied point of view. The underlying fundamental question is the reason for the alteration of the APRPs in response to

ISBN 0-12-006630-0

chronic degeneration and cancer and whether this has an advantage to the host or is an aberrant modification of various protective mechanisms that are a vital part of wound healing and the response to infection. The fundamental aspects concern the function of proteins such as α_1-acid glycoprotein (α_1AGP), C-reactive protein (C-RP), and the C_3 component of complement in the chronic tissue damage of cancer, as well as the local function of the antiproteases, α_1-antitrypsin (α_1AT) and α_1-antichymotrypsin (α_1ACT), in relation to the progression of an invading neoplasm as it infiltrates the normal host tissues. The main features of this family of proteins are shown in Table I. On the other hand, renewed interest is being taken in APRPs as components of a battery of biological tests for the monitoring of cancer. The history of investigation of APRPs in cancer shows how most of the earlier authors were content to produce a catalog of changes of a particular protein in a wide variety of cancers with very little attention to age, tumor load, or the patients' performance status. They were also handicapped by various technical problems which resulted in the proteins being quantitated indirectly as a measurement of their enzyme inhibitor activity (α_1AT), binding capacity as in haptoglobin (HP), as indirect enzyme assays as in ceruloplasmin (CPL), or as partially purified serum fractions, e.g., seromucoid. The general availability of commercial specific antisera and the introduction of the simple single radial immuodiffusion technique of Mancini *et al.* (1965) has greatly enhanced its use in laboratory medicine both for research and routine. In patients in whom there is established cancer or a high probability of cancer, the tests may be required to help assess the prognosis, in particular to increase the accuracy of prediction of probable recurrence or

TABLE I

THE ACUTE PHASE REACTANT PROTEINS WITH PARTICULAR RELEVANCE TO CLINICAL ONCOLOGY

Protein	Symbol	Molecular Weight	Amount in normal serum (gm/liter)
α_1-Acid glycoprotein	α_1AGP	40,000	0.55–1.4
α_1-Antitrypsin	α_1AT	54,000	2.0–4.0
α_1-Antichymotrypsin	α_1ACT	68,000	0.3–0.6
Ceruloplasmin	CPL	51,000	0.15–0.6
C-Reactive protein	C-RP		<10 (mg/liter)
Haptoglobin	Hp		
Type 1-1	HP 1-1	100,000	1.0–2.2
Type 2-1	HP 2-1	≈200,000	1.6–3.0
Type 2-2	HP 2-2	≈400,000	1.2–2.6
Fibrinogen		340,000	2.0–4.5

metastases in an individual. The tests may be required to help monitor therapy, especially when the tumor is no longer clinically detectable. Finally in this context is the provision of long-term monitoring of the patient at risk, so that early warning of relapse or progression can be given.

As it is quite improbable that APRPs could play any role in population screening for cancer, especially as none of the presently available tumor-related markers can seriously be advocated for this purpose (Hobbs, 1974; Neville and Cooper, 1976; Schwartz, 1978), this topic will not be discussed further.

A second general concept germane to this review is the variation in an individual's general reaction to cancer, as witnessed by such gross indicators as weight loss and a vast array of more subtle biochemical imbalances. In some tumors the metabolic disturbances in the host are gross and coupled to major defects in organ function or cachexia (Theologides, 1971; Bodansky, 1975); in others the abnormalities are often subclinical, but they can decline fairly rapidly from a metastable state to a severe life-threatening illness for what is a relatively small increase of tumor burden or additional burden on a defective system. If skin cancers are excluded, then only 50% of all cancer patients will survive (Seidman *et al.*, 1976) and since the greater proportion of deaths are associated with the effects of metastatic disease, it can be seen that knowledge of the effect of locally recurrent or disseminated tumor on biochemical homeostasis is needed as part of the information for guiding therapy on patients with advanced disease. Within the context of identifying the cancer patient's biochemical status a matrix of levels of various serum proteins, especially those with short half-lives and small pool sizes including certain APRPs, is a promising area for study.

The early studies confirmed that advanced cancer was usually accompanied by a rise of α-globulins (Winzler, 1953), and later this was attributed to APRPs but the lack of specificity tended to discourage clinical oncologists from the idea that APRPs could be of any practical value. This discouragement was strongly reinforced by the belief in the late sixties and earlier part of this decade that the age of specific tumor markers had arrived, the virtue of tests being cancer specific was then loudly proclaimed. Unfortunately, tests such as the measurement of plasma carcinoembryonic antigen (CEA), α-fetoprotein (AFP), and beta subunit of human chorionic gonadotropin (βHCG) are nowadays realized to either lack specificity or to have somewhat limited circumstances in which they can be used as an optimal marker (see Neville and Cooper, 1976; Bagshawe and Searle, 1977; King, 1978; Coletta, 1978; Schwartz, 1978; Krebs *et al.*, 1978). Indeed if the behavior of βHCG in

choriocarcinoma is the paradigm (Bagshawe, 1969), few markers can attain such sensitivity, and certainly it leaves many of the common forms of cancer without reliable specific markers, especially at the earlier stages of disease. This experience has resulted in a reappraisal of the use of some of the available nonspecific markers and examination of the value of including them in a battery of markers to monitor cancer, especially in cancers such as the kidney, bladder, and lymphomas where tumor-related products have little to offer as markers (Neville and Cooper, 1976). There are several studies in the literature that strongly reinforce the view that changes in certain fractions of the plasma proteins, especially the seromucoids (Harshman *et al.*, 1974); Randle *et al.*, 1974) and Hp (Jayle *et al.*, 1968), might be helpful in monitoring cancer, the choice of the particular protein or group being influenced by the techniques available. The advent of monospecific antisera to many human plasma proteins provided a new opportunity to examine the changes in the individual APRPs and their implications in cancer.

However, before assessing in what way APRPs could contribute to such a battery, it is important to have a clear idea of the objectives of monitoring. Essentially, monitoring is the collection of biochemical intelligence about the patient which, when taken with the appropriate clinical information, can help the clinician in decision making. The roster of markers from which a battery may be chosen are given in Table II: for a discussion of multiparametric screening in clinical chemistry see Wolf *et al.* (1973) and Galen (1975).

II. Production, Half-Lives, and Destruction of Acute Phase Reactant Proteins

The plasma half lives of several APRPs based on tracing iodinated proteins injected into healthy subjects were established in the 1960s. Thus the estimates were 5.2 days for α_1AGP (Weisman *et al.*, 1961), 2–4 days for Hp (Freeman, 1964; Krauss *et al.*, 1966), 4.2 days for CPL (Koskelo *et al.*, 1967), and 3.2 days for fibrinogen (McFarlane *et al.*, 1964); this method gives albumin a plasma half-life of 19 days (Peters, 1970).

These data in conjunction with the blood levels cannot provide accurate information on the rates of synthesis, as the blood level is the resultant of synthesis, catabolism, and partition between the blood and tissue fluids. The intravenous pool is estimated to be 40% of the total pool of albumin (Peters, 1975), 40% of the total CPL pool (Koskelo *et al.*, 1967), and about 80% of the total fibrinogen pool (McFarlane *et al.*, 1964;

TABLE II
LIST OF SOME POTENTIAL TUMOR MARKER SUBSTANCES[a]

I. Tumor-Associated Antigens
  A. Oncofetal
    α-fetoprotein
    CEA
    Fetal sulfo-glycoprotein antigen
  B. Other tumor-associated antigens
II. DNA Binding Proteins
III. Hormones
    ACTH
    Calcitonin
    hCG and βHCG
    Prolactin
IV. Enzymes

Acid phosphatase	Isocitrate dehydrogenase
Alkaline phosphatases	Lactate dehydrogenase
Aldolase	Malate dehydrogenase
Aminopeptidases	Muramidase
Amylase	5'-Nuclectidase
Aryl sulfatase	Pepsinogen isoenzymes
Aspartate aminotransferase	Phosphohexose isomerase
Glycosyltransferases	Ribonuclease
γ-Glutamyl transpeptidase	Terminal deoxynucleotidyl transferas
Glutathione reductase	
Histaminase	Tyrosinase

V. Metabolites and Degradation Products

β-Aminoisobutyric acid	Minor nucleosides
Fibrinogen degradation products	Myeloma proteins
Hydroxyproline	Polyamines
κ-casein	Protein-bound fucose
α-lactalbumin	β_2-Microglobulin
Catechol amines and metabolites	Acute phase reactant proteins

[a] Modified from the list by Dr. R. W. Ruddon, Frederick Cancer Research Center, Frederick, Maryland 21701.

Takeda, 1966). The phenotype of Hp profoundly influences the balance between the intra- and extravascular pools, with Hp 1–1 (mol. wt. 85,000) having an almost equal partition between the pools and Hp 2–2 (mol. wt. 200,000) with only 25% in the extravascular pool (Krauss, 1969). Clearly any extensive inflammatory process altering the permeability of the capillaries might influence this partition between the vascular and extravascular pool as is seen in inflammatory and noninflammatory effusions (Agostoni and Marasini, 1977). Koj (1974) has reviewed the evidence on the metabolism of APRPs in animals and humans during the course of acute and chronic elevations of the serum levels of these proteins. The

general consensus is that the fractional catabolic rate is not influenced by the level of the APRPs in the blood. In relation to cancer the fractional catabolic rate of Hp has been reported to be unchanged in Hodgkin's disease, even when it was raised to a concentration of 7.0 gm/liter (Krauss, 1969). O'Hara *et al.*, (1967) investigated the Hp half-life in three normal subjects and in three patients with cancer using radioiodinated human Hp, the $t_{1/2}$ was 1.9, 2.1, and 2.1 days in their controls and 2.4, 2.7, and 2.8 days in the cancer patients. But these results must be regarded with some reservation as there is an uncertainty as to what is the true range of normal plasma half-lives and the influence of phenotype on the distribution of Hp in disease (Putnam, 1975). A similar stability has been observed in fibrinogen catabolism in inflammation and cancer (see Koj, 1974). More recently Lyman *et al.*, (1978) have investigated the half-lives of fibrinogen in 30 cancer patients; they found that $t_{1/2}$ was 3.89 ± 1.38 days in controls and 3.01 ± 1.09 in cancer patients, and the half-life was shortest in patients receiving chemotherapy. However, compared to the other APRPs, there is the added complication of various changes in local or generalized deposition of fibrin that may influence the rate of removal of this protein.

A starting point for a new chapter in the biochemistry of glycoproteins was the observation that the activity of certain hormones appeared to be dependent on their sialic acid content. This was first demonstrated for follicle stimulating hormone (Gottschalk *et al.*, 1960) and HCG (Goverde *et al.*, 1968; Mori, 1969). Later, the studies of Ashwell and his group (see Ashwell and Morell, 1974, for review) were to extend this observation and produce a general hypothesis that the partial or complete loss of sialic acid from a plasma glycoprotein causes it to be rapidly removed from the circulation (Fig. 1). Beginning with a study of CPL, they were able to show this also applied to α_1AGP, Hp, and fetuin. Radioisotopic labeling indicated that the liver was the organ responsible for removing the asialated glycoprotein where it undergoes enzymic hydrolysis. A typical result is the finding that the half-life of human α_1AT in the circulation of the rat is 18 hours, while in asialic AT it is almost toally cleared in about 30 minutes (Yu and Gan, 1977).

The effect of the desialation seems to depend on the exposure of galactose residues which then trigger the recognition processes in the liver; this effect can be inhibited by altering the galactose group (Van den Hamer *et al.*, 1970). The binding sites on the liver cells appear to involve sialic acid, as treatment of hepatic parenchymal cells with neuraminidase removes the binding activity for asialoglycoprotein, but sialic acid also blocks the binding of glycoproteins to these cells (Ashwell, 1974); this dual function of sialic acid is still an enigma.

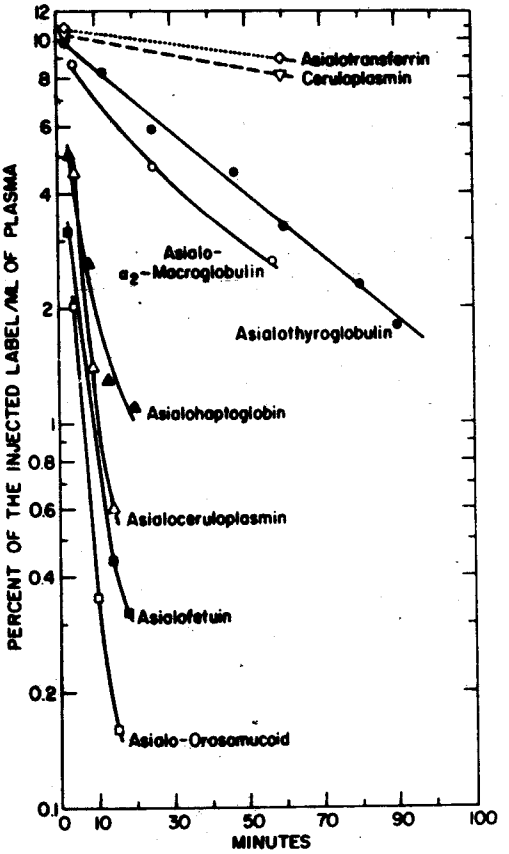

FIG. 1. Plasma survival of labeled asialoglycoproteins (from Ashwell and Morrell, 1974).

This mechanism could provide a way of distinguishing recently synthesized molecules from those that are "aged" and ready for removal, that is assuming the aging would involve loss of sialic residues.

It is of interest that, in those proteins where a biological activity can be measured, desialation is not associated with a loss of function, for example asialo-α_1AT, partially desialated α_1AT, and partially desialated α_1AT with oxidized galactose, maintain their trypsin inhibitory and chymotrypsin inhibitory potential (Yu and Gan, 1977). Hence the loss of hormone activity as the result of desialation is due to removal from the circulation rather than interference with the functional moiety.

The clearance of α_1AT in subjects with α_1AT deficiencies has shown that the protein has a half-life of 6 days (Makino and Reed, 1970), while

iodinated α_1AT in recipients of the MM phenotype indicated the half-life was 4 days, and 5.5–6.5 days in the MM and MZ phenotypes (Keuppers and Fallat, 1969). This is a somewhat unexpected result as these authors treated their preparation with neuraminidase. More recently, the behavior of the α_1AT isolated from PiM and PiZ subjects has been investigated by the simultaneous injection of the two labeled proteins into PiM subjects (Laurell *et al.*, 1977). The mean fraction catabolic rates for M protein and Z protein were 0.26 and 0.40, respectively, which is too small a difference to explain why the α_1AT content of blood in PiZ phenotypes is only 15% of normal. Following injury or estrogen stimulation in MZ and ZZ patients there is a similar proportional increase of α_1AT, but the absolute response is less in the ZZ patients. This points to either a small mRNA pool or a slower translation of the Z gene (Laurell *et al.*, 1977).

III. Prostate and Breast Cancers

It is convenient to consider these cancers together, as they are both hormone dependent and estrogens are frequently used to control their growth. This treatment produces a profound alteration in the plasma protein by its direct effects on their synthesis independent of any effect on the cancer (Musa *et al.*, 1965; Laurell *et al.*, 1968).

A. Prostate Cancer

Untreated carcinoma of the prostate has to be associated with raised serum α-globulins which are related to tumor stage and the treatment (Ablin *et al.*, 1973). An elevated pretreatment plasma fibrinogen in prostatic cancer has been significantly correlated with an increased proportion of deaths from all causes and from carcinoma of the prostate (Seal *et al.*, 1976). More recently Seal *et al.* (1978) have confirmed that serum Hp levels are raised in prostatic cancer stages III and IV, stage IV being significantly higher than state III. The pretreatment values were significantly correlated with death rates. Ward *et al.* (1977) examined the serum levels of Hp, α_1AGP, and CPL in benign prostatic hypertrophy (BPH), and untreated carcinoma of the prostate with and without bone metastases. The levels of this protein profile were not statistically different in BPH and the carcinoma without metastases, while carcinoma with bone metastases was accompanied by a significant increase in the four APRPs. They demonstrated that a mean discriminant function using the levels of the proteins in combination with the level of tartrate resistant serum acid

phosphatase (SAP) and prealbumin (PALB) could give a correct classification of cases with and without bone metastases 88.6% of the time. The evidence for the bone metastases was the uptake of technicium 99 into the bone as shown by scintigraphy. Figure 2 illustrates the separation achieved by this discriminant function. Subsequently, Houghton (1978) reexamined the discriminant on a further set of 25 prostatic cancer patients without bone metastases and 45 with bone metastases and examined the value of the levels of ACT and C-RP in addition to the variates used in the earlier study. A stepwise discrimination showed the importance of the variates in descending order was SAP, α_1ACT, Hp, PALB, α_1AT, α_1AGP, and C-RP. He obtained a 90% overall correct assignment with his discriminant function. However, the recent advances in the isolation of specific prostatic acid phosphatase (Foti *et al.*, 1977) and the finding that serum ribonuclease levels may be a guide in monitoring prostatic cancer (Chu *et al.*, 1977) may result in a more powerful discriminant which would be less liable to be influenced by the vagaries of the APRPs' response to therapy (Chu, 1978). Several forms of estrogen therapy and estrogen-containing drugs such as estramustine phosphate, a nornitrogen mustard linked to 17β-estradiol phosphate (Jönsson *et al.*, 1975), have major effects on the plasma protein profile in prostatic cancer. These effects include a rise of serum α_1AT and CPL levels (Ward *et al.*, 1978) and plasminogen (Seal *et al.*, 1976) and a fall of α_1AGP and Hp (Ward *et al.*, 1978) and fibrinogen (Seal *et al.*, 1976). Seal and his colleagues (1976) pointed out that the fall of fibrinogen was unexpected as it rises in pregnancy and a rise may be induced by several forms of estrogen-containing oral contraceptives in women. They were unable to account for the rise in patients with prostatic cancer, except to note its effect was maximal when a 1 mg/day dose of diethylstilbestrol was used, the effect being absent at 5 mg/day. Provera, another estrogen used for

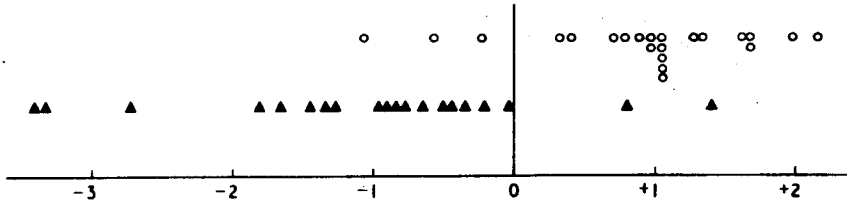

FIG. 2. Discriminant analysis scores using prealbumin, α_1-antitrypsin, α_1-acid glycoprotein, haptoglobin, serum acid phosphatase with prior logarithmic transformation of the data. Closed triangles represent metastatic carcinoma of the prostate. Circles represent nonmetastatic carcinoma of the prostate. Discriminant (D) = $-0.638(\log_e \text{SAP}) + 0.767(\log_e \text{PALB}) - 2.074(\log_e \alpha_1\text{AT}) + 0.605(\log_e \text{AGP}) - 0.911(\log_e \text{Hp}) + 4.996$. From Ward *et al.* (1977).