

# Levels of Creatine Kinase and Its BB Isoenzyme in Lung Cancer Specimens and Cultures<sup>1</sup>

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## ABSTRACT

Small-cell carcinomas of the lung (SCCL) have properties of amine-handling cells, and high levels of the key amine-handling cell enzyme L-dopa decarboxylase (EC 4.1.1.28) distinguish SCCL from most other lung cancers. SCCL tumor specimens and continuous cultures also are characterized by high levels of creatine kinase (EC 2.7.3.2) and its BB isoenzyme (CK-BB). Electrophoretic analysis of creatine kinase isoenzymes indicated that creatine kinase levels in SCCL were quantitatively but not qualitatively different from those in normal lung and other lung cancers. Supernatant fluids of SCCL cultures contained relatively modest concentrations of CK-BB but lacked detectable L-dopa decarboxylase activity. Variant SCCL cultures with altered morphology lost their amine-handling properties, including L-dopa decarboxylase activity, but retained high levels of CK-BB, indicating discordant expression of the two enzymes. CK-BB levels were measured in the sera of 67 patients having SCCL. Elevated levels were present in 16 of 41 patients (39%) having extensive-stage disease but in none of 26 patients (0%) having limited-stage disease.

## INTRODUCTION

SCCL's<sup>4</sup> have properties of APUD cells (21), and high levels of the key APUD cell enzyme DDC (EC 4.1.1.28; also known as L-aromatic amino acid decarboxylase) distinguish SCCL from most non-SCCL lung cancers (2, 5, 14).

CK (EC 2.7.3.2) reversibly catalyzes the transfer of a high-energy phosphate bond from creatine phosphate to ADP (22). The soluble cytoplasmic forms of CK occur as dimers consisting of the 3 possible combinations of 2 antigenically distinct chains, M (muscle form) and B (brain form). While CK is probably present in all body tissues and organs, CK levels and the relative proportions of its isoenzymes CK-MM, CK-MB, and CK-BB alter during embryonic development and differentiation and show considerable organ-to-organ variation (10, 17, 23, 25). Apart from striated muscle, brain, bladder, and gastrointestinal tract, most organs have low levels of enzyme, predominantly in the form of CK-BB. We now report that SCCL tumor specimens and cultures also are characterized by high levels of CK in the form of its BB isoenzyme. Variant SCCL cultures

with altered morphology lose DDC activity but retain high levels of CK-BB, indicating discordant regulation of the 2 enzymes. In addition, we demonstrate that some SCCL patients with extensive-stage disease but not those with limited-stage disease have elevated serum concentrations of CK-BB.

## MATERIALS AND METHODS

**Tumors and Cell Cultures.** Primary, non-SCCL lung cancers were obtained by surgical resection. In some instances, microscopically normal lung tissue remote from the tumor also was obtained. Because SCCL tumors seldom are resected, biopsies of metastases to lymph nodes and s.c. nodules and malignant pleural effusions were obtained. These specimens consisted almost entirely of tumor cells, as confirmed by microscopic examination. Cell cultures were initiated from similar materials, either directly or after heterotransplantation in athymic nude mice. Details of culture methods, propagation, and characterization are presented elsewhere (14). In brief, cultures were maintained in Roswell Park Memorial Institute Medium 1640 supplemented with 10% heat-inactivated fetal bovine serum. All cultures were continuous, clonable, aneuploid, and tumorigenic, and most had been in culture for more than 1 year when tested. Tumors induced by injection of cultured cells into athymic nude mice had histologies similar to those of the original tumors (with the exception of variant SCCL cultures described later). SCCL cultures consisted of floating cell aggregates having APUD cell properties (except for the variant cultures), including high levels of DDC, formaldehyde-induced fluorescence, neurosecretory granules, and high frequency of polypeptide hormone secretion. Non-SCCL lung cancer cultures consisted of adherent epithelial cells lacking APUD cell properties. All cultures were free of fibroblast contamination. Tests for *Mycoplasma* contamination were negative (performed by Microbiological Associates, Bethesda, Md.).

**Sample Preparation.** Cell pellets of malignant effusions and cell cultures were washed 3 times by centrifugation and resuspension in Dulbecco's phosphate-buffered saline, pH 7.2 (Grand Island Biological Co., Grand Island, N. Y.). Floating cultures were harvested directly, while adherent cultures were scraped with a rubber policeman. All cultures were in exponential growth phase when harvested, 24 hr after a medium change. Tumors and cell pellets were disrupted in Dulbecco's phosphate-buffered saline in a Potter-Elvehjem tissue homogenizer, clarified (30,000 × g, 15 min), aliquoted, analyzed immediately (some enzymatic CK analyses and electrophoresis), or frozen at -70° and assayed later (DDC, CK-BB, and protein assays). Supernatant culture fluids were handled similarly but not homogenized.

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<sup>4</sup> The abbreviations used are: SCCL, small-cell carcinoma of the lung; APUD, amine precursor uptake and decarboxylation; DDC, L-dopa decarboxylase; CK, creatine kinase; CK-MM, MM isoenzyme of creatine kinase; CK-MB, MB isoenzyme of creatine kinase; CK-BB, BB isoenzyme of creatine kinase.

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**Sera.** Sera were obtained from untreated SCCL patients undergoing staging procedures, and were frozen at  $-70^{\circ}$  prior to assay.

**Assays.** DDC assays were performed by our modification (4) of a published method (6). A unit is defined as 1 nmol of  $^{14}\text{CO}_2$  released per hr. Enzymatic CK activity was determined by standard UV kinetic assay with an Automatic Clinical Analyzer (DuPont Instruments, Wilmington, Del.) using the manufacturer's instructions and reagents. The assay, performed at  $37^{\circ}$  and pH 6.6, used creatine kinase and ADP as substrates and measured the formation of ATP via coupling reactions. CK values are expressed in mIU. Isoenzyme distribution of enzymatic CK was determined by electrophoresis on agarose gel (Corning Medical, Medfield, Mass.) using reagents from BioDynamics/bmc (Indianapolis, Ind.), and scanned on a Corning Model 720 fluorimeter. It utilized the same basic reactions used in the quantitative assay. Immunoreactive CK-BB was measured by a sensitive, specific double-antibody assay (26). The antibody, raised against purified human brain CK-BB, did not cross-react with human CK-MM from muscle and to a very low degree (about 1%) with CK-MB from cardiac muscle. Protein determinations were performed by a one-reagent method (Bio-Rad, Richmond, Calif.) according to the manufacturer's instructions. All samples were diluted so that the results fell within the linear ranges of the various assays.

**Exclusion of Adenylate Kinase Activity.** Adenylate kinase (EC 2.7.4.3), an enzyme present in many tissues, also can convert ADP to ATP. Both CK enzymatic assays contained effective inhibitors of adenylate kinase (a combination of AMP and diadenosine pentaphosphate in the quantitative assay and AMP in the electrophoretic assay) (24). The absence of significant residual adenylate kinase activity was formally demonstrated in the electrophoretograms. Deletion of the CK substrate creatine phosphate from the reaction mixture resulted in the abolition of the isoenzyme patterns of cardiac and heart muscle and of homogenates of SCCL and non-SCCL tumors and cultures.

**Staging of Patients.** Patients on approved therapeutic protocols were staged as described previously (8). Staging procedures included: chest roentgenograms; fiberoptic bronchoscopy with biopsies and bronchial washing cytology; bone marrow biopsies and aspirations; needle biopsies of the liver and all accessible nodes and soft-tissue lesions; and scans of bone, liver, and brain. After staging, the patients were divided into those with limited-stage disease (tumor limited to lung of origin and its regional lymphatic drainage) and those with extensive-stage disease (tumor involvement of other sites).

## RESULTS

Enzyme concentrations of lung cancer specimens and cultures are presented in Table 1. Values (other than correlation coefficients) were compared by the nonparametric 2-rank test of Mann and Whitney (18). The non-SCCL tumors consisted of 11 squamous carcinomas, 6 adenocarcinomas, 3 large cell carcinomas, and 2 mesotheliomas. The non-SCCL cultures consisted of 1 squamous carcinoma, 4 adenocarcinomas, 5 large-cell carcinomas, and 2 mesotheliomas. While most non-SCCL tumors had low or absent levels of DDC, the mean value was raised by 2 adenocarcinomas having high levels. Detectable DDC activity was present in all SCCL tumors, and the mean value was nearly 20-fold higher than in non-SCCL tumors. DDC activity in normal lung specimens was detectable but low. Low or absent DDC levels were present in non-SCCL cultures, while the mean value of SCCL cultures was over 1000-fold higher. Enzymatic CK values of SCCL tumors were approximately 4-fold higher than those of normal lung and nearly 10-fold higher than non-SCCL tumor values. The mean value of CK in SCCL cultures was more than 15-fold higher than of non-SCCL cultures. The mean values of immunoreactive CK-BB concentrations in SCCL tumors and cultures were 45- and 73-fold greater than their non-SCCL counterparts. CK-BB concentrations of normal lung were higher than in non-SCCL tumors but nearly 9-fold lower than in SCCL tumors. The values of all 3 enzymes were significantly higher ( $p < 0.01$ ) in SCCL tumors and cultures than in normal lung and non-SCCL tumors and cultures. Values for individual cultures are presented in Chart 1. There was very little overlap between values of SCCL cultures and tumors and the other groups.

Electrophoresis was used to determine the isoenzyme profile of enzymatic CK in normal lung, lung cancers, and cultures. With the exception of a few squamous carcinomas and mesotheliomas, more than 50% of the CK activities of normal lung, tumors, and cultures was in the form of CK-BB (Table 1). While the percentages of CK-BB in SCCL tumors and cultures were the highest of the mean values, they did not differ significantly from those in normal lung and non-SCCL tumors and cultures ( $p > 0.05$ ). Representative examples of electrophoretic patterns are presented in Chart 2.

While the values of enzymatic CK and immunoreactive CK-BB could not be directly compared, we explored a possible relationship by performing correlation coefficients (Table 1). Significant relationships existed for the 2 sets of values in normal lung, SCCL cultures, and SCCL tumors, although the ratio for normal lung differed from those of SCCL cultures and

Table 1  
Mean values of enzyme concentrations in lung cancer specimens and cultures

The percentages of biologically active CK in the form of CK-BB (as determined by electrophoresis) are included. The ratios of enzymatic CK and immunoreactive CK-BB are presented also.

Material	No.	DDC (units/mg)	CK (mIU/mg)	% of CK-BB	CK-BB (ng/mg)	CK/CK-BB	
						Ratio	$p^a$
Normal lung	6	0.4 (0.1-1.4) <sup>b</sup>	394 (160-606)	74 (68-85)	394 (105-625)	1.0	<0.05
Non-SCCL tumor specimens	22	2.3 (0-42)	135 (10-430)	76 (43-100)	78 (0-460)	1.7	>0.05
Non-SCCL cultures	12	0.1 (0-0.4)	128 (1-415)	76 (41-100)	103 (0-443)	1.2	>0.05
SCCL tumor specimens	8	41.2 (0.9-115)	1,258 (380-2,800)	90 (64-100)	3,491 (680-9,000)	0.4	<0.01
SCCL cultures	22	280.8 (1-1415)	2,004 (601-4,950)	88 (72-100)	7,487 (2,065-17,555)	0.3	<0.01

<sup>a</sup>  $p$  values were determined by calculation of the correlation coefficients ( $r$  values).

<sup>b</sup> Numbers in parentheses, range.

tumors. There was no demonstrable relationship between enzymatic CK and immunoreactive CK-BB values in non-SCCL tumors and cultures.

Enzyme concentrations were also measured in supernatant fluids of the cultures 24 hr after a medium change. DDC activity was not detected in any of the fluids. Enzymatic CK values for SCCL and non-SCCL cultures were 7 and 19 mIU/ml, respectively, and did not differ significantly ( $p > 0.05$ ). Immunoreactive CK-BB values were also low for both groups (18 and 1 ng/ml, respectively) but did differ significantly ( $p < 0.01$ ).

Because high levels of both DDC and CK-BB characterize SCCL tumors and cultures, we investigated whether both enzymes were regulated concordantly (Table 2). SCCL may un-

dergo alteration of morphology to other forms of lung cancer, especially large-cell carcinoma, both in the patient and after prolonged culture or heterotransplantation (1, 12, 13, 19).

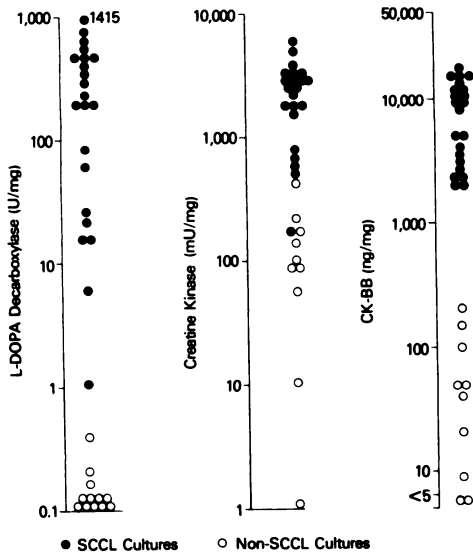


Chart 1. Enzyme concentrations of lung cancer cultures. Cell homogenates were prepared as described in the text and assayed immediately (enzymatic CK assays) or frozen at  $-70^{\circ}$  until assayed (DDC and CK-BB assays). Note log scale.

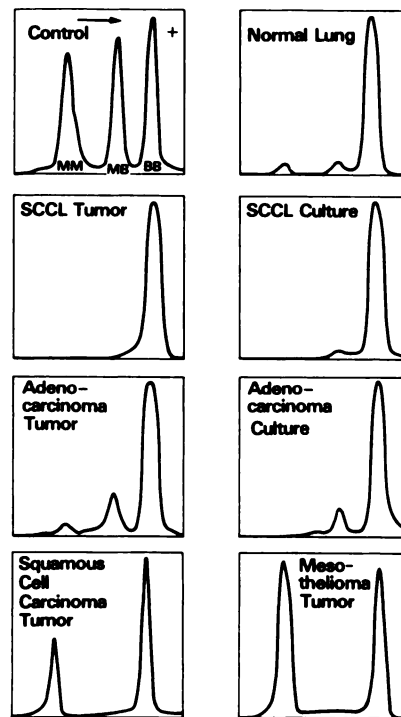


Chart 2. CK isoenzyme patterns of normal lung and lung cancer specimens and cultures. Isoenzyme distribution of CK was determined by electrophoresis on agarose gel as described in the text. Representative examples are displayed. Top left, control consisting of nearly equal amounts of the 3 cytoplasmic forms of CK. CK-BB migrates the furthest towards the anode and CK-MM migrates the least. With the exceptions of a few squamous cell carcinomas and mesotheliomas (as displayed), the predominant form in all specimens was CK-BB. The heights of the peaks in different panels cannot be directly compared since specimens were adjusted to optimal CK concentration prior to electrophoresis and because the densitometer contained an automatic gain device.

Table 2  
Discordant expression of DDC and CK-BB concentrations

The data of related cultures or tumor-culture combinations are grouped together and represent enzyme concentrations before, during, and after conversion from SCCL to LC<sup>a</sup> morphology. Culture NCI-N179, passaged approximately once every 7 days, gradually converted to LC morphology during the second year of culture. Culture NCI-H82 was initiated from a malignant effusion with mixed SCCL/LC cytology; the culture consisted entirely of LC. Two cultures were derived from another mixed SCCL/LC effusion; NCI-H60 started from the original specimen had only SCCL cytology, while NCI-N177 initiated from a nude mouse tumor induced by the same malignant effusion consisted entirely of LC. Parent culture NCI-N231 retained SCCL cytology, while its subline 417 had only LC cytology. The last data pair represents surgically resected pulmonary adenocarcinomas from different patients; both tumors had relatively high DDC levels.

Designation	Comment	Morphology	DDC level (units/mg)	CK-BB level (ng/mg)
NCI-N179	Passage 30	SCCL	110	3,081
NCI-N179	Passage 57	Mixed SCCL/LC	4.7	5,867
NCI-N179	Passage 88	LC	0.3	2,239
NCI-H82	Pleural fluid pellet	Mixed SCCL/LC	5.0	5,800
NCI-H82	Cell culture	LC	0.4	6,340
NCI-H60	Culture from pleural fluid	SCCL	15.5	12,544
NCI-N177	Culture from nude mouse tumor	LC	0	2,259
NCI-N231	Parent culture	SCCL	435	2,539
NCI-N231	Subline 417	LC	0	6,370
Patient L	Primary lung cancer	Adenocarcinoma	42	127
Patient R	Primary lung cancer	Adenocarcinoma	4.0	12

<sup>a</sup> LC, large cell.

Morphological conversion is accompanied by loss of APUD cell markers including DDC levels, neurosecretory granules, and polypeptide hormone secretion, as well as a drop in the high histaminase levels present in some SCCL tumors. We measured enzyme levels in SCCL tumors and cultures having characteristic SCCL cytology before, during, and after morphological conversion (Table 2). DDC levels were low in cultures that had converted to large-cell morphology and intermediate in culture NCI-N179 during conversion (*i.e.*, when it had mixed small cell-large cell morphology). However, high CK-BB concentrations were present before, during, and after morphological conversion. In contrast, tumor specimens and cultures of 8 large-cell carcinomas arising *de novo* had low CK-BB concentrations (Table 1 and Chart 1). Another form of discordant regulation of the 2 enzymes also was noted (Table 2); 2 surgically resected adenocarcinomas with relatively high DDC levels had low CK-BB concentrations.

We measured serum CK-BB levels in 67 untreated SCCL patients. The mean serum value of 209 adult volunteers was 3.4 ng/ml, and the 95th percentile was 6.2. Serum levels were normal (<10 ng/ml) in all 26 patients having limited-stage disease. In contrast, 16 of 41 patients (39%) with extensive-stage disease had elevated levels (mean of the elevated values was 82.3 ng/ml; range, 11.7 to 487). DDC activity was not detected in the sera of any of the 22 patients, (8 with limited-stage and 14 with extensive-stage disease), in whom it was measured.

## DISCUSSION

Our data, presented earlier (2, 14) and herein, indicate that high DDC levels readily distinguish SCCL from non-SCCL lung cancer cultures. In general, most SCCL tumors also have high DDC values, but there is some overlap with values of non-SCCL tumors. In fact, some non-SCCL tumors express the entire biochemical profile of APUD cells (2, 3). These results are explained by viewing lung cancer as a continuous, overlapping spectrum of tumor types having a common embryological origin (2, 3, 13).

In contrast to DDC, high levels of CK and CK-BB are characteristic of both SCCL tumors and cultures, and to date we have not identified high levels of these enzymes in lung cancers and cultures other than those of SCCL lineage. SCCL tumors may alter their morphology in the patient (1, 19) as well as in heterotransplanted tumors and long-term cultures (12, 13). Loss of typical SCCL morphology is accompanied by loss of many of the distinctive features of SCCL: neurosecretory granules; formaldehyde-induced fluorescence; high DDC levels; polypeptide hormone secretion; and even the high levels of histaminase present in some SCCL tumors. In contrast to the loss of these distinctive properties, high levels of CK-BB are retained in SCCL during and after conversion to large cell morphology. SCCL is usually considered, morphologically, an undifferentiated tumor. However, the constant presence of APUD cell properties, at least initially, implies biochemical differentiation (13). Loss of APUD cell properties suggests a change to a less differentiated form. Our findings indicate that DDC and CK-BB are expressed discordantly and suggest that CK-BB expression occurs at an earlier phase of differentiation. In addition, 2 adenocarcinomas expressing relatively high levels of DDC had low CK-BB concentrations. High expression

of CK, in the form of its BB isoenzyme, appears to be a more consistent feature of SCCL than of DDC. It also distinguishes large-cell carcinomas of SCCL lineage from morphologically similar tumors arising *de novo*. The relationship, if any, of elevated CK-BB levels to APUD cell properties remains to be determined. SCCL tumors are highly sensitive to chemo- and radiotherapy (8), while non-SCCL tumors are resistant. SCCL is not always identifiable by light microscopy, and poorly differentiated squamous cell carcinomas may mimic the histological appearances of SCCL (7). Biochemical markers, including CK-BB, may help to identify therapy-sensitive lung tumors.

Both DDC and CK-BB occur intracellularly and probably are not actively secreted. High levels of DDC characterize cells the primary function of which is small polypeptide hormone or biogenic amine secretion (21). CK activity is probably present in all cells, with each cell type regulating its level and isoenzyme pattern. We were not able to detect DDC activity in the supernatant fluids of SCCL cultures or in the sera of SCCL patients. However, relatively modest concentrations of CK-BB were present in SCCL culture fluids, and 39% of extensive-stage patients had elevated levels. These findings may reflect the greater sensitivity of the assay method for CK-BB.

SCCL tumors and cultures are characterized by very high levels of enzymatic CK and immunoreactive CK-BB. While the 2 sets of values cannot be compared directly, correlation coefficients indicate that a constant relationship exists between them. Similarly, in a small group of normal lung specimens, a significant relationship was also present, although the ratio of the values was very different from those present in SCCL tumors and cultures. In contrast, no relationship existed between CK and CK-BB in non-SCCL tumors and cultures. Tumors, especially lung cancers, may secrete products such as "big" adrenocorticotrophic hormone which are immunoreactive but not biologically active (20). The non-SCCL tumors and cultures that we studied represent a diverse group of at least 4 subtypes. It is possible that various subtypes, or even individual tumors within each subtype, vary in their ratios of enzymatic and immunoreactive CK.

Relatively few human or animal tumors have been analyzed for CK activity or isoenzyme profile. Shatton *et al.* (23) found that a variety of rodent tumors had CK activities at about the same levels as those present in their respective tissues of origin. However, the isoenzyme profile of some of the tumors differed from that of the tissue of origin. Normal lung and almost all of the lung cancers and cultures that we analyzed expressed most of their CK activity in the form of CK-BB. Thus, the activity of CK in SCCL tumors and cultures is quantitatively but not qualitatively different from those of normal lung and other forms of lung cancer. Data on human tumors are limited to a few reports of single or small numbers of various types (9, 15, 16). Of interest, Coolen *et al.* (9) found that 2 SCCL tumors had higher CK-BB levels than did adjacent lung tissue. Increased levels of serum CK-BB have been described in a varying percentage of patients with diverse tumor types (9, 11, 15). Using an indirect method, Coolen *et al.* (9) demonstrated an increase in serum CK-BB in 12 of 15 patients with SCCL. They did not describe the tumor stage or therapy status of their patients. Using a specific and sensitive radioimmunoassay, we report elevated levels in 39% of extensive-stage patients but in none of the limited-stage patients. Thus, the unexpected finding of an elevated CK-BB serum level in a patient believed to have

limited-stage SCCL indicates that further staging procedures are required. One possible mechanism for elevated serum CK-BB levels in cancer patients is the presence of brain metastases with destruction of surrounding brain tissue and release of its high CK-BB content (9). Our findings of high intracellular concentrations of CK-BB in SCCL tumors and cultures and relatively modest levels in culture fluids suggest an alternative explanation. Elevated serum levels in some SCCL patients having extensive-stage disease probably reflect large tumor burdens, with release of enzyme from necrotic or dying tumor tissue.

In muscle cells, high levels of CK and its substrate creatine phosphate are a mechanism for the rapid regeneration of ATP stocks depleted during contraction (22). Our findings suggest that the energy requirements of SCCL are very different from those of normal lung and other lung cancers.

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## REFERENCES

- Abeloff, M. D., Eggleston, J. C., Mendelsohn, G., Ettinger, D. S., and Baylin, S. B. Changes in morphologic and biochemical characteristics of small cell carcinoma of the lung. *Am. J. Med.*, **66**: 757-764, 1979.
- Baylin, S. B., Abeloff, M. D., Goodwin, G., Carney, D. N., and Gazdar, A. F. Activities of L-dopa decarboxylase and diamine oxidase (histaminase) in human lung cancers and decarboxylase as a marker for small (oat) cell cancer in cell cultures. *Cancer Res.*, **40**: 1990-1994, 1980.
- Baylin, S. B., and Gazdar, A. F. Endocrine biochemistry in the spectrum of human lung cancer: implications for the cellular origin of small cell carcinoma. *In*: A. Greco, P. A. Bunn, and R. Oldham (eds.), *Small Cell Lung Cancer*, pp. 123-143. New York: Grune and Stratton, 1981.
- Baylin, S. B., Stevens, S. A., and Shakir, K. M. Association of diamine oxidase and ornithine decarboxylase with maturing cells in rapidly proliferating epithelium. *Biochim. Biophys. Acta*, **541**: 415-419, 1978.
- Baylin, S. B., Weisburger, W. R., Eggleston, J. C., Mendelsohn, G., Beaven, M. A., Abeloff, M. D., and Ettinger, D. S. Variable content of histaminase, L-dopa decarboxylase and calcitonin in small cell carcinoma of the lung. *N. Engl. J. Med.*, **299**: 105-110, 1978.
- Beaven, M. A., and Jacobsen, S. A new assay for histaminase activity. *J. Pharmacol. Exp. Ther.*, **176**: 52-64, 1971.
- Churg, A., Johnston, W. H., and Stulbarg, M. Small cell squamous and mixed small cell squamous and small cell anaplastic carcinomas of the lung. *Am. J. Surg. Pathol.*, **4**: 255-263, 1980.
- Cohen, M. H., Ihde, D. C., Bunn, P. A., Fossieck, B. E., Matthews, M. J., Shackney, S. E., Johnston-Early, A., Makuch, R., and Minna, J. D. Cyclic alternating combination chemotherapy for small cell bronchogenic carcinoma. *Cancer Treat. Rep.*, **63**: 163-170, 1979.
- Coolen, R. B., Pragay, D. A., Nosanchur, J. S., and Belding, R. Elevation of brain-type creatine kinase in serum from patients with carcinoma. *Cancer (Phila.)*, **44**: 1414-1418, 1979.
- Dym, J., and Jaffe, D. Expression of creatine kinase isoenzymes in myogenic cell lines. *Dev. Biol.*, **68**: 592-599, 1979.
- Feld, R. D., Van Steirteghem, A. C., Zweig, M. H., Weimar, G. W., Narayana, A. S., and Witte, D. L. The presence of creatine kinase isoenzymes in patients with prostatic cancer. *Clin. Chim. Acta*, **100**: 267-273, 1980.
- Gazdar, A. F., Carney, D. N., Baylin, S. B., and Guccion, J. Small cell carcinoma of the lung: altered morphological, biological and biochemical characteristics in long term cultures and heterotransplanted tumors. *Proc. Am. Assoc. Cancer Res.*, **21**: 51, 1980.
- Gazdar, A. F., Carney, D. N., Guccion, J. G., and Baylin, S. B. Small cell carcinoma of the lung: cellular origin and relationship to other pulmonary tumors. *In*: A. Grecco, P. A. Bunn, and R. Oldham (eds.), *Small Cell Lung Cancer*, pp. 145-175. New York: Grune and Stratton, 1981.
- Gazdar, A. F., Carney, D. N., Russell, E. K., Sims, H. L., Baylin, S. B., Bunn, P. A., Guccion, J. G., and Minna, J. D. Establishment of continuous, clonable small cell carcinomas of the lung cultures having amine precursor uptake and decarboxylation properties. *Cancer Res.*, **40**: 3502-3507, 1980.
- Hoag, G., Amies, D. R., and Colquhoun, B. D. The production of creatine kinase isoenzyme BB in sera of a patient with prostatic carcinoma and in tumor homogenates. *Clin. Biochem.*, **11**: 38-41, 1978.
- Hoag, G. N., Singh, R., Franks, C. R., and DeCoteau, W. E. Creatine kinase isoenzymes in testicular tissue of normal subjects and a case of lymphoblastic lymphosarcoma. *Clin. Chem.*, **26**: 1360-1361, 1980.
- Jockers-Wretou, E., and Pfeleiderer, G. Quantitation of creatine kinase isoenzymes in human tissues and sera by an immunological method. *Clin. Chim. Acta*, **58**: 223-232, 1975.
- Mann, H. B., and Whitney, D. R. On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Statist.*, **18**: 50-60, 1947.
- Matthews, M. J., and Gazdar, A. F. Pathology of small cell carcinoma of the lung and its subtypes: a clinico-pathologic correlation. *In*: R. B. Livingston (ed.), *Lung Cancer: Advances in Research and Treatment*, pp. 283-306. The Hague: Martinus Nijhoff, 1981.
- Odell, W., Wolfson, A., Yoshimoto, Y., Weitzman, R., Fisher, D., and Hirose, F. Ectopic peptide synthesis: a universal concomitant of neoplasia. *Trans. Assoc. Am. Phys.*, **40**: 204-227, 1977.
- Pearse, A. G. The cytochemistry and ultrastructure of polypeptide hormone producing cells of the APUD series, and the embryologic, physiologic and pathologic implications of the concept. *J. Histochem. Cytochem.*, **17**: 303-313, 1969.
- Saks, V. A., Rosenshtraukh, L. V., Smirnov, V. N., and Chazov, E. I. Role of creatine phosphokinase in cellular function and metabolism. *Can. J. Physiol. Pharmacol.*, **56**: 698-706, 1978.
- Shatton, J. B., Morris, H. P., and Weinhouse, S. Creatine kinase activity and isozyme composition in normal tissues and neoplasms of rats and mice. *Cancer Res.*, **39**: 492-501, 1979.
- Szasz, G., Gerhardt, W., and Gruber, W. Creatine kinase in serum: 3. Further study of adenylate kinase inhibitors. *Clin. Chem.*, **23**: 1888-1892, 1977.
- Tsung, S. W. Creatine kinase isoenzyme patterns in human tissue obtained at surgery. *Clin. Chem.*, **22**: 173-175, 1976.
- Zweig, M. H., Van Steirteghem, A. C., and Schechter, A. N. Radioimmunoassay of creatine kinase isoenzymes in human serum: isoenzyme BB. *Clin. Chem.*, **24**: 422-428, 1978.