A New Proteolipid Apparently Associated with Cancer[1] (35471)

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In the course of studies of the lipid composition of human serum lipoproteins (1, 2) we observed a new lipid in thin-layer chromatograms (TLC) (3) of lipid extracts from high-density lipoproteins (HDL) from cancer patients. Similar extracts from HDL of normal subjects did not reveal this lipid. We since found that lipids extracted from transplanted animal tumors also contain a similar lipid.

This preliminary communication describes the general chemistry of this lipid, its distribution in rat tissues, its presence in sera from cancer patients, and implications of these observations.

Nature of new lipid. The new lipid was isolated from lipid extracts of Walker carcinosarcoma 256 (W256) by silicic acid column chromatography with stepwise gradient elution (chloroform–methanol mixtures). The isolated lipid had the same R_f in several TLC systems (see below) as did the new lipid in the sera HDL of cancer patients. Complete drying of the isolated new lipid from W256 *in vacuo* (with N_2 bubbling) caused partial decomposition. Decomposition products, identified by TLC (3, 4), included phosphorus and nonphosphorus containing lipids. It was inferred that the new lipid is a complex unstable under certain conditions. In addition to complete drying, contact with sodium oxalate and treatment with ultrasonic vibration also cause decomposition of this complex. Further chemical studies of the complex revealed that protein (or polypeptide) was also present. The amount of protein determined by the Lowry *et al.* (5) procedure and the Hess and Lewin (6) modification was 7–8%, and by gas-liquid chromatography analysis of the amino acids (7) in the hydrolyzate of this complex was ~6–8%. The protein–lipid complex behaves as a lipid being extractable from tissues along with other lipids by chloroform:methanol, 2:1 (v/v), 20 ml of solvent to 1 g of wet tissue. It remained in the solvent phase during purification of lipid extracts by the procedure of Folch *et al.* (8, 9). It was soluble in chloroform:methanol mixtures 2:1 to 1:3 (v/v) and in toluene. It was essentially insoluble in water or weak salt solutions. These properties indicated that the complex was a proteolipid by the Folch-Pi classification (10).

The proteolipid isolated from W256 differed in the following ways from proteolipids from bovine and human nervous tissues (10, 11) and nonneural tissues (12–14): the new proteolipid chromatographs differently on silicic acid columns (15); it contains significantly less protein (10, 13, 15), which is characterized by a higher glutamic acid and/or glutamine content, and there is a stronger association between the lipid and protein moieties. Acid hydrolyzates of the neoproteolipid contain high quantities of monosaccharides (approx 45%) which are probably present as glycolipids. Fatty acids present in neoproteolipid are predominently those with 24 carbon chains. As described below, the new proteolipid shows a markedly different distribution pattern in tissues from that reported for other proteolipids (10, 12). Consequently, the provisional term neoproteolipid has been assigned to the new proteolipid isolated from W256 to distinguish it from other types of proteolipids.

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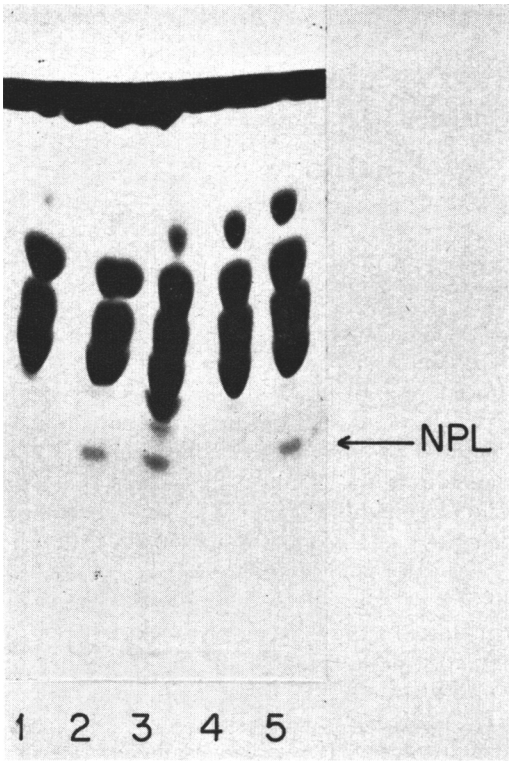

FIG. 1. Thin-layer chromatograms of lipid extracted from tissues of normal rats and tumors. Each lipid extract was applied in 1-mg quantities: (1) lung; (2) lung plus added neoproteolipid, as internal standard; (3) Walker 256 carcinosarcoma; (4) liver; (5) liver plus added neoproteolipid as internal standard. Developing solvent (b) (see text). NPL = position of neoproteolipid spot. Spots were detected with 40% sulfuric acid spray.

Distribution of neoproteolipid in tissues. Lipid extracts from nearly all tissues were tested by three different silica gel TLC systems: (a) chloroform : methanol : acetic acid : water, 100:50:18:8 (v/v) (3, 16); (b) chloroform : methanol : 2 *N* ammonium hydroxide, 90:68:20 (v/v) (16); and (c) chloroform: methanol:1 *N* ammonium hydroxide, 60:40: 6.5 (v/v) (16). When all three chromatograms revealed that lipid extracts of the tissues contained neoproteolipid, this was considered evidence for the presence of this lipid. Neoproteolipid would be detected if it is at least 0.03% of the total lipid extracted from the tissues. With extracts of human serum HDL it sufficed to use only TLC system (a) because of the simpler composition of such extracts.

Lipid extracts from several different transplanted tumors and tissues from normal rats were tested for the presence of neoproteolipid. It was present in the lipid extracts of the three different transplanted tumors tested: W256 in the rat, sarcoma 180 and the Taper liver tumor in mice; in mice TLC (a) used. Figure 1 is a chromatogram of lipid from W256 and some tissues from normal rats. The lipid extracts from a 7–14-day-old W256 tumor contained an average of 1.1% neoproteolipid as determined by densitometry of the charred chromatograms or by weighing the fraction of neoproteolipid isolated by column chromatography from tumor lipid extracts.

Of normal tissues, only spleen had significant amounts of neoproteolipid (av, 0.23% of total lipids). Whereas trace amounts were found (~0.03% of total lipids) in lung and small intestine, neoproteolipid was not detected in liver, brain, kidney, serum, muscle, and heart, within the limits of the sensitivity of the test.

Presence of neoproteolipid in serum of rats bearing W256. TLC of lipid extracts of whole serum from rats with W256 did not reveal neoproteolipid although it was detected in lipid extracts of their HDL. However, when 36 mg of whole serum lipid extract were applied to a silicic acid column and one-half the fraction of the eluate in which neoproteolipid usually emerged from the column was chromatographed on TLC, the presence of neoproteolipid was revealed. Thus, concentrating neoproteolipid with silicic acid column chromatography, this lipid can be detected when present as 0.003% of total lipid extract. It averaged 0.018% of the total lipid extracts of sera of rats bearing W256. Extracts of sera from normal rats were negative when tested identically.

Neoproteolipid in high density lipoproteins of patients with cancer. Lipids extracted from HDL isolated from sera (2, 17) of patients with cancer and from normal subjects were tested for neoproteolipid. The results are shown in Table I. The first series of experiments were performed on HDL of sera from patients with several types of advanced cancer (mostly inoperable), who were receiving no medication at the time (2, 17). Lipid extracts were tested directly on one system of TLC, system (a). Neoproteolipid was

TABLE I. Detection by Thin-Layer Chromatography of Neoproteolipid in High Density Lipoproteins from Sera of Patients with Cancer and from Normal Subjects.

Sources of sera and (sex)	Presence of neoproteolipid
Normal subjects	
15 individual samples (F)	—[a]
1 pooled sample (M and F)	—
Cancer patients, Series I (no treatment before blood samples)	
8 Carcinoma of breast patients (F)	+
2 Ovarian carcinoma (F)	+
1 Bronchiogenic carcinoma (F)	+
1 Cancer of the colon (M)	+
1 Chronic Myelocytic Leukemia (M)	±
Cancer patients, Series II (all patients under treatment)[b]	
1 Melanoma (M)	+
1 Epidermoid bronchiogenic carcinoma[b] (M)	+
1 Neuroblastoma (F)	+
1 Carcinoma of uterus,[b] metastases (F)	+
1 Carcinoma of breast,[b] bone metastases (F)	+
1 Carcinoma of breast,[b] (1 week postmastectomy) (F)	±
1 Carcinoma of breast,[b] (minimal recurrence 2 years postmastectomy) (F)	—

[a] One sample out of 15 was questionable in first test but negative on retest.

[b] Primary tumor was removed.

present in lipid extracts from 12 of the 13 patients. Results were not clear on one patient who was in remission with chronic myelocytic leukemia. Extracts of other lipoproteins tested—those with densities <1.063 g/ml or lipoproteins with densities >1.21 g/ml (2, 18)—did not contain neoproteolipid.

Neoproteolipid was not detected in one pooled sample nor in 15 individual normal control subjects; however, the results of the first sample from one control subject were questionable but a second sample showed no neoproteolipid.

In the second series of experiments with patients with cancer, five had undergone surgery for removal of the primary tumor but there were evidences of metastases. All of the patients were receiving some form of therapy. Neoproteolipid was present in the sera of five of the seven patients. The result was indefinite in one patient studied 1 week after radical mastectomy. In another patient, no neoproteolipid was detected in a sample taken at the time of minimal recurrence of breast carcinoma 2 years after removal of the primary tumor.

The data from determinations on rats, both normal and those bearing W256, and on man suggest that the presence of neoproteolipid in blood serum in measurable quantities could be specific for the presence of cancer and might eventually provide a test for cancer. In addition, the chemical composition, as thus far determined, suggests the neoproteolipid should have immunologic properties.

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