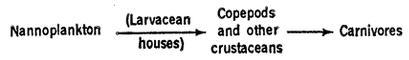
utilize most nannoplankton since they have difficulty filtering particles smaller than 5 to 10 μm in diameter (*5, 8*). This discovery of the use of concentrated nannoplankton on abandoned larvacean houses by pelagic copepods, as either a major or a supplementary food source, reveals avenues in the pelagic food web, of the form:



The abundance of these abandoned houses and the variety of crustacean species observed on them suggest that this food chain may be significant. Second, strictly herbivorous, planktonic copepods are not limited to suspension feeding or direct capture of large phytoplankters (*9*) as the only modes of obtaining food. Abandoned larvacean houses, mucus feeding webs of pelagic gastropods (*7*), and organic aggregates (*3*) provide innumerable microsurfaces that are essentially benthic in nature and suitable for benthic feeding methods. Third, the abundance of abandoned houses suggests that larvaceans may be a major source of the particulate organic matter in the sea. As they decay, the houses form organic aggregates and may provide free surfaces for the absorption of dissolved organic matter, a process already documented for surfaces produced by bubbles and Langmuir circulations (*3, 10*). Last, since larvacean houses are invariably destroyed, disintegrated, or filtered through most conventional plankton nets, direct observations must be made of many planktonic forms if their biology is to be correctly understood.

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Separation of Skin Reactive Intestinal Cancer Antigen from the Carcinoembryonic Antigen of Gold

Abstract. *Soluble fractions of human intestinal cancer and fetal intestinal cell membranes produced delayed hypersensitivity reactions in patients with intestinal cancer. These soluble fractions and perchloric acid extracts of intestinal cancer cells were fractionated by polacrylamide-gel electrophoresis. The Gold carcinoembryonic antigen was found in a region of the gels different from that of the skin reactive antigen.*

Delayed hypersensitivity reactions in patients with intestinal cancer have been elicited by a soluble fraction of membranes prepared from autologous and allogeneic tumor cells (*1, 2*). These reactions appeared to be specific since negative reactions were obtained with comparable soluble membrane fractions from normal cells. Skin reactive antigen was also detected in soluble cell membrane fractions from the intestines of 1- to 6-month fetuses.

The soluble fractions producing skin reactions were also shown to contain the carcinoembryonic antigen (CEA) of Gold (*1, 2*). Gold and Freedman (*3*) have shown that human intestinal cancers contain an antigen which is also found in embryonic entodermal tissues, during the first two trimesters of pregnancy. The CEA is a glycoprotein closely associated with the cell surface membrane (*4*).

A question raised by the previous studies (*1, 2*) was whether CEA was the same as the skin reactive antigen. One observation which cast some doubt as to whether these antigens were identical was that purified CEA produced negative skin reactions (*2*). Moreover, Lejtenyi *et al.* (*5*) have reported that purified CEA failed to induce blast transformation of the lymphocytes of patients with intestinal cancer. We have now separated active fractions by gel electrophoresis in order to ascertain the relation of skin reactive antigen to CEA.

Six patients (George Washington University Hospital) with rectal and colonic carcinomas were selected on the basis of positive tests with one or both recall (control) antigens—that is, mumps and SKSD [Varidase (Lederle), a product consisting of streptokinase (40 units) and streptodornase (10 units)]. All patients had definitive resection of localized tumor, were tested 1 to 2 weeks after operation, and were not receiving chemotherapy or other treatment. They were inoculated intradermally with 0.1 ml of the various gel fractions of sonicated cell membranes of allogeneic tumor and of fetal intestine, of CEA, or of partially purified CEA. Erythema and induration were measured at 24, 48, and 72 hours. A positive delayed reaction was defined as induration of 5 mm or greater at 48 hours. Only 20 to 30 percent of the membrane proteins were recovered in the soluble portions of the sonicated membranes. Membrane preparations, 50 to 100 μg of protein per 0.1 ml, produced delayed skin reactions; however, the pooled soluble sonicated membranes (prior to separation), of comparable or twice the protein concentration of membrane preparations, did not produce delayed skin reactions. The Sephadex fractions producing delayed skin reactivity at 5 to 25 μg of protein per 0.1 ml contained more than 50 percent of the protein recovered from the columns. The yields from the cancer extracts and from the fetal liver or intestinal material were similar. The elution patterns of the protein peaks containing the skin reactive antigen were virtually identical (ratio of elution volume to void volume of peaks was 2.4) from each tumor cell membrane preparation.

Partially purified CEA and purified CEA were obtained as follows (*6*). Homogenates of hepatic metastases of intestinal cancer were extracted with perchloric acid and separated by Sepharose 4B and Sephadex G-200, yielding partially purified CEA; the purified CEA was then obtained by preparative block electrophoresis. Both were tested within 1 month for skin reactivity. The skin reactive antigens from both the intestinal cancer and fetal cell membranes, as well as CEA and partially purified CEA, were further separated by gradient polyacrylamide-gel electrophoresis (*7, 8*). Immediately after it was used for skin testing, the partially purified CEA was subjected to electrophoresis; a similar preparation

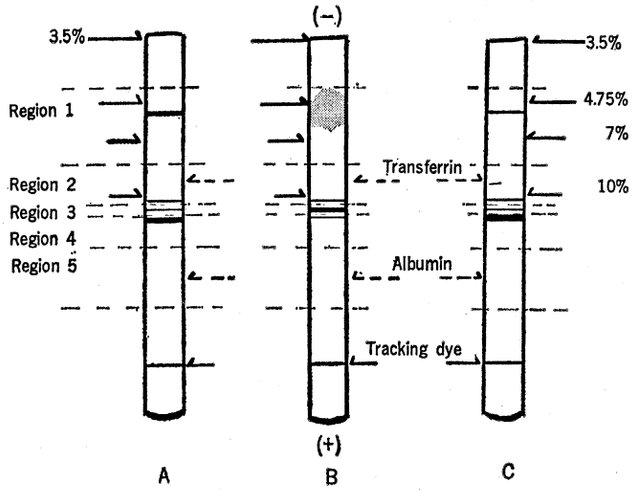

Fig. 1. The partially purified CEA preparation (B) and the skin reactive Sephadex G-200 (C) fractions of the intestinal cancer and the fetal intestinal membrane extract (A) were fractionated by gradient polyacrylamide-gel electrophoresis (3.5 to 10 percent gel). A tracking dye (bromphenol blue) was used in each preparation. Albumin (67,000 molecular weight) and transferrin (approximately 90,000 molecular weight) were run on separate gels. On each of the four gels was placed 13 μg of protein of the membrane-extracted materials and 15 μg of CEA. One gel of each preparation was stained with Coomassie brilliant blue. The others were sliced into five parts as indicated by the dashed lines and the proteins were eluted and concentrated by ultrafiltration for testing. The diffuse zone of glycoprotein is indicated by the hatching.

was stored at −60°C for 10 months and then subjected to electrophoresis. The patterns of protein distribution of three of these materials were very similar (Fig. 1). There were three lower bands in virtually the same positions (regions 2 to 4) with each preparation. These proteins migrated to positions intermediate between the albumin and transferrin reference points. The intestinal cancer and fetal preparations had an additional band near the cathodal end of the gels (region 1). In the same region with the partially purified CEA, there was diffuse staining of the gel by Coomassie blue.

Each of the fractionated gel preparations was sliced into five parts (as indicated in Fig. 1). The proteins in each region were eluted with sterile saline (*7, 8*), concentrated, diluted 100-fold, and again concentrated by Diaflo ultrafiltration. Each gel fraction was divided into two portions, which were tested (blind) for skin reactivity and for CEA content (*9*). The results of both tests are given in Table 1.

With each preparation, positive skin reactions were elicited by the proteins in regions 3 and 4. No positive skin reactions were observed with regions 1 and 2. In contrast, only region 1 of the membrane-extracted materials and regions 1 and 2 of the CEA preparation had detectable amounts of CEA.

Skin tests with the more purified CEA preparation were negative, when as much as 100 μg protein per 0.1 ml were tested in two nonanergic patients. The CEA was fractionated on polyacrylamide gels, and a diffuse zone was detected in the same region 1 area, as indicated in Fig. 1 for the partially purified CEA. No other bands were seen. Another CEA preparation was later fractionated on polyacrylamide gels. No protein bands were seen. This latter material had been in the lyophilized state at −60°C for 10 months, and some deterioration may have occurred.

In order to locate polysaccharides that might be associated with any of the protein bands, CEA, partially purified CEA, and the skin reactive fetal antigens were each again tested in quadruplicate for further separation by gradient polyacrylamide-gel electrophoresis. Polysaccharide was detected (*8*) by periodic acid–Schiff reagent staining of duplicate gels and matched with duplicate stains with Coomassie brilliant blue. In each preparation, polysaccharide was detected only in the Coomassie-stained diffuse zone of region 1.

Our results indicate that there are two separable types of antigens in human intestinal cancer. Each of these antigens was found in the fetal extracts and may, therefore, generically be called carcinoembryonic antigens. One of these antigens, CEA (the carcinoembryonic antigen of Gold) was found in regions 1 and 2 of the polyacrylamide gels of all the preparations. The other antigen, which produced delayed hypersensitivity skin reactions in patients with intestinal cancer, migrated considerably further into the gels and presumably has a lower molecular weight than CEA does.

Table 1. Assays of proteins eluted from polyacrylamide gels for skin reactivity and for CEA.

Material tested	Delayed skin reaction	CEA
Fetal intestine skin reactive Sephadex fraction		
Region 1	—	+ (2 ng/150 μl)
Region 2	—	—
Region 3	+	—
Region 4	+	—
Region 5	—	—
Adult colon cancer skin reactive Sephadex fraction		
Region 1	—	+ (0.75 ng/150 μl)
Region 2	—	—
Region 3	+	—
Region 4	+	—
Region 5	—	—
Partially purified CEA		
Region 1	—	+ (> 200 ng/40 μl)
Region 2	—	+ (50 ng/40 μl)
Region 3 + 4	+	—
Region 5	—	—
Purified CEA		
	—	+

The methods for extraction of partially purified CEA (*3, 6*) were quite different from those employed with the membrane extracts. It was quite remarkable that the different preparations gave such similar patterns on the polyacrylamide gels. The close association of the two different antigens in both types of preparations is consistent with their coming from similar locations on the cell membranes (*4*). Previous skin tests with purified CEA gave negative skin reactions (*1, 2*). It was suggested that the extraction procedures might have resulted in denaturation and loss of skin reactivity of the CEA (*1*). However, since skin reactive antigen was found in our study in the CEA preparation (Table 1), it appears resistant to perchloric acid extraction. It is now clear that, in the previously tested, purified CEA preparations the skin reactive antigen had already been separated from the CEA activity.

Our studies have raised question as to whether the skin reactive antigen circulates, like CEA, in the serum, whether each antigen has a distinct, biological role, and whether either or both of these antigens plays a role in host resistance to intestinal cancer.

Antibodies to CEA were found in the serums of some patients with intes-

tinal cancer (*10*). Some patients have cellular immunity to the skin reactive antigen (*1*, *2*). It remains to be determined how these reactions correlate with each other and with the clinical state of the patients. Purified CEA preparations have failed to produce blast transformation of the lymphocytes of patients with intestinal cancer (*5*). These CEA preparations have also been inactive in migration inhibition assays (*11*). It will be of interest to test the skin reactive antigen in these assays. Using the colony inhibition assay, Hellstrom *et al.* (*12*) have found reactivity, in the lymphocytes of patients with intestinal cancer, against antigens common to human colonic carcinomas and fetal gut. Whether the antigen detected by this assay is the same as either CEA or the skin reactive antigen, or whether there is a third type of carcinoembryonic antigen in human intestinal cancers remains to be determined.

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Cyclic Changes in Insulin Needs of an Unstable Diabetic

Abstract. *The fluctuating insulin requirements of an unstable diabetic over an 8-year period have been subjected to spectral analysis. There is evidence of cyclic changes of several different period lengths in addition to red noise. The periodicities indicate that social causes play no major role but suggest that a weather-mediated effect may exist.*

Diurnal variations in glucose tolerance have been established (*1*) and annual variations in the incidence and severity of diabetes have long been known (*2*). In addition there is a large variance in the glucose tolerance of unselected individuals from day to day (*3*), and one type of diabetic, the unstable (brittle or labile) diabetic, displays wide variations on the scale of hours to days (*4*) in the amount of insulin required to maintain normoglycemia. It is our intention to show that these variations, for one unstable diabetic, are not entirely random but exhibit deterministic changes of several different cycle periods, and a strong red-noise or $1/f$ trend (*5*) in their spectrum (f = frequency).

One of us (M.J.C., age 34) is an unstable diabetic who has kept records of the quantity of insulin, mostly NPH or Ultralente, he requires each day. The dosage has been adjusted, incrementally several times a day as required, to maintain normal blood sugar levels while the dietary intake of carbohydrate was kept constant by estimation and taken in regularly divided portions. The criterion of "normal" blood sugar was originally the absence of subjective hypoglycemia (roughly, pro-

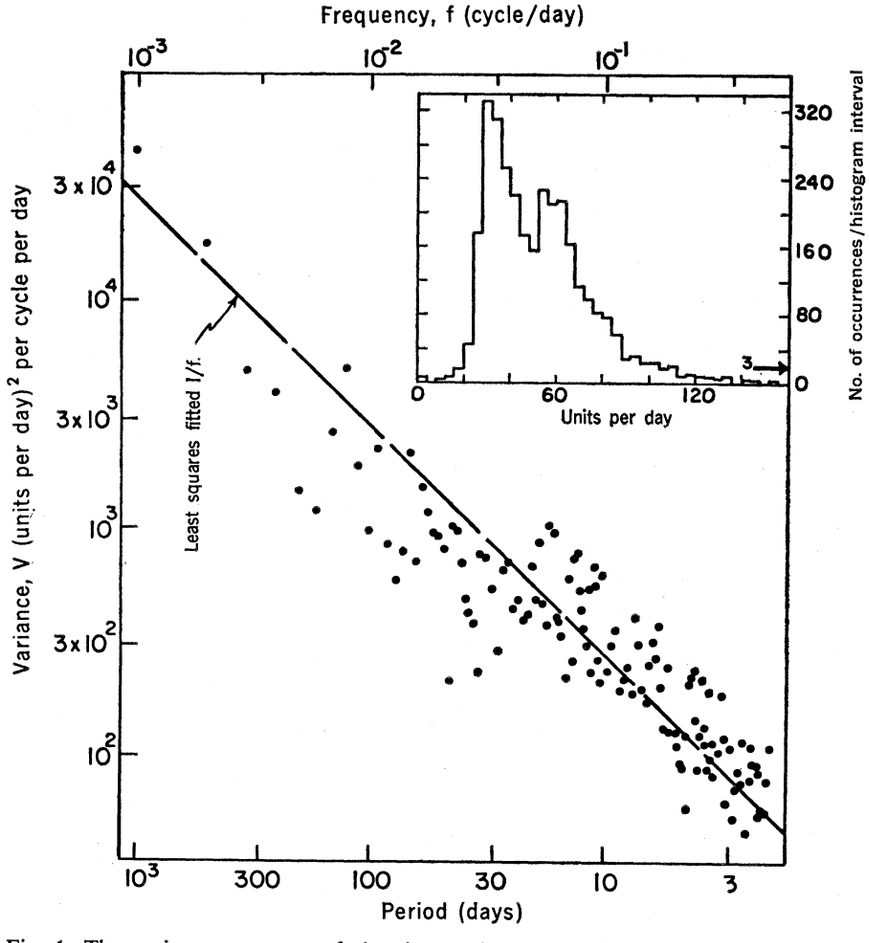

Fig. 1. The variance spectrum of the time series formed by 3072 consecutive daily insulin doses. In this log-log plot of the variance density against frequency, the 512 points of the raw spectrum have been grouped for clarity. The first 30 points are untreated; points 31 to 100 are averaged in pairs; points 101 to 300 are averaged in fives; points 301 to 512 are averaged in tens. (Inset) The distribution of 3109 daily doses of insulin, in insulin units per day. There are three doses in excess of 160 units per day.