

## SPECIFIC CARCINOEMBRYONIC ANTIGENS OF THE HUMAN DIGESTIVE SYSTEM\*

By PHIL GOLD,† M.D., AND SAMUEL O. FREEDMAN, M.D.

(From the McGill University Medical Clinic, Montreal General Hospital, and the Department of Physiology, McGill University, Montreal, Canada)

(Received for publication, April 29, 1965)

In a previous study reported from this laboratory (1), at least two common, qualitatively tumor-specific antigens or antigenic determinants were demonstrated to be present in adenocarcinomata of the human colon. These antigenic components were unrelated to iso-antigens, bacterial contaminants, or fibrin deposits. The present investigation was undertaken to determine whether the antigens found to be specific for colonic carcinomata could be detected (*a*) in other adult human tissues, or (*b*) in human embryonic and fetal tissues at various stages of gestation.

Certain investigators have suggested the existence of universal tumor-specific antigens common to all forms of human malignant tumors (2-4), while others have attempted to classify human cancers on the basis of the particular tumor-specific antigens that they may contain (5). These concepts have, however, been challenged (6) and their general acceptance awaits further experimental study (7).

### *Materials and Methods*

*Preparation of the Human Colonic Tumor Tissue Extract (T<sub>1</sub>)*—The pooled colonic tumor tissue extract was prepared according to the method previously described (1).

*Preparation of the Anticolonic Tumor-Specific Antiserum (t<sub>1</sub>-abs)*—Rabbits were immunized with the extract of pooled colonic carcinomata (T<sub>1</sub>), and the anti-tumor antiserum thus produced was absorbed with a pooled extract of normal human colon and with human blood components. The procedure followed was identical to that previously described (1).

*Extraction Procedure for the Individual Human Tissue Specimens*—Tissue specimens to be tested were obtained either at operation or at autopsy within 12 hours of death. Tissue diagnoses were determined by histological examination. A portion of each specimen was extracted by the procedure used for the preparation of T<sub>1</sub> except that the supernatants obtained following the centrifugation steps were lyophilized. Immediately before initial testing, the powdered material was dissolved in the smallest volume of distilled water which would allow complete resolution. The protein concentrations of these solutions varied from 80 to 100 mg/ml (Kjeldahl).

\* This work was supported by research grants from the Cancer Research Society, Montreal, and the National Cancer Institute of Canada, Toronto, Canada.

† Medical Research Fellow, Medical Research Council of Canada.

*Precipitin-Inhibition and Direct Ouchterlony Reactions.*—Both types of reactions were performed in agar gel plates prepared as previously described (1). The patterns were cut in the gel plates so that the central well was spaced 1 cm from each of the six peripheral wells.

In the precipitin-inhibition reactions, the central well was filled with 0.15 ml of  $T_1$  (10 mg protein/ml). A volume of 0.075 ml of  $t_1$ -abs was placed into each of the six peripheral wells. Interaction of  $T_1$  with  $t_1$ -abs was known to give rise to a reproducible pattern consisting of a single tumor-specific precipitin band (1).

To one of the six peripheral well, 0.075 ml of saline was added so that the usual precipitin band could develop. To a second peripheral well, 0.075 ml of  $T_1$  was added so that complete inhibition of the precipitin band was achieved. To each of the other four peripheral wells, 0.075 ml of an extract of an individual tissue specimen was added. If the usual single band failed to develop between the central well and one of the peripheral wells, it was considered that the tumor-specific antigen(s) of  $T_1$  and the test antigenic material shared common antigenic determinant groups. Failure of the expected precipitin band to appear constituted a positive precipitin-inhibition reaction.

The initial protein concentration of the test antigenic materials placed in the peripheral wells was 80 to 100 mg/ml. If a positive precipitin-inhibition test was obtained, the active test material was serially diluted and the precipitin-inhibition reactions were repeated until a negative test was obtained. It was therefore possible to determine the degree to which an extract could be diluted but still retain demonstrable antigenic (*i.e.*, inhibitory) activity. An active tissue extract, in the highest or next to highest dilution capable of producing precipitin-inhibition, was then used in a direct Ouchterlony reaction against  $t_1$ -abs. The use of diluted test extracts in the direct Ouchterlony reactions did much to overcome the problem of non-specific precipitation around the antigen wells which frequently occurs with concentrated tissue protein solutions. In many cases this phenomenon would have made interpretation of results difficult.

#### RESULTS

Extracts of 322 human tissue specimens were studied for their capacity to inhibit the single tumor-specific precipitin band formed between  $T_1$  and  $t_1$ -abs in agar gel. Typical precipitin-inhibition reactions are shown in Figs. 1 to 3,

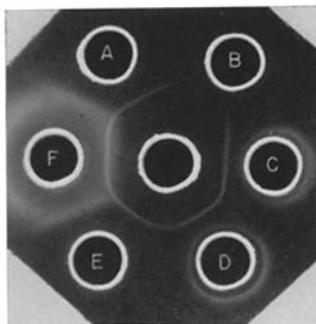


FIG. 1. Example of precipitin-inhibition reactions, all test extracts negative. Center,  $T_1$ , (A)  $t_1$ -abs + saline, (B)  $t_1$ -abs +  $T_1$ , (C)  $t_1$ -abs + normal gastric mucosa, (D)  $t_1$ -abs + renal cell carcinoma, (E)  $t_1$ -abs + female breast carcinoma, (F)  $t_1$ -abs + full-term placenta.

and the results of the precipitin-inhibition experiments are summarized in Tables I and II.

Precipitin-inhibition was achieved with all of 40 tested specimens of primary carcinomata of adult digestive system organs (esophagus, stomach, duodenum,

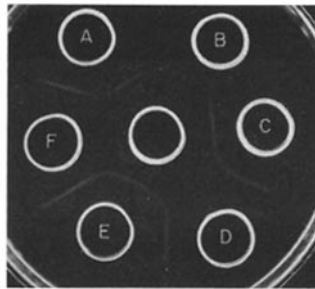


FIG. 2. Example of precipitin-inhibition reactions, high concentrations of active extracts. Center,  $T_1$ , (A)  $t_1$ -abs + saline, (B)  $t_1$ -abs +  $T_1$ , (C)  $t_1$ -abs + bronchogenic carcinoma, (D)  $t_1$ -abs + carcinoma of the rectum, (E)  $t_1$ -abs + gall bladder (cholecystitis), (F)  $t_1$ -abs + carcinoma of the stomach. Test extracts in D and F were capable of precipitin-inhibition. Bands formed between peripheral wells due to excess tumor-specific antigens in wells D and F and uninhibited tumor-specific antibodies in wells A, C, and E.

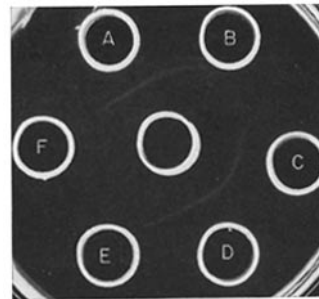


FIG. 3. Example of precipitin-inhibition reactions, highest dilution of active extracts giving positive precipitin-inhibition test. Center,  $T_1$ , (A)  $t_1$ -abs + saline, (B)  $t_1$ -abs +  $T_1$ , (C)  $t_1$ -abs + carcinoma of the thyroid gland, (D)  $t_1$ -abs + carcinoma of anterior portion of floor of the mouth, (E)  $t_1$ -ab + carcinoma of the duodenum, (F)  $t_1$ -abs + carcinoma of the colon. Test extracts in E and F were capable of precipitin-inhibition. No bands formed between peripheral wells, indicating lack of tumor-specific antigen excess in wells E and F.

pancreas, colon, and rectum). Precipitin-inhibition was also achieved with all of the tested metastatic lesions from primary carcinomata of the gastrointestinal tract to other organs (metastases from carcinomata of the stomach and colon to the lung and liver). Direct Ouchterlony reactions between  $t_1$ -abs and the extracts of these specimens, in the highest or next to highest dilutions producing precipitin-inhibition, yielded single bands in all cases. Each of the

bands thus formed showed a reaction of complete identity with the single tumor-specific precipitin band between  $T_1$  and  $t_1$ -abs (Fig. 4). The comparative effect of dilution on the inhibitory capacity of the digestive tract cancer extracts (Table I) would suggest that carcinomata of the lower bowel contained higher concentrations of inhibiting antigenic material than those of the upper digestive system organs.

TABLE I  
*Tissue Specimens Giving Positive Precipitin-Inhibition Reactions (Inhibition of Precipitin Band Formation Between  $T_1$  and  $t_1$ -abs)*

Group	Organ	No. of specimens tested	Protein concentration of inhibiting extract-mg/ml					
			80-100 Positive*	40-50 Positive*	20-25 Positive*	10-12.5 Positive*	5-6.3 Positive*	2.5-3.1 Positive*
Group I: Primary carcinomata (obtained at operation)	Esophagus	1	1	0	—	—	—	—
	Stomach	8	8	4	2	0	—	—
	Duodenum	1	1	1	1	0	—	—
	Pancreas	2	2	2	1	0	—	—
	Colon	20	20	20	20	19	8	0
	Rectum	8	8	8	8	7	3	0
Group II: Metastatic lesions (obtained at autopsy) from/to	Colon/liver	2	2	2	2	2	1	0
	Colon/lung	2	2	2	2	2	1	0
	Stomach/liver	2	2	2	1	0	—	—
	Stomach/lung	2	2	2	1	0	—	—
Group III: Fetal tissues between 2 and 6 months of gestation	Gut	7	7	0	—	—	—	—
	Liver	7	7	1	0	—	—	—
	Pancreas	7	7	1	0	—	—	—

\* Number of specimens giving positive precipitin-inhibition reaction at protein concentration indicated.

All adult tissue specimens (normal or diseased, cancerous or non-cancerous), other than those noted above, yielded negative results. It is particularly emphasized that tumors of stomatodaeal ectodermal origin (carcinomata of the anterior portion of the floor of the mouth, of the hard palate, and a malignant mixed tumor of the parotid gland) were incapable of inhibition. Similarly, a benign insulinoma of the pancreas, benign polyps of the colon and rectum, and specimens of ulcerative colitis and colonic diverticulitis yielded negative results. Furthermore, metastatic tumor masses from other organs (ovary and prostate) to the digestive system (liver, pancreas, and colon) were devoid of inhibitory activity (Table II).

Similar precipitin-inhibition experiments were performed with extracts of human embryonic and fetal tissues between 2 and 8 months of gestation. These specimens were obtained following spontaneous abortion or death after premature delivery. All tested extracts of fetal gut, liver, and pancreas between 2 and 6 months of gestation were capable of inhibiting the single tumor-specific precipitin band formed between  $T_1$  and  $t_1$ -abs (Table I). Direct Ouchterlony reactions between these extracts and  $t_1$ -abs produced single precipitin bands which showed reactions of complete identity with the tumor-specific precipitin band between  $T_1$  and  $t_1$ -abs (Fig. 4, well *F*). The concentrations of the active antigenic constituents in the fetal digestive tissues as determined by serial dilution experiments were relatively low (Table I). Fetal gut, liver, and pancreas in the third trimester and all other tested fetal tissues at any time during gestation were devoid of precipitin-inhibiting capacity.

#### DISCUSSION

*Specific Cancer Antigens of the Adult Human Digestive System.*—The results of the experiments with adult human tissues revealed that only primary or metastatic carcinomata originating in other digestive system organs (esophagus, stomach, duodenum, pancreas, and rectum) contained components identical with the colonic tumor-specific antigens. Metastatic lesions from carcinomata of the ovary or prostate to the colon, pancreas, or liver showed no cross-reactivity with the colonic tumor-specific constituents. Therefore, it may be concluded that the tumor-specific antigens detected in digestive system cancers are a manifestation of factors determined by the site of origin, rather than the site of growth of the tumors. Furthermore, these findings exclude the possibility that the tumor-specific antigens are products of an interaction between a growing, invasive mass and surrounding normal digestive system tissue, or that they are related to exogenous contamination by enteric viruses.

It was found that benign tumors of the pancreas, colon, and rectum lacked detectable tumor-specific antigens. These antigens would thus appear to have been produced only when the tumor cells had attained some degree of anaplasia; *i.e.*, loss of organization (orientation) and differentiation (specialization) (8). It was also observed that carcinomata of the lower bowel (colon and rectum) contained higher concentrations of the tumor-specific antigens than cancers of the upper gastrointestinal tract (esophagus and stomach).

The findings of Nairn *et al.* (9, 10) are of considerable interest in relation to the present investigation. These workers reported that normal human gastrointestinal epithelium, from the esophago-gastric junction to the recto-anal junction, contained a specific mucopolysaccharide antigenic material. This material was present in greater quantities in the lower bowel epithelium than in the epithelium of the upper gastrointestinal tract (stomach) and tended to disappear during tumor transformation in relation to the degree of tumor malignancy. Thus, benign colonic polyps still contained moderate amounts of

TABLE II  
*Tissue Specimens Giving Negative Precipitin-Inhibition Reactions (No Inhibition  
of Precipitin Band Formation Between T<sub>1</sub> and t<sub>1</sub>-abs)*

Organ	No. of specimens studied	Pathological diagnosis
<i>Digestive system:</i>		
Floor of mouth* (anterior portion)	1	Primary carcinoma
Parotid gland*	1	Mixed tumor (malignant)
Submaxillary gland*	1	Mixed tumor (benign)
Tongue‡	4	Normal
Hard palate§	1	Primary carcinoma
Esophagus§	1	Epithelial hyperplasia with submucosal edema
Stomach*	1	Edge of benign ulcer
Stomach*	12	Normal—partial gastrectomies for duodenal ulcers
Small bowel‡	4	Normal
Appendix*	2	Appendicitis
Gall bladder*	12	Cholecystitis—cholelithiasis
Liver‡	4	Normal
Liver‡	1	Metastases from carcinoma of the ovary
Liver‡	1	Metastases from carcinoma of the prostate
Liver*	1	Cavernous hemangioma
Pancreas‡	4	Normal
Pancreas*	1	Islet cell adenoma
Pancreas‡	1	Metastases from carcinoma of the ovary
Pancreas‡	1	Metastases from carcinoma of the prostate
Colon*	12	Normal—distal to carcinoma of the colon
Colon‡	6	Normal
Colon*	3	Benign polyps
Colon*	4	Diverticulitis
Colon*	4	Ulcerative colitis with pseudo-polyps (benign)
Colon*	1	Submucosal lipoma
Colon‡	1	Metastases from carcinoma of the ovary
Colon‡	1	Metastases from carcinoma of the prostate
Rectum‡	3	Normal
Rectum*	3	Benign polyps
Breast*	6 (5 female 1 male)	Carcinoma
Breast*	2 (female)	Fibrocystic disease
<i>Respiratory system:</i>		
Nose*	1	Nasal polyps
Lung‡	4	Normal
Lung*	1	Bronchial adenoma
Lung*	4	Bronchogenic carcinoma
Lung‡	1	Metastases from carcinoma of the prostate
Lung‡	1	Metastases from carcinoma of the ovary

Note: With regard to fetal tissues, the term "normal" appearing in the "Pathological Diagnosis" column indicates that no defects were observed in the tissues under consideration, for their particular periods of gestation.

\* Operative specimens.

‡ Autopsy specimens.

§ Obtained at parturition.

|| Venopuncture.

TABLE II—*Concluded*

Organ	No. of specimens studied	Pathological diagnosis
<i>Genito-urinary system:</i>		
Kidney‡	1	Metastases from carcinoma of the prostate
Kidney‡	4	Normal
Kidney*	1	Hydronephrosis—no primary pathology
Kidney*	3	Renal cell carcinoma
Prostate*	4	Benign hyperplasia
Prostate*	2	Primary carcinoma
Ovary‡	4	Normal—postmenopausal
Ovary*	1	Benign cystic teratoma
Ovary*	2	Benign cysts
Ovary*	4	Adenocarcinomata—various types
Uterus‡	4	Normal—postmenopausal
Uterus*	5	Fibroids
Uterus*	2	Primary carcinoma
<i>Endocrine system:</i>		
Thyroid*	3	Colloid nodular goitre
Thyroid*	2	Diffuse colloid goitre
Thyroid*	3	Adenoma
Thyroid*	2	Adenocarcinoma
Adrenal*	1	Normal—removed therapeutically for carcinoma of the breast
<i>Reticulo-endothelial system:</i>		
Spleen‡	4	Normal
Spleen*	1	Malignant lymphoma—reticulum cell type
White blood cells	2 (cases)	Acute myelogenous leukemia
White blood cells	2 (cases)	Acute lymphoid leukemia
White blood cells	2 (cases)	Chronic lymphoid leukemia
<i>Mesenchymal tissues:</i>		
Skeletal muscle‡	3	Normal
Cardiac muscle‡	4	Normal
Nerve*	1	Neurolemmoma
Peritoneum*	1	Liposarcoma
Subcutaneous tissue*	1	Lipoma
HeLa cell extract	1	
<i>Fetal tissues:</i>		
Placenta§	12	Normal
Cord Blood§	6	Normal
Lung and bronchial tissue‡ (2-8 months gestation)	10	Normal
Kidney‡ (2-8 months gestation)	10	Normal
Heart‡ (2-8 months gestation)	10	Normal
Spleen‡ (2-8 months gestation)	10	Normal
Brain‡ (2-8 months gestation)	4	Normal
Gut‡ (7-8 months gestation)	3	Normal
Pancreas‡ (7-8 months gestation)	3	Normal
Liver‡ (7-8 months gestation)	3	Normal

the specific normal antigenic component(s), while most colonic carcinomata contained negligible quantities. At first glance, these results would appear to represent another example of deletion of normal tissue antigens during the process of carcinogenesis (11-18) and to conflict with the findings of the present investigation. However, this apparent contradiction can be reconciled by a somewhat different interpretation of the experimental data. The chemical nature and distribution of the specific antigens of normal human bowel mucosa found by Nairn *et al.* showed marked similarities to the properties (1) and distribution of the tumor-specific antigens demonstrated in the present study. Thus, it is possible that normal tissue components, apparently deleted during the development of malignant tumors in the human gastrointestinal tract, may actually have been converted into, or replaced by, very similar components specific to the cancer tissue.

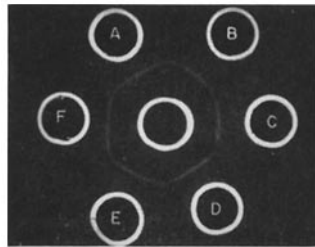


FIG. 4. Direct Ouchterlony reactions between  $t_1$ -abs and active tissue extracts, in highest or next to highest dilutions, giving positive precipitin-inhibition tests. Center,  $t_1$ -abs, (A) T<sub>1</sub>, (B) carcinoma of the stomach, (C) carcinoma of the pancreas, (D) carcinoma of the duodenum, (E) carcinoma of the rectum, (F) fetal liver at 5 months of gestation.

The tumor-specific antigens demonstrated in the present study could not be detected in malignant tumors of the uppermost portion of the digestive system; *i.e.*, in cancers of the palate, anterior portion of the floor of the mouth, or in a malignant mixed tumor of the parotid gland. A possible explanation for this finding is the difference in the germ layer of origin of the epithelium of the areas under consideration. The normal epithelium of those parts of the digestive system which developed the tumor-specific antigens upon malignant transformation were derived from embryonic entodermal tissue. This tissue in the embryo gives rise to the lining of the continuous fore-, mid-, and hindgut tract, extending cephalically to the stomatodaeum and caudally to the proctodaeum, and becomes the parenchymal tissue of the pancreas and liver. The anterior portion of the floor of the mouth, hard palate, and parotid gland, however, are derived from the stomatodaeum and are thus of ectodermal origin.

On the basis of the foregoing observations, it is suggested that cancerous lesions arising from various sites of the entodermally derived human digestive system, in different individuals, contain common tumor-specific antigens.



The findings of a number of studies, such as those of Makari (2, 3), and of Bjorklund *et al.* (4), suggested the existence of common tumor-specific components in different human cancers. Commenting on the observations of these investigators, Prehn (7) stated, "that none of these findings has as yet been shown to be readily reproducible in other laboratories, and that none of them has, as yet, been accepted by the majority of workers in this field". Kosyakov and Koresteleva (5) noted that human cancers appeared to fall into certain groups with regard to the tumor-specific antigens they contained, but did not indicate the types of tumors assigned to each particular group. However, no single tumor-specific component was found to be common to all cancerous tissues.

Similarly, the tumor-specific antigens detected in the present investigation were not universally distributed in all human cancer specimens, but were found to be present in all tested malignant tumors of the entodermally derived epithelium of the digestive system. It is believed that the results of the present investigation represent the first demonstration of system-specific cancer antigens in humans.

*The Antigens of the Digestive System of the Human Fetus.*—The localization of the demonstrated tumor-specific antigens to digestive system malignancies suggested the possibility of some relationship between them and the embryonic development of the human gastrointestinal tract and its functionally related derivatives (liver and pancreas). For this reason, various tissues of fetuses between 2 and 8 months of gestation were investigated. These studies revealed that the antigens which, in the adult, were specifically localized to entodermally derived digestive system cancers were also present in the digestive organs of fetuses between 2 and 6 months of gestation.

The possibility of an antigenic similarity between human embryonic and tumor tissues was suggested by several groups of workers during the last 35 years (19–22). The interpretation of the results of these studies is difficult since in many cases no consideration was given to the possibility that the supposed tumor-specific antibodies may actually have been directed against iso-antigens, species-specific antigens, or normal tissue antigens present in higher concentrations in embryonic and cancer tissues. Recently, however, Abelev (15) presented convincing evidence for antigenic reversion in certain mouse hepatomas.

The results of the present investigation support the possibility of a distinct relationship between the system-specific tumor antigens and certain embryonic tissue components of the human digestive system. These common antigenic constituents will henceforth be referred to as "carcinoembryonic" antigens of the human digestive system. The observations made in this study indicate that the presence of carcinoembryonic constituents in the tissues in which they were detected is probably dependent upon two factors. The importance of the embryonic derivation of the normal tissue is indicated by the finding that malignant tumors arising from the ectodermally derived portions of the

gastrointestinal tract lacked the carcinoembryonic antigens detected in malignant lesions of the entodermally derived parts of the digestive system. The second factor would appear to be the manner of differentiation and specialization which the primitive tissue undergoes in the process of forming adult structures. For example, neither fetal lung and bronchial tissue, which form from a bud of entodermally lined foregut, nor the corresponding malignant lesion of bronchogenic carcinoma contained the carcinoembryonic antigens of the digestive system. It would seem, therefore, that different modes of differentiation and specialization call for the presence of different specific cellular components during embryonic and fetal life.

On the basis of these findings, it would appear that during the embryonic and fetal processes of differentiation, specialization, and organization which take place during the major part of human gestation (23), the entodermal tissue of the digestive system contains specific antigenic components which disappear toward the later stages of gestation and normally do not reappear during adult life. However, during the anaplastic changes of disorganization (disorientation) and dedifferentiation (despecialization) associated with the process of malignant transformation, these components reappear as tumor-specific antigens absent from comparable normal adult tissue. The process of deletion of embryonic antigens from adult tissues has long been recognized (21, 24-27). However, the phenomenon of the reappearance of the deleted or repressed embryonic antigens in cancerous tissue requires further consideration.

Monod (28) postulated that it is the breakdown of the repressor mechanisms determining the differentiation of the cell which leads to malignant transformation. The suggested mechanism by which genetic repression and derepression may take place in the cell is summarized in Fig. 5 and has resulted from studies by Jacob and Monod and their associates (see references 28-31) with inducible and repressible enzyme systems in bacteria. Commenting on the possible role of repressor mechanisms in carcinogenesis, Zilber (32) proposed that mutagenic carcinogens such as ionizing radiation could act either by altering the regulator genes to derepress inherent genetic information, or by allowing the activation of repressed, additional genetic information brought into the cell by carcinogenic viruses. Non-mutagenic carcinogens, on the other hand, could inactivate repressors by acting as "substrate-inducers". Observations of impaired repressor function in neoplastic disease have been made in studies of the "minimal deviation" hepatomas (see reference 33) as well as in experiments with other cancer systems (34-36).

On the basis of the foregoing discussion, it is possible to formulate a tentative hypothesis to explain the findings of the present investigation. In the very early stages of human embryonic cellular division, each primitive cell is endowed with the totality of inherited genetic information. This genetic potential, which is passed on to all subsequent generations of somatic cells, could

theoretically allow each cell to specialize into any human cellular form. As anatomical orientation is initiated under the influence of certain "organizers" (37), distinct organ systems begin to develop. At this stage the pluripotential endodermal cells destined to become the epithelial tissue of the digestive system prepare for the events which will ultimately lead to the formation of the adult structures (gastrointestinal tract, liver, and pancreas). Systems of "regulator gene-repressor-operator" act first to suppress the areas of the nuclear genome not required for the differentiation of these cells. However, the genetic potentialities necessary to allow the cells to undergo their particular type of special-

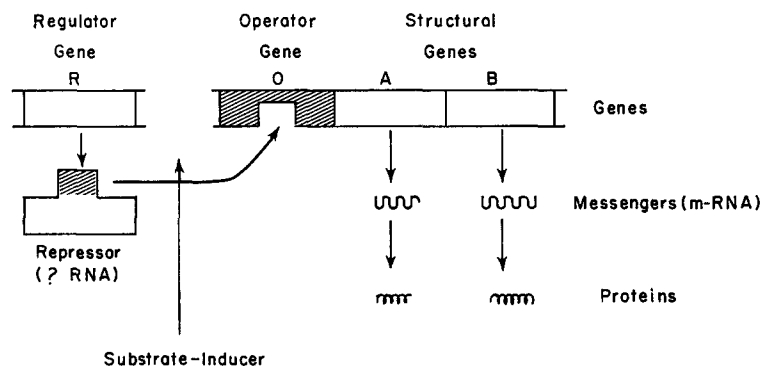


FIG. 5. Proposed model for the regulation of protein synthesis in somatic cells. Combination of repressor with the operator inhibits the production of m-RNA on the structural genes. The introduction of a substrate-inducer, into the cytoplasm, which combines with repressor leads to initiation of m-RNA production on the structural genes and thereby to the synthesis of specific protein molecules in the cytoplasm. (Adapted from information in Jacob, F., and Monod, J., Genetic regulatory mechanisms in the synthesis of proteins, *J. Mol. Biol.*, 1961, **3**, 318.)

ization continue to be expressed. Upon adequate differentiation of the digestive system epithelium prior to birth, certain of the cellular components needed during the course of specialization appear to be no longer required. At this stage further repression of genetic information occurs and the production of "primitive" components ceases. The disappearance of the carcinoembryonic antigens from fetal gut, liver, and pancreas in the third trimester of gestation and their absence from the corresponding normal tissues of the adult is compatible with this hypothetical sequence of events. During the process of carcinogenesis in the gastrointestinal tract and the pancreas, derepression of previously repressed genetic information may occur, leading to the recommencement of cytoplasmic production of the carcinoembryonic antigens.

At least two possible mechanisms may be involved in the process of derepression. Mutagenic carcinogens could derepress genetic information by specifi-

cally altering certain regulator genes or operators. On the other hand, non-mutagenic carcinogens could initiate an epigenetic (38) sequence of derepression by inactivating certain cytoplasmic repressors. It is also possible that the carcinoembryonic antigens demonstrated in this study reappear coincidentally as a consequence of generalized derepressive-dedifferentiation during carcinogenesis. Such an occurrence could result in autonomous cells containing these components by chance alone. However, since the total genetic potentiality of every somatic cell is presumably the same, it seems more than mere coincidence that the production of the same carcinoembryonic components should be activated in all digestive system cancers, but in none of the malignant tumors of any other organ system.

The system-specificity of the carcinoembryonic antigens to cancer tissues of the digestive organs must also be explained. It is conceivable that carcinogens acting upon gastrointestinal and pancreatic tissues have a selective inactivating effect on only certain repressor-operator circuits. Alternatively, the carcinoembryonic components may develop in digestive system cancer cells following malignant transformation because they are required to fulfil some essential, but poorly understood, function in the neoplastic cell. In the embryonic and fetal digestive system entoderm, the carcinoembryonic components might participate directly in the differentiation of the cells. Although the concept of the universal inability of the dedifferentiated cancer cell to redifferentiate has been challenged (31, 39, 40), there is as yet no conclusive evidence that redifferentiation of malignant cells is a general phenomenon. Thus, if the carcinoembryonic components of the human digestive system were directly responsible for cellular differentiation, they would have little function in a gastrointestinal cancer cell. Still another possibility is that these constituents are active in stimulating rapid cellular proliferation in both fetal and malignant cells. However, the absence of the carcinoembryonic antigens from fetal digestive organs during the third trimester, when rapid growth is still taking place, would be difficult to understand if this latter concept were valid.

The most satisfying explanation is that the carcinoembryonic components, in both the fetal and cancer cells, function in maintaining the viability of these cells in their incompletely differentiated state. Thus, when differentiation is completed in the fetal cells to the point where the carcinoembryonic antigens disappear, other mechanisms may become operative in order to maintain the health and function of the specialized cellular units. In cancer cells, however, where redifferentiation to the normal cell type has not as yet been generally shown to occur, these carcinoembryonic constituents would have to be produced continuously in order to maintain the life of the malignant cells.

#### SUMMARY

A wide variety of human adult and fetal tissues were studied by immunodiffusion techniques in agar gel to determine whether they contained the tumor-

specific antigen(s) previously found in colonic cancers. In the *adult* tissues it was demonstrated that identical antigens were present in all tested specimens of malignant tumors of the entodermally derived epithelium of the gastrointestinal tract and pancreas, but were absent from all other tested adult tissues. The common antigenic constituents, therefore, represent system-specific cancer antigens of the human digestive system. System-specific cancer antigens have not previously been demonstrated in humans.

Experiments with *fetal* tissues demonstrated that identical antigens were also present in fetal gut, liver, and pancreas between 2 and 6 months of gestation. These components were named "carcinoembryonic" antigens of the human digestive system. On the basis of the present findings and the recent work regarding control of the expression of genetic potentialities in various types of cells, it was concluded that the carcinoembryonic antigens represent cellular constituents which are repressed during the course of differentiation of the normal digestive system epithelium and reappear in the corresponding malignant cells by a process of derepressive-dedifferentiation.

The authors wish to express their thanks to Miss J. Jonson for invaluable technical assistance, and to Mr. H. Artinian of the Department of Medical Photography, Montreal General Hospital for his aid in the preparation of the figures.

*Addendum.*—Since submission of this manuscript a specimen of a malignant primary hepatoma has been obtained for study. Extracts of this tumor were found to be active in precipitin-inhibition and direct Ouchterlony reactions in the protein concentration range of 80 to 100 mg/ml.

#### BIBLIOGRAPHY

1. Gold, P., and Freedman, S. O., Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques, *J. Exp. Med.*, 1965, **121**, 439.
2. Makari, J. G., Recent studies in the immunology of cancer. III. Detection of cancer antibodies and auto-antibodies by an intradermal reaction, with a review of the detection in human serum of cancer antigens by the Schultz-Dale method, *J. Am. Geriat. Soc.*, 1960, **8**, 16.
3. Makari, J. G., An immunologic unitarian concept of cancer, *J. Am. Geriat. Soc.*, 1963, **11**, 167.
4. Bjorklund, B., Lundblad, G., and Bjorklund, V., Antigenicity of pooled human malignant and normal tissues by cytoimmunological technique. II. Nature of tumor antigen, *Intern. Arch. Allergy and Appl. Immunol.*, 1958, **12**, 242.
5. Kosyakov, P. N., and Koresteleva, V. S., The antigens of cancerous tumors in man, *Acta Unio Intern. Contra Cancrum*, 1963, **19**, 158.
6. Gluck, E., Immunohistochemical investigations of human neoplasia, *Acta Unio Intern. Contra Cancrum*, 1963, **19**, 154.
7. Prehn, R. T., Tumor-specific antigens and the homograft reaction, *Am. J. Surg.*, 1963, **105**, 184.

8. Berenblum, I., The nature of tumor growth, *in* General Pathology, (H. Florey, editor), London, W. B. Saunders, 1962, 528.
9. Nairn, R. C., Fothergill, J. E., McEntegart, M. G., and Porteous, I. B., Gastro-intestinal-specific antigen: An immunohistological and serological study, *Brit. Med. J.*, 1962, **1**, 1788.
10. Nairn, R. C., Fothergill, J. E., McEntegart, M. G., and Richmond, H. G., Loss of gastro-intestinal-specific antigen in neoplasia, *Brit. Med. J.*, 1962, **1**, 1791.
11. Kaliss, N., The transplanted tumor as a research tool in cancer immunology, *Cancer Research*, 1961, **21**, 1203.
12. Weiler, E., Loss of specific cell antigen in relation to carcinogenesis, *Ciba Found. Symp., Carcinogenesis, mechanism of action*, 1959, **27**, 165.
13. Sachs, L., and Feldman, M., Cytotoxic antibodies in the homograft reaction, *J. Nat. Cancer Inst.*, 1958, **21**, 563.
14. Seligmann, M., Grabar, P., and Bernard, I., Études sur la constitution antigénique des leucocytes normaux et leucémiques par les méthodes de précipitation spécifiques en milieu gélifié, *Sang*, 1955, **26**, 52.
15. Abelev, G. I., Study of the antigenic structure of tumors, *Acta Unio Intern. Contra Cancrum*, 1963, **19**, 80.
16. Burrows, D., Schultz-Dale test for detection of specific antigen in sera of patients with carcinoma, *Brit. Med. J.*, 1958, **1**, 368.
17. Khramkova, N. I., Engelhart, N. Y., and Postnikova, Z. A., Antigenic simplification of hepatomas, *Acta Unio Intern. Contra Cancrum*, 1963, **19**, 168.
18. Green, H. N., The present status of the immunological theory of cancer, *Acta Unio Intern. Contra Cancrum*, 1963, **19**, 211.
19. Hirszfeld, L., and Halber, W., Untersuchungen über Verwandtschaftsreaktionen zwischen Embryonal- und Krebsgewebe. I. Rattenembryonen und Menschentumoren, *Z. Immunitätsforsch.*, 1932, **75**, 193.
20. Hirszfeld, L., Halber, W., and Rosenblat, J., Untersuchungen über Verwandtschaftsreaktionen zwischen Embryonal- und Krebsgewebe, II. Menschenembryo und Menschenkrebs, *Z. Immunitätsforsch.*, 1932, **75**, 209.
21. Kolmykova, V. N., and Eroshkina, A. M., The similarity and difference in the antigenic composition of tumour, embryonal and adult tissues in man, *Probl. Oncol.*, 1959, **5**, 1.
22. Viazov, O. E., quoted by Kolmykova, V. N., and Eroshkina, A. M., The similarity and difference in the antigenic composition of tumour, embryonal and adult tissues in man, *Probl. Oncol.*, 1959, **5**, 1.
23. Willis, R. A., *The Borderland of Embryology and Pathology*, London, Butterworths, 2nd edition, 1962, 54.
24. Lockemann, G., and Thies, I., Über den Katalasengehalt des mütterlichen und foetalen Kaninchenblutes und über die Wirkung des foetalen Serums auf des artzue Tier, *Biochem. Z.*, 1910, **25**, 120.
25. Nattan-Larrier, L., and Richard, L., Pouvoir antigène du sang foetal, *Compt. Rend. Soc. Biol.*, 1931, **107**, 668.
26. McCallion, D. J., and Langman, J., An immunological study on the effect of brain extract on the developing nervous tissue in the chick embryo, *J. Embryol. and Exp. Morph.*, 1964, **12**, 78.

27. McCallion, D. J., and Trott, J.G., Transient embryonic antigens in the chick, *J. Embryol. and Exp. Morph.*, 1964, **12**, 511.
28. Monod, J., Summary of symposium, in *Basic Problems in Neoplastic Disease*, (A. Gellhorn, and E. Hirschberg, editors), New York, Columbia University Press, 1962, 218.
29. Jacob, F., and Monod, J., Genetic regulatory mechanisms in the synthesis of proteins, *J. Mol. Biol.*, 1961, **3**, 318.
30. Monod, J., Jacob, F., and Gros, F., Structural and rate-determining factors in the biosynthesis of adaptive enzymes, *Biochem. Soc. Symp.*, (Cambridge, Engl.), 1961, **21**, 104.
31. Monod, J., and Jacob, F., General conclusions: Teleonomic mechanisms in cellular metabolism, growth and differentiation, *Cold Spring Harbor Symp. Quant. Biol.*, 1961, **26**, 389.
32. Zilber, L. A., On the viral etiology of cancer, *Acta Unio Intern. Contra Cancrum*, 1963, **19**, 219.
33. Potter, V. R., Biochemical perspectives in cancer research, *Cancer Research*, 1964, **24**, 1085.
34. Siperstein, M. D., and Fagan, V. M., Deletion of the cholesterol-negative feedback system in liver tumors, *Cancer Research*, 1964, **24**, 1108.
35. Kidson, C., and Kirby, K. S., Recognition of altered patterns of messenger RNA synthesis in a mouse hepatoma, *Cancer Research*, 1964, **24**, 1604.
36. Taylor, W. H., Proteinases of the stomach in health and disease, *Physiol. Rev.*, 1962, **42**, 519.
37. Hamilton, W. J., Boyd, J. D., and Mossman, H. W., *Human Embryology*, Cambridge, England, W. Heffer and Son Ltd., 3rd edition, 1962, 139.
38. Luria, S., Viruses, cancer cells and the genetic concept of virus infection, *Cancer Research*, 1960, **20**, 677.
39. Pitot, H. C., and Heidelberger, C., Metabolic regulatory circuits and carcinogenesis, *Cancer Research*, 1963, **23**, 1694.
40. Kleinsmith, L. J., and Pierce, G. B., Jr., Multipotentiality of single embryonal carcinoma cells, *Cancer Research*, 1964, **24**, 1544.