The Hydrolytic Activity in Normal Human and Malignant Tissue* (33987)

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Hydrolytic activity is generally high in tissues involved in rapid turnover of cellular constituents (1, 2). On this basis, rapidly proliferating malignant tissue may be expected to have higher levels of hydrolase activity than normal tissue. The present communication reports the levels of acid proteinase, neutral proteinase, alkaline and acid phosphatases, arylamidase, tripeptidase, and dipeptidases in normal breast tissue extracts and adjacent infiltrating duct carcinoma, normal bowel and adjacent infiltrating duct carcinoma, normal bowel and adjacent adenocarcinoma of the cecum, mixed astrocytic and oligodendrogliocytic glioma, and solid and papillary ovarian adenocarcinoma.

Materials and Methods. Normal and adjacent neoplastic human tissues were obtained at frozen section biopsy or immediately after surgery and were homogenized with 9 vol (w/v) of cold 0.32 *M* sucrose solution in a Virtis homogenizer. After centrifugation at 27,000*g* for 30 min to remove particulate matter, the supernatant fractions were stored frozen in aliquots until enzyme assays were performed.

TABLE I. Hydrolytic Activity in Normal Human and Adjacent Tumor Tissue.

Patient	Diagnosis	Tissue[a]	Proteinase activity (meq of tyrosine solubilized/hr/mg of protein; $\times 10^3$)		
			Acid	Thiol-stimulated neutral	Neutral
		Breast			
S.H.	Infiltrating duct carcinoma	N	0	0	0
		C	1.94	0	0.56
B.D.	Infiltrating duct carcinoma	N	2.00	0	0
		C	4.10	0	0
S.D.	Infiltrating duct carcinoma	N	1.23	0	0
		C	4.25	0	0.07
J.R.	Infiltrating duct carcinoma with fibrosis; fibrocystic mastopathy	N	0.70	0.23	—
		C	2.84	0.14	—
L.C.	Infiltrating duct carcinoma with focal periductal mastitis and fibrous mastopathy	N	2.98	0	0
		C	3.95	0.09	0
		Bowel			
R.L.	Infiltrating carcinoma of the cecum	N	2.09	0.18	0.07
		C; cecum	4.80	0.43	0.10
D.F.	Infiltrating adenocarcinoma with serosal involvement of the sigmoid colon	N	1.54	0.12	0
		C; cecum	2.04	—	0
K.B.	Mixed astrocytic and oligodendrogliocytic glioma	Brain tumor	0.94	0	0
G.A.	Solid and papillary ovarian adenocarcinoma	C; ovary	1.17	0	0

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TABLE I (continued)

Patient	Tissue[a]	Arylamidase activity (mμmoles of β-naphthylamine liberated/min/mg of protein)						Peptidase activity (μmoles amino groups liberated/hr/mg of protein)							Phosphatase activity (mμmoles of *p*-nitrophenol liberated/min/mg of protein)	
								Leu·Gly·Gly		Gly·Gly·Gly		Gly·Gly		Ala·gly		
		BANA	GPNA	Arg-β-NA	Phe-β-NA	Ala-β-NA	Leu-β-NA	$-Mn^{2+}$	$+Mn^{2+}$	$-Mn^{2+}$	$+Mn^{2+}$	$-Co^{2+}$	$+Co^{2+}$		Acid	Alkaline
	Breast															
S.H.	N	1	1	21.8	18.8	29.1	9.1	0	6.1	0	0	0	0	0	1.52	0.61
	C	0	0	100.5	46.3	148.8	82.8	26.2	31.2	7.0	13.8	11.3	40.6	101.8	13.00	3.50
B.D.	N	0	0	56.3	37.2	120.0	57.6	20.0	22.8	12.9	15.3	0	22.7	87.8	11.90	13.10
	C	0	0.3	175.5	68.0	226.0	92.3	29.5	32.6	0	10.9	17.9	112.8	>258.5	70.40	8.45
S.D.	N	1.3	3.0	30.6	13.2	57.5	25.1	0	10.1	0	0	8.6	19.6	0	2.47	4.05
	C	2.3	1.0	74.7	41.2	119.2	53.2	22.3	21.8	2.8	6.9	7.9	28.6	80.9	8.10	10.65
J.R.	N	8.8	5.1	48.3	21.8	79.0	48.3	13.4	16.9	0	0	7.0	21.4	43.6	0.37	0.31
	C	5.1	3.7	107.9	48.9	151.0	64.7	23.9	39.6	10.5	21.6	9.8	51.0	126.8	25.90	6.30
L.C.	N	0	9.0	16.0	18.0	31.0	24.0	0	0	0	0	0	0	0	5.55	1.1
	C	5.1	0.4	122.2	43.7	165.0	81.9	19.6	26.3	10.0	14.1	30.5	64.0	165.4	23.20	10.85
	Bowel															
R.L.	N	2.8	2.5	108.2	42.2	153.6	65.6	14.7	19.7	5.3	8.8	3.7	13.4	113.5	10.92	0.90
	C; cecum	2.6	8.9	160.5	115.7	181.1	93.9	25.9	36.4	9.1	55.2	13.4	58.0	130.0	51.40	2.08
D.F.	N	2.6	2.6	128.0	60.2	109.5	69.9	18.8	17.9	0	0	7.1	51.7	36.4	7.49	4.76
	C; cecum	2.0	4.7	156.0	59.6	186.7	85.2	16.0	17.5	0	9.1	—	—	86.6	17.99	5.65
K.B.	Brain tumor	2.6	0	372.0	41.3	460.0	234.0	34.0	41.5	25.7	26.8	19.1	69.8	175.6	13.41	5.68
G.A.	C; ovary	0.8	0	71.3	49.9	102.7	51.2	26.6	32.4	26.6	50.0	7.4	21.0	56.0	14.83	3.82

[a] Abbrev.: N = normal; C = cancer.

Acid proteinase activity was measured according to the method of Anson (3) using 2% hemoglobin as a substrate at pH 3.5. The increase in TCA-soluble tyrosine was determined by the procedure of Lowry *et al.* (4). Neutral proteinase was measured both in the absence of 7.5 mM mercaptoethanol (5) and in the presence of a reducing agent (6) with casein as a substrate at pH 7.6. Arylamidase activity was measured using L-leucyl-β-naphthylamide, L-alanyl-β-naphthylamide, L-arginyl-β-naphthylamide, L-phenylalanyl-β-naphthylamide, benzoyl-DL-arginyl-β-naphthylamide (BANA), and glutaryl-L-phenylalanyl-β-naphthylamide (GPNA) (purchased from Mann Research Laboratories, New York, N. Y.) as substrates in the presence of 5 m*M* dithiothreitol and 0.1 *M* potassium phosphate buffer, pH 7.5 (7, 8). Acid phosphatase was measured according to the method of Torriani (9). Alkaline phosphatase was measured according to the procedure of Garen and Levinthal (10). Tripeptidase assays were performed on L-leucylglycylglycine and triglycine in the presence and absence of 1 m*M* Mn^{2+} (11), utilizing the ninhydrin procedure of Moore and Stein (12). Dipeptidase assays were carried out with L-alanylglycine and glycylglycine as substrates. Hydrolysis of the latter compound was measured in the presence and absence of 1 m*M* Co^{2+} (13). Protein determinations were performed according to the procedure of Lowry *et al.* (4).

Results. The hydrolytic activity in the 27,000*g* supernatant fraction of normal and adjacent neoplastic human tissue is given in Table I. The acid proteinase activity (acid cathepsin) in normal breast and normal bowel is consistently and markedly lower than that of malignant tissue. In contrast to the considerable acid catheptic activity, essentially no neutral proteinase activity is seen in the presence or absence of mercaptoethanol. Neither BANA nor GPNA are appreciably cleaved, indicating the absence of cathepsin B and C-type enzymes (14, 15). The hydrolysis of the amino acid β-naphthylamides is considerably higher in tumor tissue than in adjacent normal tissue. Similarly, higher levels of tripeptidase and dipeptidase activity are observed in tumor tissue. Peptidase activity is raised in the presence of metal ions. Acid and alkaline phosphatase activities are higher in all pathological tissue tested, with the exception of one of the five breast preparations tested. Significant hydrolytic activity is observed in tissue extracts from mixed astrocytic and oligodendrogliocytic glioma as well as solid and papillary ovarian adenocarcinoma.

Discussion. Higher levels of acid proteinase, arylamidase, tripeptidase, dipeptidases, and acid and alkaline phosphatase are found in neoplastic tissue as compared to adjacent normal human tissue. The increase may be explained by a greater susceptibility to breakdown of the lysosomal membrane of tumor tissue as compared to normal tissue. Whereas no neutral proteinase activity is seen with casein, BANA, or GPNA as substrates, arylamidase, possessing a similar thiol requirement as cathepsins B and C, but differing in specificity, is found in all tissues examined. The higher peptidase levels observed in pathological breast and cecum are in agreement with increased peptidase activity reported in astrocytoma tissue (11) and those values reported in liver neoplasms (16).

Summary. The hydrolytic activity in extracts of human breast and bowel has been compared with adjacent infiltrating duct carcinoma and infiltrating carcinoma of the cecum, respectively. There are markedly increased levels of acid proteinase, arylamidase, tripeptidase, dipeptidases, acid phosphatase, and alkaline phosphatase in the tumor extracts over comparable adjacent normal extracts. Neutral proteinase is not present to any significant degree in the preparations examined. There is considerable hydrolytic activity in human mixed astrocytic and oligodendrogliocytic glioma as well as solid and papillary ovarian adenocarcinoma.

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The Pattern of Recovery of Erythropoiesis in Heavily Irradiated Mice Receiving Transplants of Fetal Liver* (33988)

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In the past we noted differences between adult and neonatal erythropoiesis in the rodent (1–3). In the newborn rat the red cells are hypochromic and macrocytic (2) whereas, in the adult, hypochromia when it occurs, is preceeded by microcytosis. Moreover, bilateral nephrectomy (2) and starvation (1) which produced red cell aplasia in the adult animal resulted in only a modest decrease in erythropoiesis in the newborn animal. In part, these differences may relate to extrarenal erythropoietin production in the neonatal rat (4) or perhaps in the sensitivity of red cell precursors to erythropoietin. The possibility of a fundamental difference in the regulation of red cell production however, must be considered. Efforts to pursue this problem in the intact newborn animal are hampered by the inability to adequately suppress erythropoiesis prior to the tenth to fifteenth day of life as well as the rapid growth of the animal which is associated with a continuing change in metabolic requirements and hence in the tissue O_2 supply–demand relationships. In an effort to circumvent these problems we initiated studies on red cell production in heavily irradiated adult mice which were transplanted with fetal hematopoietic tissue. In such a system the problems associated with rapid growth of the animal can be circumvented as can some of the technical problems associated with those maneuvers used for suppression of erythropoiesis. In the present study the splenic and femoral iron incorporation and peripheral reticulocyte counts were used as indices of red cell production after transplantation of either fetal liver or adult bone marrow cells. In parallel experiments the numbers of colonies produced by fetal liver cells or bone marrow cells were estimated by the technique of Till and McCulloch (5).

Materials and Methods. Virgin female CF_1

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