TABLE I.
Deposition of liver glycogen

Group	Days after tumor implant	No. animals	Glucose, mg	Avg. body wt, gm	Liver glycogen, %	Total mg liver glycogen
Normal	—	27	0	19.6	0.29±0.02*	3.1
"	—	33	100	20.9	2.25±0.03	26.9
Sarcoma 180	7-10	5	0	18.0	0.19±0.02	2.3
" "	11-14	5	0	18.1	0.46±0.14	3.3
" "	7-10	17	100	20.4	1.15±0.08	14.4
" "	11-14	16	100	18.7	1.25±0.05	15.1

* Probable error of mean value.

All glycogen values are expressed in terms of glucose. Under the conditions of these experiments, the normal mice stored liver glycogen to the extent of 23.8% of the injected glucose as contrasted with only 12.0% by those animals with tumors. The probable errors of the means indicate that this is a highly significant difference whereas the difference between the mean values of the two tumor-bearing groups is not statistically significant.

Discussion. In view of the fact that, (a) the tumor-bearing mice did not lose weight; (b) their fasting liver glycogen concentrations were as high as those of normal mice; and (c) their food intake was as high as the normal, this difference can not be explained on a nutritional basis in the usual sense. Neither was the bodily activity of the tumor-bearing mice observed to be greater than that of the controls during the period between glucose injection and sacrifice. It is clear from the data that the length of time which the mice have borne the tumors, at least between 7 and 14 days, has no influence on the glycogen content. Neither was the mass of the tumor found to influence the extent of glycogen storage. A group of animals bearing tumors having an average weight of 325 mg averaged 1.32% liver glycogen whereas a group with 1,200 mg tumors were found to average 1.24%. Greenstein[4] found that the decrease in liver arginase and catalase activities of animals bearing tumors were likewise independent of the age of the tumor. It is possible that the impaired glycogenesis may result from a similar effect on the enzyme systems in the livers.

Conclusion. Male mice bearing sarcoma 180 implants exhibit an impairment in their ability to store liver glycogen after glucose administration. This is similar to the defect in patient with gastric cancer.

[4] Greenstein, J. P., *J. Nat. Cancer Inst.*, 1942-1943, **3**, 419.

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Arginase and Catalase Activity in Livers of Patients Having Benign and Malignant Gastric Lesions.*

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The comparison of activities of certain enzymes of normal adult tissues with those of tissues undergoing rapid proliferation has revealed differences. These have given rise to much speculation regarding the possible relationship of enzyme activity to neoplastic growth. One important phase of this work

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has been the demonstration that tumors may influence composition or metabolism of tissues not involved anatomically.

The most striking instance of such an effect is that reported by Greenstein.[1] He showed that the catalase activity of the livers of rats and mice bearing transplanted or spontaneous tumors was markedly decreased. On removal of the tumor, the activity promptly returned to normal and a second transplant resulted in a second depression.[2] Similar although less marked effects were demonstrated on liver arginase activities in some species.[3] These results lead one to conclude that the tumor may produce systemic effects in the host. Because of the implications of such a point of view, the extension of this type of investigation to the field of human cancer may be of great importance.

This paper presents the results of catalase and arginase determinations on liver biopsies obtained from 16 patients bearing gastric cancer and 11 patients who underwent surgery for benign lesions.

Clinical Material. The patients included in this study were unselected insofar as age, sex or nutritional state were concerned. They were all admitted to the Gastric Service of the Memorial Hospital for abdominal surgery and were given the usual pre-operative care and study. Food was withheld for at least 10 hours prior to operation. The liver biopsy was taken at the start of the operation, frozen within 15 minutes, and stored in a glass, air-tight container at –40°C until immediately before analysis. The biopsy was invariably a wedge-shaped piece taken from the edge of the right lobe and weighed from 300-1000 mg.†

Methods. The weighed, frozen tissue was dropped into 6 volumes of ice-cold 0.5 N KCl solution, containing .03 N $NaHCO_3$ and thoroughly ground in a glass homogenizer. One milliliter of this mixture was diluted to 100 ml with cold distilled water and used immediately for estimation of catalase activity. The remainder of the homogenized tissue was allowed to incubate for 18 hours at 5°C before measurement of arginase activity.

Catalase activity was determined by estimating the H_2O_2 decomposed by 2 ml of the fresh, dilute tissue suspension added to 5 ml of approximately 0.09 M H_2O_2 (Merck's superoxol diluted 1-100) buffered with 1 ml M/5 sodium phosphate at pH 7.4. The mixtures were shaken for 20 seconds at 25°C and the reaction stopped by the addition of 2 ml 10 N H_2SO_4. The remaining H_2O_2 was titrated with 0.04 N $KMnO_4$ and this value subtracted from a blank titration obtained when the acid was added before the tissue. Preliminary control experiments demonstrated the suitability of this test system with regard to linearity of H_2O_2 decomposed as a function of tissue concentration and of time. In no case was the availability of substrate a limiting factor.

The arginase estimations were performed exactly as described by Greenstein,[4] except that the whole tissue *brei* was used rather than the supernatant extract. Ten dilutions of each activated extract were selected so that in the half hour incubation time chosen, the most dilute suspensions hydrolyzed from 8 to 20% of the arginase whereas the more concentrated suspensions effected complete hydrolysis.

The total nitrogen in each homogenate was determined by a micro-Kjeldahl method.

Results. Table I shows the results of the activity estimations together with pertinent data on each patient. Catalase activity is expressed as milliliters of oxygen liberated from the test system per milligram of tissue nitrogen per second. One unit of arginase activity is chosen as that which will effect the splitting of 50% of the arginine present in the test system in thirty minutes at 37°C. The arginase activities are expressed in units per milli-

1 Greenstein, J. P., Jenrette, W. V., and White, J., *J. Nat. Cancer Inst.*, 1941, **2**, 282.

2 Greenstein, J. P., and Andervont, H. B., *J. Nat. Cancer Inst.*, 1942, **2**, 345.

3 Greenstein, J. P., *J. Nat. Cancer Inst.*, 1943, **3**, 419.

† The cooperation of Drs. G. T. Pack and G. McNeer who, with their staff, made this study possible, is gratefully acknowledged.

4 Greenstein, J. P., Jenrette, W. V., Mider, G. P., and White, J., *J. Nat. Cancer Inst.*, 1940-1941, **1**, 687.

TABLE I.
Arginase and Catalase Activities of Human Liver Biopsies.

No.*	Sex	Age	Diagnosis	Catalase ml O_2/MgN/sec	Arginase AU/mgN†
82806	m	55	Gastric Ulcer	1.00	9.1
49722	m	64	" "	0.96	8.3
83610	f	48	" "	1.14	9.1
81622	m	45	" "	1.15	6.5
82072	m	53	" "	1.36	7.1
83417	m	43	" "	1.51	9.0
82719	m	48	" "	0.97	6.7
82233	m	59	" "	1.12	5.1
83114	m	50	" "	1.16	9.0
83584	m	53	" "	0.85	8.7
81603	f	48	" "	1.46	7.4
				avg 1.15	avg 7.8
82300	m	46	Prim. Op. Ca. Stomach	1.30	6.9
81764	m	64	" " " "	0.81	6.5
79547	m	45	Op. Ca. Duodenum	0.87	7.4
82108	f	60	Prim. Op. Ca. Stomach	0.74	7.1
82391	f	50	" " " "	0.93	6.1
81954	f	63	" " " "	1.12	8.0
82018	m	51	" " " "	1.28	7.2
83016	m	49	" " " "	0.94	6.7
83444	m	62	" " " "	1.58	10.0
83273	m	63	Inop. Ca. Stomach	1.06	15.0
81473	m	64	" " "	1.26	4.8
80296	f	41	" " "	1.04	10.2
75069	m	74	" " "	0.86	7.1
25000	m	56	" " "	0.42	4.4
80928	m	69	" " "	1.02	6.5
80901	f	52	" " "	0.78	5.0
				avg 1.00	avg 7.4

* Memorial Hospital Chart Number.
† AU = arginase activity which effects 50% hydrolysis under the conditions specified.

gram of tissue nitrogen.

It is apparent that there is no significant difference in arginase activity between the two groups of livers. The catalase values are quite variable in each group and although the average control value is 15% higher than that of the experimental group, the overlapping of the individual values is such as to make this difference an insignificant one.

Discussion. These negative results are of interest with respect to the work of Greenstein.[1,2,4]

The negative results are also of interest with respect to the work of Abels[5] and coworkers who demonstrated impairment of certain liver functions as an almost invariable concomitant of cancer of the gastro-intestinal tract in humans. Liver functions found to be impaired included several which involve a variety of types of chemical reaction. Hence, it is of interest that the two functions chosen for this study should be found to be normal.

The decrease in arginase activity in the livers of animals bearing tumors may be limited to a few species since in no case were mice observed by Greenstein to exhibit this phenomenon. However, the decrease in catalase activities was found in both rats and mice with a wide variety of tumors but one exception. The actual magnitude of this decrease is not clear since later work by Greenstein indicates a 50% decrease in activity whereas in the first reports the decrease was 90% in many cases. In any event, it is clear that in humans the presence of adenocarcinoma of the stomach does not significantly influence the catalase content of the liver.

Conclusions. The catalase and arginase activities of liver biopsies from 16 patients

5 Abels, J. C., Rekers, P. E., Binkley, G. E., Pack, G. T., and Rhoads, C. P., *Ann. Int. Med.*, 1942, **16**, 221.

bearing gastric cancer did not differ significantly from those of 11 patients having benign gastric lesions. These results are in contrast to those found in animal experiments, and are in contrast to those obtained with certain other liver function tests which appear to be impaired in patients with gastric cancer.

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Activation of Serum Protease in Peptone Shock.*

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A mechanism for the release of histamine and heparin in anaphylactoid conditions has been previously postulated by one of us.[1] According to this theory, the anaphylotoxins are released as a direct result of the activation of a serum protease which has been described in the literature as "plasma trypsin,"[2] "lytic factor,"[3] "plasmin,"[4] "tryptase"[5] and "fibrinolysin."[6] The activation of this protease in peptone shock is indicated by an increase in the fibrinolytic activity of the blood taken from dogs in the severe stages of shock, the fibrinolysis being observed in the tubes of the protamine titration test for heparin.[7] Heparin has been shown to have an inhibitory effect on "plasma trypsin."[2] Hence the addition of protamine to the blood, as in the protamine titration test for heparin, provides conditions under which the activation of the serum protease may be demonstrated by the resultant fibrinolysis.

Fibrinolytic activity of the blood has been observed by Nolf[8] after the injection of peptone into the liverless and the anterior dog. Since the anterior dog has its inferior vena cava and thoracic aorta ligated just above the diaphragm, thus excluding the liver from the circulation, and since in the dog most of the heparin released comes from the liver, the anterior dog provides conditions *in vivo* similar to those provided *in vitro* by an excess of protamine. The anterior dog was used in our experiments to study the fibrinolytic activity of the blood in peptone shock. A second system which has been studied is the release of histamine from the leucocytes of the rabbit on the addition of peptone. The activity of the serum protease has been investigated by the use of the trypsin inhibitor isolated from unheated soya bean flour, which has been shown to be an inhibitor of the serum protease.[9]

Materials.

Soya Bean Trypsin Inhibitor (S.B.I.): We are indebted to Dr. M. Kunitz for a sample of this material recrystallized 5 times.

Peptone. Difco proteose-peptone.

Heparin (1000 units/ml) and *Protamine* (Salmine hydrochloride) were supplied by the Connaught Laboratories, University of Toronto.

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† Scholar of the National Council of Jewish Women of Canada for Research on Blood Plasma, University of Saskatchewan.

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2 Rocha e Silva, M., and Andrade, S. O., *Science*, 1945, **92**, 670.

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8 Nolf, P., *Medicine*, 1938, **17**, 381.

9 Tagnon, H. J., and Soulier, J. P., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 440.