subjected to partial hepatectomy.

2. The daily food consumption of exercised animals, both operated and non-operated, was slightly less than that of non-exercised animals.

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Chymotrypsin Inhibition by Human Serum in Health and Disease.*† (17948)

HENRY TAUBER

From the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, U. S. Marine Hospital, Staten Island, N. Y.

The antiproteolytic power of blood serum in the diagnostic application to cancer has been considered(1-3). Serum also inhibits the milk clotting power of pepsin and rennin. Proteinase inhibitors may be prepared from the gastric mucosa of various animals(4), and from soy beans and lima beans as reported first by Tauber(4). Two inhibitors were recently isolated from legumes in crystalline form(5,6). The present study was well in progress when West and Hilliard(7) presented a procedure for the estimation of rennin and chymotrypsin inhibitors in blood serum, using unbuffered milk, as a diagnostic method for malignant neoplasia. In the present study strongly buffered homogenized milk of a constant pH of 5.0 is employed. This substrate produces a readily recognizable flocculant precipitate with chymotrypsin. Rennin inhibition is not included in this study.

Materials and methods. (a) *Chymotrypsin solution.* 2 mg of crystalline chymotrypsin (Worthington Biochemical Laboratory) is dissolved per cc of distilled water. (b) *Acetate buffer.* To 42 g of sodium hydroxide in 700 cc of distilled water, 115 cc of an 80% acetic acid solution is added. The mixture is cooled to 20°C and diluted to 1 liter with distilled water. (c) *Buffered milk.* Equal volumes of homogenized milk (ordinary milk may also be used but not powdered milk) and acetate buffer are mixed. The pH of the buffered milk is 5.00. (d) *Standardization of chymotrypsin solution.* All solutions are placed into a constant temperature water bath and adjusted to 20°C. 0.2 cc of distilled water and 1 mg of chymotrypsin in 0.5 cc of distilled water are placed in a 102 x 13 mm test tube. The contents of the tube are mixed. Three cc of buffered milk are added and the contents of the tube are rapidly mixed. The tube is placed in the water bath and the time necessary until a flocculent precipitate appears in the buffered milk is noted. Precipitation usually appears in 4 minutes. If the chymotrypsin solution is more or less active a new enzyme solution must be made up having a clotting time of exactly 4 minutes. The several chymotrypsin samples used in the present work appeared to be of quite constant activity. *Chymotrypsin inhibition test.* 0.2 cc of fresh serum or serum stored in the re-

* Present address: Venereal Disease Experimental Laboratory, U. S. Public Health Service, School of Public Health, Chapel Hill, N. C.

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TABLE I.
Chymotrypsin Inhibitor in Patients Without Neoplasia.

	No. of cases	No. of abnormal tests	Remarks
Entire class	1556	59	
1. Pregnancy (all stages)	52	24	None of abnormal tests was below 15 wk pregnancy
2. Acute infections, active phase (bacterial and virus)	98	8	Abnormal tests included 1 epididymitis with penicillin, 1 infectious hepatitis, 4 broncho-pneumona, 1 interstitial-pneumonia, 1 tonsillitis receiving penicillin
3. Cardiovascular (entire group)	41		
arteriosclerotic heart disease	12	2	
hypertension	20	3	
others	9	0	
4. Chronic infections (tuberculosis, syphilis, bronchiectasis, etc.)	193	16	Abnormal tests included 1 gangrene, 3 advanced tuberculosis, 12 penicillin treated cases (3 cellulitis, 5 syphilis, 1 necrosis of urethra, 2 abscesses, and 1 benign hypertrophy of prostate)
5. Psychoneurosis and psychosis	11	0	
6. Traumatic damage and surgical procedures	125	0	
7. Peptic ulcer, ileitis, colitis, and pancreatitis	89	2	2 abnormal tests on 1 patient who had a chronic pyloric ulcer with obstruction, and 1 with pancreatitis under penicillin treatment
8. Metabolic (diabetes, nephritis, arthritis, hyperthyroidism, and hypercholesteremia)	53	1	1 abnormal test was a diabetic receiving penicillin
9. Benign tumors (polyps, papilloma, fibromyoma, fibroma and lipoma)	90	2	2 abnormal tests on patients receiving penicillin
10. Unclassified	140	1	
11. Healthy subjects (including routine preoperative hernia, hemorrhoids, varicose veins, fractures, etc.)	674	0	Only 14 subjects showed 11½ min. clotting time. 34 showed 11 min., 78 showed 10½ min., 338 showed 8½ to 9½ min., and 82 showed 7 to 8 min.

One test was performed on each patient.

frigerator not over 24 hours is placed in a test tube using a 0.2 cc pipette. The blood need not be from fasting subjects. 1 mg of chymotrypsin in 0.5 cc of distilled water is added. The contents of the tube are mixed. 3 cc of buffered milk are added. The contents of the tube are mixed rapidly. The tube is placed in the water bath (20°C). The time necessary for the buffered milk to flocculate is noted. In the classification of the results the clotting time of 7 to 11½ minutes was chosen as the normal range (Table I, Class 11). Abnormal sera fell in the range of 12 to 40 minutes (See Tables I to III). Thus a delayed flocculation time indicates that the serum contains an increased amount of chymotrypsin-inhibitory material.

Discussion. This study includes 1556 subjects of which 60 had some type of malignant neoplasia as proved by biopsy. These patients did not receive penicillin and were free of complications such as would affect the in-

TABLE II.
Chymotrypsin Inhibitor in Untreated Patients with Neoplasia.

Diagnosis	No. of patients	Avg clotting time in min.	No. of tests performed	No. of abnormal tests
Colon (carcinoma of sigmoid); esophagus (adenocarcinoma); lung (squamous cell with metastasis); prostate (adenocarcinoma with metastasis); rectum (adenocarcinoma); skin (basal cell); stomach (generalized carcinomatosis; adenocarcinoma); lymphosarcoma; prostate (leiomyosarcoma)	14	8–11½	22	2
Cervix (carcinoma with metastasis); colon (adenocarcinoma; carcinoma of sigmoid); prostate (carcinoma with metastasis; adenocarcinoma); skin (basal cell); stomach (carcinoma); urethra (adenocarcinoma); liposarcoma (retroperitoneal); lymphosarcomatosis; testicle (teratoma with metastasis)	12	12–17½	43	29
Colon (adenocarcinoma); lung (squamous cell)	3	18–40	11	11

TABLE III.
Chymotrypsin Inhibitor in Patients with Neoplasia Under Treatment.

Diagnosis	No. of patients	Avg clotting time in min.	No. of tests performed	No. of abnormal tests
Bladder (epidermoid carcinoma); breast (adenocarcinoma); cervix (squamous cell); lip (carcinoma); lung (carcinoma with metastasis); melanoma (malignant with metastasis); rectum (carcinoma with metastasis); skin (basal; squamous cell); leukemia (chronic)	20	8–11½	47	12
Colon (carcinoma of sigmoid with metastasis); heart (adenocarcinoma); (prostate (adenocarcinoma with metastasis); rectum (squamous cell; metastatic); thyroid (adenocarcinoma with metastasis); testicle (teratoma)	10	12–17½	68	48
Bladder (adenocarcinoma)	1	18 and over	2	2

hibition of the chymotrypsin. When a large number of tests were performed on individual patients, the tests were carried out within a period of 6 to 8 months. The fluctuations of higher values to normal values were not always due to therapy. When one abnormal value was obtained, other abnormal values followed but not always in direct order. From Table I it may be seen that only 3.8% of the patients without malignancies fell in the abnormal range of which 46% were pregnancies. Only those above 15 weeks of pregnancy, however, had an abnormal anti-chymotrypsin value. 8.1% of those with acute infections and 12% of the cardiovascular disease gave abnormal tests. Those with chronic infections showed 8.2% abnormal tests which were mostly due to the patients receiving penicillin treatment. The gastrointestinal group contained 89 patients and showed 2 abnormal tests of which 1 had received penicillin. Of the unclassified group of 140 patients only 1 showed an abnormal test. Table II lists chymotrypsin values of patients with malignancies. This data shows that one single normal test is not indicative of the absence of malignancies, however, these patients were not available for further tests. In Table III

are listed chymotrypsin values of treated patients with malignancies showing that the inhibitory power of serum decreased in patients that were responsive to X-ray treatment and surgical excision.

Summary. The antichymotrypsin content of serum, as found by this test, is not changed in early malignant disease. The presence of hidden metastatic cancer, however, can be detected. Thus the test may assist in the diagnosis of advanced malignancies or in detecting such lesions when they appear in an obscure state. False positive values with this procedure fall in the same group as with the methods which measure proteolysis.

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Effect of Hypothalamic Lesions on the Peripheral Blood Picture of the Cat. (17949)

HELEN DAWSON AND MAX D. WHEATLEY (Introduced by W. R. Ingram)

From the Department of Anatomy, State University of Iowa, Iowa City, Ia.

This investigation was carried out in an attempt to verify reports in the literature to the effect that the peripheral blood picture may be influenced by the vegetative centers of the brain. The study was made using adult male and female cats which had undergone operations to produce bilateral lesions in the hypothalamus for induction of possible behavior changes. When the behavior study was completed, the animals were killed with ether, their brains fixed in formalin, embedded and sectioned for study of the areas damaged (1).

Method. The following data consist of a series of conventional blood counts and differential counts obtained from ear vein blood of 9 male and 19 female cats. The blood samples were collected for a period both before and after the production of the hypothalamic lesions. Blood counts were made with the standard pipettes and a Spencer haemacytometer. Differential counts were made after staining the smears with Wright's stain. The blood samples were taken at approximately the same time of day in each case in order to control the diurnal variations that are known to occur(2). Time of feeding was recorded in view of the possible effect it might have on the blood picture(3,4). The animals were handled as gently and quietly as possible in order to avoid emotional disturbances which also are known to have a marked effect on the peripheral blood picture(5). The number of counts made on each animal varied somewhat, ranging from 6 to 9 in the preoperative period and from 6 to 8 counts in the post-operative period.

Findings. In the normal cats the blood picture before operation showed an average WBC count of 20,017 for the males, with a range from 10,361 to 28,950. After hypothalamic damage* the average WBC count was 25,224 with counts ranging from 10,333 to 35,117. Except for one male cat which showed a .3% decrease in the average post-operative count, the remaining males showed individual increases in WBC counts ranging from .5 to 49.1% (Table I). The average WBC count before operation for the females averaged 22,711 with a range from 13,525 to 32,786. After operation the average WBC count for this group was 22,962, an increase over the average count before operation.

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* For extent and location of hypothalamic lesions see Wheatley(1).