Determination of Serum Antitrypsin in the Study of Malignant Neoplasia. (17345)

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Before 1920 an antitryptic activity in the blood of various species had been observed, and was the subject of several theories and investigations. Following this there was a period of about 20 years during which the problem was almost entirely neglected. In recent years attention has again been focused on the serum antitrypsin phenomenon, and no less than 10 methods for its study have been reported in the literature. Several of these have been designed for use in the clinical laboratory. Clark, *et al.*,[1] Duthie, *et al.*,[2] Tallan, *et al.*,[3] and others have reported the application of their respective methods to the study of cancer. Although the results obtained by these investigators are in no sense conclusive, there is a prevailing suggestion that serum antitrypsin is a reflection of improtant physiologic mechanisms and that its alteration in disease should in some manner serve the practical objectives of clinical case study. At least 3 medical problems have been dealt with repeatedly in these reports: (1) endocrine problems, particularly pregnancy and its complications (2) infectious diseases, especially tuberculosis and syphilis, and (3) cancer.

Our interest in serum antitryptic activity persists upon the hope that certain changes may be of value in the diagnosis or study of malignant disease. The method devised in this laboratory[4] has the advantages of speed, simplicity and reproducibility, making it a suitable tool for extended clinical study. The survey reported herein will summarize our results to date.

Materials and methods. Our patients were unselected and included all those admitted to the M.D. Anderson Clinic during the interval of study. Whole blood samples of 3 cc each were drawn and transferred to centrifuge tubes containing 6 mg of crystalline lithium oxalate. The plasma was obtained and serial dilutions with physiological saline were prepared according to the method previously reported.[4] When a large number of plasma samples were to be run simultaneously we found that the procedure could be simplified by using only 4 dilutions (1:40, 1:80, 1:160, and 1:320). The linear segment of the sigmoid curve which expresses the tryosine liberation, and is important in determining the unitage of antitrypsin, almost invariably fell within this range of plasma concentration. Normal, control values were established by determination of the activity in the plasma of individuals who were clinically free of disease.

Of the 174 patients included in this series 81 had some type of malignant neoplasm. In 38 there were known metastases, and in 10 there was concomitant disease. After excluding 15 cases in which the diagnosis was not entirely clear, the remaining 159 were grouped into the several categories shown in Table I. More detailed analysis of the case records failed to reveal other correlations of importance.

Results. In the tabulation of our results we have accepted 140 units of antitryptic activity per cubic centimeter of plasma as the upper limit of normal. All our cases of acute infection, pregnancy and fibrocystic disease of the breast showed values in the pathological range. Patients with chronic infections, cardiovascular disease and diabetes showed antitryptic activity within the limits of normal. The malignant diseases were not sharply seg-

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2 Duthie, E. S., and Lorenz, L., *Biochem. J.*, 1949, **44**, 167.

3 Tallan, H. H., Cliffton, E. E., and Downie, G. R., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 667.

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TABLE I.

	Diagnosis	No. cases	Unitage Range	Unitage Avg
Antitrypsin <140 units	Non-malignant			
	No disease	52	74-141	112
	Cardiovascular disease	9	69-137	104
	Chronic inflammation	11	76-162	129
	Diabetes	2	127-135	131
	Malignant			
	Terminal malignancy	3	117-123	121
	Chronic myelogenous leukemia	2	132-142	137
	Primary skin cancer	13	98-146	125
	Primary cervical cancer	5	93-142	120
Antitrypsin >140 units	Non-malignant			
	Acute infections	9	158-229	178
	Fibrocystic disease of the breast	2	162-166	164
	Pregnancy	3	166-219	196
	Malignant			
	Primary skin and cervical cancer with acute infection	5	162-193	178
	Primary cancer other than skin and cervix	13	138-204	157
	Acute leukemia	2	155-214	185
	Metastatic cancer of all types	38	151-240	182

regated on this basis, but there are interesting and well-defined trends. Individuals having extensive malignancies in terminal phase and those having small primary lesions showed no rise in plasma antitrypsin. In the first instance we may suppose that reactivity has been lost and, in the second, that the stimulous was insufficient. Visceral cancer and advanced, but not terminal, malignancy was quite regularly attended by high levels of plasma antitrypsin.

Discussion. The antitrypsin content of plasma is not altered in the early and more limited forms of malignant disease. It fails, therefore, to have positive diagnostic value when the condition is most amenable to surgical or radiological intervention. The determination also fails as a means of excluding cancer since terminal, and primary skin and cervical cancer are associated with values within the normal range.

Potentially, the greatest advantage of the procedure may be that the presence of hidden or metastatic cancer is suggested in those patients with more than 140 units of antitrypsin. It is true that in several non-malignant conditions the antitrypsin values are elevated above normal, but these are readily diagnosed by other means. In any event, the underlying mechanisms of the phenomenon present an interesting field for further investigations.

Both clinical and experimental studies indicate that the plasma antitrypsin concentration is subject to endocrine influence. High levels are regularly found during pregnancy. One is tempted to explain the findings in fibrocystic disease of the breast as a reflection of endocrine imbalance. In this connection we have recently studied a case of seminoma of the testis with pulmonary metastases in which plasma antitrypsin values varied between 219 and 204 units prior to x-ray therapy. During x-ray treatment the antitrypsin decreased rapidly to 147 units while the patient's general condition improved markedly. Values between 147 and 154 were obtained during a period of 6 weeks. At this time stilbestrol therapy was instituted. The plasma antitrypsin increased rapidly to 209 units in less than 3 weeks while the patient showed marked progress of his disease. Since estrogen administration has failed to increase plasma antitrypsin activity in animals we are inclined to attribute these results entirely to fluctuations in growth of the tumor. Plasma antitrypsin in relation to testicular neoplasms deserves considerably more study.

Conclusions. 1) The plasma antitrypsin test

does not show changes in association with early or minimal lesions of cancer.

2) The test may aid in excluding the diagnosis of advanced but non-terminal malignancy, or in the discovery of these lesions when they present themselves in obscure form.

3) Since antitrypsin values appear to be closely related to the growth of seminoma of the testis, it suggested that the test may have unique prognostic value in the study of this condition.

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Rat Growth Assay for Vitamin B_{12}. (17346)

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The method of assay for vitamin B_{12} herein described was developed using a diet based on purified casein. The diet was made to contain 0.05% iodinated casein (Protamone) as a metabolic stimulant and 0.5% sulfaguanidine to inhibit bacterial synthesis of vitamins. Weanling rats, depleted on this diet for about 7 days, show a depression of growth rate which is counteracted by administration of vitamin B_{12}. The rate of response within a critical range is proportional to the amount of vitamin B_{12} administered, either as the purified vitamin or as concentrates of the vitamin, such as liver extract.

It is well known that liver extract and vit. B_{12} show much higher activity by injection than by oral administration in the pernicious anemia patient. We were interested in the present study to determine whether a difference could be shown between the effects of oral and intramuscular administration of the vitamin. We were interested also to determine the comparative effects of single doses at the beginning of the assay period versus divided doses throughout the assay period. Following completion of these studies, Emerson[1] reported that vitamin B_{12} is equally active by either the oral or subcutaneous route in daily doses ranging from 0.0625 to 0.5 μg per rat day, somewhat higher than the range we have studied.

Register, Ruegamer and Elvehjem[2] reported a quantitative response in rat growth in their assay in the range of 0.025 to 0.1 U.S.P. units per rat day in the form of commercial liver extract. Direct comparison with vit. B_{12} activity was not made in the Wisconsin report. We were interested to determine with a sample liver extract whether 1 μg of vit. B_{12} would correspond fairly closely by rat assay to I U.S.P. unit, a figure previously suggested by Rickes *et al.*,[3] to be in approximately the correct range.

Experimental. Weanling rats, 35-45 g, of mixed sex were placed on a vitamin B_{12} low diet for depletion. The B_{12} depletion diet has the composition shown in Table I. In preliminary assays S.M.A vitamin test casein was further purified by the method of solution and reprecipitation of Novak and Hauge.[4] Later we found that vitamin test casein gave satisfactory results without further purification.

The rate of weight gain is about 12-15 g during the second week on the depletion diet and diminishes to about one-half this rate during the third week of depletion. The depletion period used ranged from 7 to 14 days. The 7-day period is preferred because of the increased mortality which occurs on more ex-

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3 Rickes, E. L., Brink, N. G., Koniuszy, F. R., Wood, T. R., and Folkers, K., *Science*, 1948, **107**, 396.

4 Novak, A. F., and Hauge, S. M., *J. Biol. Chem.*, 1947, **174**, 647.