Precipitation of Blood Sera with a Cationic Detergent. A Clinical Evaluation.* (18811)

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After the technique of precipitation of blood sera with cationic detergents had been developed(1), a clinical evalution of this procedure was undertaken.

Material. A total of 294 tests were carried out in a group of 234 subjects, 43 cases being repeated one or more times for the purpose of substantiating results or detecting serial changes. Twenty-one of the cases were controls (tests run on resident doctors, nurses, etc.), while the remainder (213) were male patients hospitalized in a general medical and surgical veterans hospital. Although many cases were taken at random out of the hospital population, particular emphasis was placed on the testing of all available patients with a clinical diagnosis of malignancy, hepatic, or renal disease.

Procedure. Blood samples were drawn from the patients while in a fasting state and the sera obtained through centrifugation were refrigerated at 5°C. for an average period of 24 hours prior to testing. Whole serum and normal saline-serum dilutions of 9:1, 8:2, 7:3, 6:4, and 5:5 were in each case tested with 0.1 ml of a 1% Bradosol (Beta-phenoxy-ethyl-dimethyl-dodecyl ammonium bromide)† solution. The degree of turbidity of all dilutions was noted at intervals of 30″, 2′, and 5′ and compared with a set of standards of seven tubes prepared by Mayer(1). Those sera which gave a 5 to 7+ reaction in 30″, with a sustained or increased degree of turbidity at 2 minutes, were considered as positive tests. All others of lesser turbidity, as well as those considered doubtful reactions, were classed as negative. In addition to the Bradosol protein precipitation reaction, 186 of the cases were additionally tested by means of the thermal coagulation point of serum. This procedure was carried out according to the methods described by Mayer(2) and by Glass(3), 78°C. being taken as the critical temperature. Inhibition of coagulation to above this temperature was considered positive.

Results. The group of 21 normal controls, without exception, showed a negative turbidimetric pattern. Four of these had more than one test carried out during varied metabolic states, including fasting, postprandial, rest, and following exercise. Uniformly negative results were obtained in these cases and it thus seemed evident that a stable reaction exists in the sera of normal healthy individuals.

The remaining 213 cases were patients with known disease. Serial determinations were carried out in 39 of these cases, 2 to 6 tests being done at various time intervals. A stable positive or negative reaction, with two exceptions, was found to exist during the period of observation. The two cases with a varying Bradosol reaction are omitted from this series as insufficient data is available to permit conclusions regarding their significance. The data given below are thus based on the number of cases and not on the number of estimations.

Fifty-four or 25.3% of the 213 cases gave positive reactions while the other 159 cases (74.7%) revealed a negative turbidimetric pattern. Analysis of the results suggested that the distribution of the positive reactors throughout the entire series was not of a random character but rather followed a pattern.

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† Bradosol was supplied by Ciba Pharm. Prod., Summit, N. J.

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2. Mayer, R. L., *Klin. Wschr.*, 1922, v1, 1693.

3. Glass, George B., *Am. J. Med.*, 1950, v8, June.

TABLE I. Tabulation of the 234 Cases Tested by the Bradosol Reaction.

Malignancies	Total cases	Bradosol positive	Bradosol negative	% positive
Squamous cell carcinoma	8	2	6	25
Prostatic carcinoma	9	7	2	77.8
Lymphomas	8	3	5	37.5
Sarcoma	8	5	3	62.5
Visceral carcinoma	29	17	12	58.6
Bronchogenic carcinoma	6	1	5	16.7
Totals	68	35	33	51.5
Non-malignant disease				
Benign tumors	3	0	3	0
Nephritis	4	1	3	25
Nephrosis	5	4	1	80
Cirrhosis	13	1	12	7.7
Peptic ulcer	9	2	7	22.2
Myocardial infarction	13	3	10	23.1
Misc., infectious and degenerative disease	98	8	90	8.2
Controls	21	0	21	0
Totals	166	19	147	11.4

An arbitrary division of the total cases into malignant and non-malignant disease revealed that a significantly greater number of the 54 positive cases fell into the former group. For the purpose of presentation, as noted in Table I, the cases have been accordingly divided into the two categories of malignant non-malignant disease. The former group, 68 cases, were proven by histological study. The non-malignant cases, a total of 166, were investigated thoroughly and the clinical diagnosis adequately substantiated. Any case of doubtful diagnosis or suspected but unproven malignancy was deleted from the series.

Reference to Table I reveals that 35 or 51.5% of the malignant group had a positive reaction. Nineteen or 11.4% of the non-malignant cases, including the controls, were positive reactors. With elimination of the 21 controls from the latter group, to include only cases with pathology of a non-malignant variety, 13.4% of the total were positive. Of special interest were the results obtained in 2 varieties of non-malignant disease, cirrhosis and nephrosis. One of the 13 cases of cirrhosis was positive while 4 out of the 5 nephrotics tested were positive reactors.

The thermal coagulation point as well as Bradosol reaction was determined in 186 cases of which 53 were malignancies. Fifty-nine of the 186 cases were found to have serum which coagulated at 78°C. or over and were considered positive. This total includes 32 non-malignant and 27 malignant cases, the latter representing 51% of the malignant cases so tested. A comparison of the thermal coagulation point of serum and the Bradosol reaction showed that there existed an over-all correlation in 136 cases or 73.1%, *i.e.* both tests were either positive or negative by both methods.

Discussion. During the course of this investigation and by subsequent analysis of the final results, it became evident that any attempt to diagnose individual diseases solely by the precipitation reaction would be unsuccessful. It was equally obvious, however, that a definite trend with a consistency beyond the realm of chance was obtained in many of the conditions tested. Thus, although not of diagnostic value, the figure of 51.5% positive reactions in the malignancies is sufficiently higher than observed in the alternate group to suggest that certain variations from normal, as demonstrated by protein precipitation, do exist in malignancy. The number of cases of cancer with a positive turbidimetric pattern in this study is remarkably similar to the value obtained by Glass(3), who found by means of the thermal coagulation point of serum that 52.5% of the cancer cases tested were positive. Our efforts to correlate the positive reactions with the total proteins, albumin, and globulin, or other serum constituents in the patients tested were largely unsuccessful. It has been suggested by Glass(3), and by Huggins, *et al.*(4), that the abnormal reactions in their series were dependent upon qualitative as well as quantitative disturbances in serum albumin. While this could likewise be true for the Bradosol reaction, the work of Mayer(1) indicates that the globulin and fibrinogen components of the serum proteins may be specifically implicated

4. Huggins, C., Miller, G. N., Jensen, E. V., *Cancer Research,* 1949, v9, March.

as the disturbed serum constituents upon which the positive Bradosol test is dependent. This possibility is further suggested by the above mentioned variation in results obtained in cirrhosis and nephrosis. In both of these diseases a gross alteration of total serum proteins exists, with the common finding of a low albumin level. Despite this fact, the serum protein precipitation, as demonstrated by the Bradosol reaction, revealed that only 1 out of 13 of the cirrhotics tested was positive while 4 out of 5 cases of nephrosis gave a positive reaction. It thus seems likely that factors other than quantitative variations in the serum albumin level are responsible for the positive Bradosol reaction.

The precipitation of blood sera with cationic detergents would thus appear to be another of the group of non-specific tests which have been described in recent years. This group includes the thermal coagulation test of Mayer(2) and the iodoacetate index of Huggins(4) which demonstrate a definite alteration from the normal pattern in malignancy as well as in an appreciable percentage of non-malignant disease. Some evidence was noted during this work, as indicated by the previously mentioned cases with a varying Bradosol reaction, to suggest the use of this test as a prognostic guide to the progression or regression of malignant disease. This possibility is at present the subject of further investigation.

Summary. 1. The sera of 234 subjects were tested with Bradosol, a total of 294 tests being carried out. 2. A significantly greater percentage of positive reactions was noted in malignancy than in non-malignant disease.

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Effect of Aureomycin on Liver Fat and Liver Function. (18812)

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Recent observations(1-2) indicate that some patients given aureomycin or terramycin developed fatty liver. This was associated with an increase in bromsulphthalein retention in some cases. The following experiments represent attempts to study this phenomenon.

Methods. Aureomycin was administered to mongrel dogs, Sprague-Dawley rats and humans in the manner and dose indicated in Table I. Serial data from dogs and humans fed aureomycin were compared to pre-treatment data. Rats fed or injected aureomycin were compared with pair-fed or pair-injected (saline) animals. Rats and dogs were continued on commercial rations and humans on their usual diet. Histological studies including fat stains were made of liver tissue from needle biopsies(3) of dogs and necropsies from rats. Liver fat in rats was also determined chemically(4). The following liver function tests were performed on dogs and humans at weekly intervals: bromsulphthalein retention(5), serum bilirubin(6), thymol turbidity(7) and zinc sulfate turbidity(8). In

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3. Zimmerman, H. A., Personal communication.

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