Thermal Coagulation Point and Iodoacetate Index as Detectors of Abnormal Serum Proteins and Underlying Illness. (18374)

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Frequent abnormality of the thermal coagulation of the blood serum was first described in patients with malignancies(1). Since a higher temperature was required to coagulate cancer sera to a clot not susceptible to liquefaction from standardized shaking than normal sera, the gel of the sera of cancer patients appeared looser than normal. The phenomenon was not specific for cancer, since a similar abnormality was encountered in pregnancy, nephrosis, pneumonia, severe tuberculosis, Hodgkin's disease, heart failure and malnutrition. As additional cases were studied(2), it became evident that this abnormality did not constitute a cancer test but was a nonspecific serological "signature of the gravity of the disease," irrespective of the neoplastic or non-neoplastic etiology. Thus, the one-year mortality among 628 patients was 10 times as great in those with highly abnormal thermal coagulation as in those with normal coagulation(2). Apart from noting that this phenomenon depended upon quantitative and qualitative disturbances of serum proteins, particularly serum albumin(1,2), the results of 1500 determinations of the serum thermal coagulation point in a variety of diseases indicated this test had value in the detection of organic disease, an application to prognosis, and possibilities for studying disturbances of serum proteins(3). The existence of the abnormal thermal coagulation of serum in patients with cancer and some severe non-neoplastic diseases was also demonstrated by measurement of the least coagulable serum concentration(4). Krzeminska-Lawkowicz determined the least dilution of serum at which an unbreakable serum clot formed on heating. On the basis of 145 determinations in normal and pathological sera(4), she confirmed the observations mentioned above and concluded that sera of patients with cancer and some severe non-neoplastic diseases required less dilution to prevent thermal coagulation of serum than those of normal subjects or patients with other disease. The frequent occurrence of the abnormal thermal coagulation with normal total protein content, and its dependence upon qualitative changes in serum proteins, was also stressed(1-4). Other authors(5,6) tried to employ the principle of abnormal thermal coagulation of serum for screening cancer. They also determined the least coagulable serum concentration with a modified method and, in addition, measured the iodoacetate index, *i.e.* the smallest concentration of iodoacetate per unit of serum proteins sufficient to prevent the heat gelification of serum. The conclusions drawn by Huggins *et al.* could not be confirmed by other workers(7-13) who found iodoacetate test unsatisfactory for the

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TABLE I. Correlation of Thermal Coagulation Point of Blood Serum and Iodoacetate Test in 219 Pathological Sera.

Micromolarity of iodoacetate necessary to prevent thermal coagulation	No. cases examined in each group	% distribution in each group of thermal coagulation points				
		75-81	82-84	85-89	90-95	95°C
39-36	128	84.4	13.3	2.3	0	0
33-30	49	49.0	44.8	4.1	2.1	0
27-24	24	16.6	36.6	21.8	12.5	12.5
18	18	0	11.1	11.1	33.4	44.4

diagnosis of cancer and as a screening test for malignancy. More recently, Huggins *et al.* have altered their viewpoint as indicated by their recent statement of the significance of defective thermal coagulation as a "screening device for protein metabolism" and an "aid in detection of serious disease"(14) instead of a "screen for cancer" or "useful information in recognition of cancer in man" (5,6).

The clinical importance of the phenomenon of abnormal thermal coagulation of serum raises the problem of whether the thermal coagulation should be measured by the determination of the thermal coagulation point, a method which supplied valuable data concerning its clinical significance and mechanism, or by the technics recently introduced. Since no data based on simultaneous determination are available, it was of interest to compare the data obtained by the use of both technics in the same pathological sera. Moreover, since the technic of measurement of the thermal coagulation point was quoted recently(14) as an abandoned method yielding results of limited value, an evaluation of the merits of recent technical improvements for the study of thermal coagulation seemed appropriate.

Material and methods. Two hundred and forty sera were collected under fasting conditions, with the precautions described previously(3).* They were obtained from 51 cancer patients and 189 individuals with various organic, non-neoplastic, diseases. The non-neoplastic cases had, for the most part, diseases which previously were found to be associated often with abnormal thermal coagulation of serum(1-3). The diagnoses were verified by all means possible, and in cases of cancer they were confirmed by operation, biopsy or autopsy. The iodoacetate test was performed by the Huggins-Miller-Jensen technic(15), the total protein content by the method of Greenberg(16), and the thermal coagulation point of serum by the recent simplified technic of Jerzy Glass(3).

Correlation of both technics. Data were obtained with both technics in 219 pathological sera (Tables I and II, Fig. 1). The serum protein content need not be considered in comparing these results since it is the same with both technics, as will be discussed below. The data reveal an inverse relationship between the level of the thermal coagulation point and the concentration of iodoacetate sufficient to prevent the heat coagulation process. The higher the temperature necessary for heat coagulation, the less iodoacetate is required to prevent the coagulation of serum, if temperature of heating is kept constant, and *vice versa*. This is demonstrated

TABLE II. Average Concentration of Iodoacetate Necessary to Prevent Thermal Coagulation of Blood Serum at Various Levels of Thermal Coagulation Point.

Thermal coagulation point, °C	No. cases examined in each group	Avg conc. of iodoacetate in micromols, calculated with stand. error
75-81	136	36.6 ± 0.1
82-84	50	32.0 ± 0.6
85-89	12	29.7 ± 4.1
90-95	10	21.6 ± 2.3
>95	11	19.9 ± 1.0

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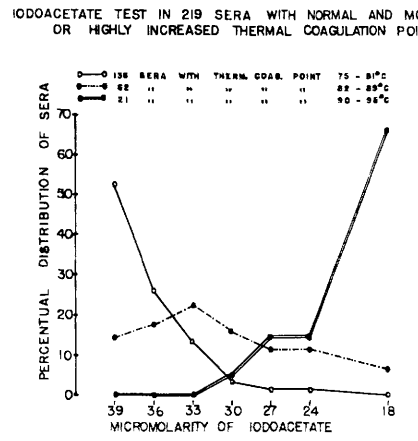

FIG. 1.

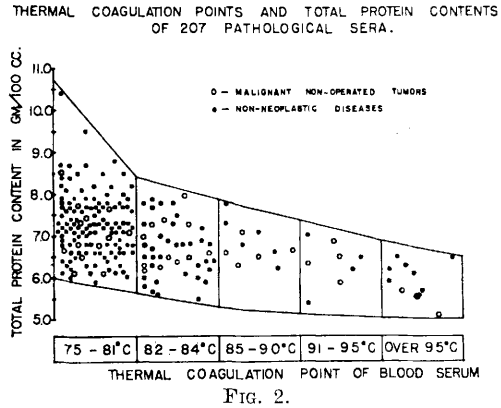

FIG. 2.

most clearly in sera showing normal thermal coagulation, as well as in those presenting a highly defective coagulation (Fig. 1, Table I). It is true also for sera showing a slightly defective thermal coagulation (thermal coagulation point of 82-84°C and iodoacetate concentration of 30-33 micromoles). There is a frequent dissociation, however, between results of individual tests performed with both technics on sera with a moderately abnormal coagulation, *i.e.* thermal coagulation point of 82-89°C and iodoacetate concentration of 24-27 micromoles. With iodoacetate values of 24-27 micromoles, the thermal coagulation point may be normal or slightly, moderately or greatly increased (Table I). That the reverse is true at moderately increased thermal coagulation points is shown in Fig. 1. The average values of both tests still show an inverse relationship, however, even at that level of thermal coagulation as listed in Table II.

Calculation of thermal coagulation defect in terms of total serum proteins. The relationship just described should also hold if both tests were calculated in terms of the relation of thermal coagulation point to total serum proteins and iodoacetate index. From the diagnostic point of view, this feature would be important, if there were statistical evidence, as contended previously by Huggins *et al.*(5), that cancer sera required less iodoacetate on the average per unit of weight of serum proteins than non-neoplastic sera. Because of the inverse correlation between the iodoacetate concentration and the thermal coagulation point of serum it could be presumed that the same phenomenon would also be evident by a higher position of cancer sera in a correlation scatter diagram, if the thermal coagulation points of neoplastic and non-neoplastic sera were plotted against their total protein content. These data (Fig. 2) indicate, however, that the cancer sera do not differ from non-neoplastic sera in this respect, confirming earlier studies(3). From the diagnostic point of view, therefore, the calculation of defective thermal coagulation in terms of total protein content of serum, is not of additional value in cancer diagnosis.

Thermal coagulation point and iodoacetate index of serum in disease. Data obtained simultaneously with both tests in 240 sera of patients with cancer and non-neoplastic disease are summarized in Table III. The abnormal iodoacetate index (below 9.0) (5,17) occurred in 32.4% of non-operated malignant tumors, and in 23.2% of non-neoplastic diseases. The superposition of the postoperative state upon the cancer, both of which increase the thermal coagulation defect (3,9), resulted in an increased frequency of abnormal iodoacetate indices in the group of patients studied shortly after operative removal of the malignant tumor, and contrasted with a normal iodoacetate index in patients studied long after a successful operation. The abnormally low iodoacetate indices in diseases

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TABLE III. Iodoacetate Index and Thermal Coagulation Point of Blood Serum in 240 Patients with Neoplastic and Non-neoplastic Disease.

Diagnosis	Iodoacetate index			Thermal coagulation point		
	No. cases studied	No. abnormal tests*	%	No. cases studied	No. abnormal tests*	%
Neoplastic diseases:						
A. Non-operated tumors						
Digestive tract, kidney, bladder, prostate, lung, thyroid	25	9	36.0	23	9	39.1
Cervix, uterus, breast, skin, bones, and brain	12	3	25.0	12	3	25.0
Total	37	12	32.4	35	12	34.3
B. Operated tumors						
<2 wk after operation	9	5	55.6	8	4	50.0
Several mos. or yrs. after successful operation	4	0	0.0	5	1	20.0
Total	13	5	38.5	13	5	38.5
Non-neoplastic diseases:						
A. Prone to defective thermal coagulation of serum†	146	39	26.7	138	31	22.5
B. Other misc. diseases‡	44	5	11.4	34	3	8.8
Total	190	44	23.2	172	34	19.8
Total of cases	240	61	25.4	220	51	23.2

* As definitely abnormal tests were considered iodoacetate index below 9.0 and thermal coagulation point increased 2-4+, *i.e.*, above 83°C.

† Non-neoplastic diseases listed in this table and reported in detail elsewhere(11) included pneumonia, pyogenic processes, pulmonary tuberculosis, complicated by infection or hemorrhage pregnancy, heart failure, postoperative states, ulcerative colitis, pyloric stenosis, and obstructive jaundice.

‡ Other miscellaneous diseases included pleurisy, infectious hepatitis, peptic ulcer, anemias of various origin, diabetes mellitus, bronchial asthma, peptic ulcer, early pregnancy, etc.

not prone to abnormal thermal coagulation (3) occurred in only 11.4% as compared to 26.7% of abnormal indices in the group of patients prone to this disturbance (Table III). The abnormally increased thermal coagulation point of serum (2-4 plus, above 83°C) was found in 34.3% of cases of non-operated cancer, 22.5% of cases of non-neoplastic disease prone to thermal coagulation defect, and in only 8.8% of other miscellaneous diseases. With this technic, also, the occurrence of abnormal thermal coagulation in recently operated cases of cancer was very frequent, and with both technics the abnormality in thermal coagulation was more frequently detected in cancer of the digestive and urogenital tract, than in tumors of genital organs, breast, skin or brain. It is evident from Fig. 3, which compares the distribution of the iodoacetate index and thermal coagulation point of blood serum in neoplastic and non-neoplastic diseases, that neither of these technics discloses a significant difference between the two groups of cases. Although the increased thermal coagulation point and low iodoacetate index (5.0-6.0) were noted with slightly greater frequency in cancer than in non-neoplastic disease, statistical evaluation of these data shows that this difference is not significant. The simultaneous determination of the thermal coagulation defect with both technics is of little additional value. Of 207 sera, including 35 patients with non-operated cancer and 172 with non-neoplastic disease, defective thermal coagulation (as indicated either by an iodoacetate index below 9.0 or a thermal coagulation point above 83°C or both) was found in 61 instances (29.4%). Since 15 sera were from cancer patients and 46 from those with non-neoplastic disease, the percentage of abnormal thermal coagulation in cancer as detected by one or the other, or both, tests was 42.9% as compared to 26.8% in the non-neoplastic group. Using the "4-fold table" and calculating the χ^2 value by the corrected Yates formula, one obtains $\chi^2 = 2.9$, at $n = 1$, and a probability value, P, of between 0.1 and 0.05. This indicates no statistically significant difference in thermal coagulation between both groups of dis-

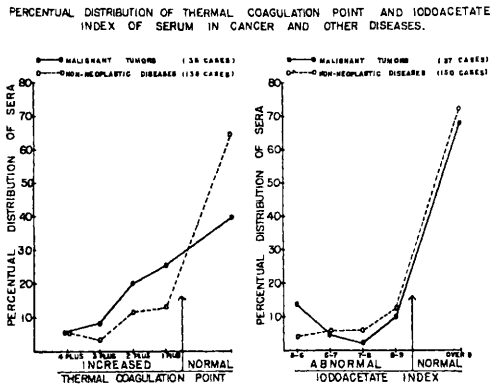

FIG. 3.

eases.

The simultaneous use of both technics slightly increases the percentage of detected abnormalities in thermal coagulation in all pathological cases as shown by the fact that 25.4% were detected with the iodoacetate index, 23.2% with the thermal coagulation point, and 30.4% with both technics simultaneously.

Comment and conclusions. The simultaneous determination of thermal coagulation of blood serum in a large series of cases by the measurement of thermal coagulation point and iodoacetate index has shown similarity of results. There was an inverse relationship, in most instances, between the level of the thermal coagulation point of serum and the concentration of iodoacetate necessary to prevent heat coagulation. The dissociation between the results of both technics appeared exclusively in the group of cases showing a moderate defect in thermal coagulation. This could have resulted, in part, from calculation of the thermal coagulation defect, in the case of the iodoacetate index, in terms of total proteins content, and the omission of the protein factor in the estimation of the thermal coagulation point of serum. It must have been due at times, however, to the results obtained with the iodoacetate technic, which, for unknown reasons, are not always reproducible(7,9). The percentage of abnormalities found in the pathological sera with both technics, singly or combined, in cancer cases and non-neoplastic diseases, was very similar. Neither technic permits a sharp differentiation between neoplastic and non-neoplastic disease. Since abnormal thermal coagulation seems to be related to decrease in sulfhydryl groups of serum proteins which occurs in cancer(18), as well as in metabolic and infectious diseases(19), there is no theoretical basis for the use of measurement of defective thermal coagulation as a diagnostic test for cancer.

The calculation of the thermal coagulation defect per unit of serum proteins, as in the Huggins technic, has not added to the specificity of this method for differentiation of neoplastic from non-neoplastic diseases, nor changed the total percentage of serological abnormalities in pathological material tested. It is possible, however, that the employment of two or more technics for determination of abnormal structure of serum gel may increase the percentage of pathological sera detected. This occurred when the serum gel was tested simultaneously by the measurement of the thermal coagulation point, and the determination of the speed of diffusion of electrolyte through the gelified column of serum(20).

Quantitative disturbances in serum proteins can be detected by many non-specific methods, including serological flocculation or turbidity reactions and sedimentation rate. Determination of qualitative alteration within individual fractions of serum proteins requires much more complicated technics(21-24, 2,6) or study of the electrophoretic pattern of serum. The chief advantage of the method of examination of thermal coagulation of serum is that it provides a very simple way

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to detect certain quantitative and qualitative alterations, which frequently escape other non-specific serological tests, which are dependent upon different abnormalities in proteins. Thus the presence of disease is easily discovered and diagnostic and prognostic conclusions may be drawn(3). Measurement of the thermal coagulation of serum with determination of the thermal coagulation point is simpler than other technics. It has a smaller intrinsic error because it does not require an additional measurement of protein concentration, nor the calculating of a ratio. The results obtained by either method are equally significant for the detection of abnormal thermal coagulation; the scope of both is determined by factors inherent in the basic principle.

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Effect of Antibiotics on Intestinal Microflora and on Growth of Turkeys and Pigs.* (18375)

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A marked increase in growth of turkey poults fed a diet supplemented with a whole dried aureomycin fermentation mash was reported by McGinnis *et al.*(1). This response was obtained under conditions where vitamin B_{12} was ineffective in promoting growth. Stokstad, *et al.*(2) reported that a similar product gave increased chick growth over that obtained with crystalline vitamin B_{12}. Subsequent reports have shown that crystalline aureomycin(3,4) and streptomycin(5) were effective in promoting growth of chicks, turkeys and pigs. Unpublished data from this laboratory confirm these reports and have also shown that penicillin, terramycin, bacitracin and 3-nitro-4-hydroxybenzene arsonic acid were effective in promoting growth of turkey poults.

Several possible explanations have been offered for the growth-promoting properties of antibiotics: (a) the inhibition of toxin-producing bacteria; (b) the reduction in total numbers of intestinal bacteria and lowering of competition between host and microflora for nutrients; and (c) the selective inhibition of a group of the microflora permits increased growth of other microorganisms that synthesize unidentified essential nutrients. The experiments described in this paper were conducted to determine the effects of antibiotics on the intestinal microflora of turkey poults and pigs and to find whether the changes could be correlated with increased animal growth.

Procedure. The turkey poults used in these studies were kept in electrically heated wire-floored battery brooders and were fed the experimental diets *ad libitum* immediately after hatching. Drinking water was available in metal pans at all times. The basal diet was composed of ingredients ordinarily used in making practical feeds; its composition is given in Table I. Turkey poults fed

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