From a diagnostic point of view, these differences in various pathological conditions seem of interest. The underlying causes will be the subject of further research.

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Inactivation of Staphylocoagulase by Trypsin and Pepsin[1]

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Staphylocoagulase is a substance, present in filtrates of cultures of pathogenic staphylococci, which has the property of coagulating plasma. Despite its name, which would imply enzymic character, little is known of either its chemical nature or its mode of action. Walston (*2*) found that the active principle was not dialyzable from crude filtrates and was present in the precipitates obtained with alcohol, dilute acetic acid, or half-saturated ammonium sulfate. He also stated that the activity is destroyed by tryptic digestion. The present report is concerned with further study of the effects of trypsin and pepsin on the activity of staphylocoagulase.

Cultures of a coagulase-positive *Staphylococcus aureus* grown for 7 days in tryptic digest medium were used as the source of coagulase. After incubation, 0.5 per cent of phenol was added as a sterilizing agent, the organisms removed by centrifugation, and the clear supernates used. Coagulase activity was titrated by a serial dilution procedure in which 0.5 ml. of each dilution was mixed with an equal amount of fresh, sterile, citrated human plasma. The titer of coagulase was considered to be the highest final dilution yielding a clot filling about half the volume of the mixture at the end of 2 hours.

TABLE 1

PROTEOLYTIC DIGESTION OF STAPHYLOCOAGULASE

Enzyme	pH of digestion	Coagulase titer		Percentage decrease in protein N
		Before digestion	After digestion	
Trypsin	8.7	128	0	77
"	8.7	128	0	74
"	8.7	128	0	81
"	8.7	64	0	80
Inactivated trypsin	8.7	64	64	0
None	8.7	128	64	0
Pepsin	2.0	64	0	70
"	2.0	128	0	64
"	2.0	128	0	72
Inactivated pepsin	2.0	64	64	0
None	2.0	128	128	0

Commercial trypsin (Pfanstiehl) and U. S. P. pepsin (Merck) were used. Five ml. of the supernate plus 20 mg. of the enzyme were incubated at the appropriate pH and 37°C. for a period of 2–2.5 hours, with occasional shaking. Controls were run for each enzyme using heat-inactivated enzyme and also with no enzyme present. Before carrying out the titration of coagulase, tryptic action was checked by heating for 5 minutes at 80°C.; peptic action, by adjusting the reaction to pH 8. The preparations were cleared by centrifugation, and the coagulase titer was then determined. The activity of the enzyme preparations was checked by measuring the protein nitrogen, *i.e.* that precipitated by 5 per cent trichloroacetic acid, before and after the incubation.

The results are shown in Table 1. There was complete destruction of coagulase activity in all experiments with active enzymes. There was a one-dilution decrease in coagulase activity in one of the controls, otherwise no decrease in the controls.

This work bears upon the question of the enzymic or non-enzymic nature of staphylocoagulase. Its extreme resistance to thermal inactivation (*1*) raises some doubt as to its being a typical enzyme. The complete destruction of coagulase activity by trypsin or pepsin indicates that it contains peptide linkages hydrolyzable by these enzymes. If coagulase activity depends upon intact protein structure, it is remarkably stable when heated, since we have been able to confirm Gengou's observations and extend them by the observation that 20 minutes at autoclave temperature (120°C.) does not abolish the coagulase activity of staphylococcal culture-supernates.

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Linkage Between the Genes for Sickle Cells and the M-N Blood Types[1]

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It has often been pointed out that the detection of instances of linkage in man is of importance not only in an academic sense but also in order to make more precise genetic prognoses for the occurrence of anomalies and diseases (*3, 4*) and to institute preventive measures on the basis of early recognition of preclinical signs (*2*). Various methods of analyzing family data for linkage have been formulated (*1, 3*).

We have recently been investigating the linkage relationships of the genes for sickle cells and for the various blood groups and types. To date, 33 families have been tested. Although we found no evidence against random assortment between the gene for sickle cells and the genes for the A-B blood groups and the Rh types, we did find evidence that the gene for sickle cells is linked with those for the M-N blood types.

In order that any family may furnish information on the linkage between two genes, it is necessary that at least one parent be heterozygous for both pairs of genes. The gene for

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sickle cells is rare enough that any sickle cell parent is almost certain to be heterozygous. Out of our 33 families there were 5 in which one parent had sickle cells and was type MN. Following the procedure of Finney (*4*), these may be arranged as follows:

Family	Finney Type	a	b	c	d	λ	K
7	4	3	0	0	1	6	6
8	4	1	0	0	2	3	3
11	4	1	2	1	0	0	6
17	4	0	1	0	1	−1	1
30	3	2	0	0	0	1	1
Totals						9	17

The total score (summation of λ) is seen to be 9, with a variance (summation of K) of 17. Since the total score exceeds 1.64 $\sqrt{\text{summation of K}}$, there is significant evidence against the hypothesis of random assortment, and we may consider that the existence of a linkage between the genes for sickle cells and for the M-N blood types has been demonstrated. As rapidly as possible we are adding to the collection of families.

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Spleen Extract and Tumor Growth

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The relationship between the spleen and neoplastic growth has been the subject of numerous papers, only a few of which can be mentioned here (e.g. *4*). Interest in the subject has been stimulated by the fact that primary carcinoma of the spleen has almost never been found and cannot be experimentally induced; nor is there growth of tumor tissue even when fragments are implanted directly into the organ (*1*). Metastases to the spleen from primary growths at other loci are also infrequent, and diffuse neoplastic infiltration of the spleen does not occur. The use of spleen extracts in tumor therapy has also been the subject of much controversial discussion, but that there is some definite inhibitory effect of spleen extract on tumor growth has been convincingly demonstrated by Lewisohn (*2*) and his collaborators in experimental animals.

An aqueous extract of calf's spleen has been prepared by the senior author and employed clinically by him for more than 18 years. He reported in 1929 (*3*) that two cases of Hodgkin's lesions (with and without previous X-ray therapy) showed decrease in size and softening of the nodes, but that results on other malignant growths were not encouraging. However, with improvement of techniques of extraction he has prepared a much more effective product which has been employed with decided clinical benefit, and two patients have a history of 12–13 years survival. A report of these clinical findings will be published elsewhere.

This same extract injected into mice bearing transplanted sarcoma 37 or methylcholanthrene-induced primary sarcoma produced cellular changes of a striking nature. Three concentrations were employed: "low" (8.0 grams of spleen solids/100 cc.), "medium" (15.5 grams/100 cc.) and "high" (26.5 grams/100 cc.). The difference between low and medium concentrations is merely one of an additional step in filtration. Resorption of both primary and implanted tumors was obtained, varying in percentage with the size of the tumor treated, the concentration of the extract, and the injection route employed. The best results were obtained with the medium concentration injected intraperitoneally three times daily (total daily dose, 1 cc.). The high concentration was toxic, even when injected subcutaneously; the low concentration appeared to stimulate growth. With the medium concentration, growth inhibition could be detected as early as 18 hours after the first intraperitoneal injection, and it was not necessary to resort to injection by the intravenous route. As early as 48 hours, at which time mice with transplanted tumors had received intraperitoneally 1.8 cc. of the medium concentration, almost complete degeneration of tumor cells was microscopically demonstrable. Nuclei had disappeared completely, leaving structurally intact only the cell body filled with small vacuoles. Similar phenomena were produced in about 5 days in small (5-mm. diameter), chemically induced tumors whose hosts received a total of 3.0 cc. of medium concentration spleen extract intraperitoneally. These tumors were characterized by fragmentation of nuclei and aberrant staining. With the low concentration, nuclei became greatly swollen by the end of the second day after initial injection, and this was a forerunner of increased mitotic activity. Tumors so treated grew rapidly, surpassing the dimensions of the controls. With all of the concentrations, mitosis was uninhibited as long as any viable tissue persisted. The destructive agent does not appear, therefore, to be a mitotic poison.

In Hahnemann Medical College and Hospital, three patients with malignancy and metastases (one metastatic hypernephroma and two metastatic bronchogenic carcinomas) have been injected with the Watson spleen extract (medium concentration) intravenously and intramuscularly, twice daily for 12 weeks—a total daily dose of 5–6 cc. There is definite improvement in the general health of all these patients and inhibition of progress of their tumors as revealed by Roentgen studies, made at regular intervals. Detailed clinical findings will be reported at a later date.

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