"The seventy-two exercises offer a wide variety of experiments from which the teacher . . . may choose a suitable number of almost any desired type." "Practical applications . . . are brought out here and there . . . to reveal to the student that chemistry is related to his personal existence." "More and more responsibility is thrown upon the student as he progresses from the earlier experiments to the later ones."

The description of materials and solutions required is complete and adequate. Each experiment has a set of "Preparatory Questions" for preliminary study, the "Procedure" with notes calling for observations to be written down and used as a guide in filling in blanks in the "Interpretation" pages, which are to be torn out and handed in. The whole book is paper-covered, with spiral binder, and all sheets are perforated and punched for reassembly with rings. The format is good, and there are few if any errors.

CECIL V. KING

# SPECIAL ARTICLES

## STUDIES ON THE ISOLATION OF THE FACTOR RESPONSIBLE FOR TISSUE INJURY IN INFLAMMATION[1, 2]

CAREFUL analysis of the various manifestations of inflammation reveals an essentially stereo-patterned reaction, irrespective of the causative irritant. The latter as well as the anatomical location of the lesion may influence the ultimate appearance of the inflamed area; but close scrutiny reveals the presence of a basic pattern.[3, 4, 5] This is characterized first by an increased fluid passage primarily referable to the liberation of leukotaxine. This substance as shown in earlier studies increases capillary permeability.[3] The alteration in the structure of the capillary endothelium allows the free passage of plasma proteins, including fibrinogen. The latter in the presence of injured tissue is precipitated as a fibrinous network.[3] The tributary lymphatics being evidently more delicate in structure than the capillaries are damaged at a relatively early stage, becoming thus occluded with fibrinous thrombi. The presence of coagulated plasma at the site of inflammation in addition to the occlusion of the tributary lymphatics induce, a lymphatic blockade which thus "walls-off" the inflammatory irritant. In this way inflammation as shown in a number of earlier studies plays an important rôle in immunity as a regulator of bacterial invasiveness.[6] Subsequently, polymorphonuclear leukocytes appear on the scene. Chemotaxis of these phagocytic cells is brought about by the liberation of leukotaxine.[3] Thus this substance is responsible for two of the basic sequences in the development of the inflammatory reaction, namely, increased capillary permeability and migration of polymorphonuclear leukocytes. The usual cytological sequence of polymorphonuclear leukocytes followed eventually by macrophages is conditioned by the local pH at the site of inflammation.[7] The developing local acidosis is in turn referable to a disturbance in the local intermediary carbohydrate metabolism.[8] The rise in number of circulating leukocytes is due to the liberation of a pseudo-globulin in the exudate. It has been termed the *leukocytosis-promoting factor*.[9] The interplay of the foregoing sequences ultimately disposes of the irritant and allows unhampered regeneration or repair.

In the last analysis the inflammatory reaction is a manifestation of severe cellular injury. Neither leukotaxine nor the leukocytosis-promoting factor induce the characteristic injury of inflammation. Besides the function ascribed above to these two substances, there is as a result of their presence in normal tissue scarcely any detectable cellular injury. An attempt has therefore been made now to identify the factor responsible for injury *per se*. Studies have been undertaken on the pleural exudates of dogs obtained as a result of turpentine injection. The results have been further substantiated by additional studies on exudative material obtained from man. In brief, it has been found that either dialysis of the exudate or its fractionation with usually one-third saturation of ammonium sulfate yields, after removal of the $SO^{=}_{4}$ by dialysis, a potent euglobulin fraction which rapidly induces severe tissue damage in rabbits and to some extent in dogs. The induced inflammatory reaction is characterized after a few hours not only by marked leukocytic infiltration but also by massive thrombosis both of lymphatics and to some extent of the small blood vessels. There is also present a fibrinous network in the tissue distended with edema. The presence of the elements inducing lymphatic blockade, which in themselves serve as a gauge of the degree of local injury, is fully substan-

[1] From the Department of Pathology, Harvard University Medical School, Boston, Massachusetts.

[2] Aided by a grant from the Jane Coffin Childs Fund for Medical Research and under a Government Contract from the Office of Scientific Research and Development.

[3] Valy Menkin, "Dynamics of Inflammation," Macmillan Company, New York, 1940.

[4] *Idem*, "Medico-Surgical Tributes to Harold Brunn," University of California Press, 1942, p. 275.

[5] *Idem*, *Physiol. Rev.*, 18: 366, 1938.

[6] *Idem*, *Am. Jour. Med. Sci.*, 190: 583, 1935.

[7] *Idem*, *Am. Jour. Path.*, 10: 193, 1934.

[8] Valy Menkin and C. R. Warner, *Am. Jour. Path.*, 13: 25, 1937.

[9] Valy Menkin, *Arch. Path.*, 30: 363, 1940.

tiated in numerous observations on rabbits. The introduction of trypan blue in an area of the axillary region, previously treated with the euglobulin fraction of exudate, is invariably followed by prompt local fixation of the dye as indicated by its inability to diffuse to the tributary lymphatic vessels and nodes. The introduction of the material in the cutaneous tissue of the abdomen of rabbits may or may not be accompanied by prompt local seepage of trypan blue previously injected intravenously; but in any case, contrary to leukotaxine, there is no diapedesis of leukocytes within the customary testing interval of approximately one hour. The collagenous material in such subcutaneous areas tends to be swollen and often appears somewhat ground-glass-like in appearance. The lymphatic blockade can be induced as early as about a half hour after the introduction of this protein substance. The material is thermolabile and non-diffusible. It can be dried by freezing in a Flosdorf-Mudd apparatus. The potency of the material is not appreciably reduced by the procedure. Some degree of fixation and manifest inflammation has been obtained following the injection of three milligrams of the desiccated material. The injection of this powerful substance obtained either from canine or human exudates is characterized, in the gross, in rabbits by intense redness, edema and frequent central necrosis. The tributary lymphatic nodes are usually erythematous and congested. The acute inflammatory reaction can not be elicited either by the pseudo-globulin (*i.e.*, the leukocytosis-promoting factor) or the albumin fraction of exudates derived from dogs or man. This fact, therefore, indicates that it is not the injection of foreign proteins into rabbits which is primarily responsible for the response. Furthermore, the effect can to a large extent be elicited by injecting the canine material into the cutaneous tissue of a dog. Finally, the acute reaction is not essentially referable to the insolubility of the euglobulin fraction, for it can be suspended as a very fine suspension in physiological saline. The injection of such a preparation elicits in the rabbit a similar effect accompanied by lymphatic blockade. As control for these findings similar fractions were obtained by treating the blood serum of dogs in precisely the same manner. It was found that fractionation or short-time dialysis of clear straw-colored serum yields a euglobulin fraction which, even though insoluble, is incapable of inducing in the rabbit a severe inflammatory reaction accompanied by lymphatic blockade. Trypan blue injected into such treated areas freely diffuses to the tributary lymphatics. Sera, however, containing large quantities of hemolyzed material or highly lipemic sera are apt to yield euglobulin fractions capable of inducing variable degrees of fixation of the dye. These facts suggest that the active fraction recovered from exudates is liberated from injured cells. Furthermore, it has been found that prolonged dialysis of serum for a period of thirty-six hours or over may give rise to an active euglobulin fraction. This is an interesting fact in view of the observation of Chick,[10] reported a number of years ago. This investigator pointed out that following prolonged dialysis a certain amount of pseudo-globulin is converted into euglobulin. One wonders whether such converted material may not be analogous to the injury factor or active euglobulin recovered from exudates. The active substance is absent in normal serum, but it can often be extracted from the blood serum of an animal with a concomitant acute inflammation. This fact points definitely to the significance of absorbed toxic material from the site of acute injury.

The foregoing facts demonstrate the presence of an injury factor in inflammatory exudates. This factor is found in the euglobulin fraction. It is therefore either a euglobulin or at least it seems to be associated with this protein fraction. The presence of such a chemical unit in exudative material warrants for the sake of convenience a name for this active biological substance. The term "necrosin" is therefore tentatively suggested. The untreated exudate *per se* induces when injected into rabbits a severe edematous inflammation characterized by lymphatic blockade. Fractionation of the exudate has yielded in the euglobulin fraction necrosin capable by itself of reproducing in an even more marked manner (undoubtedly due to the concentration and purification of the material) a similar picture as the whole exudate. The recovery of necrosin from exudates offers a reasonable explanation for the injury pattern revealed in an inflammatory reaction.[11]

The biological implications of this substance are at present being studied. The detail of this study as well as its effect on lymphatic blockade will be published *in extenso* elsewhere. In brief, necrosin injected into the circulation does not seem to alter appreciably the blood pressure of a cat. Intravenous administration of necrosin in a dog is followed by a marked leukopenia accompanied by transient toxic manifestations such as vomiting and diarrhea. Utilization of this finding has recently been employed in the further purification of the leukocytosis-promoting factor. By eliminating the euglobulin or necrosin, an active non-toxic leukocytosis-promoting factor is thus

[10] H. Chick, *Biochem. Jour.*, 8: 404, 1914.

[11] The basic pattern of injury in the development of various types of inflammation suggests that the irritant *per se* induces direct injury to the cell. The resulting deranged metabolism of the cell liberates various by-products (*e.g.*, leukotaxine, the leukocytosis-promoting factor, and necrosin) which act as common denominators in the development of a fundamentally basic pattern in inflammation.

obtained. It is conceivable that the presence or liberation of necrosin will explain, in part at least, the leukopenia frequently accompanying inflammatory processes. Finally, necrosin hastens markedly the rate of coagulation of blood *in vitro*. Whether this fact is due to thrombokinase associated with necrosin in the latter's present state of purification remains to be seen. Repeated injections of necrosin subcutaneously into rabbits induce the formation of precipitin antibodies to this substance. The implication of this finding remains to be determined.

In conclusion, the demonstration of an injury factor in the exudates of dogs and man, as brought down in the euglobulin fraction, and termed *necrosin* suggests further studies both in regard to the biological properties and the chemical purification of this substance. These investigations will form the subject of future communications.

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## COLD AGGLUTININS (AUTOHEMAGGLUTININS) IN PRIMARY ATYPICAL PNEUMONIAS[1]

THREE cases have been encountered recently in which acute hemolytic anemia occurred in patients with the prevalent type of primary atypical pneumonia of unknown etiology. In two of these patients, difficulties in determining the blood group led to the discovery of a reversible autohemagglutinin (cold agglutinin). In certain other cases phlebothromboses and pulmonary emboli occurred during the latter part of the illness or during convalescence. Further study revealed that the great majority of the patients with primary atypical pneumonia tested this season showed cold agglutinins in dilutions of serum or plasma ranging from 1:10 to over 1:10,000 at 0° C.

This preliminary report is made because of the possibility that the development of cold agglutinins may serve as a criterion for segregating some of the prevalent cases of primary atypical pneumonia until definite etiological agents are established. The mechanism producing the autohemagglutinins is not known.

The maximum titer of cold agglutinins (in most cases 1:160 or 1:320 at 0° C.) was usually obtained at or near the end of the febrile period, and a rapid decline in titer occurred during convalescence. High titers were usually but not always obtained in the clinically severest cases. Essentially the same titers were obtained in serum from clotted blood and in plasma from oxalated samples. No hemagglutination was noted when the same samples were examined at 37° C. and the titer of cold agglutinins (tested at 0° C.) was unaffected by adsorption at 37° C. with erythrocytes of each of the four major blood groups.

A few of the patients in whom cold agglutinins were demonstrated also developed complement fixing antibodies for psittacosis and for the meningopneumonitis virus,[2] but these tests were negative in most instances.

A slight increase in the osmotic fragility of the erythrocytes was noted in some instances, but this was of a significant degree only in one of the patients who had acute hemolytic anemia. Tests were negative for autohemolysins, cold hemolysins (Donath-Landsteiner test), and the hemolysis test with acidified serum (Ham[3]).

Although the three patients with hemolytic anemia all had received sulfathiazole or sulfadiazine and many of the others in whom cold agglutinins were demonstrated were also treated with these drugs, a large percentage of those showing increased concentrations of autoagglutinins did not receive sulfonamide therapy throughout the course of their illness.

A number of samples of serum obtained from cases of primary atypical pneumonia of unknown etiology during the 1941–42 season failed to show cold agglutinins after six or more months of storage at 5° C. It is not known, however, whether or not this property was originally present in these samples or, as yet, whether the present sera will retain the property after 6 months under these conditions. Control sera obtained from cases of pneumococcus pneumonia and a variety of other febrile illnesses, most of them under treatment with sulfathiazole or sulfadiazine, were also examined for cold agglutinins with almost uniform absence of the agglutinins above a dilution of 1:4.

A brief review of the literature indicates that true reversible cold hemagglutinins have been demonstrated in significant titer only very rarely in cases of pneumonia. They have been noted in a few cases of various liver diseases or blood dyscrasias and, in a few instances, have been associated with peripheral vascular manifestations.[4,5] The only other infectious disease in which cold agglutinins have been found regularly is trypanosomiasis.[6]

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[2] These tests were carried out for us by Drs. Karl F. Meyer and Thomas Francis, Jr.

[3] T. H. Ham, *Arch. Int. Med.*, 64: 1271, 1939.

[4] R. P. McCoombs and J. S. McElroy, *Arch. Int. Med.*, 59: 107, 1937.

[5] K. M. Wheeler, H. J. Gallagher and C. A. Stuart, *Jour. Lab. and Clin. Med.*, 24: 1135, 1939.

[6] W. York, *Ann. Tropical Med. and Parasit.*, 4: 529, 1910.

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