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CERTAIN ASPECTS OF THE CHEMISTRY OF INFECTIOUS DISEASES[1]

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THE discovery of microorganisms as the causative agents in infectious diseases introduced a new problem of relating the specific characteristics of a disease to the nature and behavior of the particular type of organism responsible for the pathological condition. It seemed reasonable to expect that the infecting agent would change the normal course of certain physical and chemical processes essential to the regular functions of the healthy state of the animal. A knowledge of the mechanism of the cycle of events involved would lead to a rational basis of treating the disease to eliminate the difficulties and reestablish normal conditions. Obviously, it was of prime importance to learn as much as possible about microorganisms and their pathogenicity before a comprehensive study of their rôle in disease would be instructive and profitable. Just how the infecting agent causes a specific disease, what changes occur, where these changes are initiated, the nature of the resulting products and their influence on the physical and chemical process underlying the normal cellular activity of the animal; are questions which must be answered before chemotherapy can be highly effective in relieving man of the many ills that now reduce his efficiency, limit his usefulness and endanger his life.

All these questions involve difficult problems. The infecting agents are themselves complex organisms, whose metabolic processes are poorly understood. The animal organism is much more complex and its

[1] Address delivered before the general meeting of the American Chemical Society at the Detroit meeting September 9, 1940.

cellular activities so numerous and varied as to render difficult if not impossible direct experiments at the seat of the trouble. The indirect approach thus necessitated in research of this kind leads to uncertainties in the interpretations of the results. Even if microorganisms worked like termites, boring into animal tissues and thus reducing their strength, impairing their efficiency and jeopardizing their life, it would still be a difficult task to examine the nature of the damage done and relate it to the cause of the trouble. A man can not be sawed into pieces like a log for inspection nor can he be subjected to physical and chemical tests in the ordinary laboratory equipment.

In spite of the fact that much has been learned about the chemistry of animal metabolism since the discovery of the microbe nature of the causative agents in infectious diseases, little or nothing is known about the chemistry of these diseases. What evidence there is seems to indicate that the blood may be expected to show marked changes in composition, reflecting the effect of the infection on tissue metabolism. Certain changes may occur in the serum proteins and possibly in the enzymatic processes of this and other tissues in the animal body.

Changes in the solid content of the blood with variations in the albumin-globulin ratio of the serum proteins have been observed in certain infectious diseases. In such cases the globulin increases in proportion to the severity of the disease. In acute fevers the viscosity of the blood is reported to increase.[2] Changes occur in other tissues. During the fever stage in pneumonia[3] there is a decrease in vitamin A of the liver with a correspondingly high output in the urine. The glucosamine in the blood serum is significantly higher in pneumonia.[4] The plasma lipids are said to drop to abnormal levels[5] and may be controlled by the early administration of serum. In tubercular meningitis the sodium chloride content of the cerebrospinal fluid drops to the unusually low level of about 510 mg per cent.[6] The normal value is about 700 mg per cent. Also, in tuberculosis there is a decrease in the coloring matter of the blood without a corresponding decrease in the iron.[7] The serum proteins are said to decrease during the progress of tuberculosis.[8]

The available evidence is not convincing enough to permit much stress on the significance of the chemical changes and the specificity of their nature in relation to definite causative agents of disease. A major difficulty in evaluating the results is the uncertainty that all the important factors influencing these results were known and appreciated. The infecting agent which under certain conditions would produce infection in experimental animals may at other times have little or no effect due to protective agencies which in some unknown way offset the effect. The susceptibility of the animal varies widely. The factors governing this are unknown. There is no way at present of measuring the capacity of an animal to resist infection. It is undoubtedly a complex cycle of cellular events in which highly reactive chemical substances play important rôles. Vitamins and enzymes are the chemical compensators which help to establish and maintain the immediate degree of livingness of the organism. If there was some way of measuring this it might be designated as "the living potential" of the organism.

From the available evidence it seemed likely that a significant change might be expected in the relation between sulfide and sulfhydryl sulfur of the tissue proteins in infectious diseases. Brdicka, using the Heyrovsky polarograph,[9] had shown significant differences between the polarographic waves obtained on electrolyzing blood sera of normal persons and of those having cancer.

The polarograph is essentially an electrolytic apparatus in which mercury serves as the electrode. The cathode is formed by mercury dropping at a definite rate from a glass capillary of about .05 mm diameter. The collecting layer of mercury serves as the anode. A direct current is passed through the electrolytic solution under increasing voltage and the current-voltage curve recorded photographically. The curves so obtained are reproducible with a fair degree of accuracy. Only a few cc of solution are required for the electrolysis. When protein is preesnt in the dilute electrolytic solution of ammonium chloride a characteristic wave is produced on the current-voltage curve and this effect is believed to be due to the electrolytic evolution of hydrogen catalyzed by the presence of the protein at the cathode interphase. Brdicka,[9] studying this protein effect, found that in buffered cobalt-ammonium chloride solutions a characteristic "double wave" was produced on the current-voltage curve by proteins containing sulfur. He ascribed this double wave to available sulfhydryl and disulfidic groups in the protein and showed that the height of the protein wave was a significant indication of changes occurring in the proteins during pathological conditions and could be used as a valuable aid in diagnosing cancer. It was also shown that cystine and other thio acids produced a similar catalytic effect with divalent cobalt in the buffer solution. The polypeptides and proteins

[2] Bachmann, *Deutsch. Arch. f. klin. Med.*, 94: 409, 1908.

[3] T. Lindquist, *Klin. Wochschr.*, 16: 1345, 1937.

[4] Ivar Nilsson, University of Upsala, *Biochem. Zeit.*, 291: 254–8, 1937.

[5] A. V. Stoesser, *Proc. Soc. Exp. Biol. and Med.*, 43: 168, 1940.

[6] John Ingram, *Brit. Med. Jour.*, July, 1937, p. 111.

[7] Wells and Long, "Chemistry of Tuberculosis," p. 225.

[8] *Ibid.*, p. 231.

[9] Heyrovsky, *Nature*, 142: 317, 1938.

gave the effect with both divalent and trivalent cobalt. Other investigators have confirmed the essential facts and the polarographic method has been shown to be a valuable means of studying certain biochemical reactions.

The height of the characteristic protein double wave is lower for cancer sera than for normal. Likewise, it has been shown that a more pronounced difference can be obtained by comparing the sera after controlled denaturation and precipitation of the macroproteins with sulfosalicylic acid. The height of the wave of the unprecipitated fractions, so obtained, designated "peptone fraction," of a cancer serum is greater than that of the normal serum when measured under the same conditions in the same trivalent cobalt-ammonium salt buffer. Brdicka[10] interpreted these results to mean that the two types of sera had different amounts of available sulfhydryl sulfur and that this polarographic result was in harmony with the results previously obtained by A. Purr and M. Russel, who found that carcinomatic blood was less active in certain biological reactions than normal blood. E. Waldschmidt-Leitz and his collaborators ascribed this lower activity of carcinomatic blood to the presence of fewer sulfhydryl groups in the carcinomatic serum.

A limited number of results with various inflammatory conditions indicated that this polarographic method might prove to be useful in the study of the blood sera in the infectious diseases that interested us. By this means it was thought possible to get some idea of the chemical changes occurring in the tissue protein in different infectious diseases. We elected to study first experimental pneumococcus pneumonia in dogs, using the technique of Terrell, Robertson, and Coggeshall[11] for infecting them. By this technique, that is the direct introduction of the infecting agent into the lungs, it is possible to produce in dogs a pneumococcal pneumonia which resembles closely the corresponding disease in man. In this way the disease can be studied under controlled conditions. Such a study should furnish information of value as to the nature of the disease, the chemical changes involved and the chemotherapy most likely to be successful in combating it. Until these fundamental data are established the treatment of infectious diseases in man will remain highly empirical and unsatisfactory.

A careful study of the blood sera of several normal dogs was made to establish a basis for comparison and also to demonstrate the reproducibility of the polarographic method in this case. The results were highly satisfactory and justified the further application of the method to the study of the blood sera of the pneumonia dogs.

The height of the characteristic wave of the polarogram for the whole serum in this type of pneumonia[12] is lower than normal. This is consistent with Brdicka's findings for carcinomatic serum and would seem to indicate that in both pathological conditions there is a decrease in polarographically active protein groups and in experimental pneumonia it would appear also that there is an actual decrease in serum protein since the protein nitrogen is lower than normal.

A more significant result is the change manifested in the peptone fraction of the pneumonia serum. There is a marked and progressive rise in the height of this characteristic wave accompanied by a change in the shape of the wave with the increase in the severity of the disease, reaching a maximum at the height of the pneumonia. With recovery the wave height drops continuously until it reaches the normal value and the curve assumes the normal shape. The nitrogen values rise with the corresponding increase in the height of this significant wave confirming the fact that there is a progressive change in proteins of the blood serum with the advance of the disease and this leads to increase in protein degradation products in the denatured protein.

The changes in the current-voltage curves of the blood serum of the pneumonia dogs are not confined to the fever stage of the disease. This is over, as a rule, before the maximum effects are obtained. The fever is but one symptom of the disease. It may or may not be indicative of significant physical and chemical changes in the tissues, particularly those tissues directly involved; in pneumonia, lung tissue. That definite changes occur in the metabolism of the lung tissue in lobar pneumonia is to be expected from the nature of the disease. It has been shown previously[13] that the environmental conditions of the cells change and with the rapid growth of pneumococci, the engorgement of the capillaries, and the filling up of the air cells with serous material the oxygen supply from the inspired air is diminished to a degree which makes the normal cellular metabolism difficult if not impossible. The oxidation processes in the congested area must then depend upon a supply of oxygen from the venous blood. Under these conditions the glucose content of the tissues diminishes and lactic acid increases. There must be also certain products of the bacterial metabolism which may influence the physical and chemical processes of many cellular structures throughout the animal body. Since the cellular processes appear to be regulated by a finely adjusted

[10] Brdicka, *Col. Czech. Chem. Commu.*, 5: 112, 148, 238, 1933.

[11] Terrell, Robertson, Coggeshall, *Jour. Clin. Investigation*, 12: 393, 1933.

[12] Crossley, Kienle, Vassel, Christopher. In press.

[13] Friedemann and Graeses, *Jour. Exp. Med.*, 67: 481, 1938.

mechanism in which liver cells play an important rôle, the effects of the bacterial invasion may be far-reaching and necessitate adjustments which tax the reserve power of the organism beyond the limit of its capacity.

The question is: are the effects shown by the polarograph the result of the infection or the manifestation of the chemical changes involved in the readjustment of the system to meet the new conditions imposed by the bacterial invasion. Obviously this question can not be answered fully with the available evidence. We do not yet know the full significance of the changes in protein as indicated by the current-voltage curves. From the resemblance of these with the curves obtained with cancer sera and also with the sera from a limited number of patients suffering from different inflammatory conditions it would seem that the effects we get are not entirely specific for pneumonia. Of course, further study may reveal some phase of the effect which is definitely related to the peculiar conditions of pneumonia and lead to a better understanding of the disease. We can not tell from the evidence if the changes in the proteins are of the same type and follow the same course. It is possible that the cleavage of the proteins follows a definite pattern laid down by the enzymatic tools furnished by the specific bacteria. From our results it appears that not only do the sera from the pneumonia dogs give progressively higher peptone values when denatured but also show increasing amounts of such degradation products in the whole sera with the progress of the disease.

Preliminary results indicate that the treatment of the experimental pneumonia with sulfanilamide and sulfapyridine did not affect either the height or the shape of the characteristic current-voltage curves. This was also true of normal dogs given these drugs. Their curves were similar to those previously obtained when no drug had been administered. Whatever the function of these drugs in curing the disease, there is no evidence that they influence the course of the disease as indicated by the protein changes manifested by the polarographic results. This may mean that the essential function of the drug is to aid in destroying the bacterial infection and thus bring about a check of the disease. The blood changes measured by the polarograph may be part of the result of the infection and may run a regular course which is not effected by the chemotherapy. Further information should throw more light on this phase of the problem.

Besides, the cystine values of the whole sera of the pneumonia dogs are lower than those for the normal animals. The cystine drops progressively with the advance of the pneumonia until it reaches a minimum at the height of the disease. Then, if the animal recovers, it begins to rise and continues upward until it reaches the normal level, when the dog is well. This runs parallel with the rise and fall in the typical peptone wave. It also seems from the preliminary data that with rapid onset and severity of the pneumonia there is a correspondingly rapid decline in the cystine values. The drop in cystine corresponds to the decrease in total nitrogen of the serum and is additional evidence that the sera of the pneumonia dogs contain less proteins than those of the normal animals.

These polarographic results are consistent with the evidence obtained from the x-ray photographs showing the progress of the pneumonia. With the increase in the congestion of the lungs there is a rise in the peptone wave or increase in protein degradation products. Correspondingly there is observed a fall in the cystine, this tending to reach its minimum value when the pictures show the congestion at its height. Similarly, when the x-ray pictures show the dog to be improving, that is a progressive resolution of the involved areas, the polarograms also show the animal to be returning to normal. In general the polarographic results seem to give a more accurate picture of the animal's condition, showing a steady progress toward normalcy when the x-ray pictures fail to show definite evidence of the disease. The animal appears well before the polarograms indicate this condition.

While these results are interesting and appear to be significant they are not complete enough to justify a conclusion as to the specific chemical changes in pneumonia and relate cause and effect in connection with the manifestation of the symptoms of the disease. The investigation continues and it is hoped that in time sufficient data will be secured to allow of a definite correlation of the chemical changes and the symptoms of the disease. Such results would help to provide a rational basis for chemotherapy and improve the efficiency in predicting what chemical agents will have the greatest chance of success in combating infectious diseases.

THE IMPORTANCE OF MICROORGANISMS IN VITAMIN RESEARCH

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THE beneficial relationships of bacteria and microorganisms to mankind are almost as well recognized as their harmful effects. In very recent years, however, these tiny distant cousins have become useful to man in an entirely new way.

The history of their usefulness in vitamin research