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Enzymatic activity in disease

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ENZYMATIC ACTIVITY

IN

DISEASE

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INTRODUCTION

Each decade brings new approaches to the study of medicine. Thus, there were careful observations on symptoms of disease, detailed histological examinations of pathological tissues, extensive studies on the etiological agents of infections diseases, and quantitative studies on the mechanisms underlying disease processes. We have had, then, the era of clinical observation, the era of pathology, the era of bacteriology, and the era of physiology and biochemistry.

All biochemical reactions are aided by cellular catalysts called enzymes, and during the past decade an ever increasing emphasis has been placed on the relationship of enzyme activities to disease processes. This change is partly due to increased knowledge of the chemistry of enzymes and partly to the gradual realization that the underlying normalities in enzyme systems are the roots of the genesis of the disease processes. The disturbances in cellular metabolism result in physiological changes which soon pass into pathological distortions, leading ultimately to the signs of the

so familiar to the clinician and the pathologist.

The enzyme studies alone have, as yet, allowed no profound advances in the practical treatment disease, but when considered in connection with other studies, they have helped introduce fundamental basis for much experimental work, and much of the future treatment in medicine probably depends on this research.

This paper deals with the role of enzyme activity in deficiency diseases, inflammatory and infectious diseases, metabolic and degenerative diseases, and concludes with a discussion of the role of enzymes in the therapy of disease. It does not include the vast realm of enzymatic changes in neoplastic diseases nor does it pretend to cover all diseases, but rather just those diseases in which sufficient experimental work has been done to warrant

DEFICIENCY DISEASES

A survey of the earlier experiments reveals a certain parallelism between the studies on enzymes and vitamins. Previous to 1890, the existence of a few enzymes had been suggested and several deficiency diseases had been described. It was in 1894, that Emil Fisher began his investigation, on which the present ideas of enzyme specificity are based, and it was in 1897, that Eijkman established the existence of a specific vitamin for the treatment of a definite deficiency (beriberi). It is also interesting to note that in 1906, Harden described the first organic coenzyme and Hopkins described specific accessory growth factors. In 1911, Casmir Funk, as a result of his investigations into the cause of beriberi, obtained a crystalline substance from the rice polishings which was capable of curing and preventing this nervous disease. He called the crystalline substance vitamin, believing erroneously that it was chemically an amine.

In 1921, Sidell, according to Elvehjem (47), stated that aside from a possibly significant degree of dialyzability, there was no outstanding evidence that vitamins should not be classified with enzymes.

it is known that the difference in dialyzability is an important distinction between the two groups of compounds. Furthermore, it has been shown that while the human organism can synthesize its enzymes, it is dependent upon the environment for its vitamins (141). In 1931, Neuberg and Euler proposed the term cozymase for the coenzyme of alcoholic fermentation and apoenzyme for the enzyme free from the coenzyme. Since that time a great deal has been learned about It is now known that the major part of an enzyme is protein in nature, and that under most conditions the living cell has little difficulty in synthesizing the proteins. However, the activity of these protein catalysts is greatly altered by the presence of activators. The specific activators may be divided into the kinases which are generally colloidal in nature and coenzymes which are crystalloidal. While one mole of kinase generally activates many moles of enzyme, one mole of coenzyme is related to one or a relatively few moles of the specific enzyme. Apparently the living organism has great difficulty in producing the structures that go to make up coenzymes and according to present knowledge, at least some of the vitamins are directly concerned with these

coenzymes. The facts regarding vitamin B_1 or thiamine and its role in coenzyme production will be presented first.

In 1913, Neuberg and Rosenthal, according to Bernheim, (16), reported the presence in a yeast of an enzyme called carboxylase which catalyzed the decarboxylation of alpha keto acids. 1932, Auhagen (6) split this enzyme into a protein component and a thermostable factor called cocarboxlase. In 1937, Lohmann and Schuster (118) isolated the cocarboxlase in crystalline form and showed it to be the pyrophosphoric acid ester of thiamine. In 1941, Green and his co-workers (67) isolated carboxylase from top brewers yeast and found it to be a diphosphothiamine magnesium protein. The same laboratory (68) then prepared a crude preparation of a similar enzyme from a variety of animal tissues. The richest source was found to be pig heart (64).

Apparently there is little difficulty for the living organism to combine cocarboxylase, magnesium, and protein into the active enzyme. Even cocarboxylase does not need to be supplied preformed in the diet since thiamine is phosphorylated by means of the phosphate transferring reactions with the inter-

vention of the adenylic acid system. This was demonstrated by Lipton and Elvehjem (117).

In vitamin B_1 deficiency, there is a decrease in the amount of free thiamine and a decrease in the amount of cocarboxylase. Many workers (59, 60, 116, 144, 145) have demonstrated a reduced cocarboxylase content in tissues from experimental animals deficient in vitamin B_1. Ochoa and Peters (145) reported about three micrograms of cocarboxylase per gram of normal brain tissue in rats, and this value decreased to less than one microgram per gram after the development of polyneuritis. In brain, liver, muscle, and heart the amount of cocarboxylase was much greater than the amount of free thiamine found in normal rats and pigeons. In thiamin deficient animals the amount of cocarboxylase was still greater than the amount of free thiamine present, but the differences were not as marked. uscle and heart had relatively more thiamine than brain and liver in both normal and thiamine deficient animals. The administration of thiamine to the def cient animals led to an immediate accumulation of both thiamine and its pyrophosphoric ester in the liver. This indicates the important role which the liver must enact in thiamine metabolism.

Goodhart and Sinclair (59, 60) presented a mean normal value of 7.0 micrograms of cocarboxylase per hundred c.c. of blood, and found a significant decrease in the blood cocarboxylase during thiamine avitaminosis. The cocarboxylase in the blood was in a combined form and all of it was found within the blood cells. The polymorphonuclear leukocytes contained more than the lymphocytes and the erythrocytes contained the least. None was found in the serum. This indicates that the vitamin is probably carried to the tissues as freely diffusible thiamine rather than in the form of cocarboxylase. Goodhart and Sinclair (59) present a mean normal value of 0.04 micrograms of thiamine per c.c. of whole blood.

Since cocarboxylase is more or less directly concerned with the metabolism of a single metabolite, pyruvic acid, more extensive studies have been made on the changes in the pyruvic acid content of the blood than on the coenzyme itself (161, 196, 207).

It has been found that with certain yeast preparations used in determining cocarboxylase, free thiamine stimulated the activity of the cocarboxylase. Lipton and Elvehjem (116) have explained this reaction on the basis that the yeast preparations contained a

heat-labile material which adsorbed cocarboxylase without the production of an active enzyme. The addition of excess thiamine saturated the material and thus permitted the reaction of cocarboxylase with the active apoenzyme. Whether such a condition occurs in the human organism or in the animal body is not known at the present time.

In 1941, it was shown (198) that certain tissues from fish contained an enzyme-like substance which was destructive to thiamine. This factor has not been found in tissues from mammals, but if it should be, one would have another relationship of an enzyme to the biological action of thiamine.

In 1936, Sure (203, 204) showed that in vitamin B_1 deficiency there was a decrease in the digestive efficiency of pancreatic esterase and a moderate decrease in the concentration of pancreatic and hepatic lipase which finding suggested a disturbance in the digestion of fat. There was an increase in the concentration of blood serum phosphatase. There was no disturbance in the digestion of proteins or starches in vitamin B_1 deficiency according to the same author.

Bernheim (16) stated that thiamine inhibited the action of cholinesterase, especially when high levels

of thiamine were used. The significance is unknown at the present time.

In summary, the main function of thiamine in metabolism is its action as a coenzyme by combining with phosphoric acid to form thiamin pyrophosphate or cocarboxylase which is required for the oxidative removal of pyruvic acid--a vital link in glucid metabolism (199).

The next vitamin to be discussed is riboflavin. The physiological role of riboflavin is related mately to the ability of riboflavin to serve as an integral portion of two respiratory carriers (coenzyme I and coenzyme II). Thus, as alloxazine mononucleotide and alloxazine dinucleotide, riboflavin enters into the structure of a variety of flavoproteins which are concerned with many phases of logical oxidation. At the present time, some ten flavoproteins are known to be concerned in biological oxidations, and this emphasizes the functional relationship of the vitamin riboflavin to the enzymatic oxidative mechanisms of the organism. Riboflavin is related to more enzymes than any of the other known vitamins. It is well known that Warburg and Christian (218) isolated a flavoprotein from yeast

1932, which was considered a carrier link between coenzyme II and molecular oxygen. This flavoprotein was resolved into a protein and riboflavin phosphate. The enzyme was reduced by reduced coenzyme II and reoxidized by molecular oxygen. This system, however, would have very little activity in animal tissues where the oxygen tension was at a low level. Since that time, several different flavoproteins related to pyridine coenzymes have been studied (16).

In 1938, Haas (78) described the isolation of a flavoprotein from yeast which catalyzed the reaction between coenzyme II and methyline blue. In contrast to the original compound of Warburg and Christian, this enzyme contained alloxazine-adenine-dinucleotide rather than riboflavin phosphate as the prosthetic group. At about the same time, Dewan and Green (42) and Euler (48) prepared concentrates of an enzyme which catalyzed the transfer of hydrogen from coenzyme to methylene blue. Later this enzyme was isolated from heart muscle by Straub (201) and was found to contain the same dinucleotide.

None of the previously mentioned compounds, catalyzed the reaction between the coenzymes and cycochrome C, which link is the most important

in animal tissues. Axelrod (10) believes that coenzyme I and II are linked to cytochrome by flavoproteins. Haas et al (80) isolated a flavoprotein which did catalyze the reaction between coenzyme II and cytochrome and Altschul (4) et al isolated a soluble enzyme which catalyzed the reaction between coenzyme I and cytochrome C. This work indicated the significance of flavoproteins in respiratory mechanisms but it should be emphasized that both of these compounds have been isolated from yeast and not from animal tissues. No one has yet measured the amounts of these two flavoproteins in tissues taken from animals suffering from riboflavin deficiency.

There are other flavoproteins which catalyze the direct reaction between the substrate and molecular oxygen and may therefore be classified as dehydrogenases (47). One of these is d-amino acid oxidase which catalyzes the oxidative deamination of several unnatural amino acids and which, according to Warburg and Christian (217) contains the dinucleotide as the prosthetic group. In 1940, Axelrod (11) demonstrated that a riboflavin deficiency in rats resulted in a decrease of the d-amino acid oxidase content of the liver and kidney. Ochoa and Rossiter (146) demonstrated

a similar decrease in the total riboflavin dinucleotide content of heart and liver from rats showing riboflavin avitaminosis. The dinucleotide content was rapidly restored to the normal level by the administration of riboflavin to the deficient rats. This indicated the ability of the living tissue to synthesize the nucleotide when sufficient ribolfavin was supplied. Klein and Kohn (101) have demonstrated this synthesis by human red blood cells in vitro and in vivo.

Xanthine oxidase is another flavoprotein and Ball (14) and Corran et al (34) have shown that the prosthetic group of this enzyme consists, at least in of the dinucleotide. Axelrod (8) showed that the xanthine oxidase activity of rat liver was greatly reduced in riboflavin deficiency. The xanthine oxidase activity was rapidly restored to normal when riboflavin was fed to the rats and it was shown that riboflavin-adenine-dinucleotide was the prosthetic group, which finding confirmed those of Ball (14) and Corran (34). In the same paper some evidence was presented to show that the protein component of xanthine oxidase was also decreased during the deficiency. Thus, in the absence of the prosthetic group it would seem that less of the protein is manufactured.

In 1942, Axelrod and Potter (10) demonstrated a definite decrease in the succinic acid oxidase content of the liver and heart from riboflavin deficient rats. Up to the present time no one has shown conclusively that succinic acid oxidase contains riboflavin, but their results may indicate that such is the case. This would give added significance to the role of riboflavin in metabolism, for the oxidation of succinic acid is believed to be an essential step in normal glucid metabolism. The report by Sure and Bichey (202) that riboflavin produced a profound effect on the economy of food utilization for synthesis of body tissues is further evidence that metabolism proceeds more efficiently when there is sufficient riboflavin for the optimal activity of the various enzyne systems.

It is impossible to correlate the changes in the individual enzymes with the variety of symptoms that have been associated with riboflavin deficiency in experimental animals and humans, but from the short survey given, it is evident that riboflavin plays an important role in the entire respiratory mechanisms in the animal body.

Nicotinic acid, the specific curative agent for the dermatitis, diarrhea, and dementia of pellagrins, is

the next vitamin to be discussed. Nicotinic acid deficiency is manifested chemically through the dysfunction of only two coenzymes, but the entire picture is quite complicated because these two coenzymes enter into so many different reactions.

Warburg and Christian (217) on their studies of flavoproteins used a coenzyme, now known as coenzyme II which was prepared from ed blood cells. In 1935, c enzyme II was isolated and characterized as a dinucleotide consisting of adenine, pentose, phophoric aced, and nicotinic acid amide. Euler and his co-workers (48) had been working on cozymase or coenzyme I from yeast, and in the same year both Euler and Warburg isolated nicotinic acid amide from cozymase hydrolysis. The necessity of a coenzyme for the oxidation of lactic acid by lactic acid dehyrogenase of animal tissues was demonstrated by Szent-Gyorgyi in 1925, (47). The two coenzymes differ only in the number of phosphoric acid molecules, but they show great specificity as far as the protein or dehyrogenase with which they may function. Alth ugh nicotinic acid, adenine, and ribose are constituent parts, nicotinic acid appears to be the only compound which the human body has difficulty in synthesizing. The

rat and chick can manufacture ~~acid~~ nicotinic acid while, on the other hand, certain microorganisms such as Hemophilus parainfluenza will grow only in the presence of the coenzyme and will not respond to the free acid or the amide (56, 120). Hence, the specific accessory substances vary with the biological level of the organism (141).

Some of the most interesting observations on the production of cozymase (coenzyme I) came from studies involving sulfapyridine. West and Coburn (230) found that sulfapyridine prevented the growth-stimulating effect of nicotinic acid on Staphylococcus aureus grown on a medium deficient in nicotinic acid. West (239) extended this study to dogs and found that if a black tongue dog was saturated with sulfapyridine, the dog would no longer respond to nicotinic acid therapy. However, raw liver was found to be highly efficacious in curing the black tongue. West concluded that the fresh liver supplied preformed coenzymes. Axelrod (47) repeated the experimental procedure with the same results, but reasoned differently. He believed that it was difficult to accept the suggestion that coenzyme would go through the digestive tract and be absorbed without degradation--a sound criticism. He reasoned that the fresh liver supplied a specific com-

ponent of the cozymase, the synthesis of which was prevented by sulfapyridine. Since the linkage between nicotinic acid and ribose was the most labile part of the molecule, he stated that it was probably the nucleotide that was involved. This relationship certainly needs more study especially in the clinical uses of the sulfonamides.

The limiting factor in the production of coenzyme I and coenzyme II under normal conditions is nicotinic acid. Numerous studies have been made on the changes in the coenzyme I and II content of tissue from animals and humans during nicotinic acid deficiency. It is impossible to discuss here the methods involved in these measurements but in general two types of procedures have been used. One was based on the growth responses obtained with various microorganisms under controlled conditions, and it measured both coenzyme and II, as well as possible other factors; the other was based on the specific reactions catalyzed by coenzyme I during yeast fermentation. Axelrod used the original fermentation test; Jundorf et al (95) described the method which involved only part of the fermentation mechanism. In both cases CO evolution was measured, although in the fermentation method the

CO_2 was evolved from a $NaHCO_3$ buffer by the acid produced. In spite of discrepancies in the absolute amount of cozymase (coenzyme I) in various tissues reported by Katzenelbogen (100), Handler (82), and Mann (132), all workers were agreed that the coenzyme I content of liver and muscle tissue decreased during nicotinic acid deficiency. The values reported by Kohn, Klein, and Dann (107) and Axelrod, Madden, and Elvehjen (9) indicated a reduction of about seventy per cent in the liver content and thirty-five per cent in the muscle content in the case of the dog. Axelrod, Spies and Elvehjem (12) found that the coenzyme I content the muscle of pellagrins decreased as the deficiency increased in severity. In all cases the coenzyme content increased with therapy. Axelrod and Elvehjem (7) reported no change of the coenzyme I content of the blood, brain, or kidney of pig and dog in nicotinic acid deficiency. Most of the workers agreed that the blood cozymass underwent little change during the nicotinic acid deficiency. Typical changes ranged from eighty-five micrograms per milliliter of normal blood to sixty-nine micrograms per milileter of blood from severe pellagrins. This may be explained, partially at least, by the finding of Kohn and Klein (106) that

red blood cells can synthesize cozymase (coenzyme I) from nicotinic acid in *vivo* and in *vitro*.

Although no specific measurements have been made on coenzyme II during nicotinic acid deficiency, it is safe to conclude that the function of nicotinic acid in the animal body is largely related to these two coenzymes. Much more work is necessary before one can relate the observed changes in coenzyme I and II content of certain tissues to the external symptoms observed in nicotinic acid deficiency.

Vitamin B_6 which is chemically either pyridoxine, pyridoxal or pyridoxamine has been shown recently (15, 114) to be an important component of various coenzyme decarboxylases. "Codecarboxylase" refers to all preparations which possess activity for amino acid decarboxylases. So far six amino acid decarboxylases--those of tyrosine, lysine, arginine, ornithine, glutamic acid, and dopa--have been shown to require this coenzyme. The exact structure of codecarboxlase or coenzyme is not known for certain, but the properties are sufficiently known to indicate that it is a phosphorylated derivative of pyridoxal and to suggest the possible position of the linkage. Pyridoxal phosphate was also shown by Lichstein (114)

to function as the coenzyme of glutamate-aspartate transaminase. This was accomplished by two methods. The first method consisted of growing cells in a medium deficient pyridoxal to yield transaminase apoenzyme, and then activating the enzyme with synthetic pyridoxal phosphate, while the second method consisted of resol ng--that is separation by aging and dialysis--the cell-free enzyme from organisms grown with pyridoxal, and then restoring the activity of the apoenzyme with pyridoxal phosphate. Therefore, the function of the vitamin B_6 group in protein metabolism is at least partially explained by its action in amino acid decarboxylation and in transamination. Pyridoxine is being used successfully clinically in the treatment of morning sickness and pernicious vomiting of pregnancy. Perhaps its function in protein metabolism is responsible for the improvement seen in most of the cases.

The remaining members of the vitamin B complex have not been associated with any specific enzymes although there are reasons to suspect certain relationships. It is well known that pantothenic acid and biotin are closely associated with protein in the living cell. There still are many gaps in the knowledge of the various respiratory enzyme systems, and

perhaps in time some of the gaps will the known vitamins. Axelrod (47) suggests that perhaps the unknown component in cytochrome C which contains sulfur, might be biotin. He also believes that the rest of the B complex will be found to fill the role of the enzymes specifically concerned with fat metabolism and protein metabolism, or in the resynthesis of pyruvic acid to glcogen.

Before leaving the B complex one must mention the relationship of enzymes and p-aminobenzoic acid which substance Ansbacher (5) has classified as a vitamin. Although no one has demonstrated any effect of p-aminobenzoic acid in experimental animals, it does function in the growth of certain microorganisms and it has been isolated from vitamin rich materials such as yeast and liver. Wisansky, Martin, and Ansbacher (233) have shown that p-aminobenzoic acid retards the oxidation of tyrosine and dopa by tyrosinase. Lipman (115) has reported that p-aminobenzoic acid is oxidized by hydrogen peroxidase and peroxidase, and that the reaction is inhibited by sulfonamides. It is also oxidized by phenol oxidase in the presence of catechol, but this reaction is not inhibited by sulfonamides. The antagonism between sulfonamides and p-aminobenzoic

acid activity in bacteriologic metabolism is well known and is the probable mechanism of the bacteriostatic activity of these drugs. The sulfonamides replace the p-aminobenzoic acid in the bacterial metabolism and the organisms are unable to utilize the sulfonamides.

Vitamin C, the anti-scorbutic substance, was associated with enzymes long before it was isolated because of the ease with which it was oxidized, but so far no one has proven conclusively that it is a constituent part of an enzyme. It has been suggested as a prosthetic group in liver esterase by Kraut (109) who postulated that liver esterase might be composed of a protein fraction (apoenzyme) linked with an active ascorbic acid group (coenzyme). Harrer (83) found that liver esterases were decreased progressively with vitamin C depletion to sixty-five per cent of normal in acute scurvey. Cytochrome oxidase showed a moderate decrease in activity in vitamin C deficient heart and skeletal muscle tissue.

Both ascrobic acid and dehydroascorbic acid exert stimulatory or inhibitory effects on many of the proteolytic and oxidizing enzymes. Meikejohn and Stewart (134) showed that the ascorbic acid oxidase is a

true copper protein compound and is much more active than an equivelent amount of ionic copper. Crook (37) produced evidence that a specific enzyme is involved in the reduction of dehydroascorbic acid by glutathione.

Independently of, or parallel with, the possible function of ascorbic acid as a carrier-catalyst in tissue respiration, the vitamin may be of major importance in cellular physiology as a regulating and protective agent.

When the fat soluble vitamins are studied, only indirect relationship to enzymes are found. There is no disturbance in the digestion of proteins or starches in vitamin A deficiency (203). Sure (205) reported a marked decrease in the concentration of the blood serum esterase, an appreciable decrease in the hepatic esterase, and a marked decrease in the hepatic lipase in vitamin A deficiency. These findings were confirmatory of Green's earlier report (69). A more specific relationship of vitamin A and enzyme activity comes from the work of Wald (216), who showed that visual purple is a conjugated protein with vitamin A as the prosthetic group.

Vitamin D deficiency has been recognized for twenty-one years as being accompanied by a high plasma phos-

phatase, but this is most likely a secondary effect due to the loss of phosphatase by rachitic bones (47). Sure (203) found that no noteworthy changes in the concentration of blood and tissue enzymes devoloped in albino rats on a rachitic diet when compared with enzyme concentrations of albino rats on the same diet supplemented with vitamin D. In experimental rickets there was no large increase in the concentration of blood serum phosphatase ~~which is~~ like that so characteristic of human rickets. Therefore, the author concluded that experimental rickets was not the analagae of of human rickets and he quoted the Italian worker, Scoz, as coming to the same conclusion.

It is possible to conlcude logically from the above review that certain vitamins may be constituent parts of some enzymes, and that these enzymes show a significant decrease during deficiency diseases. In other cases, the relationship between the enzyme and the vitamin is indirect or obscure. To these cases, we can apply Green's (47) trace substance (enzyme) thesis: namely, that there is no rationale explanation available of how traces of some substances can exert profound biological activity except in terms of enzyme phenomena.

PERNICIOUS ANEMIA--AN ENZYME DEFICIENCY

Several papers appear in the medical literature which tend to identify the intrinsic factor of pernicious anemia with an enzyme found in gastric juice. It was shown by Taylor (55) that normal human gastric juice contains a proteolytic enzyme capable of hydrolyzing casein to the protease stage in an alkaline medium, but not at a hydrogen ion concentration below pH4. This range of activity and certain other properties seemed to distinguish this enzyme from pepsin, while the failure of the enzyme to produce large amounts of amino nitrogen within twenty-four hours appeared to rule out trypsin and erepsin, acting in their generally accepted manner.

Although one cannot assert that this proteolytic activity is identical with that of the so-called intrinsic factor of P. A. as detected clinically, it is of interest that the proteolytic activity in question was retained or destroyed under certain conditions which effected the clinical activity of the intrinsic factor in a similar fashion. Adams (55) has shown that both are unable to penetrate a semi-permeable membrane. He stated that because of the

usual presence ~~in~~ of interfering enzymes from the intestinal tract, the *in vivo* method of study was unsatisfactory for determining in pernicious anemia the amount of proteolysis which could be ascribed to the proteolytic agent in normal gastric juice referred to above.

INFLAMMATION AND INFECTION

The biochemistry of inflammation and infection is a very recent field, but it has already yielded much knowledge and in the near future will prove even more valuable. Bacteria do not invade tissues by magical means, but rather with the aid of cellular enzymes which they produce. The enzymes interfere with cellular respiration and weaken the intercellular binding substance. This paves the way for the bacterial invasion. Some of these enzymes diffuse into the blood stream and are carried to all parts of the body where they exert their destructive effects. Pathogenic organisms produce such potent enzymes as coagulase, fibrinolysin, spreading factor, and many others which aid immensely in the bacterial destructive processes (29). Fortunately, however, the body has defensive enzymes which enable it, many times, to defeat the invading legions of bacteria with their coherts of enzymes. This brief introduction skims the surface of a vast field--the biochemical dynamics of pathogenesis. In this section of the paper, the experimental basis for some of these phenomena will be presented. The inflammatory process is the fundamental

reaction of the organism to injury and it is, therefore a logical introductory subject. Since the classic work of Opie in 1908, the proteolytic enzymes contained in leukocytes have been regarded as playing an important role in the biochemical defense of the organism.

Weiss in 1938, (227) made a study of the activity of the enzymes present in leukocytes. He injected starch, light mineral oil, and phosphatide obtained from Mycobacterium tuberculosis into the pleural cavity of experimental animals. These substances acted as irritants and invoked a defensive inflammatory response leading to the formation of a pleural exudate. The various types of leukocytes present were isolated and their cathepsin (intracellular proteinases) activity was studied using denatured hemoglobin as the substrate.

The author found that all the polymorphonuclear leukocytes, the monocytes, and the epitheliod cells contained a cathepsin which exhibited an optimum activity at a pH of 3. The leukocytes had in common a dipeptidase which hydrolyzed dl-alanylglycine at a pH of 8. The monocytes were, however, differentiated from the myelocytes by the fact that the monocytes in addition were able to split dl-alanylglycine at a

pH of 5. Furthermore, the monocytes were able to hydrolyze gelatin and casein at a pH of 8. Since epithelioid cells are, on the basis of embryological researches, derived from monocytes, it is interesting to note that they have an enzyme pattern of activity which is similar to that of the monocytes and not to that of the polymorphonuclear leukocytes.

In summary, then, the leukocytes are important agents in the defensive reactions of the body. They not only actively phagocytize the irritating agent, be it a pathogenic organism or some foreigh substance, while they are alive, but also by means of their intracellular proteinases, which are released after their death, liquify the dead tissue and digest the foreign agent. This enables the body to rid itself of the irritant much more easily and to start the process of repair earlier.

When the invasion of tissue occurs, the body attempts to wall off the destructive process by encapsulating it in fibrin. Certain bacteria, especially the streptococci, produce fibrinolysin which is an enzyme capable of dissolving the fibrin clot, and the path gens can then extend involving more tissues. It is the presence of fibrinolysin that gives the exudate produced

by streptococci the typical thin watery appearance. For many years fibrinolysin has been considered a complete enzyme produced mainly by B hemolytic streptococci. The resistance of human plasma clot to dissolution by fibrinolysin has generally been considered an immunological response to infection by this organism. Because of this, the development of resistant clots during convalescence has been used as a diagnostic test for hemolytic streptococcus disease. But, clot resistance is observed in individuals who have no, or have had no, evident streptococcal infection. Fifteen per cent of normal individuals, a number of unrelated infectious diseases such as lobar pneumonia, gonococcal arthritis, typhoid fever, and acute nephritis, newborns, and various animal species show clot resistance. Kaplan's experiments, in 1946, (98) explained some of these discrepancies.

Kaplan found that streptococcal fibrinolysis occurred only in the presence of an accessory plasma component (lytic factor) which was very similar in properties to the serum protease. Both enzymes occur regularly in an inactive state in normal blood associated with the serum euglobulin fraction. He demonstrated that the lytic factor was converted into the active protease by fibrinolysin which was shown to be a kinase.

Therefore, the activation of lytic factor by fibrinolysin is analagous to the transformation of trypsinogen into trypsin by enterokinase. Although serum protease and trypsin have many properties in common, they are not identical as there is separate specificity of activators.

Thus, fibrinolysin is really the activator in the following scheme:

1) Lytic Factor plus Fibrinolysin→Active Protease

2) Active Protease plus Fibrin Clot→Dissolution.

Inhibition can occur in either equation and resistance is due to either an antifibrinolysin substance, an anti-protease substance, or a deficiency of effective lytic factor in the plasma. All of these take place. For example: patients recovering from hemolytic streptococcal infection have antifibrinolysin; patients with pnemococcal pneumonia and various animals have antiproteases; newborns are deficient in the lytic factor. By suitable control of factors entering into the fibrinolytic reaction, Kaplan (98) devised a quantitative method for estimating the amount of serum antifibrinolysin.

One of the most powerful defenses of the body is the presence of the intact skin which is an extremely

out of the solution. They attributed this phenomena to the capacity of the injected material to increase tissue permeability. In the following years the phenomena of spreading and the properties of spreading factor (now called the Duran-Reynals Factor) were studied by a large number of other investigators.

Spreading factor has been demonstrated in extracts of streptococcus (139, 35), pneumococcus (93, 138), clostridium (99, 139, 188), snake venoms (44, 129), bee and leech venoms (32, 124), spermatazoa 81, 90, 122, 126), and certain malignant tumors (32). McClean (123) has shown that the spreading phenomena occurred when the injection was made into the isolated skin of rabbit. Duran-Reynals (45) demonstrated the same thing in muscles, tendons, walls of the stomach and intestines, and he showed that even the permeability of the vascular system can be locally or generally increased by means of preparations rich in spreading factor.

Chain and Duthie (27) reported that testicular extracts decreased the viscosity of synovial fluid and vitreous humour with the liberation of a reducing substance. Since such extracts also induced the phenomena of spreading, they suggested that spreading

factor was identical with the mucinase which had hydrolyzed the glucids in the fluid. Claude (32), in a study of leech extracts, contributed further evidence as to the nature of the spreading factor. He showed that leech extract which was rich in spreading factor contained a mucolytic enzyme which reduced the viscosity of a mucoprotein isolated from the skin and he concluded that the strength of the mucolytic enzyme paralleled the strength of the spreading factor. In 1941, Meyer and Chaffee (137) proved the existance of a mucin-like substance in the skin. It is a mucopolysaccharide (hyaluronic acid) containing sulfuric acid and it was related to chondroitinsulfuric acid. They called the enzyme that hydrolyzed it hyaluronidase and suggested that it might be the spreading factor. Hobby (89) found that all preparations containing hyaluronidase had spreading effect. Heat had the same effect on hyalurenidase and spreading factor, but there was no parallel in the degree of activity of the spreading factor and of hyaluronidase. All preparations with spreading activity did not contain hyaluronidase, and while antisera to hyaluronidase specifically and completely inhibited the activity of the enzyme, it did not inhibit the spreading factor in the same preparation. While

the minority of experimenters (89, 131, 138) believe that spreading factor is not identical with hyaluronidase, the majority (27, 32, 41, 124, 137) believe that the two are identical. From the evidence presented in the literature, the latter opinion is more sound.

Seemingly there should be a correlation between the virulence of the organism and the amount of hyaluronidase produced by the organism, but Cowley (35), Kass (99), Humphrey (93), and MacLennan (130) found no correlation in streptococcal, pneumococcal and clostridial infections. However, McClean and Rogers (127, 189) believed that the failures were due to antitoxin production which supressed the formation of hyaluonidase. According to Duran-Reynals (45) the degree of invasiveness of bacteria is largely determined by the amount of spreading factor present, and the successive phases of infection induced by bacteria include the following: (1) the hydrolysis of the mucoid ground substance of the connective tissue, a pre-existing biological obstacle; (2) spreading primarily through the interstitial system connective tissue.

From the preceding material it is seen that there is a mucopolysaccharide in the skin and that a specific enzyme, hyaluronidase, which is able to hydrolyze the

glucid, is present in various extracts. It is probable that the enzyme has an important role in the pathogenesis of invasion; the depolymerization of the mucoid ground substance of connective tissue. These facts will be elaborated on subsequently.

Contradictory observations reporting the presence of an inhibitor of hyaluronidase in blood serum have appeared in the literature. Some investigators believe that this inhibition is an antigen-antibody reaction. For example, by the use of decapsulati n of virulent bacteria as a test for hyaluronidase action, McClean (125) found that the heterol gous as well as the homologous antisera inhibited the action of the enzyme. The presence of a substance in horse serum which inhibited the action of the diffusion factor was mentioned briefly by Humphrey (94), and Hobby (89). However, experiments using the velocity of the spreading factor in the skin (45) and experiments using the depolymerization of hyaluronic acid by hyaluronidase (122) as a method of assay showed that the effect of each antiserum is limited to the preparation used for the immunization. Furthermore, the inhibition of hyaluronidase activity by antiserum was strictly specific and heterologous and normal sera exerted no effect. Haas (79), on the other hand, in

a recent article which will be presented in some detail. demonstr.ted that normal plasma contained a non-specific highly active enzyme (antivasin I).

In his article, Haas (79) reports the presence of two enzymes in the blood plasma which are part of the defense mechanism of the body. A third enzyme is described which is produced by certain bacteria and is found in some venoms. This enzyme promotes the invasion of tissues. Furthermore, the experimental findings suggest the existence of two additional enzymes; one is observed in the plasma, the other in the invading organism. The author offers invasion as a synonymous term for hyaluronidase. He suggests this because of its property of promoting invasion and spreading. Haas also introduces the terms "antivasin" and "proinvasin" for the newly described enzymes. The first enzyme is found in the normal blood plasma of mammals, birds and fish. It is a defensive enzyme which rapidly destroys hyaluronidase and it is, therefore, called antivasin I. The second enzyme, proinvasin I, is found in bacteria and venom. It rapidly inactivates antivasin I, thus paralyzing the body defense mechanism and indirectly promoting invasion, The third enzyme, antivasin II, has been observed in normal plasma. It

acts by destroying proinvasin I, thereby indirectly counteracting invasion. Quantitative tests for these enzymes are presented. All observations were made *in vitro*. The following diagram summarizes and clarifies these reactions:

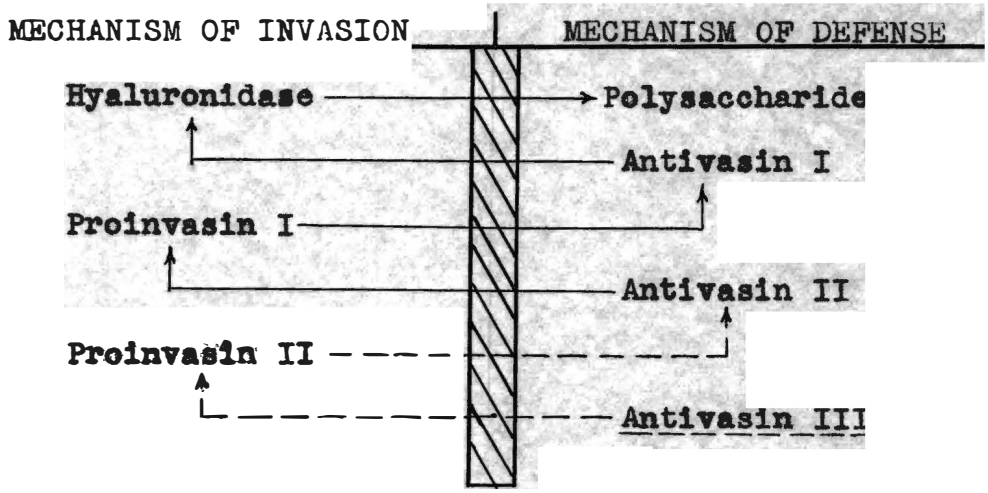


Fig. 1
(from Haas)

Dotted lines indicate postulated enzymes and postulated reactions.

The test for the assay of hyaluronidase, antivasin, and proinvasin, depends upon measuring quantitatively the fall in viscosity due to the depolymerization of hyaluronic acid by hyaluronidase. It is based on the fact that hyaluronidase decreases the viscosity of the glucid at a rate directly proportional to the hyaluronidase concentration; The rate of breakdown of the glucid is then directly proportional to hyaluronidase and proinvasin concentration or inversely proportional to antvasin I and II concentration. A phosphate compound inhibits the reaction of antivasin I and hyaluronidase, but does not interfere with the hyaluronidase-glucid reaction. Therefore, Haas put hyaluonidase and antivasin I together without phosphate for a fixed time. Then he measured the fraction of hyaluronidase remaining intact by its action on the glucid in the presence of the phosphate substance with a viscosimeter. (The phosphate prevented further reaction between the antivasin I and hyaluronidase). The conditions can be so arranged that any one of the enzymes in figure I can be made the rate-determining factor.

Haas found that contradictory to some of the reports of earlier investigators (discussed previously) the reactions were not antibody-antigen reactions, but rather

enzymatic reactions. The rate of reaction between antivasin I and hyaluronidase (~~by antivasin I~~) is directly proportional to the concentration of hyaluronidase. Normal serum has antivasin I and it has the capacity to destroy large amounts of hyaluronidase. The activity and concentration of the defense enzyme is of such a magnitude that it can deal with a high concentration of hyaluronidase, corresponding to a great number of organisms. The reaction curve follows a graph of the first order--it is an enzymatic reaction. Antivasin I activity is independent of the glucid concentration; it varies with the temperature; it is inhibited by phosphate; it is not dialyzable (high molecular weight); and it is heat labile. Therefore, antivasin I is not an antibody. Enzymatic reactions are mechanisms of natural immunity against invading organisms in distinction to acquired immunity which is represented by antibody formation following exposure of the animal to the antigen. The antibody concentration falls off rapidly after contact ceases. In contrast, antivasin is present in normal serums of all animals investigated. Antibodies react specifically with antigens while antivasin I is non-specific as it reacts with hyaluronidase from various sources. Therefore, antivasin I is an enzyme.

~~(antivasin I, therefore, is an enzyme.)~~ In those cases where the antivasin was not destroyed (it was not destroyed in some of the experiments) the author suspected the presence of an interfering substance (proinvasin I). This enzyme is found in certain pathogenic bacteria and snake venoms and it protects hyaluronidase against destruction by the body defense enzyme (antivasin I). There is a pronounced fall in the concentration of antivasin I in individuals with various infections.

Proinvasin I is produced by pathogenic organisms simultaneously with hyaluronidase, and it acts by destroying antivasin I. Hyaluronidase, although normally inactivated by antivasin I, is then left intact, since it is accompanied and protected by proinvasin I in amounts sufficiently large to cause the destruction of antivasin I. It was assumed by Haas that proinvasin I would materially enhance the inv sion of bacteria and venoms because, by eliminating antivasin I, it permitted the action of hyaluronidase to proceed unhindered.

The quantitative test for the determination of proinvasin I content, using the viscometric method, was run by first incubating the antivasin I of plasma with proinvasin I. The amount of antivasin I left intact

after this time was determined by measuring its action on hyaluronidase as previously described. Thus, a rapid decrease in viscosity indicated a high activity of proinvasin I. The results showed that the destruction of antivasin I by proinvasin I was non-specific. Proinvasin I from all sources inhibited the action of antivasin I which means that ~~one~~ the reaction is enzymatic rather than immunological.

Proinvasin I differs in chemical properties from any known enzyme and it is produced by the organisms which produce hyaluronidase. Its apparant function is the destruction of antivasin I. The relative proportions of the two enzymes, proinvasin I and hyaluronidase, varies greatly depending on the source of the enzymes. The relative proportions of the two enzymes determine the course of the reaction. If only small amounts of proinvasin I are present, hyaluronidase is unprotected and is destroyed by antivasin I and invasion by this route is prevented. With a high concentration of proinvasin I, antivasin I is rapidly destroyed,and hyaluronidase thus protected is enabled to enhance invasion. The author assumed that proinvasin I by protecting hyaluronidase from destruction by the plasma enzyme, antivasin I, contributed materially to the

the severity of the infection.

Again certain exceptions were noted. There were cases in which antivasin I was destroyed much less than usual and this according to Haas, indicated that normal plasma contained another enzyme which destroyed proinvasin I and therefore acted as a second anti-invasion factor.

The second factor in the plasma is called antivasin II in line with previous terminol gy, by Haas (79). The activity of antivasin II was determined by the same type of viscometric measurements. The depolymerization of the glucid under certain conditions is a function of the concentration of antivasin II. to its intermediate position between antivasin I and antivasin II, the action of proinvasin I is determined largely by the relative proportions of antivasin I and antivasin II. For example, with large amounts of antivasin I, such as were present in plasma of healthy individuals, proinvasin I reacted with antivasin I so fast that the reaction with antivasin II could be neglected. Under these conditions antivasin II cannot be demonstrated. Under other conditions, however, e.g. in plasma of patients with infections, the concentration of antivasin I is so low that its rate of reaction with proinvasin I

becomes negligible. In this case antivasin II will react with proinvasin I, resulting in the destruction of proinvasin I. This is apparently the physiological role of antivasin II--the destruction of proinvasin I, an enzyme employed by the invading organism. This function of antivasin II is of importance a. dit proceeded especially efficiently in th se cases in which antivasin I, the primary anti-invasive factor, had been depleted.

Antivasin II seems to be a normal constituent of plasma and its presence can be demonstrated under various conditions. Plasma of individuals with infections and plasma of cows contain antivasin II. The presence of this enzyme could be observed easily in bovine plasma because it normally has a low antivasin I content and is an especially rich source of antivasin II. Antivasin II was more resistent to denaturation than antivasin I. Due to the latter fact, it was possible to demonstrate the presence of antivasin II in every normal plasma as well.

The activity of antivasin I in bovine plasma was forty times lower than in normal human plasma. Antivasin I in human plasma was rapidly destroyed by proinvasin I of stalphylococcus aureus but adding a relatively small amoun. of bovine plasma afforded al-

most complete protection for antivasin I. Therefore, bovine plasma contains the enzyme ntivasin II capable of destroying proinvasin I. This destruction took place so rapidly that further reaction of proinvasin I of Stalphylococcus aureus with antivasin I of human plasma was prevented. The net result was that antivasin I maintained its activity despite the presence of the bacterial enzyme proinvasin I.

No direct reaction took place between antivasin II and hyaluronidase and there was no reaction between antivasin I and antivasin II. While antivasin I activity was abolished by heating for fifteen minutes, antivasin II activity was not affected.

Proinvasin I from various sources was inhibited by the antivasin II from various sources. Therefore, this was not a specific reaction, i.e., not an antibody-antigen reaction.

Haas noted that while the various plasmas used were active in destroying proinvasin I of moccasin venome, they were not effective against proinv sin I from stalphylococcus aureus. Since antivasin II is an unspecific enzyme, not an antibody, its failure to destroy proinvasin I indicated the presence of an interfering factor in the enzyme preparation from Stalphylococcus

aureus. Haas called it proinvasin II, implying that by its action, invasion of bacteria was promoted. Proinvasin II decreased the activity of antivasin II from human plasma and it completely inactivated antivasin II, from the plasma of hogs, chicken and carp. This was indic ted by antivasin II failing to destroy proinvasin I. With antivasin II removed, proinvasin I can act to promote bacterial invasion as previously described. Therefore, the enzyme system involved in the process of invasion seems to be more complex in bacteria than in venom.

Antivasin II from human and bovine plasma was much less destroyed under equal conditions than that from hog, chicken and carp plasma. This discrepancy was explained by Haas by assuming tentatively the presence in the plasma of another enzyme-antivasin III, which acted by destroying proinvasin II of Stalphylococcus aureus. A relatively high concentration of antivasin III seemed to occur in human and bovine plasma which resulted directly in the destruction of proinvasin II and which led indirectly to the destruction of proinvasin I and hyaluronidase. The existance of the last two enzymes, antivasin III and proinvasin II, is speculative.

All of these observations were based on in vitro experiments, but it is only logical to assume that this is a model of the situation prevailing in the body with its continuous interplay between aggression and defense. The action of antivasin II directed against proinvasin I of various invading organisms may possibly be of practical interest and it is desirable to investigate the role of antivasin II as a therapeutic agent in the treatment of bacterial infections and snake bites.

While Haas' paper is well written and certainly puts forth some excellent ideas and schemes, it must be borne in mind that the work is very recent (April, 1946) and it has not been confirmed by other investigators at this time. It is certainly indicative, however, of the importance of more studies of enzymes in invasion and defense mechanisms.

Many bacteria produce toxins both exotoxins and endotoxins. These substances exert their effect through some interference with the normal enzymatic equations. They act by two mechanisms. The first, is that a toxin is a substance which blocks a metabolic reaction in the host by competing with the normal substrate for the enzyme catalyzing the reaction. For example, the aerobic oxidation of succinate by minced tissues of

guinea pig and this inhibition is decreased by the specific antitoxin (129). The second mechanism is that a toxin is an enzyme exerting toxic function by attacking one or more substances which are normal constituents of a cell, and therefore it interferes with the normal metabolism of the cell in this way. Both these hypotheses of the modes of action of bacterial toxins depends on the fact that the initial lesion in the host cell is one of molecular dimensions and high chemical specificity. Since a distortion in metabolism on one type of cell or group of cells may affect the metabolism of neighboring cells or tissues, a characteristic pathological picture may develop throughout the tissues of the host as a result of this primary lesion. The total pathogenic effect of a bacterial infection is no doubt a summation of all the metabolic disturbances caused by the growth of the microorganism, including the disturbances due to various non-specific toxic substances, for example histamine.

MacFarlane (129) has shown the possible relationship of the enzyme lecithinase to the local invasiveness of Clostridium welchii. The alpha toxin from this organism contains a lecithinase which is identical, most likely, with the lethal hemolytic and necrotic

substance produced by the organism. The lecithinase decomposes lecithin into phosphocholine and a diglyceride. This hydrolysis is prevented specifically by Cl. welchii (Type A) antisera. Falsenfeld (49) has shown that Vibrio coma has lecithinase activity resulting in the production of free phosphate choline and fatty acids from lecithin. This has not been correlated with virulence or pathology at the present time. Schiff (195) has isolated a lysing ecto-enzyme from Cl. welchii which is strictly specific for blood group A. So far as is known, it is the sole pathogen and also the only anaerobeic organism producing an enzyme of this kind.

Peters (160) studied the effect of diptheria toxin upon tissue enzymes in vitro. He based this experiment on the fact that diptheria is characterized by ketonuria and other symptoms indicating serious metabolic disturbances. Many of the regular pathological symptoms, e. g; circulatory failure, fatty changes in the myocardium, interference with heart action, and hemorrhage into the adrenals are also capable of being traced back to a biochemical origin. There was no evidence of a vitamin or dietary deficiency. The study showed that the dehydrogenase systems for succinic, lactic, malic, and

B-hydroxybutyruc acids were not affected at all, while indications were that other systems studied were also unaffected. Oxidation of lecithin by glutathione was slightly accelerated. The effect on auto-oxidation was inconclusive. The author suggested that a study of the effect of the toxin on the oxidation of fats be undertaken.

E. R. Trethewie (209) investigated the chemopathology of pneumonia in cats. He found that histamine was liberated in bronchopneumonia from the isolated perfused and the intact perfused lungs of cats. Only a small amount of the histamine appeared in the blood vessels; the greater part was found in the lymphatics and tracheal fluid. By adding one ounce of adenosine per c.c. of fluid, after perfusion had been carried out, a deaminizing enzyme was detected. In bronchopneumonia the activity of the deaminating enzyme in the perfusion fluid was increased. In addition, a substance was liberated which impaired the cardiac depression produced by adenosine.

There are many studies in the literature about the enzymes involved in tuberculosis and the hosts reaction to the bacillus. In 1941, Gerstl (54) found that tubercle phosphatide was biologically the most active

fraction of the split products of the tubercle bacillus. It was responsible for the epithelial cell response and the tubercle formation. The monocytes phagocytized the phosphatide but were unable to destroy it in rabbits. However, the monocytes of mice can destroy it and they showed high resistance to tuberculosis. The important factor in tuberculosis is resistance. The enzymes responsible for the phosphatide breakdown were stated to be a phosphatase preceeded by a lipase or better by a lecithinase. The phosphatase alone was found not to be very effective.

In 1942, the same investigator (53) published a paper in which he confirmed the enzyme breakdown of tubercle phosphatide. The enzymes involved in the process were lecithinase followed by phosphatase. It was found that the fatty acids split off from the tubercle phosphatide inhibited the lecithinase. The importance of this finding will be obvious after more experimental results are presented.

Weiss (212) in the same year found that the concentration of cathepsin (endocellular proteclytic enzymes presumably conceived with cellular growth, repair, and inflammation) of the spleen and liver decreased in reinfected animals, i. e. animals immunized with non-

virulent bacilli and reinfected with virulent strains. These proteinases were not appreciably decreased in the lungs and kidneys. Reinfection with non-virulent culture resulted in little or no decrease in tissue proteinase. In vitro experiments showed that that phosphatides of human and bovine tubercle bacilli markedly depressed catheptic activity. Weiss interpreted this to mean that in the livers and spleens of reinfected animals which effectively destroyed the bacilli that invaded them, enough tubercle phosphatide was liberated from the disintegrated bacilli to inhibit catheptic activity. In the lungs and kidneys, however, where destruction of the bacilli under these conditions was less pronounced, less phosphatide was released and hence there was less depression of catheptic enzymes.

The fact that catheptic action was not affected by reinfection with non-virulent bacilli and the well known fact that non-virulent vacilli cause no macroscopic tubercles beyond the site of inoculation in highly susceptible animals were further evidences along this line of reasoning. In much more resistant vaccinated rabbits, low virulent bacilli were localized. No bacilli invaded the livers and spleens and, therefore, no phosphatide was liberated in these organs and no

depression of catheptic activity occurred. On this basis, it seemed evident that the decrease in cathepsins of the spleen and liver were due to the inhibitory action of the phosphatide which might have been liberated during the destruction of the tubercles.

In 1943, the same investigator (226) concluded from further experiments with rabbits that the spleens of the animals infected with the tubercle bacilli by the intravenous route suffered a significant decrease in the speed of their cathepsin activity; the reduction being greater when virulent bacilli were injected than when lowly virulent strains were used. Moen and Swift in 1936, had noted that cells from animals infected with virulent strains were less active in their initial growth than those removed from animals infected with avirulent cultures. They found a close correlation between the pathologic changes in the spleen and the growth capacity of these explants in normal tissue culture media.

Weiss and Halliday (225) concluded that there was no correlation between the virulence of the infection and, hence, the extent of injury of tissue and the degree of enzymic inhibition exerted by purified protein derivatives in vitro and that there was no proportionality

between the initial growth energy of tissue explants and the degree of inhibition of growth exerted on them by old tuberculin. The same authors (226) state that Lurie demonstrated that following a primary blood stream infection of rabbits with tubercle bacillus the rate of their initial multiplication was faster in lung than in the liver or spleen. Furthermore when immunity developed during the course of this first infection, the bacilli were effectively destroyed in the liver, spleen, and bone marrow, but not in the lungs and kidneys. The organ resistance to tuberculosis acquired by rabbits during the course of a first infection represented in each case an increment of the innate inhibitory capacity which the different organs possess against the initial multiplication of the bacilli. The in vivo destruction of bacilli of reinfection was a function of the acceleration and intensification of the inflammatory process. The more complete destruction, however, was associated not with a greater intensification but with a greater acceleration of the sharply localized inflammation.

Because of the important role played by proteolytic enzymes in the dynamics of inflammation and especially in tuberculous lesions, Weiss (225) studied cathepsin

activity. His results paralleled those of Lurie and furthermore, the author showed that the speed with which the enzymes of the liver and the spleen hydrolyzed the substrate benzoyl-l-arginineamide (BAA) was much greater than that of the kidneys and lungs. The rank of activity was spleen > liver > kidney > lung. The heightened physiological activity and acceleration of the local inflammation observed by Lurie as a result of reinfection with virulent bacilli was accompanied here by an accelerated rate of cathepsin II activity from the spleen lungs and kidneys. Immunization of rabbits with avirulent strain did not increase the speed of hydrolysis by the endocellular proteinases; just as it failed to increase the phagocytic capacity of the cells for tubercle bacillus in Lurie's experiments.

Weiss and Halliday (224) in some enzymologic investigation to throw light on the mechanism of the specific cytotoxic effect of tuberculin in vitro, observed that purified protein derivitive (PPD) inhibited to an equal degree proteinase (Cathepsin II) from organs of normal and infected (virulent or non-virulent strain) animals. Old tuberculin (OT) on the other hand, had but a slight effect, in tissue culture, on cells from normal animals, but severely injured cells derived from

animals infected with either strain.

The fact that there was selective inhibitory action of tuberculocarbohydrate and tuberculophosphatide upon cathepsin II derived from tissues of infected animals suggested the following mechanism according to the authors: Since cathepsin was the enzyme which was concerned with the process of cellular growth and repair, inhibition thereof lends to injury of the cells. OT was more toxic for tuberculous than normal cells in tissue culture because two of its important constituents (glucid and phosphatide) exerted a selective inhibitory action on proteinases of the cells.

This observation throws light on the mechanism of caseation and softening. Caseation in tuberculosis is a form of coagulation necrosis in which dead tissue rarely undergoes autolysis, except as a result of secondary infection. It is quite likely, therefore, that inhibition of autolysis is accomplished by the glucid and phosphatide fraction of the bacillus. The authors further suggested that it is also possible that the development of increased susceptibility to the inhibitory action of the tubercle's products which comes about as a result of infection is a defense mechanism as the bacilli usually die in caseous areas but grow in softened

areas. Furthermore, the former may undergo calcification and healing whereas in the latter the bacilli grow and from there disseminate to other sites.

In summary, then, in the rabbit the inherent (natural) capacity of the organs (lung, kidney, liver and spleen) to destroy virulent tubercle bacilli is correlated with the speed of hydrolysis of their endocellular enzyme cathepsin II. Immunization with non-virulent bacilli followed by infection with virulent organisms results in an increased capacity to destroy the bacilli and a parallel acceleration of the speed of tissue enzymes. Injection of non-virulent bacilli alone does not affect the rate of proteolysis nor does it increase phagocytosis of the organism.

These observations permit for the first time correlation of organ and species susceptibility to infection with tubercle bacillus with endocellular enzyme activity. Tuberculoglucid exerts a selective inhibitory action on cathepsin II derived from tuberculous tissue, but is inert in the presence of normal tissue proteinases. The phosphatide and protein (PPD) fraction are also inhibitory but are not selective. It is possible that these substances prevent softening of caseous tuberculous foci. It is known that in caseous foci, bacilli

tend to die, whereas in areas of softening (cavities) they survive and multiply. Since softened areas may rupture into adjacent blood vessels or bronchi and thus cause dissemination of the infection, it is important to investigate the enzyme anti-enzyme balance under these conditions. Weiss in 1946, (223) has reported that biotin and others of the B group (thiamine, riboflavin, calcium pantothenate, niacin and inositol) which accelerate growth, were found to have no influence on the speed of cathepsin activity, even though the former participated in the synthesis of proteins and in the growth process.

The next few pages will discuss the reported changes in enzymatic reactions induced by various neurotropic viruses. Of all the infectious agents viruses are the most characteristically intracellular parasites and it would seem that a good correlation would be obtainable. However, the work in this field is very recent and the enzymatic processes of the cell are many. Therefore, the problem of choosing the right enzyme system to study--that is the one that is most affected by the virus--is very difficult. The enzyme involved may be concerned with the synthesis of some essential structure in the cell, e. g.; a nucleoprotein, it may be necessary for a reaction which yields energy to the cell, e.g, phosphorylation, or for a reaction which is important in maintaining the permeability character of an essential

cell membrane. Glucid, fat, protein, and mineral metabolism may all be involved.

Since glucose oxidation is quantitatively the most important respiratory process in brain studies in vivo, most of the studies concern it. Its respiratory quotient is approximately unity. The brain contains the cytochrome oxidase system which brings about the activation of oxygen. Cyanide, an inhibitor of the cytochrome oxidase system, blocks almost completely the oxygen uptake by the brain. Apparently cytochrome oxidase is quantitatively the most important oxygen activator in the brain (234). The dehydrogenases pull off hydrogen from the substrates which are oxidized. This hydrogen is transported to the cytochrome oxidase system by carriers-- in reality vitamins that have been transformed into coenzymes. Although there is no reference to it in the literature at the present time, the possible interference by viruses of the mechanisms of transmission of nerve impulses is of future interest.

Victor and Huang (211) have shown that the Western strain of equine encephalomyelitis virus inhibited the anaerobic production of acid (anaerobic glycolysis) by embryonic chick tissue. This was not confined to the nervous tissue as it occurred throughout the embryo. They suggested that this depression of anaerobic glycolysis by tissue might be part of the mechanism

whereby viruses produced tissue death.

Racker and Kabat (176) concluded that brain tissue infected with the virus of poliomyelitis had decreased ability to metabolize glucose under anaerobic conditions while the aerobic oxidation of glucose was unimpaired. The authors and others (97, 143, 177) showed that there was a decrease in the lactic acid content of brains infected with poliomyelitis as compared to normal. This effect of poliomyelitis virus seems specific as it was not produced by other neurotropic viruses. Other changes noted by Kabat (96) in mouse brain infected with poliomyelitis was an increase in adenosine triphosphate while phesphocreatine and residual orgaic phosphate decreased. These changes cannot be explained on the basis of greater autolysis in the infected tissue, since autolysis would have decreased rather than increased the adenosine triphosphate content. The author suggested that the intracellular parasite might have, in the course of its growth and multiplication, broken down the nucleoproteins of the cell or might have interferred with the dephosphorylation of adenosine triphosphate and, thus, produced an increase of the latter compound in brain tissue. The marked change in the acid-soluble phosphorous compounds suggests a considerable

interference with the energy mechanisms and glucid metabolism of the neurons by the virus infection.

Nichle and Kabat's (143) experiments demonstrated that with a high glucose concentration present, poliomyelitic infected brain showed a decreased anaerobic glucolysis, but the oxygen consumption was unaffected. At a lower glucose concentration the oxygen consumption in poliomyelitic brains decreased while anaerobic glycolysis was not significantly below normal. The authors pointed out the very interesting fact that brain tissue infected with the virus of Western equine encephalomyelitis showed a specific difference in metabolism from the brain infected with poliomyelitic virus.

With the glucose concentration at 121 mg per cent, the oxygen utilization of poliomyelitic infected brains was significantly below normal while that of the encephalitic brain was not. With a glucose concentration of 217 mg per cent the oxygen utilization of encephalitic brain was significantly below normal and that of poliomyelitic brain was not. With lactate-glucose as the substrate the oxygen utilization of encephalitic brain was significantly below normal and that of poliomyelitis was not. With pyruvate-glucose as the substrate, neither encephalitic brain nor poliomyelitic brain differed

significantly from normal in oxygen consumption. With succinate-glucose as the substrate, oxygen utilization of poliomyelitic brain was significantly above that of the normal control. With a glucose concentration of 37.5 mg per cent, anaerobic metabolism of the encephalitic brain was significantly below normal whereas that of poliomyelitic brain was not and finally with a glucose concentration of 229.5 mg per cent, anaerobic metabolism of both encephalitic and poliomyelitic brain was significantly below normal.

It must be pointed out that Wood (235) failed to find any difference in the rate of anaerobic glycolysis of brain tissue from normal animals and animals experimentally infected with poliomyelitis. However he stated that the methods used so far in the study of the enzymes may be inadequate and no decision could be made as to the effect of poliomyelitis on anaerobic glycolysis. Utter (210) in a well organized paper concluded that only a small part of the potential anaerobic glycolytic activity had been studied in the previous experiments on the effects of the viruses on glucose metabolism. Further studies are necessary before any definite conclusions can be made, but this field of investigation should certainly prove to be productive of valuable information.

METABOLIC AND DEGENERATIVE DISEASES

Arteriosclerosis: It has been shown by Rich and Duff (184) and Mellon, Baker and McIlroy (135) that certain proteolytic enzymes and/or their degredation products were capable of producing arteriolar lesions which were characteristic of human arteriolosclerosis. Hyaline arteriolosclerosis and ateriolonecrosis occurred at the site of the injection of tryptic enzymes of plant or animal origin (pancreatic juice, commercial or crystalline trypsin and papain) within 24 hours after the injection. It was shown that neither previous medial hypertrophy nor intimal proliferation was essential for the thickening of the wall and the narrowing of the lumen. These occurred apparently by imbibition and the microscopic examination showed that the cellular media disappeared leaving a homogenious hyaline substance which was either faintly basophilic or sometimes purely eosinophilic in color. If the enzyme was inactivated by boiling in water for ten minutes, no pathological changes resulted. These articles suggest one mechanism by which arterial changes resembling the pathological findings in human ateriolosclerosis can be produced, but do not show any definite eciological

relationships.

Acute Hemorrhagic Pancreatitis: The pathogenesis of acute hemorrhagic pancreatitis will serve as an introduction to the next topic. Opie (148) by means of carefully controlled animal experiments and autopsy findings demonstrated that aside from rare instandes of primary rupture or occlusion of the pancreatic blood vessels, hemorrhagic pancreatitis occurred only as a result of the disruption of the duct-acinar system of the pancreas with a subsequent escape of the secretion into the interstitial tissue of the gland. The majority of the cases of hemorrhagic pancreatitis resulted from a partial obstruction to the outflow of secretion, causing distension and rupture of the acini and ductules behind the obstruction, with the resulting escape of pancreatic juice into the interstitial tissue. If the escaping juice was rich in tryptic ferment and contacted arteries and veins, their walls were destroyed, and extensive or localized hemorrhage resulted depending on the size of the vessels affected. If no arteries or veins were present in the vicinity of the acinar rupture, or if the escaping secretion had low tryptic power, fat necrosis, produced by the lipase of the juice, was the only result. Rupture of dilated thinned-out acini

behind obstruction usually occurred during periods of increased pressure in the system resulting from stimuli which greatly increased the production of secretion, e.g. large meal and ingesting alcohol. While the underlying obstruction in some cases of hemorrhagic pancreatitis was caused by a gall-stone lodged at or near the ampulla of Vater, in most cases, autopsy showed that the main pancreatic duct was unobstructed, and the obstruction was in branches of the duct within the pancreas. Retrojection of bile into the pancreatic duct was an infrequent cause of hemorrhagic pancreatitis and proved cases are scarce in the literature according to Opie. Even in those cases in which bile retrojection occurred, hemorrhagic pancreatitis did not result unless the duct-acinar system was ruptured and secretion with sufficient tryptic potency to produce vascular necrosis escaped.

Autopsy findings (183), in 18.6 per cent of 150 consecutive cases on individuals over 25 years old, showed metaplasia of the epithelium of the branches of the pancreatic duct, leading to partial obstruction and consequent dilation of the acini and ductules behind the obstruction. It is, therefore, a common occurrence and would be reported more often if routine serial sections of the pancreas were made. Rupture of the distended acini behind the obstructed metaplastic ducts also occurred frequently, but in most cases the

resulting damage was limited to focal fat necrosis or small hemorrhages. There was evidence that acute attacks of severe epigastric pains were associated with these accidents. Metaplasia of the duct epithelium associated with acinar dilatation was found in the routine seactions of the pancreas in thirteen out of twenty-four cases of hemmorrhagic pancreatitis. This is probably the most common etiological factor in the pathogenesis of hemorrhagic pancreatitis, though any of the numerous causes of duct obstruction may, of course, lead to the same result.

Rich and Duff (183, 185) showed that the hemorrhage in acute hemorrhagic pancreatitis resulted from a peculiar type of necrosis of the walls of the blood vessels in the substance of the gland, caused by the necrotizing action of escaped pancreatic secretion containing proteolytic ferment without contact with enterokinase (the normal activator of trypsinogen found in the normal succus entericus). They confirmed their findings (184) that the changes in the arterioles produced in human and experimental hemorrhagic pancreatitis resembled microscopically the hyaline arteriolesclerosis often associated with hypertension and nephritis in humans. This collaborates the experiments with trypsin

reported above.

While it is usually stated that the proteclytic enzyme of the pancreatic juice is secreted, under ordinary conditions in the form of an inactive proenzyme, trypsinogen, which is activated upon entering the duodenum by enterokinase contained in the succus entericus, and that the normal pancreatic secretion prior to activation possesses only a slight or no proteolytic activity in vitro; it was shown that introducing pancreatic duct juice into the tissues that had never been in contact with enterokinase, produced lesions identical with those produced by active trypsin. The authors (183) offered two possible explanations of this fact. One, that the pancreas, under certain conditions, secreted active trypsin; or two, that the trypsinogen of the pancreatic juice becomes activated and acquired proteolytic potency when it escaped into the tissues. Regarding the first possibility, Rich and Duff quote Bayliss as stating that the pancreatic secretion induced by vagus stimulation contains active trypsin. If this be true, if the juice that escaped into the tissues happened to be juice that was secreted under the influence of vagus activity, it would possess tryptic activity independent of extrapancreatic activation.

But apart from the possibility of a secretion of active trypsin, it is important to note that trypsinogen can be converted into active trypsin by means other than contact with enterokinase, and there are several circumstances which could explain the activation of trypsinogen when it escaped into the tissue spaces. In the first place, neutralization of the alkaline pancreatic juice accelerates spontaneous activation in vitro according to Bayliss (183), and its neutralization by the buffers of the tissue fluids may be expected to have the same effect in vivo according to Rich and Duff. Furthermore, the same authors quoted S. C. Prescott as having determined that the trypsinogen of pancreatic juice was converted into active trypsin on contact with calcium ions. In addition, in the experiments in which the pancreatic duct juice displayed the properties of active trypsin when introduced into the living tissues, necrosis of the tissues soon occurred as a result of the action of lipase of the juice which needed no activator: It was probable that contact with the necrotic tissue and the subsequent reduction of the oxidation potential, although not necessary for the initial activation of the juice, at least prompted further activation of the trypsinogen, for there is evidence that necrotic tissue may exert an activating effect on tryp-

sinogen. This is indicated by the familiar self-digestion of the pancreas after death.

In the last few years clinicians have been using determinations of the level of amylase in the blood serum as an aid in the differential diagnosis of acute pancreatitis. They have also used the blood lipase level to a lesser extent. The first paper to suggest that there were variations of the blood amylase during acute transient disease of the pancreas was Elman's (46) in 1937. A series of papers by different authors then followed which, in general, showed the importance of this determination in the differential diagnosis of acute pancreatitis.

The normal blood amylase range as reported by Lewison (113) is given as 40-175 units, while McCall and Rernhold (121) reported a range of 30-100 units, and McCorkle and Goldman (128) reported 80-175 as the range of normalcy.

Popper and Plotke (167) found that while the increased concentration of lipase may be of some diagnostic value in pancreatitis, the elevation was not as constant and unequivocal as that of the amylase elevation, while McCall and Rernhold (121) found both valuable, but the amylase more useful clinically because of the rapidity with which ~~can~~ the test be performed. Popper and

Plotke (167) found that a high amylase level over several days indicated an active pathological process in the pancreas. The secretory function of the damaged pancreas usually stopped in three to five days after the beginning of the disease and this was followed by a decrease of the blood amylase level. If the blood amylase level declined before that time, from their results, one can conclude that the pathologic process of the pancreas is subsiding, but others (128, 185) disagree. Rhodes (182) obtained high readings of blood amylase from a large group of patients only in those in whom the clinical picture warranted a diagnosis of acute pancreatitis (30 cases). The rise was present early in the disease process and it fluctuated remarkably within a few hours. After twenty-four hours there were temporary subnormal readings in some cases. Therefore, it is essential that repeated tests of the blood amylase level be run before one can exclude the possibility of acute hemmorhagic pancreatitis.

Lewison (113) found that age, sex, diet, vitamin deficiency, and starvation had no effect upon the serum amylase values, which in his series averaged 76 units for females and 81 units for males. Ninty-four per cent of 720 patients having clinical conditions other than

mumps or disease of the biliary system were found to have normal serum values. Patients with mumps and pancreatitis were observed to have elevated amylase levels while patients with liver disease, regardless of the kind, were often found to have depressed amylase levels. The author found that the rise in pancreatitis reached its peak in the first 48 hours and usually returned to normal in several days.

McCorkle and Goldman (128) found that the serum amylase of 43 cases of acute pancreatitis was elevated. The lowest maximum reading in any single case of this disease was 225 units, the highest was 2, 459 units, and the average maximum reading was 696 units. In two hundred other cases they used the serum amylase test to rule out the possibility of acute pancreatitis. They found that when the amylase test was done within the first 2 or 3 days of the acute illness, a low or normal reading was very valuable in eliminating the likelihood of acute pancreatitis. The authors diagnosed 14 out of 42 cases of acute cholecystitis as having acute pancreatitis as well because of elevated amylase findings, and they similarly diagnosed acute pancreatitis in 6 of 26 cases of chronic cholecystitis for the same reason. McCorkle and Goldman (128) found several variations in

the amylase level curve. In the first few hours of acute pancreatic disease there was almost always a sharp rise in the serum amylase and the level usually remained elevated for from 24 to 72 hours, and then declined sharply into the normal zone. The rise of the serum during an episode did not necessarily reflect the severity or indicate the type of pancreatic disease. The declination in the curve did not always correspond exactly to the subsidence of the acute process. In some cases there was a sharp rise and a fall to subnormal levels which again meant that early and repeated determinations were necessary. For the diagnosis of acute pancreatitis, fluctuations within the normal zone, and slightly above and below the usual normal range, occurred rather frequently during the days following an episode of acute pancreatitis. Sustained high amylase levels were associated with a continuation, or with acute exacerbations of pancreatitis occurring at very frequent intervals. Secondary elevations of the serum amylase after it had leveled out was interpreted by the authors as meaning an acute exacerbation of the pancreatitis even though in some cases there was no subjective or objective signs of this occurrence.

In conclusion, it may be stated that a considerable

elevation (over 180 units) almost always occurs at some time during an episode of acute pancreatic necrosis, acute interstitial pancreatitis, or traumatic pancreatitis, especially in the first 24-48 hours. Significant elevations in the amount of amylase found in the serum rarely occur except in certain phases of acute diseases of the pancreas, although in certain instances acute parotitis will cause a rise in serum amylase and occasionally diseases of the kidney are accompanied by a slight elevation resulting from impaired excretion. Renal disease with retention of amylase should, however, be readily recognized if determinations of both serum and urinary amylase are made in all cases in which the serum amylase is only slightly increased. An elevated serum amylase is, at the present time, the best differential diagnostic criteria of acute pancreatitis if several determinations are run early during the acute conditions.

Catarract: A. Bakker (13) has shown that while the normal lens of the eye has a high constant concentration of carbonic anhydrase, in lenses with cataract, there is a marked decrease of the enzyme amounting to complete disappearance in some cases. The significance of this finding is unknown.

Multiple Sclerosis: Although many theories on the pathogenesis of multiple sclerosis have been postulated, it can be stated that its etiology is still unknown. Enzymes of lipolytic type have been incriminated by several workers. Brickner (25), in 1930, in experiments in which he sought to find a lipolytic enzyme, immersed segments of the spinal cords of rats in blood plasma obtained from patients with multiple sclerosis. Sections of the cords twenty-four hours after such immersion showed that multiple sclerotic blood had a demyelinizing action which was not demonstrable in blood from controls (humans free of multiple sclerosis). The results were suggestive, but the difference between the two groups was not very marked.

In 1931, Brickner (23) took the serum and plasma of normal and multiple scerotic patients and incubated it with egg lecithin under various conditions. Then, he titrated for the fatty acid content to determine the amount of hydrolysis of lecithin which occurred during the incubation period. His results showed a slight difference between the serum and plasma from patients with multiple sclerosis and the normal controls. However, Richards and Wolff (186) criticized this result because the titration values were too small to be of

significance and the maximum change in acidity of any preparation over a 72 hour incubation period was about 0.03 cc--an amount near the limit of error of the method used. Also, when the CO_2 content was partially controlled by sealing the tubes before incubation, no change in acidity resulted; it seems that the slight variations plotted represented nothing more than the changes in the CO_2 content of the preparation plus the experimental error.

In 1932, Brickner (24) used lecithin as a substrate in one series and certain esters were used in a second series which were incubated with serum from patients with multiple sclerosis. He then measured the degree to which the lipoids were broken down by the serum by again estimating the amount of fatty acids produced in the mixture after a 24 hour period of incubation. His results showed some difference between the breakdown caused by the action of multiple sclerotic serum and control serum, but the difficulty was that the difference was so small that it was necessary to compare group totals to see the difference.

Weil and Cleveland (219) found that a larger number of serums from cases of multiple sclerosis than of normal serums acted destructively on the spinal cord

of rats. However, such action was demonstrated in serums from patients with other diseases, and the differences did not seem to be large enough for the authors to draw a conclusion as to the importance of the increased lipase activity in the pathogenesis of multiple sclerosis. They also showed that pancreatic lipase did not produce demyelinization.

Weil, Luhan and Basler (221) and Weil and Heilbrunn (220) demonstrated in the urine of about 70 per cent of the cases of multiple sclerosis the presence of a substance which was myelotropic but was not, however, identical with the lipolytic enzyme of Brickner. They found this substance in the urine in postencephalitic states, parinsonism, pulmonary tuberculosis, and in certain liver diseases as well and the amount of lipolytic substance varied with remissions and exacerbations of the underlying disease process.

Crandall and Cherry (36) found a blood lipase capable of splitting olive oil in 70 per cent of the cases of multiple sclerosis, 80 per cent of cases of liver diseases, and 7.6 per cent of other diseases, and they interpreted this to mean that in multiple sclerosis there was a functional disturbance of the liver.

In 1936, Brickner (32) attempted to establish a

relationship between blood esterase and the state of activity of multiple sclerosis. Using methyl butyrate as the substrate, with or without the additionof quinine (which increased the amount of hydrolysis), he concluded that in cases of active multiple sclerosis, there was a high spontaneous esterase activity while cases in the inactive phase manifested low activity. However, the differentiation of high and low esterase activity was based on the effect of quinine, which Brickner said depended on such factors as obesity and certain menstrual influences. If this is true, any differences noted by this method must have been influenced by these factors; yet the extent to which they played a role was not indicated in the results presented.

G. Rivela (187) repeated Brickner's experiments using serum, plasma, and cerebrospinal fluid frompatients with multiple sclerosis incubated with the spinal cards of both white rats and rabbits. He found no difference in lipolytic action whether the subject was normal or had multiple sclerosis. Richards and Wolff (186) confirmed Rivela (187) findings using the Warburg apparatus and the manometric method which is more accurate than the titration methods used by the previous experimenters to determine the esterase and lipase

activity.

Therefore, the more recent evidence points against a lipolytic disturbance in the etiology of multiple sclerosis, but in considering the theory that abnormal enzymatic activity plays an etiological role in multiple sclerosis, it should be kept in mind that one of the most characteristic features of an enzyme is its specificity. In view of the fact that myelin is the substance attacked in this pathologic process, it follows that myelin should be the substrate in any experiment designed to test this theory. Practically this is difficult, for there is no adequate method of measuring the hydrolysis of such a complicated substance. Also it is at least theoretically possible that disorganization of the physical structure alone, without any chemical change, would be enough to initiate the disintegration of the myelin. For the foregoing reasons, the experiments reported here are not adequate to really disprove the theory that an abnormal enzyme may be involved in the pathogenesis of multiple sclerosis, but it must be stated that the experiments using simple substrates and serum from patients with multiple sclerosis and normal patients as controls do not show any significant difference.

Hypertension: There has developed in the past decade certain ideas about essential hypertension, ischemic kidneys, and some enzyme systems of the normal and anoxic kidneys. A brief historical introduction will be presented which will be followed by a discussion of the experiments involving the kidney enzymes.

Tigerstedt and Bergmann (208) performed a bilateral nephrectomy on anesthetized animals. They then injected extracts of kidneys which produced a rise in the arterial blood pressure. They thought that the increased sensitivity was due to the animals inability to excrete the extract containing renin, the term introduced by these workers to describe the hypertensive substance.

H. Goldblatt et al (58) were the first experimenters to show conclusively that compression of the renal arteries caused a rise in blood pressure. Constriction of the renal artery was effected by means of a specially devised adjustable silver clamp. Ischemia produced in this way of one kidney, the other remaining intact, caused a moderate elevation of the blood pressure which commenced three or four days after the operation, but persisted for only a short time returning to the normal level after a month or so. If, on the other hand, both renal arteries were constricted, or if only one was con-

stricted and the opposite kidney was removed, pronounced hypertension resulted and persisted indefinitely, up to periods of five years or more. The severity of the hypertension varied with the degree to which the renal blood flow was curtailed. Unless the compression of the renal artery was extreme and, in consequence, the ischemia of the kidney very severe, the elevation of the pressure was not accompanied by any detectable impairment of renal function and the kidney showed little histological change. But with severe constriction of the artery, renal insufficiency developed and the animals died in uremia accompanied by wide-spread degenerative changes in the systemic arterioles; the arteriolar walls in many instances showing hyaline degeneration and necrosis. Such a state of the vascular system, taken together with renal insufficiency and elevated blood pressure, is closely comparable, it would seem, to malignant hypertension as seen clinically. However, Goldblatt was unable to produce arteriolar degeneration in the kidney itself, because a severe degree of hypertension could not be produced as long as one non-ischemic kidney were protected from the destructive effect of the hypertension by the compressing clamp, i. e. they were in a region of low pressure.

Page (152) found that hypertension developed within

four to six weeks after a kidney had been wrapped loosely in cellophane or silk. These materials set up a chronic inflammatory reaction (perinephritis) which led to the formation of a firm fibrocartilagenous capsule enclosing the kidney. Hypertension has also been produced by obstruction of the ureters, or by renal damage caused by exposure of the kidneys to X-rays, or the injection of a nephrotoxic agent. Clinically, it has been shown that the removal of a cystic kidney or a chronically infected kidney may cause a remarkable drop in the blood pressure if the other kidney is normal.

The hypertensive effect is not dependent upon the nervous mechanisms (e.g.; a reflex through afferent endings in the kidney, the vasomotor center, and vasoconstrictor fibers to the systemic vessels) for it occurs after denervation of the kidney, or section of the splanchnic nerves, or of the anterior spinal nerve roots. Page (153) showed that the production of experimental high blood pressure by constriction of the renal artery or x-ray irradiation was not affected by preliminary stripping of the renal pedicle of its extrinsic nerve supply. Goldblatt et al (57) excised the splanchnics and lower four dorsal sympathetic ganglia

and found that it had no effect on experimental renal hypertension. Finally, Child (31) showed that hypertension resulted if one kidney, which had been transplanted into the neck by anastomosing the renal artery to the carotid and the renal vein to the jugular, was made ischemic and the other kidney was excised. The transplanted kidney was, of course, completely isolated from nervous control. The inability of such denervation operations to prevent or modify the hypertensive effects proved conclusively that it was mediated through a pressor substance circulating in the blood stream.

However, it has been shown that total sympathectomy produces a temporary fall in the blood pressure of normal dogs (74) and a temporary moderate fall in the blood pressure of dogs with experimental hypertension (3, 51). This is due to the transient dominance of vasolilator fibers and it is the basis of the operations for essential hypertension which consists of total sympathectomy. Allen (2) reported that it lowered the blood pressure somewhat in favorable cases--those in which there was a minimum of permanent pathalogical changes in the arterioles and organs. This treatment is based on the neurogenic theory of origin of essential hypertension and it is apparent that it is not the

equivelent of experimentally induced hypertension.

Leiter (111) showed that dogs with impaired renal circulations were hypersensitive to renin when compared with normal dogs. Harrison (84) showed that in experimental hypertension the concentration of renin was increased in the kidney itself and he suggested the idea of a decreased rate of formation of a depressor substance in the ischemic kidney.

While the apparent cause of the increased production of pressor substance seemed to be ischemia, it was observed by Corcoran and Page (33) that the intra-renal reduction of pulse pressure rather than ischemia might be the cause of experimental renal hypertension. It should be noted that Page (150) stated that so far the pressor substance or sensitizing substance have not been found, in more than normal amounts, in the blood, urine, or spinal fluid of patients with nephritis and hypertension. However, this does not mean that they are not present.

Fasciolo (50) in 1938, demonstrated a vasoconstricting substance in the venous blood from an ischemic kidney. The substance was active in the absence of the adrenals, and the normal healthy kidney was capable of diminishing the action of this blood pressure raising substance.

Kohlstaedt and Pate (104) repeated the experiment perfusing an isolated kidney and confirmed the findings, and added that reduction of the mean pressure was not necessary for the production of the vasoconstrictor substance, but rather that it was necessary to reduce the pulse pressure and the rate of blood flow. In a latter experiment (103) they concluded that it was the reduction of the pulse pressure that was the stimulus for eliciting an outpouring of renin. The reduction of blood flow followed but appeared to be an effect rather than the cause of the increased liberation of renin. Page (149) showed that the cellophane perinephritis method of hypertension produced similar results.

In 1938, the first of a series of excellent papers by Kohlstaedt, Helmer and Page appeared (102). The authors showed that dialyzed renin was inactive as a vasoconstrictor agent. Undialyzed renin was moderately active in vasoconstriction. Normal heparinized dog's whole blood was inactive. Dialyzed renin plus heparinized dog's blood produced marked vasoconstriction. Undialyzed renin was potentiated by dog's blood. Heparinized plasma was inactive. Plasma plus indialyzed renin was active (as with whole blood). The protein-free ultrafiltrate

of plasma could not activate renin while the colloidal residue on resuspension activated renin. The vasoconstrictor action of undialyzed renin appeared to be due in part to non-protein materials contained in it, for the protein free fluid obtained by boling undialyzed renin produced vasoconstriction but was not potentiated by blood as was renin. Dialysis removed the non-protein substances. Boiled dialyzed renin with added plasma or protein free filtrate of boiled plasma was vaso-inactive. These results suggested that renin was an enzyme-like substance which was activated by a kinase-like material contained in the protein fraction of plasma and whole blood. This substance was called "renin-activator".

Williams (232) obtained two pressor substances from the isolated kidneys of hogs and dogs. One was heat labile, alcohol insoluble, non-dialyzable, and produced a prolonged but slight rise in blood pressure. This was stated to be "pressin" (renin). If, however, the kidney was kept out of the body for one to two hours, a second pressor agent was obtained which was resistant to boiling, was dialyzed readily, and was alcohol soluble. Its pressor effect was potentiated by cocaine and decreased by ergoamine and its blood pressure curve

followed that of adrenalin. Williams called it "perfusin", and in a following paper (231) suggested that the pressor action of kidney extract might be due to adrenalin as he had confirmed the fact that its action was potentiated by cocaine and inhibited by ergotamine as ~~has~~ is the action of adrenalin. He, however, stated that differences in his results and those of others might be due to different techniques used.

Others (52, 87, 88) confirmed Page's findings (15) that renin was not potentiated by cocaine in dogs, and ergotamine did not prevent its action. It was further shown by Landis (110) that renin was the only pressor agent which produced a rise in blood pressure without lowering the skin temperature. From the above experiments, it became obvious that the action of renin was not due to the slow liberation of adrenaline.

Page (151) in 1930, demonstrated that tachyphylaxis developed with repeated injections of renin into anesthetized, hepatectomized, suprarenalectomized, nephrectomized, or eviscerated dogs. The continuous infusion of renin into these animals produced a prolonged rise of arterial blood pressure, but it ultimately decreased despite the continuation of the infusion of renin. He also showed that tachyphylaxis developed in the response

of isolated rabbit's ear perfused with blood and a small dose of renin. The same blood perfused through a second ear produced no vasoconstriction even when renin was added to it. If, however, renin-activator was added, prompt vasoconstriction resulted. If renin, Ringer's solution, and renin-actiator were perfused, no tachphylaxis resulted. Blood from animals made tachphylactic by repeated injections of renin was lacking in activator and it also failed to cause vasoconstriction in rabbit's ear when renin and renin-activator were added, (some anti substance must have been prefused).

Page, therefore, concluded that the blood pressure may fall after a period of renin infusion despite the presence of excess renin in the blood. Injection of partially purified activator restored the activator content of the blood as demonstrated in the isolated ear experiment, but produced no effect in the animals made tachyphylactic by continuous infusion. Renin tachphylasix was due to the loss of renin activator and the development of anti-substance (anti-pressor state or the loss of some substance necessary for the chemical reaction which culminated in the pressor action of renin) in the blood. It was due in the perfused ear experiment chiefly to the lack of the renin activator

which seemed to be an essential constituent of the chemical system responsible for the renin effect. In the animal experiment it was due to the development of some anti-substance.

In the same year Braun-Menendez (19) independently in Argentina noted that neither renin itself nor the blood globulins alone were responsible for the vasoconstriction but rather their combination was responsible for the vasoconstrictor effect. The combination was called "hypertensin" and this substance was found to be inactivated by an enzyme found in the kidney and other tissues which was called "hypertensinase".

The same laboratory (20) showed that hypertensin formed by the combination of renin and the blood proteins pseudo-globulin fraction had the same chemical and physiological properties as did the pressor substance obtained from the venous blood of ischemic kidneys. Renin itself produced a rise in blood pressure but its curve of action was more gradual and prolonged. When a fixed amount of renin was incubated with various amounts of globulin, the amount of hypertensin produced was roughly proportional to the amount of globulin. The authors believed that hypertensin was probably a polypeptide and its formation was due to a specific

enzyme-substrate reaction. They found that the normal kidney produced renin but produced little or no hypertensin till the kidney was ischemic, and suggested that reducing agents were quite likely to activate renin during the anoxia. The hypertensin was destroyed by some substance in the preparation which was either a destructive enzyme--hypertensinase--or an impurity in the preparation. In a later paper the same investigators (142) found that for a given amount of activation the maximum yield of hypertensin cannot be surpassed if ten or twenty times the minimum amount of renin was used. If the reaction was stoichiometric the maximum amount of hypertensin ought to be proportional to the amount of renin.

In 1940, Page (105, 155), in this country, showed independently of Braun-Menendez that renin plus renin-activator produced a pressor substance which he called "angiotonin", (Greek: blood vessel plus strain). The substance was heat stable and crystalline. Angiotonin (hypertensin) produced a sharp increase in blood pressure similar to that of adrenaline but more prolonged in a single intravenous dose, and it differed from renin in that the rise appeared much more quickly, was steeper, and was less prolonged. Cocaine did not

potentiate its reaction. This was all confirmatory of the Argentina laboratory's experiments. The maximum yield of angiotonin resulted when three parts of renin were allowed to react with one hundred parts of activator. The experiments showed that angiotonin was an intermediate rather than the end product as further incubation of renin and angiotonin resulted in the destruction of the angiotonin. The maximum yield of angiotonin occurred at 40° C in 30 minutes. Angiotonin produced a sharp immediate blood pressure rise while renin produced a slow rise with a latent period. Renin was an enzyme according to the authors, because it was: 1. a protein; 2. heat-labile; 3. when it reacted with activator the action was slow and affected by temperature (all chemical reactions are); 4. it appeared to be required in very small amounts relative to the amount of activator. Angiotonin acted primarily on peripheral blood vessels and early indications seemed to show that it did not manifest as much tachyphylaxis as renin.

Page (154) then showed that angiotonin required an activator for its action and that there was an angiotonin-inhibitor as well The blood from intact animals made tachyphyalactic to renin or angiotonin did not produce vasoconstriction when perfused along with renin

plus activator or angiotonin plus activator in an isolated organ. When tachyphylaxis was developed by the repeated injection of renin or angiotonin into blood re-circulated in an isolated organ, the addition of renin plus activator or angiotonin plus activator produced vasoconstriction. This suggested that an inhibitor was added to the blood by the intact animal which did not occur in the isolated organ. Nephrectomy greatly increased the sensitivity of animals both to angiotonin and renin. The induction of experimental hypertension by the periniphritis method also increased the sensitivity but not as markedly. Addition of angiotonin or renin to the blood from such animals caused a tremendous amoung of vasoconstriction in perfused organs. Transfusion of normal and tachyphylactic blood into unephrectomized or hypertensive animals reduced the response to injections or infusion of renin.

It was shown that angiotonin does not exert its vasoconstrictor effect in the absence of a substance found in red blood cells and serum (angiotonin activator). This fraction was separated and shown to contain little or no renin-activator. Angiotonin tachyphlaxis occurred in the experimental animal but it developed much slower than renin tachyphylaxis. When

the angiotonin response was abolished, renin also failed to act. Large doses of renin reduced and finally abolished angiotonin response but exhaustion of renin-activator in the blood abolished the renin response without abolishing the angiotonin response. Blood from animals made tachyphylactic by the infusion of angiotonin contained greatly reduced amounts of angiotonin-activator, but there appeared to be an inhibitior in the blood as well. Bilateral nephrectomy was shown to prolong and greatly enhance the rise of arterial blood pressure following angiotonin and renin administration. The maximum response occurred in 24 to 39 hours post-operatively. Blood from these animals exhibited greatly increased ability to activate angiotonin and renin in isolated perfused organs. Large amounts of angiotonin were needed to reduce the amount of activator in their blood. Renin activator was simultaneously but little affected.

Transfusion of blood from animals made tachyphlactic to angiotonin into a nephrectomized dog decreased the response of the latter to angiotonin. Angiotonin when added to the blood of the recipient of the transfusion and perfused through a rabbit's ear also showed a decreased vasoconstriction action. Transfusion of normal

blood in large amounts into nephrectomized or hypertensive dogs reduced the recipient's response to renin. If renin tachyphylaxis was established in the donor, the transfusion abolished the response to renin in the recipient. The blood from such animals exhibited greatly decreased vasoconstrictor effect when perfused through isolated organs with renin or angiotonin. Therefore, nephrectomized dogs exhibited the greatest pressor response to infusion of angiotonin and renin; normal animals the least; and hypertensive animals in between. Renin tachyphylactic or normal dog's blood did not reduce areterial blood pressure elevated by a single injection of renin into nephrectomized dogs.

In conclusion, these experiments demonstrate that angiotonin needs an activator which is different from renin-activator to exert its vasoconstriction. The activator is in the blood and was isolated. Angiotonin tachyphylaxis occurs with repeated injections but slower than with renin. The mechanisms for the production of tachyphylaxis to angiotonin plus renin are interrelated. Tachyphylaxis appears to be due to the exhaustion of their respective activators as well as to the development of inhibitors which seem to originate in part in the kidneys and it is their loss

which accounts in a large measure for the increased sensitivity of animals to these substances after nephrectomy. Hypertensive animals may not only have increased amounts of activator in their blood but reduced amounts of inhibitor as well.

A diagramatic summary of the renin-angiotonin vasopressor system as presented by Page (154) is:

Renin + renin activator ⟶ angiotonin

Renin inhibitor + renin + renin activator ⟶ inactive substance

Renin + angiotonin ⟶ inactive substance

Antiotonin + angiotonin activator ⟶ vasopressor substance

Angiotonin + angiotonin activator + angotonin-inhibitor ⟶ inactive substance.

It would seem, therefore, that in experimental hypertension, a decrease in pulse pressure produces a relative tubular ischemia which leads to an increased permeability of the tubular cells. Less renin inhibitors and angiotonin inhibitor and more renin are produced by the kidneys and there is more angiotonin. This would seem to be the mechanism of the experimentally induced hypertension. Its purpose seems to be to maintain the proper flow of blood to the kidneys--

a compensatory mechanism. Some investigators are inclined to believe that the neurogenicly induced generalized vasoconstriction may produce a similar chemical mechanism in essential hypertension, but Smith (199), in a comprehensive paper on the role of the kidney in hypertension, concludes that the kidney is secondarily involved and is not the cause of the disease, which is still unknown. Wakerlin (213) could find no correlation between the renin content of the kidneys of normal dogs and their blood pressure levels and there is no experimental evidence showing that renin has a role in the control and maintenance of normal blood pressure.

Now that the renin-angiotonin system was worked out, experimenters began to study in more detail the nature of these substances. It was noted by Helmer, Kohlstaedt, and Page (86) that the anti-angiotonin activity of the kidney depended upon a different chemical system than that of muscle, liver and intestinal mucosa. The kidney anti-pressor extract destroyed angiotonin in vitro and probably in vivo (86).

Up to 1943, it was only speculated that angiotonin formation from renin and renin-activator was enzymatic but in that year Plenti and Page (163) showed that the

amount of the substrate decomposed was proportional to the concentration of the substrate, and that the rate of the reaction was proportional to the concentration of the renin. The logarithm of the concentration of renin activity was plotted as a function of the angiotonin pressor response against time and a straight line was obtained as required by the theory of enzymatic reaction (first order reaction). Renin was, therefore, proven to be an enzyme and the renin-activator was the substrate. The renin-activator was shown by these same workers (166) to be identical with or move with the same electrophoretic mobility as alpha$_2$ globulin. In another paper (159) they suggested that the term <u>renin-substrate</u> be substituted for <u>renin-activator</u> in the light of these enzymatic studies.

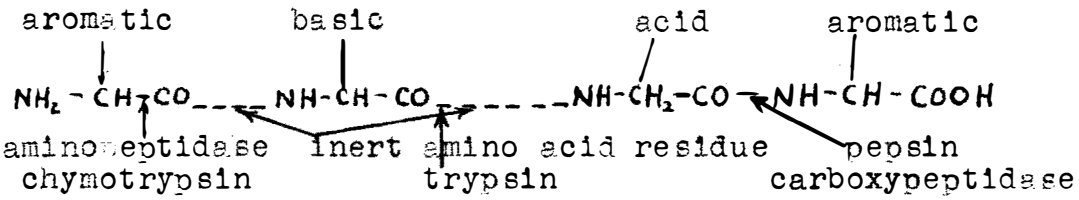
Page and Plenti (165) then proved that angiotonin was the intermediate product rather than the final product of renin and renin-activator, reaction. An enzyme destroying angiotonin was isolated ~~and~~ in the albumen fraction of the plasma and was called angiotonase (Braun's hypertensinase). The formation and destruction of angiotonin was shown to consist of two consecutive reactions both of which followed laws of first order kinetics. The reaction constant was found to be

proportional to the enzyme concentration and they suggested that it, therefore, should be used to express the activity of the enzymes: renin and angiotonase.

The Croxattos (40) showed that pepsin can be substituted for renin in the preparation of an angiotonin-like substance called "pepsitensin". This was produced during peptic digestion of proteins. Therefore, they suggested that renin might be chemically a carbonyl-proteinase. Plenti and Page (164) concluded that pepsitensin and angiotonin were not homospecific or identical and probably differed in the number rather than the nature of the amino acid residues of which they were composed. From their experiments renin could not be classified in any of the groups of Berg-mann. Schales (190) confirmed this as did Braun-Menen-dez (21) who showed that while pepsitensin and angiotoninwere related substances, they were not identical because angiotonin was inactivated by hypertensinase (angiotonase) from red blood cells while pepsitensin was resistant to this enzyme.

Plenti and Page (162), in 1944, suggested on the basis of their experiments that angiotonin had a structure containing the following: (1) three peptide linkages; (2) a free terminal amino group; (3) a free

terminal carboxyl group; (4) one basic amino acid resi-due which may be t rmi al but its carboxyl must be in-volved in a peptide linkage; (5) one central dibasic amino residue in combination with an aromatic amino acid residue; (6) an aromatic amino acid residue which may be part of five or must be terminal with its carboxyl group in a peptide linkage. The workers believe that the simplest possible formula would be tyroyl-arginyl-glutamyl-phenylalanine.



The following experiments are concerned with the oxidati ve reduction aspects of the enzymes found in the kidneys. In 1935, Krebs (108) noted that kidney tissue incubated with amino acids in the absence of oxygen but in the presence of methyline blue resulted in a deamination of the amino acids, ammonia formation, and the reduction of the methyline blue. While Mason (133) showed that there was not necessarily a decrease in the capacity of hypertensive animals to use oxygen, Levy (112) demonstrated that the renal oxygen consump-tion decressed in experimental hypertension.

In 1937, Holtz (91) demonstrated that the vasocon-

strictor principle in ischemic kidneys was identical with hydroxytyramine. In the following year, Holtz (92) found that phosphate extracts of kidney acted on dopa (dioxyphenylalanine) anaerobically to form a pressor substance identified as hydroxytyramine. However, kidney extracts which acted on dopa and hydroxytyramine in the presence of oxygen and ascorbic acid formed a substance (possibly dihydroxyphenylacetaldehyde) which lowered the blood pressure of cats. It was well known that phenolic compounds containing aliphactic amines as side chains were strong pressor substances, the most potent being aminoethane catechol which was closely allied to adrenalin.

Holtz (92) in his experiments in vitro demonstrated that while renal tissue was able to decarboxylate tyrosine and dopa in the absence of oxygen, deamination occurred only when oxygen was present. This suggested the presence of an amino oxidase in the kidney. The product of the decarboxylation of dihydroxyphenylalanine was hydroxy-tyramine which was a pressor substance. The deamination of the same substance produced di-hydroxyphenylacetic acid which was inert as a vaso pressor agent. Therefore, it appeared possible that the pressor substance responsible for hypertension was a simple pressor amine, released by the ischemic kidneys because

of an alteration in the action of some system of enzymes requiring oxygen.

In 1941, Bing (19) confirmed Holtz's findings and elaborated on them. He investigated the production of hydroxytyramine from dopa in the isolated cat's kidney perfused with a reduced blood flow and found that a similar reaction occurred. The amount of pressor substance formed varied with the rate of blood flow through the organ. In further experiments, Bing (18) showed that the decarboxylation enzymes contained in the kidneys were specific for certain amino acids and varied with the species.

In view of the marked pressor effects of some amines and the hypertension resulting from the renal ischemia produced experimentally, a study in the ischemic kidney of respiration and of the oxidative enzymes acting on amino acids was made by S. B.Raska (178). The oxidase activity of the kidney enzymes of dogs and rabbits was determined by measuring the increased oxygen consumption and ammonia formation on adding the substrates: tyramine, isoamylamine, l-epinephrine, histamine, dl-alanine, l-aspartic acid, and l-dopa. The results showed a definite decrease of tissue respiration and a marked disturbance of enzyme equilibrium in the ischemic kidney.

The author suggested that the equilibrium between the decarboxylating systems and the oxidative systems in the ischemic kidney was altered in such a way that the former was a pathological excess; i. e. the anaerobic degradation process exceeded the aerobic oxidation process. Therefore, pressor substances were formed in larger amounts than normal or new ones were made. Renin and angitonin-like substances, which might conceivably be formed at any stage of the degradation of proteins or poypeptides, accumulated owing to the reduced oxidizing power of the ischemic kidney. The author further stated that decarboxylation of the amino acids led to amines (some of them pressor amines) which the ischemic kidney could not metabolize efficiently, since there was a decrease in the oxidative systems of the ischemic kidneys. In ischemic kidneys without necrosis or atrophy, the respiration of the kidney tissue slices, their oxidative power for amines and amino acids, and also the oxidizing power of the kidney extracts were markedly reduced when compared with the normal kidney. Raska stated that it was possible that in the first stage of renal ischemia the oxidative enzymes were still present in sufficient quantity, but their activity was reduced by the lack of oxygen, and the possible lack of other

substances supplied by the blood and necessary for the utilization of the oxygen.

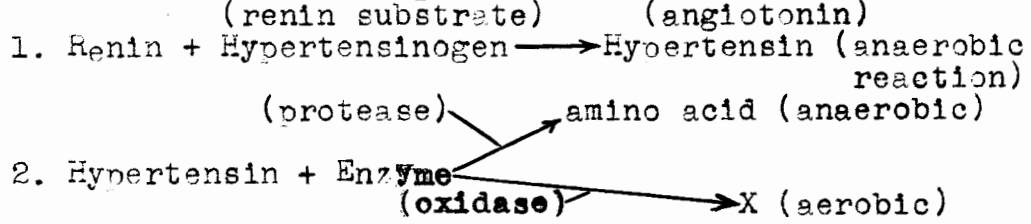
In the later stage of ischemia, through pathological changes of the cells there is probably a decrease in the enzyme content of the kidney, due either to lack of the prosthetic group or to a decreased production of enzyme protein. Levy (112) showed that a decreased blood flow through the kidney was accompanied by a decreased oxygen consumption of the whole organ. Raska concluded that since a low Q_{O_2} was found for ischemic kidney tissue in his experiments, it was logical to speculate that the functioning of the cytochrome-cytochrome oxidase system was reduced. The fact that Raska found a decreased production of ammonia would indicate that there was an interference with the ability of the kidney to regulate acid-base balance under conditions of ischemia.

Raska in further studies of the metabolism of the kidney (179) found that in experimentally produced hypertension the kidneys showed a marked decrease in the concentration of cytochrome c and also showed decreased activity of the cytochrome oxidase and succinic dehydrogenase systems when compared with normal kidneys thus confirming his last paper (178). The rate of

oxidation of pyruvate and of l-glutamate as well as the rate of glucid synthesis from these substances was greatly decreased. The rate of formation of ammonia from the oxidative deamination of l + glutamate was decreased, and the amount of protein-bound phosphorous was lower. The opposite kidney ("control") showed an increase in the cytochrome c concentration, cytochrome oxidase, and succinic dehydrogenase activity per gram from 15 to 35 per cent over the average normal values. Unilateral nephrectomy produced an increase in the concentration of cytochrome c, flavin-adenine dinucleotide and protein-bound phosphorous. Renin inhibited the action of cytochrome oxidase, succinic dehydrogenase, l-amino acid oxidase and the amine oxidase systems, but the author was hesitant to say whether this was due to renin or some other substance present in the renin extract. Kidney extracts from hypertensive dogs inhibited the same enzyme systems. Raska stated that since renin is a proteolytic enzyme, it might, if it had access to the respiratory enzymes above, be responsible directly or indirectly for at least part of the decreased activity of the enzymes in both of his papers (178, 179).

E. Cruz-Coke et al (41) found that the destruction

of hypertensin (angiotonin) was markedly accelerated by oxidized cytochrome and it was sharply retarted by reducing agents such as ascorbic acid and cysteine. They presented the following scheme:



The first reaction was inhibited by vitamin A, certain oils, and quinones, and hypertensin was destroyed by renal and intestinal prteinases, amino polypeptidases found in renal extract, blood plasma, tryosinase, and carboxypeptidase.

The possibly lowered activity of cytochrome oxidase, succinic dehydrogenase, amino acid oxidase, and amine oxidase in hypertensive kidneys might be due to an enzyme deficiency or a deficiency of carriers responsible for respiration as well as to the presence of inhibitory substances in the kidney of the hypertensive animal.

Raska (180) assumed that the alterations caused in the intracellular metabolism by the reduced power of the oxidative mechanism led to an increased formation and secretion of renin by the kidney in hypertension, and these alterations were thought to be responsible

for the appearance of large amounts of renin and angiotonin and angiotonin-like substances in the renal venous blood of these animals. By the same alteration of the oxidative mechanism in the kidney in renal hypertension, other vasoconstrictor substances might of also be produced or released. Since the oxidative enzymes were shown to be important fr the inactivation of various vasoconstrictor substances including angiotonin, a decrease activity of the oxidative mechanism not only of the kidney but also of the liver and blood might result in accumulation and prolonged circulation of vasoconstrictor substances which leads to hypertension.

From this experiment by Raska (180) which dealt with the determination of the concentration and the activity of the respiratory enzymes in the excised normal kidney and in the remaining kidney of unilaterally nephrectomized dogs, one can conclude that an increase in the concentration and activity of the respiratory enzymes proceeds hypertrophy of the remaining kidney. This was explained by the assumption that the increase of the biocatalysts acted as a stimulus for cell growth and multiplication.

Raska (180) stated that renin was present in high

concentration in the normal kidney and he assmed that the normal kidney secretes renin in small amounts. In experimental renal hypertension the rate of renin secretion increases, and Raska believes that there must occur a simultaneously increase in the rate of formation of renin. Like other proteolytic enzymes such as pepsin and trypsin, renin may have a precursur. Under the altered conditions in experimental renal hypertension, the rate of formation of renin from the precursur may increase. Furthermore, changes in the physiochemical state of the cellular membrane may lead to greater permeability for renin, thereby increasing its rate of renal secretion.

A biochemical characteristic of experimental renal hypertension and probably of renal hypertension in the human being, according to the author, seems to be a functional derangement of the cells of the parenchyma followed by a readjustment of these cells involving changes in the metabolism of many substances. All these changes can occur without any visible histopathological manifestations detectable by ordinary means. It is known that certain enzyme systems such as cytochrome oxidase, succinic dehydrogenase, amine oxidase, and l and d amino acid oxidase are associated

with insoluble cellular components. Therefore, in the future it may be possible to demonstrate pathological changes in kidney structure with renal hypertension with special stains. Raska believes that since the clinical picture of essential hypertension is similar to experimentally induced renal hypertension, it is justifiable to expect similar biochemical findings in human beings. A word of caution is again necessary for as has been mentioned previously, the two conditions are not identical and there are many workers (Smith, Goldring, Chasis (199) and others) who believe that the mechanisms of their production are not the same.

In 1946, Raska (181) pointed out that a direct relationship appeared to exist between the oxidative power of the kidney and the formation of hypotensive substances. This relationship was indicated by the increased concentration and increased activity of the various respiratory enzymes in the remaining kidney of unilaterally nephrectomized dogs when compared to the activity of the same respiratory enzyme systems of the excised kidney of the same animal or of the normal kidneys of normal dogs. There was a similar increase in the activity of the oxidative enzymes in the uninjured kidney of dogs in which the function of one kidney was modified

by silk perimephritis or partial constiction of the renal artery. In the latter there was a decreased activity of the oxidative biocatalysts. Extracts prepared from the remaining kidney of dogs or rabbits one week to three months after unilateral nephrectomy were more effective in lowering the blood pressure of hypertensive rabbits and rats when it was given parenterally than extracts prepared by the same method from the excised kidney of the same animal or from the normal kidneys of normal dogs or rabbits. Similar results were obtained with extracts of the uninjured kidneys of dogs when the function of the opposite kidney was altered by wrapping it in silk or partial constriction of its renal artery. Further study (181) showed that the increase in the hypotensive activity of these extracts was due largely to an increase of a dialyzable blood pressure lowering fraction. Raska believes that hypotensive substances of renal origin are probably the products of the oxidative process. They were formed in increasing amounts in the compensating kidney.

We have now surveyed the enzymatic systems involved in the production of experimental hypertension and we are now ready to analyze the practical application of

of this knowledge to clinical medicine by a study, in the next section of this paper, of the use of various renal extracts and enzymes in the treatment of clinical essential hypertension.

E.Werle (228) produced uretral obstruction in guinea pigs and found that the stasis produced resulted in a reduction of the concentration of histidine carboxylase and histaminase in the kidney of the guinea pig.

M. Wachstein (212) studied changes in the lipase activity and the alkaline phosphate activity under the influence of kidney damage in rats, rabbits, dogs, mice, hamsters, and guinea pigs. He found that mercury or uranium poisoning did not inactivate either enzyme in the necrotic cells of the proximal convoluted tubules, but their activities in the atrophic and regenerating cells of the proximal convoluted tubules surviving the acute stage of intoxication was decreased. Choline deficiency produced a decreased activity also. Hydronephrotic kidneys produced a decrease in activity in 10 to 12 days. The author believed it conceivable that variations of the enzyme concentrations with fat metabolism in the kidney may be significant in the regulation of lipoid metabolism of the whole organism.

ENZYMES IN THERAPY

From the preceding experimental studies on hypertension, it is logical to expect investigators to use renal extracts in the therapy of hypertension. This work serves as an introduction to the section on enzymes in therapy.

It was shown by Page, Helmer, and Kohlsteadt (158) in 1940, that extracts of kidney and muscle effectively reduced arterial blood pressure in hypertensive human beings, dogs, and rats for prolonged periods of time. The extracts simultaneously reduced the ability of the blood plasma to activate renin and angiotonin. Grollman, Harrison, and Williams (76) confirmed this finding using renal extracts orally and showed that the anti-pressor substance was deficient in the kidneys of hypertensive animals. In a later paper the same year (85) they demonstrated that the extract produced no significant effect on blood pressure when administered to normal rats. However, the animals so treated showed a decreased pressor response to renin when compared with control animals. Normal pregnant rats were less sensitive to renin than non-pregnant controls, and hypertensive

rats displayed a decreased blood pressure the last few days of their pregnancy and the blood pressure gradually increased after delivery. This suggested that the fetus elaborated an anti-pressor substance which is contrary to the clinical findings in eclampsia, where as soon as the baby is removed from the mother the blood pressure drops remarkably.

If the increased blood pressure in experimentally produced hypertension is due to the production of pressor amines by the anoxic kidney as was suggested in the previous part of this paper, enzymes capable of altering pressor amines should be effective. Tyrosinase is such an enzyme. It is very abundant in fruits and vegetables and it has the property of oxidizing mono-and-ortho-di-hydroxyphenols to quinones, (194). When certain physiologically active phenolic amines are acted upon by this enzyme, derivatives are formed which are inactive on the vascular system. The conversion of adrenalin to adrenochrome is an example (194).

Schroeder (193) injected tyrosinase into hypertensive rats and noted that it consistently lowered the blood pressure in five to fifteen minutes after intravenous administration. The use of tyrosinase

in normal control animals gave variable results, which suggests a possible non-specific effect of the enzyme. In another experiment, Schroeder (192) gave seventeen patients with hypertension daily subcutaneous doses of tyrosinase for three to four weeks. In fourteen cases there was a marked fall in the blood pressure while there was no marked changes in the other three cases. Seven of the patients' electrocardiograms tended to regress towards more normal tracings, while all of the patients experienced some subjective relief. Kidney function tests were unchanged. When the injections were stopped, the blood pressure returned to its previous level in three to six days while the symptomatic improvements lasted weeks to months. Three of the patients had local allergic manifestations, while one patient had a severe reaction consisting of nausea, vomiting, increased peristolsis, bradycardia, and a decreased blood pressure. The deposition of grey or yellow pigment at the site of injection was common.

In another paper (194), the same investigator demonstrated that tyrosinase inactivated renin, angiotonin, adrenalin, and tyramine in <u>vitro</u> and altered the response of blood pressure to these substances

in rats and dogs. The author suggested that tyrosinase acted by either oxidizing all compounds with catechol or phenolic structure to quinones, which structural change was accompanied by a change in physiological action, and in that way tyrosinase might have destroyed some yet unknown phenolic pressor substance responsible for hypertension; or that, perhaps, the orthoquinones produced by the action of the tyrosinase on the phenolic substances had special properties; being powerful oxidizing agents, they might have destroyed some pressor substance of lower oxidation-reduction potential in a manner analagous to the destruction of ascorbic acid by orthoqunones.

Croxatto (39) used amino oxidase to enzymatically destroy angiotonin and submitted this as indirect proof that the vasoconstrictor substance was a phenolic pressor amine as suggested by Holtz, Bing, and Schroeder (18,92,193).

Page, Helmer, and Kohlsteadt (156), in 1941, studied the effects of parenteral injections of extract of whole kidneys on 280 hypertensive dogs, 13 patients with malignant hypertension, and six patients with essential hypertension. Some of the patients were treated for about a year and the

arterial blood pressure was significantly reduced. Other objective as well as subjective signs of improvement occurred. Because of the occasional shocklike reaction, and the lack of standard chemical procedures to yield a uniform product of high potency, and because when the extract was discontinued the blood pressure increased, they concluded that at the time, renal extract was not a practical method of treatment.

In 1942, Wakerlin et al (215) found that large doses of vitamin A reduced the blood pressure of experimental renal hypertension but they were unable to explain the mechanism. In the next year, Grollman and Harrison (75) showed that it was not the vitamin A that was the responsible agent for the reduction of the blood pressure, but rather the oil base in which the vitamin was dissolved. They found the oil was more effective as an antipressor agen than were renal extracts. This casted further doubt on the specificity of renal extract antipressor effect.

Schales, Stead, and Warren (191) gave kidney extract intramuscularly to seven patients daily. In five patients there was a decrease in the blood pressure which however appeared related to fever, sweating, weakness, anorexia, and severe local reactions. In

two patients there was no decrease in the blood pressure and there were also no local reactions. They, therefore, prepared a hypertensinase (angiotonase) poor extract to determine whether the fall in blood pressure was produced by a specific action of the hypertensinase on a renin-hypertensin system. Their results showed a similar fall in blood pressure and they therefore, concluded that the results were due to a non-specific effect of the extract on the renin-hypertensin system rather than to any specific interference with the pressor system.

Chasis, Goldring, and Smith (30) wondered if the fall in blood pressure in hypertensive patients when renal extract was administered could be due to a pyrogenic reaction. Pyrogenic reactions are known to produce a sensation of chilliness, pulsating lumbar pain, headache, fever, renal hyperenia, and a fall in blood pressure due to a complex effect on the vascular system. Their results demonstrated that tyrosinase had a pyrogenic action but did not demonstrate that tyrosinase is without a specific effect on blood pressure because heating destroyed the hypotensive action of tyrosinase whereas pyrogens are generally heat resistant.

They warn that since tyrosinase does have a pyrogenic effect in addition to any specific effect it may have, it should be given with caution as pyrogens can induce an alarming degree of circulatory failure.

Page et al (157) isolated a more purified kidney extract and their animal and human experiments illustrated the fact that the more purified substance produced less systemic and local reaction, but that there also was a loss of antipressor activity. Wakerlin (214) also found that the partially purified extract was superior to the highly purified extract in the treatment of experimentally induced renal hypertension, but he found that the potency was specific for kidney extracts since extracts of hog liver were not effective in the treatment of the hypertension.

Stevens (200) in a paper published in 1946, on four cases of human hypertension treated with renal extract which did not contain renin or angiotoninase described a fall in the blood pressure. Although the blood pressure dropped, the patients manifested allergic and febrile reactions and because of this the authors recommend that the extract not be used generally till the toxic effects are eliminated.

They do not believe that the blood pressure lowering was due to pyrogenic activity although the depressor effect appeared coincidental with fever, because the blood pressure measured during the afternnon peak of the fever was not appreciably lower than that observed in the morning before the temperature began to rise. Therefore, vasodilation alone was not responsible for the depressor effect. The allergic and toxic effects may have played some part in the lowering of the blood pressure because the local allergic phenomena appeared in two subjects coincident with the greatest decrease in the blood pressure. These observations are in agreement with those of Chasis (30).

In conclusion, one may state that while at the present time the treatment of essential hypertension by means of antipressor enzymes is still in the experimental stage, and although there are many side effects of a toxic nature which may aid in the antipressor effect of these extracts, the experimental evidence shows that there seems to be a specific effect of the enzymes in addition to the non-specific pyrogenic effect. In the future purified antipressor enzymes may play an

important role in the treatment of essntial hypertension. It must be borne in mind that the etiology of the disease is still unknown and accurate therapy depends on knowledge of the etiology.

The next topic to be discussed is the most fascinating subject in a survey of enzymatic activity in disease, and it is a topic which in the very near future will probably be applied practically. The subject deals with the problem of tissue anoxia which is a very important problem, for it is likely that the essential mechanism of most of the degenerative diseases is, in the final analysis, dependent on chronic tissue anoxia. For example, the heart muscle gradually fails probably because the coronary blood flow becomes progressively more inadequate, and, hence, the oxygen supply is not sufficient for the metabolic needs of the organ. Another example is the kidney in which tissue anoxia has been experimentally indicated as the major factor in causing the secretion of various pressor substances responsible for resulting hypertension. The senile changes in the brain are based on chronic tissue anoxia and the same applies to other body tissues. Malignant cells grow by using an anarobic type of metabolism and if a high concentration of oxygen was obtained

in the tissues perhaps the growth of the malignancy might be inhibited. Moreover, anoxia is a very important factor in aviation medicine.

At the present, there are, in general, three practical methods of approaching the problem of tissue anoxia clinically. (1) If the circulation is adequate, attempts may be made to increase the oxygen supply by having the patients breath gas mixtures rich in oxygne. (2) If the anoxia is due to inadequate circulation, that is if the arterial blood is fully saturated with oxygen but there is too little of this blood reaching the tissues, attempts may be made to improve the circulation. Nature makes this attempt to some extent by developing collateral circulation as in for example; while man has attempted this by various surgical procedures. The clinical use of vasodilators is directed towards the same end, namely increasing the oxygen supply to tissues deficient in oxygen by increasing the supply of the arterial blood which carries this oxygen. (3) The need for oxygen may be decreased by rest.

There is still another approach to the problem of tissue anoxia; that of enabling anoxic tissues to utilize more effectively available oxygen even though this supply may appear to be inadequate. Fully saturated

arterial blood normally carries with it about 20 volume per cent of oxygen. After tissue utilization of oxygen, about 12 to 14 volume per cent is returned to the lungs by way of the veins. Only about one-third of the oxygen which reaches the tissues is, therefore, utilized. So far as is known, the fairly large amount of oxygen unused by the tissues and returned by the venous blood serves no useful purpose. In other words, there seems to be additional oxygen available locally in the tissues if it can only be put to use. This is true even under conditions of apparent tissue anoxia.

In the past decade, considerable knowledge has accumulated regarding the mechanisms of biological oxidation. Oxidation is not the simple process which until fairly recently it was thought to be. Actually, the process of oxidation involves the transfer of electrons through a series of pyridine nucleotides and flavoproteins, probably through a group of C_4 dicarboxylic acids, through a series of cytochromes, and finally to the substance to be oxidized. It is in the transfer of electrons from substance to substance that the energy of the oxidative process is released. Many enzymes are required for these reactions, and those in turn require coenzymes. Tissue cells have two mechanisms

for the liberation of energy. When the final products are water and carbon dioxide all the available energy has been released and this process is true oxidation, but under anaerobic conditions, the original molecule is split into two parts both of which finally yield pyruvic or lactic acids. The amount of energy released by this anaerobic fermentation is much less than that available in true aerobic oxidation. In the presence of oxygen, the fermentation reaction is depressed in favor of complete operation. This is called the Pasteur effect.

Cellular respiration depends, first on the fact that cellular metabolites are oxidized when they lose hydrogen after activation by their specific dehydrogenases. Second, on the fact that the main function of molecular oxygen in the respiratory process is concerned with the oxidation of the ferrous iron of cytochrome oxidase; and third, on the fact that the cytochrome is an essential mediator for the transfer of electrons between the dehydrogenase systems and the cytochrome oxidase. Practically, however, this fact emerges: In this process, there are certain chemical substances which act as respiratory catalysts, i.e. they will catalytically promote tissue oxidation. If it were

possible to increase the tissue utilization of oxygen by one or more of these respiratory catalysts, it would provide a fourth clinical method of treating anoxia.

Proger (172) using the fact shown by Szent-Gyorgyi that a group of C_4 dicarboxylic acids (succinic, fumaric, malic and oxaloacetic) were concerned in the process of biological oxidation showed that with decreased oxygen tension, the catalytic effects in vitro of these acids was even greater than under conditions of normal or high oxygen tension. His results showed that in some cases in a ten per cent oxygen environment, the oxygen uptake of minced heart muscle of dog was increased by more than one hundred per cent by these substances.

In the intact anesthetized dogs, and under ten per cent oxygen mixture, certain changes in the electrocardiograms which usually occurred under these conditions were sometimes apparently prevented by the previous intravenous administration of sodium succinate. One explanation for the effect of the succinic acid action was that it might increase the local tissue utilization of oxygen sufficiently to overcome some of the effects of anoxia. The author showed that it was not a simple vasodilator effect by the fact that nitro-

glycerine did not produce the same result. In a following paper, Proger (171) demonstrated the fact that succinic acid produced an increased arterio-venous oxygen difference which gave support to the idea that it did promote tissue oxidation. The arterial oxygen contents were the same in the control and the succinic acid administrated animals, but the venous content of the succinic acid animals was diminished indicating a greater utilization of oxygen from the arterial blood. The total oxygen consumption of both groups remained the same and, therefore, each unit of blood supplied more oxygen, which meant that the succinic acid treated animals had a decreased cardiac output. Therefore, succinic acid should be useful in the treatment of cardiac weakness.

E. R. Gubner (77) made a comparative study of the effects of salicylates, succinates, sodium bicarbonate, sulfathiazole, and penicillin in the treatment of acute rheumatic fever. Sodium bicarbonate, sulfathiazole, and penicillin were found to be of no value. The salicylates were given to sixty-five patients in daily dosages varying from 4.0 to 6.7 grams, while the succinates in the form of the double calcium salt of benzoic acid and succinic acid benzyl ester were

given to fifty-five patients in dosages varying from 4.0 to 5.3 grams daily. The average age and the severity of the disease in the two groups were identical as judged by the degree of fever, leukocytosis, accelerated sedimentation rate, degree of polyarthritis, and the lowering of the plasma ascorbic acid level.

It was found that on all points analyzed, the cases receiving the succinate compound responded more favorably than did those receiving salicylates. The duration of acute symptoms, fever, luekocytosis, accelerated sedimentation rate, and the total days of hospitalization were uniformly and significantly abbreviated. Signs of carditis developed in 69 per cent of the salicylate treated cases, compared with 19 per cent of the succinate treated cases. Relapses of rheumatic activity occurred in seven of the salicylate treated cases, whereas no relapses developed in the succinate treated group. Drug toxicity was noticed in 19 per cent of the salicylate treated cases, but in only 2 per cent of the cases receiving the succinate compound.

Gubner based his experiment on the previously described findings of Proger (171, 172), and the fact that Govier (61) had shown that sodium succinate in dogs protected to a great extent against the breakdown of coenzyme I in cardiac muscle rendered ischemic by

ligation of the coronary artery. Succinic acid functions as a catalyst in the intermediate metabolism of glucids and fatty acids through the Krebs citric acid cycle and it is also believed to transfer hydrogen to cytochrome c. According to Gubner, there is no evidence that a deficiency of dicarboxylic acids exist in rheumatic fever, but he states that numerous studies have indicated a wide spread interference with various enzyme systems involved in tissue oxidation. The author further states that there is a marked decrease in porphyrin excretion, that ascorbic acid concentration was reduced, and that the vitamin A level of the plasma was also reduced.

Indirect evidence of enzymatic inhibition in rheumatic fever stems from the fact that sulfonamides, known inactivators of a variety of respiratory enzymes particularly the dehydrogenases, exert a deletorious effect in the disease. Therefore, it appears possible that in acute rheumatic fever administration of succinates helps to prevent inhibition of succinic dehyrogenase, thereby maintaining cytochrome in its reduced form, and probably prevents the oxidation and inactivation of other respiratory enzyme constituents. On the basis of theoretical grounds and this experiment, succinates

appear to be better than any other known theraputic agent in the treatment of acute rheumatic fever, and certainly more clinical experiments should be undertaken using this chemical as a theraputic agent in the treatment of acute rheumatic fever.

Even under conditions of anoxia, the venous blood returning from the tissues contains considerable unused oxygen. This means that additional oxygen is available but not utilized. Proger (175) attempted to increase the utilization of the available oxygen through the catalytic effect of the important cytochrome respiratory enzymes. He found that the cytochrome C content of heart, brain, liver, and kidney was far below that required for the maximal activity of the cytochrome oxidase present. Cytochrome C was prepared from beef hearts and was found stable, non-toxic in large doses in man and animals, recoverable from the blood, and found in increased amounts in the organs after intravenous and intramuscular injection. It was apparently broken down before excretion because it was not recovered in the urine. By daily intravenous injections of 25 mgs. he maintained a satisfactory blood level (20 to 30 micrograms per cent) in human beings. Following

the intravenous injection of cytochrome C there was a considerable increase in the content of this substance in the heart, liver, and kidney of the rat and rabbit. A similar though delayed increase followed intramuscular injection. Proger (175) demonstrated by in vitro experiments using the Warburg apparatus that an increase of cytochrome C, such as can be quantitatively produced in living organs by injection, was sufficient to produce a significant increase in the tissue uptake of oxygen.

Under conditions of anoxia, the organ content of cytochrome C increases after injection, whereas the blood level decreases. With release from anoxia there is a decrease in the organ content and an increase in the blood level. This suggests to Proger that the cytochrome supply circulating in the blood stream acts as a reservoir to be called upon as the need arises and is replenished when the need no longer exists.

It is known that the easily hydrolyzable phosphorus compounds are largely responsible for the phosphorylation which is so basic to the transfer of energy in tissues. The content of easily hydrolyzable phosphorous in the hearts and kidneys of rats underwent a sharp drop (one-half to one-third of the original content)

when the rats were placed in an atmosphere containing three per cent oxygen for five minutes (174). If, however, the rats were previously injected with cytochrome C, there was little or no drop in the organ content of hydrolyzable phosphorous following similar exposures to low oxygen tension. Proger concluded that the disturbance of phosphorylation resulting from anoxia may be largely prevented by the previous injection of cytochrome C. Under conditions of severe anoxia (three per cent) there was frequently a striking increase in the survival time of the rats previously injected with cytochrome C compared to the control animals (174).

Electrocardiographic changes produced in humans by ten per cent oxygen anoxia can be prevented by the previous intravenous injection of 25 to 50 mgs. of cytochrome C. Proger (170) also found that the extreme subjective disturbances which occasionally occurred with anoxia induced by a mixture of ten per cent oxygen and ninty per cent nitrogen could be prevented by the same dosage. With the aid of Drs. Forbes and Niven, he obtained evidence which suggested that the impairment of visual discrimination which was produced by low oxygen tension might also

be largely prevented by the injection of cytochrome C.

Since visual discrimination is one of the earliest functions to become impaired with anoxia, one would anticipate that cytochrome C would prevent its development. Proger in a subsequent paper (173) demonstrated that the impairment of visual discrimination produced by moderate anoxia (12 to 15 per cent oxygen) could be completely overcome in five to ten minutes by the intravenous injection of 60 to 70 mgs. of cytochrome C. The restoration to normal vision which occurred while the subject was still under the anoxic condition was, in fact, so complete that an atmosphere containing 100 per cent oxygen produced no further improvement.

Anoxia produces an easily measurable effect on the ability to transliterate letters into corresponding codes, the time required for transliteration becoming increasingly longer as the subject goes beyond a certain critical degree of anoxia. The second portion of this paper (173) dealt with a study of this particular cerebral function. The experiments were carried out in a pressure chamber with decompression corresponding to an elevation of 16,500 feet. In three subjects, the increase in the time required for decoding the

words together with the subjective distress accompanying this degree of anoxia (11 per cent oxygen) were completely overcome in five to ten minutes by the intravenous injection of 65 mgs. of cytochrome C. In three subjects electocardiographic changes induced by anoxia were largely prevented by 50 mgs. of intravenous cytochrome C.

Intermittent claudication is a clinical condition in which anoxia plays an important role. In a review of 13 patients with intermittent claudication treated with cytochrome C, Proger (169) found that three patients showed no improvement, three patients showed definite moderate improvement, while the remaining seven improved strikingly. Improvement was measured by means of exercise tolerence which were of two types; namely, stair climbing and level walking. The following case report is quoted.

"B. S., a man 53 years of age, for five to six years had had pain in the calves on walking. This pain was relieved by rest. He had also noticed some pallor and coldness of the lower extremities. Three years ago a diagnosis was made of peripheral vascular disease, arteriosclerotic, with vasospasm, and he was subjected

to bilateral lumbar symathectomy at another hospital. There was some improvement for several weeks after the operation, but the pains soon returned. His condition grew worse so that shortly before he was referred to us he could not walk one city block without having to stop because of the pain. He had had most of the current theraputic procedures for intermittent claudication to no avail."

"His blood pressure was 115/75 mm. Hg.. There was no evidence of heart disease. The dorsalis pedis and posterior tibial pulsations could not be felt on either side."

"During a control period of eight days (intravenous injections of an inert solution with a color similar to that of cytochrome C were given), the number of trips which he could take on the two-step stair-climbing test before the development of pain varied between 17 and 21. After four days of cytochrome C (daily injection of 50 mgs. intravenously) he could still make only 23 trips. During the next six days, however, there was a progressive improvement so that after ten days of treatment he could make 50 trips before the pain developed. The patient was impressed

by the fact that whereas formerly he developed pain when he attempted to walk one block, he was able, after the cytochrome treatment, to walk 18 blocks without pain. A possible further effect of the treatment was indicated by the considerable improvement in his general well being, i.e.; his appetite was better, he was more vigorous and active. Such an effect may, of course, by psychic but it was noted in many of our patients."

The characteristic response to cytochrome C treatment consisted of a steadily increasing exercise tolerance over a period of about ten days. After about ten days of steady improvement, there was usually no further improvement despite the continued injections of cytochrome C, but the improvement was generally of such a magnitude as to enable a patient who was seriously handicapped before the treatment to carry on practically normal activities after the treatment. The improvement continued after the treatment was stopped. In the first patient treated the improvement lasted up to the time the paper was written (one year later). Proger has no explanation for this except that it is possible that the series of injections restored certain chemical equilibria which thereafter maintained themselves.

A clear distinction must be made between anoxia and ischemia. The beneficial effects of cytochrome C observed so far by Proger have been associated with relieving the anoxia and probably have had no or little effect on the ischemia, outside of the anoxia. With ischemia there is not only an oxygen deficiency to the tissue, but also a deficiency of everything else which the circulating blood carries. One can expect stimulation of tissue uptake of oxygen to be helpful only to the extent that the anoxia is responsible for the clinical disturbance.

Despite the most intensive and widespread chemical studies in the past twenty years on the mechanisms of biological respiration, there have been comparatively few attempts to apply the enormous volume of chemical facts thus elaborated to clinical problems. The most obvious of such problems deal with tissue anoxia both acute and chronic. The clinical conditions concerned with anoxia include such diverse subjects as high-altitude flying, shock, degenerative diseases, malignancy, fatigue, and many peculiarities in tissue behavior which have their basis in chemical disturbances which are in turn due to various degrees of tissue anoxia.

Malignant cells are known to metabolize anaerobically and there is a known disturbance in the respiratory enzyme systems of organisms with neoplastic growths. Might not the use of cytochrome C injections raise the tissues ability to utilize oxygen and to metabolize normally at the expense of the neoplastic cells? This question is certainly worthy of experimentation.

It is now widely believed that tissue anoxia plays a central or at least an important role in the mechanism of shock. Proger (173) found that hemmorrhagic shock in dogs was favorably influenced by cytochrome C. Govier and Greer published a series of papers showing the importance of vitamins in the treatment of shock (61, 62, 63, 64, 65).

Govier and Greer (65) induced shock in a number of dogs by fractional bleeding and thiamine was administered to half of them with an apparent beneficial result in that the thiamine treated dogs lived longer than did the controls. There was a significant difference in the blood pressures of the two groups. The results led the authors to wonder whether thiamine was acting in its normal manner as a coenzyme in tissue metabolism because an animal in shock was in some way thiamine deficient or whether thiamine was acting in some other manner. A diagnostic test for

thiamine deficiency is the estimation of the level of pyruvic acid in the blood. Since the breakdown of pyruvic acid requires phosphorylated thiamine or cocarboxylase as a coenzyme, a deficiency of thiamine will cause pyruvate to pile up in the circulating blood. Govier and Greer (64) did blood pyruvate determinations on a number of animals in shock, and the pyruvate level of the circulating blood rose from a normal amount of 1.0 to 2.0 mgs. per 100 c.c. to 4.0 to 5.0 mgs. per 100 c.c. of blood. This level is actually higher than that seen clinically in most cases of beriberi. Thus, it appeared either that these animals became thiamine deficient as shock was induced or that their thiamine became incapable of functioning in a normal manner.

By way of an attack from a slightly different angle, it was thought of possible interest to find out whether the amount of thiamine initially present in the dog might have some relationship to the ease with which shock was induced in the animal. Consequently, avitaminosis B_1 was produced in a number of dogs, whereas others were given a stock diet fortified with vitamin B_1. An index of deficiency was given by the

determination of the plasma thiamine levels. The difference in ease of the production of shock was very striking. The results (63) showed that seven of the nine thiamine deficient dogs went into shock after a total bleeding of less than 4.0 per cent of their body weight, whereas all of the sixteen vitamin B_1 fortified dogs required more than this amount of bleeding and 44 per cent of them required even more than five per cent of body weight in blood to be removed before prolonged hypotension was produced. Other pronounced differences were noted between these two groups of animals. The blood pressure of the low thiamine dogs dropped percipitously after the first few bleedings and remained low (45 to 60 mm. HG) throughout the experiment; whereas the blood pressure of the thiamine fortified dogs showed a constant tendency to rise even after more than five per cent of the body weight in blood had been removed in some cases. Hemoconcentration was also much less as well as the amount of intestinal hemorrhage in dogs with high plasma thiamine levels.

With reference to the use of thiamine in the treatment, surprisingly, best results (80 per cent recovery) occurred in those dogs having intermediate or normal plasma thiamine levels. None of these dogs were

given fluids to replace the blood withdrawn, and one could not expect the high thiamine animals to live without transfusion when half or more of their circulating blood volume was removed. The extensive pathologic changes in the low thiamine group as exemplified by intestinal hemorrhage was blamed for preventing their response to thiamine alone when it was used. In most cases, the plasma thiamine level rose as the animal went into shock.

Greig and Govier (73) interpreted this to mean that, since thiamine must be phosphorylated to diphosphothiamine or cocarboxylase in order to be effective as a coenzyme in pyruvate metabolism, in the tissues there was probably an equilibrium: thiamine plus phosphorous $\rightleftharpoons$ cocarboxylase. Under normal conditions most of the thiamine was in the phosphorylated form, but it seemed possible that under abnormal conditions, such as shock, the cocarboxylase might become dephosphorylated, thus shifting the equilibrium to the left and concomitantly decreasing the amount of metabolically "active" thiamine. Greig and Govier (73) determined both the cocarboxylase and the total thiamine in the skeletal muscle, liver, and duodenum of dogs before and after shock, and after thiamine therapy.

Dephosphorylation of cocarboxylase occurred in 92 per cent of the cases in muscle, 62 per cent of the cases in the duodenum, and 46 per cent of the cases in the liver. The magnitude of the dephosphorylation was variable; there being a tendency for more dephosphorylation to occur in dogs which went into shock with relatively small amounts of bleeding. These findings were confirmed by Alexander (1).

Thus, animals in shock, although well supplied with thiamine, were in a sense vitamin B_1 deficient, since their thiamine was in a form which was useless in tissue metabolism. The administration of more thiamine in large doses to these dogs as treatment resulted in a resynthesis of the cocarboxylase. Large doses of thiamine are probably required in order to raise the intracellular concentration of thiamine so that resynthesis may occur, even when oxidative processes supplying energy for phosphorylation of thiamine are greatly reduced. Greig and Govier (72) found that dogs' serum contained a phosphatase which hydrolysed cocarboxylase. Therefore, if any cocarboxylase did diffuse into the plasma with thiamine it would be rapidly destroyed. This means that shock cannot be combated with cocarboxylase--thiamine must be used. There is normally no cocar-

boxylase in serum as a result of this.

As dogs go into shock the animals' plasma thiamine rose due to the free diffusion of free thiamine from the tissues to the circulating plasma after cocarboxylase is dephosphorylated as thiamine was more diffusible. There was no reason to suppose that cocarboxylase was the only coenzyme which was broken down in anoxic conditions or that shock and anoxic anoxia should be the only conditions in which coenzyme should be broken down. Indeed, Greig (70) showed a pronounced breakdown in coenzyme I, the nicotinomide containing coenzyme which is essential for the metabolism of many substrates such as lactate, malate, bethahydroxybutyrate, and diphosphoglyceraldehyde, and that the resynthesis of the coenzyme occurred after the administration of nicotinic acid. She also demonstrated a similar breakdown in alloxazine adenine dinucleotide (flavin adenine dinucleotide), the riboflavin containing coenzyme which is essential for the reoxidation of reduced coenzyme I and for the metabolism of amino acids. Alloxazine adenine dinucleotide was resynthesized in the animal in shock when riboflavin was administered.

Long and his co-workers (119) had noticed an accumulation of amino acids in the peripheral blood

in shock and this was explained by Greig (71) demonstration of the breakdown of riboflavin containing coenzymes. Long found that in vitro experiments the respiration of liver slices from shocked animals was much less than that of the controls, and that the addition of coenzyme containing boiled liver extract increased the oxygen consumption of mildly shocked tissue. Greig (71) incubated tissue in nitrogen and produced a marked decrease in its ability to oxidize amino and lactic acid. Analysis for cozymase--coenzyme for the oxidation of lactic acid--and alloxazine adenine dinucleotide--the coenzyme required for amino acid oxidation--showed only a slight difference between anoxic and normal tissue, but adding coenzymes to anoxic tissue produced some increase in the rate of oxidation but did not increase it to the normal value.

The summary of the facts that show that coenzymes are broken down in shock as presented by Govier (62) are: (1) The resistence of dogs to shock was significantly greater in those with high plasma thiamine levels than those with low values of plasma thiamine; (2) Animals which were susceptible to shock show a diffusion into the plasma of large amounts of thiamine,

indicating cocarboxylase breakdown in tissues. (3) Cocarboxylase was found to decrease in muscle, liver, and duodenum in animals subjected to hemorrhage and anoxic anoxia; (4) Some degree of correlation was found between the amount of bleeding necessary to go into shock and the degree of the destruction of cocarboxylase; (5) Cozymase and alloxazine adenine dinucleotide were found to decrease frequently in the brain, muscle, and liver in shock; (6) Dogs requiring more than the average amounts of bleeding to go into shock showed less destruction of tissue cozymase than did dogs which went into shock with small amounts of bleeding.

It is obvious then, that the prevention and treatment of coenzyme breakdown is of the utmost importance if normal metabolism, or in fact any metabolism, is to be maintained in anoxic conditions such as shock. High doses of vitamins are an essential part of the modern treatment of shock. In addition, the important work of Proger indicates a great future for cytochrome C in preventing the anoxia of shock. I do not mean to imply, however, that other means of therapy are unimportant, and certainly in the treatment of shock one should employ all of the remedies available.

Pancreatic enzymes in indigestion: Oelgoetz, Oelgoetz, and Wittekind (147) published a series of papers in which they attempted to show that indigestion secondary to pancreatic hypofunction was a fairly common cause of digestive disturbance. They found that food allergy might be caused by a decrease in the concentration of the serum enzymes which permit whole, unracemized proteins to reach the body cells, where, because they cannot be used as food they exerted their own physiological action which was essentially that of irritation. The administration of pancreatic substance by mouth was "theraputically justified and clinically effective" according to their conclusion.

They explained that the stomach and duodenum were not the essential organs of digestion. During absorption in the duodenum the food protein was split to various derived proteins and amino acids. The bulk was changed in varying degrees to alkaline-metaproteins and they pass into the blood stream where the essential process of digestion supposedly occurred. Normal serum always contains amylase, protease, and lipase. In disease there was a decreased concentration in the serum and the whole unracemized protein reached the body cells where it gave rise to "indigestion--food allergy".

Pancreatic extract is not destroyed by stomach digestion, but it is ineffective in the acid media. However, when it reached the duodenum, it is activated and absorbed raising the blood enzyme level and thus preventing the food allergy and the resulting indigestion.

This article was written in 1935, and there are no articles which either confirm or deny the accuracy of the findings. The mechanism certainly could explain some food allergies.

CONCLUSIONS:

1. The main function of thiamine in metabolism is its action as a coenzyme by combining with phosphoric acid to form thiamine pyrophosphate or cocarboxylase which is required for the oxidative removal of pyruvic acid-- a vital link in glucid metabolism.

2. Riboflavin plays an important role in the entire respiratory mechanisms of the animal body.

3. The function of nicotinic acid in the animal body is largely related to coenzyme I and coenzyme II.

4. Pyridoxine functions in protein metabolism are at least partially explained by its action in amino acid decarboxylation and in transamination.

5. Although so far no one has conclusively proven that ascorbic acid is a constituent part of an enzyme system, there is much indirect evidence associating

it with enzymatic activity.

6. Only indirect relationships are found between the fat soluble vitamins and enzymes. Visual purple is a conjugated protein with vitamin A as the prosthetic group. The high plasma phosphatase accompanying rickets is most likely a secondary effect.

7. Certain vitamins are constituent parts of some enzyme systems, and these enzymes show a significant decrease during deficiency diseases. To those cases where the relationship between the enzyme and vitamin is indirect or obscure, Green's trace substance enzymethesis can be applied: Namely, that there is no rationale explanation available of how traces of some substance can exert profound biological activity except in terms of enzyme phenomena.

8. Leukocytes are important agents in the defensive reactions of the body. They not only actively phagocytize the irritating agent, be it a pathogenic organism or some foreign substance, while they are alive, but also by means of their intracellular proteinases released after their death, liquify the dead tissue and digest the foreign agent.

9. Fibrinolysin is a kinase which activates the lytic factor of the serum forming an active protease capable of dissolving fibrin clots.

10. The results of the study of experimental tuberculosis and enzymes permit for the first time correlation of organ

and species susceptibility to infection with tubercle bacillus with endocellular enzyme activity.

11. Considerable elevation (over 180 units) of the blood amylase almost always occurs at some time during an episode of acute hemorrhagic pancreatitis.

12. A definite enzyme system (the renin-angiotonin-pressor system) is incriminated in the etiology of experimental renal hypertension.

13. At the present time the treatment of essential hypertension by means of antipressor enzymes is still in the experimental stage, and although there are many side effects of a toxic nature which may aid in the antipressor effect of these extracts, the experimental evidence indicates that there seems to be a specific effect of the enzymes in addition to the non-specific pyrogenic effect. In the future, purified antipressor enzymes may enact an important role in the treatment of essential hypertension.

14. Intravenous succinates or cytochrome C can prevent some of the electrocardiographic changes caused by anoxia in animals and man.

15. Succinates on theoretical and experimental grounds are the best agents in the treatment of acute rheumatic fever.

16. Cytochrome C combats the effects of cerebral anoxia as measured by visual discrimination and ability to transliterate letters.

17. Cytochrome C appears to be effective in the treatment of intermittent claudication.

18. High doses of vitamins are an essential part of the modern treatment of shock.

SUMMARY:

This paper has reviewed the role of enzyme activity in deficiency diseases, inflammatory and infectious diseases, metabolic and degenerative diseases, and concluded with a discussion of the role of enzymes in the therapy of disease. It did not include the vast realm of enzymatic changes in neoplastic disease nor did it pretend to cover all diseases, but rather just those diseases in which sufficient experimental work has been done to warrant inclusion.

In a recent communication from Dr. S. Proger, I was informed that cytochrome C appeared to be effective in the treatment of angina pectoris. Patients afflicted with this disease on whom cytochrome C was used experienced much relief and there was a decided decrease in the frequency of attacks. The value of cytochrome C in the treatment of acute and chronic tissue anoxia should not be underestimated.

It is the sincere hope of the author that this thesis conveys to clinicians the importance of enzyme activity in disease and the tremendous possibilities of the enzymatic treatment of disease processes, especially anoxia.

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