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Metabolic Functions of Vitamin B Complex

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I. METABOLIC FUNCTIONS OF THE VITAMIN B COMPLEX*

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1. Introduction

The definition of vitamin function depends to some extent on the degree of refinement exercised. It may be found, for example, that an organic substance is required in small concentration for the optimal growth of a biological species, or, conversely, that its absence from the diet results in sub-optimal growth or certain definitive deficiency signs. Obviously, then, the function of this substance is to permit growth. On closer inspection, however, it may be found that this substance or vitamin is required for the proper conduct of certain enzymatic reactions necessary for the growth of the organism. This then is the metabolic or enzymatic function of the vitamin.

The precise locus of activity of a particular vitamin in metabolism represents far more than an academic problem, since this is the only method by which the primary biochemical dysfunction resulting from a deficiency in the vitamin may be elucidated.

On the basis of a variety of criteria it has become increasingly manifest that the B vitamins differ from ascorbic acid and the fat soluble vitamins in several respects. In general, it is found that the B vitamins are intimately involved in the metabolism of all living cells, while the other vitamins appear to have more specialized functions.

According to Williams,[1] the B vitamins are those which occur as indispensable constituents of all living matter, which act catalytically in all living cells, and which function nutritionally for at least some of the higher animals.

2. Metabolic Functions of B Vitamins

There is abundant evidence that these vitamins act in the form of coenzymes, or component parts of the enzyme systems carrying out certain reactions necessary for the life of the organism.

Many of the enzymes studied have been found to be composed of two components, an apoenzyme and a coenzyme or prosthetic group. The apoenzyme is the protein, exhibiting the usual characteristics of high molecular weight, heat lability, and non-dialyzability, while the coenzyme is a relatively low molecular weight organic substance, relatively heat stable, and in some instances dialyzable. The intact enzyme or holoenzyme requires both components for activity, and in some cases an inorganic ion is also required. The apoenzyme is synthesized from the amino acids, while the coenzyme is derived from the particular B vitamin involved. Since enzymes have a limited period of life due to their gradual destruction during activity, one can readily appreciate the constant need for amino acids to be used for the formation of apoenzymes, and of B vitamins to synthesize coenzymes. This is not, however, meant to infer that

all coenzymes are derivatives of B vitamins.

In Table I, I have summarized briefly with representative references some of the metabolic or enzymatic functions of several members of the B complex group. It may be seen that thiamine functions as a coenzyme for several reactions of pyruvic acid, which is a key compound in the intermediary metabolism of all forms of life. Riboflavin and niacin are essential in biological oxidations by virtue of their ability to transfer

TABLE I

Metabolic Functions of Representative Members of the Vitamin B Complex

Vitamin	Function	Coenzyme Form	Selected References
Thiamine	several reactions of pyruvate	diphosphothiamin	Lohman and Schuster[2]
Riboflavin	hydrogen transport	flavin-adenine-dinucleotide flavin phosphate	Warburg and Christian[3]
Niacin	hydrogen transport	diphosphopyridine nucleotide triphosphopyridine nucleotide	Warburg and Christian[4] von Euler, et al.[5]
Pyridoxin	coenzyme of several reactions of amino acids	pyridoxal phosphate	
	1. transamination		Lichstein, et al.[6]
	2. decarboxylation		Gunsalus and Bellamy[7] Umbreit, et al.[8]
	3. alanine racemase		Wood and Gunsalus[9]
	4. tryptophan metabolism		Umbreit, et al.[10] Dawes, et al.[11]
Pantothenate	various acetylation reactions	coenzyme A	Lipmann, et al.[12-16] Nachmansohn and Berman[17]
Biotin	1. oxalacetate decarboxylase	demonstrated but not identified	Lichstein and Umbreit[18] Lardy, et al.[19-20]
	2. deamination of aspartate, serine and threonine		Lichstein and Umbreit[21] Lichstein and Christman[22-23]
	3. succinate decarboxylase		Delwiche[24]

hydrogen or electrons in the system of mediators between the hydrogen of the substrate and molecular oxygen. Pyridoxine is required for a wide variety of reactions involving the degradation and synthesis of amino acids, while pantothenate is necessary for many reactions of acetate, including the formation of acetylcholine, an important compoind in nerve physiology. The role of biotin as a coenzyme is perhaps not as clear as the other vitamins listed in this table, but it is intimately involved in two important areas of metabolism, namely, the utilization of carbon dioxide for the synthesis of essential dicarboxylic acids, and the utilization of ammonia, which represents the ultimate source of nitrogen for the synthesis of amino acids.[25]

Other members of the B complex group are also involved in various areas of metabolism. Folic acid and its derivatives, as well as vitamin B_{12}, are in some manner concerned with reactions in which single carbon units are utilized for synthesis of amino acids, nucleic acids, porphyrins, etc., while inositol appears to function as an essential component of the enzyme, alpha-amylase.[26]

It is pertinent to emphasize that in all cases, where sufficient evidence has accumulated, it is found that the structure of the metabolically active form of the vitamin differs from the one ordinarily produced industrially. In all instances these active or coenzyme forms are more complex, and are phosphorylated. Results of growth experiments, particularly with microorganisms, have shown that many species are capable of synthesizing the coenzyme form from the added vitamin, while others require preformed the complex structure. By analogy, it is at least provocative to suggest that metabolic dysfunctions may exist in man wherein an individual may be unable to convert effectively a vitamin present in his diet to the coenzyme form required for its proper biochemical performance.

The entire area of nutrition provides excellent examples for the thesis of comparative biochemistry which states in essence that there is a similarity, indeed identity, in the fundamental metabolic processes of all biological species. The B vitamins are required or are synthesized by all species from microbe to man, and further, the B vitamins function in exactly the same manner in the microorganism and in the human animal.

3. Contributions of Microbiology to Our Knowledge of B Vitamins

The microbiologist is perhaps the oldest experimental nutritionist, since it has been with him a day to day problem in the isolation of microorganisms. Inasmuch as the very foundation of microbiology rests on the ability to grow organisms in pure culture on artificial media, it became apparent very early that the nutritional requirements of these organisms must be understood. Although the entrance of the microbiologist into the field of modern nutrition was looked upon with some misgivings by the animal nutritionists, it may be stated that today the use of microbes as tools in nutritional research occupies a position equal to that of the animal.

a. Discovery of Vitamins

As early as 1919, Williams[27] concluded that the substance or substances which stimulate the growth of yeast are identical with the substance or substances which prevent beriberi in animals. Since that time there has been a gradual recognition that the growth factors of bacteria are identical with the vitamins of man.

In the discovery of the various members of the B complex, microorganisms have played a tremendous role. For example, pantothenic acid, pyridoxal, para-aminobenzoic acid, biotin, inositol, and B_{12} were discovered as growth factors for microorganisms before their importance in animal nutrition was recognized.

If one may be permitted speculations, it is probable that bacteria and other

microorganisms will play an even more important role in the future. The complex growth requirements of certain fastidious microorganisms such as the lactic bacteria, plus the low concentration of yet unrecognized factors in natural materials, suggest this possibility. In addition, one may predict that we are coming into an era of discovery of functional factors uncovered as a result of the study of cellular metabolism, particularly microbial cells, and that these factors may or may not be required for the growth of all species, but are required for the proper activity of certain enzyme systems. Such factors would remain unknown if the investigator imployed only growth studies.

b. Synthesis of B Vitamins

Among the microorganisms one finds varying degrees of nutritional dependence. With regard to the B vitamins, these are either required or are synthesized so that all microorganisms have a full complement of these factors. It has been concluded, therefore, that these vitamins are actually required by all organisms and are recognized as growth-factors only when the cell is unable to accomplish synthesis or can do so only at a suboptimal rate.

Inasmuch as animal species require preformed vitamins, suggesting their inability to synthesize these compounds, the study of the biochemical pathways of vitamin synthesis by certain microorganisms has already contributed to the understanding of the metabolism of the vitamin itself.

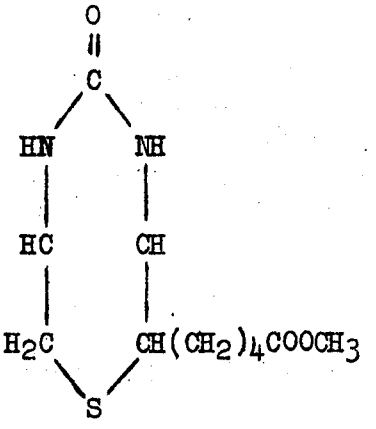
c. The Study of Vitamin Function

The use of microorganisms in the localization and elucidation of vitamin function offers several advantages over other types of cells. It is relatively easy and inexpensive to maintain pure cultures of well defined organisms; the cell source is a rapidly multiplying one, thus aiding materially in the accumulation of data; the exact nutritional requirements for many microorganisms are known so that a chemically defined or synthetic medium is available; and the synthetic abilities of many of these organisms often makes possible the recognition of a more active form of a particular vitamin prior to its isolation or synthesis.

As an example of the methods employed in the study of vitamin function, I should like now to discuss with you work done by our group and others on the metabolic functions of biotin, the structure of which is given in Figure 1.

Figure 1

Methyl Ester of Biotin

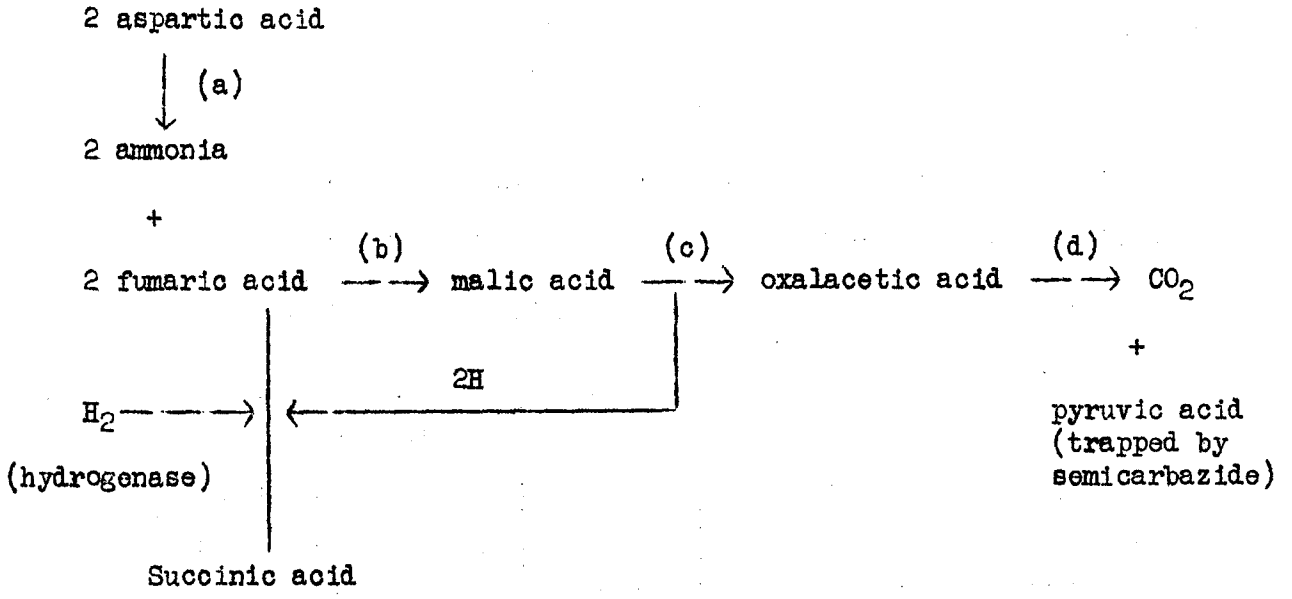


During studies on the metabolism of aspartic acid in Escherichia coli, it became evident that cells harvested from a complex medium rapidly lost activity after exposure to phosphate buffer at pH 4, and that the lost activity could be restored by the addition of a mixture of the known members of the vitamin B complex. By restricting the conditions of measurement, the active agent among these vitamins proved to be biotin, and by separating the various reactions concerned, this vitamin was shown to function in the oxalacetate decarboxylase system (Wood-Werkman reaction).[18]

The degradation of aspartic acid in this organism follows the series of reactions given in Figure 2. Localization of biotin activity in this series was demonstrated by studying the individual reactions manometrically, employing con-

Figure 2

Degradation of Aspartic Acid in E. Coli



(from Lichstein and Umbreit[18])

ventional Warburg techniques. Evidence that the biotin effect resides in the oxalacetate decarboxylase system was obtained in several ways, one of which is given in Table II. It may be seen that deficient cells show no biotin effect when malate is oxidized in the presence of large quantities of cyanide to bind the oxalacetate, whereas a definite biotin stimulation of carbon dioxide production is noted when malate is permitted to proceed normally to pyruvic acid via the decarboxylation of oxalacetate. More direct evidence was obtained by studying CO_2

TABLE II

Decomposition of Malate to Oxalacetate or to Carbon Dioxide

(a) Malate to CO_2 in N, cells exposed for 30 minutes before use, about 0.8 mg. of cell nitrogen per cup. (b) Malate to oxalacetate, value given is O_2 uptake; cells at pH 4 in refrigerator 60 minutes, then at 37° for 30 minutes; reaction run in 0.3 M phosphate, 0.02 M semicarbazide, pH 4, 37°; about 0.8 mg. of cell nitrogen per cup gas phase air.

Experiment No.	Conditions	Q (N)		
		No Additions, 0 biotin	Vitamins, 5 mγ biotin	Biotin, 50 mγ biotin
456	(a) Malate to CO_2	42	91	89
	(b) " to oxalacetate	146	146	146
461	(a) " to CO_2	32	56	56
	(b) " to oxalacetate	127	127	

(from Lichstein and Umbreit [18])

production from added oxalacetate itself, which showed clearly that biotin increases markedly the rate of CO_2 production from this dicarboxylic acid (Table III). Since this was studied in the presence of cyanide which inhibits the decomposition of pyruvate, it was concluded that biotin does indeed function in the conversion of oxalacetate to pyruvic acid.

TABLE III

Influence of Biotin upon Oxalacetate and Malate Decarboxylation

(a) Oxalacetate decarboxylation; cells at pH 4 at 37° for 60 minutes, centrifuged, adjusted to pH 6.5; reaction run in 0.3 M phosphate, 896 microliters of added oxalacetate, 0.005 M NaCN, inorganic salt mixture, gas phase N_2. Controls were boiled cells. (b) Malate decomposition; pH 4.

Experiment No.	Cell treatment	Additions mγ	Biotin 3 per ml.	Oxalacetic acid Q_{CO_2} (N)		Malate Q_{CO_2} (N)
				Observed	Less killed cells	
476	37°, pH 4, 60 min.	None	0	362	225	187
		Vitamins*	20	600	463	350
		Biotin	100	545	408	350
	heated, 100°, 10 min.	Vitamins*	20	137		
		"				
478	37°, pH 4, 60 min.	None	0	250	138	12
		Vitamins*	20	375	263	112
		" less biotin†	0	204	92	12
		Biotin	10	375	263	112
	heated, 100°, 10 min.	Vitamins	20	112		

* Vitamins added per cup; 0.5 γ each of riboflavin, thiamine, pantothenic acid, nicotinic acid, folic acid, p-aminobenzoic acid, and 20 mγ of biotin.

† The same as above, biotin omitted.

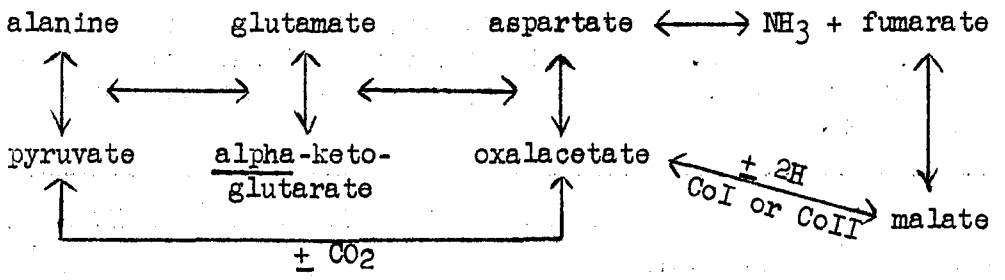
(from Lichstein and Umbreit[18])

At about the same time three other laboratories reported their results using other techniques and concluding that biotin functions in some manner in the oxalacetate decarboxylase system, which is under certain conditions reversible, thus constituting an important carbon dioxide pickup mechanism.[25]

Although the demonstrated role of biotin in the Wood-Werkman reaction does finally lead to the synthesis of aspartic acid by transamination of oxalacetate with glutamate (Figure 3) and does therefore provide an explanation for the biotin-aspartate relationship discovered by Koser et al.,[28] it occurred to us that biotin

Figure 3

Transamination - Aspartate Cycle



might also be involved in a more direct reaction of this amino acid, namely the direct amination of fumaric acid. By use of the same aging technique (exposure of living bacterial cells to M phosphate at pH 4 for 30 minutes at 27 - 37°) the aspartic deaminase system as well as other deaminases was followed by studying ammonia production upon the addition of the respective amino acid to the cell suspensions.[21] The data presented in Table IV demonstrate biotin activation of the aspartate, serine, and threonine deaminases. Further studies with other deaminases were uniformly negative, showing that this vitamin is not required for the deamination of all amino acids. The role of biotin in the aspartic acid deaminase system has been confirmed by Wright et al.[29]

The deaminase systems were chosen for study in order to delve further into the mode of action of biotin in these enzymes. Gale[30] working with the aspartic acid deaminase system reported that washed suspensions of bacterial cells lost activity on standing and that the activity could be increased by adding several materials, the most active of which was adenylic acid. It was therefore of interest to determine whether adenylic acid would stimulate the deficient cells obtained by our aging technique, which respond to biotin. The results of such experiments (Table V) show that muscle adenylic acid (adenosine-5-phosphoric acid) can replace biotin in these deaminases.[22] We were thus confronted by a very interesting and somewhat confusing situation, namely, that these three enzyme systems can be activated either by biotin or adenylic acid. Experiments designed to elucidate this phenomenon resulted in data which were interpreted by us to mean that adenylic acid is intimately associated with these deaminases and may have the function of either specifically phosphorylating biotin to an active coenzyme form, by virtue of its known role in phosphate transport, or by combining chemically with biotin to form a coenzyme similar to diphosphopyridine nucleotide or triphosphopyridine nucleotide.

By analogy with other vitamins, it might be expected that biotin does not function in these various reactions as such, but is converted into a more active coenzyme form. In studies on the aging technique it was found that, although in some instances partially resolved cells were not activated by biotin or adenylic acid or even a combination of both agents, they were consistently stimulated by yeast extract. This was taken as an indication that certain natural materials contain the preformed coenzyme of the reactions studied. Certain additional data were accumulated which were interpreted to show the existence in yeast extract of a coenzyme form of biotin. It was found, for example, that yeast extract was approximately 100 fold more active in the stimulation of the aspartate deaminase system than could be accounted for by its assayable biotin content, using Saccharomyces cerevisiae as an assay

TABLE IV

Biotin Activation of Aspartic Acid, Serine, and Threonine Deaminase

Cells aged at pH 4 in M phosphate at 25-30°. Reaction run at pH 7, 37°, 20 to 30 minutes; volume 2 ml. Amino acids added at 0.005 M final concentration. The increase in ammonia over samples stopped at zero time was taken as an index of deamination.

Organism	Amino acid	Ammonia nitrogen produced			
		No additions	Vitamins less biotin*	Vitamins†	Biotin, 0.1γ per ml.
		γ	γ	γ	γ
E. coli (Gratia)	L-Aspartic	8.1	7.7	10.1	10.0
		8.3	9.2	28.4	25.8
		11.0	8.8	23.5	25.0
		9.3	10.6	24.5	23.9
		6.6	8.4	23.5	21.6
		10.9	11.2	46.5	40.1
	DL-Serine	23.0	23.0	31.9	30.5
		21.6			31.3
		25.1	25.2		46.4
		8.8			15.7
P. vulgaris	L-Aspartic	1.5			14.8
		5.9	6.5		20.3
		3.9			6.8
	DL-Serine	5.3			10.4
		14.6	14.4		27.2
E. coli (10B3)	L-Aspartic	5.4			10.4
	DL-Threonine	7.8			14.9
		6.2	6.5	12.8	12.9
B. cadaveris	L-Aspartic	3.6			8.9
		4.1	4.5		12.7
		0			7
	DL-Serine	2.9			7
		6.3	6.3	12.9	13.3
	DL-Threonine	2.2			5.8
		2.4	3.0	8.9	8.6

* Vitamins added per ml., micotinic acid 2.5γ, p-aminobenzoic acid 1γ, riboflavin 0.5γ, pantothenic acid 0.5γ, thiamine 1γ, folic acid 0.5γ, pyridoxal 5γ.
† As vitamins less biotin with free biotin added to yield 0.1γ per ml. of the reaction mixture.

(from Lichstein and Christman[22])

organism.[31]

Also, by grinding vacuum-dried preparations of cells of E. coli, it has been possible to obtain a cell-free preparation of aspartic deaminase in a partially resolved state. These preparations were not stimulated (as shown in Table VI) by biotin alone, but could be activated by biotin and adenylic acid (adenosine-5-

TABLE V

Adenylic Acid Stimulation of Aspartic Acid, Serine, and Threonine Deaminase

Conditions as for Table IV

Organism	Amino acid		Ammonia nitrogen		
		No additions	Biotin, 0.5 γ per ml.	Adenylic acid 50γ per ml.	Adenylic acid + biotin
		γ	γ	γ	γ
E. coli (Gratia)	L-Aspartic	8.3	25.5	23.8	23.1
		11.0	25.0	27.0	23.6
		8.1	10.0	10.6	10.9
		4.4	6.9	5.5	
	DL-Serine	6.3	12.1	11.6	11.9
B. cadaveris	L-Aspartic	0	5.7	5.3	
		0	7.0	12.1	
	DL-Serine	12.6	12.6	20.9	
		6.5	10.9	10.8	
	L-Aspartic	6.8	9.2	10.1	
	DL-Threonine	4.4	8.8	8.8	
	L-Aspartic	6.6	9.9	8.6	
	DL-Serine	4.8	7.3	7.0	
	DL-Threonine	4.2	7.0	6.2	
P. vulgaris	L-Aspartic	3.9	6.8	6.8	
	DL-Serine	7.7	12.1	11.5	

(from Lichstein and Christman [22])

phosphate), and by yeast extract. In preparations A and B, the biotin plus adenylic acid was initially as effective as yeast extract, but after refrigeration for 24 hours the preparations were activated only by the yeast extract. The activity of the yeast is not therefore attributable to its adenylic acid content. The data can be interpreted to mean that during the refrigeration the enzyme system utilizing biotin and adenylic acid for activation has been destroyed, while the yeast extract maintains its activity because it contains the preformed coenzyme. In preparations C and D the grinding alone has destroyed the ability of biotin and adenylic acid to activate the system, but yeast extract remains effective. Since ashed yeast extract was completely inactive, it was definitely shown that the stimulatory material is organic in nature. These results suggested to us that a coenzyme form of biotin does exist, that it is either more active than biotin or not assayable by S. cerevisiae, and that adenylic acid is somehow concerned with its formation.[31]

More conclusive evidence was obtained while studying the stimulation of partially resolved aspartate deaminase in Bacterium cadaveris (Figure 4). It may be seen that the stimulation produced by yeast extract continues linearly to completion, whereas that produced by biotin or adenylic acid levels off soon after the initial activation. This latter phenomenon varies somewhat with the degree and type of resolution obtained by the aging procedure, and may be due to a deficiency of the enzyme system necessary for the conversion to the coenzyme form.[23]

TABLE VI

Aspartic Acid Deamination in Cell-Free Preparations

Preparation	Treatment	Ammonia nitrogen produced				
		No. addition	Biotin, 0.05γ per ml.	Adenylic acid, 50γ per ml.	Biotin + adenylic acid	Yeast extract* 0.5 mg. per ml.
		γ	γ	γ	γ	γ
A	None	2.4	2.6	2.1	4.5	5.5
"	Refrigerated 24 hrs.	2.1	2.1	2.2	2.0	6.7
B	None	2.1			7.9	7.9
"	Refrigerated 24 hrs.	2.0			2.5	6.0
C	None	3.4	3.8	3.2	4.0	7.0
D	"	3.6	4.0	3.4	4.2	8.9

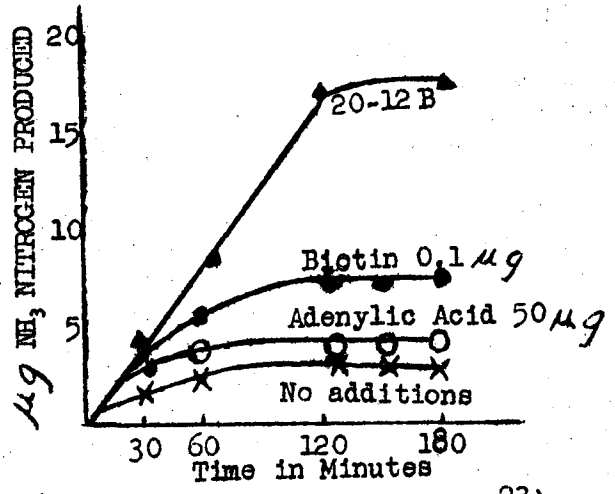
Cells of Escherichia coli (Gratia) grown 17 hours at 27° in media containing 1 per cent each of yeast extract and tryptone, 0.5 per cent phosphate, and 0.6 per cent glucose. Cells harvested by centrifugation, washed once with water, and dried in vacuo over Drierite. Dried cells ground in a ball mill in vacuo 12 hours, suspended in water, centrifuged, and clear supernatant used as cell-free juice. Reaction run at 37°, pH 7, in 0.5 M phosphate for 60 minutes; cell-free juice per tube equivalent to 20 mg. of dried cells; stopped with trichloroacetic acid, centrifuged, and an aliquot of the supernatant analyzed for ammonia by nesslerization with a Klett-Summerson photoelectric colorimeter. The increase in ammonia over the samples incubated for 60 minutes without aspartic acid was taken as an index of deamination.

* Equivalent to 0.005 γ of biotin by assay with Saccharomyces cerevisiae.

(from Lichstein[31])

Figure 4

Effect of Yeast Extract (20-12 B), Biotin, and Adenylic Acid on Aspartic Acid Deaminase in Bact. Cadaveris.



(from Lichstein and Christman[23])

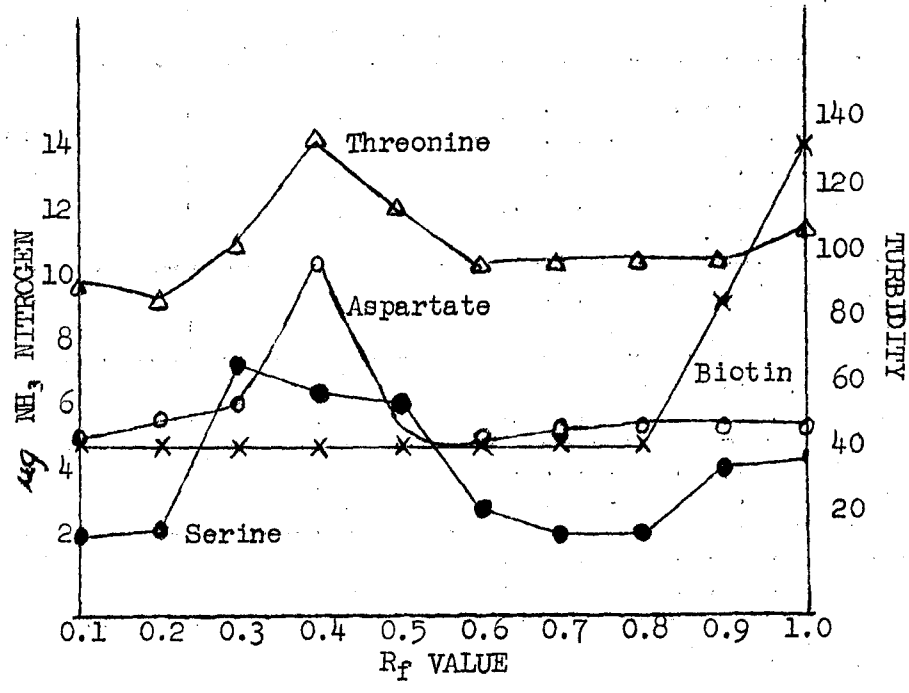
On the basis of these observations we studied the possibility of separating the coenzyme of these deaminases from yeast extract.[23] Although a variety of techniques were investigated, the most satisfactory proved to be that of paper partition chromatography employing a phenol-water mobile phase in a water saturated atmosphere. It can be seen from the data plotted in Figure 5 that biotin moves to an Rf value of approximately 0.9, while the activators of the three deaminases fall between Rf 0.3 and 0.5. Since the activators of all three enzymes fall on approximately the same spot, it was suggested that they are either closely related chemically or identical. It was concluded further that this material is relatively pure with respect to free biotin as assayed with S. cerevisiae (139), and that this organism does not respond to the coenzyme fraction. The relative stimulatory effect of coenzyme samples obtained by this technique on the three deaminases is shown in Figure 6.

What is the relation of this coenzyme to biotin? Since our assay organism, S. cerevisiae (139), did not respond to the coenzyme fraction, it was suggested that either there is notbiotin in the fraction or that the biotin is not available to this organism. The existence of bound forms of biotin, namely forms that may be microbiologically unavailable, has been demonstrated by several investigators.[32] The experimental approach to this problem resulted in the demonstration that a particular strain of yeast, S. cerevisiae (Java), responded to the coenzyme fraction in a chemically defined medium devoid of biotin, and that S. cerevisiae (139), which does not respond to the native fraction, does respond after hydrolysis (Table VII). It was concluded that either the coenzyme is a bound form of biotin or the relationship is due to contamination of the coenzyme with bound biotin of yeast extract.

The relationship of biotin to this

Figure 5

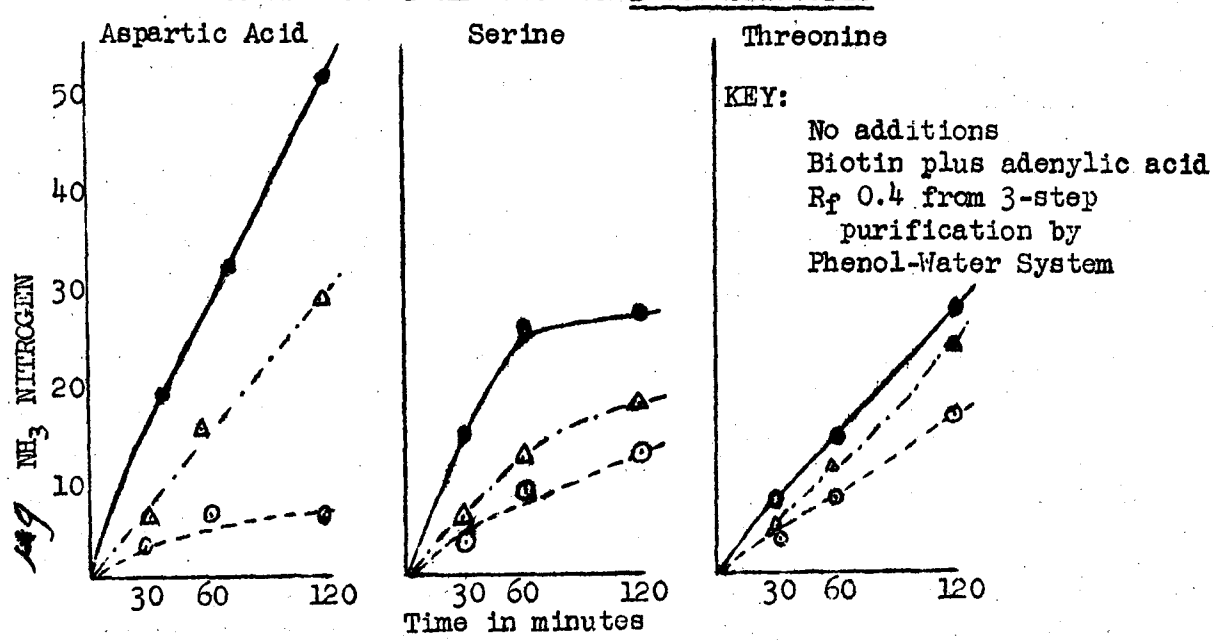
Paper Strip Chromatogram Distribution of Biotin and Coenzyme from Yeast Extract. (Phenol-water system.)



(from Lichstein and Christman[23])

Figure 6

Relative Stimulatory Effect of Several Agents on Amino Acid Deaminases in Bact. Cadaveris



(from Lichstein and Christman[23])

TABLE VII

Relative effect on growth of two strains of Saccharomyces cerevisiae by yeast extract after one-step purification on phenol-water chromatogram

Sample	Turbidity	
	S. cerevisiae 139	S. cerevisiae Java
No additions	30	30
Biotin 10^{-5} μg	32	33
Biotin 10^{-3} μg	90	90
Biotin 10^{-1} μg	239	154
Biotin 10^{1} μg	242	160
Pooled R_f 0.1, 0.2, 0.6, 0.7, 0.8.	35	40
Same hydrolyzed*	28	32
Pooled R_f 0.3, 0.4, 0.5	35	65
Same hydrolyzed*	50	48
Pooled R_f 0.9, 1.0	90	85
Same hydrolyzed*	26	25
Biotin 1 μg.	260	175
Same hydrolyzed*	145	140

* 6 n H_2SO_4, 121 C, 2 hours.

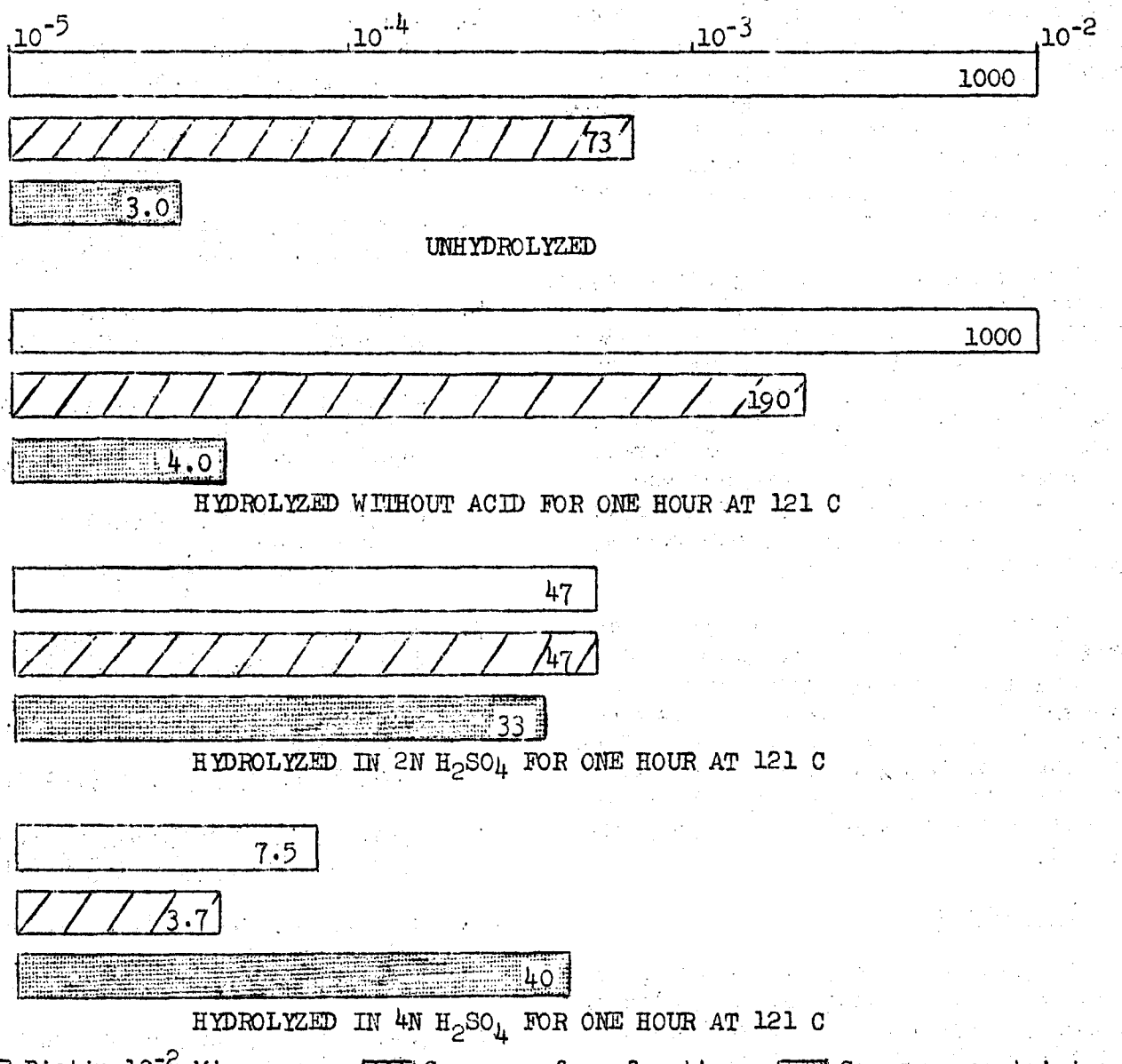
(from Lichstein and Christman[23])

coenzyme is perhaps more clearly demonstrated by the hydrolysis data given in Figure 7 for liver extract.[33] It is seen that biotin is markedly destroyed by acid concentrations of 2 to 6 N at 121° C. The coenzyme-free fractions concentrated by paper partition chromatography contain assayable biotin and exhibit a pattern following very closely the degradation shown by biotin. On the other hand, the coenzyme-containing fraction follows a very distinctly different pattern; a substance is liberated after acid hydrolysis that stimulates the growth of S. cerevisiae (139) in a medium free of biotin. It was concluded that these observations suggest strongly that a component of the coenzyme can act as a substitute for biotin and that this component is actually an acid-stable derivative of biotin that may be an intermediate in the synthesis of the coenzyme form from biotin.

The most convincing evidence that the material separated from yeast or liver

Figure 7

Biotin Assay of Liver Extract Chromatogram



(from Christman and Lichstein[33])

extract by these techniques is the biotin coenzyme was the demonstration that it stimulates partially resolved aspartic acid, serine, and threonine deaminases, and the decarboxylases of oxalacetate and succinic acid.[34] Since this material replaces biotin in five of the enzyme systems controlled by this vitamin, it would appear that it is actually the coenzyme form of biotin.

Other investigators[32, 35, 36] have isolated bound forms of biotin and one of these, namely biocytin, has been crystallized and shown to be a conjugate of biotin with the amino acid lysine.[37] The material obtained by Bowden and Peterson[32] and that investigated by Hoffmann et al.[36] have not been studied with respect to coenzymatic function. Biocytin, on the other hand, has been tested in the aspartic deaminase system, and although active as demonstrated by Wright's group, it was shown by us to be of low activity when compared to our coenzyme fraction.[38]

Although some controversy still exists, it appears quite probable that biotin functions coenzymatically in the oxalacetate decarboxylase system and in the deaminases of aspartic acid, serine, and threonine. However, the demonstration of biotin or a derivative thereof in these enzyme systems has not been made, and conclusive proof of the role of biotin in these systems may have to await such a demonstration.

Perhaps the most significant strides have been made in the partial elucidation of the bound complexes of biotin present in nature. Although it may be that these complexes are indeed different forms of bound biotin, it is also possible that they represent different stages of complexity of one native component.

4. Conclusion

The purpose of this presentation has been to review briefly the metabolic or enzymatic functions of the members of the vitamin B complex, and to suggest the fundamental importance of such knowledge. We have tried further to demonstrate the contributions which studies of the nutrition of microorganisms can make to a general understanding of vitamin function, vitamin synthesis, and the discovery of new factors, and to point out that on the basis of comparative biochemistry much of the information so obtained may be directly applicable to other more complex biological species. Biotin was discussed in detail, not only because of its importance and somewhat controversial status, but because the author is most conversant in this vitamin.

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II. MEDICAL SCHOOL NEWS

Coming Events

Jan. 18 Special Lecture; "Biochemistry of Bone Formation"; Dr. Marcel J. Dallemagne, University of Liege, Belgium; Owre Amphitheater; 4:00 p.m.
Jan. 21-25 Continuation Course in Electrocardiography for General Physicians
Jan. 22 Minnesota Pathological Society Meeting; "The Problem of Intracellular Parasitism in Brucellosis," Dr. Wesley W. Spink; Owre Amphitheater, 8:00 p.m.
Jan. 28 - Feb. 9 Continuation Course in Clinical Neurology for General Physicians and Specialists
Feb. 14-16 Continuation Course in Therapy of Cardiovascular Diseases for General Physicians

* * *

Continuation Course in Clinical Neurology

A continuation course in Clinical Neurology will be presented January 28 - February 9, 1952, at the Center for Continuation Study. The course is intended for doctors of medicine who are working in the fields of neurology, psychiatry, internal medicine, pediatrics, and general practice. Emphasis throughout the course will be placed upon the diagnosis and management of neurological disorders. Basic medical sciences will be briefly reviewed as they apply to this field of medicine. Distinguished visting physicians who will participate as faculty members for the course include Dr. Benjamin Boshes, Professor and Acting Director, Department of Neurology and Psychiatry, Northwestern University Medical School, Evanston, Illinois; Dr. H. Houston Merritt, Professor of Neurology, Columbia University, and Director of the Service of Neurology, Neurological Institute, Presbyterian Hospital, New York City; Dr. Henry G. Schwartz, Professor of Neurosurgery, Washington University School of Medicine, St. Louis, Missouri; and Dr. Harold G. Wolff, Professor of Medicine and Associate Professor of Psychiatry, Cornell University Medical College, New York City. Dr. A. B. Baker, Professor and Director, Division of Neurology, University of Minnesota, is Chairman for the course.

Brucellosis Conference

On January 25 and 26 there will be a Conference on Brucellosis at the Center for Continuation Study at the University of Minnesota. Participants include the Committee on Brucellosis of the National Research Council of which Dr. Wesley W. Spink is Chairman. Representatives who will participate in the meeting include individuals from the Mayo Clinic, the University of Iowa, the University of Wisconsin, and the University of California. Dr. M. Ruiz Castaneda of Mexico City will be present, as well as Dr. M. Kaplan of the World Health Organization of the United Nations, who is in charge of brucellosis control. The purpose of the two-day session will be to discuss basic problems relating to animal and human brucellosis. In addition, the visiting participants will have an opportunity to see the work on brucellosis that is being carried out with relation to the World Health Organization Center of Brucellosis Research, University of Minnesota, which will include the Minnesota State Laboratories of Health, the Division of Veterinary Science, and the University of Minnesota Hospitals.

* * *

Faculty News

Dr. Owen H. Wangensteen, Professor and Head of the Department of Surgery, attended the quarterly meeting of the Surgical Study Section of the U. S. Public Health Service, in Bethesda, Maryland, on January 9 and 10. Dr. Wangensteen is chairman of this section. On January 12 and 13, he attended the dedication ceremonies for the new nine-story addition to the Buffalo General Hospital in Buffalo, New York. Here he presented a paper entitled, "The Role of Research in the Modern Voluntary Teaching Hospital." His itinerary also includes Washington, D.C., where he will attend a meeting of the Committee on Growth of the National Research Council and Chicago, where he will attend a meeting of the regents of the American College of Surgeons.

III. UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
WEEKLY CALENDAR OF EVENTS

Physicians Welcome

January 21 - 26, 1952

Monday, January 21

Medical School and University Hospitals

9:00 - 9:50 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 10:50 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; W-612, U. H.

10:00 - 12:00 Neurology Rounds; A. B. Baker and Staff; Station 50, U. H.

11:30 - Tumor Conference; Doctors Kremen, Moore, and Stenstrom, Todd Amphitheater, U. H.

12:15 - 1:20 Obstetrics and Gynecology Journal Club; Staff Dining Room, U. H.

12:30 - Physiology Seminar: Observation on Venule Pressures; Francis J. Haddy; 214 Millard Hall.

1:30 - 2:30 Pediatric-Neurological Rounds; R. Jensen, A. B. Baker and Staff; U. H.

4:30 - 5:30 Dermatological Seminar; M-346, U. H.

4:30 - Public Health Seminar; 15 Owre Hall.

5:00 - 6:00 Urology-Roentgenology Conference; C. D. Creevy, O. J. Baggenstoss, and Staff; Eustis Amphitheater.

Minneapolis General Hospital

10:30 - 12:00 Tuberculosis and Contagion Rounds; Thomas Lowry; Station M.

Veterans Administration Hospital

9:00 - G. I. Rounds; R. V. Ebert, J. A. Wilson, Norman Shrifter; Bldg. I.

11:30 - X-ray Conference; Conference Room; Bldg. I.

2:00 - Psychosomatic Rounds; Bldg. 5

3:30 - Psychosomatic Rounds; Bldg. 1, C. K. Aldrich

Tuesday, January 22

Medical School and University Hospitals

8:30 - Conference on Diet Endocrines and Cancer; M. B. Visscher; Physiology Library.

Tuesday, January 22 (Cont.)

Medical School and University Hospitals (Cont.)

9:00 - 9:50 Roentgenology-Pediatric Conference; L. G. Rigler, I. McQuarrie and Staff; Eustis Amphitheater, U. H.

9:00 - 12:00 Cardiovascular Rounds; Station 30, U. H.

12:30 - 1:20 Pathology Conference; Autopsies; J. R. Dawson and Staff; 102 I. A.

12:00 - 1:30 Selected Topics, Permeability and Metabolism; Nathan Lifson; 129 Millard Hall.

3:15 - 4:20 Gynecology Chart Conference; J. L. McKelvey and Staff; Station 54, U.H.

4:00 - Pediatric Seminar; The Problem of Reading and Writing Disabilities in Children; H. O. Reynolds; Sixth Floor West, U. H.

4:00 - 5:00 Pediatric Rounds on Wards; I. McQuarrie and Staff; U. H.

4:30 - Clinical-Medical-Pathological Conference; Todd Amphitheater, U. H.

5:00 - 6:00 X-ray Conference; Presentation of Cases by General Hospital Staff; Drs. Lipschultz and Von Drashek; Eustis Amphitheater, U. H.

* 8:00 p.m. Minnesota Pathological Society Meeting; The Problem of Intra-Cellular Parasitism in Brucellosis; Wesley W. Spink; Owre Amphitheater.

Ancker Hospital

1:00 - 2:30 X-ray Surgery Conference; Auditorium.

Minneapolis General Hospital

10:30 - 12:00 Medicine Rounds; Thomas Lowry and Staff.

Veterans Administration Hospital

7:30 - Anesthesiology Conference; Conference Room, Bldg. I.

8:30 - Infectious Disease Rounds; Dr. Hall.

8:45 - Surgery Journal Club; Conference Room, Bldg. I.

9:00 - Liver Rounds; Drs. Nesbitt and MacDonald.

9:30 - Surgery-Pathology Conference; Conference Room, Bldg. I.

10:30 - Surgery Tumor Conference; Conference Room, Bldg. I.

1:00 - Surgery Chest Conference; T. Kinsella and Wm. Tucker; Conference Room, Bldg. I.

2:00 - 2:50 Dermatology and Syphilology Conference; H. E. Michelson and Staff; Bldg. III.

3:30 - 4:20 Clinical Pathological Conference; Conference Room, Bldg. I.

Wednesday, January 23

Medical School and University Hospitals

8:00 - 8:50 Surgery Journal Club; O. H. Wangensteen and Staff; M-109, U. H.

8:00 - 9:00 Roentgenology-Surgical-Pathological Conference; Allen Judd and L. G. Rigler; Todd Amphitheater, U. H.

11:00 - 12:00 Pathology-Medicine-Surgery Conference; Surgery Case; O. H. Wangensteen, C. J. Watson and Staffs; Todd Amphitheater, U. H.

12:30 - 1:20 Radio-Isotope Seminar; Specific Problems in Radio-isotope Instrumentation; D. A. Cole; 12 Owre Hall.

1:30 - Conference on Circulatory and Renal Systems Problems; M. B. Visscher; 214 Millard Hall.

5:00 - 5:50 Urology-Pathological Conference; C. D. Creevy and Staff; Eustis Amphitheater, U. H.

5:00 - 6:00 Vascular Conference; Todd Amphitheater, U. H.

5:00 - 7:00 Dermatology Clinical Seminar; Dining Room, U. H.

7:00 - 8:00 Dermatology Journal Club; Dining Room, U. H.

8:00 - 10:00 Dermatological-Pathology Conference; Review of Histopathology Section; R. Goltz; Todd Amphitheater, U. H.

Ancker Hospital

8:30 - 9:30 Clinico-Pathological Conference; Auditorium.

3:30 - 4:30 Journal Club; Surgery Office.

Minneapolis General Hospital

10:30 - 12:00 Medicine Rounds; Thomas Lowry and Staff.

Veterans Administration Hospital

8:30 - 10:00 Orthopedic X-ray Conference; Conference Room, Bldg. I.

8:30 - 12:00 Neurology Rehabilitation and Case Conference; A. B. Baker.

2:00 - 4:00 Infectious Disease Rounds; Main Conference Room, Bldg. I.

4:00 - 5:00 Infectious Disease Conference; W. Spink; Conference Room, Bldg. I.

7:00 p.m. Lectures in Basic Science of Orthopedics; Conference Room, Bldg. I.

Thursday, January 24

Medical School and University Hospitals

9:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

Thursday, January 24 (Cont.)

Medical School and University Hospitals (Cont.)

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Todd Amphitheater, U. H.

12:30 - Physiological Chemistry Seminar; Chemical Structure of Insulin; M. Goldfine; 214 Millard Hall.

1:30 - 4:00 Cardiology X-ray Conference; Heart Hospital Theater.

4:00 - 5:00 Physiology-Surgery Conference; Todd Amphitheater, U. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling W. Hansen and Staff; E-534, U. H.

5:00 - 6:00 X-ray Seminar; Report of Meeting of Radiological Society of North America; Eustis Amphitheater, U. H.

7:30 - 9:30 Pediatric Cardiology Conference and Journal Club; Review of Current Literature 1st hour and Review of Patients 2nd hour; 206 Temporary West Hospital

Minneapolis General Hospital

10:30 - 12:00 Medicine Rounds; Thomas Lowry and Staff;

1:30 - 3:00 Clinical Medical Conference; Thomas Lowry; Main Classroom.

Veterans Administration Hospital

8:00 - Surgery Ward Rounds; Lyle Hay and Staff; Ward 11.

9:15 - Surgery Grand Rounds; Conference Room, Bldg. I.

11:00 - Surgery Roentgen Conference; Conference Room, Bldg. I.

Friday, January 25

Medical School and University Hospitals

8:30 - 10:00 Neurology Grand Rounds; A. B. Baker and Staff; Station 50, U. H.

9:00 - 9:50 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U. H.

10:30 - 11:50 Medicine Rounds; C. J. Watson and Staff; Todd Amphitheater, U. H.

10:30 - 11:50 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Department, U. H.

11:45 - 12:50 University of Minnesota Hospitals Staff Meeting; Roentgen Diagnosis of Benign Gastric Ulcer -- Preliminary Report of 133 Cases; Harold O. Peterson and C. J. Corrigan; Powell Hall Amphitheater.

1:00 - 2:50 Neurosurgery-Roentgenology Conference; W. T. Peyton, Harold O. Peterson and Staff; Todd Amphitheater, U. H.

Friday, January 25 (Cont.)

Medical School and University Hospitals (Cont.)

2:00 - 3:00 Dermatology and Syphilology Conference; Presentation of Selected Cases of the Week; H. E. Michelson and Staff; W-312, U. H.

3:00 - 4:00 Neuropathological Conference; F. Tichy; Todd Amphitheater, U. H.

3:30 - 4:30 Advanced Neurophysiology Seminar; E. Gellhorn; 111 Owre Hall.

4:00 - 5:00 Dermatology Seminar; W-312, U. H.

5:00 - Urology Seminar and X-ray Conference; Eustis Amphitheater, U. H.

Ancker Hospital

1:00 - 3:00 Pathology-Surgery Conference; Auditorium.

Minneapolis General Hospital

8:00 - Pediatric Allergy Rounds; Dr. Nelson; 4th Floor.

11:00 - Pediatric Rounds; F. H. Top; 7th Floor.

11:00 - Pediatric-Surgery Conference; Drs. Wyatt and F. H. Adams; Classroom, Sta. I.

12:00 - Surgery-Pathology Conference; Drs. Zierold and Coe; Classroom.

1:30 - Pediatric Rounds; R. Ulstrom, 4th Floor.

Veterans Administration Hospital

10:30 - 11:20 Medicine Grand Rounds; Conference Room, Bldg. I.

1:00 - Microscopic-Pathology Conference; E. T. Bell; Conference Room, Bldg. I.

1:30 - Chest Conference; Wm. Tucker and J. A. Meyers; Ward 62, Day Room.

3:00 - Renal Pathology; E. T. Bell; Conference Room, Bldg. I.

Saturday, January 26

Medical School and University Hospitals

7:45 - 8:50 Orthopedic X-ray Conference; W. H. Cole and Staff; M-109, U. H.

9:00 - 10:30 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amphitheater, U. H.

9:15 - 10:00 Surgery-Roentgenology Conference; J. Friedman, O. H. Wangensteen and Staff; Todd Amphitheater, U. H.

9:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; Heart Hospital Amphitheater.

Saturday, January 26 (Cont.)

Medical School and University Hospitals (Cont.)

10:00 - 11:30 Surgery Conference; Todd Amphitheater, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff; Station 44, U. H.

11:30 - Anatomy Seminar; The Pathologic Anatomy of Body Irradiation, Albina Yakaitis; Effects of Inanition upon Distribution of Lymphocytes in the the Gastro-Intestinal Epithelium of the Hamster, Roland Meader; 226 Institute of Anatomy.

Minneapolis General Hospital

11:00 - 12:00 Medical-X-Ray Conference; O. Lipschultz, Thomas Lowry, and Staff; Main Classroom.

Veterans Administration Hospital

8:00 - Proctology Rounds; W. C. Bernstein and Staff; Bldg. III.

8:30 - Hematology Rounds; P. Hagen and E. F. Englund.

* Indicates special meeting. All other meetings occur regularly each week at the same time on the same day. Meeting place may vary from week to week for some conferences.