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Metabolic disorders in hepatolenticular degeneratio

METABOLIC DISORDERS IN HEPATOLENTICULAR DEGENERATION

Andrew Huvos,
BUSM III

Mr. Huvos

COMMENTARY ON THIRD YEAR THESIS BY A. HUVOS

Mr. Huvos, in choosing the "Metabolic Disorders in Hepatolenticular Degeneration, has proven himself a capable and critical reviewer of a complicated biochemical disorder.

The reviewer has successfully maneuvered his way through the vast literature sorting out the theoretical from the actual, while tracing the concepts of the evolution of this disorder. The following points are worthy of mention, specifically:

1. The choice of one facet of a complicated subject, has been strictly followed, which obviates the confusion which frequently ensues if many facets are discussed.

2. The review is well planned in that the two major parts, amino-aciduria and copper metabolism are discussed separately, then as interrelated to each other in the pathogenesis of the disease.

3. While discussing each segment separately, the progress and contributions of various investigators are chronologically presented.

4. Using major and minor divisions of the two segments has successfully kept the reader constantly aware of the aims of the paper.

5. Unquestionably the relevant literature has been searched.

6. The reviewer has shown the ability to break down and resynthesize the material.

Mr. Huvos has shown an understanding of the metabolic defects in this disorder beyond his stage in medicine. There are a few points in the paper which could be improved upon:

1. The introduction needs to have some of the longer, rambling sentences rewritten. They do not read smoothly.

2. One does not need to include a sentence such as "In order to enable the reader to check the validity of criticisms and conclusions offered or to note the possible omissions of such, -- etc." in a statement about the bibliography. The bibliography is obviously for that purpose.

3. When a reviewer is expressing his opinion of the concepts of those who have done extensive research in a field, he should not flatly state that the investigator is incorrect, but should <u>suggest</u> his own interpretation.

Specifically, there are also one or two points that should be checked by Mr. Huvos:

1. The work of Cooper, Davidson, et al, Reference No. 7, should be reviewed. (page 8, paragraph 1 of the review) The authors state, and I believe their figures show, that the degree of urinary amino-aciduria is not at all an inconstant factor, while the blood levels are. There may be a low or normal blood level with amino-aciduria.

2. In the discussion of the appearance of Copper in bile, no mention is made by the reviewer of one important part of the experiment of van Ravestryn, e.g. collection of bile through a T-tube before and after administration of Copper.

3. It might be expected that ciruloplasmin levels are elevated in cirrhosis, since the total globulin fraction is usually increased.

I should be glad to discuss the paper with Mr. Huvos.

R. Aycock, MD

Grade 95

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METABOLIC DISORDERS
IN HEPATOLENTICULAR DEGENERATION

A. INTRODUCTION

This thesis is written on a subject about which a great deal is yet unknown. Although the disease, hepatolenticular degeneration, has been described in 1912, and an extensive literature exists on its clincal manifestations, it is only within the last decade, that advances in biochemistry and a better understanding of human metabolism enabled investigators to uncover some of the factors that underlie the outward functional and structural picture of the disease.

The problem proved to be too complex to be completely solved in less than 10 years, Relative scarcity of material, due to the low incidence of the disease, technical difficulties in dealing with trace elements, requiring an accuracy of chemical determinations in the order of micrograms, have so far defied an all-out solution. The body of knowledge is constantly being extended by new discoveries, leading to the formulation of new hypotheses and the discarding of some of the older ones. Various workers investigate different aspects of the problem, often unrelated to each other on appearance.

In view of this, a comprehensive review cannot be restricted to a condensation of the literature. It must, in addition, try to unify the diverse findings and hypo-

theses as much as possible and permissible without omitting anything or getting involved in undue speculation. Such unifying presentation was attempted in this paper by -rather than taking up and discussing the different articles in an additive manner- breaking the subject down to its component parts, critically reviewing the various contributions -factual and theoretical- to each of these parts, and finally correlating these units with one another. A chronological basis is used, to show how the concepts took their present day forms.

As the title indicates, the discussion is confined to the biochemical and metabolic abnormalities found in hepatolenticular degeneration. Other aspects of the disease will be touched upon very lightly, only to an extent that is necessary for the better understanding of the main topic. In order to enable the reader to check the validity of criticisms and conclusions offered, or to notice the possible omission of such, important observations and experiments will be outlined in some detail with regard to number of subjects studied, normal range of values vs. those found under pathological conditions, etc.

While covering of the English literature is believed to be complete, only those articles being omitted that are abstracts of the ones included in the bibliography, the foreign literature, that fortunately has a historical interest only, was reviewed from secondary, English sources and is contained in a separate, supplementary bibliography. To

make the paper more readable, articles will be referred to by using the name of the first author only, the names of co-authors appearing in the bibliography.

B. HEPATOLENTICULAR DEGENERATION[1,2,3]

Hepatolenticular degeneration is a progressive, familial cirrhosis of the liver, affecting nearly all the siblings in one generation, which in some members of the family is associated with tremor and rigidity. While the cirrhosis is the primary process, having as its essential basis a subacute hepatitis leading to the cirrhosis, it usually gives rise to mild digestive symptoms only. In most cases the clinical picture is dominated by the nervous symptoms which follow either one of two general patterns, according to which the disease can be subdivided into two chief types.

The so-called "progressive lenticular degeneration of Wilson" is characterized by rigidity and distortion of posture, with or without tremors. It appears in adolescence and runs an acute course of a few months' to 1 or 2 years' duration. The brain shows bilateral softening of the lenticular nuclei, the putamen in particular, that may reach the stage of cavitation. In the so-called "pseudosclerosis of Westphal-Strümpell" the predominant symptom is a tremor of a peculiar type, consisting of rhythmic flexion-extension at the wrist joints when the arms are extended, the "wing-beating" tremor. The predominant sign is a zone of golden-brown pigmen-

tation near the outer margin of the cornea, called Kayser-Fleischer ring. This ring is present only in about 60 % of the cases of the progressive lenticular variety, while it is a constant feature of the pseudosclerotic type. This latter appears between the second and fourth decades of life and runs a chronic course of several years' duration. The brain shows only microscopic alterations, consisting of widespread proliferation of astroglia especially in the region of the lenticular nuclei. This glial change is also present in progressive lenticular degeneration. Much more common than the two extreme types of disease just described, are intermediate cases, showing a mixture of "progressive lenticular" and "pseudosclerotic" elements, both clinically and pathologically.

As a rule, signs of portal obstruction such as ascites and hematemesis occur only late in the disease, after the appearance of the nervous symptoms, although a few patients die before that, presenting only the earliest signs of gliosis in the brain. Cirrhosis of the liver is an invariable pathological finding. It is of a coarsely nodular type without any apparent specificity. Still, the presence of cerebral degeneration and corneal pigmentation, unencountered in other forms of hepatic cirrhosis, indicate that the underlying disorder in hepatolenticular degeneration is highly specific.[1]

This paper is written on these underlying factors. Two of them have been discovered so far: an aminoaciduria and a disturbance of copper-metabolism.

C. AMINO-ACIDURIA IN HEPATOLENTICULARDDEGENERATION

I. Demonstration of the amino-aciduria.

Amino-aciduria in hepatolenticular degeneration was discovered in 1948, by Uzman[3]. The investigation was made in an effort to determine whether a disorder of amino-acid metabolism exists and might cause the cirrhosis in hepatolenticular degeneration, since it is known that such disorder, particularly a lack of cystein, may lead to a fine lobular cirrhosis in experimental animals[3,4].

Uzman studied a patient suffering from a chronic form of the disease, in whom liver function tests and liver biopsy showed only a minimal liver involvement. Examination of the urine for amino-nitrogen /by Soerensen's formol titration and by Van Slyke's gasometric ninhydrin method/ over a 2-week period, revealed a daily amino-nitrogen excretion of 790 to 1560 mg, while normal subjects, tested with the same methods, were found to excrete 100 to 200 mg of amino-nitrogen per day, only.

Confirmation of this observation came fast from various workers, and up until today a total of over 30 cases have been reported[5,6,7,8,9,10,11,12,13,14,15,16,17,18]. Even more significant than this relatively large number of confirmatory studies, is the fact that amino-aciduria was found absent in only one reported case, in Cumings' series of 4 patients[11], which, according to Denny-Brown can probably be attributed to a day-to-day fluctuation in the urinary amino-nitro-

gen level, such as this latter author found in 1 of his 5 patients[12], and that Cooper also observed[7].

This evidence indicates that amino-aciduria is a constant feature of hepatolenticular degeneration.

II. Characteristics of the amino-aciduria.

1. Relationship to the two types of the disease.

As it was stated earlier, two, more or less distinct types of hepatolenticular degeneration are recognized clinically: the more acute progressive lenticular degeneration and the more chronic pseudosclerosis. Since Uzman made his discovery in a patient suffering from the pseudosclerotic type of the disease, it may be asked whether amino-aciduria is a feature of lenticular degeneration too. While most workers do not specify which type of the disease they are dealing with, Cooper[7] reports in 6 of his cases patients with both varieties of the disorder, who all had amino-aciduria of a similar nature and degree.

2. Nature of the amino-acids involved.

In certain metabolic abnormalities, such as cystinuria, a single amino-acid is excreted in excess, while the excretion of others is apparently undisturbed. It had to be determined whether the excess amino-nitrogen appearing in the urine in hepatolenticular degeneration represented one, a a few, or many amino-acids.

Uzman[3] showed by means of paper partition

chromatography that the urine of his patient contained at least 10 to 12 different amino-acids, none of which was present in excess over the others. Eckhardt[5] studied 5 patients, using microbiological methods, and found that each of the essential amino-acids were excreted in larger than normal amounts. Cooper[7] demonstrated that while all the amino-acids normally found in the urine are present in increased quantities, the excretion pattern of the 10 essential acids is roughly the same in patients as in normal controls /6 cases/. Similar results were reported by de Verdier[8], Matthews[14] and Bearn[16]. Regarding the quantitative excretion of individual amino-acids, the few data available are too contradictory to allow any conclusions. However, it can be said with certainty, that the possibility of a metabolic defect involving the excretion of a single amino-acid can be excluded.

3. Relationship to blood amino-acid concentrations.

As it will be pointed out later, from the standpoint of determining the causative mechanism of aminoaciduria it is very important to know whether or not it is accompanied by elevated blood amino-acid concentrations. Most authors state that a hyperamino-acidemia is not present in hepatolenticular degeneration. However, a review of the data warrants more caution.

Uzman found the blood amino-nitrogen levels slightly elevated[3]. Eckhardt[5] also obseved slightly elevated

plasma values /figures not given/. Cooper[7], investigating 6 patients found the range of fasting plasma amino-nitrogen levels 3.3 to 5.7 mg %, against a normal range of 3.1 to 4.7 mg. Simultaneous urine and plasma determinations showed that the urinary amino-nitrogen was consistently high, while plasma levels were "usually" -but not always- within normal limits. Cumings reported the results of serum studies in 4 patients as normal in two, and "slightly elevated" in two others[11]. Matthews[14] observed normal serum levels in 2 patients.

I think that all one can say safely on basis of these data is that <u>in hepatolenticular degeneration there is no gross hyperamino-acidemia</u>, such as seen in terminal liver failure, for example. However, to conclude that blood amino-acid levels are normal would be erroneous in view of the inconsistency of the findings, that do not allow for ruling out a slight but significant elevation. The importance of these considerations will be seen in the section dealing with the possible causes of amino-aciduria.

4. <u>Relationship to protein intake and urine volume</u>.

Uzman[3] observed a slight decrease in the degree of amino-aciduria by placing the patient on a low protein diet, however this change could have been due to a day-to-day variation in the urinary amino-nitrogen level, unrelated to the diet. Cooper[7] calculated the theoretical daily fasting amino-acid excretion of 6 patients, by determining the 1-hourly fasting excretion and multiplying that by 24. The average cal-

culated value accounted for about 100 % of the observed daily excretion, and it was concluded that dietary intake and post-prandial rises in the blood amino-acid concentration contributed little if anything to the amino-aciduria.

I question the validity of this conclusion. Looking at the individual data rather than their average, one sees that there is a great variation from patient to patient, so that the calculated value accounts for 50 to 230 % of the observed daily excretion. In view of such variation, the mean is hardly significant and method of calculation itself is probably not well grounded.

The important point here is not the extent to which daily protein ingestion contributes to the amino-aciduria, but rather that the contention that such contribution does not exist is unjustified. Indeed, experiments by Cooper himself[7] and Matthews[14], consisting of feeding amino-acids to patients, showed a definite rise in the amino-acids excreted in the urine, indicating that post-prandial elevations of blood levels do increase the amino-aciduria. The implications of this on the mechanism of amino-aciduria will be discussed later.

Urine volume was shown to have no effect on the degree of amino-aciduria.[7]

5. Relationship to the duration of the disease and degree of hepatic involvement.

Although the magnitude of amino-aciduria varies considerably in individual cases, it is unrelated to either of these two factors.[5,7,9,11,13,15,17]

III. Mechanism and cause of the amino-aciduria.

Having reviewed the characteristic features of amino-aciduria in hepatolenticular degeneration, two more subjects must be considered before discussing its possible cause: normal amino-acid excretion and amino-aciduria in general.

1. Normal renal excretion of amino-acids.

Amino-acids, liberated by digestion of dietary proteins, are absorbed from the intestin into the portal vein, and are distributed in the general circulation[19]. Reaching the kidneys, they filter through the glomerulus, being present in the glomerular filtrate in the same concentrations as in plasma. But the urinary loss is very small, about 100 to 200 mg daily, thus an extensive tubular reabsorption must take place. Very little is known about the tubular reabsorption mechanism in man. It may or may not involve deamination and transamination; probably the same mechanism operates for several amino-acids if not for all, which thereby show mutual interference, the concentration of one affecting the reabsorption of others. The value of Tm /maximal rate of tubular reabsorption/ is not known. Most experimental has been carried out on dogs, and as Neuberger[20] points out, the capacity for reabsorption being variable from species to species, it is not permissible to assume that tubular reabsorption in man resembles that of the dog.

2. Amino-aciduria in general.

Amino-aciduria may result from 3 possible mechanisms according to Brick[21] and can be classified as pri-

mary or secondary. Secondary amino-aciduria is one where an etiologic factor can be ascertained, while such factor is not demonstrable with our current knowledge in primary amino-aciduria. The amino-aciduria /primary or secondary/ may be general, the excretion of all amino-acids being increased, or specific, involving only one acid.

Regarding the 3 mechanisms mentioned above, two may be encountered in secondary amino-acidurias. In the so-called "overflow" type, amino-acids get into the urine as a consequence of abnormally high blood concentrations. This may result from hepatic insufficiency with a failure of deamination, or from increased protein catabolism as in muscular atrophies, burns, etc. Another type of secondary amino-aciduria is characterized by normal blood levels, and results from extensive renal tubular damage with failure of reabsorption, as in tubular necrosis. In primary amino-acidurias a third mechanism is operating, which occurs with normal blood levels and with no demonstrable renal lesion. Cystinuria, phenylpyruvic oligophrenia and tyrosinosis are examples of the "specific" subdivision of this class, while the de Toni-Fanconi syndrome may be classified as primary general amino-aciduria.

3. <u>The amino-aciduria of hepatolenticular degeneration</u>.

The above classification serves as a framework for defining the causative mechanism of amino-aciduria in hepatolenticular degeneration. In its terms, two questions must be answered:

a. Is it a primary or secondary amino-aciduria?

b. If it is secondary, what condition is it secondary to? -or:
If it is primary, where does the "biochemical lesion" lie?

a. Primary vs. secondary amino-aciduria.

To settle this question, the possibility of secondary amino-aciduria was to be ruled in or out.

Uzman[3] found no evidence for renal involvement in his patient, this finding was also confirmed by subsequent workers, carrying out repeated urinalyses and renal function tests. Similarly, no patient showed any clinical evidence of increased protein breakdown. Thus it became clear, that if amino-aciduria was "secondary" to anything, it was to the cirrhosis occuring in the disease.

It is known that in cases of severe hepatic involvement of any kind, amino-acids appear in the urine in excessive quantities, indicating presumably a failure of deamination by the diseased liver. Earlier in this discussion it was pointed out that the magnitude of amino-aciduria in hepatolenticular degeneration was unrelated to the severity of the liver damage. Still, since the actual stage of liver disease in which failure of deamination begins to occur is unknown, it seemed possible that such failure preceeded and caused the amnio-aciduria. One would not expect to see normal blood levels if this were the case,-however, it will be recalled that the presence of a slight hyperamino-acidemia could never be satisfactorily excluded, and it is known[22] that in certain instances a very

small increase in blood concentrations may result in a renal "overflow". As a result, liver disease as the cause of the amino-aciduria could not be "a priori" excluded. In order to do that, one needed:

i. To demonstrate the absence of amino-aciduria in /early/ liver disease other than hepatolenticular degeneration;

ii. To demonstrate the presence of amino-aciduria in people showing no evidence of the disease, but who have a good chance of developing it later in their lives.

The second requisite could be answered /theoretically at least/ because of the familial nature of the disease. As it was stated earlier in this paper, nearly all siblings in one generation /though not in the preceding one/ are affected. Efforts were directed toward finding a large family suffering from the disease, in which the /yet/ unaffected members could be investigated for having or not having amino-aciduria. Hood[9] in 1951 reported from Sweden a family of 10 siblings /5 girls and 5 boys/, 3 of which died of hepatolenticular degeneration and 1 of cirrhosis of the liver, at the ages of 8 to 13. The living children /3 girls and 3 boys/ ranged from 8 to 22 years of age. One of them showed at the time of investigation clinical and laboratory evidence of liver dysfunction, without any neurologic signs or Kayser-Fleischer ring. All other children were totally asymptomatic. Examination of the urine for amino-nitrogen revealed that the child with liver involvement excreted 430 to 1030 mg of amino-acids daily, and

that 4 of the 5 symptomless children had an amino-aciduria with values ranging from 204 to 384 mg daily. Only one child /the oldest one, incidentally/ showed no sign of excessive urinary amino-nitrogen excretion. The mother and a child resulting from a previous union of the mother were completely normal.

Although this is the only study of its kind recorded so far -other authors could find no abnormality in the siblings of some of their patients[7]- on its basis the possibility of the amino-aciduria being secondary to hepatic insufficiency appeared highly unlikely. Further evidence against this explanation came from workers investigating the other aspect of the problem, i.e. the presence or absence of amino-aciduria in liver diseases other than hepatolenticular degeneration.

Eckhardt[5] studied urinary amino-acid excretion in 8 normals, 5 cases of hepatolenticular degeneration and 7 cases of other liver diseases /alcoholic cirrhosis, hemochromatosis and subacute yellow atrophy/. Although the last group showed evidence of severe liver involvement their daily amino-nitrogen excretion averaged 175 mg, falling within the normal range /120 to 200 mg in this series/, while patients with hepatolenticular degeneration excreted an average of 400 mg daily. Cooper[7] compared 6 patients with 6 cirrhotics in a "stabilized" state, having considerable hepatic derangement. Results showed that even in these advanced cases of cirrhosis amino-acid excretion was not increased above normal, while it definitely was in patients with hepatolenticular degenera-

tion whose liver function tests showed little or no impairment. Cumings[11] found no amino-aciduria in a patient with non-specified liver dysfunction, in contrast with 3 cases of hepatolenticular degeneration. The absence of any contradicting report among investigators should be emphasized here. This is important in evaluating studies on a disease of rare occurence, where significance of findings has to be measured in terms of use of controls, adequacy of laboratory methods, and concurrence of results by various workers, rather than in terms of number of cases alone.

It is concluded that the amino-aciduria of hepatolenticular degeneration is not secondary to the cirrhosis occuring in this disease, and is different from the amino-aciduria observed in hepatic failure.[3,5,7,13,14]

A logical counterpart of this issue had to be considered: If amino-aciduria cannot be attributed to the cirrhosis, could it represent the other lesion in hepatolenticular degeneration,-neuronal degeneration?

Porter[6] in 1949 studied the urinary amino-acid excretion in the following diseases characterized by neuronal degeneration: hepatolenticular degeneration, Huntington's chorea, paralysis agitans, dystonia musculorum deformans and familial spastic paraplegia - 1 case of each. Except for hepatolenticular degeneration, in which an average of 350 mg of daily urinary amino-nitrogen excretion was found, in none of the conditions was the normal range /average: 120 mg daily/ exceeded.

Entirely similar results were found by Spillane[15] who investigated 1 case of hepatolenticular degeneration, 4 Huntington's chorea, 6 dystonia musculorum deformans, 2 congenital choreoathetoses and 9 normals. /All of Spillane's values, including the normal ones, are higher than those reported by other workers, due to the fact that he used the method of Albanese and Irby, which, as Matthews[14] points out, gives higher figures than either Sorensen's formol titration or Van Slyke's gasometric ninhydrin method, used by the other investigators/. Thus no correlation was found between amino-aciduria and neuronal degeneration "per se".

The first of the two questions required for defining the causative mechanism of amino-aciduria can then be answered: It is a primary amino-aciduria, not caused by any of the known mechanisms giving rise to secondary amino-aciduria.[21]

b. The site of the "biochemical lesion".

This second question is a much more difficult one to answer, and at this moment the answer is unknown. The hypotheses proposed will be discussed now, together with their experimental support, but their hypothetical nature should be constantly realized.

The first hypothesis was introduced by Eckhardt[5] and Copper[7], and postulated "a renal mechanism of undetermined type operating, which allows increased spillage of amino-acids in the fasting state but which normally accomodates increased loads of amino-acids to the kidneys, as following the

the ingestion of food or the infusion of amino-acids." Incrimination of the kidney as the site of the abnormality was arrived at by simple exclusion. From the lack of correlation between the severity of liver disease and the degree of amino-aciduria it was inferred -as well as from the absence of amino-aciduria in other kinds of cirrhosis- that the liver could be excluded as the site of the lesion. Next, a suggestion of Uzman[3] was disproved, according to which the defect lay in the enzyme-system involved in the utilization of amino-acids, leading to the excretion of those acids that could not be used up.

Patients and normal controls were infused intravenously with a mixture of amino-acids. Plasma amino-nitrogen determinations showed that the infusion resulted in nearly identical rise and fall of amino-acid concentrations in patients and normals. If there were a defect of cellular absorption and deamination of amino-acids, one would expect that following a rapid infusion plasma concentrations would rise higher and return to previous levels more slowly than in normals. Similarly, no difference was found between the two groups regarding the intestinal absorption and disappearance from the blood of orally administered glycine and glutamic acid. Thus, by exclusion, the existence of a lowered renal threshold for amino-acids was postulated.[7]

"Lowered renal threshold" means a reduction of the level of tubular reabsorption beyond which no further material is reabsorbed. Eckhardt and Cooper thought that this

pattern -the only one known in renal physiology for lowering the renal threshold- could not operate in hepatolenticular degeneration, and had to invoke a "mechanism of undetermined type"[5,7] to sustain their concept. The reason for doing so was this: If such pattern were in operation, an elevation of blood amino-acid levels would result in increased amino-aciduria. This was thought not to take place in hepatolenticular degeneration, because it seemed to contradict two observations:

i. That post-prandial rises of blood amino-acid concentrations had no influence on urinary amino-acid excretion.

ii. The infusion experiment referred to earlier. Examination of the urine for 4 hours following the infusion of 500 cc of a 10 % solution of amino-acids revealed that there was no significant difference between patients and normal controls in the percentage of excretion of the infused acids. This was interpreted as showing that the patients' tubular reabsorption is not reduced, when it has to "accomodate increased loads".[5,7]

Since subsequent work showed that the amino-aciduria can be explained by a defect of tubular reabsorption /see below/ without invoking any mechanism not already known in kidney physiology, one must try to account for the above observations. I already discussed the first one and indicated its dubious validity, cited experiments showing that ingestion of amino-acids does increase the degree of amino-aciduria /page 9/. Regarding the infusion experiment, I suggest that the identical results in patients and normals can be ex-

plained by postulating, instead of a non-reduced tubular reabsorption in the patients, the reaching of the maximal tubular reabsorption capacity or Tm in both groups during the infusion. Cooper[7] recognized this possibility, but termed it unlikely in view of Wright's experiments[23] who found that in dogs the plasma levels following the intravenous administration of amino-acid mixtures are too low to reach the Tm. However, this is not sufficient evidence against its possible occurence in man. Indeed, Uzman[3] in a different connotation, but referring to the same experiments, writes: "Recent renal clearance estimation of amino-acids on dogs are certainly not in accord with observed facts in normal humans, since the normal adult excretes about 15 mg arginine daily, and normal urine gives a strong reaction for histidine, whereas in dogs, even with plasma levels of up to 10 to 50 times that of the post-absorptive state, there was no evidence that the Tm for histidine had been reached, while the Tm for arginine was estimated as 11 mg per minute."

The validity of the "renal hypothesis" was demonstrated by Matthews[14] in 1952. Two patients and normal controls received orally large amounts of glycine and alanine. The urine was examined during the next 8 hours, determining the percentage excretion of the ingested acids. It was found that while the normal group excreted about 8 % of the administered glycine and 18 % of the alanine, the urine of the patients contained 16 and 28 % respectively of the total dose of these two substances. /Doses were the same for the two groups on a mg per

kg of body weight basis/. Thus, by using different methods, results, that Eckhardt[5] and Cooper[7] expected but did not get, were obtained. The reason for this might lie in the fact that individual amino-acids rather than a mixture of them were used. But maybe a more likely possibility is, that, as I suggested, during the infusion experiment the Tm for amino-acids had been reached, while this did not occur after oral administration. Indeed, plasma amino-nitrogen levels after the infusion were over 20 mg %[7], while they did not exceed 12 to 13 mg following the ingestion of glycine and alanine[14].

Matthews[14] also presents direct evidence that the increased amino-acid excretion is due to a diminished tubular reabsorption. Amino-nitrogen clearance values at different serum levels were determined in both patients and normal individuals, and simultaneously the creatinine clearance, representing the glomerular filtration rate, was measured. By expressing the amino-acid clearance as a percentage of the creatinine clearance, the fraction of amino-acids filtered by the glomerulus and not reabsorbed by the tubules was calculated. This clearence ratio was found to be statistically significantly higher /more than 2 standard deviations/ in patients than in normals at all serum levels ranging from normal to about twice the normal value. Thus, a <u>decreased renal tubular reabsorption of amino-acids in hepatolenticular degeneration</u> was demonstrated.

This however does not prove that the "biochemical lesion" is a defect in the renal tubular epithelium,

although that would be the simplest explanation. Uzman on the other hand considers the lowered renal threshold for amino-acids "a descriptive term rather than an explanation of the 'modus operandi'"[13]. This hypothesis stems from his discovery of abnormally large amounts of peptides in the urine of the members of the Scandinavian family, originally reported by de Verdier[8] and Hood[9], showing amino-aciduria. In addition to their being present in excessive quantity, these peptides differed from those normally found in the urine, by 80 to 90 % of them containing aspartic or glutamic acid residues at the ends of the peptide chains. /normal urinary peptides contain a random distribution of amino-acids/. The selective nature of the peptides excreted is interpreted as an argument against both the peptiduria being a concommitant of the generalized amino-aciduria, and the primacy of a renal tubular defect. Indeed, if the latter mechanism operated, one would expect all of the normally present peptides to be spilled in the urine in an increased quantity.[13]

The following alternative is postulated by Uzman: The "biochemical lesion" is a deficiency of dicarboxylic-carboxypeptidases /i.e. enzymes splitting off glutamyl and aspartyl residues from that end of the peptide chain containing a free carboxyl-ion/ in the body tissues. This results in a high excretion of peptides having terminal dicarboxylic amino-acid residues, leading further to amino-aciduria by competitive reabsorption of such peptides in preference to, and to the partial exclusion of, free amino-acids by the renal tubules[13]. While

the suggestion is too hypothetical in nature for a discussion of any length, one or two points in its favor might be cited.
a/ A competition between individual amino-acids for tubular reabsorption is well known[20,23] /see page 10/. It is entirely conceivable that peptides also participate in this competition.
b/ Very recently the presence of these urinary peptides has been demonstrated in overt cases of hepatolenticular degeneration where they were not found previously. /As it will be recalled, the original observations were made on members of an affected family who showed no other evidence of the disease/. This apparent discrepancy was shown to be due to the fact that in the former group the peptides are combined to copper in the form of a chelate complex that does not show up on paper chromatographic studies used for their detection,- while copper being absent from the urine of the latent cases /as it is in normal individuals/, the peptides are free and detectable by that method[24]. The significance of this finding will be discussed in detail in the section dealing with copper metabolism.
Despite these points supporting it indirectly, the hypothesis lacks experimental backing, as of today, on such important issues as plasma peptide levels and tissue enzyme concentrations in diseased and normal subjects, and there can be hardly more said about it.

This sums up the present day status of the theories about the site of the"bio-chemical lesion" resulting in the amino-aciduria of hepatolenticular degeneration.

IV. The significance of the amino-aciduria.

Having reviewed the characteristics and the possible causative mechanisms of amino-aciduria, it remains to be seen the role it plays in hepatolenticular degeneration.

1. Relationship to the disease in general.

From the studies on the asymptomatic members of a family stigmatized by hepatolenticular degeneration[8,9,13] one can hardly escape the conclusion, that whatever defect underlies amino-aciduria, it occurs prior to the onset of hepatic and neurological disorder, as well as of the cupruria[13,24]. However, demonstration of the actual development of the clinically recognizable disease in these latent cases, as well as studies on more unaffected siblings of patients is needed to prove the validity of this conclusion beyond doubt.

2. Relationship to the hepatic lesion.

Himsworth[4] believes that the hepatic cirrhosis in hepatolenticular degeneration is caused by the chronic loss of amino-acids in the urine, the hepatic lesions being identical with those produced by experimental dietetic deficiency. This opinion is shared by Uzman[13] and Brick[21], and supported by the observation of the high incidence of "pure" cirrhosis in families affected by hepatolenticular degeneration.

Direct proof, however, is lacking.

3. Relationship to the cerebral lesion.

This relationship is not well understood. ~~The biochemical abnormality responsible for the brain lesion,~~ disturbed copper metabolism will be discussed in the next section of this paper.

D. COPPER METABOLISM IN HEPATOLENTICULAR DEGENERATION.

I. History.
/After Denny-Brown[12] and Matthews[14]/.

An abnormality of the metabolism of heavy metals in hepatolenticular degeneration has long been suspected, because of the pigmentation of cornea, skin and other organs, found in some cases of the disease. Rumpel[a] in 1913 observed increased amounts of silver and copper in the liver and kidney of a patient dying of pseudosclerosis. Siemerling[b] reported a a case of chalcosis -presence of cataracts of a special kind /so-called "sunflower cataracts"/, seen when a foreign body containing copper lodges in the eye- associated with hepatolenticular degeneration. He suggested that the disease was due to an accumulation of copper salts in the viscera, eyes and the nervous system. Vogt[c] considered the disease a form of argyria or silver intoxication, however Lüthy[d] pointed out that the finding of increased amounts of silver in the tissues was due to the use silver salts for the treatment of nervous diseases in that period. He and Haurowitz[e] found elevated concentrations of copper in the liver and the brain. At the same time /1930/, some doubt was cast on the significance of the hepatic findings by Herkel[f], who reported increased amounts of copper in cirrhotic livers not associated with hepatolenticular degeneration.

Maybe it is because of the inconclusive nature of these early studies that the problem became all but forgotten for the next 15 years, until 1945, when iterest was suddenly reawakened.

II. Indications of a disturbance in copper metabolism.

1. Copper contents of the liver and brain.

Glazebrook[25] described in 1945 a case of hepatolenticular degeneration with "a marked excess" of copper in the liver and the basal ganglia. No controls were used and the determinations were performed on wet tissues, a method which does not give satisfactory accuracy. The validity of the observation was demonstrated by Cumings[26] in a controlled study. The copper content of the liver in 3 cases of hepatolenticular degeneration was found to average 83.6 mg per 100 gm of dry tissue, that of 9 normal livers ranged from 3.7 to 17.2 mg. The putamen of the brain in the patients contained an average of 65 mg of copper per 100 gm of dry tissue, against a range of 6 to 12 mg obtained from 27 normal brains. Spillane[15] reports very similar results in one controlled case, although both normal and abnormal values are lower than found by Cumings.

Despite the small number of cases, the difference between patients and controls is so marked in every instance, that one may safely conclude that the copper content of the liver and brain /particularly the lenticular nucleus/ is increased in hepatolenticular degeneration.[12]

It should be pointed out however, that proof is lacking about the specificity of the condition, i.e. its absence in "pure" hepatic cirrhosis /only one recorded case[26]; normal values in the brain, slightly elevated in the liver/, and other degenerative diseases of the nervous system /no studies/.

2. Urinary copper excretion.

Much of the present day knowledge about the metabolic error in hepatolenticular degeneration is due, indirectly, to a chance observation. Mandelbrote[27] in 1948, interested by a finding that showed that two demyelinating diseases of young lambs -enzootic ataxia and swayback- were associated with copper deficiency, investigated copper turnover in a somewhat analogous human condition, multiple sclerosis. For control purposes, other chronic nervous diseases, among them one case of hepatolenticular degeneration, along with normal individuals were included in the study. Results in multiple sclerosis were entirely negative, however it was noted that the patient with hepatolenticular degeneration excreted much larger amounts of copper in the urine than any other subjects, this excretion being greatly increased, as it was in normals, too, by an injection of British Anti-Lewisite /the effects of BAL will be discussed later in this paper/. The resting urinary copper excretion was 21 ug per hour against 5 ug in normals. Following this report, confirmation of the observation was rapid, various workers describing it in a total of 25 cases up until today[6,28,11,12,16,29,14,15,30,31]. It was found absent in only one recorded case[11]. Thus the evidence is strong that urinary excretion of copper is increased in hepatolenticular degeneration.

Some data are available about the specificity of the condition. Mandelbrote[27] found no cupruria in multiple sclerosis, neuromyelitis optica, Parkinson's syndrome,

motor neuron disease and retrobulbar neuritis. Spillane[15] observed normal urinary copper excretion in four non-specified diseases of the basal ganglia, other than hepatolenticular degeneration. Thus cupruria appears to be absent in these chronic nervous disorders. On the hepatic side there is less specificity. Contrarily to Matthews' belief,[14] Bearn[29] pointed out that in many cases of cirrhosis, particularly in long-standing biliary cirrhosis, urinary copper excretion may be as high as it is in hepatolenticular degeneration.

III. Metabolism of copper.

Increased copper contents of the brain, liver and urine indicated a defective handling of the element by the organism. The exact nature of this defect is not known yet, however, several aspects of copper metabolism -normal as well as in hepatolenticular degeneration- have been elucidated.

1. Absorption and excretion.

Copper is contained in practically all foodstuffs and in drinking water. van Ravesteyn[32] and Cartwright[33] estimated ~~estimated~~ the daily copper intake around 2 to 3 mg on an average diet. ~~Since~~ The organ of absorption, the intestinal mucosa, also serves as a route of excretion, together with the bile and urine. The feces contain 1 to 3 mg, the urine 15 to 50 ug of copper daily.[32] After intravenous administration of copper salts, urinary copper excretion remains unchanged, while there is a significant increase in the fecal copper, per-

sisting for many days, according to van Ravesteyn[32], who studied copper metabolism in three normal subjects. Samples of bile, obtained by duodenal drainage, also showed definite, persistent elevation of copper concentration following the injection, which however did not seem to parallel the fecal copper levels. This is interpreted to indicate that part of the copper appearing in the feces is excreted through the intestinal wall, presumably the colon, known to excrete other heavy metals. Because of the crudeness of the biliary determinations /only 1 or 2 samples analyzed daily, total volume of bile not determined/ the relative importance of the intestinal vs. biliary excretion is not known. Radioactive tracer studies by Schubert[8] /article available only in form of an abstract[34]/ confirmed the existence of these two routes.

Since the ingested copper is excreted almost entirely through the feces, intake-and-output studies cannot reveal the extent of absorption, for it is impossible to determine what part of the fecal copper had completed an absorption-excretion circuit and what part of it results from simple passage through the intestinal tract[32]. Thus, as of today, the normal degree of copper absorption is not known. Very recently, Zimdahl[31] reported to "have shown that there is an increased intestinal absorption of copper in patients with hepatolenticular degeneration". The conclusion was based upon copper balance studies, consisting of copper determinations of the food intake and of urinary and fecal excretions, done on 3 patients and 3 normal

controls. The results showed that the patients:

a/ Excreted much smaller amounts of copper through the feces /about 40 % of the total copper intake/,than the normals /ca. 95%/.

b/ Excreted about 30 times as much copper through the urine as did normals.

c/ Were in a markedly positive copper balance /normals showed slightly positive balance/.

While the demonstration of a decreased fecal excretion of copper,wresulting in a positive copper balance in hepatolenticular degeneration is of great value, I think that the conclusion reached on its basis is unjustified. From the fore-going discussion it is clear that the explanation given by Zimdahl,increased absorption of copper, is but one of two possible interpretations of the results - the other one being a decreased excretion of the metal. Indeed, an inability to excrete copper through the bile,resulting in deposition of copper in the liver causing liver dysfunction, was already suggested by Glazebrook[25] in the paper that re-opened investigations of hepatolenticular degeneration in 1945. This supposition is no longer held, as Denny-Brown[12] found the copper concentration of the bile falling within normal range in 3 patients; also, there is evidence that the liver lesion preceeds rather than follows the disturbance of copper metabolism /see further in this discussion/. However, no work has yet been done on the intestinal phase of copper excretion in hepatolenticular degeneration,

a failure of which could account for Zimdahl's findings just as well as the proposed increased absorption.

Thus, it may be concluded, that despite the greatly increased urinary copper excretion in hepatolenticular degeneration there exists a marked copper retention, resulting from either an increased intestinal absorption or a decreased intestinal excretion -or a combination of both- of the metal.

2. Copper in the body.

After absorption into the portal system, copper is probably deposited temporarily in the liver, according to van Ravesteyn[32]. This conclusion was arrived at by failing to observe any elevation of blood copper levels following oral administration of copper salts to normal subjects, despite that absorption took place,as shown by a rise of the biliary copper following the ingestion of the salt. Up until today this is the only study of its kind on humans; most studies about the fate of copper in the body are centered around copper in the blood.

The normal range of blood copper concentration is from 70 to 150 ug per 100 ml, according to most investigators, using a variety of methods[14,16,29,32,33,35]. Somewhat higher values were obtained by Mandelbrote[27]. Since the concentration of copper in the plasma approximately equals that in the red cells[33], the results are not significantly influenced by the use of whole blood, plasma or serum as medium for determinations. The factors regulating the blood copper level are not known.

Most of the copper contained in blood is bound to protein. Mann[36] in 1938 isolated from red blood cells a copper containing protein which he named hemocuprein. Hemocuprein has a molecular weight of about 35,000 and contains 0.34 % of copper. Holmberg[37] found a similar, blue-colored protein in the serum, containing 90 % or more of the total serum copper. This protein, named ceruloplasmin because of its blue color, is an a-globulin, has a molecular weight of about 151,000 and contains 0.35 % or 8 atoms of copper per mdecule[38]. Its relation to hemocuprein is obscure, but since the latter is known to comprise 2 copper atoms per molecule and in view of the molecular weights of the two compounds, Holmberg suggests that ceruloplasmin is composed of 4 hemocuprein units. The function of ceruloplasmin is not known, ~~as~~ Like other copper containing proteins, it has enzymatic properties, such as catalyzing the oxidation of a chemical, paraphenylenediamine[39]. Scheinberg[40] found the normal range of ceruloplasmin concentration between 23.6 and 38.7 mg per 100 ml of serum, with an average of about 30 mg.

Copper content of the blood in hepatolenticular degeneration was a controversial issue for a long time. Glazebrook[25] in his original paper reported increased blood copper levels in the patient under his study. As it is not uncommon in research, this isolated piece of information has been incorporated into many subsequent articles and hypotheses[12], even recent ones[31], as a generally accepted finding /"Serum levels of the patients tend to be elevated."[31]/. Actually, in cases

where blood copper determinations were carried out, values were found to fall within normal range[41,11,14] or slightly below it[15] /total of 8 cases/. Recently, Bearn[16,29] examined 17 patients together with normal and cirrhotic controls and found the serum copper concentration to be lower than normal in 15 of them, /range: 35 to 100 mg per 100 ml; mean:60 ug/,the remaining 2 were in the low normal range /normal range: 90 to 140 ug; mean: 110 ug/. In view of this last massive evidence it seems that the copper content of the blood is decreased or normal in hepatolenticular degeneration. Glazebrook's high figure might be explained -besides technical error- by the presence of advanced hepatic lesion, in my opinion. Indeed, Matthews[14] and Bearn[16] found that in cirrhotics blood copper levels are elevated, which probably also accounts for the cupruria observed in certain cases of cirrhosis /see page 27/.

Scheinberg[40] studied the ceruloplasmin level of blood in hepatolenticular degeneration. In 8 patients the serum concentrations ranged from 4 to 18.3 mg per 100 ml, against a normal range of 23.6 to 38.7 mg. Bearn[29], employing different methods, found a similar decrease of ceruloplasmin activity in patients. Scheinberg points out that ceruloplasmin is decreased relatively to a greater extent than copper is, so that it cannot account for all of the copper present in the serum. The non-ceruloplasmin-bound copper is not dialyzable, hence it must be combined to some larger molecules, other than ceruloplasmin. Studies on cirrhotics[29,40] showed that in this condition

ceruloplasmin concentration of the plasma is increased or in the high normal range, indicating that the ceruloplasmin deficiency is not simply a reflection of the liver cirrhosis. Scheinberg[40] suggests that hepatolenticular degeneration is caused by the congenital deficiency of this specific plasma protein -such as occuring in hemophilia-,leading to the deposition of copper in the various organs, because the absorbed metal could not be held in combination by the diminished amounts of ceruloplasmin. This hypothesis will be discussed in the next section of this paper. Here, it may be concluded that ceruloplasmin content of the blood is decreased in hepatolenticular degeneration.

IV. Mechanism of the disturbance in copper metabolism.

At the present time there is no satisfactory explanation of the mechanism behind the various disturbances of copper metabolism, reviewed above. In the following, the different hypotheses offered up to now will be discussed.

Chronologically the first one was proposed by Denny-Brown[12,42], postulating an overabsorption of copper which would cause the metal to appear in the serum in excessive amounts, to be deposited in the tissues and to overflow the renal threshold. The hypothesis rested on Glazebrook's report on increased blood copper levels, which suggested an analogy between hepatolenticular degeneration and hemochromatosis. As later studies showed that serum copper concentrations were not increased and since elevated serum iron is a characteristic feature of

hemochromatosis, this analogy cannot be accepted[29]. Similarly, no renal overflow mechanism can operate, by definition, in presence of normal or decreased plasma levels, - the excessive urinary excretion of copper has to be explained by a lowered renal threshold.

Demonstration of a decreased renal tubular reabsorption of copper by Matthews[14] led to the formulation of another hypothesis. Patients with hepatolenticular degeneration and normal and cirrhotic controls were given intravenous injections of a diffusible copper salt solution. Blood copper levels returned to normal within 2 hours in all subjects, and in the controls no rise of urinary copper followed the injection. On the other hand, in the patients, who still retained about 98 % of the administered copper, urinary copper excretion increased to about four times above the resting level, indicating that the renal threshold for copper is lowered in hepatolenticular degeneration. To be decided was, what this lowered threshold represented. As it will be recalled, normally about 90 % of the serum copper is bound to ceruloplasmin, a protein of a molecucular weight sufficient to not be filtered through the glomerulus. In hepatolenticular degeneration there is no evidence for glomerular damage that would allow the escape of protein-bound copper into the urine. Hence, for copper to get through the glomerular barrier, it must be either in free state or combined to a plasma component smaller than ceruloplasmin. Matthews[14] found, that when a diffusible copper salt was added to serum -regard-

less whether belonging to normals or to patients- most of it became undialyzable through a collodion membrane, indicating that a combination with a larger molecule took place. Holmberg[38] showed that ceruloplasmin is saturated with copper, thus the substance taking up the excess copper must be different from ceruloplasmin. Matthews[14] postulated that the copper appearing in the urine is derived from the small dialyzable fraction of plasma copper, which would be bound to amino-acids in the form of a chelation complex. Cupruria, then, would be attributable to the decreased renal tubular reabsorption of amino-acids existing in hepatolenticular degeneration /see page 20/. The hypothesis was supported by the fact that amino-acids are known to form such complexes with copper and other heavy metals, and experimentally, by showing that an increase in amino-aciduria induces increased urinary copper excretion. Oral administration of glycine and alanine to patients and to normal and cirrhotic controls,with a resulting amino-aciduria,produced a significant rise of copper excretion in the patients but not in the control group. The capacity of the urine to dissolve copper increased considerably after the ingestion of the amino-acids in all subjects, corresponding to the amino-aciduria, but the amount of copper excreted increased only in the patients - indicating a defective reabsorption of the copper-amino-acid chelate./Cirrhotic values were intermediate between patients and normals/. However, no attempt has been made to isolate such a complex from the urine, thus the actual proof of its excretion was lacking.

The latest evidence indicates, that while the correlation between amino-aciduria and the disturbance of copper metabolism in hepatolenticular degeneration postulated by Matthews[14] is a real one, his hypothesis was partially incorrect. It is possible now to integrate, to some extent at least, the diverse findings reviewed in this paper, for a long time seemingly unrelated to each other.

Uzman[24] in 1953, investigating a patient with hepatolenticular degeneration, discovered that the urinary copper formed indeed a chelate complex - but not with amino-acids, but peptides; peptides of a very specific nature, having terminal dicarboxylic amino-acid residues. As it will be recalled, these peptides were isolated by the same author[13] from the urine of asymptomatic siblings of patients, having marked amino-aciduria. /page 21/. At that time it was postulated, that these specific peptides, accumulating in the tissues, the plasma and glomerular filtrate -due, presumably to a deficiency of dicarboxylic carboxypeptidases- competed for reabsorption by the renal tubules with the amino-acids, resulting in partial elimination of the latter in the urine. Speculating over its implications, Brick[21] developed the hypothesis further, suggesting: "It is not improbable that the accumulation of copper in the tissues is the result of a complex formation between these peptides, having known affinities for copper, and accumulated in the tissues because of the metabolic deficiency, and copper." The recent demonstration of such complexes in the urine of patients, although

by no means proves the validity of this working hypothesis, nevertheless gives it substantial support. Similarly,the combined findings of Matthews[14] and Uzman[24], i.e. that ~~that~~ the ingestion of amino-acids enhances the cupruria in patients, but not in normals, ~~yet~~ at the same time the copper excreted is being bound not to amino-acids but to peptides, supports the original contention of tubular competition between peptides and amino-acids leading to amino-aciduria[13]. Indeed, administration of amino-acids results in an elevation of their concentration in the plasma and the glomerular filtrate, where the equilibrium between them and the peptides would be upset in their favor, which would result in a decreased reabsorption of the peptides and peptide-copper complexes, thus in cupruria[24]. In normal subjects no cupruria follows the ingestion of amino-acids. In terms of the hypothesis outlined above, this would indicate that the peptide-copper chelate is absent from normal plasma and glomerular filtrate.

I suggest to correlate this with the earlier presented findings of Scheinberg[40] and Bearn[29], that while normally over 90 % of the serum copper is bound to ceruloplasmin[37], in hepatolenticular degeneration the ceruloplasmin content of blood is so decreased that it cannot account for all of the copper present in serum /page 32/. This,would be in accordance with the postulate that a copper-peptide complex, not present in normal plasma, contains part of the serum copper in hepatolenticular degeneration. Since serum copper is almost entirely in an undialyzable form[14], the peptide chelate must be either of a size in-

termediate between the size of the pores in the dialyzing membrane /collodion/ and those in the kidney glomerulus, or present in extremely small amounts. Matthews[14], commenting on his dialysis experiment, points out that the urinary copper excretion could be accounted for by as little as 1 ug of diffusible copper per 100 ml of serum, a quantity far beyond the capacity of detection of the analytical methods used.

Even if the presence of the peptide-copper complexes were demonstrated in the blood and tissues /which it is not/, several questions would be left unexplained. Thus nothing is known yet about the cause of the increased copper retention, be it due to an increased absorption or decreased intestinal excretion. Bearn[29] believes that it is probably secondary to the ceruloplasmin deficiency, however offers no suggestion about the mechanism by which this would occur. Similarly, the cause of the ceruloplasmin deficiency is entirely obscure. Scheinberg[40] postulates that it is congenital, however the absence of cupruria in asymptomatic siblings of patients who did have amino-aciduria[24], might be interpreted as indicating that the disturbance in copper metabolism is secondary to that of amino-acid metabolism. Finally the cause of peptiduria, the presumed deficiency of dicarboxylic carboxypeptidases, is entirely hypothetical at this moment.

Thus, while some pieces of the metabolic jig saw puzzle start fitting together, our understanding of the "inborn error of metabolism" of hepatolenticular degeneration is far from being complete. As one of the leading investigators,

L.L.Uzman writes it in his last article[24]: "...These considerations serve to underscore the necessity for simultaneous studies on tissue, plasma, and urine in hepatolenticular degeneration for clarification of the nature of the amino-aciduria and cupruria on one hand and the increased tissue deposition of copper on the other....The studies reported from different parts of the world in recent years have presented so far a detailed scrutiny of individual facets of the entity, without offering a clear picture of the whole."

V. The significance of the disturbed copper metabolism

Having reviewed the changes in copper metabolism that take place in hepatolenticular degeneration, together with their possible causes, there remains to be seen the rôle these changes play in the disease process.

1. Relationship to the disease in general.

While cupruria, deposition of copper in the liver and brain, copper retention and ceruloplasmin deficiency are of course essential features of the clinically manifest disease, it is not known whether these biochemical abnormalities represent a primary, congenital lesion or are secondary to the disturbance in amino-acid metabolism. So far,only one of them -cupruria- was looked for in subjects who might be considered latent cases, coming from families stigmatized by the disease and exhibiting amino-aciduria; in these individuals cupruria was absent[24], suggesting that the changes in copper metabolism

occur after the onset of amino-aciduria. However, the following points must be kept in sight:

a/ It is not certain that cupruria is a reliable index of the early changes in copper metabolism. Ceruloplasmin determinations on these subjects would be of great interest.

b/ Even though disturbances of copper metabolism may start later than those of amino-acid and peptide metabolism, this does not prove a cause and effect relationship. The absence of copper disorders in other amino-acidurias is against the likelihood of such a relationship.

Although admittedly the evidence is not conclusive, I find it to be suggestive that that the disturbance in copper metabolism is congenital, becoming manifest probably after the onset of amino-aciduria.

2. Relationship to the hepatic lesion.

The classical view, held by Denny-Brown[42] among others, regards the disturbance in copper metabolism as the cause of the cirrhosis in hepatolenticular degeneration. It is based on the accumulation of copper in the liver in the disease, on Mallory's [43] work showing that copper may experimentally produce cirrhosis, and on similar studies with manganese by Hurst[44] and Mella[45], the latter producing, besides the cirrhosis, lesions in the basal ganglia in rhesus monkeys, by manganese injections. A more recent trend however is to ascribe the cirrhosis to the chronic loss of amino-acids, rather than to the deposition of copper/see page 23/[4,13,21]. The reasons for this are

the following: First, a hepatic cirrhosis, similar to the one found in hepatolenticular degeneration, occurs in another primary, generalized amino-aciduria unassociated with disturbances in copper metabolism: the de Toni-Fanconi syndrome[4,21]. Second, as it will be pointed out subsequently, there is little doubt that copper is responsible for the neurologic lesion in hepatolenticular degeneration. It is also known that cirrhosis of the liver is the primary process in the disease, preceding the onset of the neurological symptoms[1,46] /see page 3/, and in many instances -in siblings of patients with the full picture of the illness- it is the only finding[13]. /In the one such individual investigated so far -a member of the Scandinavian family reported by de Verdier[8], Hood[9] and Uzman[13]- gross amino-aciduria, but no cupruria was found/ /see page 13/. If copper, known to give rise to the brain lesion, were also responsible for the hepatic disorder, such a dissociation of symptoms would not be expected. Thus, while again the proof is not absolute, it seems that <u>copper contributes only secodarily to the hepatic cirrhosis</u>, caused by the chronic loss of amino-acids.

3. <u>Relationship to the cerebral lesion</u>.

Elucidation of the rôle of copper in the production of the neurological symptoms in hepatolenticular degeneration is connected with the discovery and development of the first rational treatment for the disease. The compound 2,3-dimercaptopropanol /British Anti-Lewisite or BAL/ was originally developed to combat arsenic poisoning, which the drug

achieves by forming a soluble combination with arsenic, excreted in the urine, thus removing the offending metal from the body. McCance[47] in 1946 studied the effect of BAL on the heavy metals, copper, zinc and iron, normally present in the serum. It was found, that administration of BAL increased the urinary excretion of copper about 20 times, that of zinc about 5 times, while it had no effect on urinary iron, in normal subjects. Mandelbrote[27] in an experiment referred to earlier in this paper /page 26/, showed that a similar increase in copper excretion following the injection of BAL also occurs in hepatolenticular degeneration. This observation was confirmed up until now in a total of over 30 cases, by different investigators[6,11,14,12,15,29,30,31]. The increase induced by BAL in hepatolenticular degeneration is greater in absolute amount than in normal subjects receiving the same dose, although the proportionate increase is less, due to the high basal excretion[14]. Zimdahl[31] found that the marked positive copper balance characteristically exhibited in patients is greatly reduced or totally reverted by BAL.

In view of the enhancement of cupruria by BAL on one hand, and of the observations about increased copper contents of the brain and liver in hepatolenticular degeneration on the other, Cumings[26] in 1948 suggested the therapeutic use of the drug "in an attempt to reduce the copper content of the liver and the brain, and possibly thereby to prevent further damage to the tissues." The first such trial was reported 3 years later by the same author[11], on 4 patients having severe neurolo-

gical disorders. Following BAL therapy of various length and intensity, objective improvement occured in 2 of the 4 subjects, persisting for 3 months. Working independently from Cumings, Denny-Brown[12] in the same year published the results of a 2-year BAL-therapy, more intense and systematic than that of Cumings, in 5 patients suffering from hepatolenticular degeneration, who at the onset of the treatment all had tremors, rigidity, dysarthria, titubation of the head and trunk, and required assistance in eating, dressing, toilet and walking. After 2 years, all of the patients with the exception of one -who had far-advanced changes at the beginning of the treatment- showed definite clinical improvement, as judged by the signs and symptoms described above, far beyond what might be ascribed to a natural fluctuation of the disease. Later reports by other workers describe the effect of BAL as varying from "striking improvement"[17] to "only minimal changes"[30], indicating that some cases are more resistant to its action than others. It should also be mentioned that BAL does not alter the course of amino-aciduria[6]; thus it is not a cure of the disease. However,in view of the high degree of correlation between administration of BAL, and increased cupruria, establishment of a negative copper balance and improvement in the neurological symptoms, together with the known deposition of copper in the brain and liver, it is hard to escape the conclusion that the cerebral lesion in hepatolenticular degeneration is due to the abnormal accumulation of copper[12,42], although the mechanism through which copper achieves this action is not known /?poisoning of respiratory enzymes?[25]/.

E. CONCLUSION.

From the diverse findings and hypotheses of the various investigators, reviewed in this thesis, a composite picture of the metabolic disorders underlying hepatolenticular degeneration may be drawn. This serves as a summary of the main points contained in the paper, rather than an attempted "explanation" of the problem, which has so far escaped a complete solution.

At least two congenital metabolic defects are present in full-blown cases of hepatolenticular degeneration. One is a deficiency of tissular dicarboxylic-carboxypeptidases/?/ resulting in an accumulation of peptides having dicarboxylic amino-acid residues at the ends of the peptide-chains, in the liver, brain and blood /?/. This defect is present very early in life, maybe at birth, before the appearance of clinical symptoms. It may be the only defect present, resulting in cirrhosis unaccompanied by neurological involvement, such as occuring in some members of affected families. The cirrhosis -regardless whether complicated by neuronal degeneration or not- is the result of the chronic loss of amino-acids through the urine, which is due to their decreased renal tubular reabsorption, caused by peptides competing for the same reabsorption mechanism.

In cases where neurological symptoms develop in addition to the cirrhosis, i.e. the "classical" cases of hepatolenticular degeneration, another metabolic defect, ceruloplasmin deficiency, is present besides the one just described.

Although it becomes manifest only later in the course of the disease, it is improbable that it would be secondary to the liver damage, since it is unencountered in any other hepatic disorder. Ceruloplasmin deficiency leads to /?/ retention of copper in the body, either throurgh an overabsorption or a faulty intestinal excretion of the metal. The excess copper becomes fixed to the peptides accumulated in the brain and liver, through formation of chelate complexes. In the liver it probably contributes to the cirrhosis, and in the brain it leads to degeneration of neurons, possibly through inhibiting respiratory enzymes. From the serum it is excreted in the urine, combined to the peptides. BAL mobilizes copper from the tissues, having more affinity for it than do the peptides, and, in cases that are not too far-advanced, reverses the neurological symptoms due to the deposition of copper in the basal ganglia.

Criticism of each of these points was made while they were discussed, and will not be reiterated this time. The importance of the work done on the metabolic disorders in hepatolenticular degeneration lies elsewhere than in the questionable accuracy of the above outlined concept. This importance is three-fold. First, it became possible to reverse, temporarily at least, the march of a disease, held irreversibly progressive before. Second, the significance of trace elements in human metabolism and disease has been demonstrated. Third, the concept of nervous degeneration itself could be reappraised in biochemical and eventually remediable terms.

In the words of D.E.Denny-Brown: "Such studies open an extraordinary vista of possibilities in neurology. We are fairly certain that disorder of metallic metabolism is not related to diseases such as Parkinsonism, Huntington's chorea, presenile psychoses and familial ataxias, yet it is equally certain that some cumulative metabolic process underlies such 'degenerative' diseases. In each of such diseases a progressive cell change documents the gradual failure of specific metabolic processes without gross anatomic lesions. The conception of abiotrophy, or premature senility of cells, now has no real foundation. The slight degree of anatomic damage in relation to symptoms lends high hope of eventual reversibility when chemical clues are found." /The Shattuck Lecture, 1952/

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