ON THE QUESTION OF THE ORIGIN OF URINARY AMMONIA.

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A few years ago we (1) presented evidence in favor of the view that urinary ammonia is formed by the kidney and that acid is not neutralized by ammonia during transportation in the blood. In subsequent communications (2) we discussed later experimental findings of others bearing on the question of the origin of urinary ammonia. During the past 2 years further work has appeared which is more or less directly related to this question and we feel that a discussion of some of these recent findings is desirable. Embden (3) and Parnas and Mozolowski (4) have studied ammonia formation in muscle tissue in relation to contraction, and later Embden and his collaborators reported extensive studies along this line (5). Recently Bliss (6-8) has reported work related to the problem of the origin of urinary ammonia.

Bliss has frankly adopted our view that the traces of ammonia found in the blood are of no significance in connection with the neutralization of acids in the body. Obviously this view involves acceptance of the theory that the urinary ammonia has its origin in the kidney. Bliss has presented evidence from which he concludes that the amide nitrogen fraction in blood protein is subject to quantitative variation under certain definite conditions. As a result of this work and the further finding that the kidney possesses an enzyme capable of liberating ammonia from the amide nitrogen of the blood proteins, Bliss draws the inference that this amide nitrogen fraction is closely related to the process of neutralization of acids in the tissues, or at least to the neutralization of lactic acid in muscle, and to the ammonia which is found in the urine.
In connection with the presentation of his work Bliss has frequently made statements which we feel may tend to mislead the reader in regard to the real significance of what he reports. The following is quoted from one of Bliss' recent papers (7 p. 130).

"The excessively low values for the ammonia of blood lead one to suspect that ammonia which is formed for acid neutralization in the tissues may be masked in its transit through the blood to the kidney for excretion. . . .

"The behavior of compounds of glucose and ammonia is suggestive. The writer has prepared crystalline compounds of glucose and ammonia . . ., and found that when this substance is subjected to the ordinary aeration method for determining the ammonia content of blood only 1 per cent, or less, of the nitrogen is obtained as ammonia. However, a few minutes contact with 0.1 N acid is sufficient to free the ammonia, and aerations of samples so treated yield 100 per cent of their nitrogen as ammonia by the direct aeration method as used for blood."

We disagree with the view here expressed that the behavior of glucose-ammonia condensation products is suggestive in connection with the problem of transportation of ammonia in blood. In our first paper on ammonia formation we showed, through the addition of large amounts of lactic acid to the blood, followed by the failure to find any increase in ammonia, that there was no compound present in blood which would yield ammonia for acid neutralization when treated with an acid which is a normal physiological compound. Later in the same paper Bliss refers repeatedly to an ammonia complex in the blood. We deem the use of this term "ammonia complex" unjustifiable and misleading. An amide firmly bound in protein and requiring 90 minutes heating with 4 N sulfuric acid for its hydrolysis to ammonia is certainly no more an ammonia complex than is, for example, urea.

In his papers which have appeared so far Bliss stresses the neutralization of lactic acid in the muscle by means of ammonia. He assumes that just as soon as the ammonia reaches the blood stream it is detectable only as firmly bound protein amide nitrogen. Bliss realizes that if this view is adopted, it is necessary to postulate the existence of ammonia in tissues, and he makes the following statement: "So far as the writer is aware, the presence of ammonia in tissues has never been seriously questioned" (8 p. 139). Bliss then cites two references to work carried out from
25 to 35 years ago and includes no reference to the clear cut more
recent experiments of Gad-Andreasen who found that in a number
of tissues, including muscle, the concentration of ammonia is
exactly the same as it is in the blood. In view of these findings
of Gad-Andreasen, we had not given serious consideration to the
possibility of acid neutralization by ammonia within the tissues.

The question of the neutralization of lactic acid in muscular tissue
by means of ammonia has been studied by several investigators
prior to Bliss’ indirect work on this subject. In 1927, Embden
(3) and Parnas and Mozolowski (4) both reported an increase in
ammonia content of muscle tissue following work, and later Emb-
den and his collaborators reported extensive studies along this
line. Parnas and Mozolowski believed there was a relationship
between the lactic acid and ammonia content of the muscle, indi-
cating a neutralization of the one by the other. Embden and
Wassermeyer on the contrary (9) failed to find any such rela-
tionship.

Bliss’ study of this question is indirect and we should hesitate
to conclude with Bliss that because an increase in amide nitrogen
may be demonstrated in the blood circulating through muscle
tissue following exercise, there is a neutralization of lactic acid
within the muscle by means of ammonia. A direct study of the
question by means of determinations of the ammonia content of
muscle under varying conditions would appear to be the desirable
mode of attack in a study of this problem.

The figures reported by Embden and his coworkers for the
ammonia content of frog muscle are approximately twice as high
as the values found by Gad-Andreasen. This latter investigator
cooled the muscle tissue to \(-20^\circ\) immediately after its excision.
Embden and his coworkers on the contrary appear to have made
little effort to maintain the muscle tissue at low temperatures
during its preparation for analysis. It would seem possible that
the higher acid concentration of exercised muscle might lead to
more rapid postmortem ammonia formation. Unfortunately the
actual technique for the determination of ammonia employed by
Gad-Andreasen is not available. Embden and his coworkers used
a complicated procedure involving distillation of the tissue with
magnesium oxide at temperatures of 35–38\(^\circ\), the ammonia being
received in 0.03 N sulfuric acid. A double titration with iodine
and thiosulfate was then employed to determine the amount of acid neutralized. In spite of this indirect and complicated method of analysis, Embden, Riebeling, and Selter (5) reported a most astonishing agreement in a series of figures for the ammonia content of the right and left leg muscles from the same frog. The maximal difference reported in their table is 0.05 mg. of ammonia per 100 gm. of muscle with an average variation of less than half this figure. Our experience with ammonia determinations leads us to state frankly that the apparent degree of accuracy of the method employed by Embden, Riebeling, and Selter is quite beyond our comprehension.

Obviously the question of ammonia formation in muscle during exercise is in no way settled at the present time. Bliss' finding of increased amide nitrogen in the blood following exercise is a type of indirect evidence which cannot be accepted as conclusive. Even assuming such ammonia formation in muscle during work, Bliss' position that the ammonia appears in the bloodstream as firmly bound amide nitrogen is a corollary which we feel many could not accept. How the ammonium ion on one side of the cell membrane appears as firmly bound protein amide nitrogen on the other side, it is difficult to imagine. We would point out that Bliss' latest position in regard to the transportation of acids in the blood and the origin of urinary ammonia is practically in exact accord with our own views. Since the amide nitrogen is firmly bound in the protein molecule and the acids are transported in the non-protein portion of the blood, it is obvious that there is no direct neutralization of acid transported in the blood by means of ammonia. Furthermore, as a corollary of Bliss' present position, there is the direct inference that the urinary ammonia arises through action of the kidney on a non-ammonia precursor in the blood exactly as we suggested. It may be noted in this connection that we suggested an amide, namely urea, as the probable source of the urinary ammonia, though we offered no evidence that urea was the particular compound involved. Whether urea or some other non-ammonium compound is the source of urinary ammonia does not affect the fundamentals of the theory. We would point out, however, that the finding of an enzyme in the kidney capable of splitting ammonia from protein amide does not constitute evidence that urea is not the source of urinary ammonia.
Urea being a highly diffusible compound, this amide would presumably be more available as a source of ammonia during the rapid passage of blood through the kidney than would amide nitrogen which is firmly bound in the non-diffusible protein molecule in the blood. The fact that the kidney can form ammonia from protein amide nitrogen does not constitute evidence that it cannot also form ammonia from urea.

Indeed, there is now direct evidence in favor of the view that the urinary ammonia arises from urea. Thus, Mann and Bollman (10), in a recent paper on ammonia formation following complete removal of liver, make the following statement.

"The cessation of the formation of urea in dogs following complete removal of the liver provides a means of studying the formation of ammonia. Following hepatectomy the urea in the blood and tissues decreases progressively, and at the same time its excretion in the urine decreases markedly. As the body becomes depleted of preformed urea, its concentration in the urine becomes extremely low. The ammonia excreted after hepatectomy is equivalent to that excreted by similarly treated normal animals, and it would appear that the amount is related to the acid-base equilibrium of the animals. However, when the urine of the dehepatized dog becomes extremely low in urea, it is always found that the ammonia excretion has likewise diminished. Intravenous injection of urea at this time causes a definite increase in the excretion of ammonia."

We believe that experimental data reported up to the present time warrant the following conclusions.

1. The urinary ammonia is formed by the kidney.
2. Urea is probably the precursor of the ammonia found in the urine.
3. Ammonia plays no part in neutralization of acids transported in the blood.
4. Satisfactory evidence is lacking that ammonia is utilized for the intracellular neutralization of acids within the organism.

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