LIQUID AMMONIA AS A DIETARY NITROGEN SUPPLEMENT

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That urea and ammonium carbonate may be effectively used as dietary nitrogen supplements in ruminant animals has been reported (1) (2) (3). Also it has been shown that proteins are ammonolyzed in liquid ammonia, and thereby increase their nitrogen content (4). This report is concerned with the use of ammonolyzed proteins and other ammonolyzed foods as a source of nitrogen in the diet of non-ruminant animals such as albino rats. Earlier work has shown that ammonolyzed casein causes a loss of weight in young albino rats when it is used to replace other proteins in a standard diet, (Protein 18%, starch 46%, butter 15%, moist yeast 15%, salt mixture, Harris 3%, cod liver oil 3%). Later work has been done in which different constituents of the diet were treated with liquid ammonia or ammonium hydroxide, or the whole diet was mixed with ammonium carbonate and kept in a closed container. Preliminary reports have been made (5).

The method of treating casein, starch and dry powdered yeast with liquid ammonia is simple. The material is placed in a suitable container and anhydrous liquid ammonia is run in until the thick paste which forms at first becomes fluid-like with stirring. On settling, a layer of liquid ammonia should rise above the thin paste. The container is then closed, and attached to a mercury seal. This protects the contents of the container from absorbing moisture from the air. The excess liquid ammonia boils off in about 24 hours, being recovered if desired, and a hard mass of the semi-transparent material remains. This can be powdered, and the fumes of ammonia gas can be removed with a vacuum pump. The material has a very faint odor of ammonia, but contains a considerable amount of the latter, as shown by Kjeldahl determinations, bound in the form of a derivative. That derivatives have been formed is evident since the excess nitrogen cannot be removed entirely after heating the materials in a vacuum oven at 100° C. for 24 hours. For example, as shown by the data in table I, the nitrogen content of starch (group 6)

TABLE I

Groups	Modified diet and method of feeding		Increased wt. of N as % of total wt.	Results
1	Casein treated with liq. NH_3	Mixed with rest of diet before feeding	2.20	Lost weight. Spastic paralysis.
2	Casein treated with liq. NH_3	Placed in vacuum oven 100°C. 24 hr. Fed mixed	0.40	Gained weight.
3	Casein treated with liq. NH_3	This casein was fed in a separate cup	2.20	Gained weight.
4	Casein treated with liq. NH_3	Casein fed mixed. Moist yeast, separate cup	2.20	Gained weight.
5	Casein treated with liq. NH_3	Casein fed mixed. Dry yeast, separate cup	2.20	Gained weight.
6	Starch treated with liq. NH_3	Mixed with rest of diet before feeding	0.75	Lost weight. Spastic paralysis.
7	Starch treated with liq. NH_3	Placed in vacuum oven 100°C. 24 hr. Fed mixed	0.31	Gained weight.
8	Starch treated with liq. NH_3	This starch was fed in a separate cup	0.75	Gained weight.
9	Starch treated with liq. NH_3	Starch fed mixed. Moist yeast, separate cup	0.75	Gained weight.
10	Starch treated with liq. NH_3	Starch fed mixed. Dry yeast, separate cup	0.75	Gained weight.
11	Dry yeast treated with liq. NH_3	This yeast was fed in a separate cup	2.00	Lost weight.
12	Moist yeast treated with liq. NH_3	This yeast was fed in a separate cup	2.00	Gained weight.
13	Dry yeast treated with NH_4OH	This yeast was fed in a separate cup	2.00	Gained weight.
14	Ammonium carbonate	Mixed with rest of diet before feeding	0.75	Lost weight. Spastic paralysis.
15	Ammonium carbonate	Mixed with moist yeast, fed in separate cup	0.75	Gained weight.
16	Ammonium citrate	Mixed with rest of diet before feeding	0.75	Gained weight.

In groups 14, 15 and 16 the percent of nitrogen increase does not refer to the compounds themselves, but to mixtures of them and starch, the excess nitrogen 0.75% being the same as for group 6.

has been increased 0.75%, from 0.21% to 0.96%, by treatment with liquid ammonia, and that after heating as described it still contained 0.52% nitrogen or an excess of 0.31% nitrogen (group 7). With casein (table I, and group 1) the excess nitrogen after treatment is even greater. Ammonia that is held by absorption only can be removed by the process mentioned. Moist yeast when treated with ammonia does not form a hard, translucent mass but instead a soft opaque dough.

Control animals were fed in a manner similar to the experimental animals. For example, when casein (group 3) which had been treated with liquid ammonia was fed in a separate container, untreated casein was also fed in a separate container to the control animals. This is done so that the feeding conditions are similar in each case, and one can watch the amounts of food eaten both treated and untreated. Litter mates were used and also an equal number of males and females. Each animal was kept in a separate cage. At least 4 rats were used as experimental animals and an equal number for control animals. In all about 200 rats were used. The results are uniform in every member of each group in regard to loss or gain in weight and the development of spastic paralysis. The control animals remained in good health, and continued to gain weight in every case. The food was given ad libitum, and the animals ate the treated foods as readily or even more so than the untreated food. Due to the kind of feeding cups used very little food was wasted. Harris vitamin free casein and starch were used. Fleischman's bakers moist yeast and Northwestern's powdered yeast were used. The yeast when fed in separate containers was weighed each day since it is known that vitamin B in excess will accelerate the growth of young rats.

The excess amount of nitrogen received per day by an experimental rat as compared to a control rat amounted on the average to be 0.066 gm. In general 4 rats eat 500 gm. of food per week or 17.8 gm. per rat per day. On a diet containing 46% starch with an increase of 0.75% nitrogen due to the liquid ammonia (table I, group 6) each experimental rat received an excess of 0.0615 gm. of nitrogen per day. For a treated 18% casein diet (table I, group 1) each experimental rat received 0.0704 gm. of excess nitrogen per day. This represents an average increase of 13.8% in the protein nitrogen. The amount of ammonium carbonate and ammonium citrate to be used was calculated from a Kjeldahl determination of the ammonolyzed starch which showed an increase of 0.75% nitrogen and was a sample that caused paralysis (table I, group 6). Therefore 16 gm. of ammonium carbonate or 23.2 gm. of ammonium citrate were added to each 500 gm. of the food which is made up to contain 230 gm. of starch in 500 gm. of the diet. Therefore, groups 14, 15 and 16 have the same nitrogen increase in their diets as those in group 6, or 0.0615 gm. of nitrogen per rat per day.

The results are summarized in Table I. They prove that the deleterious effect of liquid ammonia and ammonia gas is due to the action of the ammonia on the vitamin B complex, when the latter is in a fairly dry state. It has been shown (6) that liquid ammonia tends to form addition compounds under such circumstances and Williams has shown that liquid ammonia acts on vitamin B_1 (7). That the effect is not due to a basic action is indicated by the negative results or growth obtained with ammonium hydroxide. (Group 13). That sodium hydroxide however, partially inactivates vitamin B has been shown by others (8). The negative results with ammonium citrate (Group 16) would rule out the ammonium ion as such. The results also show that the ammonia-vitamin B derivative is easily hydrolyzed giving negative results with wet yeast, (group 12) but positive results or paralysis and loss of weight with dry yeast (group 11). Further, it is shown that the compounds formed with casein and starch are not entirely stable in a vacuum oven at 100° C. (Groups 2 and 7), but evolve a considerable amount of ammonia. However, a certain amount of ammonia remains fixed: 0.4% as nitrogen in the case of the casein and 0.31% as nitrogen in the case of the starch, and this fixed ammonia seems harmless since growth was obtained in groups 2 and 7.

The failure of the rats to gain weight on ammonolyzed casein referred to in the introduction was not due to any kind of toxicity caused by the action of ammonia

on the protein, but to inactivation of the vitamin B present. Ammonolyzed food has less tendency to spoil and become mouldy than untreated food (Mould is found on untreated food within 2 weeks; no mould on treated food in 5 weeks), and it caused a more rapid growth in albino rats, (Groups on treated foods gain weight twice as fast as controls for two month periods), providing an additional source of vitamin B is available.

The preponderance of opinion as indicated in the literature favors the use of ammonium salts as dietary nitrogen supplements. Liquid ammonia as compared to non-volatile ammonium salts has certain advantages and certain disadvantages. One disadvantage is its tendency to inactivate vitamin B. Another is that it cannot be used to increase the protein nitrogen more than about 20% and still be edible. On the other hand it is comparatively cheap, acts as a preservative against moulds and possibly plays a dual role in nitrogen assimilation; first by increasing the nitrogen content of foods by forming addition products, and second by increasing the digestibility of the original protein. McChesney and Roberts (9) have shown recently that ammonolysis increases the digestibility of some proteins to the action of trypsin in vitro. It has been assumed that in ruminants bacteria of the digestive tract play an intermediate part in the assimilation of non-protein nitrogen. Whether such an argument can be used in favor of non-ruminants is not known, but the more efficient digestion and absorption of the protein nitrogen of ammonolyzed proteins should apply as well to non-ruminants including humans. Mitchell and Hamilton (10) in reviewing the literature of the use of non-protein nitrogenous compounds, such as urea, for protein supplements in the diet, find that the evidence is inconclusive although it is sometimes indicated that urea increases the digestibility of proteins. Recently, Schoenheimer (11) has made use of the nitrogen isotope N^{15} and the mass spectrograph for detecting it, to follow the metabolism of such salts as ammonium citrate in albino rats. He finds that some of the N^{15} of the ammonium citrate fed can be located later as alpha amino nitrogen $N.^{15}$ From our own work we conclude that liquid ammonia properly used can serve as a dietary protein supplement.

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