# A TYPE OF UREA-SPLITTING BACTERIUM FOUND IN THE HUMAN INTESTINAL TRACT

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In the course of an investigation (1921) on the origin of ammonia dermatitis of the gluteal region of infants, it was shown that ammonia in the diaper is the result of a splitting of urinary urea by a bacterium which infests the gluteal region and diaper from the feces. This organism has been isolated from the stools of a number of infants and children and is probably one of the common saprophytes of the intestinal tract, not only in children but also in adults. Its occurrence in infants is of interest to the pediatrist on account of the not infrequent infestation of the gluteal region, with the production of ammonia in the wet napkin. In addition, its relatively widespread occurrence in the human intestine would indicate that the organism is one of the chief agents in the conversion of urea into ammonia in sewage. The marked urea-splitting action of this saprophyte, the fact that no record has been made of any previous study of the organism, and the observation that it is a common inhabitant of the human intestinal tract seem sufficient justification for recording the observations made in its study.

The conversion of urea into ammonia is a function possessed by a number of bacteria. The earlier observations of Pasteur, Miquel and others showed that various large spore-forming "urobacilli" could be isolated from air, soil, and sewage, and, later, many other bacteria were found able to form small amounts of ammonia from urea. No attempt will be made to discuss these ammonifiers except to mention that many Gram-negative bacilli are capable of producing small amounts of ammonia,

and that staphylococci also have this property. We wish to emphasize, however, that the organism here described differs from the above-mentioned strains of ammonifiers and especially from other organisms of the human gastro-intestinal flora, in being an exceptionally strong ammonia producer or urea fermenter. It is capable of growth and continued ammonia production in a medium so alkaline that other organisms are inhibited or destroyed. It seems probable that this particular bacterium has not been brought to the attention of other workers on the intestinal flora because suitable selective media were not used, and because their interest was not directed towards finding a strong ammonifier. Certain clinical observations on ammoniacal excoriation of the buttocks of infants prompted our search for a urea-splitting organism, and we were somewhat surprised to find that the form responsible was not one of the better known members of the intestinal flora. The only organism we have encountered having similar marked ability to form ammonia from urea is a yellow chromogenic sarcina, probably *S. lutea*, which has been found several times in air-contaminated ammoniacal urine after standing.

The organism here described was isolated in pure culture from the stools of more than 50 infants and older children, and was the only strong ammonia producer regularly found. *B. pyocyaneus* and *B. proteus*, both of which form moderate amounts of ammonia, were found a few times. The technique was simple and allowed an easy and rapid isolation. A small portion of the feces was inoculated into a tube of fresh, slightly acid urine, to which phenolsulphonephthalein (phenol red) had been added as an indicator, and which had been sterilized by passage through a diatomaceous filter. After incubation for twenty-four hours, if the reaction had become alkaline, several loopfuls were inoculated into a fresh tube of the same medium. When production of alkali had persisted through several tubes, some of the material was seeded on the surface of an agar plate. Colonies from this were transplanted into a urea medium consisting of 1 per cent urea and 0.2 per cent each of calcium chloride, monosodium phosphate, dipotassium phosphate and magnesium sulphate with

phenol red added as an indicator, and sterilized by filtration. Production of an alkaline reaction in this medium was always due to ammonia production.

The organism isolated has the following characteristics:

Morphologically, the cells from young cultures are non-motile rods with rounded ends and have a fairly uniform size. The majority have a diameter of 0.8 microns and a length which varies from 1.4 to 1.7 microns. A few shorter coccoid forms occur and occasionally an organism 2 to 3 microns long with a diameter of about one micron, but the large majority of elements are of almost uniform size. In older cultures, larger forms are somewhat more easily found and occasional irregularly pear-shaped organisms occur. There is also less uniformity in size. No characteristic grouping occurs and the morphology is similar on all culture media. Neither spores nor capsules occur. The organisms stain well and uniformily with the usual stains, retain the Gram stain, and are not acid fast.

On agar and gelatin the surface growth is filiform, fairly abundant, opaque, smooth, flat and glistening, with a butyrous consistency. No odor is produced and the medium remains unchanged. Gelatin is not liquefied. In stab cultures the growth is filiform and more abundant near the top. Although as a rule the cultures are not pigmented, several strains isolated have a faint yellow pigment. These chromogenic strains are otherwise indistinguishable from the non-chromogenic strains. Stab cultures give a filiform growth along the line of puncture which is more abundant near the surface.

The individual colonies on agar develop rapidly at 37°C. from 1 mm. diameter in twenty-four hours to 6 or 7 mm. in a week. They are round and flat, with a smooth surface and entire edge. The internal structure is amorphous.

In nutrient broth, cultures are without odor and show a moderate clouding, more marked in the upper layers, with an abundant flocculent sediment. Litmus milk is unchanged except for the production of a slight alkalinity. There is no diastatic action on starch jelly, blood serum is not liquefied, and none of the commonly used laboratory sugars are fermented.

Growth on all media is abundant at 37°C. although multiplication is somewhat more rapid at 30°C. The thermal death point is 55°C. in ten minutes. Cultures are rather resistant to drying and retain their vitality for several months in cultures. The most rapid development of cultures occurs on media with a reaction of pH 7.0 to pH 8.5 while greater degrees of acidity or alkalinity cause a marked inhibition of growth.

Considerable amounts of cultures were injected intravenously and subcutaneously into rabbits and guinea pigs without the production of any lesion.

The fermentation of urea is the function of this organism which has especially interested us and the following summarizes the results of observations on this property.

1. In unsealed cultures, urea is completely destroyed after a number of days, while in sealed flasks the destruction is incomplete. The alkalinity in such sealed flasks reaches a pH of 10 and at this point the bacterial growth is almost completely inhibited, although some viable organisms remain for a number of days.

2. No extracellular urease is present in the filtrates of young or old cultures.

3. Dried, powdered bacterial bodies contain a small amount of urease, usually considerably less than an equal weight of Jack Bean meal.

4. Salts of heavy metals such as copper and mercury, which have a marked inhibitory effect on urease activity even in very small amounts, do not inhibit ammonia formation in cultures unless added in amounts sufficient to prevent bacterial growth.

It is apparent that the formation of ammonia from urea is a function intimately associated with the vital activity of the organism, and does not depend on the formation of an extracellular ferment. In this respect it resembles other bacterial ferment activity.

According to the Committee of Classification of the Society of American Bacteriologists (1920), the organism belongs to Family 5, the Bacteriaceae, and in Genus 6, Bacterium. The name suggested is *Bacterium ammoniagenes.*

It may be remarked that the discovery of this organism in infants' stools, and of the mechanism of ammonia formation in the diaper with consequent dermatitis of the buttocks, suggested the cure of the condition by an impregnation of the diaper with an antiseptic which prevents further bacterial growth and ammonia formation. Thus an irritating skin eruption in infants, of common occurrence and sometimes rather distressing severity, has been completely controlled.

## SUMMARY

The characteristics of a microörganism commonly found in the intestinal tract of infants and older children, and having exceptional power of splitting ammonia from urea, are described. The name *Bacterium ammoniagenes* is suggested for it.

## REFERENCES

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