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# THE GROWTH FACTOR REQUIREMENTS OF *SALMONELLA DUBLIN*

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Most strains of Salmonella are able to develop in simple media consisting of ammonium nitrogen, mineral salts and glucose (*Kauffmann*, 1954). A few, however, are unable to synthesize certain essential growth factors, and these must be supplied in the culture medium. The nutritional requirements of several Salmonella strains have been described (*Johnson* and *Rettger*, 1943; *Gilfillan* et al., 1955; *Stokes* and *Bayne*, 1958 a, 1958 b). Also, *Lederberg* (1947) has reported deficient strains of Salmonella, including a thiamin-requiring strain of *S. dublin*.

*Salmonella dublin* is the most prevalent Salmonella species in cattle (*Edwards* et al., 1948) and an important cause of food poisoning in humans (*Conybeare* and *Thornton*, 1938; *Tulloch*, 1939).

The isolation of three different variants of *S. dublin* from a carrier cow initiated a study of the antigenic dissociation phenomenon of *S. dublin* in vivo and in vitro (*Thal* and *Holmquist*, 1957). The differences in electrolytic susceptibility, serological properties and virulence of these organisms have been reported (*Lehnert* and *Thal*, 1959; *Thal* and *Lehnert*, 1962). The stepwise antigenic dissociation of the bacteria may be part of the breakdown process that takes place in an invaded host able to fight invasion by cellular and humoral antibodies.

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This is a report of studies designed to determine whether Intermediate and R strains of *S. dublin* (stripped of surface antigens 0 or 0 and H) have growth-factor requirements different from those of S strains, whether all or some strains of *S. dublin* have special requirements with regard to amino acids or vitamins, and whether the requirements differ depending on animal or geographical sources of isolation.

## MATERIALS AND METHODS

Nine strains of *S. dublin* were investigated. Seven of them were isolated at the State Veterinary Medical Institute, Stockholm, Sweden, and two strains were obtained from the Laboratory of Bacteriology, State of California Department of Health, Berkeley. The Swedish isolates were: 344-9 (S strain), 344-1 (Intermediate strain), 344-42 (R strain, non-motile), and one strain each from a cow, calf, chicken and mink. All No.'s 344 strains were isolated from a single cow and are OH variant. The two California strains were from human and bovine sources. In several experiments, *S. typhimurium* Tm-1 was used for comparison of growth activities. Stock cultures of all strains were maintained on trypticase soy agar slants.

The basal medium (*Stokes* and *Bayne*, 1958 b) consisted of the following ingredients: glucose, 1.0 per cent; $(NH_4)_2SO_4$, 0.1 per cent; Na citrate $2H_2O$, 0.05 per cent; Mg $SO_4 \cdot 7H_2O$, 0.05 per cent; phosphate buffer pH 7.1, 0.05 M; and distilled water. The glucose was autoclaved separately and added aseptically to the medium after the latter had been prepared, distributed in appropriate vessels, and sterilized.

To determine whether *S. dublin* had an amino acid requirement, the basal medium was enriched with 0.3 per cent vitamin-free casein hydrolysate, and also 25 µg. l-cystine and 100 µg. DL-tryptophan per ml. of medium.

The B-vitamins employed and their final amounts per ml. of medium were: thiamin, riboflavin, nicotinic acid, and pantothenic acid, 0.2 µg. each; pyridoxin, 1.0 µg.; p-aminobenzoic acid, 0.4 µg.; folic acid, 0.01 µg.; and biotin, 0.0002 µg.. Purines and pyrimidine were supplied as a mixture of adenine, guanine and uracil and at a concentration of 10 µg. of each per ml. of medium.

The details of the preparation of the stock solutions of vitamins, purines and pyrimidine are described in the report of *Stokes and Gunness* (1945).

Inocula were prepared by transferring a small amount of growth from agar slants to 10 ml. of sterile distilled water. Experimental media usually prepared in 5-ml. amounts were inoculated with 0.1 ml. of this turbid suspension.

The manometric methods used are those described previously (*Stokes and Bayne*, 1961).

## RESULTS

*Nutritional Requirements.* Initial experiments showed that strains of *S. dublin* grow well in a complex medium such as trypticase soy broth, but not in the basal medium even when supplemented with asparagine. The effect of the addition of vitamins, purines, pyrimidine and casein hydrolysate to the basal medium as individual groups and in combinations was observed. All strains grew well in the basal medium supplemented with vitamins and the other factors. No growth occurred in the absence of the vitamin mixture. The growth of the three variants of *S. dublin* and *S. typhimurium* after 24 hours is shown in Table 1. There was no change in this growth pattern after 72 hours.

Table 1.
Growth Response of *Salmonella dublin* Variants in Various Media.

| Strain | Basal Salts Medium | | | | Trypticase soy broth |
|---|---|---|---|---|---|
| | Alone | Plus casein hydrolysate | Plus casein hydrolysate, adenine, guanine, and uracil | | |
| | | | No vitamins added | Vitamins[1]) | |
| *S. dublin* 344-9 (smooth) | — | — | — | + | + |
| *S. dublin* 344-1 (intermediate) | — | — | — | + | + |
| *S. dublin* 344-42 (rough) | — | — | — | + | + |
| *S. typhimurium* Tm-1 (control) | + | + | + | + | + |

+ = growth — = no growth

[1]) Thiamin, riboflavin, nicotinic acid, pantothenic acid, folic acid, biotin, pyridoxin, p-aminobenzoic acid.

To determine the specific vitamin or vitamins essential for growth of these strains, one vitamin of the eight used was omitted at a time from the basal medium-vitamin mixture and the effect on growth noted. None of the nine strains of *S. dublin* grew in the absence of nicotinic acid (Table 2). This was the only vitamin required.

Investigations were conducted to determine the level of nicotinic acid required to support minimal growth. The basal medium

Table 2.
Vitamin Requirements of *Salmonella dublin.*

| Vitamin omitted[1]) | Concentration when used μg./ml. | Growth of various isolates observed at 48 hours | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Cow (variants) | | | Chicken | Cow | Mink | Calf | Human | Bovine |
| | | 344-1 | 344-9 | 344-42 | | | | | | |
| None | | + | + | + | + | + | + | + | + | + |
| Thiamin | 0.2 | + | + | + | + | + | + | + | + | + |
| Riboflavin | 0.2 | + | + | + | + | + | + | + | + | + |
| Nicotinic acid | 0.2 | — | — | — | — | — | — | — | — | — |
| Pantothenic acid | 0.2 | + | + | + | + | + | + | + | + | + |
| p-aminobenzoic acid | 0.4 | + | + | + | + | + | + | + | + | + |
| Folic acid | 0.01 | + | + | + | + | + | + | + | + | + |
| Biotin | 0.0002 | + | + | + | + | + | + | + | + | + |
| Pyridoxin | 1.0 | + | + | + | + | + | + | + | + | + |
| All | | — | — | — | — | — | — | — | — | — |

+ = growth — = no growth

[1]) = basal medium + vitamins.

was prepared containing nicotinic acid in concentrations from 0.05 μg. to 5.0 μg. per ml. of medium. The minimal amount of nicotinic acid necessary to support growth was 0.20 μg. per ml. of medium.

*Manometric data.* Two amino acids, DL-alanine and DL-serine, that are rapidly oxidized by most Salmonella strains (*Bayne* and *Stokes*, 1961) were used in studies with the three variants of *S. dublin.* Data on the rates of oxidation of these amino acids and glucose are given in Table 3. There was no significant difference in the rates of oxidation of these substrates by the different strains.

Table 3.
Rates of Oxidation of Two Amino Acids and Glucose by Variants of *Salmonella dublin.*

| | $O_2$, μl./hr consumed per vessel | | |
|---|---|---|---|
| | Variant 344-9 | Variant 344-1 | Variant 344-43 |
| DL-Alanine | 48 | 53 | 42 |
| DL-Serine | 46 | 53 | 51 |
| Glucose | 113 | 137 | 126 |

## DISCUSSION

Our results indicate that these nine strains of *S. dublin* are growth-factor dependent. However, they require only the one factor, nicotinic acid. They are unable to synthesize this essential vitamin in a simple salts medium. The presence of seven other vitamins in no way substitutes or compensates for the required factor. The addition of other nutrients, such as casein hydrolysate, to the basal medium may lead to greater cell yields of *S. dublin*, but does not eliminate the strict requirement that these organisms have for the vitamin nicotinic acid.

It is of interest to note that nicotinic acid is frequently required by other growth-factor dependent strains of Salmonella (*Stokes* and *Bayne*, 1958 b). *Lederberg* (1947) reported a strain of *S. dublin* that needed thiamin, another frequently required growth factor. Thus, it appears that strains of the same serotype may not have requirements for the same growth factor.

Although our strains were isolated from animals of different generic origin and from different geographical loci, little or no difference was found among the strains in their requirements for nicotinic acid. Artificial and natural strains were found to have similar nutritional patterns.

There is no significant difference between variants in their ability to oxidize two amino acids and glucose. The oxidation rates were somewhat less than those for most of the Salmonella strains reported by *Bayne* and *Stokes* (1961).

It appears unlikely that differences in nutritional requirements are critical in the metabolic processes of variants. Further studies will be needed to provide a clear understanding of the metabolic processes of variants and other serotypes of the Salmonella.

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## SUMMARY

Nine strains of *S. dublin* isolated from animals of different species and from different countries were unable to grow in a glucose-mineral salts medium. All strains grew well, however, when nicotinic acid was added to the medium. Intermediate and R strains do not differ qualitatively or quantitatively from S strains. The minimal amount of

nicotinic acid necessary for growth was shown to be 0.20 μg. per ml. of medium.

The rates of oxidation of DL-alanine, DL-serine and glucose were essentially the same for all *S. dublin* variant strains.


## ZUSAMMENFASSUNG

*Wachstumsfaktoren bei Salmonella dublin.*

Neun von verschiedenen Tierarten in Amerika und Europa isolierte *S. dublin* Stämme waren nicht in der Lage sich in einer Mineralsalze und Glukose enthaltenden Nährflüssigkeit zu entwickeln. Sie erfordern Nikotinsäure — in einer Mindestmenge von 0.20 μg. per ccm. Nahrungsflüssigkeit. Der Zusatz von 7 anderen Vitaminen konnte diesen Wachstumsfaktor nicht ersetzen. Die Nährungsbedürfnisse von intermediären und R-Stämmen unterschieden sich in dieser Beziehung weder qualitativ noch quantitativ von S-Stämmen. Nikotinsäure ist demnach ein unentbehrlicher Wachstumsfaktor für die untersuchten *S. dublin* Stämme. In ihrer Oxidationsgeschwindigkeit zeigten die untersuchten S-, I- und R-Varianten von *S. dublin* keinen signifikanten Unterschied.

## SAMMANFATTNING

*Salmonella dublin's behov av tillväxtfaktorer.*

Nio *S. dublin* stammar isolerade från olika djurslag i olika länder kunde ej fås att växa i ett substrat innehållande glykos och mineralsalter. Stammarna växte endast när nikotinsyra tillsattes ett sådant substrat. Den minsta mängd nikotinsyra som erfordrades för att erhålla växt visade sig vara 0,20 μg. per ml. substrat. Nikotinsyran kunde ej ersättas genom tillsats av 7 andra vitaminer. Intermediära och R-stammars näringskrav i detta hänseende skiljde sig ej kvalitativt eller kvantitativt från S-stammars. Någon signifikant skillnad i oxidationshastigheten för de undersökta S-, I- och R-varianterna av *S. dublin* var ej påvisbar.


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