part of the stratum griseum intermedium, and it receives fibres from the retina and visual cortex. The other comprises the rest of the stratum griseum intermedium and the stratum album intermedium, and receives fibres from cortical areas other than visual cortex, and from the spinal cord and other regions. How the individual inputs in each group are interrelated and what relation the two groups bear to one another are problems which are now under investigation.

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SUCCINATE METABOLISM IN *Salmonella typhimurium*

Utilization of Succinate as Carbon and Energy Source by *Salmonella typhimurium*

SEVERAL mutants of *Salmonella typhimurium* were found to have a specific growth requirement for relatively high concentrations of aspartic acid when tested in the Davis–Mingioli medium[1] containing glucose, sodium citrate, and mineral salts. In the absence of glucose, however, the mutants grew readily without aspartic acid using citrate, fumarate, succinate, malate, or acetate as the sole carbon source. The results were similar to those reported by Gilvarg and Davis[2], in which a mutant requiring glutamic acid grew on citrate in the absence of glucose, the metabolic lesion being in the condensing enzyme which forms citrate. The growth of some of our mutants on the four carbon acids was repressed by glucose; others were not affected. Since the requirement in the present mutant was satisfied by 4-carbon as well as 6-carbon compounds, the results suggest that this strain lacked the ability to fix carbon dioxide via the reaction mediated by phosphoenol pyruvic carboxykinase, a condition previously described in a similar mutant[3]. In contrast, the wild-type parent *S. typhimurium* grew only very slowly with succinate, fumarate, malate or acetate as the sole carbon source; the mutant had gained the ability to utilize these organic acids in addition to losing the ability to grow with glucose. This paper presents some preliminary results which may explain this metabolic difference.

The *S. typhimurium* (wild type) used was a typical strain virulent for mice; the mutant (*X*39) was one of several auxotrophs isolated from the wild type after treatment with ultra-violet light[4]. The mutant grew with succinate either in the presence or absence of glucose but not with glucose alone. The basal medium, *p*H 7, contained (in per cent as w/v) 0·27 per cent $(NH_4)_2\,SO_4$, 0·5 per cent K_2HPO_4, 0·01 per cent of a solution of trace minerals which consisted of 10 per cent $MgSO_4$, 1 per cent $MnSO_4$, 0·1 per cent $FeSO_4$ and 3 per cent HCl. The carbon source was added in the amount of 0·2 per cent, and for investigations using solid media 0·85 per cent 'Ion Agar No. 2' (Oxoid) was included. Inocula were grown in the basal medium containing glucose and L-aspartic acid as the carbon source. Broth cultures were incubated in 250-ml. flasks on a rotary shaker at 37° C. The optical density was measured at 425 mμ; respiration studies were made using the Warburg respirometer with air as the gas phase.

Both the mutant, *X*39, and the wild type respired succinate at approximately the same rate; neither strain respired citrate, although both could utilize it as a growth substrate. This was in keeping with the findings of other workers that bacterial cells are impermeable to citrate unless a specific permease has been induced. The inability of the wild type to grow rapidly on succinate as the sole carbon source could not be ascribed to the impermeability of the cells to succinate.

The ability of both strains to grow readily on citrate as the sole carbon source suggested that the tricarboxylic acid cycle was functional in both organisms. A number of supplementary compounds were added to the succinate minimal medium to determine if any would enhance growth. Of the compounds tested, acetate, pyruvate and glutamate stimulated the growth of the wild strain, acetate being most effective. Rapid growth occurred only on the combination; the organism grew slowly on acetate alone (Table 1).

Table 1. GROWTH RESPONSE OF THE WILD-TYPE *Salmonella typhimurium* TO SUCCINATE AND ACETATE

Addition to basal medium (%)		*O.D.** after 9 h
Sodium succinate	0·2	0·03
Sodium acetate	0·1	0·06
Sodium succinate	0·2	0·47
+ sodium acetate	0·06	
Sodium succinate	0·2	0·80
+ sodium acetate	0·1	

* Measured at 425 mμ.
Note: a comparable culture of the mutant *X*39 reached an *O.D.* of 0·80 in a similar period of time in the medium containing 0·2 per cent sodium succinate.

These results suggested that the wild-type *S. typhimurium*, when grown on succinate, lacked sufficient endogenously formed acetate to permit rapid growth. Pyruvate was considered a precursor to acetate. Since both strains oxidized succinate at about the same rate, and to about the same extent, it seems unlikely that acetate functioned solely as a precursor in the essential formation of citrate in the growing culture. The economy of acetate in the wild type may have been such that the rate of those biosynthetic pathways dependent on acetate became limiting.

Finally, on occasions, rapidly growing strains of the wild type (prototrophs) were obtained which behaved on succinate media as did mutant *X*39 except that they also grew on glucose as the sole carbon source. This observation confirmed our belief that gaining the ability to utilize succinate for growth was independent of the ability to utilize glucose. Studies are being continued in hopes of clarifying the metabolic distinction between these strains.

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Succinate Metabolism and Virulence in *Salmonella typhimurium*

Two kinds of strains of *Salmonella typhimurium* which can be distinguished by their behaviour on succinate as sole carbon source have been described in the preceding communication. One kind grows rapidly, yielding colonies