Table 1. ISOCITRITASE DISTRIBUTION (AEROBIC CELLS)

Organism* and strain	Isocitritase (units†/mgm. protein)
1. *P. aeruginosa*, 9027	1·55
2. *P. fluorescens*, A3.12	1·32
3. *P. fluorescens*, B.6bs	0·76
4. *P. putrifaciens*, 207	1·77
5. *Azotobacter agile*, 4.4	1·68
6. *Azotobacter vinelandii*, 0	1·35
7. *Acetobacter aceti*, F.4	0·86
8. *Escherichia coli*, Crooks	0·93
9. *Serratia marcescens*, 3M4	0·65
10. *Micrococcus lysodeikticus*, 4698‡	2·10

* Cells 1–9 were grown with aeration in an acetate 0·5 per cent, glutamate 0·2 per cent, ammonium salts medium. Cell paste (at 200 mgm. per ml. of *M*/50 phosphate buffer, *p*H 7·0) was oscillated 15 min. in a 10 kc./s. Raytheon oscillator and centrifuged 1 hr. at 16,000 *g* to obtain a cell extract.

† 1 unit = 1 μmole glyoxylate produced per 10 min. Reaction mixture contained: 20 μmoles DL-isocitrate, 3 μmoles magnesium chloride, 10 μmoles glutathione, 200 μmoles tris-(hydroxymethyl)-aminomethane buffer *p*H 7·6, extract 3–5 mgm. protein.

‡ Grown on a DL-malate 0·5 per cent, NZ amine 0·2 per cent, salts medium, cell extract obtained by lysis with lysozyme.

In extending earlier studies of diverse metabolic pathways in micro-organisms, the isocitritase content of several bacterial extracts under study in our laboratory was measured. The results for organisms grown aerobically on organic acids as energy source are shown in Table 1. The aerobic Gram-negative rods of the *Pseudomonas* and *Azotobacter* genera were all high in isocitritase activity, as was the related acid-tolerant Gram-negative aerobe *Acetobacter aceti*—which also possesses a complete oxidation system[5]. The two facultative Enterobacteriaceae possessed lower, but comparable, levels of isocitritase. The aerobic Gram-positive coccus *Micrococcus lysodeikticus* possessed the highest activity of any strain reported; whether the altered growth conditions, malate plus organic nitrogen source, were contributing factors has not so far been determined. The isocitritase-level in *M. lysodeikticus* would imply a suitable source of enzyme for fractionation.

The very marked alteration in enzymatic content demonstrated, in facultative and aerobic organisms, in response to growth conditions prompted us to assay extracts of representative strains from several growth conditions. *E. coli*, Crooks strain, was harvested from three media: the acetate-salts medium used for isocitritase production[2], the glucose glutamate salts medium used for keto-acid oxidase production[6], and the glucose tryptone yeast extract medium used for anaerobic growth to induce citritase formation[6]. As shown in Table 2, only the *E. coli* cells grown aerobically with organic acid as source of energy contained isocitritase. With glucose, as source of both aerobic energy and of anaerobic (fermentative) energy in the presence of citrate to induce citritase formation[6], the cells formed were devoid of isocitritase (Table 2). Thus in *E. coli*, citritase and isocitritase appear to operate anaerobically and aerobically respectively. It is also noteworthy that the isocitritase content of *P. aeruginosa* devoid of fermentative pathway to sustain growth is reduced about 50 per cent by growth on glucose instead of acetate.

Table 2. ISOCITRITASE FORMATION; EFFECT OF GROWTH CONDITIONS

Aerobiosis and growth substrate	Isocitritase (units/mgm. protein)		
	P. aeruginosa, 9027	*A. aceti*, F.4	*E. coli*, Crooks
Aerobic			
Acetate	1·55	0·86	0·93
Glucose*	0·80	—	nil
Glycerol†	—	nil	—
Anaerobic			
Glucose‡	No growth	No growth	nil

* Medium, same as for acetate (Table 1), except 0·3 per cent glucose substituted for acetate.

† Glycerol 2 per cent, NZ amino 1 per cent, yeast extract 1 per cent.

‡ Glucose 1 per cent, glutamate 0·2 per cent, yeast extract 0·5 per cent ammonium salts.

In addition, extracts of two *Acetobacter* strains, *suboxydans* and *aceti*, grown on glycerol as source of energy and of carbon in the presence of organic nitrogen[5], were devoid of isocitritase. *Acetobacter suboxydans* does not possess a complete oxidative cycle, lacks condensing enzyme, and isocitric and α-ketoglutaric acid dehydrogenase[5], and requires vitamins and at least traces of glucose or glycerol for growth[5]. This strain does, however, possess aconitase, which may imply an alternative pathway of citrate formation and/or utilization under altered growth conditions—thus the assumption should not as yet be drawn that *A. suboxydans* is devoid of an isocitritase-forming mechanism. The *Acetobacter aceti* strain, when grown on an acetate-salts medium, contains isocitritase (Table 1) and, like *E. coli*, does not form isocitritase when grown on carbohydrate (glycerol) medium.

While further studies will be required to clarify these diverse metabolic patterns, including the source of the biosynthetic precursors, it is tempting to suggest that the C_2 precursor of glycine and of C_1 may arise from both the tricarboxylic acid cleavage, presumably via isocitritase, and from glucose, possibly from 'glycolaldehyde-DPT' via transketolase reaction —the source in a given case depending on growth conditions and enzymatic patterns.

These observations also lend further credence to Krebs's suggestion of the tricarboxylic acid cycle as a precursor of biosynthetic intermediates, even though by alternative reactions of cycle intermediates[2,6], and further to emphasize that diverse pathways in the oxidative, as well as in aerobic and anaerobic carbohydrate metabolism, remain to be enumerated and clarified.

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Concentration of Iodine-131 across the Placenta of the Guinea Pig and the Rabbit

DURING the course of an investigation on the structure and function of the guinea pig fœtal thyroid, it was noted that a concentration gradient of iodine-131 is established across the placenta after a single injection of iodine-131 is given to the mother. 3 mgm. of propylthiouracil per 100 gm. body-weight was injected subcutaneously in the pregnant animals, thus preventing organic binding of iodine. Forty to forty-five minutes later the dose was repeated

and 100 μc. of carrier-free sodium iodide-131 in 2 ml. of distilled water was injected subcutaneously in the right axilla. One hour later the animal was anæsthetized with 'Nembutal' and ether, a midline abdominal incision was made, the uterus opened and the fœtuses exposed and individually bled from the umbilical cord or from the heart. Maternal blood was collected by heart puncture before and after the removal of the fœtuses. Both maternal and fœtal blood was collected in heparinized centrifuge tubes. Either 0·5- or 1-ml. samples of plasma were diluted with distilled water to a volume of 10 ml. in 12-ml. glass containers and the radioactivity counted in a Geiger–Müller multiple counter[1].

Of the ten guinea pigs studied so far, all of which were between forty and sixty days pregnant, the iodine-131 concentration in the fœtal plasma was 1·5–5 times higher than the iodine-131 concentration in the maternal blood. Because the concentration of iodine-131 in the maternal blood, as indicated by heart puncture samples before and after removal of fœtuses (a 15-min. interval), was on a rising, steady or early falling curve, the ratio found of iodine-131 in fœtal plasma to that in maternal plasma reflects a true high plasma concentration of inorganic iodide in the fœtal blood.

The iodine-131 in the fœtal blood was not precipitable by protein precipitants, and when the fœtal plasma was allowed to come to equilibrium with its maternal plasma across a semi-permeable collodion membrane, the iodine-131 concentration was found to be equal in both. The results, together with the further findings that the ratio of iodine-131 in fœtal plasma to that in maternal plasma was reversed in two guinea pigs given 100 mgm. of sodium thiocyanate, indicate that there is a transport mechanism for iodide in the guinea pig placenta comparable to that in the thyroid gland, salivary glands and gastric mucosa.

It was possible to demonstrate the same high ratio of iodine-131 in fœtal plasma to that in maternal plasma in three pregnant rabbits, all of which had a steady level of iodine-131 in the plasma at the time of removal of the fœtuses; but the existence of a similar ratio could not be demonstrated in rats.

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Urinary Excretion of a Substance after a Single Dose

The method of Stern[1] for representing experimental data seems to be useful. However, the equation of Haigh and Reiss[2] has good theoretical justification, and it seemed worth while to see whether there was any simple relation between the two formulæ. Stern derives the equation

$$x \log 2 = Q \log \frac{t}{t_0} \qquad (1)$$

to represent the data where Q and t_0 are constants and x is the cumulative excretion as a function t. Haigh and Reiss's equation is

$$x = x_{\max.} \{1 - \exp(-\lambda t)\} \qquad (2)$$

where $x_{\max.}$ and λ are constants. Plotting Haigh and Reiss's equation in the manner of Stern gives an effective straight line over the range $t = 0{\cdot}2/\lambda$ to $t = 4/\lambda$, and this curve then has a discontinuity and becomes the horizontal straight line $x = x_{\max.}$ for greater times. This straight line yields values of $Q = a\, x_{\max.}$ and $t_0 = b/\lambda$; tentative values of the constants a and b are $a = 0{\cdot}25$ and $b = 0{\cdot}1$. For values less than $0{\cdot}2/\lambda$, the curve tends to the straight line $x = 0$ as t tends to zero. This behaviour is more satisfactory than the straight line of Stern, as the latter will yield negative excretions for the period immediately after the injection. Thus it appears that in fact Stern's method is merely a convenient way of plotting a Haigh–Reiss formula.

In the case of Stern's curves (C and D), there appears to be some evidence for a discontinuity of the type predicted by a Haigh and Reiss formula, except that the second straight line is not horizontal but instead has a smaller slope than the first line. Our own experiments with inulin give curves of the same type as D, and in this case a study was also made of the plasma concentration, C_1, which could be represented by an equation

$$C = C_1 \exp(-\lambda_1 t) + C_2 \exp(-\lambda_2 t) \qquad (3)$$

where C_1, C_2, λ_1 and λ_2 are constants. Assuming simple glomerular filtration, equation (3) would yield a double Haigh–Reiss formula of the form

$$x = x_1 \{1 - \exp(-\lambda_1 t)\} + x_2 \{1 - \exp(-\lambda_2 t)\} \qquad (4)$$

where x_1 and x_2 are constants. Such a formula when plotted in the manner of Stern yields a curve of type D, and it is possible to evaluate the four constants x_1, x_2, λ_1, λ_2, from an analysis of the curve.

In the case of inulin, equation (3) is a mathematical representation of the combined effect of passage of inulin into (and out of) the interstitial fluid and glomerular filtration. Thus the constants in equations (3) and (4) are related to physiological factors in the case of inulin, and probably in the cases studied by Stern.

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I am grateful to Hough, Barnard and Bassir for suggesting that my equation may be considered as an approximation, over a limited range of t, to that described by Haigh and Reiss. This explanation may provide some logical justification for what was an empirical finding.

Fig. 1 shows a graphical representation of the Haigh and Reiss equation (solid curve), plotted on a log time-scale, together with the limits over which Hough *et al.* suggest a line of the form of my equation may be considered as a good approximation (marked G). Their suggestion that $x = x_{\max.}$ for large values