Table 1. Effect of Iron on the Formation of α-Alanine and Pyruvate by a Strain of *Corynebacterium diphtheriae*. Four-day Still-incubation at 32–33° C.

Iron added (μgm./l.)	Bacterial growth (μgm. N/ml.)	Toxin (Lf./ml.)	Pyruvate formed (μgm./ml.)	Alanine formed (μgm./ml.)
0	543	60	220	617
16·5	592	55	196	547
33	561	52	172	553
66	634	44	95	373
100	627	47	120	313
133	561	23	57	307
330	546	3	11	127
660	563	0	11	97

almost one-twentieth of the quantity formed in the absence of iron. A similar variation will be seen in the amounts of toxin and α-alanine formed.

Although the primary metabolic sequence affected by iron deficiency still remains uncertain, these results clearly indicate that traces of iron exert a distinct influence not only on the liberation of toxin but also on the pyruvate metabolism of the organism. Our previous work[6] has shown that significant quantities of glutamate–pyruvate transaminase were present in the cell-free extracts of *SM*–1 culture. Consequently, the accumulation of pyruvate due to iron deficiency may result in the appearance of large amounts of α-alanine in the filtrates. Further work is now in progress, and the details will be published elsewhere.

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Thyroxine Metabolism by Extracts of Rat Liver

The *in vitro* de-iodination of thyroxine has been reported in a variety of biological systems, including slices, homogenates, and extracts of mammalian organs[1–3]. In particular, a system present in rat liver has been fairly extensively investigated and a technique evolved whereby quantitative measurements of de-iodination can be readily obtained[4]. At the outset of the present work, this technique was employed to re-investigate the properties of the de-iodinating system of rat liver. The heat stability of the homogenate[3] and the heat lability of the extract were confirmed, and the system shown to be inhibited by potassium cyanide. The rate of de-iodination was fastest at the beginning of the incubation period and fell off with time, levelling off after 1–2 hr. when 50–70 per cent de-iodination had occurred. Substrate specificity was not absolute, tri-iodothyronine being metabolized to a lesser extent. In all these respects the system used resembles that previously described by other workers[4].

All these preliminary experiments employed thyroxine labelled with iodine-131 in the 3′ : 5′ positions and chromatography of the incubation mixture failed to reveal any radioactive metabolites apart from iodide and a varying amount of excess thyroxine. Such chromatograms were frequently run together with a marker of stable tri-iodothyronine, but no trace of this compound could be identified among the labelled reaction products. It seems likely, therefore, that the two iodine atoms in the 3′ : 5′ positions are removed simultaneously, or very nearly so. It was thereupon decided to synthesize radioactive thyroxine labelled with iodine-131 in the 3 : 5 positions, and to trace the metabolic fate of these atoms in the same system. It was immediately apparent from the first trials that de-iodination from these positions was slight; 80–95 per cent of the radioactive label remained adhering to the protein of the extract, and was thus presumably still in organic combination, iodide passing through the filter. An experiment was also carried out in which the samples of thyroxine contained the label in varying proportions on the two rings. The results show a proportionality between the extent of de-iodination and the percentage of the label present in the 3′ : 5′ positions.

Percentage of label in 3′ : 5′ positions	De-iodination (per cent)
0	8·0
20	19·5
40	29·9
60	35·0
80	43·6
100	53·9

Thyroxine is thus apparently metabolized to a compound (or compounds) which contains the 3 : 5 iodine atoms but from which the 3′ : 5′ iodine atoms are absent. Work which is still in progress indicates that this unknown compound can be separated from excess thyroxine by butanol extraction. Its chemical structure is apparently such that the alanine side-chain and proximal ring are preserved intact, because, on treatment by hydrolytic procedures, a labelled degradation product is obtained having the chromatographic properties of 3 : 5 di-iodotyrosine.

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The Biuret Complex of Gelatin and the Mechanism of Gelation

The gelation of gelatin results from a thermally reversible cross-linking process involving hitherto unknown functional groups. In studies of gelation–molecular weight correlations of chemically modified gelatins (amino-acetylated, carboxyl-esterified, hydroxyl-acetylated, guanidino-nitrated) and of hypobromite-treated gelatin we have found that the polar and charged side-chain groups are of little importance in gelation[1]. Further, by study of the effects of