Table 3. Results of a pure culture thermophilic cellulose fermentation with addition of $CaC^{14}O_3$.

Products	mg	mg carbon	counts per mg and minute	total counts per minute
Ethanol	61	32	23	1.400
Formic acid	10	3	81	810
Acetic acid	87	35	4.5	390
Lactic acid	67	27	88	5.900
Succinic acid	69	28	808	56.000
Total	294	125		64.500
Carbon dioxide, added	102	38	54.700	5.580.000
Cellulose, consumed	473	210		

2 pro mille. The succinic acid formed by means of CO_2-fixation corresponds to 1.6 per cent of the total succinic acid formation. Experiments are being planned in order to establish if more CO_2 would be fixed when the fermentation is carried out under higher CO_2-pressure than in the present case.

It should be noticed that Virtanen [6] and Koistinen [7] have demonstrated CO_2-fixation in cellulose fermentation with mixed cultures, but it was not proved that the fixation was not performed by the symbionts.

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Chemistry of the Histochemical Phosphatase Reaction

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Gomori's [2] and Takamatsu's [3] reaction for the histochemical detection of phosphatase in tissues is based on the following principle: The tissue is fixed in alcohol or acetone, dehydrated, embedded in paraffin and sectioned on the microtome. The deparaffinized sections are then incubated at pH > 9 with the phosphate ester (usually glycerophosphate) in the presence of calcium ions. Upon liberation of inorganic phosphate by enzymatic cleavage a precipitate of hydroxy-apatite is formed. Then the tissue is treated with cobalt nitrate in order to convert the precipitate to cobalt phosphate, and finally with ammonium sulfide in order to convert the cobalt phosphate into cobalt sulfide. The latter precipitate is black and reveals upon inspection in the microscope the presumed sites of phosphatase activity.

The technique is widely used and various variants of the method have been developed employing other combinations of substrates, salts and pH. In all of these, however, the principle is the same, since a colourless product of the enzymatic reaction is by suitable treatment converted into a colored substance which can be observed in the microscope. A number of critical investigations of the technique have been carried out by various authors, in order to clear up certain points which are vital for its validity, such as inactivation of the enzyme during the preliminary histological treatment, secondary effects of diffusion and re-adsorption, *etc.* As far as we are aware, however, the question of the conversion of the initial precipitate into the stained end-product has not received similar attention. In order to test this

point, the following experiment has been carried out:

A calcium phosphate precipitate was prepared from Sørensen's secondary phosphate and a slight excess of calcium nitrate. After standing overnight the supernatant was decanted and an excess of cobalt nitrate added. The mixture was stirred over night, the supernatant was decanted, the precipitate resuspended in water and divided into two parts.

One part was treated with sodium sulfide and the resulting black precipitate filtered, washed and dried. The other part was washed and dried and then suspended in a 5 % agar solution. After gelation a cube of 2 cm side length was cut from the agar and immersed in sodium sulfide solution. Within 30 minutes the black coloration of cobalt sulfide had extended to the core of the cube. Accordingly, the main portion of the agar suspension was sliced in 2—3 mm thick discs and treated with sodium sulfide for 3—4 hours. Then the blackened precipitate was recovered by melting, diluting and decanting. Both preparations were then analyzed with the following result (values in % of water-free precipitate).

	Water	Agar
Sulfide-S	3.5	2.4
Co	22.0	26.5
PO_4	21.5	18.4

Besides, there was a very heavy qualitative reaction for SO_4^{--} and a somewhat slighter one for Ca^{++}. Calculated for CoS: Co 64.8 %, S 35.2 %, for Co_2S_3 : Co 55.1 %, S 44.9 %.

From the above experiments we have to conclude that the conversion of calcium phosphate into cobalt sulfide is by no means quantitative. Moreover, a substantial part of the cobalt sulfide, even if formed originally, seems to be oxidized to sulfate. Since this is the case even in model experiments it is to be assumed that in tissues the process of conversion will be still more complicated. As long as cobalt sulfide is formed at all, the value of the reaction as a qualitative method of phosphatase detection will scarcely be influenced by the results reported here; but in all cases where the intensity of blackening is taken as a quantitative indicator of the amount of phosphatase present, the greatest caution seems to be necessary. Numerous such attempts have been reported in the literature.

The only case that we know of in which the formation of sulfide has been evaluated quantitatively is an investigation by Doyle [1], where the sulfur is extracted and incorporated into methylene blue which is measured colorimetrically. The detailed communication about this method has not yet appeared, but Dr. Doyle has informed us that in this case, too, the conversion into sulfide has been found to be incomplete.

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Note on the Crystallisation of Chondrosamine

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In working with mucopolysaccharides it is often necessary to use chondrosamine (galactosamine) as a reference substance. However this substance is very difficult to obtain pure in any reasonable yield. The best method is that originally described by Levene [1], in which the lead salt