Thus the addition of phlorhizin brings about a change in the relative effective amounts of the two enzymes.

By allowing a mixture of potato phosphorylase and potato isophosphorylase to act on glucose-1-phosphate in the presence of increasing amounts of phlorhizin, we have obtained a series of polysaccharides which give with iodine a range of coloration from blue-violet, violet, purple to brown, that is to say, which have increasing degrees of branching, ranging from that of amylopectin to that of glycogen.

The degree of branching of the polysaccharide obtained by the combined action of phosphorylase and isophosphorylase depends, therefore, on the proportion of the two enzymes. The larger the relative amount of isophosphorylase the more branched is the polysaccharide formed.

We wish to thank Prof. K. H. Meyer for his interest in this investigation.

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Microbiological Oxidation of Sterols

In a previous communication[1], we described a method by which we oxidized cholesterol with *Azotobacter*. Our present experiments had a double purpose: (1) to limit the direction of the oxidation as narrowly as possible; (2) to work out a simple reproduction method, for our *Azotobacter* react with extreme sensitivity to antagonistic bacteria, so that their culture can be made only under the strictest sterile conditions.

For this double purpose we used bacteria which grow selectively on a certain medium, and investigated bacteria which decompose pyridine. We found that for oxidation of cholesterol *Proactinomyces roseus* was the most suitable[2]. This bacterium simultaneously decomposes the pyridine most effectively, and in the presence of a source of carbon multiplies fairly intensively. The presence of pyridine prevents the growth of other bacteria in the culture, so we could always carry out these experiments without any special sterilization. If we used cholesterol as source of carbon in the pyridine medium and aerated it for a suitable time, oxidation occurred and 7-oxycholesterol and cholestenone formed as oxidation products.

The formation of 7-oxycholesterol means that in the sterol series the $-CH_2-$ group can be oxidized to alcohol bacteriologically. As under suitably selected experimental conditions principally 7-oxycholesterol is formed, the results have a practical significance for the manufacture of the 'D_3' provitamin, as we succeed in obtaining it from cholesterol in very good yield in one step.

The experiments were made under the following conditions. We dispersed 0·5 gm. cholesterol in a litre of 0·2 per cent pyridine synthetic culture, and inoculated the mixture with *Proactinomyces roseus*. The culture was kept 14 days in a thermostat at 34° C. with constant aeration, and the pyridine used up was replaced from time to time. At the end of the cultivation period we extracted the medium with petrol-ether. The products were separated by chromatographic absorption on aluminium oxide. Besides the remaining minimal cholesterol we isolated as chief product 7-oxycholesterol and 4,5-cholestenone. Other oxidation products also formed in minimal quantities, identification of which is now in progress. We are continuing these experiments with *Proactinomyces roseus* in the sexual hormone series.

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The Sommelet Reaction

In a recent communication, Angyal and Rassack[1] show that the Sommelet reaction[2] is an oxidation-reduction process with the aldimine Ph.CH=NH as a probable intermediate,

$$PhCH_2NH_2 + PhCH_2N:CH_2 \xrightarrow{H_2O} PhCHO + NH_3 + PhCH_2NHCH_3,$$

and does not involve, as originally suggested by Sommelet[2], and stated by Graymore and Davies[3], prototropic conversion of methylenebenzylamine (I) to benzylidenemethylamine (II).

$$(I)\quad Ph.CH_2 - N = CH_2 \rightleftharpoons \left[Ph.CH \overset{\frown}{-} N \overset{\frown}{-} CH_2\right]^- \rightleftharpoons Ph.CH = N - CH_3 \quad (II)$$

It is desirable to point out that this mechanism, now disproved, was inherently improbable. Ingold and Shoppee[4] showed that the methyleneazomethine system is one of the least mobile of triad prototropic systems; mobility in the phenylmethylene-azomethine system (I—II) is only achieved in the catalytic presence of hydroxide ions at 300° or of a considerable concentration of ethoxide ions at 190°, 2·92 *N*-sodium ethoxide in ethanol at ~ 85° being ineffective. These results were considered by Graymore and Davies, who, however, dismissed them with the remark that they are not comparable with those obtained by them in the presence of hexamine in dilute aqueous solution (boiling approx. 0·3 *N*-hydrochloric acid), and who state that under these conditions isomerization of methylenebenzylamine (I) to benzylidenemethylamine (II) ensues. While suitable prototropic systems are capable of catalysis by acids, methylenebenzylamine (I), like other Schiff's bases, is readily hydrolysed by hot dilute mineral acid[4]; even if, owing to the presence of an excess of formaldehyde, some proportion of methylenebenzylamine is always present under the experimental conditions used by Graymore and Davies, facile catalysis of a prototropic system so refractory as (I — II) under these conditions seems in the highest degree unlikely.

Graymore and Davies also suggest that the conversion of only some 10 per cent of methylenebenzylamine (I) to benzylidenemethylamine (II) in the presence of catalytic anions, reported by Ingold and Shoppee, is due to the removal of methylenebenzylamine (I) from the reaction by polymerization. It seems more probable that the proportion of isomerides, (I) 90 per cent : (II) 10 per cent, observed experimentally, is an example of the kinetic control of ion-recombination as opposed to the thermo-