Similar results were achieved by using different combinations of pathogenic, penicillin-sensitive bacteria such as *Pasteurella* bacteria or β -*streptococci* and penicillinase-producing, non-pathogenic bacteria such as *coli*-bacteria or *pyocyaneus* bacteria.

Some experiments on the pathogenicity of the *staphylococci* used in the above-mentioned investigations gave some rather surprising results.

Eighty rabbits of the same age and weighing about 2 kgm. each were infected subcutaneously with 12-hr.-old broth cultures of penicillinase-producing *staphylococci*. The doses were 1 ml., 0.5 ml. and 0.1 ml. The mortality figures are shown in the accompanying table.

Dose	Treatment					
	Penicillin 30,000 units per day			No penicillin		
	Number	Death	Per cent	Number	Death	Per cent
1.0 ml.	10	6	60	10	0	0
0.5 ml.	20	5	25	20	0	0
0.1 ml.	10	4	40	10	0	0
Total	40	15	37.5	40	0	0

The rabbits which were treated with a daily dose of 30,000 units of penicillin had an average mortality-rate of 37.5 per cent. In the control group there were no fatal cases. The difference in mortality is statistically significant. The treated animals also had a higher rise in body temperature and a greater loss of weight than the control animals.

It is evident that the treatment with penicillin in these experiments caused an enhanced pathogenicity of the penicillinase-producing *staphylococci* for rabbits.

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Splitting of the Hæm-Globin Linkage in Hæmoglobin with Methanol

DURING an investigation of the biosynthesis of hæmoglobin in erythrocytes from rabbits intoxicated with phenylhydrazine, it was observed that the hæm-globin linkage in hæmoglobin was broken by methanol without addition of acids. The first extract was almost colourless or slightly yellowish in colour; but on further extraction with new portions of methanol the extracts became more and more reddish in colour, and by continuous extraction in a Soxhlet apparatus a dark red solution of hæm was obtained.

This observation was rather surprising, as no references could be found in the literature to such an effect of methanol on hæmoglobin.

To ensure that the methanol effect was not due to some peculiarity of the hæmoglobin molecule from the phenylhydrazine-intoxicated rabbits, erythrocytes from normal rabbits, pigs, horse, man and duck

were extracted with methanol in the same way and with the same result.

The spectral absorption curves of the methanol-hæm solutions from these species measured in a Beckman spectrophotometer were found to be identical, and showed three maxima, one at 400 m μ , one at 495 m μ and one at 600 m μ , with decreasing intensity of the maxima in the same order.

The method of sampling the erythrocytes and preparing the hæmoglobin precipitate was found to be of high importance. I have found that the best way to get a smooth and fine precipitate from which the hæm group can be easily extracted is to use cells from citrated blood. The cells are washed twice with a slightly hypertonic sodium chloride solution, the protein is precipitated with 10 vol. of acetone, washed once with 5 vol. of methanol, brought into a funnel and extracted continuously in a Soxhlet apparatus overnight. This procedure gives a highly concentrated solution of hæm and leaves a protein powder which is only slightly coloured. The reagents used are Merck analytical preparations. The acid content of the methanol measured as formic acid was found to vary between 0.0025 per cent in newly opened flasks to 0.005 per cent in flasks which stood for a couple of weeks after opening the seal.

When methanol was added to erythrocytes, hæmolysates or to oxyhæmoglobin precipitated with acetone, gas was evolved and the colour changed from bright red to dark red. This indicates that the oxyhæmoglobin present in the system is transformed into hæmoglobin by methanol.

According to the modern view on the formation of bile pigments¹, bilirubin is bound to globin in the normal bilirubin-globin complex in the same way as that proposed by Holden² for the hæm-globin linkage in hæmoglobin. The bilirubin-globin complex is split by methanol into bilirubin and denatured globin³, and one might expect a similar effect of methanol on the hæm-globin linkage in hæmoglobin. The following scheme for the methanol effect is suggested:

Hamsik⁴ has suggested that methanol is able to dislodge oxygen from oxyhæmoglobin, forming loose compounds with the hæm group, which is easily split off with acid methanol or acetone. A more reasonable assumption seems to be that methanol denatures the globin part of the molecule in such a way that the covalent linkage between the hæm-iron and the imidazole groups of globin is split, with release of oxygen and formation of denatured hæmoglobin, which then, in accordance with the above considerations, is split into hæm and denatured globin.

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² Holden, H. F., "Ann. Rev. Biochem.", **14**, 599 (1945).

³ Hijman van den Bergh, "Das Gallenfarbstoff im Blute" (2nd edit., Leyden, 1928).

⁴ Hamsik, A., *Z. physiol. Chem.*, **190**, 199 (1930).