Auto-reactivity to Haemoglobin in the Sera of Cattle and Sheep infected with Haemolytic Bacteria

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Three naturally occurring proteins in human serum bind native or altered haemoglobin or its components. Haptoglobin binds haemoglobin (Jayle & Morretti, 1962); the complexes are stable but do not precipitate. Haemopexin (Grabar, de Vaux St-Cyr & Cleve, 1960) binds haematin but not haemoglobin, and albumin binds haem from methaemoglobin (Fairley, 1938). There is also evidence for a β -protein binding haemoglobin (Tombs, 1960) but this may be identical with haemopexin.

Human serum precipitates in agar with haemolysates (Peetoom, Rose, Ruddy, Micheli & Grabar, 1960; Wilson & Jobling, 1961). This precipitation occurs in sera both with and without haptoglobin and appears to be a reaction between albumin and methaemoglobin leading to release and precipitation of partially denatured globin (Harris & Fairley, 1962). Similar reactions occur with other haptoglobin-containing sera, e.g. from pigs and horses.

Bovine and goat sera have no haemoglobin-binding protein in the α_2 region corresponding to haptoglobin, although it was claimed that bovine serum show some haemoglobin binding in the fibrinogen region (Liang, 1957). Subsequently evidence was presented for a β -protein binding haemoglobin after turpentine injection in calves (Bremner, 1964).

Studies in this laboratory have shown that in cattle and sheep with acute inflammatory processes there is rapid production of a protein precipitating with haemoglobin. The protein, which in this paper will be termed haemoglobin-reactive protein (HRP), has been shown to be a macroglobulin, travelling with immunoglobulin M (IgM) antibody on sucrose-density-gradient ultracentrifugation and being eluted from Sephadex G-200 and DEAE-cellulose with IgM. Like IgM its activity is destroyed by treatment with 2-mercaptoethanol. On the basis of this evidence and the fact that the precipitation in agar appears to be in optimum proportions it was thought likely to be immunoglobulin (Spooner, 1969), but more recently it has been found that the protein does not react with specific rabbit anti-(bovine IgM) or anti-(bovine light chain) sera and is insoluble in water, and the complex does not fix complement. Also, because of its size it cannot be examined successfully in starch gel. It is possible to prepare rabbit antisera specific for the protein by immunizing with sera containing HRP and then

absorbing the resulting antiserum with normal serum.

Reverse immunoelectrophoresis of bovine, sheep, human, pig, horse and rabbit sera developed with bovine erythrocyte lysate showed a benzidine-staining precipitate in the β region with bovine and sheep serum, whereas with the remaining sera there was a more diffuse benzidine-staining zone in the α_2 region. This latter reaction may be due to haptoglobin and cannot involve methaemalbumin as in the reaction studied by Harris & Fairley (1962), as there is no albumin present in the benzidine-stained region.

HRP reacts with all of the mammalian haemoglobins tested including all of the known bovine haemoglobin variants and bovine haemoglobin F. It also reacts with human haemoglobin C and haemoglobin S but not with methaemalbumin. It precipitates with bird haemoglobin but not with lizard, frog or fish haemoglobin, whereas human, pig, horse and rabbit sera gives a benzidine-staining band in agar with lizard lysates just as with human lysates.

HRP does not react with β -chains prepared by *p*-mercuribenzoate treatment of human haemoglobin (Tyuma, Benesch & Benesch, 1966) but reacts very weakly with α -chains. Bovine HRP precipitates weakly in agar with bovine globin but it appears that slight denaturation of the globin rapidly destroys its ability to bind with HRP. HRP does not precipitate with haematin. It is stable at 60°C for 30 min.

Acute infections in mice produce a haemoglobin-binding protein (Williams & Wemyss, 1961), which these workers called haptoglobin and which is produced in the liver (Williams, Asofsky & Thorbecke, 1964). This protein may be similar to, and the β protein described by Bremner (1964) may well be identical with, HRP, but the physical characteristics of these proteins were not studied and it was not shown with either the bovine or mouse proteins whether they precipitated with haemoglobin.

Precipitation between serum and haemoglobin has not been found in over 800 normal cattle and 100 sheep. Experimental infection with haemolytic bacteria causes a very rapid increase in HRP. It is first detectable at about 28h and subsequently increases over 4–6 days so that precipitation in agar can be obtained with serum diluted 1:256; on recovery it rapidly disappears again.

It thus appears that in bovine and sheep sera, and probably in the sera of other ruminants, the protein involved in binding haemoglobin, although bearing many similarities to haptoglobin, differs from the human protein in several ways, namely its size, electrophoretic mobility, specificity and particularly its ability to precipitate with haemo-

globin. HRP bears a resemblance to the acute-phase proteins such as C-reactive protein (Hedlund, 1961) showing many of the characteristics of an antibody without being immunoglobulin.

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COMMUNICATIONS

Biosynthesis of *N*-Acetylanthranilic Acid by Aromatic Auxotrophs of *Aerobacter aerogenes* and *Escherichia coli*

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N-Acetylanthranilic acid has been isolated from culture filtrates of *Aerobacter aerogenes* N5-36 (a mutant lacking phosphoribose anthranilate transferase) utilizing either shikimic acid or quinic acid (Ratledge, 1967, 1968). The role of this compound in the biosynthesis and metabolism of aromatic compounds was not clear. Present work has shown that during growth of this mutant on quinic acid medium supplemented with 10 μ M-L-tryptophan anthranilate accumulation commences immediately whereas that of *N*-acetylanthranilate begins about 90 min later. Maximum concentrations are reached at the beginning of the stationary phase of growth and are 190 μ M and 100 μ M respectively. By increasing the tryptophan concentration to 30 μ M anthranilate accumulation is decreased and that of *N*-acetylanthranilate is completely arrested. At 50 μ M-tryptophan or above no anthranilate accumulates but if 70 to 360 μ M anthranilate is added into such a medium from *N*-acetylanthranilate can be detected once more in the culture filtrates.

Extracts of this mutant catalyse the synthesis of *N*-acetylanthranilate from anthranilate in the presence of either acetyl-CoA or acetate, CoA and ATP. No deacylation of the product could be demonstrated. The apparent K_m for anthranilate in crude extracts is about 10 mM. Similar acetylating enzymes are also present in extracts prepared from *A. aerogenes* NCW (parent wild-type), *A. aerogenes* 62/1 (a multiple auxotroph: phe⁻, tyr⁻, try⁻) and *Escherichia coli* B37 (a mutant lacking phosphoribose anthranilate transferase). These organisms also accumulate *N*-acetylanthranilate in the growth medium (but for *A. aerogenes* NCW 360 μ M-anthranilate had to be included in the medium;

and *Escherichia coli* B37 was grown on glucose with 10 μ M-tryptophan). *N*-Acetylanthranilate is not found, however, when *A. aerogenes* NC3 (a mutant lacking anthranilate synthetase) is grown on quinic acid medium but does accumulate upon the addition of 360 μ M-anthranilate into the medium.

All available evidence suggests that *N*-acetylanthranilate arises from, and is not a precursor of, anthranilate. The acetylase appears to be a constitutive enzyme as it is not repressed in *A. aerogenes* N5-36 by up to 150 μ M-tryptophan and is present in cells of *A. aerogenes* NCW and NC3 which do not, under normal conditions, accumulate anthranilate. The high K_m value suggests that this enzyme may well have a broad specificity although this has yet to be tested.

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The Precursors of Nicotinic Acid in Bacteria

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Tryptophan is the precursor of nicotinic acid in fungi (Bonner & Yanofsky, 1951) and mammalian tissues (Nishizuka & Hayaishi, 1963), but does not appear to be a precursor in most bacteria and plants.

In studies with *Escherichia coli*, the carbon skeleton of nicotinic acid was found to be derived from a C₄ dicarboxylic acid and a C₃ unit such as glycerol (Ortega & Brown, 1960). Similar findings were reported with *Mycobacterium tuberculosis*, when it was found that [1,3-¹⁴C]glycerol was