since the extracts of hydrolyzates showed on chromatography a yellow streak which hardly moved in the phosphate-buffer system of Levy [4]. Chromatography of the aqueous phase in *tert*-amyl alcohol revealed the presence of ninhydrin-positive yellow spots which were obviously DNP-peptides of varying size. In addition, the yield of ε-DNP-lysine was very low. The incomplete hydrolysis was, therefore, one reason for the rather poor yield of glycine, which corresponded to 21 % of the total glycine present as an end group. The hydrolysis of the DNP-derivative of the faster component with 6 and 12 N HCl gave such low yields of DNP-glycine that it could not be measured. In comparison, the hydrolyzates of the DNP-derivative of the slower component showed on chromatography no derivatives which could be assumed to be DNP-peptides. The recovery of DNP-valine in the slower component was found to be 60 % after a 24 h hydrolysis at 105°C in 6 N HCl. According to Porter and Sanger [7], DNP-valine was 64 % unchanged in a 24 h hydrolysis in 5.7 N HCl. Using this value as a correction for the recovery of DNP-valine, a yield of 0.93 moles of valine was obtained for one mole of protein.

Discussion. The number of free α-amino groups gives the number of the open peptide chains present in the protein molecule. From the above findings it is evident that the electrophoretically-faster component of Lhb consists of one peptide with N-terminal glycine, whereas the slower one consists of one chain terminating in valine. These findings agree well with recent conclusions drawn from studies on the amino acid contents of the two components. The total lack of sulfur-containing amino acids, and accordingly the absence of S—S bonds that could form bridges between different peptide chains, led to the conclusion that the two components consist of one peptide chain only. Comparative studies on the N-terminal amino acids of normal and fetal human hemoglobin, as well as on the red cell pigments of various animal species, have shown that the number of peptide chains varies from two to six per molecule. It also seems that all hemoglobins contain at least one invariant chain terminating in valine [7,8]. In the case of myoglobins, which have a single peptide chain, the data are limited. Horse myoglobin has been found to terminate in glycine [7,9] in all the fractions (Mb I, Mb II and Mb III), while whale myoglobin [10] terminates in valine. The interchange of glycine and valine in the N-terminal position as found above in the two Lhb components gives additional support to the assumption recently put forward [2] that the synthesis of the two components is controlled by systems which are genetically different.

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Derivatives of 4-Amino-2-hydroxybenzenesulfonic Acid

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In connexion with researches [1-6] on derivatives of 4-amino-2-hydroxybenzoic acid (PAS) we wished to investigate the tuberculostatic activity of the analogous 4-amino-2-hydroxybenzenesulfonic acid and its derivatives. According to Lespagnol, Sevin and Beerens [7] the free acid is without tuberculostatic effect, but Youmans (*cf.* Doub *et al.*[8]) found both the acid and its amide to be slightly active. As the tuberculostatic effect of PAS is — at any rate in part — connected with an antagonism to *p*-aminobenzoic acid [1,9,10], it seemed not improbable that introduction of heterocyclic substituents into the sulfonamide group of 4-amino-2-hydroxybenzenesulfonamide might enhance the bacteriostatic activity of this compound very significantly just as it does with sulfanilamide. Our attempts to

Table 1.

Substance	Tuberculostatic effect			
	MA3	MA3(SM-res.)	MA3(PAS-res.)	MA3(INH-res.)
SM	6.8	5.8	6.8	6.9
PAS-Na	7.5	7.0	6.0	7.0
INH	7.8	7.8	7.5	5.3
II(amide)	<4	<4	<4	<4
IV(2-pyridylamide)	4.1	<4	4.0	4.3
V (anilide)	5.8	5.0	4.6	5.0

prepare the desired heterocyclic derivatives met with extraordinary difficulties, and at that time the problem was not pursued further. However, without the preparation of a PAS-analogue of sulfapyridine or sulfathiazole, *etc.*, we still considered our investigations on PAS-derivatives incomplete, and recently we succeeded in preparing the 2-pyridylamide of 4-amino-2-hydroxybenzenesulfonic acid in a pure state. Attempts to prepare derivatives of 2-aminothiazole, 2-amino-1,2,4-thiadiazole or 2-amino-4-methylpyrimidine by the same method yielded only small amounts of unidentified substances, which were not investigated further. It is remarkable that these difficulties are only met with when heterocyclic amines are used; the anilide of the sulfonic acid could be prepared without difficulty.

Recently Lora-Tamayo *et al.*[11] have prepared the hydrazide and some amides of 4-acetamido-2-hydroxybenzenesulfonic acid — also with a view to investigating their tuberculostatic activity — but have apparently not succeeded in deacetylating the amino group. A free amino group would, however, appear to be essential for any bacteriostatic effect.

By courtesy of Mr. L. Tybring, Bacteriological Department of the Leo Pharmaceutical Company, the bacteriostatic activity of 4-amino-2-hydroxybenzenesulfonic acid, its amide, anilide and 2-pyridylamide was tested against a *Mycobacterium tuberculosis* strain (MA 3) and against SM-resistant, INH-resistant and PAS-resistant variants of this strain in Dubos' medium (SM = streptomycin, INH = *iso*nicotinic hydrazide, PAS = *p*-aminosalicylic acid).

In Table 1 the tuberculostatic effect, a, is expressed as minus the logarithm of the dilution of the substance (1 g per 10^a g of the solution) that causes a definite but incomplete inhibition.

Accordingly 4-amino-2-hydroxybenzenesulfonic acid and its derivatives have a much less pronounced tuberculostatic effect than PAS, streptomycin, or *iso*nicotinic hydrazide, so the possibility of finding chemotherapeutics of practical importance in this group of substances seems to be excluded.

The activity of these substances against staphylococci, streptococci, gonococci, meningococci, and various Gram-negative bacteria was also found to be low (in most cases $a < 4$).

Experimental. 4-Amino-2-hydroxybenzenesulfonic acid. The acid was prepared by sulfonation of *m*-aminophenol (200 g) with conc. sulfuric acid (350 ml) containing a small amount of fuming sulfuric acid (1 ml), *cf.* Thorpe and Williams[12] and Miller *et al.*[13]. Yield 305 g of recrystallised product (88 %).

Pyridinium 4-acetylamino-2-acetoxybenzenesulfonate was prepared according to Thorpe and Williams[12] and recrystallised from ethanol. Yield 500 g from 330 g crude sulfonic acid (from 200 g *m*-aminophenol, *i.e.* 78 % calculated on a *m*-aminophenol basis).

4-Acetylamino-2-hydroxybenzenesulfonylchloride (I). The foregoing pyridinium salt (100 g) was finely powdered and placed in a roundbottom flask provided with a $CaCl_2$-tube. Phosphorus pentachloride (100 g) was added and the mixture shaken vigorously for 3—5 min and then warmed for 5—15 min on a water bath until a fairly homogeneous paste had formed. Then the flask was cooled rapidly and the mixture poured slowly, in small portions and with stirring, onto ice (*ca.* 700 g). The acid chloride separated as a crystalline white solid; it was filtered with suction and washed with water (100 ml). The product was spread on filter paper and dried at room temperature. The dry product was extracted with 500 ml of boiling chloroform, and filtered with suction. On cooling of the chloroform solution 10—15 g of pure chloride separated. The mother liquor was used again for extraction of the remaining product, *etc.*, until almost all the crude product had dissolved. Eventually the chloroform solu-

tion was concentrated to 100 ml and cooled. Total yield 52 g (63 %); m.p. 169°C. (Found: C 41.25; H 3.20; N 4.87. Calc. for $C_{10}H_{10}ClNO_5S$: C 41.17; H 3.43; N 4.80.)

4-Amino-2-hydroxybenzenesulfonamide (II), was prepared according to Thorpe and Williams [12] and recrystallised from propanol. M.p. 154–155°C. (Found: C 38.60; H 4.46; N 14.56. Calc. for $C_6H_8N_2O_3S$: C 38.30; H 4.28; N 14.89.)

2-(4'-Acetamido-2'-acetoxybenzenesulfonamido)-pyridine (III). The above acid chloride (5 g) and 2-aminopyridine (5 g) were dissolved in chloroform (50 ml), and the solution was refluxed for 10 min. The cooled solution was extracted three times with sodium carbonate solution. On acidification and cooling the sulfonamidopyridine separated as a white solid; yield 2 g. A better yield was obtained by the following procedure: The chloride (5 g) and 2-aminopyridine (2.1 g) were dissolved in pyridine (9 ml), and the solution was heated at 60–70°C for 10 min. The solution was diluted with 100 ml of water, acidified with acetic acid, and then neutralised with sodium bicarbonate. Yield 4 g (60 %). The product was recrystallised from glacial acetic acid; m.p. 247–249°C. (Found: C 51.05; H 4.20; N 11.68. Calc. for $C_{15}H_{15}N_3O_5S$: C 51.58; H 4.28; N 12.03.

2-(4'-Amino-2'-hydroxybenzenesulfonamido)-pyridine (IV). The foregoing compound (2 g) was dissolved in 2 N sodium hydroxide (10 ml), and the solution was refluxed for 4 h. On neutralisation of the cooled solution the deacetylated compound separated as a white solid. Yield 1.6 g. The compound was recrystallised from water with addition of carbon. It contained approximately half a molecule of water of crystallisation; it melted at 97–100°C, then solidified and melted again at 205°C. (Found: C 48.70; H 4.43; N 15.24, and after drying over phosphorus pentoxide: C 49.80; H 4.40; N 15.63. Calc. for $C_{11}H_{11}N_3O_3S.\frac{1}{2}H_2O$: C 48.20; H 4.80; N 15.15; and for $C_{11}H_{11}N_3O_3S$: C 49.81; H 4.18; N 15.84.)

4-Amino-2-hydroxybenzenesulfonanilide (V). The acetyl derivative was prepared in the same way as II. Yield 3.85 g from 5 g of the sulfonyl chloride (I). As analyses indicated a partial deacetylation, the compound was recrystallised from acetic anhydride. M.p. 208–213°C. (Found: C 54.80; H 4.70; N 7.98. Calc. for $C_{16}H_{16}N_2O_5S$: C 55.17; H 4.63; N 8.04.

The acetyl derivative (3 g) was dissolved in 1 N sodium hydroxide (20 ml) and refluxed for 2 h. On neutralisation and cooling the deacetylated compound separated. Yield 2.1 g; it was 1.3 g after three recrystallisations from water + ethanol. M.p. 175–176°C. (Found: C 54.60; H 4.66; N 10.27. Calc. for $C_{12}H_{12}N_2O_3S$: C 54.54; H 4.58; N 10.60.)

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Derivatives of *p*-Chlorobenzenesulfonic Acid

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In the course of investigations on compounds with potential diuretic or antidiabetic activity we had to use N-(β-chloroethyl)-*p*-chlorobenzenesulfonamide as a starting material. A product supposed to have this composition has been described by Kulka [1]. However, we found that the product prepared according to Kulka and having the indicated melting point contained no aliphatic chlorine, and it was soon shown actually to be di-β-(*p*-chlorobenzenesulfonylaminoethyl)-sulfite, $(ClC_6H_4SO_2NHCH_2CH_2O)_2SO$. Kulka gives no Cl analysis of his product, but only C, H, and N values, with respect to which the chloride does not differ very much from the sulfite. The desired chloride was prepared from the corresponding hydroxy compound