S_{20^0} = ca 250×10^{-13} cm. sec.$^{-1}$ dynes^{-1}. If this papilloma protein has about the same shape in solution as the tobacco mosaic virus protein molecule,[7] it will have a molecular weight somewhat in excess of 20,000,000; such a particle is about 40 millimicrons in diameter.

Practically the same yield (0.22 to 0.26 mgr per gram) of heavy protein was derived from all materials except one, which was notably richer (0.81 mgr per gram). In 3 experiments the effect of each centrifugation upon the infectious principle was determined. To do this, serial dilutions of the original saline extracts, supernatant fluids and solutions of the sedimented pellets were titrated in domestic rabbits.[8] The minimum amount of purified protein needed to produce warts visible 17 days after inoculation was between 10^{-7} and 10^{-8} grams, whereas between 10^{-5} and 10^{-6} grams of total protein in the saline extracts was required for comparable infection. The heavy protein was several thousand times as infectious as the wart tissue from which it was derived. These results show that there was no appreciable loss of viral activity at any point in the preparation, that it followed the heavy protein at every step and was concentrated with it.

There is other evidence that this protein is intimately associated with the viral activity. Active extracts of cottontail rabbit papillomas produce exuberant growths in domestic rabbits. These warty masses, however, usually yield no active virus.[9] We have subjected the extract from ten grams of domestic rabbit wart tissue, found in repeated tests by Shope to be non-infectious, to the ultracentrifugal concentration and analysis described above. No heavy protein was found.

We wish to express our indebtedness to W. M. Stanley for the invaluable advice he has given.

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ACETYLATION OF PARA-AMINOBENZENESULFONAMIDE IN THE ANIMAL ORGANISM[1]

PARA-AMINOBENZENESULFONAMIDE has been shown to have a remarkable protective and curative action in β-hemolytic streptococcic infections in animals,[2,3,4,5] and is being used in the treatment of such infections in human beings. We became interested in studying the pharmacology of para-aminobenzenesulfonamide and have accumulated considerable data on its absorption and excretion. Quantitative determination can be made by diazotizing, coupling in acid solution with dimethyl-α-naphthylamine and comparing the color obtained with that obtained from standard solutions. With this method, we have shown that in the dog the substance appears to be excreted mainly or entirely in unchanged form, while in the rabbit and human it is excreted partly as a conjugated compound from which the original substance can be obtained by hydrolysis with dilute acid.[6] We present here data on the isolation and identification of a conjugated compound obtained from the urine of rabbits and humans after the administration of para-aminobenzenesulfonamide by mouth. We have also isolated the unchanged sulfonamide from the urine of dogs and humans.

A sample of urine obtained from a dog, which had received 1.0 gm per kgm of para-aminobenzenesulfonamide, deposited crystals on cooling in the ice box. These on recrystallization from dilute alcohol melted at 167–8°, a mixture with pure para-aminobenzenesulfonamide (M.P. 166–7°) melted at 167–8°. These crystals from the urine when analyzed by the colorimetric method checked a standard solution of the pure substance within 2 per cent.

A rabbit weighing 3.5 kgm received 3.5 gms of sulfonamide by mouth. Urine collected for the next 24 hours deposited crystals on standing over night. These were filtered off, recrystallized several times from water and dilute alcohol and finally from water. The final product consisted of beautiful needles melting sharply at 219°. Gelmo[7] gives the melting point of para-acetylbenzenesulfonamide as 219°.

Analysis:

Found N by micro-method 12.72 per cent.; 12.82 per cent.
Theoretical N for $CH_3CONHC_6H_4SO_2NH_2$ = 13.05 per cent.

Acetic acid was identified after hydrolysis as silver acetate, which was analyzed for silver with the following results.

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[1] This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

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Found Ag = 63.4 per cent.
Theoretical for $C_2H_3O_2$ Ag = 64.04 per cent.

A solution of these crystals gave no color after diazotization on the addition of dimethyl-α-naphthylamine (no free NH_2-group on the benzene ring). After hydrolysis with dilute hydrochloric acid, the substance gave (by colorimetric method) 80.0 per cent. and 78.8 per cent. of para-aminobenzenesulfonamide. Theoretical for

$CH_3CONHC_6H_4SO_2NH_2$ = 80.3 per cent.

The above data prove the conjugated compound obtained from rabbit's urine to be para-acetylaminobenzenesulfonamide.

This acetyl derivative has been obtained in several other experiments from the urine of rabbits given large doses of the sulfonamide. The following experiment gives a rough idea of the recovery.

A rabbit weighing 1.7 kgm received by mouth 1.7 gms of para-aminobenzenesulfonamide. Ninety cc of urine were secreted in the following 24 hours. This urine was heated just to boiling to dissolve a precipitate, and, while hot, 5 cc were taken and diluted for analysis. From this analysis the remaining 85 cc were calculated to contain 235 mgms of free para-aminobenzenesulfonamide and 1,010 mgms of the conjugated form (calculated as the acetyl compound). The 85 cc of urine were allowed to remain in the ice box for 2 days, the deposited crystals filtered off and dried. Six hundred mgms were obtained. On analysis, these crystals were found to contain 2 per cent. of free sulfonamide and 95 per cent. of the conjugated compound (calculated as the acetyl derivative). After three recrystallizations from water, the compound melted at 218°, and when mixed with para-acetylaminobenzenesulfonamide (the sample which had been identified) melted at 218°. The second 24-hour urine sample contained a considerable amount of the conjugated compound (by colorimetric analysis).

From the urine of a patient being treated with the sulfonamide para-acetylaminobenzenesulfonamide has been isolated and identified.

A 24-hour specimen of urine measured 980 cc. Analysis of a small sample showed it to contain 1.20 gm of free and 1.25 gm of conjugated compound (calculated as acetyl derivative). The urine was treated with 5 gms of charcoal (Norit), shaken and allowed to stand in the ice box for 8 days (a shorter time is sufficient). The charcoal was removed by filtration, and the filtrate analyzed. The filtrate contained 1.03 gm of the free and 0.25 gm of the conjugated compound. The charcoal was treated with 75 cc of 95 per cent. alcohol, heated for a few minutes on a water bath and allowed to stand over night. The charcoal was removed by filtration, the filtrate evaporated to about 15 cc, several volumes of hot water added and the solution placed in the ice box for 4 hours. The crystals obtained by filtration weighed 0.34 gm (dried at 100°). Colorimetric assay showed only a small trace of free sulfonamide, but after hydrolysis the sulfonamide content was increased to 77 per cent. The substance was nearly pure para-acetylaminobenzenesulfonamide. After solution of the 0.34 gm in hot alcohol, hot water was added and the solution was placed in the ice box over night. After recrystallization from water and drying at 90°, the needles melted at 219°, and a mixture of them with the acetyl compound from rabbit's urine melted at 219°. On the colorimetric assay after hydrolysis, the purified compound gave 80.6 per cent. para-aminobenzenesulfonamide, the theoretical being 80.3.

From the urine of another individual receiving the sulfonamide, both the unchanged sulfonamide and the acetyl derivatives were isolated in small amounts by evaporation and fractional crystallization and identified by mixed melting points. This method is laborious and was done before the selective adsorption of the acetyl derivative by charcoal was discovered.

We can conclude that in the rabbit and man the conjugated compound found in the urine after administration of para-aminobenzenesulfonamide by mouth is mainly, if not entirely, the acetylated derivative. It is interesting to note that this is another example of an aromatic compound containing an amino group attached to the benzene ring which the rabbit and man can acetylate but the dog can not.[8]

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DIFFRACTION OF X-RAYS AT VERY SMALL ANGLES BY CELLULOSES AND RAYONS

FROM our laboratory have been reported already the measurements of very large spacings for a number of natural materials. These include 171 A.U. in living nerve,[1] 440 A.U. in collagen, 48 A.U. for radially oriented natural wax in intestinal wall collagen,[2] 81 A.U. in keratin, 58 A.U. in gel rubber and 75 A.U. in chitosan.[3]

By extending the experimental technique to its fullest possibilities, many attempts have been made to resolve interferences at very small angles corresponding to very large spacings in cellulose and its deriva-

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