give a proof? It is probable that he visualized the two quotient groups side by side in two columns and "saw" that the resulting intransitive group would be simply isomorphic to the given abstract group, and propositions seen in this way are sometimes awkward things to put down in black and white.

The two papers in which the primitive groups of degree 15 and degree 16 are determined are models of their kind. In the second, page 270, it is shown in Miller's easy, graceful, flowing style that all the primitive groups of degree 16 (not alternating or symmetric) contain a self-conjugate subgroup in which every permutation is of order 2. This result suggests how inaccessible are the groups of degree 32, and how pitifully few are the distinct families of primitive groups we know or can reasonably hope to know.

The volume is very handsome. Paper and typography are all that could be desired, and the editing and proofreading is as near perfection as is humanly possible. As to misprints, it ties the present record of Lehmer's list of primes, as far as the reviewer was able to discover in an extensive but not complete reading.

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SPECIAL ARTICLES

THE ISOLATION OF A HOMOGENEOUS HEAVY PROTEIN FROM VIRUS-INDUCED RABBIT PAPILLOMAS

Two years ago[1] a crystalline protein was obtained by chemical treatment of the juice of plants diseased with tobacco mosaic virus. Numerous chemical, biological and physical experiments[2] indicate that this protein is the agent responsible for the disease. Similar chemical procedures have not yielded pure virus proteins from plants infected with other viruses. Recently, however, the development of methods involving differential ultracentrifugation has made possible the purification of proteins associated with the activity of certain of the less stable plant viruses.[3] The effectiveness of these methods has suggested the desirability of similar studies with animal viruses. The unusual stability of the virus causing infectious papillomatosis (Shope) recommends this agent as a favorable subject for such a study.

We have isolated from the virus-induced warty masses[4] from western cottontail rabbits a high molecular weight protein with which is associated the infectiousness of the disease. The following procedure has been adopted in preparing this protein. From 5 to 10 grams of glycerolated wart tissue known to be infectious were ground with sand and extracted with 100 cc of normal saline. After preliminary clarification by low-speed centrifugation extracts were ultracentrifuged[5] in 17 cc tubes for about two hours in a maximum field of 60,000 times gravity. The pellets thus thrown down were pooled and taken up in 7 cc of 0.1 M phosphate buffer solution, cleared of aggregated colloidal matter by low-speed centrifugation and again ultracentrifuged at 60,000 g to yield a pellet of heavy matter. This process was continued 3 to 4 times, or until tests with the analytical ultracentrifuge showed that all light-weight impurities had been lost in the supernatant fluids and all fine colloidal matter had been aggregated and eliminated through the intermediate low-speed centrifugations. Sixty grams of wart tissue derived from 5 different sets of warts were treated in this fashion. In 3 instances the papillomas were the result of "natural" infections; in the other 2 the growths had been induced by experimental inoculation. These tissues had different degrees of infectivity, suspensions of the most active producing rapidly growing papillomas in domestic rabbits 7 days after inoculation of saline extracts, the poorest requiring 13 days for the production of scattered warts.

Differential ultracentrifugation in each case provided a heavy protein free from colloidal impurities and detectable amounts of light-weight contaminants. A solution containing one mg per cc of this purified substance was opalescent and gave positive color reactions with the Millon, xanthoproteic and biuret reagents. A portion of the same solution failed to yield an immediate positive Molisch test for carbohydrate, but a faint violet ring of color developed on standing. The material was found to contain about 15 per cent. nitrogen by Kjeldahl analysis. The heavy protein is completely coagulated at a temperature of 66–67° C. and leaves a supernatant that is free of protein; the activity of papilloma extracts[6] begins to diminish at 67° C. and is completely destroyed at 70° C.

In the analytical ultracentrifuge the heavy protein from each sample sedimented with the sharp boundary that characterizes a single molecular species. In every instance the sedimentation constant was the same—

[1] W. M. Stanley, SCIENCE, 81: 644, 1935.

[2] W. M. Stanley, *Amer. Jour. Bot.*, 24: No. 2, 1937.

[3] W. M. Stanley and R. W. G. Wyckoff, SCIENCE, 85: 181, 1937.

[4] We are indebted to R. E. Shope of this Institute for the material used in this investigation.

[5] R. W. G. Wyckoff and J. B. Lagsdin, *Rev. Sci. Instr.*, 8: No. 3, 1937.

[6] R. E. Shope, *Jour. Exp. Med.*, 58: 607, 1933.

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.

[7] I. Eriksson-Quensel and T. Svedberg, *Jour. Am. Chem. Soc.*, 58: 1863, 1936; R. W. G. Wyckoff, J. Biscoe and W. M. Stanley, *Jour. Biol. Chem.*, 117: 57, 1937.

[8] J. G. Kidd, J. W. Beard and P. Rous, *Jour. Exp. Med.*, 64: 63, 1936.

[9] R. E. Shope, *Proc. Soc. Exp. Biol. and Med.*, 32: 830, 1935.

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

[2] J. Tréfouël, Mme. J. Tréfouël, F. Nitti and D. Bovet, *Compt. rend. Soc. de biol.*, 120: 756, 1935.

[3] G. A. H. Buttle, W. H. Gray and D. Stephenson, *The Lancet*, 230: 1286, June 6, 1936.

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[5] L. Colebrook, G. A. H. Buttle and R. A. Q. O'Meara, *The Lancet*, 231: 1323, December 5, 1936.

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[7] P. Gelmo, *Jour. für praktische Chemie*, 77: 369, 1908.