

is very different from the comparatively insoluble indol, and so this result is not unexpected.

These results have a considerable bearing on the way in which the lytic process observed by Rhoads and his coworkers may be brought about, for the flooding with indol of the blood stream of a dog on a deficient diet is equivalent to the flooding of the blood stream with a weak lysin, or, perhaps more significantly, with a powerful accelerator for whatever intravascular lysins may be present. It is true, of course, that the *in vivo* effect of indol will be much less than the *in vitro* effects, partly because no great concentration of indol can occur in the blood stream, and partly because of the great inhibitory effects of the serum proteins and the great number of red cells present. The kinetics of *in vivo* lysis, acceleration, and inhibition, are at present completely unknown, so it is impossible to say exactly what the effect of establishing a *continually maintained* concentration of indol in the blood stream would be; it is safe to say, however, that the result of continually maintaining an accelerator, even in small amounts, in the blood stream would be to hasten any normal lytic process occurring there, and would, therefore, in the long run, tend to bring about an anemia.

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Ultracentrifugal Isolation of High Molecular Weight Proteins from Broad Bean and Pea Plants.

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Characteristic high molecular weight proteins have been isolated by ultracentrifugation from the juices of plants diseased with several of the less stable viruses.¹ Various lines of evidence indicate that virus activity is a property of these high molecular weight proteins. The viruses thus far studied are easily transmitted by mechanical means. Certain viruses, however, require specific insect vectors and are difficult to transmit by ordinary mechanical methods. We have made an ultracentrifugal examination of infectious juice containing such a virus, choosing for the purpose pea mosaic (pea

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¹ Stanley, W. M., and Wyckoff, R. W. G., *Science*, 1937, **85**, 181; Loring, H. S., and Wyckoff, R. W. G., *J. Biol. Chem.*, 1937, **121**, 225.

virus 1).² In nature the pea and potato aphids² carry this disease but under favorable conditions it may be transmitted mechanically to about 75% of inoculated plants.

When the juice from infected broad bean plants, *Vicia faba* L., was ultracentrifuged³ in a field of 40,000 g. for one and a half hours, appreciable amounts of a heavy material were obtained. The pellets were 2 or 3 times the size of those found in the case of the latent mosaic virus. After 3 ultracentrifugations, a solution of the pellets in water gave the usual qualitative tests for protein and the kind of absorption diagram in the analytical ultracentrifuge that is typical of purified heavy proteins. Nearly all the light "unsedimentable" proteins had been eliminated, and there were present sharp boundaries characterized by the sedimentation constants $s_{20} = 76 \times 10^{-13}$ cm. sec⁻¹ dynes⁻¹ and $s_{20} = 112 \times 10^{-13}$. Certain samples, in which there seemed to be some decomposition, gave a third boundary with $s_{20} = 54 \times 10^{-13}$.

Activity measurements were made on the juice before ultracentrifugation, on the supernatant liquid after ultracentrifugation, and on the solution of the sedimented pellets. In one experiment in which 20 young broad bean plants were inoculated with the filtered juice before centrifugation, 20 with the supernatant liquid, and 20 with the solution of the pellets obtained after one ultracentrifugation, 6 of the first group, 4 of the second, and 11 of the third showed pea mosaic disease. In a second test, after 3 ultracentrifugations the supernatant liquid failed to infect any of 10 plants, while the solution of the pellets caused infection in 4 of 10 inoculated plants. Though there was in no case a high percentage of infection, the results demonstrated some concentration of the virus in the sedimented pellets. The low specific activity of the sedimented protein indicated that it was not a pure virus protein. This was confirmed when ultracentrifugation of the juice from healthy broad bean plants yielded a similar non-infectious heavy protein. The sedimentation constants measured from this healthy protein solution were the same, within the limits of experimental error, as those from the infectious solution. It is thus apparent that either the concentration of the virus principle in the final solutions was too low to permit detection with the analytical ultracentrifuge or the virus had the same sedimentation constant as one of the normal constituents.

This demonstration of heavy proteins in healthy broad bean plants suggested the examination of another legume. After 3 ultra-

² Osborn, H. T., *Phytopath.*, 1935, **25**, 160.

³ Wyckoff, B. W. G., and Lagsdin, J. B., *Rev. Sci. Instr.*, 1937, **8**, 74, 127.

centrifugations a purified protein giving sharp boundaries with $s_{20} = 77 \times 10^{-13}$, $s_{20} = 117 \times 10^{-13}$ and sometimes $s_{20} = 54 \times 10^{-13}$ was obtained from the juice of healthy pea plants, *Pisum sativum* L. var. *arvense* Poir. It gave the usual protein color tests, contained a pentose, and was probably a nucleo-protein. Both the broad bean and pea plant proteins were pigmented and of limited stability. Solutions of the former were dark green; after repeated sedimentations in the cold the pigment became insoluble leaving a colorless opalescent suspension of inhomogeneous material. Solutions of the pea protein were light green in color and, after standing for several days in the refrigerator, they too became inhomogeneous and no longer sedimented with sharp boundaries.

Similar ultracentrifugal procedures have not isolated homogeneous macromolecules from the juice of healthy tobacco plants. If such proteins exist, they are either highly unstable or are present only in minute amounts.

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Toxicity of Nicotinic Acid.

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Nicotinic acid (I), chemically pyridine- β -carboxylic acid, was prepared as early as 1867 by Huber¹ from the oxidation of nicotine (II). During the early investigation of vitamin B, nicotinic acid was isolated from rice polishings,^{2, 3, 4} and suspected to have an antineuritic action in pigeons, although subsequent tests definitely excluded this possibility.⁵ A renewal of interest was aroused by the publication of Elvehjem, Madden, Strong, and Woolley,⁶ who showed that nicotinic acid or its amide isolated from the liver extract cures canine blacktongue of the Goldberger type.⁷ Their

¹ Huber, C., *Liebigs Ann. Chem.*, 1867, **141**, 271; *Ber. deut. chem. Gesellsch.*, 1870, **3**, 849.

² Funk, C., *J. Physiol.*, 1911-12, **43**, 395; 1912-13, **45**, 489; 1913, **46**, 173.

³ Suzuki, U., Shimamura, T., and Odake, S., *Biochem. Z.*, 1912, **43**, 89.

⁴ Drummond, J. C., and Funk, C., *Biochem. J.*, 1914, **8**, 598.

⁵ Funk, C., *J. A. M. A.*, 1937, **109**, 2086.

⁶ Elvehjem, C. A., Madden, B. J., Strong, F. M., and Woolley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767; *J. B. C.*, 1938, **123**, 137.

⁷ Smith, D. T., *J. A. M. A.*, 1937, **109**, 2086.