TABLE I
FATAL DOSE AND PERSISTENCE OF ACTION OF TINCTURE DIGITALIS IN RABBIT

| Rabbit no. | Weight kgm | Period of injection min. | M.L.D.* mgm per kgm | M.L.D. cat units | Remarks |
|---|---|---|---|---|---|
| 1 | 2.89 | 106 | 730 | 8.58 | |
| 2 | 2.98 | 61 | 408 | 4.8 | |
| 3 | 2.28 | 95 | 802 | 9.4 | Received 1.5 cat units per kgm (specimen A) daily for two days; on third day a similar dose 8 hours prior to final testing. |
| 4 | 2.57 | 95 | 708 | 8.3 | Received 2.5 cat units per kgm (specimen A) 8 hours prior to final testing. |
| 5 | 3.07 | 93 | 650 | 7.6 | Received 1.5 cat units per kgm (specimen A) daily for two days; on third day 5.5 cat units per kgm (specimen B) in two doses 5 to 7 hours prior to final testing. |
| 6 | 2.08 | .. | ... | 3+ | Survived 3 cat units per kgm (specimen B) in one injection. |
| 7 | 2.92 | .. | ... | 4–6 | Received 4 cat units per kgm (specimen B) in one dose and 2 cat units per kgm in a second injection 5 minutes later; caused death with ventricular fibrillation. |

* All these tests were made with specimen B, a tincture of digitalis with a cat unit potency of 0.85 cc. Specimen A was a tincture of digitalis with a cat unit potency of 0.75 cc. All injections were made intravenously. The doses in the table refer to the leaf.

ment caused the death of three of the five animals in four hours. These doses are extremely small for the rabbit. In our experiments (see table) the M.L.D. for the albino rabbit proved to be about eight times as much as that for the cat by the same method of slow intravenous injection.[8] Sudden intravenous injections of three and four units per kgm did not prove fatal. Accordingly, the subcutaneous doses which Nahum and Hoff gave eighteen hours prior to the calcium and which they referred to as 50 to 75 per cent. of the "calculated lethal dose" of digitalis, were in fact only about 6 to 9 per cent. of the lethal dose for the rabbit, and the additional intravenous doses (0.25 cat unit per kgm) were only about 3 per cent. of the lethal dose for the rabbit. In view of the rapid excretion of digitalis in the rabbit, the dose which proved fatal to three of five animals was only about 3 per cent. of the intravenous fatal dose when the animal was treated with calcium in the manner of their experiments.

Such facts constitute the standard evidence for the phenomenon of synergism. But the authors conclude: "In the normal unanesthetized rabbit heart the effects of calcium and digitalis are not additive." In view of the unusually high degree of synergism shown by the death of these three rabbits and the long interval before death, confirmation of these experiments would be desirable.

As matters stand, the synergism between calcium and digitalis remains an established fact. From the practical standpoint it needs to be borne in mind that the rapid intravenous injection of calcium is a dangerous procedure, and that the danger is greater when the subject is under the influence of digitalis.

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[8] The tincture of digitalis with the alcohol evaporated off was diluted five times with physiological salt solution and administered slowly intravenously so as to complete the injection in about ninety minutes, a method similar to that used in the cat method of assay.

## THE pH STABILITY RANGE OF THE ELEMENTARY BODIES OF VACCINIA

THE causative agents of known virus diseases range in size from the elementary bodies of the pox diseases, which are almost as big as the smaller microorganisms, downwards to particles comparable in weight with the larger protein molecules. Since it is now possible to obtain concentrated and purified preparations of both large and small virus particles, a comparison of their properties becomes one of the most immediately interesting problems in the study of viruses. Recently[1] we reported preliminary results of a comparison of the pH stability ranges of the virus activity of infectious papillomatosis in rabbits and of the molecules of the purified virus protein[2] of this disease. In this investigation it was found, by animal titration and by ultracentrifugal analyses, that the protein molecules were disrupted at exactly those pH's at which the virus activity was immediately lost. The present note records the results of a similar comparison of the pH stabilities of the virus activity of vaccinia and of its purified elementary bodies.

The vaccine virus employed was obtained as calf lymph from the North Carolina State Board of Health. This virus was passed repeatedly through rabbits by the methods of inoculating and harvesting described by Craigie.[3] The destruction of virus activity was determined by suspending the virus in 0.04M salt buffer mixtures and inoculating the suspensions intradermally into susceptible rabbits after standing in the buffer for an hour, a day and a week. The actual pH of each suspension was measured with a glass electrode. The resulting lesions were charted daily for eight days after inoculation. Three experiments of this kind yielded practically identical results.

[1] R. W. G. Wyckoff and J. W. Beard, *Proc. Soc. Exp. Biol. and Med.*, 36: 562, 1937.

[2] J. W. Beard and R. W. G. Wyckoff, SCIENCE, 85: 201, 1937.

[3] J. Craigie and F. O. Wishart, *Brit. Jour. Exp. Path.*, 15: 390, 1934; etc.

The findings showed that the activity of the virus is preserved for at least a week at all pH's between 5 and 9.5, that inactivation proceeds rapidly at pH 4 and pH 10.5 and is practically instantaneous at pH's below 3 and above 11.5.

Elementary bodies, pure and concentrated enough for ultracentrifugal analysis, were obtained after several passages of the virus in rabbit skin. The ultracentrifuge was the same air-driven machine[4] arranged for absorption measurements that has been used in previous studies. Photographs of the rates of sedimentation of products of the first few passages starting with calf lymph showed no sharp boundary characteristic of particles of uniform size. Instead the pictures gave evidence of much heavy colloidal and light "unsedimentable" ultra-violet-absorbing material. After these preliminary passages, however, a sharp sedimenting boundary with a constant of ca $5{,}000 \times 10^{-13}$ cm $sec^{-1}$ $dynes^{-1}$ could be seen. At first the homogeneous matter was accompanied by colloidal and "unsedimentable" substances, but after two or three additional passages and minor improvements in the technique of washing the bodies, the ultracentrifugal pattern was that of a pure homogeneous suspension. Smears stained according to the method of Morosow[5] then showed little material other than numerous elementary bodies of uniform size. Undoubtedly the particles giving the sharp ultracentrifugal boundaries are those that serve as nuclei for the stain.

Both infectivity tests and the results of ultracentrifugal analysis indicate that solutions or suspensions of the elementary bodies of vaccinia are stable only in very dilute salt solutions. If fresh elementary bodies in 0.005M buffer are washed two or three times with distilled water, their final suspension no longer produces a good boundary. Some of the elementary bodies are injured by the agglutination that accompanies this washing. This is shown by the fact that resuspension in 0.005M buffer gives both a weaker and a more diffuse boundary. The homogeneity of elementary body suspensions in 0.1M neutral buffer rapidly disappears. After a few days in the cold such a suspension will no longer yield a sharp boundary in the ultracentrifuge nor can one be recovered by resuspension in 0.005M buffer.

Because of this sensitivity to strong salt, the suspensions for ultracentrifuging at different pH's were in 0.005M buffers. At all pH's between 5.5 and ca 10 suspensions of the elementary bodies are stable for days and give sharply sedimenting boundaries with $s_{20°} = \text{ca } 5{,}400 \times 10^{-13}$ cm $sec^{-1}$ $dynes^{-1}$. At pH's between 3 and ca 5.5 agglutination is so nearly complete that sedimentation pictures show nothing. A boundary is again poorly discernible at pH 2.6 and good photographs can be obtained at pH 1.2. The boundaries in these acid solutions are more diffuse than those from active preparations and correspond to a substance that is perhaps 15 per cent. lighter. Further evidence of decomposition in the inactive acid suspensions is the fact that the quartz windows of the analytical cell become covered with a waxy deposit which interferes with the ultra-violet transparency of the air bubble. The decomposition in strongly alkaline solution is even more interesting. No trace of the sharp boundary with $s_{20°} = 5{,}400$ could be found in a suspension brought to pH 11.8. In its place was much unsedimentable material and a faint but fairly sharp boundary coming down more slowly. Some of the substance causing this boundary was isolated with the quantity ultracentrifuge. In pure solution it gives excellent sedimentation photographs corresponding to $s_{20°} = \text{ca } 1900 \times 10^{-13}$, a value about one third that of the active elementary bodies.

These experiments bring out a general parallelism between the stabilities of the virus and of the elementary bodies. The sedimentation constant of the bodies is uninfluenced by pH in that region where the activity is unaffected. In inactive acid and alkaline solutions the elementary bodies are broken down, leaving fragments some of which are very large and of a degree of homogeneity that compares well with that of the active bodies. The many similarities between these results and those obtained with the papilloma protein are evident.

A further study is being made of the properties of these inactive degradation products and of the loss of virus activity in the especially instructive range between pH 9.5 and 11.5.

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[4] J. Biscoe, E. G. Pickels and R. W. G. Wyckoff, *Jour. Exp. Med.*, 64: 39, 1936; R. W. G. Wyckoff and J. B. Lagsdin, *Rev. Sci. Instr.*, 8: 74, 1937.

[5] Morosow, *Centralbl. f. Bakt. I Orig.*, c: 385, 1926.

## REDUCTION OF THE METHYL ESTER OF 2:3:4-TRIMETHYL α-METHYL-d-GALACTURONIDE TO 2:3:4-TRIMETHYL α-METHYL-d-GALACTOSIDE

IN order to facilitate the analysis of the structure of the derivatives of uronic acids (such as pectins, aldobionic acids, etc.), it seemed desirable to convert the