succinic oxidase system, the activity of which is not affected by these agents, is able to compete even more successfully with other oxidation systems for oxygen than in unpoisoned hearts, with the result that the negative inotropic action of succinate becomes more intense and prolonged.

*Summary.* When succinate is administered to the heart-lung preparation of the dog at sufficiently high rates (0.2 g or more of unhydrated succinate anion per kg heart-lung system per minute) the phosphocreatine concentration in the myocardium declines markedly and the heart goes into failure. The concentration of ATP declines to a lesser extent or remains unchanged. Heart failure in the heart-lung preparation caused by pentobarbital and chlorobutanol is intensified by succinate. These findings are discussed in the light of existing information on the coupling of succinate oxidation with phosphorylation and on the metabolism of cardiac tissue poisoned with anesthetics.

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## Amino Acids of the Shope Papilloma Virus. (18364)

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Among the symptoms attributable to viral infections is the production of tumors, such as those, for example, of infectious papillomatosis (Shope papilloma) of rabbits(1). Interest in the Shope papilloma virus is heightened by the possibility that findings gained from studies of it may, in addition to contributing to knowledge of host-virus relationships, possess implications for the cancer problem(2). A desirable basis for further studies on rabbit papillomatosis is provided by investigation of the physical and chemical nature of the agent inducing the disease. Progress in this direction has been made by Beard and associates who, from studies made on purified preparations, have reported the virus to be composed of desoxypentose nucleic acid, protein, and perhaps a small amount of lipid(3-8).

In the present investigation, several purified preparations of papilloma virus have been obtained, and the 2 largest of these have been analyzed for amino acid content by means of microbiological methods of assay. It was not possible to perform so exhaustive an analysis as was done with some strains of tobacco mosaic virus(9), owing to the relatively greater difficulty in procuring substantial quantities of highly purified virus; nevertheless, this report is presented with the hope that the data given may guide others working or proposing to work in this field.

*Methods and Findings. Preparation of virus for assay.* The source of virus for the experiments described herein was the warty growths removed from cottontail rabbits trapped in Kansas. The papillomas, which had been stored in glycerin-saline solution(4) upon harvest were drained on a fluted filter in a

* This investigation was begun in the laboratories of the Rockefeller Institute for Medical Research, Princeton, N. J.

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room held at 4°C, and then freed of hair and connective tissue. The naked warts thus obtained were deposited in a previously weighed amount of Sorenson's M/15 phosphate buffer at pH 6.6. A final amount of buffer was added to each lot of warts to give about a 20% suspension. The warts were then ground for 5 minutes in a Waring blendor at room temperature. The mash thus obtained was allowed to stand overnight at 4°C and then was spun in an angle centrifuge for 10 minutes at 5,000 R.P.M. The supernatant fluid was saved and the angle pellets were resuspended in buffer, and ground in the blendor for 5 minutes. The pellets from this low-speed centrifugation were discarded, and the supernatant fluid was pooled with the first lot. The combined fluids were spun once more in the angle centrifuge and were then spun at 25,000 R.P.M. (about 60,000 g) for 1 hour. The pellets obtained from the high-speed run were taken up in buffer solution at pH 6.6 and spun in the angle centrifuge at 5,000 R.P.M. for 10 minutes.† The brown pellets obtained upon angle centrifugation were discarded, and the supernatant fluid, containing the virus, was returned to the high-speed centrifuge. This process of alternate low- and high-speed centrifugation was repeated 4 to 5 times to yield clear gelatinous pellets of virus. The buffer concentration was progressively reduced, and the final pellets were taken up in distilled water from which the material was obtained in a white fluffy form by drying in the frozen state. Before analysis the final product was further dried to constant weight *in vacuo* over $P_2O_5$ at 65°C.

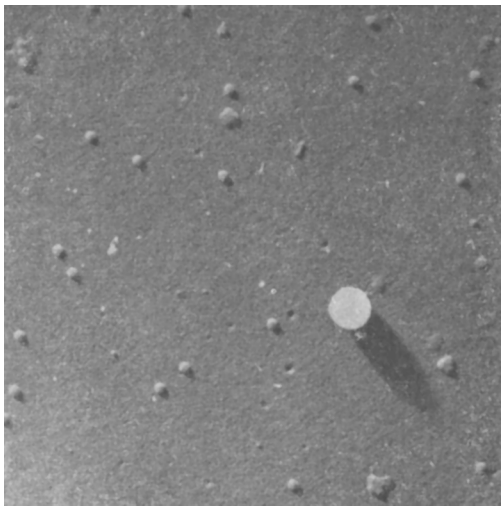

FIG. 1.
An electron micrograph of a purified preparation of Shope papilloma virus, shadowed with uranium. No attempt was made to remove all of the salt from the preparation. The diameter of the largest object shown, a polystyrene particle, is taken as 260 mμ.

*Characterization of the virus preparations.* The yields of virus obtained from different lots of warts ranged from about 0.1 to 0.3 mg of virus per gram of warts. The highly purified material induced warts on domestic rabbits to a dilution of $10^{-9}$ grams of virus. Examination of some of the virus preparations in the analytical ultracentrifuge and in the Tiselius electrophoresis apparatus by Dr. H. K. Schachman indicated the presence of a small amount of material differing in properties from the bulk of the substance(10). However, the bulk of the substance appears to coincide with the material shown by Beard and associates to represent the virus.

Several preparations of the virus were examined in the electron microscope. The resulting micrographs showed circular particles which appeared to possess considerable uniformity of size. One of these, kindly taken by Dr. R. C. Backus of this laboratory, is reproduced in Fig. 1. Included in the picture is a polystyrene particle, which coincides in size with other polystyrene particles not

† In some of the preparations it was observed that appreciable amounts of clear gelatinous material sedimented with the brownish pigment in the angle centrifugation, and, furthermore, that much of this could be redissolved by mixing with more buffer; the ultracentrifuge pattern of this material was essentially the same as that observed for the supernatant fluid of the angle centrifugation, which is presumed to represent virus. Therefore, it seemed likely that the virus has a limited solubility under the above conditions and that this situation could be improved, as it was in one later preparation, by employing a buffer at a higher pH. 0.18 M sodium chloride—0.02 M sodium veronal at pH 7.4 was used for this purpose.

10. Schachman, H. K., in press.

illustrated and hence on the basis of previous studies(11) can be taken as a spherical reference object with a diameter of about 260 mμ. From this, the diameters of the papilloma virus particles can be calculated to be about 73 mμ. However, from an inspection of the size and shape of the virus shadows as contrasted with that of the polystyrene particle, it is apparent that the virus particles are considerably flattened, as was also noted by Sharp and co-workers(12), and hence it is not possible to assign an accurate figure for the diameter of the unflattened particles. It is assumed from the uniformities of diameter and orientation that the particles in solution are essentially spherical. Further evidence suggesting this was kindly obtained for us in the following manner by Dr. R. C. Williams of this laboratory. An appropriate dilution of the preparation of virus illustrated in Fig. 1 was deposited upon a glass slide, allowed to dry, and then was washed twice with water to remove salt and dried again. The dried film was shadowed with palladium, and a replica was made in the customary manner. Several clusters of viral particles were found upon examination of the replica in the microscope. If the original viral particles were essentially spherical, it would be expected that the particles in a cluster, which are prevented from flattening to the same extent as isolated particles, would possess smaller diameters than those not in a cluster. Measurement across several particles in clusters yielded a figure of 62 mμ for the average diameter. This is significantly smaller than the diameters of isolated particles on the same micrographs. A still smaller diameter, 52 mμ, was found by a third technic in which the particles were sprayed and lyophilized on the microscope screen. The details of this method will be described later by Dr. Williams. Even with this last procedure there was some evidence for flattening of the particles, and hence the figure of 52 mμ should be taken only as a tentative value until more data, obtained with improved or other technics, can be brought to bear on the problem. It should be noted that the apparent flattening of the viral particles on electron microscope screens is not peculiar to papilloma virus, but is a phenomenon observed with all of the presumably spherical viruses thus far examined. Further investigations of the physical nature of the particles in papilloma virus preparations are being made and will be described later.

TABLE I. Amino Acid Content of Preparations of Shope Papilloma Virus.

| Amino acid | Preparation 1 % | Preparation 2 % |
|---|---|---|
| Alanine | — | 3.1 |
| Arginine | 6.7 | 7.0 |
| Aspartic acid | 11.1 | 10.7 |
| Cystine | — | 6.4* |
| Glutamic acid | 11.8 | 11.4 |
| Glycine | — | 3.1† |
| Histidine | 1.9 | 1.8 |
| Isoleucine | 4.2 | 4.1 |
| Leucine | 7.0 | 7.5 |
| Lysine | 7.0 | 7.0 |
| Methionine | 2.2 | 2.1 |
| Phenylalanine | 4.5 | 4.6 |
| Proline | — | 5.5 |
| Serine | — | 4.0 |
| Threonine | 5.2 | 5.1 |
| Tryptophane | — | 1.0 |
| Tyrosine | 6.0 | 5.8 |
| Valine | 5.4 | 5.4 |

* A calculated value using Beard's figure for sulfur (4), subtracting methionine sulfur, and expressing the residual sulfur in terms of cystine. Preliminary colorimetric tests suggest that part of this sulfur is present as cysteine.

† No attempt was made to correct for the small amount of glycine possibly contributed from decomposition of nucleic acid constituents during hydrolysis of the virus.

Amino acid analyses—The dried preparations of papilloma virus were hydrolized and analyzed for amino acid content by the methods previously described(9). The results of these analyses are summarized in Table I. It should be noted that the two preparations analyzed were obtained in different years and were derived from warts obtained from many rabbits.

*Discussion and summary.* The results of the amino acid analyses indicated that at least 18 amino acids are represented in the

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12. Sharp, D. G., Taylor, A. R., Hook, A. E., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1946, v61, 259.

protein of the Shope papilloma virus. None of these appear to be present in extraordinary amounts. In common with other viruses examined thus far(9,13,14), the protein of the papilloma virus has a preponderance of acidic amino acids over the basic ones, and hence differs from the proteins combined with nucleic acid in such substances as sperm. A summation of the amino acid residues and the nucleic acid in the papilloma virus leaves about 7% of the material unaccounted for. However, the nucleic acid figure taken from the data of Taylor *et al.*(7) is admittedly a crude estimate and, as judged from the phosphorus content of his preparations, is almost surely low. Further investigation of the nucleic acid content together with additional amino acid analyses may resolve the discrepancy, but at present the possible existence in papilloma virus preparations of material in addition to protein and nucleic acid cannot be excluded with the same degree of assurance as in the case of certain plant viruses. Finally, the amino acid values reported here must be considered subject to revision owing to the small number of analyses made.

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## Regular Diurnal Physiological Variation in Eosinophil Levels in Five Stocks of Mice.* (18365)

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Characteristic eosinophil levels for inbred, mature, male mice were defined in a previous study. They were found to be distinctive for some of the strains investigated(1). It was emphasized, however, that the determinations were made in the morning, at a time when the activity of the mice had presumably declined over a period of several hours from its high point around midnight(2). Furthermore, it was noted that the determinations made during the regular periods of maximal activity may differ from the "characteristic" level.‡

An additional study was therefore undertaken, designed to compare the midnight levels of circulating eosinophils in the tail blood of mice with the "characteristic" morning level.

*Procedures* and materials were comparable to those specified for the determination of the characteristic eosinophil morning level(1). The blood samples in which the night levels were determined had to be collected—for practical reasons—over a period of time from 9:00 P.M. to 1:00 A.M. Two independent series of determinations were made: both in midsummer, with an interval of a week between them.

The *results* are listed in Table I and indicate that under standardized conditions the numbers of circulating eosinophils show remarkable but regular variations. The significance

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† With assistance of Arthur G. Litman, Victor M. Riddle and Paul C. Royce.

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2. Bänder, A., *Z. f. d. ges. exp. Med.*, 1950, v115, 229.

‡ For the $B_1$ subline of the $C_{57}$ Black strain the P value for the significance of the difference between the midnight level of 223 ± 23 cells per cu cm and the morning level of 1022 ± 44 cells per cu mm was smaller than 0.001.