atic diabetes represented only overproduction of glucose, as was once claimed, removal of most of the liver should reduce hepatic gluconeogenesis and hence amount of urinary glucose. The exacerbation of glycosuria is consistent with the view that insulin deficiency causes failure of glucose utilization; the liver plays a primary role in uptake of dietary glucose, its storage as glycogen, and its conversion to fat. The fact that rats which were already wasting the equivalent of all the dietary carbohydrate into the urine did not develop more severe glycosuria following partial hepatectomy is also consistent with this hypothesis. Since partial hepatectomy was followed by an increase in NPN excretion which was roughly equivalent to that caused by a sham operation, it seems improbable that the glycosuria was caused by increased glyconeogenesis from protein. The explanation offered above, although plausible, is hypothetical. We have not studied the causes of the phenomenon and will not do so.

Summary. Partially depancreatized rats were tube-fed a medium carbohydrate diet. Removal of approximately 70% of the liver did not increase already severe glycosuria, but it did cause a marked exacerbation of glycosuria in moderately diabetic rats and in partially depancreatized rats without spontaneous glycosuria. A sham operation failed to cause excretion of more glucose although there was increased excretion of non-protein nitrogen.

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Preparation of a Purified Vaccinia Virus Freed from Soluble Antigens. (29175)

JÜRGEN MARQUARDT, STIG E. HOLM AND ERIK LYCKE
(Introduced by T. Francis, Jr.)
The Municipal Virological Laboratory, Gothenburg, and Department of Bacteriology, University of Gothenburg, Sweden

For the preparation of purified vaccinia virus material differential centrifugation, treatment with fluorocarbon and sedimentation in sucrose density gradients have been successfully employed(1,2,3). These preparatory methods were modified and used in the present study. For testing the degree of purity electron microscopy and the double diffusion-in-gel technique were employed.

Material and methods. Virus. The vaccinia virus used was a rabbit skin-adapted strain obtained from Dr. D. Peters, Inst. of Trop. Diseases, Dept. of Virology, Hamburg, Germany.

Production of crude virus material. The skin of rabbits weighing 3-4 kg was prepared and inoculated principally according to Hoagland *et al*(4). Each rabbit received 15 ml of a virus suspension containing approximately 10^8 plaque forming units (PFU) per ml. The skins were harvested 3 days after infection. The material obtained from each skin was suspended in 50 ml of 0.05 M Tris buffer pH 7.5 containing 200 IU of penicillin and 200 μg of streptomycin per ml.

Pooled suspensions from 6 rabbits were homogenized. They were treated 2 $\times$ 1 minute at maximum speed with an Ultra-Turrax homogenizer in an ice-bath. The homogenized material, the crude virus suspension, was designated preparation Cr.

Purification of virus. To 100 ml of the Cr preparation 5 ml of Trifluorotrichloroethane were added and the mixture was homogenized as mentioned above. The material was centrifuged at 1,500 *g* for 15 minutes; the sediment was homogenized with 4 volumes of buffer and then centrifuged. The supernatants obtained were pooled and designated preparation Tr.

The Tr preparation, 375 ml, was spun in the Spinco model L centrifuge, rotor 30, for

45 minutes at 3,500 *g*. The supernatant was pipetted off and designated preparation Sr.

The deposit was resuspended in the Tris buffer to 1:10 of the volume of the Tr preparation. The deposit was resuspended by pipetting and subsequent ultrasonic treatment for 10 minutes at full power in a Raytheon 200 watt 10 kc Oscillator. It was cleared by centrifugation at 1,500 *g* for 15 minutes. The cleared suspension was designated preparation Dr.

To the Dr preparation sucrose was added to a final concentration of 19%. Subsequently, the preparation was ultrasonicated for 20 minutes. Aliquots of 5 ml of the preparation were then superimposed on 25 ml of a 20-50% continuous sucrose density gradient prepared according to Britten and Roberts(5). Centrifugation was performed at 23,000 *g* in the Spinco SW_{25} rotor for 45 minutes. A small deposit and one clearly visible band located 13-15 mm from the bottom of the tube were obtained.

Four fractions were obtained by collecting droplets from the punctured tube. The first consisted of the fluid above the band, the second corresponded to the band, the third was the fluid below the band and the fourth consisted of the deposit resuspended in one ml of distilled water and dialyzed against distilled water for one day to remove the sucrose. The dialyzed fraction 4 was called preparation Gr 4. Fractions 2 and 3 were diluted with equal volumes of distilled water and centrifuged for 30 minutes at 35,000 *g* in the SW_{25} rotor. The sediments were taken up, each in one ml of distilled water, and dialyzed (preparations Gr 2 and Gr 3). The supernatants and fraction 1 were dialyzed and subsequently concentrated to one ml volumes by pervaporation with unheated air at room temperature. The preparations obtained were designated Gr 2 Sup, Gr 3 Sup and Gr 1, respectively. If not otherwise stated, all the preparative work described was performed at 4°C.

Electron miscroscopy. Electron micrographs were made of unfixed and unwashed preparations on formvar-filmed copper grids coated with carbon. The preparations were examined unshadowed in a Siemens-Elmiskop I.

Titration of PFU. The infectivity was assayed on chorioallantoic membranes of 11-day-old chick embryos. The material to be tested was ultrasonicated and serial dilutions were made in sterile beef heart infusion broth. Six relevant quarter $\log_{10}$ dilutions were, each, inoculated on 8 eggs with 0.2 ml per egg. The eggs were harvested after 2 days of incubation at 36.5°C and the number of PFU per ml was calculated.

Estimation of number of virus particles. The number of elementary bodies in purified suspensions was calculated on the amount of DNA, found by the method of Burton(6). Extraction of the material was performed, however, with 10% $HClO_4$. The number of virus particles was then estimated by relating the DNA-values to a particle weight of 5.69×10^{-15} *g* and a DNA-content of 5.43% according to Marquardt *et al*(7).

Titration of hemagglutinin. The material was diluted in 2-fold serial steps with buffered saline at pH 7.0. To 0.5 ml of each dilution, beginning with dilution 1:10, 0.5 ml of a 0.2% suspension of sensitive chicken erythrocytes from a selected donor was added. The mixtures were kept overnight at 4°C before reading. The titers were expressed as the reciprocal values of the highest dilution causing hemagglutination and were recorded as hemagglutination units (HAU) per ml of the tested preparation.

Titration of complement fixing capacity. The micro-technique according to Fulton and Dumbell(8) was used. One batch of complement and one antivaccinia serum were used throughout. The antiserum and the different preparations to be tested were diluted in 2-fold steps. As diluent a veronal buffered saline (pH 7.6) was used, containing Mg^{++} and Ca^{++}.

The fractions were tested in box titrations beginning with a dilution of 1 in 4 against an initial serum dilution of 1 in 16. The complement fixing capacity of the preparations was expressed as the reciprocal of the highest dilution of the fraction showing 3^+ or 4^+ fixation with the highest dilution of

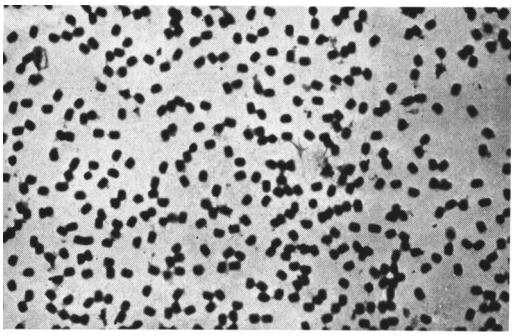

FIG. 1. Electron micrograph of a purified vaccinia virus material (preparation Gr 2). Unwashed and unshadowed material. Magnification 10,000 ×.

serum and was recorded as complement fixing units (CFU) per 0.1 ml of the tested preparation.

Immune serum. Hyperimmune sera were produced in rabbits. A partially purified virus material was inoculated into the skin of the back, so that an area of approximately 120 cm^2 was covered with confluent pocks. Three weeks later the rabbits received one intravenous and one intraperitoneal injection with virus, 3 weeks apart. Two weeks after the last injection the animals were bled by heart puncture.

The double diffusion-in-gel method. The micro-modification described by Wadsworth (9) was used. Ultrasonic treatment was performed on all preparations before testing. Photographic registration was made by means of Polaroid panchromatic film reproduction(10).

Results. Five batches of crude virus material were processed according to the technique described. In the following the results of one typical experiment are reported.

The electron microscopy showed that after the fractionation in the sucrose gradient the virus was mainly present in preparations Gr 2, Gr 3 and Gr 4. In contrast to preparation Gr 2, the preparations Gr 3 and Gr 4 contained huge amounts of aggregated virus and certain amounts of impurities. The virus found in preparation Gr 2 (the band) was well dispersed and only a few minute impurities were visible (Fig. 1). The infectivity titer of Gr 2 (1 ml) was 6.8×10^9 PFU while that of the original material (Cr, 300 ml) was 9.0×10^7 PFU per ml, indicating a recovery of 25% of PFU. The calculated content of virus was 2.13 mg. This corresponds to 3.75×10^{11} elementary bodies and 55.5 particles per one PFU.

Table I shows the hemagglutinating and complement fixing antigen activities of the different preparations. Some of the hemagglutinating activity disappeared during the treatment with fluorocarbon. After the density gradient centrifugation, hemagglutinin was recovered only in the top portion (Gr 1), while no hemagglutinin could be traced in the other preparations tested in a 1:10 dilution.

Complement fixing antigen was found in all 4 fractions of the gradient. Some complement fixing activity was not separable from the virus particles by washing. With regard to the low concentration of virus particles in Gr 1, most of the complement fixing activity found in this preparation seemed, however, to be in the form of soluble antigen.

The results of the diffusion in-gel tests are illustrated by Fig. 2 and 3. In the experiment used for illustration, the Cr preparation produced at least 5 precipitation lines against the hyperimmune serum. Two of these precipitates represent superimposed pairs of lines, thus at least 7 precipitinogens were demonstrated. In the process of preparing the Dr fraction, which was to be centrifuged in the sucrose density gradient, at least one of the precipitating factors disappeared. After the gradient separation the top fraction (Gr 1) contained at least 3 factors. The concentration of precipitinogens in preparation Gr 4, the resuspended deposit, was below that permitting the formation of a visible precipi-

TABLE I. Hemagglutination and Complement Fixing Antigen Activities.

Preparation	Vol (ml)	HAU per ml	CFU per .1 ml
Cr	300	80	16
Tr	375	20	8
Sr	360	<10	8
Dr	37	80	32
Gr 1	1	640	128
Gr 2	1	<10	32
Gr 2 sup	1	<10	<4
Gr 3	1	<10	64
Gr 3 sup	1	<10	<4
Gr 4	1	<10	16

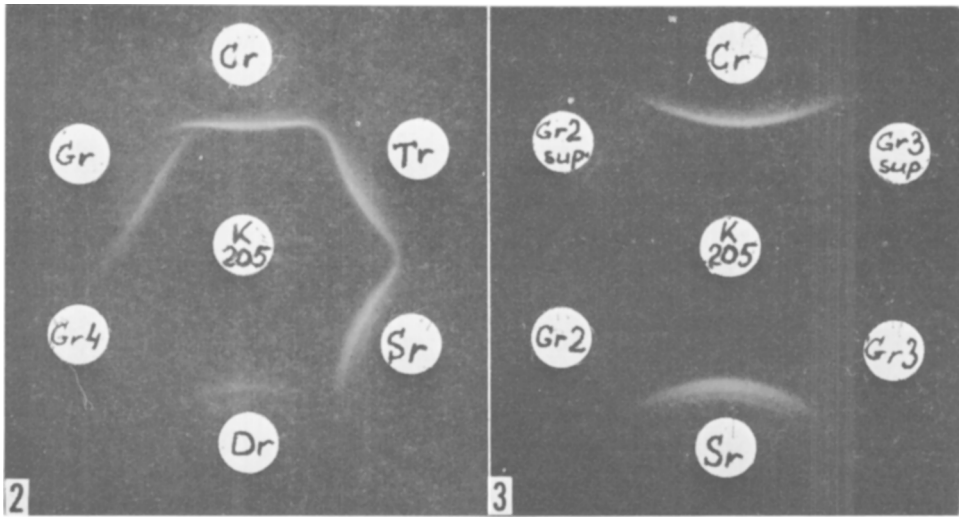

FIG. 2 and 3. Precipitation pattern of preparations obtained in the process of purification of a vaccinia virus material tested against a vaccinia hyperimmune serum (center well). Designation of preparations see text.

tate. The precipitating factors present in the banded material and in the sample collected from the fluid below the band could be separated from virus by centrifugation.

Discussion and summary. The reported experiments form part of a major study of the pattern of virus particle-associated and of soluble antigens, related to vaccinia infection. To study the antigenic composition of the vaccinia virus particle a purified virus material freed from soluble antigen was required. Principally, the method of purification described is a combination of the one used by Marquardt *et al*(7) and fractionation in a sucrose density gradient. Electron microscopy indicated that very little particulate impurity was present in the virus material banded in the gradient and washed by centrifugation. In the purified material, containing about 2 mg of virus particles per ml, no hemagglutinin and precipitinogenic factors were demonstrable. Ultrasonic treatment immediately preceding the diffusion-in-gel tests was found important as it increased the reactivity of the antigenic preparations.

The lowest concentration of vaccinial precipitinogens necessary for giving a visible reaction in the double diffusion-in-gel test is unknown. With a purified LS preparation (11) we found, however, that only 0.6 μg protein was necessary to cause precipitation with a homologous antiserum, indicating that the micro double diffusion test is highly sensitive for demonstration of vaccinial antigen-antibody precipitating systems. The high sensitivity of the micro double diffusion plate test has been documented by Crowle(12).

According to Rondle and Dumbell(13) using a macro plate technique at least 9 precipitating factors are present in cowpox antigenic preparations. The present study demonstrated the existence of at least 7 precipitinogens in crude virus suspensions produced with a rabbit skin adapted strain of vaccinia virus. The presence of additional precipitating factors is indicated by further experiments to be reported.

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Correlation Between Genetic Differences and Development of Polycythemia-Anemia in Mouse Parabionts.* (29176)

LOUISE LEONARD (Introduced by U. C. Luft)
Research Service, Veterans Administration Hospital, Albuquerque, N. M.

F_1 hybrid mice, placed in parabiosis with parental strains, suffer from a disease referred to as parabiotic intoxication(1). These animals have a wasted appearance and are extremely anemic. Billingham(2) has called attention to the similarity between this phenomenon and homologous disease. The latter syndrome can be produced in F_1 hybrid mice inoculated with lymphoid tissue from a parent that differs from the other parental strain at the H-2 histocompatibility locus (3). The injected cells form antibody against the hybrids causing the animals to become anemic and eventually die. Eichwald(1) and his colleagues expressed doubt that this syndrome was comparable to parabiotic intoxication. From their data they concluded that the symptoms of intoxication in parabionts are due to a shift of the red cell mass from intoxicated mice to their pure partners.

Data presented here indicate that a shift of red cells produces anemia and various degrees of polycythemia in both related and unrelated mouse parabionts. Fatal parabiotic intoxication was most severe in the DBA/2 partners of DBA/2:BALB/c parabionts, 2 strains of mice that are identical at the H-2 histocompatibility locus(4).

Materials and methods. Adult mice of both sexes, 3 to 6 months of age, were placed in parabiotic union of the coelomic type as described by Martinez(5). Eight combinations of parabionts were used: 22 pairs of BALB/c:BALB/c; 16 BALB/c:DBA/2; 17 BALB/c:A; 22 BALB/c:C3H; 17 BALB/c:CBA; 18 BALB/c:C57BL/6; 20 BALB/c:C57BR; and 14 BALB/c (tolerant to DBA/2 skin grafts):BALB/c. Parabionts were matched as to sex and weight, housed in 5 × 7-inch plastic cages, and given free access to Purina Laboratory Chow and water. All mice were bled from the retro-orbital sinus at 2-day intervals for a total period of 10 days. Groups of individual animals from each of the strains served as controls and were bled at the same intervals. Blood (0.03 ml) was collected at each bleeding in micro-capillary tubes which were spun in an International Micro-Capillary Centrifuge, model MB, for 5 minutes and read on a Micro-Capillary Reader.

Individual hematocrit readings of the parabionts were combined according to strain and day of bleeding and average values were determined; hematocrits of control groups were averaged in the same manner. Mean hematocrits and standard errors were calculated for day 0, day 4, and day 8.

Results. The hematocrits of the strains of mice differed noticeably (Table I). Russell (6) has reported lower levels for these strains, but the differences may be attributed to alti-

TABLE I. Effect of Continued Bleeding (at 2-day intervals) on Hematocrits of Inbred Strains of Mice.

		Mean hematocrits with standard errors of mean					
		Day 0		Day 4		Day 8	
Strain	No. of Mice	Mean	S.E.	Mean	S.E.	Mean	S.E.
BALB/c	16	50.0	.59	50.2	.54	47.6	.24
A	17	50.8	.78	46.7	1.01	46.2	.81
C3H	15	46.2	.70	44.5	.50	44.4	.95
CBA	20	47.0	.92	44.2	.46	43.0	.49
DBA/2	16	50.5	.47	47.8	.22	43.8	.30
C57BL/6	16	46.9	.39	43.5	.65	43.1	.78
C57BR	16	54.0	.40	50.4	1.42	50.7	1.12

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