taken up by intact splenic cells and bound to the 40S ribosomal subunits. It has been suggested(8) that SM (and DHSM) exerts its effect on antibody synthesis to phage MS-2 in splenic cultures from immunized rabbits by either combining with the phages and altering their antigenic specificity or by combining with ribosomes and altering the reading of the messenger for antibody synthesis. These experiments show that DHSM, and presumably SM, binds to splenic ribosomes and therefore may be capable of altering antibody synthesis at the ribosomal level.

Summary. Tritiated dihydrostreptomycin (H^3-DHSM) was taken up by intact splenic cells cultured from rabbits immunized with bacteriophage MS-2 and was bound to the 40S ribosomal subunit of the 76S splenic ribosome. Splenic cells that had been maintained in the presence of 200 μg/ml unlabeled DHSM took up as much H^3-DHSM as cells not previously exposed to the antibiotic. However, only cells that had not been cultured in the presence of DHSM bound H^3-DHSM in cold trichloracetic acid-insoluble form. The binding of H^3-DHSM by splenic cells was reversible and dependent on the concentration of Mg^{++} in the suspending medium.

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Multiplicity Reactivation of Vaccinia Virus in the Cells of the Chorioallantoic Membrane.* (31416)

D. G. SHARP AND RUTH C. DUNLAP
Biophysics Laboratory, Bacteriology Department, School of Medicine, University of North Carolina, Chapel Hill

Multiplicity reactivation (MR) which involves the infection of a cell by the cooperative effort of 2 or more radiation-damaged virus particles, none of which is completely functional alone, has been demonstrated for vaccinia virus in several kinds of tissue culture cells(1,2) but not in the cells of an organized host. If tissue culture cells can find, among the damaged DNA or other parts of 2 or more such virus particles, the stimulus to produce normal virus progeny, one would expect to observe some such effect on the chorioallantoic membrane (CAM). The experimental difficulty encountered in previous attempts to demonstrate such effects was due in part to the exceedingly large and difficult-to-determine number of CAM cells that are available to the virus inoculum. This paper describes a means of observing and measuring the effect of MR without the need of determining or estimating the number of cells involved. We,

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and Abel(1) have observed MR in tissue culture experiments in which the average multiplicity was low but the incidence of multiple infection was made appreciable through the use of aggregated virus preparations. We have employed the electron microscope to count the virus particles and determine the degree of particle aggregation as well. This technique facilitates a clear demonstration of MR for this virus in the CAM.

Materials and methods. Vaccinia virus WR (mouse neurotropic strain) was grown in culture of L cells(3). Seventy-two hour cultures yielding 3000 to 5000 particles per cell were pooled and stored frozen at —60°C until needed. After thawing and mechanical lysis of the infected cells by 20 KC waves from a Branson Sonifier (Model 75) the dispersed virus was diluted with phosphate buffered saline (PBS at pH 7.2). It was sedimented and resuspended in fresh PBS to remove protein and other light-absorbing soluble material from the suspending fluid. Further treatment with 20 KC waves was applied to disperse the aggregates produced by pelleting of the virus in the washing procedure. Virus dilutions (in PBS) containing about 10^9 particles per ml were irradiated at an incident intensity of 30 μw per cm^2 from a low pressure mercury vapor discharge lamp emitting over 95% of its effective energy in the 2537 Å spectral line. The depth of fluid irradiated was 1-3 mm. All operations after irradiation of the virus were performed in dim incandescent light to avoid possible photoreactivation.

Virus particle counts were made by the agar sedimentation method of preparing samples for the electron microscope(4). Analyses of aggregation were made from the same electron micrographs by methods already described (5).

Virus titration was made by introducing 0.1 ml of the appropriate PBS dilution onto the dropped CAM by the method of Westwood *et al*(6), using 10 eggs per dilution. Embryos 12-13 days of age were used and pocks were counted 48 hours after inoculation.

Dispersed virus was induced to aggregate by sedimenting it in a round-bottomed test tube at 10,000 *g* for 30 minutes in a horizontal centrifuge rotor (Type HS—Ivan Sorvall, Inc.). The quantities of virus sedimented were too small to produce substantial pellets but resuspension of the virus by drawing up the fluid and expelling it from the pipette 20 times yielded the desired increase in particle aggregation without addition of aggregating agents such as $MgCl_2$ that might introduce extraneous complicating effects.

Experiments and results. The experiment plan was to treat well dispersed virus suspensions with ultraviolet rays until the pock titer was reduced by a factor of 20 to 100, then induce the particles to aggregate. Particle counts and aggregation analyses were made at each step, then the aggregated virus was redispersed with a brief application of 20 KC waves. Titrations and aggregation pictures were repeated on the final sample. The results of 4 such experiments are shown in Table I, in which the conspicuous rise in the titer of the irradiated virus, following aggregation, appears in all four. This rise was 7-fold at the least and 16-fold at maximum in spite of the fact that the aggregation reduced the number of suspended units of virus to about half. This was brought about by the incorporation of many single particles into groups, a process that would have reduced the titer of unirradiated virus to half.

TABLE I. Pock Titration and Physical Data Showing the State of Aggregation Among the Virus Particles in 4 Radiation Experiments. The irradiated virus is shown dispersed (D), subsequently aggregated (Agg) and redispersed (Agg) (D).

Exp	Sample	% Singles	Slope LLL	Pocks per 10^5 v.p.
A	Fresh (D)	91	—3.73	380
	Irrad. (D)	91	"	9.5
	" (Agg)	40	—2.76	92
	" (Agg) (D)	97	—5.52	20
B	Fresh (D)	88	—4.75	500
	Irrad. (D)	88	"	28
	" (Agg)	50	—2.65	190
	" (Agg) (D)	91	—4.38	14
C	Fresh (D)	72	—3.33	462
	Irrad. (D)	81	"	7.3
	" (Agg)	27	—2.04	78
	" (Agg) (D)	89	—4.43	7.0
D	Fresh (D)	72	—3.33	462
	Irrad. (D)	65	"	4.4
	" (Agg)	27	—1.96	68
	" (Agg) (D)	87	—3.92	2.8

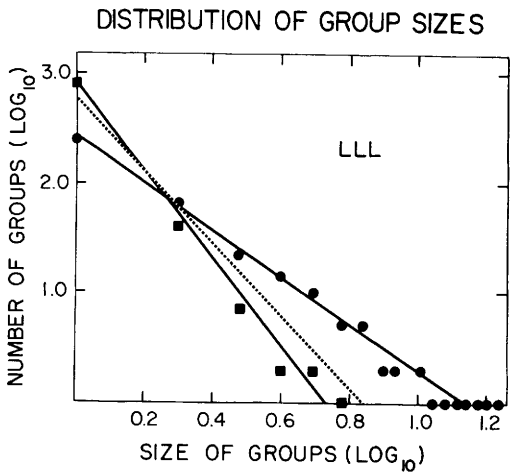

FIG. 1. Linear log-log (LLL) plots of group number or frequency *vs* group size for Exp. C, Table 1. The fresh dispersed virus (broken line), the aggregated virus (circles) and the redispersed virus (squares) show, by their slopes, the degree of aggregation among the virus particles.

While the percentages of single particles shown in Table I give a rough measure of the state of aggregation of virus preparations, much more definitive information is available in the electron micrographs. Totals of group frequency (Ni) for each group size (i) were plotted in log-log form as suggested by Geister and Peters(7) and the points fell tolerably well on straight lines for the dispersed as well as for the aggregated preparations (Fig. 1). Thus it is possible to describe the distribution quite precisely by a single statistic, the slope, which is steep for dispersed preparations and less steep for aggregated ones. These slopes are included in Table I.

The rise in titer which occurred when the virus was aggregated was quite completely reversed when the particles were redispersed. It is clearly an effect of aggregation, not the result of photoreactivation or some repair mechanism that might have taken place in the time required for the manipulation.

Discussion. The term multiplicity reactivation was used by Luria(8) to describe the phenomenon of infection of *E. coli* cells when they were attacked simultaneously by 2 or more phage particles that had been previously "inactivated" by exposure to ultraviolet rays. Their experiments and others with animal viruses have been arranged to present to the host cells a high enough concentration of virus particles to insure the adsorption of more than one to a substantial fraction of the cells which were subsequently observed to note the fraction infected. Such experiments with animal viruses are more difficult than with phage for two reasons. Many animal virus particles never make plaques, even before irradiation, and some animal viruses, particularly vaccinia virus, show a strong tendency to aggregate in suspensions of high particle concentration(9,2). The latter difficulty has been avoided in the present work by the use of dilute inocula containing measured amounts of aggregation. The present demonstration that these aggregates, induced after irradiation of the virus, are clearly more potent pock producers than single particles, gives evidence for MR of vaccinia virus in the cell of the CAM. The extent of the reactivation is comparable with that experienced in L cells (2,10), but the present data, although providing a demonstration, are insufficient for computations of the detailed nature of those in the latter publication. However, Fig. 1 also shows the change in slope of the linear log-log (LLL) frequency distribution that takes place when the dispersed particles become aggregated. Single particles decrease in number while the number of groups larger than 3 increases. We draw attention to the fact that the line pivots about an average group size, roughly 2, which does not change in frequency. This will be described more completely in a later report. It is not a unique case; it occurs also when dispersed virus reaggregates spontaneously. In the present experiments it implicates groups of size at least as large as 3 and probably as large as 5 or 6 as the chief contributors to the increase in titer. Little is known of the method of entry of vaccinia virus into CAM cells but these observations indicate strongly that groups of particles of at least this size are taken into the cell. The mechanism of MR is, of course, not revealed in this work. The concept of random damage wrought in a limited number of critical structures of the virus particle, proposed by Luria(8) for bacteriophages, is not violated by our obser-

vations of the MR of vaccinia virus in the cells of the CAM. In fact it remains a most plausible explanation of the results.

Summary. Irradiated vaccinia virus preparations increase in pock titer when the virus particles are subsequently induced to aggregate. Measurements by electron microscopy of the degree and mode of aggregation indicate the operation of a highly efficient multiplicity reactivation of the virus in the cells of the CAM. There is evidence for penetration of the cell and pock production by groups of 5 or more virus particles.

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Effects of Porcine Thyrocalcitonin on Serum Calcium, Phosphorus And Magnesium in the Monkey and in Man.* (31417)

NORMAN H. BELL, RICHARD J. BARRETT, AND ROY PATTERSON

Metabolism and Endocrine Section and Allergy-Immunology Section, Department of Medicine, Northwestern University Medical School, Chicago, Ill.

Thyrocalcitonin is a polypeptide hormone which acts to lower the serum calcium(1-3). It has been isolated from the thyroid glands of a number of animal species including man (4).

In the present work studies are presented to show that porcine thyrocalcitonin is active in the monkey and in normal human subjects.

Materials and methods. A crude extract of thyrocalcitonin was prepared from porcine thyroid glands by the method of Tenenhouse, Arnaud and Rasmussen(3). The trichloroacetic acid (TCA) precipitate was suspended in 0.1 M acetic acid and the TCA was removed by titrating to pH 3.4 with an excess of Dowex 1X2 acetate resin. The mixture was stirred for one hour, filtered and lyophilized. In the studies in monkeys, the powder was dissolved in 0.15 M sodium chloride and the pH was adjusted to 2.5 with 0.1 N hydrochloric acid. In the studies with human subjects, the powder was washed in a sterile funnel with acetone, and taken up in a sterile 0.15 M solution of sodium chloride with 0.25% phenol and acidified by addition of hydrochloric acid. Before use, it was shown to be free of bacterial contamination. The extract was assayed in young male Sprague-Dawley rats weighing between 100 and 120 g. Before the assay they were fed a low calcium diet for 4 days and fasted for 16 hours. Five or six rats were used to assay each dose. The mean fall in serum calcium 1½ hours after subcutaneous injection was 1.8 mg % for 1.0 mg of the extract and 0.8 mg % for 0.1 mg.

Studies in monkeys. One male and 5 female rhesus monkeys, *Macaca mulatta,* were used. Weights ranged from 3.0 to 5.9 kg. The monkeys were fasted overnight and were given phenobarbital, approximately 30

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