didymides in this investigation was due to circulating antibodies to BLH which inhibited endogenous gonadotropin.

Convincing confirmation of this postulate is found in our cross reactivity studies. We found 4 antigenic components of NIH-LH-B3. One of these antigens was a gamma globulin contaminant, while BSA was not a contaminating antigen. A pituitary factor in RP extracts showed reaction of partial identity with one of the antigens of BLH, and the cross reaction was not due to an NBS contaminant of BLH. We cannot be certain that the cross reacting factor is LH, and it will not be possible to know this until contaminant-free gonadotropins are available.

To our knowledge, this is the first demonstration of an autoimmune phenomenon in which antibodies produced in response to heterologous gonadotropin were shown to cross react with homologous pituitary extract.

Summary. Injections of purified bovine LH (NIH-LH-B3) in rabbits produced circulating antibodies which were associated with atrophy of the testes and epididymides. Bovine LH and crude rabbit anterior pituitary extract contained a similar antigen which was not a component of normal bovine serum. Results were attributed to an autoimmune phenomenon with circulating antibodies common to NIH-LH-B3 and endogenous gonadotropin.

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Infectious Virus Particles.* (32175)

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Most animal viruses produce plaques representing not over 10% of the particles in the inoculum(1,2,3). Ten percent of well adapted vaccinia virus particles produce visible plaques by standard procedures on monolayers of L cells, but independent evidence indicates the ability of all the particles of these preparations to infect L cells(4). It seems likely, therefore, that some virus particles do infect monolayer cells in areas where no visible plaques appear. This note provides evidence that they do, as well as a measure of the extent to which virus grows in these cells.

Materials and methods. The vaccinia virus of WR (mouse neurotropic) strain as well as the L cells, culture media, plaque titration procedure and counting of virus particles by electron microscopy have been previously described(5). Virus of L cell passage No. 22 was chosen because its average plaque yield was 1 per 125 virus particles (VP), much better than earlier passage virus but still about 1/10th of that attainable on further passage.

Experiments and results. Experiment A. A sonic lysate of infected L cells was diluted with phosphate-buffered saline (PBS) to contain 200 VP per 0.1 ml. Several 6 cm plates containing monolayers of L cells were in-

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oculated with this volume of virus. After a 3 hour adsorption period during which the plates were frequently tipped to distribute the inoculum evenly, the first agar overlay was applied. After 3 days at 37° in a humidified incubator containing 4% CO_2, a second overlay containing neutral red was added. Under these conditions the maximum number of plaques has been reached by the 6th day. Six plates were chosen that contained one plaque each. From each of these, 2/3 of the agar was removed with care, leaving the remainder with the plaque at the center undisturbed. All the cells that could be removed by washing this agar as well as scraping and washing the glass from which it came were collected in 1 ml of growth medium containing 10% horse serum. These 6 cell samples were treated with 9 KC sonic waves until all cells were lysed. Then the lysate was added to 1 oz bottle cultures of L cells, one ml to each bottle. These were incubated without overlay for 6 days, after which all the cells were scraped from the glass, resuspended in the growth medium, and treated with 9 KC waves to lyse the cells. Virus particle counts were made by electron microscope on all 6 with the results shown in Table I. The bottle cultures contained about 3 million L cells each at time of inoculation and the yields averaged 7 × 10^9 per bottle or 2.3 × 10^9 VP per million cells.

Experiment B. Fresh virus of the same passage as that of Experiment A was inoculated into bottle cultures of L cells at 6 different input multiplicities ranging from 0.0005 to 50 in steps of 10. These were treated in every way like those of Exp. A except that harvest was made at 7 days. The yields of these control cultures are shown in the chart (Fig. 1). The maximum yield occurred between M = 0.5 and 5.0 at about 3.0, which is the value given by the Poisson function for 95% of the cells to receive one or more particles from a perfectly dispersed, completely adsorbed inoculum. The level of 2.3 × 10^9 VP, reached in Exp. A, occurred at 2 points on the control graph, at M = 35.5 and at M = 0.117.

TABLE I.

One plaque plate culture No.	Virus particle yield per million cells of the bottle culture	Quality of this progeny virus, PFU/1000 VP
1	3.6 × 10^9	10.6
2	2.1 × 10^9	9.9
3	2.6 × 10^8	×
4	1.1 × 10^9	9.3
5	4.2 × 10^9	
6	2.7 × 10^9	

Monolayer bottle cultures of L cells (column 2), seeded with sonic-treated washings from the blank areas (non-plaque bearing areas) of previous plaque plate cultures, produce a copious yield of virus which, on subsequent plaque titer proves to be just as efficient in plaque production (column 3) as the starting material.

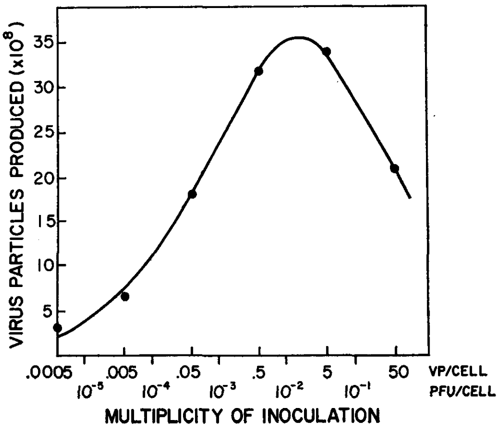

FIG. 1. Growth of vaccinia virus in monolayer of L cells inoculated at different input multiplicities. These yields are from 1 million cells.

Corollary tests. Subsequent plaque titrations of the virus from the final cultures of Exp. A showed it to be of quality indistinguishable from the starting virus (1 plaque per 125 VP, Table I). Also virus picked from the plaque areas of the plates and grown in bottle cultures to increase the number to reach the range of concentration countable in the electron microscope, then tested for plaque formation, yielded the same efficiencies as the others.

Discussion. Each of the plates chosen for these tests was inoculated with 200 VP, of which one made a visible (about 1 mm diameter) plaque. The remaining 199 VP may have remained inactive during the 6 days at 37°. If so, they would be expected to decrease by a factor of 8 from their original potency(6). This, together with the method of sampling, would reduce their effective number to 199 × ⅔ × ⅛ = 16, but we have seen from the control growth that an input multiplicity of at least 0.117 or 3.5×10^5 VP is needed to bring 3 million L cells to the observed yield of 7×10^9 VP in 7 days. Clearly there must have been cell infection and virus increase in the blank areas of the plaque plates. Furthermore, this increase, from ⅔ of 199 to 3.5×10^5, would mean that every particle infected a cell and each cell produced 2650 VP. However, one might expect some incompleteness in the collection of infected cells from the original monolayer under the agar as well as less than 100% adsorption of virus to the cells of the test cultures. These would raise the estimate for VP production in the few infected cells under the agar above 2650 per cell but not necessarily to a figure greater than those indicated in the control cultures of Fig. 1. It is therefore possible that all of the 199 VP that did not produce plaques, did infect one cell each and these provided the minimum 3.5×10^5 VP necessary to bring the bottle cultures to full yield. It is, of course, possible that the yield of virus from these few initially infected cells was not so great but that a few adjacent cells became infected, producing a microplaque and yielding the observed number of virus particles.

On further passage, the virus recovered from the blank areas between the plaques has shown the same efficiency of plaque formation as that recovered from the plaque area. Why, then, did it not make plaques on the initial monolayer? The population of L cells has given evidence of uniformity in VP yield per cell when inoculated at an input multiplicity of 10(2) but there is no data yet that shows uniformity of yield from singly infected individual cells. It is possible that a few cells, perhaps 1 in 200 in these experiments, yield a superior number of VP, a number sufficient to infect several adjacent cells and produce a visible plaque while the large majority of infected cells do not provide this critical number.

Summary. Although only a few plaques may appear when many vaccinia virus particles are put upon a monolayer of L cells, many cells become infected in areas remote from the plaques. A large fraction of the particles of the inoculum do infect cells and harvest of these from "blank" areas shows that substantial virus growth has occurred.

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Absorption of Taurocholate-24-^{14}C Through the Canine Gastric Mucosa.* (32176)

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When the gastric mucosa is damaged by salicylates or fatty acids it becomes abnormally permeable to H^+, Na^+, K^+, glucose, histamine and plasma proteins(1). In the course of unpublished work on the effects of natural and synthetic detergents upon the barrier function of the gastric mucosa, irrigation of the mucosa with neutral or acid solutions of conjugated bile salts was found to increase the mucosa's permeability. The possibility that the abnormally permeable mucosa permits bile salts to pass from gastric

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