Summary. In dogs performing graded exercise on a treadmill, there was a reproducible relationship between heart rate and oxygen consumption in the same dog. This relationship was linear up to moderately severe grades of work. Arterial blood pressure likewise increased with the severity of the exercise but the relationship was less direct. No decrease in arterial pressure was noted at the start of exercise or when the work load was suddenly increased during a continuous run.

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Effect of Interferon on RNA Synthesis in Sindbis Virus Infected Cells. (30848)

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The step in viral replication that is the site of action of interferon has not been determined. In the case of some RNA viruses it has been demonstrated that viral RNA is not made in the presence of interferon(1,2). This effect on viral RNA synthesis may be the consequence of a still earlier action, as suggested by the observations of Levy(2). Interferon delayed the cutoff of cellular RNA synthesis normally induced in suspension cultures of L cells by infection with Mengo virus within ½ hour after infection. In these experiments, interferon reduced the yield of Mengo virus in a one-step growth cycle by 99.9%. If the effect of interferon on viral-induced cutoff of cellular RNA synthesis is a general one and not restricted to the Mengo virus L cell system, then interferon's action could be related to a very early step in virus replication. (It is, of course, true that such effect on RNA cutoff could be a reflection of a still earlier event.) We, therefore, have tested for the presence of a similar cutoff of RNA synthesis during Sindbis virus infection of chick embryo (CE) tissue culture.

Experimental. Materials and methods. RNA was determined by the method of Mejbaum(3), protein by the method of Lowry (4). Radioactivity was measured on Schleicher & Schull No. B-6 membrane filters in the Packard Tri-carb scintillation counter. Chicken interferon was prepared in allantoic fluid of embryonated eggs by infection with the WS strain of influenza virus, and mouse serum interferon by intravenous injection of mice with Newcastle disease virus, as previously described(5,6).

Interferon was assayed by a plaque reduction method, using vesicular stomatitis virus (VSV). One unit of interferon is defined as that amount which reduces by one-half the number of plaques of a challenge of approximately 50 pfu of VSV on CE cells(5).

The medium used was either Eagle's basal medium (BME) or BME modified for suspension culture, hereafter called "spinner" medium.

Results. Preliminary experiments were performed to determine if monolayers of CE cells infected with high multiplicity of Sind-

bis virus could be used to study virus-induced cutoff of cellular RNA synthesis. Sindbis virus induced no detectable changes in RNA metabolism up to 5 hours after infection. This observation is in accord with the observations that Mengo virus infection does not induce a readily detectable cutoff of cellular RNA synthesis in monolayers of L cells but does in suspension cultures very soon after infection(2, and unpublished observations). Since no established suspension cultures of growing CE cells are available, we used CE cells which were placed in suspension by treatment of monolayers with versene. Primary CE cultures were prepared by placing 50 ml of trypsinized cells at 2×10^6 cells/ml in BME with 5% calf serum into Blake bottles. The bottles were plugged with cotton and incubated in 96% air, 4% CO_2 at 36°C. After 1 or 2 days of growth the medium was poured off, the cell sheet washed once with phosphate buffered saline (without Ca^{++} or Mg^{++}) (PBS) and treated at 36° with 10 ml of 0.02% versene in PBS. When the cells had come off the glass they were agitated by gentle pipetting, centrifuged at low speed, and washed once with 50 ml of "spinner" medium. The cells were then suspended in "spinner" medium at a concentration of about 5×10^5 cells/ml, and stirred with a magnetic stirrer. Other treatment of the cells, both before and after removal from glass, is indicated in the tables.

That such cells are competent to synthesize virus is shown in Table I, where the sensitivity of such virus multiplication to interferon is also shown.

TABLE I. Effect of Interferon on Growth of Sindbis Virus in "Suspended" CE Cells.

Time	Virus yield (pfu/ml)	
	No interferon	With interferon
Zero time	$10^{4.4}$	$10^{4.2}$
24 hr	$10^{7.3}$	$10^{4.4}$

Monolayers of CE cells in Blake bottles were exposed to interferon at 10 units/ml, or to an equal volume of comparably treated normal allantoic fluid for 17 hours at 36°C. The cells were treated with versene and washed as described in the text. About 4×10^7 interferon treated and control cells were exposed to 2 ml of Sindbis virus containing 4×10^8 pfu of virus, for 30 min at room temperature. The cells were washed 3 times with 50 ml Spinner BME, and diluted to 100 ml. Samples were taken to determine the amount of residual virus, and the remainder of the cells were incubated with stirring for 24 hr. Cells and fluids were frozen and thawed 3× before determining virus yield.

Experiments to determine whether or not Sindbis virus induced an early decrease in cellular RNA synthesis, and, if so, whether the inhibition was affected by interferon, were performed as indicated in Table II.

It can be seen that infection caused a 35-50% inhibition of cellular RNA synthesis. This viral-induced inhibition was not present in cells pretreated with interferon of chicken origin, but was present in cells treated with either interferon of mouse origin or normal allantoic fluid or mouse serum prepared in the same way as the interferon preparations. This specificity of action is in accord with the species specificity of interferons.

The inhibition of cellular RNA synthesis induced by Sindbis virus was found in 12 of 16 experiments performed, and varied in extent from 20-50% of the controls. In the other 4 experiments, the rats of RNA synthesis were the same in the infected cultures as in the uninfected controls. In each experiment where infection produced this inhibition, pretreatment of the cells with chick interferon prevented its development.

Discussion. The foregoing data show that, under suitable conditions, infection of CE cells with Sindbis virus can lead to an inhibition of cellular RNA synthesis, and that the development of this inhibition is prevented by pretreatment of the cells with interferon of chicken origin. This inhibition by virus could be demonstrated with some degree of regularity only on CE cells that had been put into suspension with versene. Under these conditions the cells were competent to produce virus, and interferon prevented such production.

No satisfactory explanation has been found for the fact that virus failed to produce inhibition of cellular RNA synthesis in 25% of the experiments. There was, in general, a correlation between the finding of inhibition and a high rate of RNA synthesis, but this correlation was only approximate and not sufficient to account for the lack of inhibition in 4 experiments. It is possible that inhibition might have been seen at a later

TABLE II. Effect of Sindbis Virus With and Without Interferon on RNA Metabolism of CE Cells.

Infection	Treatment	Specific activity cpm/mg protein	% inhibition by virus
No	Normal allantoic fluid	5793	
Yes	" " "	2806	52
No	Chicken interferon	5324	
Yes	" "	4847	9
No	Normal mouse serum	5111	
Yes	" " "	3416	35
No	Mouse serum interferon	4951	
Yes	" " "	2803	43
No	Medium	4708	
Yes	"	2819	40

Monolayers of CE cells, in Blake bottles containing 50 ml of fluid, after 2 days of growth were treated for 17 hr at 36° with 1000 units of either mouse serum interferon or chicken allantoic fluid interferon or comparable materials from uninfected mice or eggs. The cells were removed from the glass with versene, and washed, as described. Packed cells were exposed to either Sindbis virus in BME at an input multiplicity of 10, or to normal medium in 1 ml amounts, for 30 min at room temperature. The cells were then diluted with warm BME to a cell concentration of 3×10^5/ml and stirred with a magnetic stirrer for 75 min. H^3 uridine was added to a concentration of 0.2 μc/ml and the cells stirred for an additional 15 min. The cells were centrifuged, washed twice with PBS, extracted twice with 10 ml of cold 10% perchloricacid, and once with alcohol. The residual was dissolved in 0.1 N NaOH and aliquots used for determination of protein and radioactivity.

time or that for some reason there was no virus growth in those four experiments.

Thus, in 2 systems, Mengo virus in L cells and Sindbis virus in CE cells, interferon prevents or delays the inhibition of cellular RNA synthesis caused by infection. The significance of these observations needs examination. It is assumed that the viral induced cutoff of RNA synthesis seen in these experiments is a result of some step in virus replication, and that whether or not this step causes a detectable inhibition of RNA synthesis is dependent upon the metabolic state of the cells. The latter aspect of this assumption has been demonstrated for the infection of HeLa cells with poliovirus, and for the infection of L cells with Mengo virus(2,7,8). The effect of interferon, then, would be on a step in virus replication preceding in time the inhibition of cellular RNA synthesis. The phenomenon responsible for the early cutoff seen with Mengo, Sindbis or poliovirus is an early event in virus infection; in fact, so early as to suggest that it may be a function of the input virus rather than of some already replicated components of the virus. If so, then the prevention of cutoff by interferon might be taken to mean that interferon is affecting the expression of some aspect of input virus genome.

Summary. It has been shown that CE cells put into simulated suspension cultures by versene are capable of supporting the growth of Sindbis virus, and that chicken interferon prevents this virus growth. Under these conditions, an inhibition of cellular RNA synthesis is generally detectable within 1½ hours after infection in the absence of interferon. Pretreatment of the cells with chicken interferon, but not mouse interferon, prevents the development of this virus-induced inhibition. It is suggested that the action of interferon may be on a very early phase of virus replication.

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