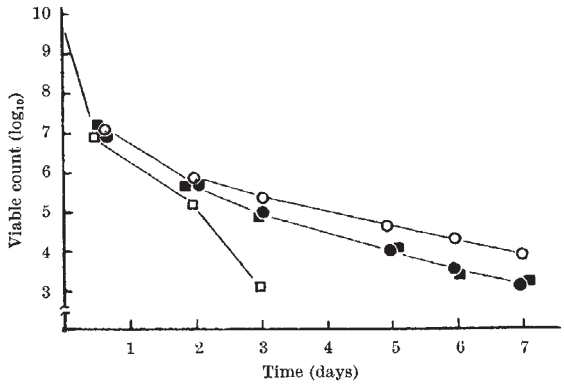


Fig. 1. Recoveries of *Salmonella ndolo* after drying and subsequent exposure to 100° C with 'dry' $P_2O_5$ *in vacuo* (□); 'dry' $P_2O_5$ in air (■); 'wet' $P_2O_5$ *in vacuo* (○); 'wet' $P_2O_5$ in air (●). Values are means from two ampoules. Viable count before drying, $4{\cdot}8 \times 10^9$ organisms/ampoule. Viable count after drying and before exposure to 100° C, $2{\cdot}3 \times 10^9$ organisms/ampoule

DA and DV ampoules occurred as water was extracted from the desiccates. The DV desiccates became progressively browner after two days and bubbles developed, expanding gradually day by day to attain diameters up to 5 mm. No such changes occurred in the WV, DA or WA desiccates during the period of these experiments or for the several further weeks that some test ampoules were left at 100° C.

The changes occurring in the DV desiccates may well have been due to vapour of $P_2O_5$ which could be expected to sublime readily at 100° C from the dry solid. From extrapolation[4] the vapour pressure of $P_2O_5$ at 100° C is $2{\cdot}5 \times 10^{-3}$ mm. Although the saturation vapour pressure of $P_2O_5$ is the same in air as it is *in vacuo*, its diffusion through the stationary air to the desiccate would be relatively slow.

McLeod gauge measurements on 'wet' $P_2O_5$ samples, both in the presence and in the absence of the desiccates, showed the water vapour pressure at 100° C to be $10^{-4}$–$10^{-5}$ mm, attained within 12 h and remaining constant for a further four days. For comparison[5], the water vapour pressure over orthophosphoric acid ($H_3PO_4$) at 100° C is 2·62 mm (extrapolated value). Between $H_3PO_4$ and solid $P_2O_5$ there are complex series of involatile acids on which there are no data for equilibrium vapour pressures of water at 100° C.

That the DA and WA recoveries were only slightly lower than those of the WV desiccates is of interest because of the practical problem of the need, or otherwise, to seal desiccates *in vacuo*[6].

The need for more precise information on water-vapour levels and for data concerning equilibrium attainment in this work is obvious. The results so far obtained, however, do provide some information concerning the behaviour of a *Salmonella* species dried and heated under reasonably defined conditions.

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## VIROLOGY

### Macromolecular Synthesis in Cells infected with Herpes Simplex Virus

A report from this laboratory[1] has shown that in *HEp*-2 cells infected with herpes simplex virus the incorporation of nucleosides into cellular DNA progressively decreases with time after infection. The purpose of this communication is to report on two other aspects of macromolecular synthesis, namely RNA and protein, in infected *HEp*-2 cells.

*RNA synthesis.* *HEp*-2 cells were infected with strain *MP* of herpes simplex virus and suspended in a maintenance medium according to procedures described elsewhere[2]. Immediately afterwards and at intervals of 30 min–2 h, suspensions consisting of $10^7$ infected or of uninfected cells received sufficient tritiated uridine (Nuclear-Chicago, Chicago, Illinois, spec. act. 0·7 c./mmole) to yield 10 μc./ml. Unlabelled thymidine and deoxycytidine in molar concentrations 10 times greater than that of tritiated uridine were also added. After incubation for 30 min at 34° C, the cells were washed in 0·01 M sodium acetate buffer (*p*H 5·1) containing $10^{-4}$ M uridine. The RNA was extracted according to a modification[3] of the procedure of Scherrer and Darnell[4], and its absorbancy was read at 260 mμ. The tritiated uridine was measured in a Nuclear-Chicago liquid scintillation spectrometer. For each pulse labelling, the ratio of:

$$\frac{\text{counts/min/unit of absorbancy at 260 m}\mu\text{ (infected cells)}}{\text{counts/min/unit of absorbancy at 260 m}\mu\text{ (uninfected cells)}}$$

was determined and plotted at the mid point of the pulse interval as shown in Fig. 1.

The data recorded in Fig. 1 show that between 1 and 3 h after infection the incorporation of tritiated uridine into RNA is reduced by a half relative to that of uninfected cells. Between 3 and 6 h after infection, the rate of uptake of tritiated uridine remains relatively level; afterwards, it declines again to approximately 20 per cent of that of uninfected cells. Reduction in RNA synthesis may be a characteristic feature of productive infection of mammalian cells with herpes simplex virus; a similar reduction was observed in canine cells infected with a mutant of the *MP* strain of herpes simplex virus[5].

*Protein synthesis.* *HEp*-2 cells infected at a multiplicity of 10 plaque-forming units/cell were suspended so as to yield $1{\cdot}2 \times 10^5$ cells/ml. of a medium containing a balanced

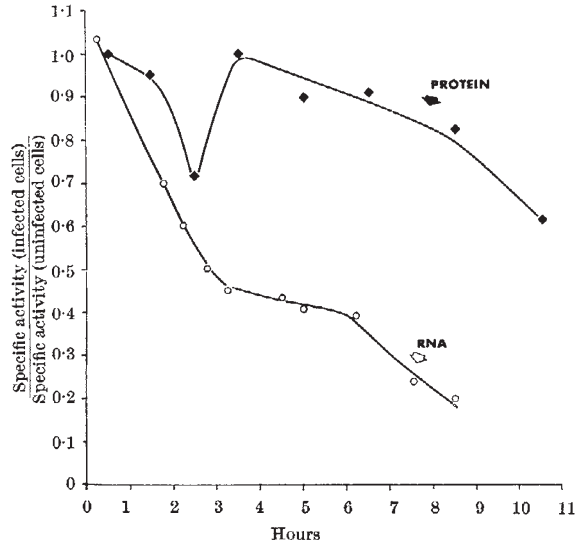


Fig. 1. Pulse labelling of RNA with tritiated uridine, and of protein with arginine labelled with carbon-14, at intervals after infection of *HEp*-2 cells with herpes simplex virus. The duration of the pulse was 30 min for tritiated uridine and 60 min for arginine-$^{14}$C

salt solution, 0·3 per cent methyl cellulose (15 c.p.s. U.S.P. grade, Fisher Scientific Co., Fair Lawn, New York), and amino-acids and vitamins at a concentration twice that recommended by Eagle[6]. Immediately afterwards and at intervals of 1–2 h, 10 ml. aliquots of infected and uninfected cell suspensions were centrifuged. The sedimented cells were suspended in a medium identical with that already described here except that the unlabelled arginine was replaced with arginine labelled with carbon-14 (0·1 μc./0·73 μg/ml., New England Nuclear Corp., Boston, Massachusetts). After 1 additional hour of incubation at 34° C, the cells were sedimented and washed with medium containing excess unlabelled arginine. They were then suspended in 3 per cent sodium lauryl sulphate solution containing cold arginine and incubated for 30 min at 37° C. At that time, an aliquot was removed for protein determination by the Lowry method; the proteins in the remainder were precipitated on filter paper disks with trichloroacetic acid. The disks were washed with trichloroacetic acid, dried, and then immersed in toluene base scintillation fluid for measurement of carbon-14 disintegrations. For each 1 h pulse labelling, the ratio of:

$$\frac{\text{counts/min/mg protein (infected cells)}}{\text{counts/min/mg protein (uninfected cells)}}$$

was determined and plotted at the mid point of the pulse interval as shown in Fig. 1.

Arginine labelled with carbon-14 was chosen because of the report by Tankersley[7], confirmed in this laboratory, that the amino-acid must be furnished in the medium for Herpes virus multiplication. However, experiments with labelled leucine and with media supplemented with labelled yeast hydrolysate yielded results similar to those recorded in Fig. 1. The pattern of protein synthesis in infected cells as revealed by amino-acid incorporation shows three distinct stages: During the first 3 h after infection, protein synthesis decreased to about 70 per cent of control rate. The decrease was observed in all experiments; the rate of decrease, however, varied with the multiplicity of infection. Between 3 and 6 h after infection, incorporation of amino-acid was generally stimulated. Lastly, between 6 and 10 h after infection, incorporation of amino-acid declined to approximately 60 per cent of that of uninfected cells.

The results presented in Fig. 1 reveal two distinct cycles of inhibition of macromolecular synthesis in infected cells. These cycles occur between 0 and 3 h after infection and from 6 h after infection. It is possible that the first cycle is brought about by a product specified by the virus early in infection, whereas the second cycle is caused by a sub-unit of the capsid or by some other product specified after the onset of DNA synthesis[2]. However, the results are insufficient to determine whether the two cycles are the expression of the same or different viral genes.

Inhibition of host macromolecular synthesis has been observed in animal cells infected with a number of RNA[8–10] and DNA[11,12] viruses. In relation to the data presented in this paper, two comments should be made. First, the inhibition produced by herpes simplex virus takes place much sooner after infection than that produced by vaccinia virus[11,12]. The difference may be due to the fact that vaccinia virus replicates in the cytoplasm, whereas herpes simplex replicates in the nucleus. Secondly, inhibition of host RNA and protein synthesis is not a general characteristic of all viral infections[13]. It is uncertain precisely what selective advantages accrue to the virus which modifies host metabolism over the one which does not. With respect to herpes simplex, the early inhibition of host RNA synthesis may be a prerequisite for the synthesis of viral constituents. Thus, infection of canine cells, under conditions which preclude early inhibition of host RNA synthesis, results in the production of interferon only. Viral constituents but no interferon, on the other hand, are made in cells the RNA synthesis of which is inhibited very early in infection[5].

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## PSYCHOLOGY

### Cortical Evoked Potentials and Appetite Drive

A NUMBER of investigators have observed the attenuation of evoked potentials during attention and conditioning[1–4].

In each study, the stimulus used has been exteroceptive and capable of producing an 'attentive' state or 'arousal' in the animal. Steiner[5] deprived rats of water for varying lengths of time and demonstrated that appetitive drive states can also produce EEG signs of arousal. In the present experiment, six cats were deprived of food for 30 h, and changes in the amplitude of auditory-evoked potentials and general EEG activity were observed at various times.

The recordings were taken from animals with monopolar stainless steel electrodes (insulated with 'Teflon' except for the very tip) chronically implanted in the auditory cortex. Uninsulated stainless steel wires attached to metal screws in the frontal sinus were used as indifferent and ground electrodes. After the experiment, the cortical electrode placements were verified histologically. One week before testing, the animals were placed on a once-per-day feeding regimen. The animals were placed in the experimental box for 0·5 h during which cat food ('Puss-in-Boots') was given until they stopped eating; they were then removed to the home cages for the intervening 23·5 h. On the testing day the animal was placed in the box and fed. Immediately after the animal stopped eating the first test session (pre-deprivation) was run. There was a total of seven test sessions: pre-deprivation and 3, 6, 12, 24 and 30 h of deprivation; the animal was then fed again until it stopped eating and the final test (post-deprivation) was administered. The animals were kept in the experimental box through the entire test sessions. A test session consisted of three trials. During each trial, 10 clicks (2,500 c.p.s., 30 msec duration) with 1-sec intervals between clicks were presented. These clicks, produced by an audio-oscillator, were of constant intensity and clearly audible to a human. In the six cats used for this report such stimulus produced well-developed evoked potentials in a rather restricted area of the posterior sylvian gyrus All six animals were subjected to this procedure once