Psychotomimetic Agents Advisory Committee of the US National Institute of Mental Health.

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Stimulation of DNA Synthesis in Resting Stage Human Fibroblasts after Infection with Rous Sarcoma Virus

ROUS sarcoma virus (RSV) suspensions can induce the synthesis of deoxyribonucleic acid (DNA) and mitosis in resting stage human fibroblasts[1]. The virus can also induce DNA synthesis in undividing myotubes[2] and DNA synthesis and mitosis in resting stage chick embryo fibroblasts[3,4]. The stimulatory activity may not be identifiable with the virus particle, for when the virus suspension was treated with RSV anti-serum the focus forming capacity was inhibited but the stimulatory action was not eliminated[1]. We have now extended these findings. The WI-38 human embryonic line[5] was used maintained in Eagle's minimal essential medium[6] supplemented with 10 per cent calf serum and aureomycin (50 μg/ml.). RSV suspensions were obtained from the supernatants of infected chick embryo fibroblastic cultures, and had a titre of 10^5 FFU/ml. DNA synthesis was estimated by adding 0·1 μCi/ml. of labelled thymidine (^{3}H-TdR) (specific activity, 14 Ci/mmole) to the cultures. The cells were fixed 24 h later, DNA was extracted[7] and the radioactivity incorporated was measured in a liquid scintillation counter.

The mucopolysaccharide used was isolated from *Mycobacterium tuberculosis* Penrois by a technique described earlier[8]. WI-38 cells were pooled and subcultured in new 60 mm plastic Petri dishes and used in the experiment 7 days later when mitotic activity was negligible. Medium was removed, kept at 37° C and 0·5 ml. of the following suspensions were added to duplicate cultures: RSV, RSV + 25 U/ml. of hyaluronidase, 100 μg/ml. of mucopolysaccharide and 100 μg/ml. of mucopolysaccharide + 25 U/ml. of hyaluronidase. All these suspensions were kept in a water bath at 37° C for 30 min before the experiments were carried out. To other cultures was added 0·5 ml. of RSV suspension, and the mixture was kept frozen until use. All cultures were kept for 1 h at 37° C in a humidified atmosphere with 5 per cent CO_2. The medium in which the cultures had been grown (used medium) was then added, supplemented with tritium-labelled thymidine. Fresh medium, supplemented with ^{3}H-TdR, was added to sister cultures, some which had been treated with hyaluronidase and some which had not. Finally, ^{3}H-TdR was added to two untreated cultures which were kept as controls.

The results in Table 1 show that significantly more radioactivity was incorporated into DNA after the addition of RSV suspensions, thawed or kept at 37° C for 30 min before adding to the cultures, than in the controls. The same virus suspension, treated with hyaluronidase, did not stimulate the incorporation of radioactive precursor into DNA compared with the DNA of the controls. More labelled precursor was incorporated after the cultures had been treated with a mucopolysaccharide but not if the same suspension was also treated with hyaluronidase. The enzyme itself was not responsible for the diminished incorporation on ^{3}H-TdR because a change of medium which is known to induce DNA synthesis in resting stage fibroblasts[9] had a stimulatory effect in spite of the treatment of the cultures with hyaluronidase. Thus the results indicate that the stimulation of DNA synthesis after infection with RSV may be caused in part by mucopolysaccharides present in the suspensions. To find out whether any other polysaccharides can induce DNA synthesis, used medium was removed and 0·2 ml. of Eagle's medium without serum (EE) containing 100 μg/ml. of DEAE cellulose was added to ten cultures. Another ten cultures received 0·2 ml. of EE. The cultures were incubated at 37° C for 0·5 h and the used medium was replaced with 0·1 μCi/ml. of ^{3}H-TdR. As Table 2 shows, cultures treated with DEAE contained significantly more radioactivity than the controls.

Table 1. TOTAL C.P.M. AFTER 24 h OF LABELLING IN DUPLICATE CULTURES TREATED AS INDICATED

Controls	11,460 (±115)
RSV thawed immediately before the experiment	29,400 (±294)
RSV kept at 37° C for 30 min before the experiment	30,660 (±307)
RSV + Hy	12,300 (±123)
mps	21,660 (±217)
mps + Hy	9,570 (±96)
New medium	93,060 (±930)
Hy + new medium	115,380 (±1,160)

Hy, hyaluronidase; mps, mucopolysaccharide.
The 95 per cent confidence limits are indicated in parentheses.

Table 2. AVERAGE C.P.M. FOUND AFTER 24 h OF LABELLING IN CONTROLS AND CULTURES TREATED WITH DEAE

Cultures treated with	c.p.m.
EE	28,650 (±2,578)*
EE with DEAE	46,740 (±4,206)*

* ($t = 8{\cdot}52$; $P < 0{\cdot}001$).
The 95 per cent confidence limits are indicated in parentheses.

Our results show that treatment of resting stage cultures with RSV suspensions stimulates DNA synthesis during the following 24 h. A similar effect is obtained with a mucopolysaccharide extracted from a bacterium and with a synthetic polysaccharide. Hyaluronidase eliminates the stimulatory action of the former and of RSV suspensions. Cell division is necessary for the successful infection by RSV[10], and so the stimulation of cell division by RSV suspensions might be brought about by some factor present in the suspensions but not identical with the virus, which influences viral replication.

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