Serum Requirement for Interferon Production by Suspended Human Leucocytes: Studies on Action of Serum

By GY. HADHÁZY, H. STRANDER AND K. CANTELL
Department of Virology, State Serum Institute, Helsinki, Finland

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SUMMARY

Suspensions of human leucocytes purified by NH₄Cl produced interferon in the same time after induction by Sendai virus whether the medium contained serum or not. In the absence of serum the interferon titre levelled off after as little as 5 hr, and the yield was less than 10% of that obtained when serum was present. The production of interferon was completely inhibited by 2 μg./ml. of actinomycin D added simultaneously with the virus. Interferon synthesis gradually became insensitive to actinomycin during the first 4 to 5 hr after induction in both the presence and absence of serum. Large amounts of interferon rapidly appeared after addition of serum to cultures induced and incubated in serum-free medium. The cells responded to added serum by interferon production up to 16 to 20 hr after induction. The rate of interferon production after addition of serum was not affected by actinomycin. Removal of serum from the medium resulted in a reduced yield of interferon; which would not be prevented by addition of actinomycin. The low yields of interferon in a medium devoid of serum were not due to reduced synthesis of the DNA-dependent RNA needed for interferon production.

INTRODUCTION

The presence of serum in the medium is needed for substantial development of interferon in human leucocyte suspensions (Falcoff et al. 1966; Strander & Cantell 1967). Attempts to find a suitable substitute for serum were not successful (Cantell et al. 1968) until it was observed that good yields could be obtained in media containing albumin and certain buffers (Strander, 1969). The present paper deals with experiments aiming to reveal why serum is required for the production of interferon by purified human leucocytes in vitro.

METHODS

The methods for the preparation of interferon in purified human leucocyte suspensions have been published in detail (Strander & Cantell, 1966, 1967). No serum was used during the purification of the cells by NH₄Cl treatment. The cells were suspended in Eagle's minimum essential medium (MEM) and incubated in MEM or in MEM supplemented with 10% heat-inactivated (56° for 30 min.) pooled calf serum. The cell concentration was 10⁷/ml. and the dose of Sendai virus used for the induction of interferon was 100 HA units per ml. Thirty ml. tubes were used for incubation of the cells in a roller wheel at 37°.

All centrifugations were done at 160 g for 10 min. and pre-warmed (37°) MEM or MEM+10% serum was used for resuspension of the cells.
Interferon was assayed by VSV plaque reduction in a line of human amnion cells as described by Strander & Cantell (1966, 1967).

Actinomycin D was obtained from Merck, Sharp and Dohme Ltd.

**RESULTS**

*Addition of serum at different times after induction*

Earlier studies (Strander, 1969) indicated that serum was not needed during the first 1½ hr of induction with Sendai virus in order to obtain good yields of interferon. The aim of the following series of experiments was to ascertain the length of the period during which a high yield of interferon could still be obtained by addition of serum. A number of leucocyte cultures were induced with Sendai virus in the absence of serum, and serum was added after incubation for different periods at 37°C. The tubes were then further incubated for 24 hr, when interferon was assayed (Fig. 1). Full yields of interferon were obtained when serum was added within 16 to 20 hr after induction. After this the yield in response to addition of serum decreased by tenfold in less than 10 hr. The production of interferon in cultures from which the extracellular Sendai virus was removed 1 hr after induction followed the same general pattern.

![Fig. 1. Yield of interferon following addition of serum at different times after induction. The cells were induced in the absence of serum, 10% serum was added after different intervals and interferon was assayed 24 hr later. •, △, ■. Three experiments. In one of them (△) the cells were washed twice by centrifugation 1 hr after induction to remove extracellular Sendai virus.](image)

In a similar experiment the cell suspension was transferred to a new tube 15 hr after induction. The cell suspension was supplemented with serum and the emptied tube was re-filled with medium containing serum. Both tubes were incubated for a further 24 hr and then assayed for interferon. The emptied tube contained no detectable activity, whereas the other tube contained 1000 interferon units per ml. Serum does not act by preventing adsorption of interferon to the walls of the tube.
Rate of interferon production in the presence and absence of serum

The kinetics of the production of interferon in the presence and absence of serum was analysed in several experiments (Fig. 2). Production started at the same time under both conditions. However, in the serum-free medium the concentration of interferon did not increase beyond 5 hr after induction, whereas in the cultures containing serum the concentration rapidly increased during the next few hours to a level more than ten times greater. Similar results were obtained in one experiment in which cells preincubated for 24 hr were induced with or without serum in the medium.

![Graph](image)

**Fig. 2.** Rate of interferon production in the presence and absence of serum. ●—●, 10% serum, mean values from six experiments; O—O, no serum, mean values from three experiments.

Experiments with actinomycin D

Different doses of actinomycin D were added to leucocyte cultures at the time of induction or at hourly intervals thereafter and the interferon present 24 hr later was assayed. All cultures contained serum. Two µg. of actinomycin per ml., added together with Sendai virus, inhibited all detectable interferon production (Fig. 3). Lower concentrations gave incomplete inhibition. The system gradually became more and more insensitive to actinomycin and 4 to 5 hr after induction the addition of the drug was ineffective.

Actinomycin (2 µg./ml.) was used to study whether insensitivity to the drug also occurred when the cells were induced and incubated in serum-free medium. Two groups of cultures were induced with Sendai virus, one in the presence and the other in the absence of serum. Actinomycin was added to both groups at different times after induction and the cultures which did not contain serum also received 10% serum at the same time. Insensitivity to actinomycin developed in exactly the same fashion in the absence as in the presence of serum (Fig. 4).
When actinomycin was administered to cultures induced and incubated in a serum-free medium for 4 or 5 hr and serum was added 1 hr later, full yields of interferon could be recovered repeatedly after further incubation for 24 hr.

Fig. 3. Yield of interferon following addition of actinomycin D at different times after induction. Concentrations of actinomycin: ●—●, 0.5 μg./ml.; ▲—▲, 1 μg./ml.; ■—■, 2 μg./ml.; ▼—▼, 4 μg./ml. Control: no actinomycin.

Fig. 4. Development of actinomycin insensitivity after induction in the presence or absence of serum. ●—●, Induction in the presence of 10% serum. Actinomycin was added at different times to give a concentration of 2 μg./ml., and interferon was assayed 24 hr later. ○—○, Induction in the absence of serum. Actinomycin and serum were added at different times to give final concentrations of 2 μg./ml. and 10%, respectively, and interferon was assayed 24 hr later.
Two types of control experiments were done to study the effect of serum and incubation on the sensitivity of the leucocyte suspensions to actinomycin. (1) Serum-free and serum-containing cultures were incubated for 24 hr in the presence of different concentrations of actinomycin. Thereafter, the cultures were induced, serum was added to the serum-free suspensions and the interferon present at 24 hr was assayed. The yield was reduced more than 90% by addition of 0.25 μg./ml. of actinomycin, and complete inhibition was obtained with 0.5 μg./ml. of the drug. The serum-free and serum-containing suspensions were equally sensitive to the drug. (2) Different doses of actinomycin were added together with Sendai virus to cultures incubated for 24 hr in the presence or absence of serum. Two μg./ml. of actinomycin were needed to inhibit interferon production completely.

![Graph showing interferon production](image)

**Fig. 5.** Rate of interferon production following addition of serum or serum and actinomycin 4 hr after induction in the absence of serum. ●—●, 10% serum from 0 to 24 hr; ○—○, no serum from 0 to 24 hr; ▲—▲, 10% serum from 4 to 24 hr; ■—■, 10% serum and actinomycin 2 μg./ml. from 4 to 24 hr. The arrow indicates the addition of serum or serum and actinomycin.

**Table 1. Yield of interferon following removal of serum at different times after induction**

<table>
<thead>
<tr>
<th>Exchange of medium</th>
<th>Interferon units at 24 hr</th>
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<tbody>
<tr>
<td>No serum</td>
<td>10% serum</td>
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<tr>
<td>Time (hr)</td>
<td>Interferon units</td>
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<td>8</td>
<td>200</td>
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<td>1000</td>
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To study the rate of interferon formation, after addition of serum, by leucocytes induced in serum-free medium, serum was added to serum-free cultures 4 hr after induction.
induction and at hourly intervals a tube was removed from the roller wheel for assay of the interferon. To another set of cultures serum and actinomycin (2 µg./ml.) were added at 4 hr, and the interferon in these cultures was assayed in hourly samples as above. The rate of interferon production was also followed in two types of control cultures, one induced and incubated in a medium containing serum and the other induced and incubated in serum-free medium (Fig. 5). The addition of serum resulted in the appearance of considerable amounts of interferon in the medium within 1 hr. The rate of interferon production was not affected by actinomycin and was of the same order as in the cultures which contained serum continuously.

In another experiment of the same type serum or serum and actinomycin were added 12 hr after induction. The interferon response was slower than in Fig. 4, but again the rate was the same in the suspensions receiving serum and in those receiving serum and actinomycin,

*Removal of serum at different times after induction*

To test the possibility that the presence of serum might not be necessary once the production of interferon had started in the medium containing serum, a number of cultures were induced in the presence of serum. At intervals, one tube was centrifuged and the supernatant saved for interferon assay. The cells were resuspended in MEM, divided into two tubes and again centrifuged. The cell pellet in one of the tubes was resuspended in half the original volume of MEM and the cells in the other tube were likewise resuspended in MEM + 10% serum. The tubes were incubated for 24 hr and the supernatant fluids collected for interferon assay. The cultures from which serum was removed contained, at the most, only 10% to 20% of the amount of interferon present in their serum-containing counterparts (Table 1).

The effect of administration of actinomycin on the decrease of interferon production following the removal of serum was tested by adding the drug (2 µg./ml.) to interferon-producing suspensions after serum had been removed at different times. The actinomycin was without effect.

**DISCUSSION**

The results obtained confirm the earlier findings (Hadžázy et al. 1967) that the production of interferon by human leucocytes exposed to Sendai virus *in vitro* is inhibited by actinomycin. In addition, we have observed that puromycin and cycloheximide completely block the production of interferon (unpublished). Therefore, it is likely that *de novo* synthesis of interferon is induced by Sendai virus in human leucocyte suspensions.

Although the induced leucocytes release little interferon into a serum-free medium, the early stages of interferon induction seem to proceed in normal fashion, since the first interferon activity was detected as early as after induction in the presence of serum, the development of insensitivity to actinomycin was not affected by serum, and addition of serum, even under the influence of actinomycin, resulted in the very rapid appearance of interferon. It seems that the DNA-dependent RNA synthesis needed for interferon production does not require the presence of serum in the medium. A considerable proportion of this DNA-dependent RNA appears to be synthesized within 4 to 5 hr after induction, whether serum is present or not.
Serum and human leucocyte interferon

The results of the present study do not explain why the yields of interferon remain low in serum-free media. At least the following possibilities should be considered:

(1) Active interferon molecules are formed at a good rate, but are rapidly destroyed intra- or extracellularly or adsorbed to cells or cell debris in the absence of serum. This cannot be definitely excluded but it is difficult to reconcile it with the experimental evidence available (e.g. Fig. 1).

(2) The DNA-dependent RNA needed for interferon formation is highly labile in serum-free leucocytes and therefore only negligible amounts of interferon are synthesized. This is rather unlikely, however, since the DNA-dependent RNA seems to be largely formed within the first 4 hr (Fig. 4) and yet addition of serum as late as 10 to 16 hr after induction resulted in full yields of interferon (Fig. 1). Large amounts of interferon also developed when serum was added 1 hr after actinomycin. Accordingly, the required DNA-dependent RNA formed in the absence of serum can hardly be very labile.

(3) The messenger RNA required for interferon production is not translated when the leucocytes are incubated in serum-free medium. This could be due to alterations in the ribosomes. It has been observed that the ribosomes derived from serum-deprived chick cells incorporate less amino acids in vitro and are less responsive to polyuridylic acid than ribosomes from normal cells (Soeiro & Amos, 1966). It is also possible that the transport of interferon from the leucocytes requires serum. An intracellular accumulation of interferon molecules could then conceivably lead to reduced translation of interferon messenger RNA. However, in our preliminary studies (unpublished) only small amounts of interferon were recovered from induced leucocytes which were disintegrated by freezing and thawing or sonic treatment, and cells devoid of serum did not harbour more interferon than normal cells. Both removal and addition of serum exerted a rapid influence on the production of interferon. These influences were not affected by actinomycin and hence the action of serum is probably not mediated by the cellular genome.

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G.H. was on leave from the Institute of Microbiology, University Medical School, Debrecen, Hungary, H.S. on leave from the Institute for Tumor Biology, Karolinska Institutet, Stockholm, Sweden.

REFERENCES


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