The sera, taken 7 days after the last injection, were pooled and used for conjugation. The globulin fraction of the serum was conjugated with fluorescein isothiocyanate on celite[5] according to the method described by RINDERKNECHT[6]. The unconjugated dye was removed by passing the serum through a column of Sephadex G25[7]. The serum was then absorped twice against rabbit liver powder before use.

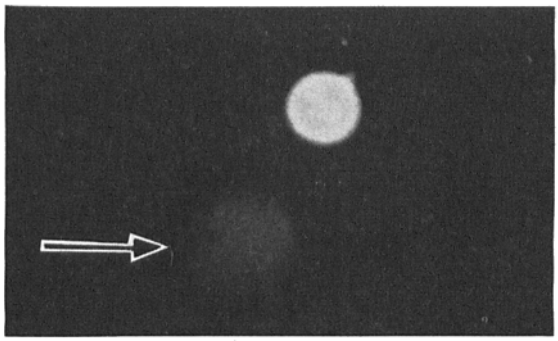

Fig. 1. The difference in the intensity of fluorescence between the cysts and the trophozoites after treatment with anti-cyst serum. The trophozoite is indicated by an arrow.

Fig. 2. Arrow marks the trophozoite emerging from a large aperture in the cyst which has been treated with anti-cyst serum. The remaining cyst wall is brightly fluorescent but the trophozoite shows negligible fluorescence.

The cysts fluoresced brightly after being treated with anti-cyst serum for 30 min in a serum dilution of 1:5 made with physiological saline. This reaction has been described previously[8] and is a true antigen-antibody reaction as it does not occur with normal serum and is inhibited by pre-treatment of cysts with unconjugated anti-cyst serum.

The trophozoites of *E. invadens* also showed some fluorescence when suspended in anti-cyst serum in a dilution of 1:5. However, it was possible to modify this reaction by absorping anti-cyst serum against a heavy suspension of trophozoites. The absorped sera then produced a negligible fluorescence of trophozoites but continued to produce an intense fluorescence of the cysts. Figure 1 shows the difference in the intensity of fluorescence between a cyst and a trophozoite after being treated with anti-cyst serum. In some cases excystation of the amoeba occurred during the treatment with anti-cyst serum and Figure 2 shows a trophozoite emerging from a large aperture in the cyst. The trophozoite shows negligible fluorescence but the remaining cyst wall is brightly fluorescent. This study indicates that the surface antigens of the cyst are different from those of the trophozoites and that this difference is recognizable by the fluorescent-antibody technique. Investigations are at present in progress to compare the internal antigens of the cysts with that of the trophozoites using the gel-diffusion technique.

Résumé. La technique des anticorps fluorescents a été appliquée à l'étude de la relation antigénique des cystes et des trophozoïtes d'*Entamoeba invadens*. Le sérum anticyste préalablement absorbé par les trophozoïtes a montré chez ces derniers une fluorescence négligeable mais il continua a produire une fluorescence intense chez les cystes, ce qui indique que les antigènes de surface des cystes sont différents de ceux des trophozoïtes.

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The Implications of the Temperature-Independent Binding and the Temperature-Dependent Action of Interferon

A number of vertebrate systems have been shown to respond to virus infection by producing interferon[1]. Interferon is the name given to a group of proteins which are capable of initiating the development of an antiviral state in cells of the appropriate species. Many studies have shown that interferon itself is not directly antiviral. Rather, interferon induces the formation of an antiviral state in treated cells. This induction is known to require both DNA dependent RNA synthesis and protein synthesis[2,3], but its details are not known. In fact, even the nature of initial interaction between cells and interferon is unclear. BARON and BUCKLER were unable to show that the induction of an antiviral state by interferon involved detectable loss of interferon from the medium[4]. This raised the question of whether interferon binding or

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absorption was required for interferon action. Logic led most workers to assume that it was at least bound, but experimental support for this deduction has been wanting. I would like to report results that support the conviction that cells must bind interferon as a necessary prologue to the induction of an antiviral state.

In these experiments, monolayer cultures of primary chick embryo cells were exposed to interferon at either 0 °C or 37 °C for 1 h. At the close of this hour, the cells were washed twice with cold Eagle's MEM. Immediately after washing, selected 0 °C and 37 °C samples were treated with 40 °C, 0.25% trypsin for 3 min. The trypsinization was stopped by the addition of cold MEM containing 10% fetal calf serum (FCS). This medium was immediately removed and replaced with warm (37 °C) MEM + FCS. The cell layer was allowed to 'heal' in this medium for 2 h at 37 °C under humidified 5% CO_2–95% air. At the close of this incubation the medium was removed and 1 ml of Semliki Forest Virus preparation (26 pfu/ml) was added. After 30 min, the virus was removed and replaced with a 1% agar in nutrient medium F-10 overlay[5]. After 48 h of incubation at 37 °C in a humidified 5% CO_2–95% air atmosphere, the layers were stained with INT (0.1% Iodonitrotetrazolium violet, 0.8% NaCl, 2% glucose solution) and the plaques counted.

As shown in the Table, exposure to interferon at 0 °C for 1 h, followed by washing and subsequent trypsinization, resulted in a marked reduction in the development of antiviral activity. On the other hand, incubation at 0 °C for 1 h, followed by an incubation at 37 °C for 30 min resulted in the development of substantial antiviral activity. Clearly, after 1 h incubation at 37 °C, the development of the antiviral state is insensitive to the proteolytic action of trypsin. Also, as indicated in the Table, experiments to determine the effects of temperature and trypsinization on plaque formation demonstrated that these factors were without any significant effect of infectivity.

These results demonstrate that the initial stages of interferon action may be separated into 2 steps; the first temperature independent, the second temperature dependent. The initial step in interferon action is a process in which interferon binds to the cell. Since this binding occurs at 0 °C, it is independent of metabolism driven membrane activity. Once bound at 0 °C, the interferon is not removed by washing, and remains on the surface of the cell, readily susceptible to attack by trypsin. The second step, during which the induction of antiviral activity becomes insensitive to the action of trypsin, is temperature dependent. One may conclude from these observations that, when cells are exposed to interferon, some of it becomes tightly bound to their surface. Therefore, some interferon, however small the amount, is in a sense taken up by the cells. Whether or not it is subsequently taken *in* is quite another matter.

The loss of susceptibility to trypsin at 37 °C does not have a unique interpretation. This loss could reflect the movement of interferon from its vulnerable position on the cell membrane to a trypsin inaccessible site inside the cell. Such an explanation would be analogous to the loss of sensitivity to neutralizing antibody as a virus penetrates from the outside to the inside of the cell in a temperature dependent process[6]. Alternatively to penetration, the interferon molecule may, in a temperature dependent process, initiate the chain of events leading to the development of the antiviral state while outside the cell. If the initiation process were completed after only a few min at 37 °C, subsequent destruction of the interferon molecule by trypsin would be without effect. The suggestion that a protein molecule attached to the outer surface of a cell can profoundly affect intracellular metabolism, finds analogy in the behavior of a group of proteins known as Colicins[7]. The attachment of a colicin molecule to the wall of a susceptible *E. coli* bacterium can cause the death of that cell. This colicin induced death does not involve the entry of the colicin molecule into the cell, since treatment of colicin treated cells with trypsin soon after the binding prevents the action of the colicin, and thus spares the cells[8]. However, after a sufficient incubation at 37 °C, treatment with trypsin does not prevent the lethal effects of colicin on treated bacteria. A decision as to whether interferon triggers the formation of an antiviral state from an extracellular or intracellular location must be deferred until further experiments have been conducted[9].

Effect of incubation temperature and trypsin on interferon action

Sample	Interferon[a]	Incubation temperature	Trypsin	Plaques	% Control[b]
1	—	0 °C	+	*26* (24-29-26)	100
2	—	37 °C	+	*26* (25-27)	100
3	+	0 °C	—	*2* (3-2-1)	8
4	+	0 °C	+	*19* (21-16-19)	77
5	+	0° (1 h) to 37° (30 min)	+	*7* (5-8)	27
6	+	37 °C	—	*0* (0-1-0)	0
7	+	37 °C	+	*1* (1-0-1-)	4

[a] 25 units of crude chick interferon. [b] Calculated on the basis of 26 pfu/ml under standard assay conditions.

Résumé. Des expériences utilisant la température et la trypsine sont décrites; elles démontrent que la phase initiale de l'action de l'interferon comporte deux étapes. La première consiste dans la fixation de l'interferon. Elle a lieu a 0 °C et n'est pas sous la dépendance du métabolisme cellulaire. La deuxième étape, au cours de laquelle l'interferon lié amorce le développement de la résistance antivirale, dépend du métabolisme cellulaire et est terminée au bout de 45 min. Deux modèles de l'étape sensible à la température sont présentés et discutés.

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