*THE ROLE OF HORMONES IN VIRAL INFECTIONS, II. ACCELERATION OF VIRAL ADSORPTION AND PENETRATION INTO CELLS TREATED WITH THYROID HORMONE IN VITRO**

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In the first communication of this series,[1] it has been shown that parathyroid or thyroid hormone added immediately after infection of HEp-2 cells with *herpes simplex* virus (HSV) does not decrease the number of polykaryocytes and therefore was ineffective in preventing viral development. However, cells pretreated for 3 to 24 hr with 0.5 or more units per ml of parathyroid hormone were shown to adsorb virus and "transport" it through the cytoplasmic membrane more slowly than untreated cells. In this communication it will be shown that HSV adsorption and penetration into cells treated with thyroid hormone are accelerated.

Materials and Methods.—Materials and methods are the same as described in the preceding paper.[1]

Results.—The effect of pretreatment of HEp-2 cells with thyroid extract on the number of polykaryocytes induced by HSV: In two experiments, HEp-2 cultures in groups of 2 received each 5 ml of maintenance medium (MM) containing 0.05 mg/ml of lyophilized thyroid extract 60, 20 and 5 min immediately preceding infection. In other experiments, MM containing thyroid extract (0.04–0.0006 mg/ml) was added to cultures 3 or 24 hr before infection. In all of these experiments, the cells were exposed to HSV for 30 min.

The thyroid extract had no effect on the number of polykaryocytes formed in cultures of cells treated for one hr or less. The dose-response relationship between the concentration of thyroid extract and the formation of polykaryocytes in cultures treated for 3 and 24 hr before infection is shown in Figure 1. It is evident that the thyroid extract at concentrations greater than 0.001 mg/ml causes an increase in the number of polykaryocytes. In cultures pretreated with high (0.04 mg/ml) concentrations of the extracts, the increase in the polykaryocyte count ranged from 1.33 to 1.84 times the number of polykaryocytes in untreated cultures. The thyroid extract appears to be equally effective in cultures treated for 3 and 24 hr before infection.

Adsorption and penetration of HSV into HEp-2 cells pretreated for 24 hours with thyroid extract: Untreated cells and cells exposed for 24 hr to 0.01 mg/ml of thyroid extract were washed and exposed to HSV. At 7, 15, and 30 min after infection, respectively, the inoculum in each of 3 treated and untreated cultures was removed for assay and replaced with 5 ml of maintenance medium containing human pooled gamma globulin (MMγG). The results of this experiment (Fig. 2) show that virus disappears more rapidly from the inocula of pretreated than untreated cultures. Concurrently, treated cultures show more polykaryocytes than untreated cultures. The data indicate that both adsorption and penetration are accelerated in cultures of pretreated cells. The titer of the virus is not increased. This conclusion emerged from another experiment.

One group of 15 cultures received each 5 ml of MM containing 0.04 mg/ml of

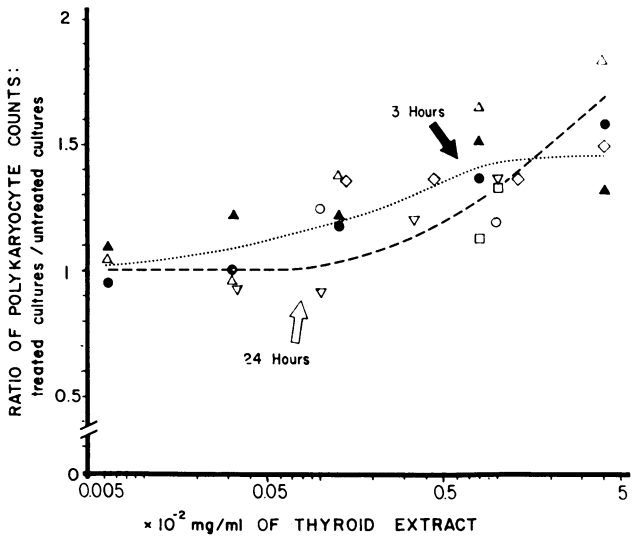

FIG. 1.—Formation of polykaryocytes in HEp-2 cell cultures pretreated with thyroid extract. Each symbol represents a different experiment.

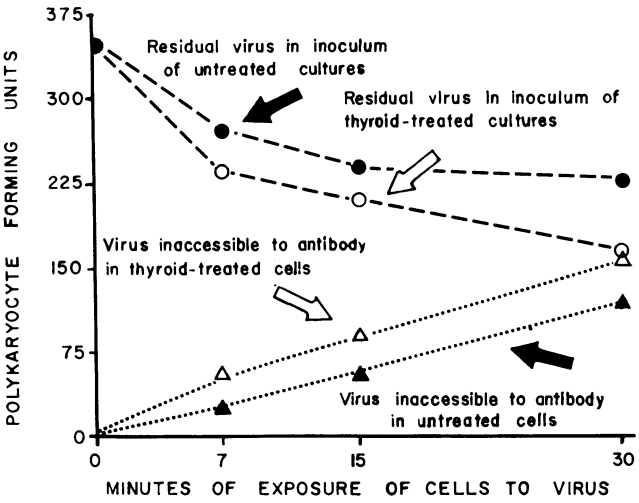

FIG. 2.—Effect of pretreatment of HEp-2 cells for 24 hr with 0.01 mg/ml of thyroid extract on adsorption and penetration of HSV.

thyroid extract. Another group of 15 cultures received each 5 ml of MM only. After 24 hr of treatment with thyroid extract, the cells were washed with phosphate buffered saline (PBS) and exposed to virus as usual. At 30 min intervals, the inoculum in 3 treated and 3 untreated cultures was replaced with MMγG. The results (Table 1) show that the final polykaryocyte counts obtained in treated and untreated cultures are identical. However, in treated cultures the maximum count is reached more rapidly than in untreated cultures.

The formation of polykaryocytes in HEp-2 cell cultures pretreated for 24 hours with both thyroid and parathyroid hormones: Replicate monolayer cultures in groups of 3

TABLE 1

POLYKARYOCYTE COUNT IN TREATED AND UNTREATED CULTURES EXPOSED TO VIRUS FOR DIFFERENT INTERVALS

Cultures	Minutes of exposure to virus before addition of MMγG				
	30	60	90	120	150
Pretreated with thyroid extract	80.7	139.3	161.3	159.0	164.0
Untreated	52.0	96.0	134.3	156.7	162.7

received MM containing thyroid extract (0.04 mg/ml), parathyroid hormone (2 units/ml), both hormones, or MM only. The cells were incubated for 24 hr, then washed and exposed to virus for 30 min. From the results summarized in Table 2,

TABLE 2

THE FORMATION OF POLYKARYOCYTES IN HEp-2 CELL CULTURES PRETREATED FOR 24 HR WITH BOTH THYROID AND PARATHYROID HORMONES

Pretreatment	No. of polykaryocytes
Maintenance medium only	93.0
Thyroid extract (0.04 mg/ml)	122.0
Parathyroid hormone (2 units/ml)	15.0
Thyroid and parathyroid hormones	35.7

it is clear that, in cultures exposed to both hormones, a balance may be achieved between effects of each hormone alone.

Discussion.—The data show that HEp-2 cells pretreated with thyroid hormone become infected with HSV more rapidly than untreated cells. Conversely, as shown earlier,[1] cells pretreated with parathyroid hormone become infected more slowly than untreated cells. It was also shown,[1] once the cells are infected both hormones cause a diminution only in the size of polykaryocytes. Very likely, the diminution of the polykaryocytes is a manifestation of an effect distinct from that which causes pretreated cells to become infected more rapidly or more slowly than untreated cells. It should be noted that the concentrations of hormones required to bring about these effects may be much greater than average *in vivo* levels. This could be due to the fact that HEp-2 cells are of neoplastic origin; it may be recalled that thyroid hormone is more effective on mitochondria obtained from normal cells than neoplastic cells.[2]

In order to understand how hormones alter the rate of infection of HEp-2 cells with HSV, we must consider briefly the mechanism by which cells become infected. The data presented in this and the preceding paper[1] show that cells become infected in two successive steps. First, virus becomes bound to the cell but may still be neutralized by antibody. Second, virus becomes inaccessible to antibody and may be said to have been "transported" inside the cell. The evidence in support of this conclusion emerges clearly from the comparison of the pattern of virus removal from the inoculum by untreated cells with the pattern of formation of infectious centers (manifest as polykaryocytes). The two patterns are not complementary and, moreover, the appearance of infectious centers seems to lag behind the disappearance of virus from the inoculum.

The effect of the two hormones on each of the two stages now becomes apparent. It is clear that pretreatment of cells with either hormone causes changes in the rates of both adsorption and penetration of virus. However, it was shown[1] that to a limited extent, parathyroid hormone may affect adsorption and penetration independently of each other. With respect to the thyroid extract, additional

analysis of the data is needed. Earlier it has been noted that there is a lag between the adsorption and penetration. The effect of the thyroid extract on the magnitude of this lag may be determined by comparing the ratios of virus adsorbed to virus inaccessible to antibody in pretreated and untreated cultures at different intervals after infection. Analysis of the ratios obtained in several experiments suggests that the average time an adsorbed virion is accessible to neutralizing antibody is about the same in both pretreated and untreated cultures. It seems reasonable therefore to conclude that the binding of virus to membranes is the rate-limiting step in the infection of untreated cells and that thyroid hormone affects the rate of binding rather than the rate of "transport" of the bound virus. Concerning the mechanism by which these effects of hormones are brought about, it may be postulated that the binding of virus to cell surfaces takes place at specific receptors. Hormones may cause an increase or decrease in the number of exposed receptors by altering the surface area of the cell or the structure of the cytoplasmic membrane. In general, the mechanism by which viruses penetrate into cells is uncertain. The mechanism by which hormones alter the rate of penetration of virus into cells and the rate of recruitment of cells into polykaryocytes are unknown.

It was pointed out in the introduction of the preceding paper[1] that various hormones alter the susceptibility of experimental animals as well as the course and outcome of natural and experimental viral infections. A clue to the nature of the modification in the host caused by hormones emerged from the current studies. It was demonstrated that pretreatment of a cell with thyroid or parathyroid hormone increases or decreases, respectively, the probability that a cell will become infected with HSV during a limited interval of exposure. The effects of the hormone would not become manifest if the exposure of the treated cells to HSV were unlimited in duration. The findings are applicable to situations *in vivo*, particularly in view of the fact that the number of cells of the host far exceeds the number of virions in the inoculum in natural as well as in experimental infections. The probability that during the limited exposure of cells to the virions contained in the inoculum a specific vital cell will become infected depends on the accessibility of the vital cell to the virus and the number of suitable receptors on its surface. The hormonal balance of the host may well determine both the accessibility of the vital cell (collateral effects on the organism) and the availability of suitable receptors (direct effects on the cell). It is likely that the effects demonstrated in this study will be observed with other cells, viruses, and hormones. Moreover, it may be predicted that some hormones will be found to act only on a specific cell-virus system whereas others may affect the ability of a wide variety of cells to become infected with many diverse viruses.

Finally, it seems pertinent to consider the possibility that the postulated role of hormones in rendering cells relatively resistant or susceptible to infection may prove to be the solution to four well-known phenomena for which no adequate explanation has yet been advanced. First, it is well known that cells of tissues which *in vivo* appear to be insusceptible to several viruses acquire the capacity to become infected when grown in monolayer culture or maintained as chunks of tissue *in vitro* in a medium that may lack adequate or balanced hormone concentration. Second, it has been observed that many viruses readily infect and frequently cause destruction of tumor cells *in vivo*. The "oncolytic" property of many viruses has in fact been tested for possible use in cancer therapy. In the light of the data presented

here, the so-called "tropism" of viruses to tumor cells may be explained by postulating that tumor cells, which may be less sensitive to hormones than normal cells, expose more appropriate viral receptors on their surface. Third, several tumor-inducing agents have not yet been adapted to multiply in cells maintained *in vitro*. In view of the apparent relationship between tumor induction by viruses and specific hormones as cited earlier, it seems quite plausible that these viruses may require either for infection or for maturation receptors present only in membranes of some specific differentiated cells. The specific stage in the differentiated cell exhibiting the appropriate receptors may be evoked *in vivo* by a hormone lacking *in vitro*. Lastly, it has been known for years that the host response to infection with many viruses becomes modified with age.[3] The young host is either more susceptible than the adult, or develops different symptoms. It could be that the change in the host response with aging is due to an alteration in the amount and pattern of hormonal secretions.

Summary.—Cells pretreated for 3 to 24 hr with thyroid extract adsorbed *herpes simplex* virus and affected its penetration more rapidly than untreated cells. The thyroid hormone affects the rate of adsorption and penetration; it does not alter the titer of the virus. The effect of the thyroid hormone is exactly opposite of the effect of parathyroid hormone described earlier.[1]

The significance of the findings with respect to the role of hormones in viral infections was discussed. It was pointed out that hormones may play a determinant role in maintaining a selective competence of cells to become infected with viruses. The hypothesis concerning the role of hormones in viral infections offers an approach to the investigation of several phenomena for which no adequate explanation has yet been given.

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[1] Roizman, B., these PROCEEDINGS, **48**, 795 (1962).

[2] Emmelot, P., and C. J. Bos, *Exptl. Cell. Res.*, **12**, 191 (1957).

[3] Andervont, H. B., *Texas Repts. Biol. & Med.*, **15**, 462 (1957).