amines by heparin and other substances has been reviewed(9). Dolowitz and Dougherty (10) reported the clinical use of heparin in treatment of patients with allergic inflammation and suggested that the prompt relief of symptoms in such cases was due to the *in vivo* binding and inactivation of histamine by heparin.

Studies reported above indicate that heparin is capable of suppressing gastric secretion stimulated by food or by exogenously administered histamine. The mechanism for this action may be the *in vivo* binding of histamine by heparin. If this is the case, the suppression of food-stimulated gastric secretion would support the concept(11) of histamine as the final common pathway in the stimulation of gastric secretion.

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## Effect of X-Rays on Cultured Monkey Kidney Cells Infected with a Myxovirus.* (28154)

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The effect of x-rays on the susceptibility of cells to several animal viruses has been studied by various investigators using different systems. The results have varied greatly but several reports indicate that irradiation can increase the cellular susceptibility to certain virus infections(1-6).

The present study is concerned with the effects of x-irradiation on rhesus monkey kidney cells infected with a recently isolated myxovirus, the DA virus(7). This agent regularly multiplies in monkey kidney cultures but produces no distinct cytopathic effect. Its presence can be demonstrated, however, by the agar overlay plaque technic(8) or by the hemadsorption method described by Vogel and Shelokov(9).

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*Materials and methods. Virus.* "DA", a myxovirus originally isolated from human blood(7), was used after 95 serial transfers in rhesus kidney cultures. This agent is serologically indistinguishable from the simian myxovirus $SV_5$ which is considered to be a parainfluenza virus(10).†

*Cell cultures.* Suspensions of trypsinized rhesus (Rh) monkey kidney cells were prepared according to standard methods. Hanks' balanced salt solution, containing 0.5% lactalbumin hydrolysate and 2% calf serum, was used for growth medium. *For infected cells,* 1 ml of infected tissue culture fluid containing approximately $2 \times 10^7$ PFU of DA virus was added to 9 ml of freshly trypsinized cell

† The designation of parainfluenza virus type 5 has been proposed for the DA-$SV_5$ group of viruses at the VIII International Congress for Microbiology, Montreal, 1962.

TABLE I. Effects of X-Rays on Cellular Changes.

| Cultures 7 days after seeding | Irradiation* (1000r) | No. normal cells per 1000 | No. of giant cells per 1000 | | | % of giant cells |
|---|---|---|---|---|---|---|
| | | | Mono-nucleated | Bi-nucleated | Multi-nucleated | |
| Normal rhesus cells | None | 993 | 7 | 0 | 0 | .7 |
| *Idem* | Irradiated | 948 | 49 | 3 | 0 | 5.2 |
| Rhesus cells infected with DA virus | None | 901 | 72 | 19 | 8 | 9.0 |
| *Idem* | Irradiated | 581 | 225 | 84 | 110 | 41.9 |

* Cells were irradiated 24 hours after seeding.

suspension containing $3 \times 10^5$ cells/ml. *For non-infected control cells,* 1 ml of growth medium was used instead of virus infected fluid. Both infected and non-infected cell suspensions were seeded into 3 oz prescription bottles (10 ml per bottle) or Leighton tubes containing 11 × 22 mm coverslips (2 ml per tube). All cultures were incubated at 37°C.

*X-irradiation.*‡ All cells were exposed to doses of 500r or 1000r at room temperature 24-48 hours or 7-8 days after seeding. A General Electric *Maxitron* deep therapy x-ray machine was used at 250 kv and 30 ma, with an aluminum filter of 1 mm thickness, and a distance of 40 cm from the target to give a rate of 500r per minute. The culture medium was changed immediately after irradiation. Fluids in non-irradiated cultures were also changed at the same time in order to assure uniform conditions.

*Staining procedure.* Coverslips with infected or non-infected cells, both irradiated and non-irradiated, were removed from the Leighton tubes at specified times, fixed in Zenker's (acetic) solution and stained with hematoxylin and eosin as described by Reissig *et al.*(11).

*Virus assay.* Virus infectivity titers were determined by the plaque counting technic (8) using rhesus monkey kidney bottle cultures. Virus hemagglutinin was detected by a method described previously(7) using guinea pig red blood cells.

*Results. Behavior of cells infected with DA virus.* Inapparent infection of rhesus monkey cell cultures with DA virus was easily accomplished in repeated experiments. Both infected and non-infected cells grew out equally well, and complete monolayers were obtained 7-8 days after seeding. Microscopically, it was difficult to differentiate infected cultures from the non-infected ones in wet preparations, but cell-sheets of the non-infected cultures were usually more compact than those of the infected cultures as observed in stained coverslips (Fig. 1, A & C). Virus multiplication was detected in the infected culture fluids both by the presence of a hemagglutinin and by the infectivity plaque titration. Such infected cultures could be kept in good condition and continue to yield high titers of virus for 10-12 days. At no time was virus activity demonstrated in cultures which had not been inoculated with DA virus. However, cell sheets generally fell off the glass in the infected cultures earlier than in the non-infected cultures.

*Cytology of infected, irradiated cultures.* Stained preparations of infected and non-infected, irradiated and non-irradiated cultures were examined microscopically after 7-8 days. Depending on the size of the cells, counts were made on 10 to 39 fields. The number of cells with normal dimensions was compared to the number of giant cells. These giant cells were classified as mononucleated, binucleated, and multinucleated.

A normal Rh culture showed an average of 0.7% giant cells none of which was found to be multinucleated (Fig. 1-A) (Table I). When such cultures had been exposed to 1000r x-rays, a 5.2% incidence of giant cells was obtained, but again no multinucleated cells were observed (Fig. 1-B). On the other hand, if the cultures were not irradiated but infected with DA virus, 9% abnormally large cells were obtained and 0.8% of the large cells

‡ Measurements made by Dr. E. H. Y. Chu, Dept. of Botany, Yale University showed that 30% and 12% of x-ray intensity was adsorbed or scattered by the glass of the 3 oz. bottles and the Leighton tubes, respectively.

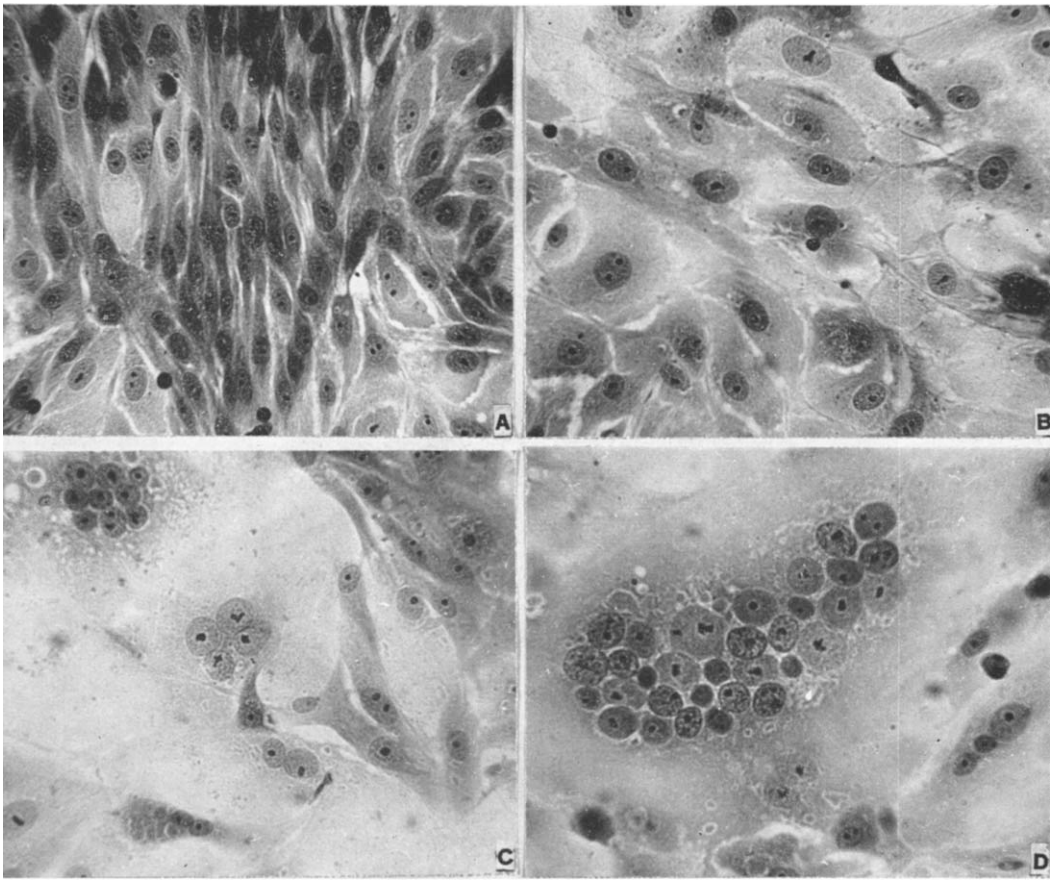


FIG. 1. Hematoxylin and eosin preparations (×2,000).

A. Normal Rh monkey kidney cell culture control—8 days after seeding. Note compact cell sheet.

B. Same cell suspension as in A, but culture was exposed to 1000r 48 hr after seeding. Complete but thin cell sheet with 5.2% mononucleated giant cells.

C. Same cell suspension as in A, with addition of DA virus at time of seeding. Complete but thin cell sheet with 9% binucleated and multinucleated giant cells.

D. Infected cell suspension as in C, but culture was exposed to 1000r 48 hr after seeding as in B. Thin cell sheet with 41% giant cells, of which 11% were multinucleated. Note unusually large multinucleated giant cell.

were multinucleated (Fig. 1-C). If such infected cultures were exposed to 1000r x-rays, the proportion of giant cells increased to 41.9% of which 11% were multinucleated cells (Fig. 1-D). Unusually large multinucleated giant cells containing 20-30 nuclei were observed only in infected cultures after exposure to irradiation. Cells exposed to a smaller amount of irradiation (500r) showed similar effects, but in the latter case the differences between irradiated and non-irradiated cultures were not so obvious. Monolayer cultures that had been completed prior to infection, generally 7-8 days after seeding, did not show such effects when exposed to x-rays as high as 10,000r.

*Discussion.* Complete monolayers were obtained equally well in cultures seeded with infected and non-infected cells; therefore, the rate of cell growth apparently was the same in both types of cultures. However, the metabolism of the cells in the infected cultures was somewhat altered by the presence of virus, for their life span was shortened and the cells generally fell off the glass earlier than did those in non-infected cultures.

Although no obvious CPE was observed at any time in DA infected cultures, x-rays ap-

parently enhanced the susceptibility of Rh cells to DA virus and made it possible to recognize the infection more readily by the presence of multinucleated giant cells in stained preparations. These findings suggest the possibility that DA virus might promote cell division in x-irradiated cells during the early phases of infection, such as is observed in human kidney cells infected with croup-associated virus(12). Since x-irradiation induces mononucleated giant cells in uninfected cultures, it is conceivable that the walls of such cells are more delicate and subject to lysis by DA virus. Both processes, that is, rapid cell division and fusion of infected cells, could occur simultaneously in the irradiated, infected cultures resulting eventually in a high percentage of multinucleated giant cells. Regardless of the mechanism involved in formation of syncytial cells in the infected irradiated cultures, x-rays apparently produce changes only when the cell system is not totally resistant to infection with a given virus. Thus, with DA virus, which multiplied well in Rh cells, cytopathology of infected cells could be enhanced by exposing cultures to x-rays; however, this exposure must take place during the *early* phases of growth.

*Summary.* Rhesus monkey kidney cells were found to be capable of forming a uniform monolayer even though they were highly infected with DA, a myxovirus. Irradiation did not result in readily observable CPE in virus infected cultures, although examination of stained preparations revealed definite changes as compared with control cultures. Increased numbers of multinucleated giant cells were observed in infected cultures exposed to x-rays, whereas considerably fewer and only mononucleated giant cells were obtained in irradiated cells without virus infection.

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## Mechanism of Anti-Hypertensive Action of Thiazides.* (28155)

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The basis for the anti-hypertensive action of chlorothiazide or its derivatives remains obscure. The preliminary explanations that blood pressure is depressed through loss of sodium or reduction of plasma volume have been, generally, discounted. These mechanisms seem even more untenable in view of the recent demonstration that a benzothiadiazine analogue, diazoxide (7-chloro-3-methyl-1, 2, 4-benzothiadiazine-1, 1-dioxide), has a potent anti-hypertensive effect in the absence of diuresis(1).

It seemed worthwhile, however, to examine the possibility that diazoxide might alter the aorta wall electrolytes of hypertensive rats. This investigation seemed pertinent in view

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