

BIOCHEMICAL AND MORPHOLOGICAL INVESTIGATIONS ON IRRADIATED WITH ULTRAVIOLET RAYS AND INFECTED WITH GRIPPE VIRUS CHICK EMBRYOS

P. Panayotov, G. Kaprelian, P. Todorov, L. Shickova and D. Stoyanov

The study of biochemical and morphological changes occurring under the combined effect (interaction) of vira and macroorganisms is an important problem in virology. Biochemical investigations have been reported in literature under various aspects. Thus *Voluiskaya* (2) investigates the sugar in the pulmonary tissue of infected with grippe virus (GV) mice and finds out 20—50 % increase of sugar as compared to control animals. *Tovarnitzki* (14) studies the biochemical alterations in experimentally produced grippe infection of white mice and comes to the conclusion that the pathological process in grippe conditions is generalized and involves a number of visceral organs and the central nervous system. *Knight* (17) investigates the aminoacid content of the allantoic fluid (AF) in chick embryos (CE), not contaminated and contaminated with grippe virus. *Killborne* and *Horsfall* (16) established an increased protein content in the AF of the CE, infected with GV. *Lutikova* (6) finds out an increase of the total nitrogen and phosphorus in the chorioallantoic membranes of CE, infected with GV. *Panayotov* (8, 9) studies in CE substrates, injected with different vira, the following indices: Ph, aminoacid content, presence of RNA and DNA, phosphatase activity, aldolase, pyrophosphatase etc. The effect of ultraviolet rays (UVR) on the GV has been investigated by numerous authors. Thus *Wells* and *Brown* (21) carry out follow-up studies on the effect of UVR upon aerosol of GV.

Salk and associates (cited by *Levin* — 20) investigates the action of the UVR on the virulence of the GV.

Vashkov (1), *Rosiisky* (11) and *Smorodintzev* (12) study the influence of UVR on the pulmonary suspension, containing GV. *Ermeev* and *Chalkina* (4) study the effect of UVR on purified GV, type A. *Zakastelskaia* (5) proves the infectious and toxic action of the allantoic fluid containing GV. *Manolova* (7) studies the effect of UVR on purified and non purified grippe vira B, A and A-1. *Rappoport* (10), *Dyhno* and assoc. (3), *Panayotov* and assoc. (19) and *Stoyanov* (13) follow the morphological changes in CE treated with microorganisms.

In the pertinent literature surveyed no informations were found concerning the effect of UVR on the CE infected with GV; hence the study of the biochemical indices and pathohistological alterations in CE irradiated with UVR and infected with GV is of utmost interest.

The purpose of the present work is determination of the biochemical characteristics, the presence of hemagglutination activity for the GV and the morphological alterations in the AF of the CE infected with GV.

Method

For the purpose of the investigation, GV A-2 was employed as well as 550 11-day-old developing CE. The latter were contaminated by means of 0,2 ml injection of allantoic fluid (containing GV), allantoically. The embryos underwent incubation for 48 hours at 37°C. In the experiments CE were utilized, divided into 11 groups. The first group was irradiated for 30 min. with UVR and thereafter infected with GV A2. The second group CE, previously irradiated for 30 min. with UVR, were inoculated with GV and after staying for 24 hours in thermostate, were repeatedly irradiated for 30 minutes. The third group CE, infected with the same virus, were treated for 30 min. with UVR, commencing on the 24th hour following the contamination. The fourth group CE is not treated with UVR, being merely inoculated with GV. The fifth group, 11-day-old growing CE were irradiated for 30 minutes, were not inoculated and were left intact for further development. The sixth group 11-day-old CE were irradiated for 30 min. on the 11th and 12th day of life (age). The seventh group 11-day-old developing CE were irradiated for 30 min. on the 12th day age. The eight, ninth and tenth groups of 11-day-old CE were treated in an identical manner as I, II and III groups with the only difference that they were treated with AF not containing GV. The eleventh group comprises 11-day-old developing CE not subjected to inoculation and untreated.

As a source of UVR the bactericidal lamp BUV-30 was utilized. The CE were placed in cardboard egg cases and irradiation was effected from a distance measuring 50 cm. The experiments were carried out in various seasons of the year. For the investigations allantoic fluids, chorioallantoic membranes and CE were utilized. The AF was studied for sugar after the method of *Hagedorn* and *Jensen*; residual nitrogen — after the method of *Rappoport* and *Eihorn*; chlorides — after *Schaless* as modified by *Natelson*; inorganic P — after *Greenberg*; total protein — after *Brandenberg* and *Stolnikov*; amylase — after *Volgemuth*; protein fractions — by means of paper electrophoresis. Hemagglutination activity was determined by the hemagglutination reaction (HAR).

The CE and their chorioallantoic and amniotic membranes were investigated macro- and microscopically.

Results and discussion

Through investigations according to the method of *Hagedorn* and *Jensen*, the following inferences are reached: the mean value of allantoic sugar in irradiated prior to inoculation HE is increased ($M = 81 \text{ mg}\%$) as compared with that of non-irradiated and non-infected HE ($M = 59,1 \text{ mg}\%$); allantoic sugar in CE irradiated prior to and after inoculation is lower as compared to the level in the first group ($M = 77,75 \text{ mg}\%$).

The results of investigations carried out according to *Rappoport* and *Eihorn* reveal that residual nitrogen in the AF of irradiated and infected CE is quantitatively smaller ($M = 28,97 \text{ mg}\%$) in comparison to not irradiated and not inoculated CE ($M = 47,27 \text{ mg}\%$).

The quantity of chlorides in the irradiated and inoculated CE compared to the group not subjected to irradiation and inoculation is almost equal ($M = 503,34$ of the former and $M = 498,87$ of the latter).

The total protein in the allantoic fluid of irradiated before inoculation and irradiated previous to and after inoculation HE is increased ($M = 98$ of the former and $M = 250$). Its quantity in the AF of not irradiated and contaminated CE is smaller ($M = 30,4$) than in those not irradiated and not contaminated ($M = 142$). The total protein has a lower value in CE, infected and irradiated before inoculation ($M = 98$) as compared to its content in non-irradiated and non-infected ($M = 142$).

Inorganic P in treated and untreated CE does not fluctuate substantially ($M = 0,9$ in the former and $M = 0,79$ in the latter).

Amylase activity is inhibited in the irradiated infected ($M = 2$) as compared to the non-irradiated and non-infected CE ($M = 3$).

Glutaminic-pyruvate transaminase in irradiated infected and in non-irradiated not contaminated CE does not differ greatly ($M = 11,4$ in the I group; $M = 10,9$ in the II; $M = 11,5$ in the IV and $M = 12,6$ in the XI group).

α and γ fractions were established in the allantoic fluids by means of paper electrophoresis. Quantitative differences in the protein fractions in AF of the various groups are not found.

The hemagglutination titer is highest in the irradiated and contaminated with GV hen embryos.

In the sheaths and organs of the CE investigated, grossly no malformations and morphological alterations were established. The weight of the CE was within normal limits. The microscopic investigations were carried out on preparations stained with hemalaun-eosin.

Desquamation is noted of the epithelium in the allantochorione of the I group. The majority of epithelial cells are with large protoplasm. At various points in the mesoderm infiltrations are found with pseudoeosinophile leukocytes and histiocytes; part of the infiltrated cells are with hyperchromic and pyknotic nuclei. Round-cell infiltration is observed in the submucosa of the esophagus.

The mesoderm of the II group exhibits hyperemia and edematosis, in some places with slight vacuolation of the ectoderm. The intercellular substance of the mesoderm reveals weak basophilia. In certain points of the mesoderm infiltrates are observed of pseudoeosinophilic leukocytes and histiocytes. In between the cells of the infiltrates chromatinic fragments are encountered.

The allantochorione of the III group exhibits weak edema of the mesoderm and slight proliferation of the ectodermal epithelium in certain regions. The epithelium of the yolk vesicle is vacuolated. Numerous nucleus cells are observed in the surroundings of its blood vessels. In the CE histiocytes 3—4 vacuoles and basophilic fragments are observed.

The allantochorione of the IV group is hyperemized, at places with hemorrhages. Its epithelium is proliferated at different areas. The mesoderm is edematous in various regions. Slight eosinophilia is detected in the intercellular substance of the mesoderm.

The allantochorione of the V group is hyperemized, with slight edema. Here and there vacuolated epithelial cells are observed, and in other lo-

calities — activation of connective tissue cells. The epithelial cells of the yolk vesicle of some CE are vacuolated, whereas in others-chromatin fragments are disclosed.

The mesoderm of group VI is strongly edematized. In separated areas it is infiltrated by protein fluid. In the ectodermal epithelium small band-shaped proliferative foci are present.

In group VII small proliferative foci are observed in the ectodermal epithelium.

In the control group of CE, untreated with virus, nor with UVR, no patophysiological changes are observed whatsoever.

The results of the biochemical, morphological and virological investigations prove that treatment of CE with UVR leads to biochemical and morphological alterations. The biochemical and morphological changes observed in various annual seasons did not show substantial variations.

REFERENCES

1. Вашков Ц. В. — *Труды ЦНИДИ*, 8, 7, 1954.
2. Волуйская Е. И. — *Вопросы медицинской вирусологии*. М., Медгиз, 1954.
3. Дыхно М. М., П. Г. Сорокина, Л. Л. Шимкевич — *Пробл. туб.*, 12, 51, 1963.
4. Еремеев В. Г., О. М. Чалкина — *Труды обед. научной сессии АМН СС СР* 43, 1952.
5. Закастелская Я. А. — *Токсичность вируса гриппа*, 1953.
6. Лутикова Т. О. — *Физиология и биохимия вирусов*, М., Медгиз, 1959.
7. Манолова Н. — *Известия на микробиологическия институт*, X, 161, 1959.
8. Панайотов П. — *Научни трудове ВМИ — Варна*, т. 1, св. 1, 1962.
9. Панайотов П., В. Желев, Н. Петрова — *Доклады Болг. акад. наук*, 11, 4, 313—316, 1958.
10. Рапопорт Л. Я. — *Архив патологии*, 11, 3, 1958.
11. Росийски М. Д. — *Грипп и острые катары верхних дих. путей*, 1951.
12. Смородицев А. А. — *Труды АМН СССР*, XXVIII, 49, 1953.
13. Стоянов Д. — *Пробл. туб.*, 12, 1964.
14. Тварницкий И. В. — *Физиология и биохимия вирусов*, М., Медгиз, 1959.
15. Вгеегер Е. — *Fortschr. Tuberk.*, 4, 236, 1951.
16. Kilbogne E., D., Horsfall — *Proc. Soc. exper. biol. and med.*, 71, 708, 1949.
17. Knight C. — *J. exper. Med.*, 86, 2, 1947.
18. Lange F., W. Ehrlich, O. Schn — *J. exp. Med.*, 52, 65.
19. Panajotov P. — *Comptes rendus de l'Academie bulgare des sciences*, t. 13, N 3, 1960.
20. Levin J. — *Exp. Med.*, 72, 6, 729, 1940.
21. Weels W. F., W. H. Brown — *Zbl. t. Bakt., Ref.*, Bd. 123, 481, 1936.

**БИОЛОГИЧЕСКИЕ И МОРФОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ
ОБЛУЧЕННЫХ УЛЬТРАФИОЛЕТОВЫМИ ЛУЧАМИ КУРИННЫХ ЭМБРИОНОВ
ЗАРАЖЕННЫХ ГРИППОЗНЫМ ВИРУСОМ**

П. Панайотов, Г. Капрелян, П. Тодоров, Л. Шикова и Д. Стоянов

РЕЗЮМЕ

Результаты исследований показывают, что алантоисный сахар в облученных до инокуляции гриппозным вирусом куринных эмбрионах по сравнению с таковым у необлученных и незараженных увеличен; остаточный азот в аллантоисной течности (АТ) в облученных и зараженных куринных эмбрионов (КЭ) в меньшем количестве чем в необлученных и незараженных; количество хлоридов в АТ облученных и необлученных КЭ почти одинаково; общий белок в АТ облученных и инокулированных КЭ увеличен; неорганический фосфор в АТ и у облученных и необлученных КЭ не показывает существенных колебаний; гемоаглютинационная активность АТ больше у облученных и зараженных.

У исследованных КЭ не установлено уродств. В алантохорионе облученных и зараженных наблюдалась местами десквамация эпителия, местами пролиферация эпителия. В мезенхиме на некоторых местах наблюдалась гиперемия, отек и геморрагии. Местами в мезодерме определялись инфильтраты состоящие из псевдозинофильных лейкоцитов, гистиоцитов. Между клетками инфильтратов встречаются хроматиновые фрагменты.