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THE PRESENCE OF SUGARS IN THE ALLANTOIC LIQUID OF HEN EMBRYOS INOCULATED WITH INFECTIVE MATERIAL FROM INFECTIOUS (VIRAL) HEPATITIS PATIENTS

(Preliminary Report)

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The literature survey provides no evidence about the presence of sugars in the allantoic liquid of hen embryos, both healthy and inoculated with material, obtained from patients with viral (infectious) hepatitis (VH).

In the present report the results are discussed of the chromatographic investigation of allantoic liquid for sugars, following inoculation of hen embryos with infective material from VH patients.

Material and method

3 ml blood is obtained from each VH patient, undergoing treatment at the Clinic of Infectious Diseases — Higher Medical Institute — Varna.

All patients were in the icteric phase of the disease, with jaundice dating back one day prior to prelevation of the material. The affections were of middle degree severity as per general clinical assessment.

The blood was subjected to centrifugation at a speed 2500 revolutions/min, for a duration of 10 minutes. Hen embryos at the 12th day of developmental stage were inoculated with 0.2 ml serum. They were of the Leghorn species. Contamination was effected in the allantoic cavity. Cultivation was performed in a thermostat maintaining the temperature at 37°C.

The allantoic liquid for investigation was taken on the 13th, 14th, 15th, 16th, 17th and 19th day at the rate of 5 samples per day, and on the 18th and 20th days — at the rate of 3 samples from different inoculated embryos.

An equal number samples of non-inoculated embryos served as controls, at the same stage of development as well as further 4 samples from the 12th day of embryonal development.

2 ml allantoic liquid was employed for chromatography, accordingly deprived of proteins with 8 ml 96° ethanol (alcohol). Next desalination was carried out according to the method of White and Hess, as modified by Kopecky (8, 6). Onedimensional descendent chromatography on paper FN No. 1 was carried out at 20°C. The paper bands measured 18 cm in width and 43 cm in length. The start was distant 9,5 cm from the edge of the band. The drops were with diameter ranging from 0,3 to 0,7 cm. A test of 1% solution of 11 sugars: raffinose (melitose), lactose, maltose, saccharose, galactose, glucose, fructose, arabinose, xylose, ribose and rhamnose was plotted on each band.

Table 1

Number of hen embryos	Non-inoculated hen embryos		Inoculated hen embryos	
	Number of samples	Number of sugars	Number of samples	Number of sugars
12	4	3	—	—
13	5	6	5	7
14	5	8	5	10
15	5	10	5	14
16	5	10	5	15
17	5	13	5	20
18	3	7	3	9
19	5	20	5	28
20	3	11	3	15

Table 2

Type of sugars	Number of allantoic liquids in which sugar is detected	
	Non-inoculated hen embryos	Inoculated hen embryos
Raffinose	0	0
Lactose	0	1
Maltose	0	2
Saccharose	0	6
Galactose	3	3
Glucose	36	36
Fructose	4	7
Arabinose	29	31
Xylose	7	15
Ribose	3	11
Rhamnose	6	10

The system N-butanol: ethanol: water = 40:11:19 was used as a solvent.

The mixture 0,5 gr benzidine, 20 ml icy acetic acid and 80 ml 96° ethanol was used as detector number one. It produced the yellow-brownish tinge of the hexoses and disaccharides, whereas pentoses — red-brown (1).

The mixture used as detector two contained 5 gr carbamide dissolved in 20 ml 2-normal acid salt and 80 ml 96° ethanol. The latter mixture stains the fructose and oligosaccharides, containing in their molecule fructose blue, whereas the rest of the sugars — pale yellow (5).

The chromatograms were dried at room temperature, and thereafter kept at 105° for further 10—15 minutes till full detection.

Thirty six samples were obtained from the inoculated and 40 from the control embryos. Two chromatograms were obtained of each sample, stained with developer number one and two. In all, 152 chromatograms were performed.

Results

1) It is obvious from table 1 that the number of sugars within the allantoic fluid of inoculated and non-inoculated embryos rises with the lapse of time after the inoculation day — for example, on the 15th, 16th, 17th day.

The table shows moreover, that in the allantoic fluid of inoculated embryos, in comparison with control embryos, a greater amount of sugars are established at the same post-inoculation day and equal number of samples.

2) Table 2 shows that in the allantoic liquid of controls the disaccharides are absent. They are met in the liquid of inoculated embryos, with a growing incidence of lactose, maltose and saccharose in particular.

3) The hexoses — glucose and galactose — are encountered with equal incidence both in experimental and control liquids, whereas fructose is more frequent in the liquid of inoculated embryos.

4) The pentoses — xylose, ribose and rhamnose — are discovered two-three-fold more frequently in the liquid of inoculated embryos as compared to controls.

5) Arabinose is found very often and in equal quantity both in controls and experimental embryos.

6) Raffinose is not detected in any of the samples, neither experimental, nor control liquids.

Inferences

Through chromatographic analysis of the sugars found in the allantoic liquid of hen embryos, inoculated on the 12th day of their development with 0,2 ml serum, obtained from patients with viral hepatitis in the icteric phase of the disease, it has been established that the amount of sugars in the liquid of the inoculated embryos is higher and their appearance more variable as compared to those found in the controls. Within the liquid of experimental embryos, disaccharides — maltose, lactose and saccharose — are detected which are absent in the liquid of controls. Fructose is found rather often in the liquids of inoculated embryos. The pentoses: xylose, ribose and rhamnose are encountered two-three-fold more frequently: in the liquid of inoculated embryos. No relevant evidence was found for raffinose.

НАЛИЧИЕ САХАРОВ В АЛЛАНТОИСКОЙ ЖИДКОСТИ КУРИНЫХ ЭМБРИОНОВ, ИННОКУЛИРОВАННЫХ МАТЕРИАЛОМ ОТ БОЛЬНЫХ ВИРУСНЫМ ГЕПАТИТОМ

(Предварительное сообщение)

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РЕЗЮМЕ

При помощи хроматографического анализа сахаров в аллантоисной жидкости куриных эмбрионов, иннокулированных на 12-ый день их развития 0,2 мл сыворотки крови от больных вирусным гепатитом в желтушной фазе болезни устанавливается, что число сахаров в жидкости иннокулированных эмбрионов больше, а их вид более разнообразный. В жидкости подопытных эмбрионов обнаружены дисахариды: лактоза, мальтоза и сахароза, которые не устанавливаются в жидкости контрольных эмбрионов. Довольно часто в жидкости иннокулированных эмбрионов имеется фруктоза. Пентозы ксилоза, рибоза и рамноза встречаются в 2—3 раза чаще в жидкости иннокулированных эмбрионов. Рафиноза не была доказана.