A Chromosome Study in Leucocyte Cultures from Serum Hepatitis Patients[1),2),3)]

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(With 7 Text-figures and 2 Tables)

Pioneer work of Nichols *et al.* (1962) finding a high percentage of chromosome breakages in leucocyte cultures from the patients with measles, has attracted special attention of current workers and stimulated investigators to establish the cytogenetic relationship between chromosome aberrations and diseases of viral origin (Aula 1963, 1965, Nichols 1963, Tanzer *et al.* 1963, Hardnen 1964, Makino *et al.* 1965). Evidence has hitherto been presented that chromosome breaks induced by infections of viruses, such as measles, chickenpox and aseptic meningitis, occurred with random distribution on the chromosomes (Aula 1963, Nichols 1963, Makino *et al.* 1965). Since a chromosome study in this field has as yet been at the young stage of development, it is essential to collect data on a large scale from as many subjects with viral diseases as possible, in order to provide criteria for understanding the relationship of chromosomal changes to virus-associated diseases.

Serum hepatitis is generally known as a virus-induced disease and transmitted exclusively by blood transfusion. So far as the author is aware, no cytogenetic investigation has been made in the patient with this disease. The present study concerns with chromosome aberrations in 17 cases with serum hepatitis with special concern to the incidence as well as to the type of chromosome changes.

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Materials and methods: Seventeen serum hepatitis patients supplied materials for study. All patients had the history of blood transfusion at their surgical operations in the past. Diagnoses given to the patients at the time of blood transfusion were gastric ulcer, gastric, bone and uterine tumors and pulmonary tuberculosis. Early clinical symptoms of the patients with this disease were obscure. The blood samples were obtained on the 7th to the 635th day of disease, since the latent period of this disease differed from case to case. In patients no. 2,4,5,6 and 8, chromosome study was made 2 to 3 times with blood samples taken at ten-day-interval.

Chromosome analyses were exclusively made on metaphase chromosomes in leucocyte cultures. Blood cultures were done according to Hungerford *et al.* (1959) with a slight modification. Culture medium used was 5 gram of lactalbumin hydrolyzate, 1 gram of yeast extract, 200 mg. of glutamine and a small amount of penicillin dissolved in 1000 ml. of the slightly modified Earle's saline solution. Slides for chromosome study were made following the modified air-drying technique of Rothfels and Siminovitch (1958).

Six to 200 well-spread metaphases in each case were scanned for chromosome aberrations. Cultured leucocytes derived from six healthy males provided control materials. Chromosomes were observed in special reference to the frequency as well as to the type of chromosome aberrations.

In analysis of chromosome breaks, chromosomes were divided into seven groups according to Denver nomenclature system, and the incidence of chromosome breaks in each chromosome group was obtained. Chromosome or chromatid type break was regarded as one break. The relative length of chromosomes in each group was based on the data given by Makino and Sasaki (1961) with slight adjustments. Expected incidence of chromosome breaks in each chromosome group was obtained following the system proposed by Aula (1963).

Results

With one exceptional case, in the majority of cells all the patients under study had 46 chromosomes along with a normal complement. The exceptional case showed no abnormality in the phenotype having 46 chromosomes, but detailed analysis revealed the occurrence of a remarkable balanced B/C reciprocal translocation in all cells studied. A preliminary note on this abnormality was given in another paper by Makino *et al.* (1965).

The incidence of chromosome aberrations in 17 serum hepatitis patients together with that of the controls is presented in Table 1.

The average frequency of chromosome breaks in the control specimens was 5.3 per cent. The mean value in the 17 cases with serum hepatitis was obtained as 13.4 per cent. Among them 13 cases showed a higher frequency of chromosome breaks than that of the controls. The highest incidence of breaks was observed in patient no. 1 showing 32 per cent.

In Figure 1 is shown the relationship between incidence of chromosome aberrations and days of disease. The onset day of the disease was estimated by clinical examinations. At the early stage of disease, the frequency of chromosome aberrations was around 15 per cent. Higher frequencies of chromosome aberrations

Table 1. The incidence of chromosome aberrations in 17 patients with serum hepatitis, and in controls

Case no.	Sex	Age	Sampling time after onset of disease (day)	Chromosome aberrations				Total no. of cells observed
				No. of cells with				
				Breakages	Fragments	Inter-changes	Dicentrics	
1	M	36	60	30 (32%)				94
2	M	31	90	14 (28%)	2			50
			101	25 (25%)	1			100
			111	0 (0%)				50
3	M	57	270	41 (21%)	5			200
4	F	26	16	10 (20%)	1	1		50
			19	5 (10%)				50
5	M	55	120	2 (18%)				11
			130	2 (-%)*	1			6
6	F	25	365	9 (18%)	1			50
			381	2 (2.5%)				80
7	F	43	575	8 (16%)	1		1	50
8	M	47	100	15 (15%)	4			100
			115	25 (14%)	1			180
9	M	31	9	14 (14%)		3		100
10	M	42	635	10 (10%)	1			100
11	F	50	70	1 (9.1%)				11
12	M	26	7	3 (6%)	1	1		50
13	F	57	10	3 (6%)	2			50
14	F	36	180	8 (4%)	3			200
15	M	29	80	4 (4%)	1			100
16	M	58	70	7 (3.5%)				200
17	M	40	100	3 (3%)	1			100
Control				30 (5.3%)				566

Numerals in the parentheses indicate per cent of cells with breaks.
* The number of cells scored is unavailable for statistical analysis.

occurred mostly on the 60th to 120th day of disease. Chromosome studies were examined repeatedly in patients no. 2,4,5,6 and 8 at about a 10-day-interval. The blood samples from patient no. 2 were taken on the 90th, 101st and 111th day of disease. The frequencies of chromosome breaks were 28 per cent in the first sample, 25 per cent in the second, and within the control level in the last sample. Repeated examinations in patient no. 5, provided no statistically sufficient data. The same tendency was obtained in other cases with the passage of time.

Expected frequency values of chromosome breaks were calculated on the basis of the relative length of chromosomes and compared with the number of chromosome

breaks observed. Data as given in Table 2 indicate that chromosome breaks seem to occur at random on the whole chromosomes.

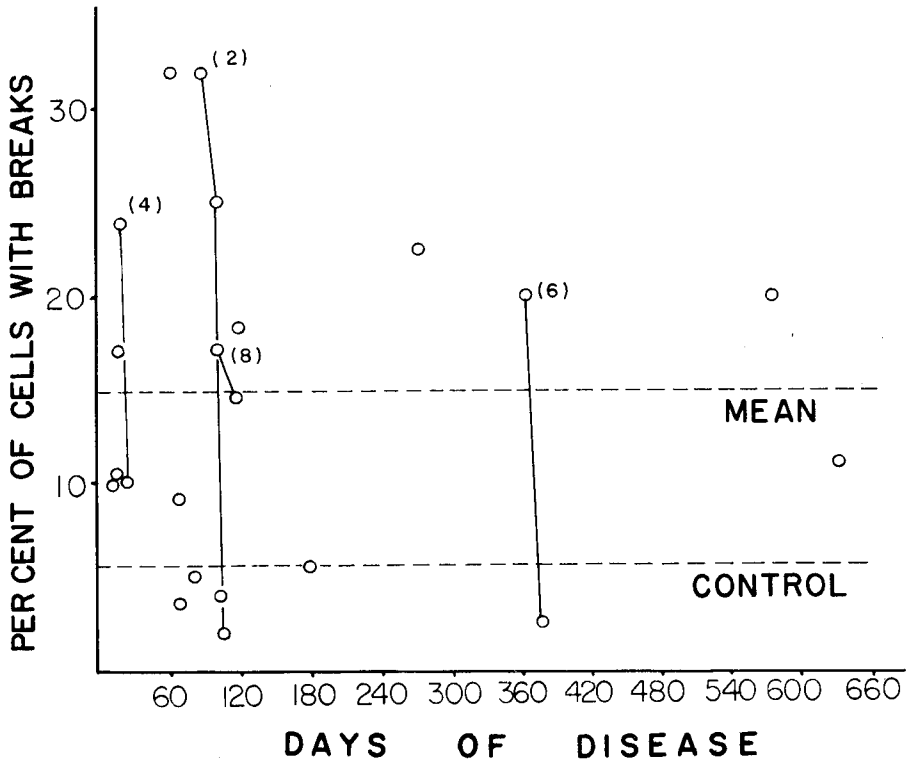
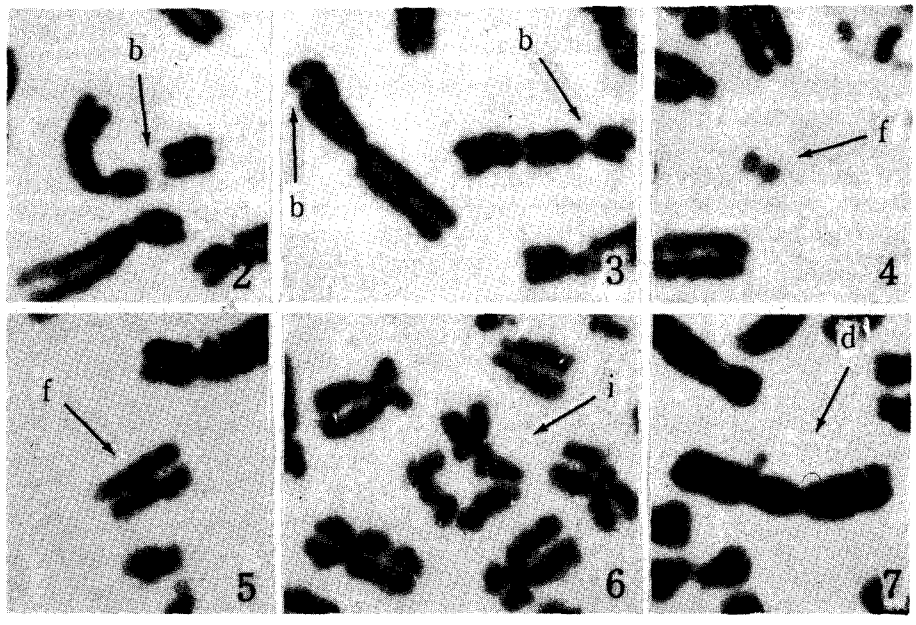


Fig. 1. Frequencies of cells with chromosome aberrations in 17 patients with serum hepatitis after the onset of the disease. The numerals in the parentheses indicate the case numbers of the patients.

The chromosome aberrations detected in blood specimens from serum hepatitis patients were mostly breaks (Figs. 2–7). The type of breaks was exclusively single chromatid breaks. The breaks of chromosome (iso-chromatid) type and acentric fragments were rare in occurrence (Figs. 2,4–5). Some cells showed chromatid interchanges and a dicentric chromosome as a severe type of chromosome damages (Figs. 6–7). In striking contrast, simple chromatid breaks and/or gaps were scored in the control materials.



Figs. 2–7. Various chromosome aberrations in cultured leucocytes from 17 serum hepatitis patients. Figs. 2 and 3, chromosome and chromatid type breaks. Figs. 4 and 5, acentric fragments of chromosomes. Fig. 6, a chromatid interchange. Fig. 7, a dicentric chromosome. b: break, f: acentric fragment, i: chromatid interchange, d: dicentric chromosome.

Table 2. Incidences of chromosome breaks in seven chromosome groups and expected values of breaks calculated based on relative length of chromosomes

Chromosome group	Relative length*	Number of chromosome breaks	
		Observed	Expected on chromosome length
A 1- 3	229	77	63.5
B 4- 5	119	36	33.0
C-X, 6-12	371	107	102.8
D 13-15	109	41	30.2
E 16-18	87	11	24.1
F 19-20	46	7	12.8
G-Y, 21-22	60	4	16.6
Total	1021	283	283.0

*These were adjusted from the data reported by Makino and Sasaki (1961).

Discussion

It has been shown in the present study that the leucocytes from the patients with serum hepatitis are characterized to some extent by a significant increase in number of chromosome breakages. The highest incidence of chromosome breaks obtained was 32 per cent, the value is higher than that observed in the leucocytes of the patients with chickenpox (Aula 1963, 1965, Harnden 1964), mumps (Harnden 1964, Aula 1965), herpes zoster (Harnden 1964) and German measles (Harnden 1964), while lower than that in cases with aseptic meningitis (Makino *et al.* 1965) and measles (Nichols *et al.* 1962, Nichols 1963, Aula 1965).

Cultured leucocytes from aseptic meningitis patients showed a high incidence of chromosome aberrations including chromosome breakages, translocations, dicentrics and acentric fragments of chromosomes (Makino *et al.* 1965). El-Alfi *et al.* (1965) observed significant chromosome breaks in leucocyte cultures from healthy adults received the plasma inoculation from patients with infectious hepatitis. In the present serum hepatitis patients, the single chromatid breaks were common in occurrence. Further, the breaks showed at random distribution on chromosomes. High incidence of breaks was observed around on the 60th and 120th day of disease. The frequency of chromosome aberrations showed a decrease with the passage of time. The occurrence of the cases showing high and low incidences of breaks remains unknown at the present status of knowledge. Further cytogenetic studies are requested for understanding many etiological problems adherent to viral diseases.

Summary

Seventeen patients with serum hepatitis were subjected to chromosome analyses in cultured leucocytes. Information was obtained that leucocytes from serum hepatitis patients showed random chromosome aberrations significantly higher in frequency than the control series. The most common type of the aberrations observed was a chromatid type break. Iso-chromatid breaks, chromatid interchanges and acentric fragments were less frequent in occurrence.

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