does not exhibit any absorption bands around 2050 and 750 cm.$^{-1}$. This is a strong argument in favour of the ionic structure for the compound.

An alternate procedure for the preparation of the supposed MTCA was attempted starting from a mixture of morpholine and ammonium thiocyanate containing morpholine in slight excess than corresponding to 1 : 1 molar ratio. Ammonia, being more volatile than morpholine, was displaced by morpholine from ammonium thiocyanate by heating the mixture on the water-bath. The crystals obtained on cooling the concentrated solution were washed with acetone and recrystallised from anhydrous ethanol. The melting point, chemical behaviour and infrared spectrum of this compound were identical with those got for the compound which was prepared by adopting Henry and Dehn's procedure. Thus we conclude that both methods of preparation yield only morpholinium thiocyanate and not MTCA.

Keeping the morpholinium thiocyanate at the melting point for long periods and cooling it also did not bring about any conversion to thiocarbamide. Such conversions sometimes do occur as in the well-known case of ammonium thiocyanate to thiourea. It is strange that morpholine alone, among many of the secondary amines should behave in this manner to give morpholinium thiocyanate and not thiocarbamide.

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CHROMOSOMAL ANALYSIS OF YOSHIDA ASCITES SARCOMA IN RATS

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INTRODUCTION

CHROMOSOMAL patterns of cancer cells have been studied extensively to elucidate correlation if any, between abnormalities of structure and number of chromosomes with the biological characteristics of the tumour.[1]

While the chromosome constitution of some tumours remain fairly stable during serial animal passages and different transfer generations, very often the derived sublines from the parent strain show considerable variation. This type of variations become more frequent with environmental changes like the strain of the animals used for maintenance of the tumours, the source material transplanted, whether ascites cells or cells obtained from solid forms of the same type of tumour, etc.

The present investigation was undertaken to study the chromosomal pattern of the Yoshida ascites sarcoma maintained in our laboratory and their stability or variability in different transfer generations during animal passage over the last few years.

MATERIALS AND METHODS

Yoshida ascites sarcoma (YAS) obtained from the Indian Cancer Research Centre, Bombay, who in turn had received the tumour from Professor Druckeray of Germany is being maintained in our laboratory since four years in inbred strain of wistar rats A/I.I.Sc. Intraperitoneal transplantation of 25 to 30 million cells every 5th day is the maintenance routine. The ascites developed with this dose becomes hæmorrhagic by 5th day and the average survival period of the animal is seven days.

At one stage, after about 80 transplant generations, the ascites form had to be regenerated from the solid tumour by homogenising the tumour mass and injecting the suspension intraperitoneally. Since then the ascites form is transplanted serially for over 208 generations.

Specimens for chromosomal analysis were prepared as follows: Colchicine in physiological saline (0·035 mg./kg.) was injected intraperitoneally into ascites tumour of five

days growth; ascites tumour was aspirated 3½ hr. after the injection, mixed with fifteen times its volume of 0·04 M sodium chloride solution, shaken well and incubated at 37° C. for 10 minutes.[2] The material was centrifuged, supernatant discarded and the sedimented tumour cells were fixed with Carnoy's fixative (ethanol, acetic acid, 3 : 1). The cells were then processed for spreading on glass slides as per the method of Moorhead *et al.*,[3] stained with Giemsa and mounted in canada balsam. A large number of colchicine arrested metaphase plates were observed.

One hundred well spread metaphase plates were examined for each specimen. All metaphase chromosomes were examined by microphotography and camera lucida drawings.

The chromosome numbers of 44th, 66th and 111th generations of tumour cells were determined and a morphological study and karyotyping was carried out.

Results and Discussion

The chromosome number of Yoshida ascites sarcoma at different transfer generations is shown in Table I and Fig. 1. Karyotype at 111th generation is shown in Fig. 2. Chromosomes are classified morphologically according to the location of the centromere into metacentrics, sub-metacentrics and telocentrics. A change in the modal number has been observed at different transfer generations such as 37 at 44th, 35 at 66th and 40 at 111th generations.

TABLE I

Distribution of chromosomes in Yoshida ascites sarcoma at different transfer generations

Chromosome Numbers	Generation number		
	44	66	111
4	..	..	2
5	..	..	2
6	..	..	2
7	..	..	8
8	..	..	2
9	..	..	6
10	..	..	6
11	..	..	4
13	..	..	6
15	..	2	..
16	..	..	4
17	..	..	2
19	..	..	2
20	..	2	2
21	..	2	..
22	3	..	..
23	2	2	2
24	2	..	2
25	3	2	4
26	2	8	..
27	5	4	..
28	4	2	..
29	9	4	..
30	5	8	..
31	9	6	4
32	5	4	..
33	6	10	2
34	6	8	..
35	6	14	..
36	6	4	2
37	10	12	4
38	4	4	..
39	4	..	10
40	3	2	16
41	2	..	2
42	1	..	4
49	1	..	..
72	1	..	..
76	1	..	..

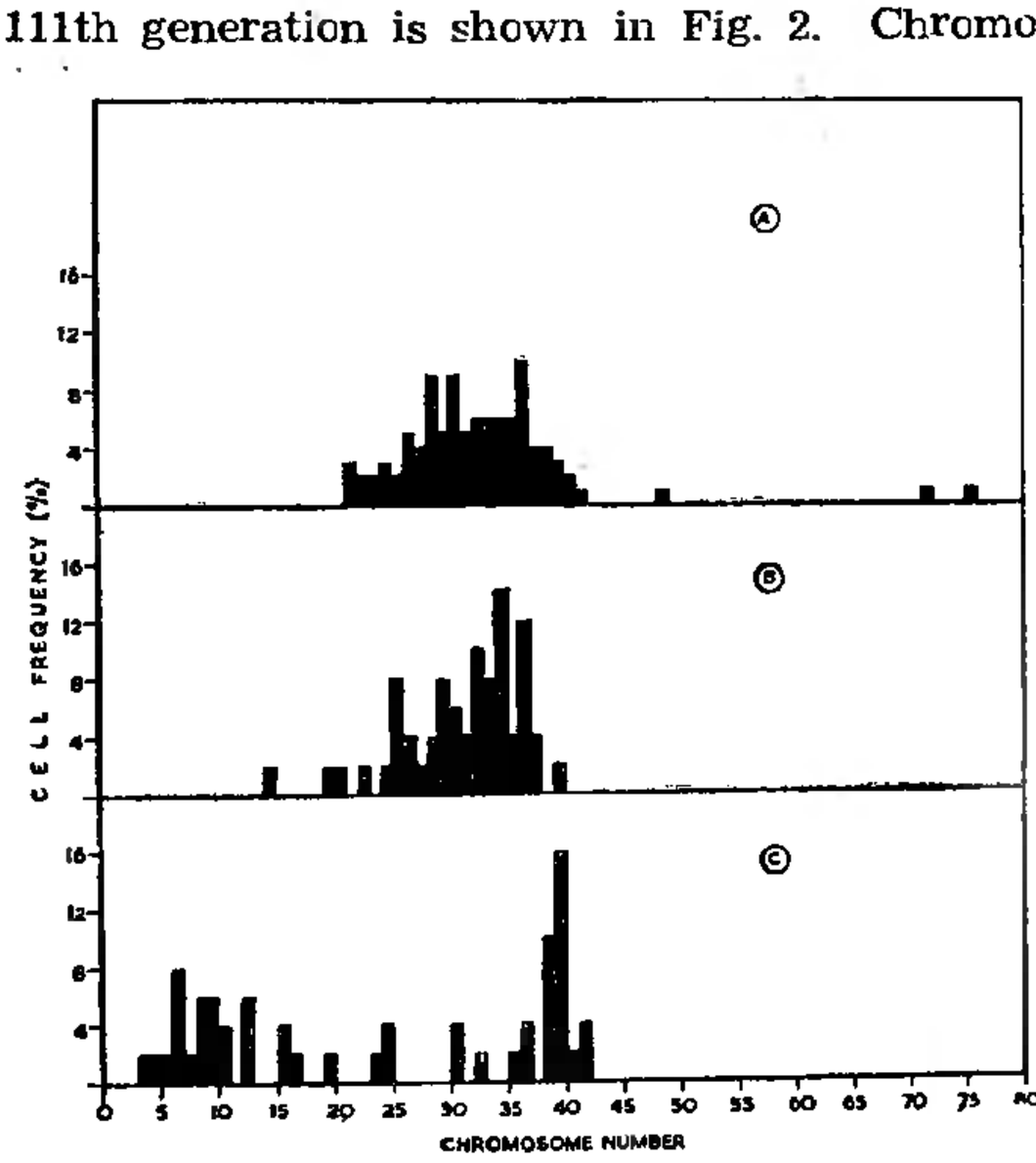

FIG. 1. Histogram showing the distribution of chromosomes in Yoshida ascites sarcoma. A, B and C represent the chromosome numbers of 44th, 66th and 111th generations respectively.

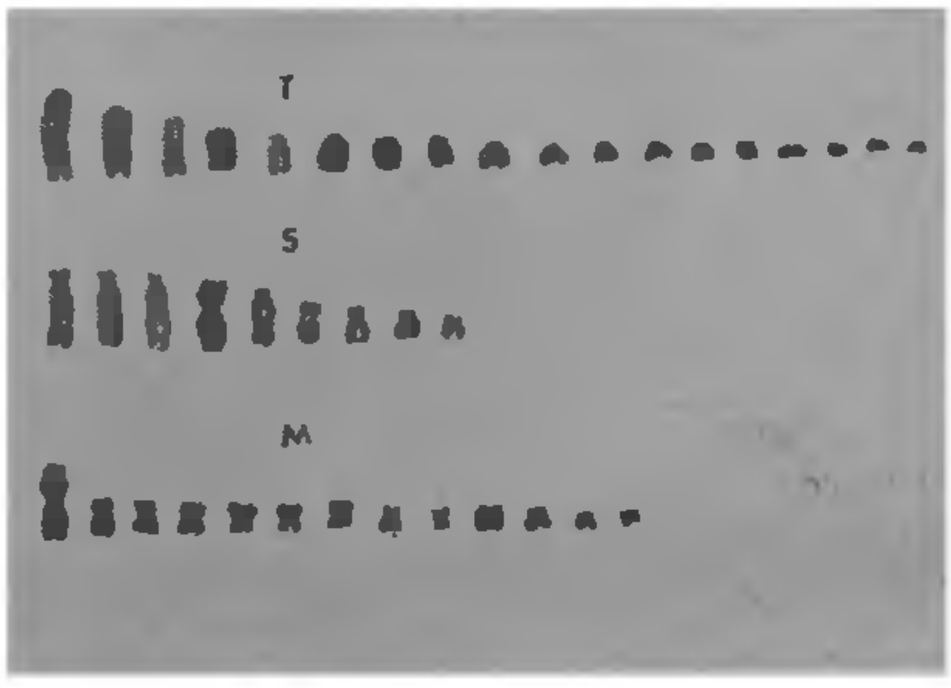

FIG. 2. Karyotype of the cells in the 111th generation. T, Telocentrics; S Sub metacentrics; M, Metacentrics.

In the earlier studies, the Japanese workers have described Yoshida sarcoma as a near diploid tumour with forty elements as the modal chromosome number.[4] They have also described the establishment of a polyploid clonal subline with 30 chromosomes as the modal number which has maintained this character for over 200 transfer generations.[2]

Varied events have occurred during the transplantation of this tumour since the time of its discovery. Proliferation of a single clone by the transplantation of single cell, heterotransplantation in mice, preservation of cells in the cold, regeneration from a nodule day after the death of the animal, exposure to 60° C. for half-an-hour and chemicals, have all been reported during its maintenance. Sublines of Yoshida sarcoma with large number of chromosomes with marked slow down in growth accompanied by remarkable changes in the ascites characteristics of the tumour have been obtained.[5]

The present study indicates that no chromosome number is absolutely predominant in any one of the transfer generations. From karyological studies it is observed that even though the modal number in the 111th generation is 40, as in the original Yoshida ascites sarcoma, it is different in its chromosomal make-up by possessing more of the telocentrics and metacentrics whereas submetacentrics are less in number.

Variations in the modal number has occurred at the different transfer generations. The tumour appears to be undergoing a continuous change in its chromosomal constitution, though transplantation is being done in isogeneic strains of rats and has not yet attained stabilization.

In spite of these chromosomal variations there has not been any noticeable difference in the character of the tumour development and its progression,[6] thus confirming the general observation that visible chromosomal changes may not always reflect change in biological activity.

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BIOCHEMICAL CHANGES IN RELATION TO THE BREEDING CYCLES OF *NAUSITORA HEDLEYI* SCHEPMAN (BIVALVIA : TEREDINIDAE)

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THE present study was initiated to elucidate the general biochemical make up of *Nausitora hedleyi*—a typical estuarine boring bivalve and more specifically to determine whether systematic variations of any of the constituents might be correlated with the annual reproductive cycle. The estimations have been made on entire animals only, collected from the Cochin Backwaters for a period of twelve months. All biochemical estimations were done on material dried to constant weight. Water content, ash content, glycogen, protein, total nitrogen and non-protein nitrogen, lipids, calcium, phosphorus and chloride content were estimated. Glycogen estimation was done according to the method outlined by Mendel and Hoogland and adopted by Raymont and Krishnaswamy[1] and expressed as percentage of glucose in dry

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