Lymphocyte Cultures from Chinese Hamster (*Cricetulus griseus*)

Owing to their relatively low number ($n = 11$) and their well analysable pattern, the chromosomes of the Chinese hamster are often used in cytogenetic research[1,2]. Mostly direct preparations from spleen or bone marrow or cells from established lines are used for this purpose. Because in certain cases, e.g. the analysis of mutagenic activity of substances in vivo, it is necessary to check a great number of mitoses derived from exposed body-cells, we have tried to work out a method for the culture of peripheral lymphocytes. On a second approach it appeared that the basic experience we gained by these experiments could be used for making mixed lymphocyte cultures. This will be the subject of another publication.

Materials and methods. Cell culture: 0.2 ml blood was taken by puncture from the retrobulbar venous plexus and collected in a heparinized culture tube. This blood was suspended in culture medium supplemented with streptomycine (100 µg/ml) and penicillin (100 IU/ml). The following media were tested: 199, F_{12}, F_{10}, Minimum Essential Medium (Eagle) for Suspension Cultures (MEM S), Trowell T8 and R.P.M.I. 1640. The medium was supplemented with fetal calfserum in concentrations varying between 5 and 20% (the fetal calfserum was inactivated by heating at 56 °C for 30 min.). Finally phytohaemagglutinin (Wellcome MR 10) was added. The concentrations tested were: 3, 6, 9, 12 and 15 µl per ml cell suspension. The final volume of the cultures was 6 ml, they were incubated at 37 °C for 72 h in tightly capped 10 ml glass bottles. 2 h before harvesting, vincristine sulfate (Oncovin, Eli Lilly & Co) was added.

The concentrations 0.04, 0.4, 4 and 40 γ/ml culture were tested. At the end of this treatment the cultures were centrifuged for 5 min at 1000 rpm (170 g). The pellet was resuspended in 5 ml hypotonic sodium citrate (2H2O) solution. Here again, different concentrations were tested: 0.5, 0.6, 0.7, 0.9 and 1 g/100 ml. This suspension was centrifuged at once for 5 min at 1000 rpm and the pellet resuspended in a fresh citrate solution and incubated at 39 °C for different periods of time. Several combinations of citrate concentration and time of incubation were tested. Thereafter the pellet obtained by centrifugation for 5 min at 1000 rpm was fixed by slowly adding 6 ml of fixative (3 parts absolute alcohol to 1 part glacial acetic acid) and gently shaking. The suspension was centrifuged immediately for 5 min at 1000 rpm and the pellet was resuspended in fresh fixative and left at room-temperature for 20 min. Finally after a last centrifugation, the pellet was pipetted onto clean object-slides, air dried and coloured with May-Grünwald solution, using a modification of the method of MOORHEAD[3]. The frequency of metaphases in the material was checked by counting 100 blastcells in each of 3 replicate cultures, which received 0.4 γ vincristine/ml culture for the last 2 h.

Cell labeling: 6 µC of H^3 thymidine (The Radiochemical Centre, Amersham, England, s.a. 2 C/mM) were added to each culture for the final 24 h. At termination the cells were spun down at 200 g for 10 min, washed twice with cold ammoniumchloride 0.83% w/v, twice with cold 5% TCA, and once with cold absolute methanol. The precipitate was dissolved in 0.5 ml N.C.S. at 56 °C for 1 h, and transferred to counting vials, 10 ml liquid scintillation solution, consisting of toluene with PPO 5 g/l and POPOP 50 mg/l was added and the vials were counted for 10 min in a scintillation counter. The results reported, representing the mean values from 3 replicate cultures, are corrected for quenching and are given in dpm.

Results: The different combinations of media and complements were tested in about 1000 different cultures. The highest H^3 thymidine uptake was found with the media R.P.M.I. 1640, Trowell T8 and MEM S using a fetal calfserum concentration of 10%. An example of one such experiment is shown in Table I. The best concentration for phytohaemagglutinin was 6 µl/ml culture medium. The results of 2 experiments are given in Table II. The most satisfactory blocking of metaphases was arrived at by a treatment of 2 to 3 h with vincristine at concentrations between 0.4 γ/ml and 4 γ/ml culture. Of the all citrate concentrations used, 0.7 g/100 ml during 7 min gave the best spread metaphases. R.P.M.I. 1640 supplemented with 10% fetal calfserum and 6 µl/ml phytohaemagglutinin gave the richest harvest of well spread mitoses. Table III shows that the frequency of metaphases counted in our preparations parallels the results of the H^3 thymidine uptake experiments shown in Table I. On the whole R.P.M.I. 1640 gave mostly better or at least as good results as the 2 other media MEM S and Trowell

Table I. dpm × 10^3 in one series of lymphocyte cultures labelled with H^3 thymidine using different media and fetal calfserum concentrations

Medium	Serum concentration 5%	10%	15%
R.P.M.I. 1640	633 ± 50	866 ± 64	422 ± 35
Trowell T8	530 ± 41	817 ± 31	497 ± 39
MEM S	498 ± 4	771 ± 168	668 ± 51
F 10	246 ± 49	454 ± 9	342 ± 92
F 12	309 ± 16	427 ± 158	385 ± 45
199	106 ± 45	333 ± 35	241 ± 94

Table II. Effect of different phytohaemagglutinin concentrations in µl/ml culture on H^3 thymidine uptake (dpm × 10^3). The results of 2 independent experimental culture sets I and II with R.P.M.I. 1640 and 10% fetal calfserum are given

	3 µl/ml	6 µl/ml	9 µl/ml	12 µl/ml	15 µl/ml
I	574 ± 31	1,053 ± 218	446 ± 140	245 ± 27	187 ± 6
II	664 ± 51	788 ± 114	640 ± 110	167 ± 28	114 ± 4

Table III. Number of metaphases in counts of each 300 blastcells in 2 independent cultures I and II fed with different media, 10% fetal calfserum and 6 µl phytohaemagglutinin per ml cell suspension; vincristine, 0.4 γ/ml culture, added for the last 2 h

	R.P.M.I. 1640	MEM S	Trowell T8	F 10	F 12	199
I	27	12	8	5	3	5
II	21	17	5	4	2	3

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T8. The other media gave only exceptionally comparable results.

Zusammenfassung. Lymphozyten des chinesischen Hamsters (*Cricetulus griseus*) wurden in verschiedenen Media mit verschiedenen Zusätzen gezüchtet. Die beste Mitosenausbeute wurde erhalten mit dem Medium R.P.M.I. 1640, bei einem Zusatz von 10% fötalem Kalbsserum und 6 µl/ml Phytohämagglutinin.

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Free Amino Acids in the Haemolymph of *Eoperipatus weldoni* (Onychophora)

Arthropods belonging to the class Onychophora are of special zoological interest in that they are considered to be the most primitive terrestrial arthropods closely related to the myriapodan-insectan stem[1]. A characteristic feature of Insecta and Myriapoda is the presence, in their haemolymph, of free amino acids in very high concentrations, ranging from 219 mg to 2340 mg per 100 ml[2,3]. It will therefore be of interest to find out if the haemolymph of Onychophora also has a similar feature.

The species investigated was *Eoperipatus weldoni*, collected from Templer's Park, Malaysia, as given by SUNDARA RAJULU and SINGH[4]. They were collected, brought to the laboratory and maintained as described elsewhere[5].

The haemolymph was obtained from living animals by the method described by SUNDARA RAJULU[6]. The sample of haemolymph obtained from each animal was kept separately and centrifuged at 5°C for 15 min at 2000 *g*/min to separate haemocytes. The clear supernatant was collected and deproteinized following the method of STEIN and MOORE[7]. The Folin method, as given in the publication of HAWK et al.[8], was adopted for quantitative estimation of total free amino content. For quantitative estimation of individual free amino acids, samples of 1 to 2 ml of the deproteinized haemolymph were analyzed in an automatic amino acid analyzer[9]. Quantitation was based on the ninhydrin colouring intensity of the effluent from ion-exchange columns. The absorbancy of the colour developed was estimated at 570 and 440 nm in a spectrophotometer. The peaks on the recorded curve were integrated for loads varying from 0.1 to 3.0 µmoles for each amino acid.

From the results recorded in Table I, it is evident that *Eoperipatus weldoni* has an average of 261.67 mg of free amino acids per 100 ml of haemolymph, the minimum being 254 mg/100 ml and the maximum being 271 mg/100 ml. This value is much higher than the values reported for crustaceans[10] and arachnids[11], but comparable to that

Table I. Concentration of total free amino acids in the haemolymph of *Eoperipatus weldoni* in mg/100 ml

No. of specimen	Free amino acids
1	263
2	271
3	254
4	259
5	268
6	255
	Average 261.67

Table II. Free amino acids in the haemolymph of *Eoperipatus weldoni* in mg/100 ml

No.	Amino acids	Quantity
1	Alanine	7.9 ± 1.6
2	Aspartic acid	15.1 ± 1.9
3	Arginine	2.2 ± 0.4
4	Cystine	3.1 ± 0.3
5	Glutamic acid	18.7 ± 0.8
6	Glycine	56.6 ± 5.2
7	Histidine	23.2 ± 1.8
8	Isoleucine	3.9 ± 0.2
9	Leucine	12.4 ± 2.1
10	Lysine	8.2 ± 0.6
11	Methionine	4.7 ± 1.2
12	Phenylalanine	13.3 ± 1.6
13	Proline	42.1 ± 3.3
14	Serine	9.2 ± 1.1
15	Threonine	11.5 ± 1.8
16	Tyrosine	18.4 ± 1.7
17	Valine	9.8 ± 0.7
		Total 260.3 ± 11.8

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