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COLONY-FORMING ABILITY OF CELLS FROM DIFFERENT HEMATOPOIETIC ORGANS OF THE QUAIL EMBRYO

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KEY WORDS: colony-forming unit; hematopoietic stem cell; anlage of the heart; limb bud; yolk sac.

One of the most successful methods used to study the origin, dynamics of distribution, and quantitative aspects of the population of hematopoietic stem cells (HSC), their properties, and their relations with other tissue cells of the internal medium, is the method of splenic colonies, although it is applicable only to mice [7].

It is only recently that a method of cloning avian HSC in the bone marrow of lethally irradiated chicks has been developed [5]. The writers showed previously that a combination of this method with the method of natural cell markers [4] using sublethally irradiated chicks enabled the fate of both the donor's and the recipient's HSC, forming colonies in the bone marrow, to be studied [1].

In the present investigation colony-forming ability of yolk sac cells and of the quail embryo itself, at the stage corresponding to 48 h of incubation, and also of cells from the yolk sac, limb bud, and anlage of the heart of a quail embryo at the stage corresponding to 60 h of incubation.

EXPERIMENTAL METHOD

Hens of the Russian White breed and embryos of the Japanese quail Coturnix japonica L., of the Pharaoh breed, were used.

Full details of the experimental technique were described previously [1]. Chicks age 3 weeks, irradiated in a dose of 750 R (dose rate 50 R/min), received an injection of 0.6 ml of a suspension of embryonic quail cells 24 h after irradiation, into the marginal vein of the wing. Data on the sources of the cells of the suspension injected and the number of donor's cells injected into the irradiated chick are given in Table 1.

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TABLE 1. Number of Donor's Cells Injected into Irradiated Chicks

Source of cells of suspension injected (quail embryos)	Incubation time, h	Experiment 1		Experiment 2	
		number of experimental chicks	number of cells injected	number of experimental chicks	number of cells injected
Embryo proper	48	15	$2,2 \cdot 10^6$	13	$1,5 \cdot 10^6$
Yolk sac	48	14	$2,25 \cdot 10^8$	15	$3,5 \cdot 10^8$
Yolk sac	60	10	$5,6 \cdot 10^8$	9	$3,9 \cdot 10^8$
Limb bud	60	12	$1,6 \cdot 10^6$	9	$2,6 \cdot 10^6$
Anlage of heart	60	7	$1,9 \cdot 10^6$	7	$2,3 \cdot 10^6$

The bone marrow of the chicks was fixed 7-10 days after injection of the donor's cells and the number of benzidine-positive erythrocytes of the macroscopic colonies was counted. After macroscopic counting of the colonies in the bone marrow, detected by the benzidine test in toto, a microscopic analysis was made of the composition of the hematopoietic colonies in the organ [1]. As a result of analysis it was possible to calculate the relative percentages of exogenous (quail) and endogenous (hen) colonies for each variant of the experiment.

EXPERIMENTAL RESULTS

Microscopic analysis of the hematopoietic colonies in the bone marrow of the irradiated chicks, into which quail cells were transplanted, revealed a high proportion of exogenous colonies in each variant of the experiment. On the basis of the results it was possible to calculate the relative numbers of colony-forming units (CFU_{bm}) in the suspensions of donor's cells studied and to compare colony-forming ability of the cells from different organs of the quail embryo (Table 2).

It will be clear from Table 2 that the concentration of CFU_{bm} in the quail embryo proper at 48 h of incubation was two orders of magnitude higher than in the yolk sac at both 48 and 60 h of incubation. However, the total number of CFU_{bm} in the embryo proper was only 1.5-2.5 times greater than in the yolk sac, for at this stage of incubation the embryos proper contained about 10^6 cells and the yolk sac 10^8 cells. The number of CFU_{bm} in the yolk sac was practically unchanged from 48 to 60 h of incubation, although the number of cells in the organ increased almost two-fold during this period. This is evidence of the relative constancy of the number of CFU of the yolk sac in the period studied. However, from the end of the 3rd day of incubation after establishment of the circulation the number of CFU on the yolk sac increased and, in the opinion of many workers this takes place on account of its population from intraembryonic sources [2, 6].

Jurdic et al. [3] showed that the chick embryo at 48 h of incubation contains 33.7 ± 11 CFU_{bm}. The number of CFU_{bm} both in the quail embryo and in the chick embryo can be seen to agree closely at this stage of incubation, although these values were obtained by the use of different methods of counting the number of colonies in the chick bone marrow [1, 3].

The concentration of CFU_{bm} in the limb bud and anlage of the heart in the quail embryo was about equal (Table 2). The total number of cells in the anlage of the heart at this stage of incubation averaged $0.5 \cdot 10^6$. The total number of cells in both anlagen was the same as the number of cells in the quail embryo proper at 48 h of incubation. Hence it follows that the number of CFU_{bm} detectable in the embryo proper at 48 h of incubation corresponds with the total number of CFU_{bm} determined in the anlage of the heart and in the limb buds. Considering the exponential increase in the number of CFU_{bm} in the period from 36 to 96 h of incubation [3], it can be tentatively suggested that most CFU_{bm} contained in the embryo proper at 48 h of incubation were

TABLE 2. Relative Numbers of CFU_{bm} in Different Organs of Quail Embryos ($M \pm m$)

Source of cells of suspension injected (quail embryos)	Incubation time, h	Experiment 1			Experiment 2	
		percent of exogenous (quail) colonies	number of benzidine-positive colonies per 10^6 cells	number of quail colonies (CFU_{bm}) per 10^6 cells	number of benzidine-positive colonies per 10^6 cells	number of quail colonies (CFU_{bm}) per 10^6 cells
Embryo proper	48	58,2±2,7	86,00±4,03	50,05±4,67	67,08±9,42	39,05±7,29
Yolk sac	48	64,2±2,5	0,52±0,04	0,33±0,04	0,24±0,02	0,16±0,02
Yolk sac	60	68,2±2,6	0,25±0,04	0,17±0,03	0,21±0,03	0,15±0,02
Limb bud	60	66,9±2.7	88,80±7,3	59,41±7,28	24,33±3,85	16,28±3,23
Anlage of heart	60	63,6±1,8	67,88±2,36	43,17±2,72	45,86±8,51	29,17±6,24

identified in the present investigation in the composition of the anlage of the heart and the limb buds. The remaining CFU_{bm} evidently were located in other regions of the embryo.

The results thus indicate that the region of the anlage of the heart and limb buds of the quail embryo can serve as the main source for HSC populating both intraembryonic and extraembryonic hematopoietic organs. It can also be postulated that the anlage of the blood, which initially is located in a restricted region of the visceral layer of the mesoderm (in the caudal part of the developing embryo), in the course of morphogenesis is dispersed and distributed in the mesenchyme of the anlagen of the embryonic and definitive hematopoietic organs.

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STEM CELLS AND T AND B LYMPHOCYTES IN ACUTE HYPOXIA

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The effect of hypoxia on functional systems of the body is currently being intensively studied [1, 2, 5]. However, the immunologic reactivity of the body during hypoxia has hardly been studied at all [3, 11]. Analysis of immunologic reactivity at the present-day level must include a study of the basic cell processes responsible for immunogenesis [7, 8].

The object of this investigation was to study colony-forming stem cells (CFU) in the bone marrow, spleen and peripheral blood, and the migrating ability of CFU and also of T and B lymphocytes of mice exposed to graded rapid acclimatization to hypoxia.

EXPERIMENTAL METHOD

$(CBA \times C57BL)F_1$ hybrid mice obtained from the "Stolbovaya" Nursery of Laboratory Animals, Academy of Medical Sciences of the USSR, were used in the experiments. Female animals age 3-4 months, weighing initially 18-20 g, were used.

Graded rapid acclimatization to hypoxia was carried out in a pressure chamber; the animals were "raised to an altitude" of 4000, 5000, 6000 and 8000 m at the rate of 50 m/sec, they were kept at each of the above "altitudes" for 1 min, and they were then "lowered" in the course of 2 min 40 sec. Raising and lowering under these conditions were repeated 15 times. Training was given on three successive days, and the animals were investigated during the 3-4 h after the last training session. The control (intact) group of mice was kept in an atmosphere with a normal oxygen concentration.

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