Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Morphofunctional Changes of the Spleen When Correcting Hamsters' Body with Bee-keeping Products in Ontogenesis

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THE aim of our research is to study the indicators of growth, development, immunomorphological alteration in the structural components of the spleen during correction of the body of hamsters under experimental loading with biologically active bee-keeping products (BABP), "Microvitum" preparation and their composite forms and without it. There was conducted comprehensive research to identify experience of growth, development, proliferation processes, differentiation, alteration of structural and functional components of the spleen in postnatal ontogenesis of hamsters and under experimental loading on the body with BABP, "Microvitum" preparation and their composite forms. The study found: the dynamics of change in the spleen weight, the absolute values of the average daily liveweight gain of hamsters in postnatal ontogenesis and on the background of introduced BABP (pollen, royal jelly), amino acid-mineral-vitamin preparation "Microvitum" and their composite forms to the diet; macro- and microstructure as well as immunomorphological alteration in the spleen of hamsters in postnatal ontogenesis and under the effect of BABP, "Microvitum" preparation and their composite forms; the relationship of the structural components of the spleen, T- and B-lymphocytes and their populations in the blood that condition immunobiological response of the harmster body in postnatal ontogenesis and ways to correct it with BABP, "Microvitum" preparation and their composite forms; the effect of the experimental loading on immunomorphological response of the spleen, the cellular and humoral component of the immune system and against the background of BABP, "Microvitum" and their composite forms introduced into the diet; ways to correct performance efficiency of hamsters, prevention of cannibalism and female hibernation with BABP, "Microvitum" and their composite forms.

Keywords: Spleen, Immune status, Rabies vaccine, "Microvitum", Biologically active bee-keeping products, Postnatal ontogenesis.

Introduction

Hamsters are widely used to accumulate the virus to produce an antirabic vaccine from laboratory animals [1]. However, due to sharp change of the habitat and living conditions natural defensive mechanisms of laboratory animals are weakening [2,3]. The latter is one of the main reasons for the slowdown in growth and development, different diseases, the birth of weak offspring that does not gain enough live weight for a certain period. As a result, the development of laboratory mammals being helpful in solving current problems of modern biology, medicine and veterinary medicine is of great practical interest. The range of laboratory animals is expanding due to the development of cellular breeding of new species. In Russia breeding hamsters of this species started in the mid 1940s. Hamsters are born blind, with closed ear holes and underdeveloped limbs [4-6].

On the other hand, female hamsters often cannibalize their offspring and hibernate (especially in the period from October to February) [7-9]. Animals suffering from cannibalism lack macromicroelements in the body that leads to

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Received 16/10/2019; Accepted 17/11/2019
DOI: 10.21608/EJCHEM.2019.18281.2120

the development of suppressive reactions of the immune system [10].

However, from immature laboratory animals only hamsters were not studied for cell proliferation, immunomorphological alteration in the structural components of the spleen at different time of postnatal ontogenesis [11,12]. These aspects are the most relevant, as the spleen takes an active part in ensuring the immunological response of the body, iron recycling in t red blood cell dissolution, proliferation of immune competent cells in T - and B-dependent areas of the body that determine the immune status and health of developing animals [13-15].

The purpose of the work is to study the growth, development, immunological alteration in the structural components of the spleen, the processes of proliferation of T - and B-lymphocytes and their populations in the blood against the background of hamster body correction under experimental loading with biologically active bee-keeping products, "Microvitum" preparation and their composite forms and without it.

Method

In accordance with the purpose of the given paper during first series of experiments hamster body growth and development, spleen macro- and micromorphology in postnatal ontogenesis as well as corrected with biologically active bee-keeping products, "Microvitum" preparation and their composite forms were studied in 6 groups of hamsters at the age of 1-270 days [16].

Group 1 was the control hamsters. Their main diet (MD) consisted of a feed mixture of summer and winter periods used in the nursery of laboratory animals. Animals of group 2 received the MD+pollen, group 3 - MD+royal jelly, group 4 — MD+amino acid-mineral-vitamin preparation "Microvitum", 5 group - MD+pollen+"Microvitum", 6 group — MD+royal jelly+"Microvitum".

Animals of each age group were weighed [17]. Blood collection for immunological, hematological and biochemical studies was carried out by decapitation. Then there were performed full opening and harvesting of organs for macroscopic, histological, immunological studies [18, 19].

To study spleen macromorphology methods of preparation, macroscopic examination, weighing, taking fingerprints (to the nearest hundredths mg) were used.

For histological examination fragments and whole organs were fixed in 10% neutral formalin solution, Zenker and Carnoy's solution. Then the samples was embedded in paraffin. Then there were made sections 5-7 μm thick. The general structure of organs was studied on slices coloured with hematoxylin–eosin according to Van Gieson and Mallory. An iron-containing pigment, hemosiderin, in the spleen parenchyma was found by Perl's method. To study the cellular composition the splenic sections were stained with azure-eaosin by Romanowsky – Giemsa and Brachet's methyl-green-pyronine staining for better visualization of cellular elements [20,27].

Different tissue structures of organs were measured with an eyepiece micrometer under a microscope MBSU-3. Areas of red and white pulps, capsule, trabeculae, T - and B-dependent zones were measured using an ocular reticule [21,22].

The influence of biologically active bee-keeping products on the performance of the body, immune morphological restructuring of the spleen components was studied on hamsters exposed to swimming by the method of Makarova V.G. (1986). According to the similarity principle 90-day-old animals were divided into 7 groups of 12 heads in each. Animals of groups 1-6 were subjected to swimming, hamsters in group 7 didn't swim [26].

Groups 1 and 7 were control ones. They were also fed with the main diet used in the nursery of laboratory animals. Animals of groups 2-6 received the same extra nutrition as in the first series.

In accordance with the given method, the swimming time in groups was determined before the experiments (initial indicator), and then on the 10th, 20th day after beginning of BABP reception and 5 days after the course termination. A relative measure of the swimming time indicating changes in animal health was calculated according to the formula of Makarova V.G.:

$$P=(T\ exp. - T\ control): (T\ control) \times 100\%;$$

Where: T exp. is the average swimming time of experimental animals, T control is the average swimming time of control animals [7].

The received data were statistically processed by methods of variation statistics with the results reliability checked using the Student's t-test and the significance level (P), according to the developed programs on IBM PC [23-25].

Analysis and Results

The live weight of newborn male hamsters ranged from 1.79 to 1.83 g. This indicator of hamsters in ontogenesis tended to change in age aspect and in groups. Hamsters grow intensively in the first two months of life. In the next 3 and 4 months of life, this process is noticeably slowed down, and by 5 and 6 months (to the periods of maturation of all organs and systems of the body) decreases. In the period of mating activity of hamsters (7 and 8 months), there is a second wave of increase in live weight of animals. By the age of 9 months, a decrease in the described indicator is recorded. Similarly, the live weight of hamsters in experimental groups 2-6 changes. However, indicators of live weight of male hamsters in experimental groups, in all terms of postnatal ontogenesis, exceeded the data of animals of the control group. Similarly, the dynamics of live weight of female hamsters changed in ontogenesis.

The spleen weight of newborn males ranged from 5.1 to 5.3 mg. By puberty (45 days), it increased by 23.07 times (114.8 mg). By the active reproductive period (120 days) it was higher by 27.88 times (139.8 mg). With extinction of the reproductive period (240 days) it was more by 42.5 times (215.8 mg). At the same time, the maximum increase in the spleen weight index was observed up to 45 days of age of animals. The main diet of animals supplemented with pollen (group 2), royal jelly (group 3), "Microvitum" preparation (group 4), and, especially, pollen, royal jelly combined with the "Microvitum" (5 and 6 groups) contributed to a significant increase in spleen weight of both male and female hamsters.

The spleen length of the newborn hamsters ranged from 0.53 to 0.61 cm. Its size in hamsters of the control group 1 increased, compared with the initial indicator of newborn animals by the fifth day of life by 1.23 times (0.14 cm), by the tenth day by 2.16 times (0.7 cm), by the 30th day by 3.16 times (1.3 cm), by the 45th day by 5.0 times (2.4 cm), by the 60th day by 5.33 times (2.6 cm), by the 90 day by 6.66 times (3.4 cm). At this age, the length of the spleen was the maximum. In subsequent periods the spleen length of hamsters has slightly decreased, compared with the index of the 90-day experience. By the 120th day its size in length exceeded the indicator of newborn animals by 5,83 times (2,9 cm), at the 150th day by 6,16 times (3,1 cm), at the 180th day by 5,66 times (2,8 cm), at the 210th day by 5,83 times (2,9 cm), at the 240th day by 6,0 times (3,0 cm), at the 270th day by 5,66 times (2,8 cm). The similar trend was recorded for female hamsters' spleen linear dimensions. The length and width of the hamster spleen in experimental groups 2, 3, 4, 5 and 6 tended to increase significantly compared to the control one. The maximum spleen length in animals of experimental groups as well as in the control one was by the 90th day of life. By this period of the study, the indicator of the hamster spleen length in group 2 exceeded the control values by 1.05 times (0.2 cm), in group 3 by 1.1 times (0.4 cm), in group 4 by 1.17 times (0.7 cm), in group 5 by 1.22 times (0.9 cm), in group 6 by 1.25 times (0.1 cm). In subsequent periods the spleen length in all groups slightly decreased.

The study of the spleen microstructure showed that in 1 day old hamsters the organ parenchyma, consisting of reticular tissue, is not differentiated into red and white pulp (see Fig. 1). White pulp differentiation is recorded from the 10th day of life. It occurs as the result of the reticular tissue induration and formation of lymphatic nodules, where maturation and antigenic differentiation of lymphocytes takes place. From the age of 30 days there appeared light, germinative centers (centers of cell reproduction). The number of the last is growing intensively up to 90 days of animals' age. In relation to the light center of the lymphatic nodules in its cortex there revealed eccentrically located artery and vein. On the 5th day of newborn male and female hamsters' life there are no lymphatic nodules on spleen sections . 10-day-old male hamsters had different number of lymphatic nodules of the spleen sections depending on the groups. There were only 2 nodules in the control group, and 4, 5, 3, 6 and 8 nodules in animals of the groups 2, 3, 4, 5 and 6 respectively. The number of lymphatic nodules in the spleen of hamsters dramatically increased from the 30th day.

This process progressed up to the age of 240 days. In the postnatal ontogenesis, there is an active increase in the size of the lymphatic nodules that affects the size of the white pulp of the spleen.

The size of lymphatic nodules in the spleen of

male and female hamsters of experimental groups differed from the control figure from the 10th day of their life. A significant jump in this indicator was registered when animals were 30 days old. The maximum area of lymphatic follicles in the spleen of male hamsters reached by the age of 150 days.

There was a slight decrease in the area of the lymphatic follicles in the spleen on the 240th day of the experiment. This tendency was typical for both experimental and control groups and continued until the end of the study (270 days). Similarly, there was a change in the reactivity of lymphatic follicles in the spleen of female hamsters. They were slightly different from indicators of males in terms of their manifestation and severity.

The performance of hamsters was evaluated by the swimming duration. 1-3 minutes before the test there was attached a load of 7.5% of the live weight to the root of animals' tail . Time (min) from the beginning of swimming to the inability to stay on the water surface was considered as a criterion of "refusal" from work and development of fatigue. The swimming time duration of hamsters of the control group 1 ranged from 89.6 to 106.1%. This indicates that in the control series of experiments as a result of swimming training there is an increase in the duration of swimming by 5.8%. Analysis of experimental results by the experimental groups shows that biologically active bee-keeping products with "Microvitum" preparation have greater effect on the performance of animals. In particular, the maximum values of efficiency were recorded in animals of group 5 and, especially, group 6 . Here health indicators of hamsters of the groups described exceeded targets by 1.58 and 1.76 times on the 10th day of the experiment and by 1.45 and 1.53 times on the 20th day. After five days of the termination of the course of biologically active bee-keeping products, preparation "Microvitum" and their composite forms performance of hamsters in groups 2, 3, 4, 5 and 6, defined according to the swimming test , remained above the initial level and the record of the control group animals that received the basic diet with physiological solution. Thus, introducing composite forms pollen+"Microvitum" and especially royal jelly +"Microvitum" into the diet of 90 days old male hamsters contributed to their performance.

The structural components of the hamster spleen in the control and experimental groups were measured on the 20th day of intense physical activity. The analysis of the received digital data showed that intensive physical activity of the control group I hamsters getting only the main diet compared to the similar data of the control group 7 animals that were not subjected to intensive swimming, contributes to deep destructive changes, which are manifested primarily by rearrangements in the white and red pulps of the organ. The relative area occupied by the red pulp in the described group was 13.3% more, and the white pulp, on the contrary, was 11.3% less. At the same time, in the white pulp of the organ there was not only a decrease in the number, but also in the area of lymphatic nodules without light and with light centers and perivascular lymphoid couplings. The area of the T-zone of the spleen was lower, compared with the control figure by 1.27 times (2.1%), and B-zone - 1.88% (9.2%). Inclusion in the diet of animals BABP and preparation "Microvitum" on intense physical activity had a positive impact on the ratio of the structural components of the spleen. However, the level of their impact on the structural alterations of the organ described was uneven. A marked increase in the area of white pulp compared to the control group 1 was found in animals of experimental groups 2 and 3 with pollen and royal jelly included in their main diet. The white pulp area in the spleen of group 2 animals was 25.1% and 26.4% of group 3 animals. They were higher than the control value of group 1 animals by 1.37 and 1.45 (6.9 and 8.2%) times, respectively. However, they did not reach the background values of animals that were not exposed to swimming by 4.4 and 3.1%., respectively, In the structure of lymphatic nodules the number of nodules with light centers increased. The proportion of lymphatic nodule area with light centers increased by 0.9% (4.0 times) in group 2 , by 1.0% (4.3 times)in group 3. The area of perivascular lymphoid couplings (T-zone) in the described groups also increased by 1.07 times (0.6%) in group 2 and 1.15 times (1.2%) in group 3. Introducing "Microvitum" preparation into the diet of hamsters caused a modest alteration In the B-dependent area of the spleen. Their value exceeded that of group 1, by 1.65 times (0.7%) respectively. The area of the red pulp in this group decreased in comparison with the same indicator of group 1 by 1.07 times (5.2%). The area of the white pulp, on the contrary, increased by 1.34 times (6.2%). The

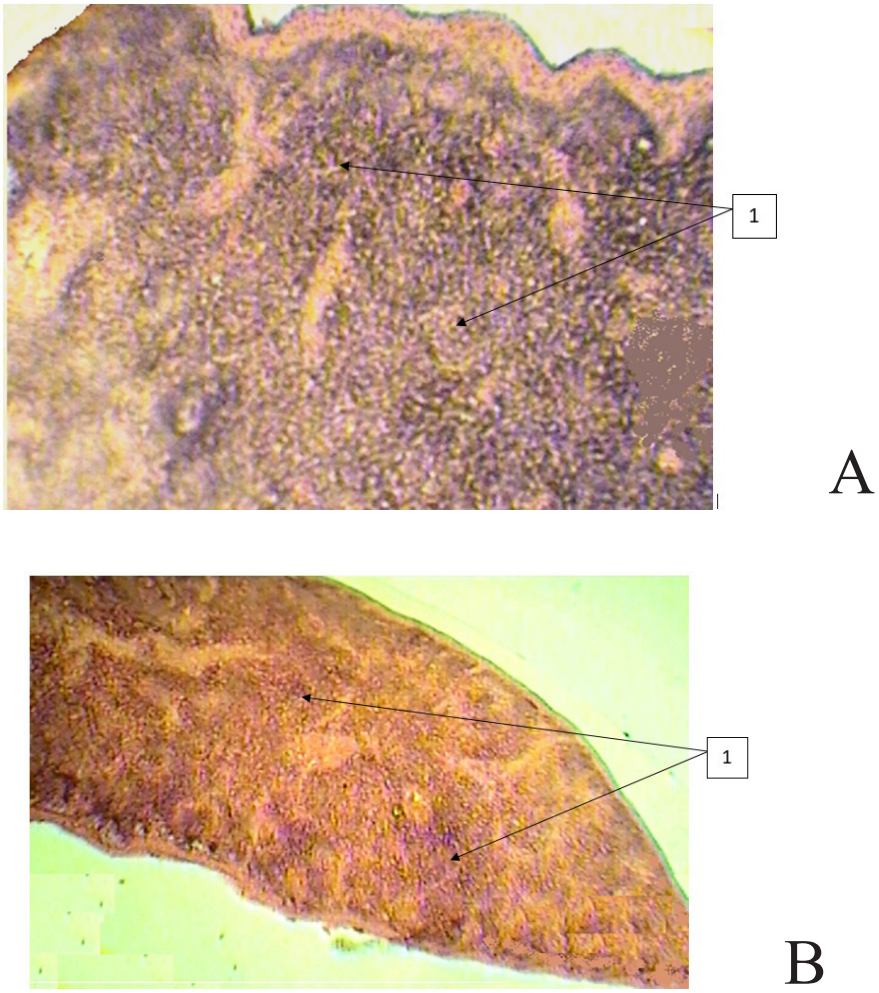

Fig. 1. The spleen of a one-day-old hamster: male (A), female (B). The Spleen parenchyma consisting of reticular tissue (1) is not differentiated into red and white pulps. Hematoxylin and eosin. Increase: A – 100 (lens – 10, eyepiece – 10), B – 40 (lens – 20, eyepiece – 2).

most pronounced positive changes in the T - and B-dependent areas of the spleen were recorded in hamsters of group 5 and, especially, group 6. Their diet included pollen and royal jelly in combination with "Microvitam" preparation. The area of perivascular lymphoid couplings in the hamster spleen of the described groups was close to the parameters of physiological norms and amounted to 9.8%, respectively. It exceeded the control figure of group 1 animals by 1.25 times (by 2.0%). The area of lymphatic nodules with light centers was the highest in group 6 being 1.8%. The white pulp area of the spleen exceeded the same indicator of group 1 hamsters by 1.58 and 1.61 times (10.6 and 11.2%). The red pulp value, on the contrary, was lower by 1.14 times (9.4%) and 1.15 times (10.1%), being corresponding to physiological parameters. Therefore, BABP with "Microvitum" preparation in complex introduced in the diet of animals on intense physical activity have a positive impact on productivity as well as contributes to the maintenance of the structural components of the spleen at the level of the physiological norms.

Discussion

Live weight gain and spleen development of hamsters in postnatal ontogenesis goes in three phases: the first – intense (1 and 2 months before puberty), the second – slowing growth (from 3 and 4 months of life), the third – fading (7-8 months).

Pollen, royal jelly and especially their composite forms with "Microvitum" preparation stimulate live weight gain, larger linear dimensions and the spleen weight . The described indicators in male animals are higher than in females.

The formation of immunobiological function, morphological alteration and differentiation of the spleen parenchyma into the red and white pulps occurs by the age of 10 days. The immunological reactivity of the white pulp increases with the appearance of lymphatic nodules with light (germinative) centers by the age of 30 days.

Composite forms of "Microvitum" preparation with pollen (group 5), and especially with royal jelly (group 6) contribute to a significant immune morphological alterations in the spleen of male and female hamsters. Herewith: (a) the spleen weight enlarges by 49.42-49.43 times in males and 52.98-53.13 times in females from birth to 270 days of life,

b) the spleen length increases by 8.6 (by the 90th day) and 8.67 times (by the 150th day) in male hamsters and by 8.07-8.0 times in females by 240 th day,

(a) the spleen width grows by 6.06-6.53 times in males by the 180th day and 7.5-7.0 times in females by the 60th day of life,

d) the spleen capsule thickness increases by 9.27-9.3 times in males by the 180th day and 9.87-9.73 times in females by the 210th day,

e) the area of the white pulp expands due to the formation of lymphatic nodules by 9,37- 9,0 times in males by the 270th day and 6,26-6,1 times in females b y the 270th day,

e) the area of the red pulp reduces by 1.58 and 1.59 times in males by the 270th day and 1.56 and 1.62 times in females by the 270th day.

In postnatal ontogenesis of hamsters of all age groups there is more red pulp in the spleen parenchyma. Its area is considerably reducing by the age of 120 days.

On forced swimming test (extreme physical effort) pollen, royal jelly, "Microvitam" preparation and their composite forms added into the diet of hamsters contributes to the efficiency of males and females by 34.2 and 28.6% in group 2 , 50.07 and 35.6% in group 3, 33.4 and 16.3% in group 4, by , 52.07 and 48.7% in group 5 and 68.7 and 56.7% in group 6.

Introducing composite forms of pollen and royal jelly with "Microvitum" into the diet of hamsters during their intense exercise, maintains the structural components of the spleen at the level of physiological norms, with significant activation of immunocompetent T - and B-dependent zones in the white pulp of the organ.

Conclusion

To improve metabolic processes in hamsters' bodies, prevent sleepiness and cannibalism of female hamsters in order to expand opportunities for preserving and increasing hamster population, required to receive the rabies vaccine, nurseries for laboratory animals are recommended to add composite forms of pollen or royal jelly of bees with "Microvitum" preparation into the diet of hamsters.

Pollen is recommended to be included in the diet at the rate of 1 g/head. Royal jelly should be given at the rate of 0.005 g/head in the tableted apilac. "Microvitum" must be introduced at the rate of 15 mg/head. Preparation should be added to the feed daily 2 times a day during 20 days with repeated application after 15 days.

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