BEHAVIOR OF RAT SERA IN THE GEL-PRECIPITATION REACTION DURING REGENERATIVE PROCESSES

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UDC 612.6.03:612.017.1

After removal of two-thirds of the liver or kidney in rats, the precipitating activity of the blood serum relative to extracts from autologous or homologous liver and kidney is modified: 24-48 h after the operation it is reduced or completely lost, but by 120 h after the operation it recovers. The decrease in precipitating activity of the serum is accompanied by a fall in the complement titer.

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There are reports in the literature that during intensified growth of certain tissues, a factor appears in the sera of the experimental animals which can be detected by immunological reactions [4, 6]. Darcy [4], for instance, using immune sera, demonstrated the appearance of such a factor in experimental animals by means of the gel-precipitation reaction during growth of malignant tumors, growth of young animals, during pregnancy, and so on.

In the present investigation the precipitating activity of the sera of animals was investigated during regeneration of the liver and compensatory hypertrophy of the kidney.

EXPERIMENTAL METHOD

Experiments were carried out on 84 Wistar rats and on noninbred male rats weighing 200-250 g. The animals were divided into two series. By the usual technique [3] two-thirds of the liver was removed from the rats of series I (48 animals). One kidney was removed from the rats of series II (17). The control consisted of 19 rats undergoing mock hepatectomy or nephrectomy or no operation whatever. Blood for investigation was taken from the experimental animals before and 4, 8, 12, 24, 48, 72, 96, and 120 h after the operation. The precipitating activity of the animals' sera was determined with saline extracts from the liver and kidney tissues in the gel-precipitation reaction by Ouchterlony's method and by immunoelectrophoresis [1]. Extracts for the reactions were prepared from autologous and homologous tissues under strictly standardized conditions: 1 g tissue was triturated for 10 min in a sterile mortar with glass sand, and 3 ml physiological saline was added. Before the experiment the protein in the saline extracts was determined by the methods of Lowry and Kjeldahl. The protein content in the extracts varied from 30 to 35 mg/ml. Difco agar was made up in medinal–veronal buffer, pH 8.6. The results of the reactions were read in all cases after 24 and 48 h. The complement titer was also determined in the sera of the control and experimental animals.

EXPERIMENTAL RESULTS

The sera of the control and most experimental animals reacted before partial hepatectomy and unilateral nephrectomy with extracts from liver and kidney tissues, forming 1 and, in some cases, 2 precipitation bands (Fig. 1a), in agreement with published data [2, 5, 7].

Precipitation bands were formed by the animals' sera with extracts from both autologous and homologous tissues. The sera of some experimental animals formed precipitation bands before the operation with only one of the extracts tested. For instance, the sera of 19 rats of series I (39.7%) formed precipitation bands before removal of two-thirds of the liver with extract from kidney tissue, but failed to react completely with extract of liver tissue (7 rats) or reacted only slightly (12 rats). Conversely, sera of 7 of

Laboratory of Immunology of Growth and Development, Laboratory of Growth and Development, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Active Member of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 3, pp. 67-69, March, 1969. Original article submitted September 15, 1967.

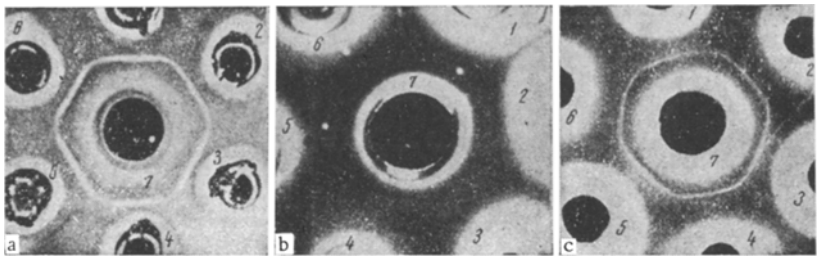

Fig. 1. Results of gel-precipitation reaction of rat sera with extracts from liver and kidney tissues. 1, 3) Extract from autologous liver tissue; 2, 5) extract from homologous kidney tissue; 4, 6) extract from homologous liver tissue; 7) sera of rats: a) before hepatectomy; b) 24-48 h after partial hepatectomy; c) 120 h after partial hepatectomy.

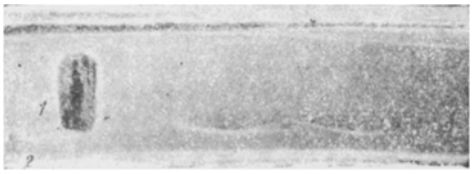

Fig. 2. Immunoelectrophoresis of rat serum (1) taken 24 h after partial hepatectomy with extract from liver tissue (2) during the period of increased precipitating activity of the serum.

the 17 rats of series II gave precipitation bands before unilateral nephrectomy with extracts from liver tissue and very weak bands with extracts from kidney tissue.

The precipitation reaction given by the 19 control animals remained without significant change at its initial intensity. A slight weakening of precipitating activity of the serum relative to extracts from liver and kidney tissue was observed in only one rat after mock hepatectomy. Neither frequent taking of blood nor the mock operation was thus accompanied by any significant change in intensity of the precipitating activity of the tested sera.

Investigation of the immunoelectrophoretic mobility of the factor responsible for the positive precipitation reaction of the sera of normal rats (10 animals) with extracts from these organs showed it to be localized in the zone of α_2- and β-globulins.

In the animals undergoing partial hepatectomy and unilateral nephrectomy changes in the precipitating activity of the sera from the level established before the operation were observed. For instance, the sera of rats reacting weakly or not at all before the operation with extract from liver tissue formed precipitation bands with this extract 4-24 h after partial hepatectomy. The intensity of the reaction of these sera with extracts from kidney tissue was unchanged in this period. During immunoelectrophoresis, the precipitation bands given by sera of some animals of this group with extract from liver tissue were located not only in the zone of α_2- and β-globulins, but also in the zone of γ-globulins (Fig. 2).

The sera of all animals of this group lost their ability to react with liver and kidney extracts 48-96 h after partial hepatectomy (Fig. 1b).

The sera of rats which, before operation, formed intense precipitation bands with extract from liver tissue, lost their ability to react with extracts from liver and kidney tissues 24-48 h after partial hepatectomy, as revealed by the gel-precipitation reaction and by immunoelectrophoresis.

The precipitating activity of the sera of this group of experimental animals was restored 120 h after the operation (Fig. 1c).

Similar changes were observed in the rats after unilateral nephrectomy. The sera of animals reacting slightly before the operation with extracts from kidney tissue became capable of reacting 4-24 h after the operation, but lost this power 48-120 h after the operation. The sera of animals reacting strongly with extracts from liver and kidney tissues before unilateral nephrectomy, on the other hand, lost this power at different times after the operation (24-72 h).

In these experiments the early periods of recovery were thus characterized by the fact that the sera of the experimental animals became capable of reacting with extract from the removed organ. In the period

corresponding, according to data in the literature [3], to a decrease in mitotic activity of the regenerating organ, the precipitating power of the sera from animals undergoing the operation was lost. A definite correlation perhaps exists between changes in serologic indices and the proliferative activity of the organ.

It is interesting to note that, parallel with the disappearance of or diminution in precipitating activity of sera from the hepatectomized animals (10 rats), a decrease in complement titer took place (24-48 h after the operation).

It should be mentioned in conclusion that the question of the nature of the factor responsible for the positive gel-precipitation reaction of sera of normal and experimental rats with extracts from autologous and homologous liver and kidney tissues remains unanswered.

The possibility is not ruled out that the changes observed in the properties of the sera during regeneration of internal organs are due to immunologic reactions. However, this problem requires further analysis.

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