REVIEWS

Unity and Opposition of Cytogenetic Lymphocyte Activity and Antibody Formation during Regenerative Processes

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Here we present a comparative temporal analysis of the appearance and dynamics of cytogenetic and immune activity of lymphocytes during regenerative processes in different organs based on own and published data. The changes in these activities are coupled and codirected, depend on experimental conditions, but are realized via different pathways. These similarities depend on changes in the balance between immunoregulatory cells (T helpers and T suppressors).

Key Words: *lymphocytes; cytogenetic activity; antibody formation; regeneration; compensatory hypertrophy*

During the last 3 decades studies of the role of the immune system in the regulation of regeneration in different organs were focused on: 1) examination of cytogenetic lymphocyte activity and the role of these cells in proliferative stage of reparation, and 2) analysis of the immune state of the organism during regeneration and changes in immunoreactivity under these conditions. Ample experimental data were reviewed separately and in combination in order to compare these lymphocyte activities [1,2,14,16,18,19,26].

This comparison revealed three main regularities. First, both types of activities depend on surgery-induced changes in the functional state of T lymphocytes. Second, changes in the dynamics of regeneration and immunoreactivity are provided by different T lymphocyte populations. Third, both activities depend on the same conditions, particularly, damaged organ and volume of surgical intervention [1,2,6,13, 15,26].

Despite apparent similarity in the development of these activities their cause-effect relations remain poorly studied.

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Comparison of the dynamics of these activities and the sequence of stimulation can answer this question. The method of adoptive transfer of lymphocytes provides data, which allow to estimate their capacities for realization of both activities at each stage of regeneration. To this end, lymphocytes are isolated from the spleen at different terms postoperation and transferred to healthy syngenic recipients [1].

In the recipients, lymphocytes perform the same functions as in donors. Since cytogenetic activity appears 43-50 h after transfer and antibody formation on days 7-8 after transfer with sheep erythrocytes (SE), it is possible to analyze these activities [1].

Lethally irradiated animals are routinely used for estimation of antibody production after adoptive transfer. Complete immune inactivation of host lymphocytes allows to evaluate this function in donor lymphocytes, because they are the only cells responding to the antigen. However, these data were insufficient and had to be supplemented with the results of experiments on operated nonirradiated donors immunized at different terms postoperation [3,17]. In both cases the animals were immunized with SE, the most widely used thymus-dependent antigen, because T lymphocytes responsible for this type of immune reaction are

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most affected by regenerative processes. We analyzed antibody production as the most studied regenerative immune reaction [1,10].

Cytogenetic activity of lymphocytes is studied on nonirradiated animals. Sublethal irradiation enhances proliferation and organ sensitivity to stimuli, which interferes with the evaluation of the period and degree of proliferation stimulated by transplanted lymphocytes [1].

The periods reported here are connected with potential capacities of lymphocytes determined at standard time for each method. In other words, the time of donor sacrifice (donor interval) is variable, while that of recipient (recipient interval) is constant in both methods.

Direct correlation between the volume of resected tissue and both types of lymphocyte activity allows to consider changes in lymphocytes under conditions of complete identity of surgical interventions [1,5,6,24].

These data were accumulated in experiments with liver regeneration after resection of $^2/_3$ hepatic tissue and compensatory hypertrophy of the kidney after unilateral nephrectomy. The test parameters can be easily analysed because the time of appearance of cytogenetic and increased immune activity in lymphocytes is relatively constant and independent on the operated organ. In particular, examination of SE-induced antibody production showed that lymphocyte stimulation caused by resection of $^2/_3$ liver was observed only 4 h after operation. Three hours postoperation lymphocyte response to SE did not differ from the control. Antibody generation peaked 17 and 21 h postoperation, 2.6- and 3.5-fold exceeding the control, and returned to normal 48 h postoperation [1,24,26].

Four hours after this operation, cytogenetic lymphocyte activity also increases 3-fold. During the early postoperation period this activity in operated did not differ significantly from that in control animals. After 17 h cytogenetic activity remained at a high level (3.5-fold above the control), decreased by 26 h (1.7-fold of the control) and reached the control level 48 h after operation [1,24,26]. Thus, both activities showed no complete identity, but their initial periods and dynamics were similar (Fig. 1, *a*).

Correlations between these activities were observed in experimental unilateral nephrectomy. In this case, lymphocyte reaction to SE was delayed compared to that induced by $^2/_3$ liver resection [5,13]. Four hours after nephrectomy, the number of antibody generating precursors in the spleen of operated donors did not differ from that in intact or sham-operated animals. Significant (1.7-fold) increase in the number of these precursors was observed only 9 h postoperation, after 12, 17 and 19-21 h this parameter increased 5-fold, and then gradually decreased, but 3- and 2-fold exceeded the control level after 24 and 48 h, respectively; 72 h postoperation this parameter reached the control level [5,13,26]. In other model of nephrectomy-stimulated antibody production including immunization of the mice 72 h after operation, the enhanced lymphocyte reaction to SE persisted [3].

These data show that nephrectomy-stimulated antibody-generating capacity of lymphocytes appeared later and persisted longer than after $^2/_3$ liver resection.

There are no published data on the time of lymphocyte-stimulated proliferation in renal tubular epithelium of recipients after unilateral nephrectomy. However, no stimulation was observed 4 h after unilateral nephrectomy [6].

Thus, cytogenetic activity, as well as immune responsiveness of lymphocytes developed later than

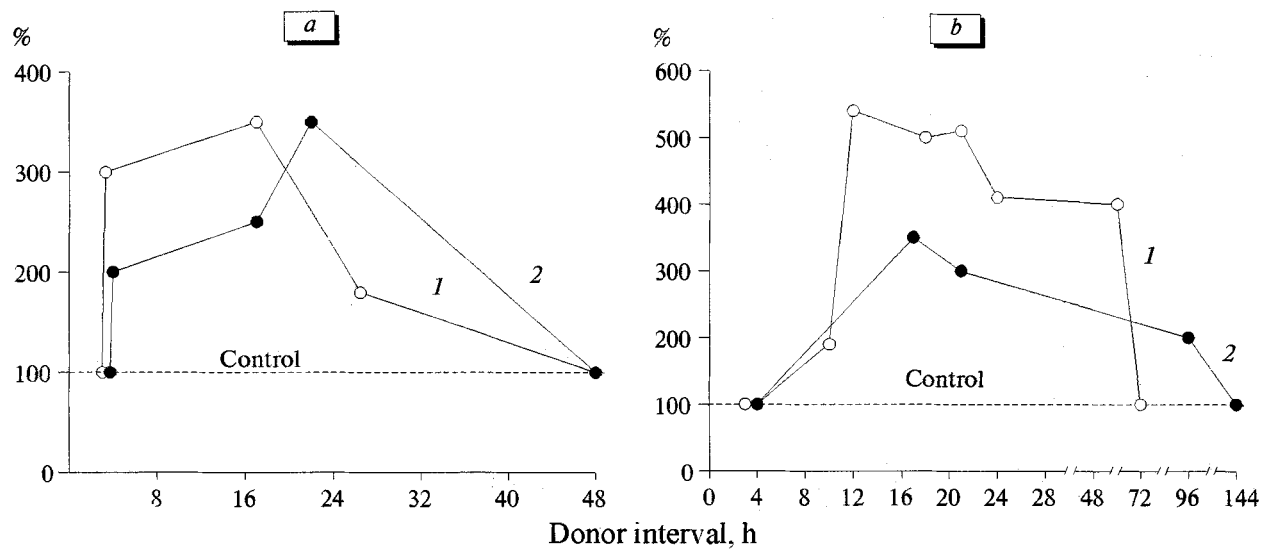

Fig. 1. Dynamics of antibody production (*1*) and cytogenetic activity (*2*) of donor lymphocytes at different terms after $^2/_3$ liver resection (*a*) and unilateral nephrectomy (*b*) compared to the corresponding controls. Recipient interval: 7 days (*1*), 43-45 (*a, 2*), and 48-50 h (*b, 2*).

after $^2/_3$ liver resection. Published data suggest that 17 and 19-21 h after operation, donor lymphocytes enhanced proliferation of tubular epithelium in recipient kidneys 3.5- and 3-fold, respectively [1,26].

After unilateral nephrectomy, cytogenetic activity of lymphocytes persists for 4 days: the number of DNA-synthesizing renal epitheliocytes in nonoperated sublethally irradiated recipients 1.5-fold surpassed that in control recipients [28,30]. Prolonged persistence of cytogenetic lymphocyte activity in comparison with their antibody generating capacities can be explained by the fact that irradiated animals used in the experiments were more sensitive to stimuli, which was confirmed on the model of liver regeneration [1]. The dynamics of these activities did not coincide in this model, but their similarity was apparent: in contrast to surgical intervention, lymphocyte activation was delayed, the plateau was stable, and the decrease to the initial level occurred later. We assume that these changes are determined by the same mechanism. However, despite similar character of these changes, the activities develop independently. Similar but fragmentary data on the prolongation of cytogenetic and antibody generating activities after phlebotomy [11,17] were published. It was only reported that lymphocyte-dependent cytogenetic activity of peritoneal cells did not decrease 2 h after bloodletting [11], while antibody production exceeded the control level after immunization of anemic mice 4 days after blood loss [17].

It is interesting to compare the changes in cytogenetic and antibody-generating activities under different experimental conditions, in particular, after different resection volume.

This comparison showed that smaller volume ($^1/_4$) of liver resection induced no changes in cytogenetic lymphocyte activity 17 h after operation, whereas $^2/_3$ liver resection markedly stimulated this activity [1,7, 24,26]. There are no published data on similar changes of antibody production. However, less extensive resection of renal tissue was associated with a pronounced delay in the stimulation of cytogenetic activity and antibody formation. Thus, half-kidney resection does not change lymphocyte cytogenetic activity 17 h postoperation, while stimulation of antibody production was observed after 17 h compared to 9-12 h after unilateral nephrectomy [1,13].

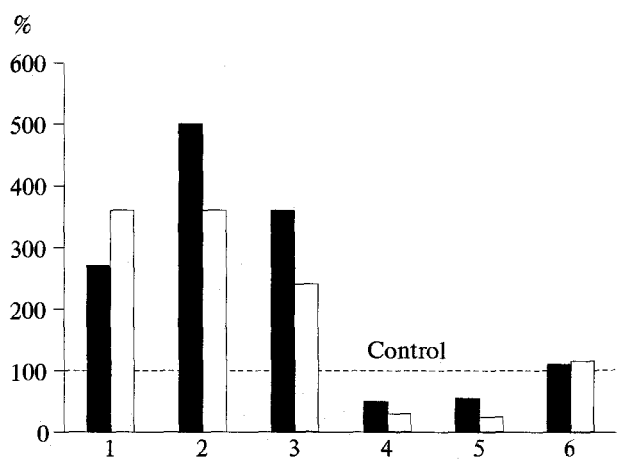

Fig. 2. Antibody formation (dark bars) and cytogenetic activity (open bars) of lymphocytes 17-18 h after $^2/_3$ liver resection (*1*), unilateral nephrectomy (*2*), blood loss (*3*), partial splenotomy (*4*), uni- (*5*) and bilateral (*6*) sialoadenectomy.

On the contrary, increasing the volume of resected tissue reduced the latency of manifestation of examined lymphocyte activities.

Bilateral nephrectomy induced pronounced cytogenetic lymphocyte activity as soon as after 4 h. Recipients of these lymphocytes demonstrated more than 4-fold enhancement of proliferation of renal epithelium, while lymphocytes of sham- and nonoperated animals and injection of culture medium had no effect on proliferation [6].

Bilateral nephrectomy reduced the latency of stimulation of lymphocyte antibody production. The number of antibody generating precursor cells in the spleen of operated animals 2.5-fold surpassed that in non- and sham-operated animals as soon as 1 h postoperation. Four hours later this difference increased to 5-fold [5].

These data point to the same tendency in changes of cytogenetic activity and antibody production in lymphocytes. These activities are not synchronous, but appeared in individual experiments with a short interval. Fourfold increase in the proliferation of renal epitheliocytes induced by lymphocytes from bilaterally nephrectomized donors suggests that this property was acquired before 4 but later than 1 h postoperation [6].

Other experiments also point to the same tendency of operation-induced changes in cytogenetic activity and antibody generation in lymphocytes (Fig. 2).

Some surgical interventions did not enhance but inhibited or induced no changes in lymphocyte activity [1,3, 21,23,25,29]. Thus, resection of the spleen and unilateral resection of the submandibular and sublingual salivary glands, incapsulated together, inhibited antibody production (Fig. 2), which was shown in the experiments with adoptive transfer of lymphocytes isolated 4-24 h postoperation and with direct immunization of operated animals at different terms postoperation [1,3,21,24,25,29].

Uni- and bilateral resection of such physiologically isolated organs as testicles and bilateral sialoadenectomy induced no changes in immunoreactivity (Fig. 2) [3].

Suppressive activity of lymphocytes induced by splenectomy and unilateral sialoadenectomy involved not only antibody production, but also proliferation-inducing capacities of lymphocytes (Fig. 3): lympho-

cytes acquired pronounced cytostatic properties. They inhibited proliferation in all examined recipient tissues with relatively high mitotic activity, including corneal epithelium (4-fold), small intestine (3-fold), and regenerating hepatocytes after $^1/_4$ liver resection [3,15].

Cytostatic lymphocyte activity was tested in organs distinct from those subjected to surgical intervention in donors, because inhibition of proliferation can be easily studied in tissues with high mitotic activity. In the spleen this evaluation is complicated by specific function of this organ, while in the salivary glands – by extremely low mitotic activity even during regeneration. Since, in contrast to stimulation of proliferation, suppressive properties of lymphocytes were not organ-specific, the effect of suppression on regenerating liver was examined after $^1/_4$ tissue resection [3, 15]. Moderate proliferation observed under these conditions allowed us to reveal both the suppressive and stimulatory effects.

Lymphocytes isolated from the spleen of operated animals 17 h after unilateral sialoadenectomy 8-fold inhibited mitotic activity in regenerating liver. This inhibitory effect decreased 2.5- and 3.3-fold 48 and 72 h postoperation, respectively. Antibody production in animals immunized 17 h after operation decreased 0.5-fold, while in those immunized on day 3 it increased 2.5- and 3.7-fold compared to the control and animals immunized after 17 h, respectively (Fig. 3) [3,4].

These data agree with the idea about the same directions of the changes in these activities.

This can be explained by differences in proliferative capacities and sensitivity to stimulatory and inhibitory factors of lymphocytes and hepatocytes. Being cells of a stable organ (those include liver, kidney, and salivary glands) hepatocytes are characterized by extremely low proliferation in adult organism, and their mitotic activity are stimulated only under the effect of strong stimuli like tissue resection.

On the contrary, lymphocytes can realize their function only through proliferation and are highly sensitive to various stimuli. Therefore, the number of antibody-forming cells reaches thousands, while mitotic activity in the liver even after extensive resection amounts to few percents. However, this proliferative activity is sufficient for rapid recovery of liver volume.

These data suggest that hepatocytes are more sensitive to inhibition, while lymphocytes — to stimulation. Besides, the comparison of lymphocyte effect on various parenchymatous organs revealed organ specificity of their stimulatory activity [1,26]. Significant decrease in the number of inhibitory lymphocytes accompanied by an increase in the number of stimulatory cells induces no stimulation in the organs nonhomologous to those in operated donors, but only attenuates suppression via reducing the number of inhibitory cells.

This is not true for lymphoid cells, which respond even to insignificant adequate stimuli by enhanced antibody production depending on the intensity of stimuli, which modulates cytogenetic activity of lymphocytes.

Because of different lymphocyte and epitheliocyte sensitivity, the number of stimulating lymphocytes in the spleen is not quite clear. The number of suppressors inhibiting proliferation of both lymphoid and nonlymphoid cells [18,23] increases in the spleen after its partial resection. T helpers stimulate division of both lymphoid and epithelial cells [18].

Comparison of antibody formation and cytogenetic activity of lymphocytes from partially hepatoectomized recipients injected with lymphocytes from animals subjected to bilateral sialoadenectomy showed coincidence of these activities during the examined period. Transfer of lymphocytes 18-20 h after bilateral sialoadenectomy induced no changes in hepatocyte proliferation in regenerating liver. Antibody formation was not affected by immunization of mice, while unilateral sialoadenectomy induced inhibitory lymphocyte activity (Fig. 2) [3,4].

Thus, the obtained data point to the same tendencies and coupled changes of both lymphocyte activities in various organs after trauma and during reparation.

Different dynamics of both activities after surgery is associated with variations in the helper/suppressor ratio in the spleen [10,12]. Normally, the number of T supressors 2-fold exceeds that of T helpers. After

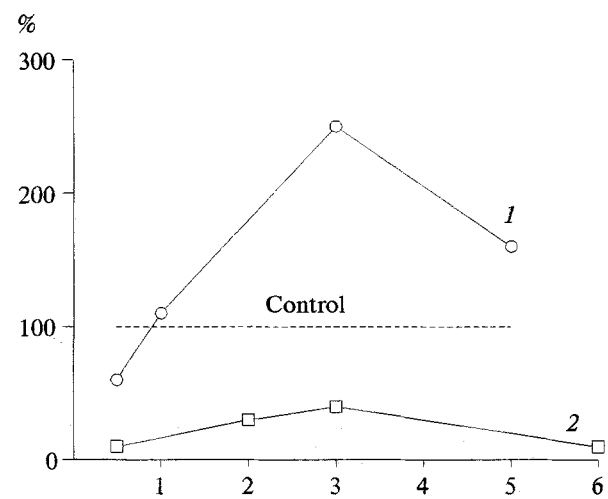

Fig. 3. Dynamics of antibody production and cytogenetic activity of lymphocytes in animals immunized at different terms after unilateral sialoadenectomy compared to the corresponding control. Abscissa: time of immunization after operation (*1*) and donor interval (*2*), days. Ordinate: number of antibody-producing cells in the spleen of immunized mice (*1*) and mitotic index of regenerating hepatocytes in the liver of recipients injected with lymphocytes from operated donors (*2*).

operation on kidneys, the number of T helpers increases 2.0-1.7-fold 2, 4, 17 and 48 h after surgery. At the same time, the number of T supressors does not change, therefore the T helper/T suppressor ratio changes preceding the appearance of cytogenetic activity and antibody formation in lymphocytes. The dynamics of T lymphocyte subpopulations after liver resection was examined only 4 h after operation and later, while the initial stages were not yet studied. However, by the moment of manifestation of cytogenetic lymphocyte activity (4 h) the number of T helpers increased 2-fold [12]. There is direct evidence for stimulation or inhibition of mitotic activity in the epithelium by T helpers and T suppressors, respectively, accompanied by stimulation or inhibition of proliferation in a number of other organs [18].

The absence of immune reaction to bilateral extirpation of the testes and salivary glands can be explained by specific interrelations between their antigens and immunocompetent cells. Antigens of these organs are separated by tissue-blood barrier from immune cells and not recognized by the organism, which was shown for testes [22]. Antigens of salivary glands and isolated because of high content of nerve growth factor (venom analog) [8]. However, physiological isolation of the salivary glands requires confirmation, therefore different reactions to uni- and bilateral sialoadenectomy remains unexplained.

Uni- and bilateral extirpation of the testes induces no immune response [3]. It is well known that immune response depends on the ratio of immunoregulatory cells. Surgery can increase or decrease this ratio, thus enhancing or attenuating or even blocking antibody formation, which depends on the content of T suppressors after surgery.

Thus, activation of both lymphocyte functions is underlain by the same mechanism, associated with changes in the number and ratio of T helpers and T suppressors. This can explain the same direction, coupling, and phasic changes in these activities during regeneration, since they are triggered by the same immunoregulatory cells: T helpers and T supressors. Therefore, these regularities are characterized by the same peculiarities and depend on the same conditions. However, cytogenetic and cytostatic activities preserve cell number via replicative and inhibitory functions of lymphocytes, while immune activity is associated with destruction, which preserves cell types. Lymphocytes protect the organism from alien information by killing its own mutant cells, alien cells, and microorganisms. Cytogenetic lymphocyte activity is realized via interaction with the cells of other histotypes, whereas the immune response is initially realized within one histotype.

Coupling of these activities aimed at rapid elimination of injury consequences associated with penetration of microorganisms is of great biological importance.

This coupling appeared in evolution in invertebrates, in whom protective and morphogenetic functions are combined in one cell or in cells of one histogenetic lineage. In the course of evolutionary divergence of these vital functions, wide morphogenetic capacities and primitive protection of amoebocytes was replaced by moderate morphogenetic capacities manifested as cytogenetic activity and wide immunocompetence of lymphocytes [20,27]. Coupling of these two functions is essential for life.

REFERENCES

1. A. G. Babaeva, *Regeneration and Immunogenesis System*, Moscow (1985).
2. A. G. Babaeva, *Byull. Exp. Biol. Med.*, **119**, No. 3, 301-303 (1995).
3. A. G. Babaeva and V. V. Arsent'eva, *Ibid.*, **112**, No. 8, 169-170 (1991).
4. A. G. Babaeva and V. V. Arsent'eva, *Ibid.*, No. 9, 328-330.
5. A. G. Babaeva and E. I. Gimmel'farb, *Ontogenesis*, **19**, No. 1, 42-47 (1988).
6. A. G. Babaeva and E. I. Gimmel'farb, *Byull. Exp. Biol. Med.*, **124**, No. 7, 109-110 (1997).
7. A. G. Babaeva, V. G. Nesterenko, and N. V. Yudina, *Ibid.*, **93**, No. 6, 98-99 (1982).
8. A. G. Babaeva and E. A. Shubnikova, *Structure, Function, and Adaptive Growth of Salivary Glands*, Moscow (1979).
9. A. G. Babaeva, A. N. Shutko, N. N. Shatinina, *et al.*, *Arkh. Pat.*, No. 1, 62-64 (1993).
10. A. G. Babaeva, N. V. Yudina, and T. A. Druzhkova, *Ontogenesis*, **19**, 573-581 (1988).
11. E. I. Belan, *Role of Cell-Cell Interactions in Transmition of Regenerative Signals during Bloodloss-Induced Reparative Erythropoiesis in Red Bone Marrow*, Abstract of Cand. Biol. Sci. Dissertation, Moscow (1992).
12. M. S. Blyakher, N. M. Gutorova, I. M. Fedorova, *et al.*, *Byull. Exp. Biol. Med.*, **121**, No. 3, 301-303 (1996).
13. E. I. Gimmel'farb, *Morphofunctional Characteristic of Immune System after Surgical Trauma of Mouse Kidney*, Abstract of Cand. Biol. Sci. Dissertation, Moscow (1990).
14. I. I. Dolgushin, L. Ya. Ebert, and R. I. Livshits, *Immunology of Trauma*, Sverdlovsk (1989).
15. T. A. Druzhkova, *Effect of Partial Splenotomy on Proliferation of Epitheliocytes in Nonlymphoid Organs*, Abstract of Cand. Biol. Sci. Dissertation, Moscow (1989).
16. Yu. I. Zimin, in: *Advances in Science and Technology. Immunology*, **8** (1979), pp. 173-198.
17. V. A. Kozlov, V. P. Lozovoii, and I. N. Zhuravkin, *Byull. Exp. Biol. Med.*, **83**, No. 3, 303-305 (1977).
18. N. A. Kraskina, E. M. Veger, N. M. Gutorova et al., Ibid., **105**, No. 4, 464-466 (1988).
19. A. Ya. Kul'berg, *Regulation of Immune Response*, Moscow (1986).
20. E. Kuper, *Comparative Immunology*, Moscow (1980).
21. R. Ya. Meshkova, *Graft-against-Host Reaction during Reparative Regeneration of the Spleen*, Abstract of Cand. Med. Sci. Dissertation, Moscow (1982).

22. S. S. Raitsina, *Trauma of Testicle and Autoimmunity*, Moscow (1970).
23. G. V. Kharlova, *Regeneration of Lymphoid Organs in Mammals*, Moscow (1975).
24. N. V. Yudina, *Reaction of Lymphoid Tissue to Damage and Regeneration of Organs with Different Regenerative Reaction,* Abstract of Cand. Biol. Sci. Dissertation, Moscow (1980).
25. N. V. Yudina, *Byull. Exp. Biol. Med.*, **88**, No. 12, 712-714 (1979).
26. A. G. Babaeva, in: *Recent Trends in Regeneration Research*, N.Y.-Lond. (1989), pp. 121-128.
27. M. D. Candia Carnevali, F. Bonasaro, A. Biale, *Cell Tiss. Res.*, **289**, 363-374 (1997).
28. M. Fox and G. E. Wahman, *Investig. Urol. (Berl.)*, **5**, No. 6, 521-538 (1968).
29. K. Hatakeyama, M. Hiramatsu, and N. Minami, *Experientia*, **36**, 1219-1220 (1980).
30. M. E. Pliskin and R. T. Prehn, *Reticuloendothel. Soc.*, **17**, No. 5, 290-299 (1975).