Action of Long-Chain Polymers on Kidney Juxtaglomerular Cells and Connective Tissue Mast Cells

By Aldo N. Corbascio, M.D.

With the technical assistance of Marta Keller

EVER since the kidney has been implicated as a major factor in the genesis of hypertension, search for morphologic alterations which could account for this relationship has been intensive. However, Castelman, Smithwick, and Palmer, who published, in 1941, the results of renal biopsies in 100 cases of hypertension, were unable to detect any specific abnormality except changes in blood vessels similar to those observed in other organs (intimal hyalinization, medial hypertrophy, endothelial hyperplasia). Other clinicopathologic studies have been equally nonrevealing.

The discovery by Ruyter, in 1925, of granular cells around the preglomerular artery of the kidney, juxtaglomerular cells (J-G cells fig. 1), and the production of renal hypertension by clamping of the renal artery or by constriction of the kidney by means of cellophane wrapping, furnished impetus for a great number of researches which extended over a period of 30 years and gave rise to a voluminous literature. One type of investigation which attracted considerable attention had to do with morphologic changes of the J-G cells. The studies of Goormaghtigh, Elaut, Donihue and Candon, Bohle, and the more recent contributions of Hartroft and Tobian have attempted to establish a link between the stages of granulations of these cells and hypertension in man and experimental animals.

A complete review of this subject can be found in the recent monograph by Hennebert. Most of this work, however, is entirely morphologic in nature, is unsupported by any physiologic evidence, and is strongly influenced by 1 or the other of the prevailing concepts of the cause of hypertension. We therefore decided to reinvestigate the problem in a more systematic and unbiased fashion.

The present study began as an attempt to elucidate, by histochemical methods, the nature of the cytoplasmic granulations contained in the J-G cells, their distribution in the kidney, and their possible presence in the vasculature of other organs. Subsequently, the action of some long-chain polymers (dextran and ovomucoid), which show a marked "granulolytic" action towards connective tissue mast cells in the rat, was utilized in connection with the J-G cells of rat kidneys in the attempt to confirm a possible analogy between these 2 types of elements. It was found that the J-G cells promptly lose their granulations when subjected to the action of dextran or ovomucoid. This response by connective tissue mast cells is attended in the rat by histamine liberation; therefore histamine assays of kidney cortical tissue were performed to establish whether histamine liberation would also occur in J-G cells after experimental degranulation.

Methods and Results

Fixation

After repeated trials with conventional chemical fixatives (Helly's fluid, Bouin, Ciaccio), we resorted to fixation by physical means, i.e., the freeze-drying technic of Altmann-Gersh. Accuracy of detail, absence of streaming artifacts due to the slow penetration of the fixative in the tissue, preservation in situ of cytoplasmic constituents, particularly...
granules, justify the relative complexity of the procedure.

Essentially, the method consists of the rapid immersion of the tissue specimen in an isopentane bath, cooled with liquid nitrogen to a temperature of —160 C, allowing an almost instantaneous freezing of all cytoplasmic water. The low temperature of the bath and the rapid heat exchange favored by the isopentane prevent the formation of large ice crystals in the tissue, thus preserving the integrity of cellular constituents while stopping all chemical reactions taking place within the cells. Immediately after freezing, the tissue is inserted in a closed system in which a high vacuum (10^-5) is maintained by means of an oil diffusion pump, coupled to a mechanical backing pump. The low pressure in the system allows the water in the tissue to sublime and condense on a cold trap situated at a short distance from the specimen. Over a period of several hours, the tissue loses all its water except a small fraction chemically bound to protein. Subsequently, the specimen is embedded in vacuo in degassed paraffin; it can thus be sectioned and directly applied to glass slides. After paraffin is removed, the sections can be stained directly or exposed for a short period of time to the action of a mordant (potassium dichromate or formaldehyde vapors). Staining time has to be considerably shortened, owing to the increased affinity of tissue proteins for histologic dyes.

Specimens fixed in the conventional way (Helly or Bouin), as well as frozen dried material, were concurrently used in our study in order to double-check our results and compare them with the work of others.

**Histochemical Techniques**

A systematic study with several histochemical methods was undertaken to gain a better insight into the nature of the cytoplasmic granulations of the J-G cells of the rat, rabbit, and cat kidney. Initially, we employed a simplification of Wilson’s method or the phosphotungstic hematoxylin of Mallory which, after chromic fixation, brings out the granules quite distinctly. In the course of our experience with material prepared with standard fixatives (Helly’s fluid), it was noticed that prolonged washing of the specimen after fixation would remove all traces of granules from J-G cells. When washing was omitted, visualization of J-G granules was more likely to succeed. The impression that the granules are readily soluble in water was strengthened by their susceptibility, during differentiation, to alcohol-water mixtures rather than absolute alcohol alone, as used in Wilson’s method. By varying the exposure to water-alcohol, it was possible to induce a change to red of the J-G granules which, after treatment with ethyl violet-scharlach rot (Bowie’s mixture), usually stain deep purple.

**Metachromasia**

This peculiarity, strongly indicative of a metachromatic effect, induced us to try 3 groups of dyes which specifically produce metachromasia, namely the thiazines (thionin, toluidine blue, polychrome methylene blue), the azines (neutral red), and some triphenylmethane derivatives (gentian violet, methyl violet). It was found that, following fixation with Helly or freeze-drying, J-G granulations could be promptly stained with a dilute solution of gentian violet (1:10^3), methyl or crystal violet, while staining with toluidine blue was positive at a neutral pH. A marked metachromasia was also obtained with
Juxtaglomerular cells of the rat kidney—normal stage of granulation. The specimen was fixed by freeze-drying and stained with Bowie’s stain (Wilson’s modification, X 600).

the Sylvèn modification of the Lyson method for mucopolysaccharides. Metachromasia is usually explained in terms of polymerization of the substrate attacked by the dye, which induces a structural change in its molecular arrangement, with formation of polymers that modify the absorption spectrum of the dye, thereby producing a different color. It is also known that water-alcohol mixtures reduce the metachromatic form of the dye to its original orthochromatic state, thus inducing a reversal of metachromasia. This could account for the change from purple to red seen in the J-G granules exposed to water-alcohol mixtures after staining with Bowie’s method.

These properties of J-G cells (water solubility, metachromasia, metachromasia reversal) are shared by connective tissue mast cells, which also have a granular cytoplasm. A series of parallel tests on kidney cortical tissue and tissues with a high mast-cell content were undertaken to elucidate the analogy further. Fragments of subcutaneous tissue from the snout and paws of rats and kidney cortices of the same animals were subjected to identical histologic manipulations and stained with dilute solutions of gentian violet, crystal violet, Lyson’s method for mucopolysaccharides (Sylvèn modification), or our simplification of Wilson’s method. In almost all instances, a successful staining of both J-G cells and connective tissue mast-cell granules was obtained (figs. 2 and 3), the only noticeable difference being the difficulty in staining J-G granules following alcoholic or lead acetate fixation. The presence of potassium dichromate in the fixative appeared to be practically indispensable for a good visualization of the J-G granules. A similar observation was reported by Harada, who suggested that this may be due to the presence in the J-G granules of a “chromotropic” lipid that requires for staining the action of a strong oxidizing agent like potassium dichromate.

Vital Staining

A further step towards establishing an analogy between J-G cells and connective tissue mast cells was accomplished by means of supravital staining of both elements with neutral red. It was found in rats that within 1 hour after an intraperitoneal injection of 1.5 ml. of neutral red saline solution, both J-G cells and connective tissue mast cells would selectively concentrate the dye in their cytoplasmic granulations. The uptake of neutral red does not seem to be due to phagocytosis or to precipitation of dye particles at some cellular boundaries, but rather to a specific affinity of J-G and mast cell granules that contain a molecular complex carrying a strong negative charge capable of binding the molecules of the circulating dye at its anionic sites. The metachromatic properties of the granules indicate that the substance most likely to be responsible for such an effect could be a sulfonated mucopolysaccharide ester of the heparin type, whose presence could also account for its multiple histochemical reactions. The presence of other nonsulfonated acid mucopolysaccharides like hyaluronic acid was carefully excluded by incubating kidney cortical tissue for 48 hours at 37 C., with high concentrations of testicular hyaluronidase (10,000 T.R.U. per ml.) at optimum pH. This procedure did not affect the staining properties of the J-G granules; mast-cellular granulations were also unaffected by the treatment.

These findings suggest that the J-G cells of
the kidney glomerulus contain a complex of sulfonated mucopolysaccharide esters of the heparin type associated with a "chromotropic" lipoid, which facilitates staining after fixation with chromium salts. The substance contained in the J-G granules is quite similar from the histochemical point of view to that present in the cytoplasmic granulations of connective tissue mast cells. Granules present in both types of elements can be stained supravitaly with neutral red. Incubation with a highly potent testicular hyaluronidase extract does not affect the staining properties of the J-G granules, indicating thus that their histochemical reactions are not due to presence of hyaluronic acid.

**Experimental Degranulation**

In order to secure additional information concerning the possible analogy between J-G cells and connective tissue mast cells, we performed a series of experiments in which both elements were subjected to the action of dextran or ovomucoid, two long-chain molecules which display a marked lytic action on rat mast-cell granules.

Dextran,* a glucose polymer formed by bacterial action from a sucrose-containing medium, attains a molecular weight of $4 \times 10^6$. It is sometimes used in therapy as a plasma expander, in which case only polymers of molecular weight, ranging from 35,000 to 150,000, are employed. Ovomucoid is a glycoprotein obtained from egg white by alcoholic precipitation after previous elimination of all other proteins by trichloracetic acid. Its molecular weight is about 29,000. After peritoneal or subcutaneous injection in rats, both compounds have been shown to produce a peculiar syndrome, the main features of which are a marked edema of the paws and snout of the animal. Selye, who first described this effect after ovomucoid injections, called it "anaphylactoid reaction." The mechanism of the anaphylactoid reaction following dextran administration was described and elucidated in detail by Halpern. This reaction is brought about by dextran in amounts as low as 30 mg./100 Gm. of body weight. The same picture is seen in dosages corresponding to 1 mg. of N/100 Gm. of body weight of ovomucoid. The subcutaneous injection of 1 of these compounds provokes within 20 minutes, a conspicuous edema of the paws, snout, tongue, and eyelids in the injected animal. The effect reaches its peak within 60 minutes and recedes after 4 to 5 hours.

We have been able to ascertain that the subcutaneous or intraperitoneal injection of dextran or ovomucoid, in doses which produce the anaphylactoid reaction, cause complete disruption of mast-cell granules with total loss of metachromasia and related histochemical reactions (fig. 4). In rats, sacrificed 60 minutes after an intraperitoneal injection of dextran, connective tissue mast cells are no longer visible; only in some areas is it possible to see a faint metachromatic halo around the nucleus of few scattered elements which have been able partially to escape the profound chemical injury caused by the 2 substances. Likewise, the intravenous administration of 0.5 ml. of a 6 per cent dextran solution in the superficial vein of the hind leg of normal, non-anesthetized rats provokes, within 30 minutes, complete degranulation of the J-G cells of the kidney (fig. 5). Degranulation of the J-G cells can be induced, also, by intraperitoneal and subcutaneous injections of dextran; the effect, however, is less prompt and complete.

---

*Abbott Laboratories, Chicago, Ill.

*Circulation Research, Volume VIII, March 1960*
Biological Assay

Riley and West have established that degranulation of mast cells by various agents will liberate considerable quantities of histamine which, together with heparin, are present in mast-cell granules. For this reason, we tried to detect, by means of direct histamine assays of subcutaneous tissue from rat skin and from kidney cortical tissue, before and after treatment with dextran or ovomucoid, whether or not a fall in tissue histamine occurred after "dextran-induced" degranulation of J-G cells and connective tissue mast cells.

Histamine assays, employing the atropinized guinea pig ileum according to the technic of Barsoom and Gaddum, modified by Code, were performed on skin and kidney cortex of normal albino rats. The kidney cortices, after careful stripping of the capsule and all remnants of perirenal fat (which may contain a considerable number of mast cells) and the removal of a thin fragment for microscopic examination, were ground with quartz sand and extracted with hot HCl. The homogenate was subsequently neutralized to pH 7 with NaOH, filtered, and suspended in phosphate buffer. The assay was performed on isolated segments of guinea pig ileum suspended in a 20-ml bath containing Tyrode solution at 37 °C, to which 1:10⁶ atropine per ml had been added. The histamine values found by this method of assay were rather low, averaging 2 µg./Gm. (range 3 to 15 µg./Gm.) of kidney cortical tissue. The values obtained for rat skin, treated in the same manner with the same assay, were much higher, averaging 67 µg./Gm. (range 58 to 75) (see table 1).

A slice of tissue from each skin and kidney specimen was subjected to dry-freezing and stained with toluidine blue at pH 7, or with our modification of Wilson's method which promptly stains both J-G cells and connective tissue mast cells. Counts per area of tissue were carried out under low power in order to establish a numerical relationship between the number of mast cells in the skin and J-G cells in the kidney and the respective histamine content of both tissues. We preferred to count cells in 10 contiguous fields from each section. The ratio between the total count and the number of fields gave us an approximate idea of the number of mast cells and J-G cells in that tissue, which somehow could be related to the histamine value obtained by the assay. The ratio between the mast-cell number for a given sample of skin agreed quite well with the histamine values. The correlation between J-G cells and histamine content was less obvious, owing to the low yields of hista-
Table 1
Histamine Content of Rat Skin and Kidney, Using Code's Method Employing Isolated Guinea Pig Intestine (20-ml bath containing Tyrode solution + 1:10^(-6) atropine/ml.)

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Skin histamine μg./Gm.</th>
<th>Kidney histamine μg./Gm.</th>
<th>Juxtaglomerular Mast cells number</th>
<th>Juxtaglomerular Mast cells number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal 6.5</td>
<td>2</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>2</td>
<td>Normal 7.2</td>
<td>1.5</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Normal 5.8</td>
<td>3</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>4</td>
<td>Normal 6.0</td>
<td>1</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>5</td>
<td>Normal 6.3</td>
<td>Not dosable</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>6</td>
<td>Normal 5.9</td>
<td>3</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>7</td>
<td>Normal 6.9</td>
<td>1.5</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>Normal 7.0</td>
<td>2</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>9</td>
<td>Normal 7.5</td>
<td>3</td>
<td>+++</td>
<td>***</td>
</tr>
<tr>
<td>10</td>
<td>Normal 7.0</td>
<td>2</td>
<td>+++</td>
<td>**</td>
</tr>
<tr>
<td>Average</td>
<td>6.6</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5.8-7.5</td>
<td>3-1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Histamine Content of Ovomucoid-Treated Rat Skin and Kidney, Using Code's Method Employing Isolated Guinea Pig Intestine (20-ml bath containing Tyrode solution + 1:10^(-6) atropine/ml.)

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Skin histamine μg./Gm.</th>
<th>Kidney histamine μg./Gm.</th>
<th>Mast cells degranulation and clumping</th>
<th>Juxtaglomerular cells degranulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>8</td>
<td>1</td>
<td>++</td>
<td>***</td>
</tr>
<tr>
<td>23</td>
<td>10</td>
<td>2</td>
<td>+++</td>
<td>*</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>1</td>
<td>++</td>
<td>*</td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>1</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>26</td>
<td>7</td>
<td>3</td>
<td>+++</td>
<td>**</td>
</tr>
<tr>
<td>27</td>
<td>5</td>
<td>2</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>28</td>
<td>7</td>
<td>—</td>
<td>—</td>
<td>***</td>
</tr>
<tr>
<td>29</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>**</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>1</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>31</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>***</td>
</tr>
<tr>
<td>Average</td>
<td>6.3</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>10-3</td>
<td>1-3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

mine obtained from the kidney (table 2). This may be due to the fact that the kidney contains little histamine, while it holds a large quantity of diamine oxidase which could promptly inactivate histamine as soon as it is liberated from the cells. Though special precautions were observed (all kidney homogenizations were carried out at low temperature), it is possible that significant amounts of histamine were lost during the procedure, owing to enzymatic inactivation. Moreover, if J-G cells contain histamine, the ratio between these cells and the remaining cortical parenchyma is such that, even under the best of conditions, their normal histamine content would probably border the threshold of sensitivity of the assay (0.5 μg./ml.).

Histamine assays, however, were repeated with the same technic on skin and kidneys of rats injected intraperitoneally with dextran (30 mg./100 Gm.) for 5 consecutive days. The results are reported in table 3.

We were, therefore, able to establish a definite correlation between the presence of granulated connective tissue mast cells and the histamine content of the skin, degranulation being followed by a significant decrease in histamine content. The results of assays of kidney cortical tissue were less impressive and statistically not significant, although a decrease in histamine content was suggested.

Discussion
The results of our investigation indicate striking analogies between J-G cells and connective tissue mast cells (figs. 2 and 3). The failure, however, to demonstrate a statistically significant decrease in histamine content of the kidney of rats treated with dextran or ovomucoid deprived us of a good way to show that J-G cells share an important biologic property with connective tissue mast cells.

Another discrepancy emerging in our experience with both types of cells was the fact that staining of J-G cells is somewhat more critical than connective tissue mast cells, being seldom successful without preliminary treatment with chromium salts, while toluidine blue staining of J-G cells was possible only at a neutral pH. From a morphologic viewpoint, it was noted that only in the cat kidney did J-G cells normally show enough coarse granulations to fill the entire cytoplasm, completely obliterate the nucleus, and give the "mulberry" appearance characteristic of connective tissue mast cells. Discrete stages of granulations are also seen, however, in connective tissue mast cells, as their morphology varies...
considerably with their location in tissues and organs and the hormonal influences to which the animal is exposed, such as stress, diet, parasitosis, etc. On the other hand, the well-known susceptibility of J-G cells to the manipulations which are unavoidable during the removal of the specimen from the kidney could account for the fact that the granularity is less intense than that seen in mast cells, which are embedded in a more elastic medium (connective tissue) and therefore can better withstand mechanical injury.

Other considerations of a more general nature, however, point to a remarkable similarity, if not identity, between J-G cells and connective tissue mast cells. In the course of our study, it was incidentally observed that granular cells with the same morphologic characteristics and histochemical affinities of J-G cells can be found in close relation to blood vessels elsewhere in the body (myocardium, spleen, tongue, and corpora cavernosa). It appears, therefore, that J-G cells are not a specialized feature of the kidney, but are a fairly common finding in the subintimal layer of arterioles in other organs. J-G cells also do not appear to be exclusively located around the afferent glomerular artery, as they can be seen sometimes in the efferent vessel as well (the criteria for distinction between afferent and efferent glomerular arteriole being extremely vague and based mostly on size differences which are, at best, difficult to evaluate).21

In the kidney itself it can actually be seen how some arterial segments, particularly the interlobar arteries, are surrounded over their entire length by strands of granulated cells with the same morphologic characteristics and histochemical properties as J-G cells (fig. 6). It appears, therefore, that the assumption that the J-G cells are the site of production of renal pressor or depressor substances is an unwarranted and restricted view of the possible functions of these cells, merely reflecting the overemphasis on the kidney as the *primum movens* of hypertension.

It would be logical, therefore, to assign to the J-G cells in the kidney a more general but no less important role than has been done so far. The type of evidence obtained in our study seems to indicate the mesenchymal nature of these elements and suggests that the substance contained in their cytoplasmic granules is in all probability a mixture of polysulfuric esters of mucopolysaccharides held in a protein moiety containing lipoids. Their behavior in the rat, when exposed to granulolytic substances like dextran or ovomucoid, is analogous to that of connective tissue mast cells. Further research intended to compare the rates of incorporation of 35S by mast cells and J-G cells by means of autoradiographic techniques will provide additional evidence.

It is appropriate, at this point, to speculate on the possibility that J-G cells may actually be identical with connective tissue elements of the mast-cell type and to consider the decisive role that cells of such nature might play at the origin of the vascular bed of the glomerulus. The presence at this site of mast-cellular elements, which are a unicellular storehouse of powerful pharmacodynamic agents like heparin, histamine, and serotonin, would open an entirely new approach to our understanding of glomerular circulation and to the whole physiopathology of the kidney. One of the open questions of renal physiology is the manner in which the cellular elements of blood,
subjected to a pronounced inspissation in the capillary loops of the glomerulus, escape coagulation and thrombosis. The release of heparin at this site could be of paramount importance to this end, while the presence of substances like histamine and serotonin could readily influence the rate of flow, permeability, and pressure in the glomerular loops, thus profoundly influencing the composition and rate of formation of the glomerular filtrate.

Summary

The histochemical nature and the biologic characteristics of the specific cytoplasmic granulations of the juxtaglomerular (J-G) cells of the kidney have been reinvestigated. In the rat, it appears that these cells are mesenchymal in origin and capable of reacting as part of the reticuloendothelial system when exposed to the action of long-chain polymers like dextran or ovomucoid.

The substance contained in the granules is probably constituted by a mixture of polysaccharide sulfate esters and lipoids, accounting for their staining properties with metachromatic dyes. Hyaluronic acid is not responsible for the latter reaction.

The J-G granules respond in a manner strikingly similar to that of mast-cell granules after treatment with dextran or ovomucoid. The attempt to quantitate the amount of histamine liberated by degranulation of J-G cells has been less successful than with degranulated connective tissue mast cells, owing to the low J-G cell/parenchyma ratio in the kidney.

The presence of mast-cell-like elements at the vascular pole of the kidney glomerulus is stressed in view of their possible role in the functional activity of the nephron, owing to the fact that degranulation of these cells is capable of releasing anticoagulant and vasomotor substances like heparin, histamine, and serotonin proximal to 1 of the most important circulatory districts of the body.

The finding of elements with analogous characteristics in the subintimal layer of arterioles in other organs raises the question as to whether J-G cells play a less specialized role in the kidney than hitherto supposed.
de lo rollo posible en la actividad funcional del nefron. Le fakto que debe esser rememorato in iste connexion es que le differagulation de iste cellulas es capace a liberar substantias anticoagulante e vasocative del typo de heparina, histamina, e serotonina in le proximitate de un del plus important districts circulatori del corpore.

Le constatation de elementos con caracteristicas analoge in le strato subintimal del artcriolas de altere organos subleva le question si le cellulas juxtaglomerular non ha possibilemente un rollo minus specialisato in le ren que lo que on ha supponite.

References
Action of Long-Chain Polymers on Kidney Juxtaglomerular Cells and Connective Tissue

Mast Cells

ALDO N. CORBASCIO

Circ Res. 1960;8:390-398
doi: 10.1161/01.RES.8.2.390

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1960 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:

http://circres.ahajournals.org/content/8/2/390

An erratum has been published regarding this article. Please see the attached page for:

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/