Comparative analyses of capsular fluid and interstitial fluid

H. HALJAMÄE, A. LINDE, AND B. AMUNDSON

Departments of Histology and Anatomy, University of Göteborg, Fack, S-400 33 Göteborg 33, Sweden

Comparative analyses of capsular fluid and interstitial fluid. Am. J. Physiol. 227(5): 1199-1205. 1974.—Interstitial fluid from rabbits was collected from implanted capsules and with a “liquid-paraffin cavity” technique. The fluids were analyzed for K⁺, Na⁺, Cl⁻, proteins, and glycosaminoglycans. The rate of transvascular exchange of albumin¹³¹I to the fluids was determined. The results show that the electrolyte composition is different from that of an ultrafiltrate of plasma due to the existence of negatively charged macromolecules in the interstitium. Thus the K⁺ and Na⁺ concentrations are higher and the Cl⁻ concentration is lower in interstitial fluid than in plasma. The observed protein content of the fluids was in agreement with expected interstitial protein levels. Furthermore, significant differences in electrolyte, protein, and glycosaminoglycan content between the capsular fluid and the directly sampled interstitial fluid are demonstrated. The exchange of labeled albumin was also extremely slow to capsular fluid as compared to interstitial fluid proper. These differences between the two fluids are discussed on the basis of current concepts of the interstitium and a suggestion of interstitial- and capsular-phase submicroscopic structure is presented. It is concluded that the compositional and functional characteristics of the directly sampled fluid fit the requirements of a hypothesized, true interstitial fluid far better than does capsular fluid.

MATERIAL AND METHODS

Elliptical perforated titanium capsules were deposited subcutaneously into the groins of adult male albino rabbits weighing 2-3 kg. Titanium was selected on the basis of good interface characteristics with living tissue. According to Levine (22) very little reactive granulation ensues upon deposition. The capsules were approximately 3 cm long, with a widest diameter of 1.5 cm. There were approximately 15 holes per square centimeter, each 1 mm in diameter. Prior to implantation, the capsules were boiled in concentrated orthophosphoric acid, carefully rinsed in distilled water, and sonicated in absolute ethanol before being autoclaved, and finally filled with sterile physiological saline. At the implantation of the capsules a subcutaneous tunnel was created by blunt dissection while bleeding was carefully avoided. The capsule was finally deposited approximately 5 cm from the incision in the skin. A minimum period of 3 wk was allowed for healing and no capsule was analyzed later than 6 wk after implantation.

Samples of the capsular fluid were obtained by puncture with a thin quartz pipette through one of the holes via a small incision in the skin. Prior to the sampling another quartz pipette in connection with external air was inserted upon expected Gibbs-Donnan distribution across the capillary wall, although the protein content was in agreement with a hypothesized tissue fluid (19). Therefore, it was suggested that charged anionic macromolecules in the interstitial ground substance could affect the distribution of cations in the local tissue fluid. In situations in which there is disturbance of the normal tissue perfusion and hydration it has also been possible to show, with this direct technique, that significant compositional changes concerning electrolytes occur within the interstitium (16, 18); these results have later been reproduced by others adapting the same technique (8).

The present study deals with comparative analyses of the composition and dynamics of capsular fluid and tissue fluid obtained with the liquid-paraffin cavity technique. The objective is to determine which fluid, if any, that in reference to found parameters, most readily fulfills the concepts of a “true” interstitial fluid. The results indicate that there are basic compositional as well as functional differences between the two fluids. The reasons for such differences are discussed and a critical evaluation of the possibilities of both employed techniques is presented.
in another hole to prevent pressure disturbances within the capsule during the sampling. Capsules showing signs of hemorrhagic content were excluded from analysis. Each capsule was sampled only once. Immediately afterward venous blood samples were drawn and subcutaneous tissue fluid was collected from corresponding contralateral sites with the liquid-paraffin cavity technique according to Haljamäe (17). With this technique nanoliter quantities of free tissue fluid from the subcutaneous fasciae could be obtained. The sampling was carried out under liquid-paraffin cover to prevent fluid changes due to contamination or evaporation. For the electrolyte studies very thin-pointed quartz pipettes were used. Usually about 50 nl but sometimes up to more than 100 nl of tissue fluid were obtained. The samples were handled on quartz plates under liquid paraffin.

Electrolyte analysis. The potassium and sodium contents of capular fluid, tissue fluid, and serum were determined by flame microphotometry according to Haljamäe and Larsson (20). The chloride content was determined with a silver-precipitation technique according to Haljamäe and Wood (21) with use of flame ultramicrospectrophotometry.

Protein determinations. Total protein content of the different fluid samples was determined according to Lowry et al. (24) using crystalline bovine serum albumin as standard. Albumin and globulin distributions in the tissue fluid, capsular fluid, and plasma were determined after electrophoretic separations. Those were run: a) on cellulose acetate strips in 0.1 M Tris buffer (pH 8.2) at 4°C for 30-40 min (10 V cm⁻¹) followed by amid-schwarz staining and microdensitometer scannings after the strips were made transparent (for details see ref 19), and: b) on polyacrylamide gels according to Ornstein (27) and Davies (9) on 5-mm cylindrical gels. A 5% spacer gel (pH 6.7) and a 7.5% running gel (pH 8.9) were used. After electrophoresis for 2 h (4°C, 130 V) either amid-o-schwarz or brilliant blue staining were used, and after 24 h destaining the gels were photographed and the photographic negatives were scanned.

Glycosaminoglycan content. Samples of capsular fluid and blood plasma were treated according to the following: 150 µl each of 11 different capsular fluid samples and 5 different plasma samples were freeze-dried. Remaining capsular fluid was pooled and freeze-dried. The former samples were digested for 8 h at 65°C with 20 µl papain (EC 3.4.4.10, 2 × crystallized; Sigma Chemical Company) in 1 ml of digestion buffer (0.1 M phosphate buffer, pH 6.5, containing 50 mM Na₂-EDTA and 5 mM cysteine-HCl). This was followed by precipitation with 10% trichloroacetic acid, dialysis overnight, precipitation with 4 vol of ethanol for 48 h, and finally centrifugation at 4,000 × g for 1 h and freeze-drying. From each sample, half of the crude glycosaminoglycan (GAG) pellet was hydrolyzed in a boiling water bath for 8 h in 6 M HCl; this was followed by freeze-drying overnight and quantitative hexosamine determinations with the Elson-Morgan reaction as described by Antonopoulos and Gardell (1). The remaining half of the crude GAG pellet was used for hexosamine acid determinations according to the method of Bitter and Muir (3).

The pooled capsular fluid sample was digested as above but in 2 ml of the digestion buffer. The crude GAG pellet obtained was dissolved in distilled water to give a final concentration of about 20 µg hexosamine per 50 µl. Such 50-µl samples were applied onto etiopropylidinium chloride-cellulose (CPC) microcolumns for separation of glycosaminoglycans (GAGs) according to Antonopoulos et al. (2) and Antonopoulos and Gardell (1), but with minor modifications. The columns were eluted at 27°C with 1-ml volumes of the following solutions: 1% CPC; 0.3 M NaCl containing 0.05% CPC; 0.275 M MgCl₂ containing 0.05% CPC; propanol-methanol-acetic acid-distilled water (40: 20: 20: 3.5 by volume) containing 0.04% CPC; 0.75 M MgCl₂ containing 0.05% CPC and 0.1 M acetic acid 0.75 M MgCl₂ containing 0.05% CPC; and finally 6 M HCl. After precipitation or evaporation, hydrolysis for 8 h in 6 M HCl, and freeze-drying of the separate fractions as above, the GAG content in the fractions was determined as hexosamine with the Elson-Morgan reaction.

It was not possible to perform similar analyses of the tissue fluid samples due to the very small sample volumes obtainable. To get some information concerning differences in the GAG content of capsular fluid and tissue fluid, the following approach was used. Equal nanoliter volumes of capsular fluid, tissue fluid, and blood plasma were applied as small dots onto cellulose acetate strips. Staining in 0.5% Alcian Blue 8GS (Chroma Gesellschaft, Stuttgart) was performed followed by destaining in several rinses of 5% acetic acid and the color intensities of the dots were compared.

Protein exchange. 3H-labeled human serum albumin (KHISA; from AB Atomenergi, Studsvik, Sweden) was given intravenously as an isotonic solution (0.5 ml). Repeated small blood samples (0.3 ml) were drawn to follow the disappearance rate from the vascular compartment. After various periods of time capsular fluid and tissue fluid were taken and the activity in known volumes was determined (Baird-Atomic scintillation counter). Each animal was sampled only once to prevent uncontrollable effects of previous samplings.

Pressure registrations. The pressure within the capsule was registered via a saline-filled needle connected to a fine-caliber mercury U-tube manometer.

RESULTS

Figure 1 shows the loose connective tissue lining the interior wall of the capsule. The connective tissue thus penetrates the holes and forms this lining, but within the central parts of the capsule a fluid pool always remained without any ingrowth of formed connective tissue.

In Table 1 the potassium, sodium, and chloride contents of the capsular fluid, tissue fluid, and serum are given. As can be seen from Table 1, the potassium content of both tissue fluid and capsular fluid was significantly (P < 0.001 and P < 0.05, respectively) higher than that of serum. The potassium content of the tissue fluid was also significantly (P < 0.01) higher than that of the capsular fluid. A similar difference concerning sodium also existed. Only the sodium content of tissue fluid, however, was significantly (P < 0.05) higher than that of serum. Concerning chloride, the reverse distribution was observed with higher serum values than those of capsular fluid or tissue fluid.

The fluid values in Table 1 include interindividual
TABLE 1. Potassium, sodium, and chloride contents in capsular fluid, tissue fluid, and serum

<table>
<thead>
<tr>
<th></th>
<th>Potassium (n = 8)</th>
<th>Sodium (n = 8)</th>
<th>Chloride (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsular fluid</td>
<td>3.91 ± 0.36†</td>
<td>145.4 ± 8.3</td>
<td>113.5 ± 2.4</td>
</tr>
<tr>
<td>Tissue fluid</td>
<td>4.67 ± 0.43†</td>
<td>151.5 ± 7.9*</td>
<td>103.1 ± 6.2</td>
</tr>
<tr>
<td>Serum</td>
<td>3.10 ± 0.64</td>
<td>143.8 ± 4.8</td>
<td>127.7 ± 7.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. †P < 0.05 vs. serum. ‡P < 0.01 vs. serum. §P < 0.001 vs. capsular fluid.

The above results show that the potassium and sodium contents of capsular fluid as well as of tissue fluid are higher than the corresponding plasma levels. The content of anions such as protein and chloride, on the other hand, is considerably lower in the two extravascular fluids. This indicates that there must be some other anion in the capsular and tissue fluids for electroneutrality. Since the extracellular space is known to contain GAGs which at physiological pH have a high negative-charge density, it was considered necessary to determine differences in GAG distribution.

A preliminary screening test, using application of small volumes of the fluids as dots onto cellulose acetate strips followed by Alcian Blue staining, was carried out. Such stainings showed higher contents of stainable material in capsular fluid than in tissue fluid, while only minute quantities of tissue fluid available. Albumin:globulin ratios as well as total protein content from all three compartments were, however, obtained through electrophoretic separation as shown in Table 3. The total protein content of tissue fluid was significantly (P < 0.01) lower than that of capsular fluid. The albumin:globulin ratios of the two fluids were similar, but considerably higher than the corresponding value for plasma (1.98).

The above results show that the potassium and sodium contents of capsular fluid as well as of tissue fluid are higher than the corresponding plasma levels. The content of anions such as protein and chloride, on the other hand, is considerably lower in the two extravascular fluids. This indicates that there must be some other anion in the capsular and tissue fluids for electroneutrality. Since the extracellular space is known to contain GAGs which at physiological pH have a high negative-charge density, it was considered necessary to determine differences in GAG distribution.

A preliminary screening test, using application of small volumes of the fluids as dots onto cellulose acetate strips followed by Alcian Blue staining, was carried out. Such stainings showed higher contents of stainable material in capsular fluid than in tissue fluid, while only minute quantities of tissue fluid available. Albumin:globulin ratios as well as total protein content from all three compartments were, however, obtained through electrophoretic separation as shown in Table 3. The total protein content of tissue fluid was significantly (P < 0.01) lower than that of capsular fluid. The albumin:globulin ratios of the two fluids were similar, but considerably higher than the corresponding value for plasma (1.98).

The above results show that the potassium and sodium contents of capsular fluid as well as of tissue fluid are higher than the corresponding plasma levels. The content of anions such as protein and chloride, on the other hand, is considerably lower in the two extravascular fluids. This indicates that there must be some other anion in the capsular and tissue fluids for electroneutrality. Since the extracellular space is known to contain GAGs which at physiological pH have a high negative-charge density, it was considered necessary to determine differences in GAG distribution.

A preliminary screening test, using application of small volumes of the fluids as dots onto cellulose acetate strips followed by Alcian Blue staining, was carried out. Such stainings showed higher contents of stainable material in capsular fluid than in tissue fluid, while only minute quantities of tissue fluid available. Albumin:globulin ratios as well as total protein content from all three compartments were, however, obtained through electrophoretic separation as shown in Table 3. The total protein content of tissue fluid was significantly (P < 0.01) lower than that of capsular fluid. The albumin:globulin ratios of the two fluids were similar, but considerably higher than the corresponding value for plasma (1.98).

The above results show that the potassium and sodium contents of capsular fluid as well as of tissue fluid are higher than the corresponding plasma levels. The content of anions such as protein and chloride, on the other hand, is considerably lower in the two extravascular fluids. This indicates that there must be some other anion in the capsular and tissue fluids for electroneutrality. Since the extracellular space is known to contain GAGs which at physiological pH have a high negative-charge density, it was considered necessary to determine differences in GAG distribution.
TABLE 2. Potassium, sodium, and chloride ratios for capsular fluid/serum and tissue fluid/serum; and mean absolute differences between capsular fluid and tissue fluid as compared to serum

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Capsular/serum</th>
<th>Sodium</th>
<th>Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 3)</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.28±0.19*</td>
<td>1.01±0.04</td>
<td>0.89±0.02</td>
</tr>
<tr>
<td>Average difference</td>
<td>0.76±0.45†</td>
<td>1.53±6.2</td>
<td>-14.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Tissue fluid/serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.54±0.15*</td>
</tr>
<tr>
<td>Average difference</td>
<td>0.15±0.05‡</td>
</tr>
</tbody>
</table>

Values are means ± SD. * P < 0.001 vs. ratio 1.0. † P < 0.001 vs. zero diff. ‡ P < 0.01 vs. ratio 1.0. § P < 0.01 vs. zero diff.

TABLE 3. Total protein and albumin/globulin ratios for capsular fluid and tissue fluid as compared to plasma

<table>
<thead>
<tr>
<th></th>
<th>Total Protein</th>
<th>Albumin/Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsular fluid</td>
<td>0.43 ± 0.03*</td>
<td>3.90 ± 0.28</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue fluid</td>
<td>0.32 ± 0.02</td>
<td>4.27 ± 0.43</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. * P < 0.01 vs. tissue fluid.

The albumin-131I studies were performed to get some functional exchange characteristics between plasma and the two different types of fluids. The results are shown in Fig. 2. The usual at least two-phase type of slope for the disappearance of albumin 131I from plasma was observed. The exchange to the capsular fluid was slow and after 6 hr only 0.62% of the simultaneous plasma activity was recovered. The exchange to the tissue fluid was much faster and already after 2 hr the corresponding recovery was 6.8% as compared to 0.20% for capsular fluid after the same time period.

The pressure registrations showed a negative pressure, on the average -2.9 cmH2O (SD ± 0.99). The titanium capsule used thus seems to have pressure characteristics similar to those previously reported for other types of implanted subcutaneous capsules.
DISCUSSION

Prior to a more detailed interpretation of the results of the present investigation it is necessary to try to analyze in greater detail the following question: "What is true interstitial fluid and to what extent is capsular fluid representative of such a fluid?" Until now there has been no generally accepted answer to this question. Because of the difficulties in sampling true physiological tissue fluid, the capsular technique was introduced as a possible approach to studies on interstitial dynamics, and there are studies in which capsular fluid is considered to be a true sample of interstitial fluid (5, 15, 23). The present results clearly demonstrate that there are basic differences between tissue fluid sampled according to Haljanac (17) and capsular fluid. Apparently they are not both true exponents of interstitial tissue fluid.

The concept "tissue fluid" is still not fully understood from morphological, compositional, and functional points of view. The early ideas from Starling's time involved the assumption of an existence of tissue clefts or slitlike tissue cavities with fluid, whereas later, a thin film of fluid around connective tissue fibers and cells was hypothesized (25). Manery herself (25) defined the interstitial fluid as "the extravascular or tissue space fluid which occurs between and surrounds all organized structures comprising a tissue."

She considers it a free fluid available for the solution of solutes and she points out that it should not be confused with formed or amorphous intercellular substances, such as connective tissue fibers or ground substance. In most discussions, however, due to lack of precise information, the whole interstitial phase is looked upon, from the functional point of view, in a deliberately simplified manner as a homogeneous and relatively inactive space. The original concepts suggested by Catchpole, Enge1s, Grish, Joseph, and Laskin (see ref 6) indicate, nevertheless, that more complex physiochemical and physiological properties are at hand explaining a participation of the interstitial space in the homeostasis of the tissues. Part of the functional characteristics of the interstitium seems to be governed by the content of nondiffusible aggregates of macromolecules such as glycoproteins and proteoglycans. The latter substances have isoelectric points at pH 2-3 and are therefore, negatively charged at physiological pH, constituting a considerable fixed net negative charge in the interstitium. The concentration and degree of aggregation of such nondiffusible colloidal material will affect the physiochemical characteristics of the interstitium and affect the distribution of all other diffusible cations and anions. As a result of physiochemical studies combined with approaches to the submicroscopic organization of the ground substance, Catchpole et al. suggested that the interstitial phase is a heterogeneous system. At least two phases, i.e., one "colloid-rich, water-poor" phase and one "colloid-poor, water-rich" phase, seem to coexist. These two phases are in functional equilibrium with each other. On the basis of this interstitial "two-phase system" hypothesis, it may be suggested that the organization of the studied interstitial phase is schematically according to Fig. 3. In Fig. 3A represents the colloid-rich, water-poor phase more in contact with the structural components of the connective tissue, i.e., different types of fibers and membranes. This phase contains a highly aggregated, water-insoluble ground substance. B represents the colloid-poor, water-rich phase containing smaller quantities of dis-aggregated and thus more soluble mucopolysaccharides (11). The interstitial phase is, therefore, conceived as a heterogeneous colloidal system in which an equilibrium is maintained between the two phases and in which phase B may be considered as the faster transport route through the interstitium (32). The interstitium interposed between the vascular bed and the cells is thus the true milieu interieur through which substances to and from cells have to pass. It is also obvious that due to the existence of relatively high amounts of negatively charged ground substance a milieu interieur with a composition different from that of plasma or an ultrafilterate of plasma is created.

Corresponding structural considerations concerning capsular fluid is schematically shown in Fig. 4. In this case an enormous artificially created pool of fluid exists, the exchange possibilities of which are limited to the capillary-containing tissue lining formed on the inner wall of the capsule. The capillary density per volume of interstitium must, therefore, be extremely low in the capsule in comparison to that in the interstitium proper. The absence of organized tissue within the capsular content also makes a submicroscopic organization of the ground substance into two different phases highly improbable and, therefore, a homogeneous distribution of ground substance according to Fig. 4 is suggested.

To what extent do the experimental data of the present study agree with the above-given hypothesis concerning "true" tissue fluid and capsular fluid? It may be assumed that during the sampling of tissue fluid mainly the colloid-poor, water-rich phase, being more movable, is collected, while in the case of the capsule, a fluid with a relatively homogeneous glycosaminoglycan distribution will be obtained. The GAG-specific Alcian Blue staining of equal volumes of the two fluids, as compared to plasma, is in agreement with such a difference. The content of stainable material is much higher in the capsular fluid than in the inter-
The binding capacity in the metabolism through this phase composition of the interstitial phase will also be affected by the exchange and distribution of products from cellular metabolic processes. Different charge density due to the coexistence of sulfate and carboxyl groups of the ground substance. The significantly higher K⁺ and Na⁺ content and lower Cl⁻ content of tissue fluid as compared to capsular fluid, however, do not agree with the demonstrated higher content of glycosaminoglycans in the capsular fluid. Apart from the obvious difficulties in obtaining samples from the colloid-rich, water-poor phase other possible reasons for such a distribution difference could be qualitative differences in the types of GAGs present and effects of adjacent cell metabolites on the binding properties. The capsular fluid mainly contained hyaluronate. According to other data on GAG content of skin, however, sulfated mucopolysaccharides should also be present (29). It is, therefore, probable that in the interstitium proper a mucopolysaccharide content qualitatively different from that in the capsular fluid is at hand. Unfortunately too small amounts of tissue fluid were available for such qualitative analysis in this study, but a different charge density due to the coexistence of sulfate and carboxylate groups in the tissue fluid is proposed. The composition of the interstitial phase will also be affected by the exchange and distribution of products from cellular metabolism through this phase (10), contributing to a different binding capacity in the tissue fluid as compared to capsular fluid, as the latter fluid, because of the enormous distance to cellular tissue, will be much less affected by local cellular metabolic processes.

The observed protein levels of both fluids are in agreement with previously reported values for interstitial fluid (7, 12, 19, 26), but a significant difference in total protein content also existed between capsular fluid and tissue fluid; this further demonstrates basic compositional differences. The most striking difference, however, was observed concerning albumin-¹³¹I exchange. It has previously been shown that the disappearance rate of proteins injected into a capsule is very low. Injection of a 5% protein solution has been shown to result in a remaining capsular protein concentration as high as 4.1% 1 mo later (13). The passage of intravenously deposited albumin into and through the interstitium proper has quite different equilibrium characteristics. Already 7-10 min after injection labeled albumin appears in the thoracic lymph, indicating a passage through interstitial spaces (31). Equilibrium time between plasma and lymph is in the range of 7-13 h. In the cutaneous tissue an equilibration time of 13 h has been reported (30) and also a two-phase distribution in agreement with different equilibrium rates for the colloid-poor, water-rich and the colloid-rich, water-poor phases. In the present study the exchange rate of albumin-¹³¹I was very low to the capsular fluid. After 2 h the demonstrable activity in the interstitial tissue fluid was 34 times higher than that in the capsular fluid. Assuming that the albumin that has disappeared from plasma during the 2 h is relatively equally distributed within the extravascular space, one may calculate the approximate volume of distribution. In the case of interstitial tissue fluid the extravascular space 4.7 times that of the plasma volume was obtained. This figure agrees rather well with accepted physiological dimensions of the volume of the interstitial phase. These results also indicate that the tissue fluid obtained with the liquid-paraffin cavity technique cannot be influenced to any significant extent by transvascular exudation caused by the sampling procedure (19). Corresponding calculations using the capsular fluid activity after 2 h yields an interstitial phase volume 160 times that of plasma volume, indicating that the capsular fluid pool is far from equilibrium at this point. The capsular fluid can thus not be considered to participate sufficiently rapidly and actively in exchange processes to be representative of real interstitial tissue fluid.

Although the capsular fluid does not represent an entirely inactive collection of fluid, the present results indicate that factors such as capillary density per unit volume of fluid, lack of submicroscopic organization of the ground substance, and large distances to actively metabolizing cells make it an insufficient approach for functional studies of the interstitium. The fluid sampled with the liquid-paraffin cavity technique seems more suitable for such studies. A major drawback is, however, the very small quantities obtainable. On the basis of the presented evidence and discussion, it is our conviction that the tissue fluid obtained with this latter technique is representative of the colloid-poor, water-rich movable phase of the interstitium, and that this is the fluid which most truthfully corresponds to the concept of interstitial tissue fluid.

This work was supported by grants from the Swedish Medical Research Council (Project B74-17X-127-10) and from Göteborgs Läkaresällskap.

Received for publication 19 November 1973.
REFERENCES


