as adenomas and the other one as an adenocarcinoma. Grossly and histologically these tumors were identical to those described by other investigators[13,14]. Other tumors. In a number of instances other types of neoplasms were seen in the various groups shown in table 2. Since they occurred in low incidences, their appearances cannot be attributed to the treatment.

Discussion. The present findings demonstrate that lifelong administration of 0.0625% 1,2-DBH in drinking water to 6-week-old Swiss mice produced tumors of the lungs, lymphoreticular tissue, and kidneys. In treated females, the tumor incidences in these 3 tissues were 72 ($p > 0.000001$), 42 ($p > 0.003$), and 0% respectively, while in the treated males, they were 76 ($p > 0.000001$), 12 and 8% ($p > 0.03$), respectively. In untreated controls the corresponding tumor incidences were 25, 18 and 1% in females and 26, 8 and 0% in males. Statistical analysis was carried out by Fisher's exact probability test for 2×2 tables[15]. Histopathologically, the tumors were classified as adenomas and adenocarcinomas of the lungs, various types of malignant lymphomas, and adenomas and adenocarcinoma of kidneys. The current study is an integral part of our structure activity relationship inquiry. Specifically its aim is to reveal whether the disubstituted derivatives of hydrazines are more active carcinogens than the monosubstituted derivatives. In an earlier experiment *n*-butylhydrazine hydrochloride, a monoalkylhydrazine, administered as a 0.0125% solution in drinking water to Swiss mice induced only lung tumors[6]. The presently reported 1,2-DBH elicited the development of tumors of the lungs, lymphoreticular tissue, and kidneys. Therefore it seems evident that this dialkylhydrazine analogue is a stronger cancer-inducing agent than the corresponding monoalkyl derivative, since 1,2-DBH gave rise to tumors in 2 additional tissues at a lower dose. Previous investigations have clearly shown that symmetrical and unsymmetrical dimethylhydrazines are much more potent carcinogens than monomethylhydrazine[1-3]. Formylhydrazine, however, roughly exhibited tumors similar in type and incidence to those induced by 1,2-diformylhydrazine[4,5]. This study confirms the earlier findings that dialkylhydrazines are more potent carcinogens than the corresponding monoalkyl analogues. This does not appear to be true for mono- and disubstituted hydrazides however since formyl- and 1,2-diformylhydrazine exhibit similar activity. Further studies in this area are currently in progress. Hydrazines, hydrazides and hydrazones are known tumorigenic agents in laboratory animals. To date, well over 40 such compounds were shown to induce tumors in more than 2 dozen organs and tissues of mice, hamsters and rats[16,17]. The human population is exposed to approximately one-half of these chemicals in the form of drugs, agricultural herbicides, industrial chemicals and as naturally occurring ingredients of edible mushrooms and tobacco[18-23]. Therefore, this group should be considered an environmentally important class of chemicals.

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Ultrastructural study of patch-graft re-endothelialization[1]

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Summary. On the 8th post-operative day, an endothelial layer covers the 'venous patch' luminal part when the adventitia of the patch is sutured to a 5–8-mm-long arteriotomy performed in the common carotid of the rat. The probable origin of this secondary endothelium is discussed.

During the first post-operative hours, the endothelium of 'venous patch' grafts undergoes great alterations which lead to its disappearance by desquamation[2]. In areas where the endothelium has disappeared, the subendothelial connective tissue, especially the collagen fibres, is consequently in direct contact with the vascular lumen and becomes a thrombogenic surface where thrombocytes and fibrin can easily be deposited[3]. Great damage must occur in the endothelium in order to stimulate re-endothelialization[4]. The 'venous patch' re-endothelialization is completed during the 5th and 6th days when the endothelium of the venous patch is oriented toward the arterial lumen[2].

The controversy about the origin of secondary endothelial cells has not yet been solved. Some investigators have proposed that endothelial cells of the host vein may be responsible for the secondary endothelium (endothelial re-endothelialization)[4-13], while smooth muscle cells have been reported to originate secondary endothelial cells of the venous patch (muscular re-endothelialization)[14-22] and other studies[23-28] suggest that hematogenous re-endo-

thelialization exists due to leucocytes in the blood stream. To clarify this controversy, we performed experiments with venous patch grafts in arteries 1 mm in external diameter, with the patch adventitia oriented toward the arterial lumen.

Material and methods. In 20 Wistar rats weighing about 200 g, surgery was performed with the aid of an operating microscope on the common carotid artery (external diameter 1 mm), which was longitudinally incised for 5–8 mm. An autogenous venous patch, taken from the external saphenous vein, was grafted to the carotid incision using interrupted sutures with 10-0 Nylon monofilament. The animals were sacrified at different times during the first 2 weeks after surgery, under Nembutal i.p. anaesthesia, and fixation was done by retrograde perfusion through the abdominal aorta with a mixture of glutaraldehyde and paraformaldehyde[29]. For conventional electron microscopic study, the venous patches and adjacent arterial wall were then fixed for 2 h in an osmium tetroxide solution (2%) in a cacodylate buffer (0.1 M, pH 7.2). The specimens were dehydrated in graded alcohols and treated either with propylene oxide or contrasted in a block with uranyl acetate before dehydratation. The tissues were embedded in epon 812. Semithin sections of about 0.5 µm were cut with a glass knife and the most interesting areas were cut with a diamond knife prior to examination with a Phillips 301 electron microscope. For the SEM study, the specimens were dried with CO_2 at the critical point[30]. Following gold coating, the luminal part of the venous patch and the adjacent arterial area were viewed through a JEOL JSM 50A electron microscope.

Results and discussion. It is known that the venous patch re-endothelialization process takes longer when the adventitia is in contact with the blood (seen on the 8th post-operative day) than when the intima is oriented towards the arterial lumen (seen on the 6th day)[2]. On the 5th post-operative day, a quantity of endothelial cells are observed, which move concentrically forward to the venous patch from the proximal arterial endothelium, crossing over thrombus residues previously formed in the suture line (fig. 1). 6 days after surgery, arterial endothelial cells can be distinguished from those of the venous patch. Indeed the endothelial cells covering the venous patch are polygonal and present a flattened luminal surface, while the arterial endothelial cells are fusiform, rising toward the vascular lumen (fig. 2). The venous patch endothelial cells show ultrastructurally a long and irregular nucleus with condensed chromatin in the periphery (fig. 3). The cytoplasm is moderately rich in RER. Microfilaments appear in the luminal and abluminal parts of the cytoplasm. Microfilaments and microtubules

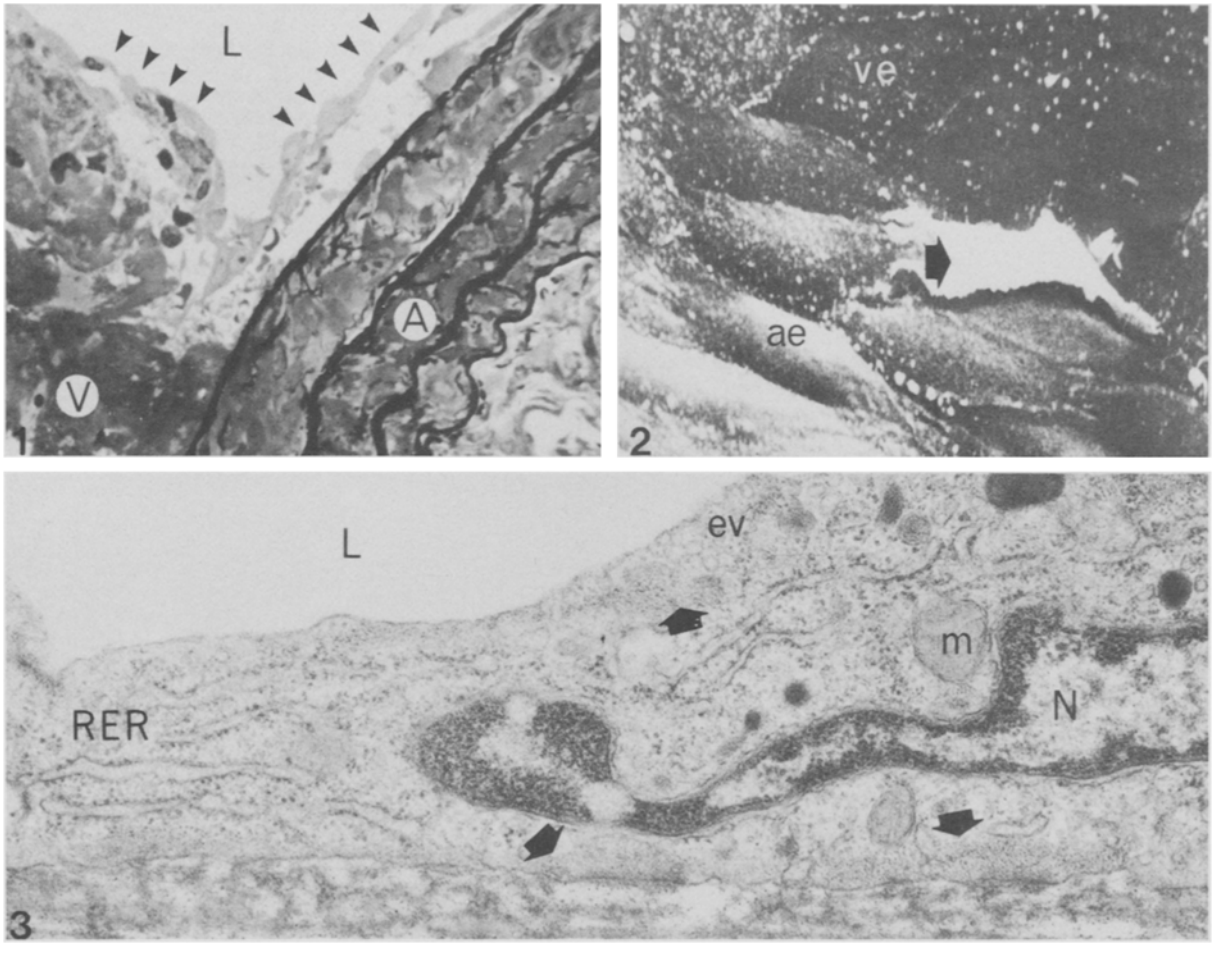

Figure 1. Confluence area between artery (A) and venous patch (V). Arrows point out the endothelium. L, lumen. Rat in the 5th day after surgery. × 1380.

Figure 2. Rat in the 6th day after operation. ae, arterial endothelium; ve, venous endothelium; an isolated (primary?) endothelial cell is pointed out by the arrow. × 2500.

Figure 3. Rat in the 6th day after operation. N, nucleus; RER, rough-surfaced endoplasmic reticulum; m, mitochondria; ev, endocytotic vesicles. Arrows indicate the microfilaments in the luminal and abluminal part of the endothelial cytoplasm. × 35,500.

seem to be prerequisites for the maintenance of cell shape and polarity of cell movement in relation to the substratum[31]. Endocytotic vesicles exist, mainly in the cytoplasm luminal part. It is known that the plasminogen activator is associated with vascular endothelium[32] and is released from these cells into the surrounding medium[33]. It is now generally accepted that this is the mechanism by which intravascular fibrin is lysed, so it can be thought that re-endothelialization of venous patch graft may be necessary for lysis of deposited fibrin in the vascular graft wall. Recently[34] endothelial cells have been shown to produce a growth factor in vivo which may play a part in the induction of smooth muscle cell movement and differentiation. Our recent work confirmed such a role of endothelium[2], for the formation of a new endothelium is immediatly followed by the appearance of smooth muscle cells in the venous patch wall.

Present findings do not indicate that the endothelial cells which cover the venous patch after the 2nd postoperative week originate from the smooth muscle cells of the patch, since the thrombus formed on the luminal part of the patch prevents cell migration from the medial layer of the patch wall, although a hematogenous re-endothelialization may occur at the level of the suture line[25]. Our results indicate that secondary endothelial cells originate mainly in the adjacent arterial endothelium. Future studies using radioactive labelling should clarify this postulate.

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Action of group A streptococcus extracellular product(s) on the connective tissue of the human heart valve

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Summary. The cultivation of a 'rheumatogenic' strain of group A streptococcus in presence of human heart valve connective tissues leads to the release of substance(s) reacting with antistreptococcal polysaccharide and antihuman glycoprotein antibodies.

We have previously reported[1] that when certain group A streptococcus strains, isolated from rheumatic patients, were grown in the presence of bovine heart valves, the culture supernatants contained substance(s) showing immunological cross reactions with rabbit antisera to both group A streptococcal polysaccharide and a structural glycoprotein isolated from the connective tissue of bovine heart valves. This observation supports the hypothesis that an immunological mechanism may be involved in cardiac lesion pathogenesis in rheumatic fever. However, to uphold this view, it was necessary to show that similar results could be obtained with human heart valves; the present study reports on these complementary experiments.

Material and methods. Materials, methods and techniques were described in detail in a previous publication[1]. Strain A 5205, type 5, was cultivated in phytone-yeast medium, as specified. Human heart valves were obtained during necropsy on patients deceased with no known cardiac disease. Approximately 4 g of valves were dissected free of cardiac muscle, cut into small pieces, sterilized with β-propiolactone[2] and divided equally between 2 diffusion chambers, prepared as described previously[1]. Four 250-ml vials of phytone-yeast medium were prepared and inoculated as shown in the table. The vials were incubated at 37 °C for 62 h. The cultures were centrifuged and the supernatants precipitated by acetone[3]. Human urea-soluble glycoprotein was prepared as previously described by Goldstein et al.[1], using human, instead of bovine, heart valves. Rabbit antisera to group A streptococcal polysaccharide (a/PS) and to human glycoprotein (a/GP)