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AN INVESTIGATION INTO THE IMMUNOGENICITY OF VARIOUS COMPONENTS OF OSTEOARTICULAR GRAFTS

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Summary.—Rats have been grafted subcutaneously with heterografts of sheep osteoarticular tissues. Antibody against sheep transplantation antigens has been sought. The results of the studies indicate that articular cartilage alone is poorly immunogenic and if it does lead to an immune response, this response is delayed; it is the bone components of the composite osteoarticular grafts which are highly immunogenic and in most recipients cause a brisk production of antibodies.

It is suggested that the cartilage matrix forms a protective barrier to both the afferent and the efferent arms of the immune response.

OSTEOARTICULAR allografts have been the subject of study in recent years, but the results of these studies have been somewhat variable. In animal experiments some groups have found that a high proportion of allogeneic osteoarticular grafts survive for over two years (Chesterman, 1968; Hamilton, Barnes and Gibson, 1969; Pap and Krompecher, 1961). Others have found that degenerative changes and resorption of cartilage did occur in a majority of grafts (Campbell *et al.*, 1963; De Palma, Sawyer and Hoffman, 1962; De Palma *et al.*, 1963; Silver, 1969). In the human subject, osteoarticular allografts have been used at the hip and knee with encouraging results (Laurence, 1969; O'Garra, 1969; Pap and Krompecher 1961).

The reason for the failure of osteoarticular grafts is at present not clear. Clearly mechanical factors will play some part in determining the success or otherwise of a graft. It has been suggested that immunological factors play a part in the resorption of grafted cartilage (Silver, 1969; De Palma *et al.*, 1963). This aspect has, however, received little attention. In the sheep, osteoarticular grafts to the femoral condyles have been found to lead to the production of cytotoxic antibodies in the serum of the recipient (Elves, 1971; Elves and Ford, 1971).

To ensure union of graft with its bed, and to achieve mechanical stability, these grafts must consist of bone, with its contained marrow, as well as articular cartilage. They are therefore composite tissue grafts. The source of antigen in these grafts is not known and therefore it was decided to investigate the ability of each component of the graft to elicit an immune response (*i.e.* their immunogenicity). In order to achieve a maximum degree of antigenic disparity between host and donor, and so giving maximum opportunity for an immune response, the heterograft situation was chosen for this study.

MATERIALS AND METHODS

Heterografts of osteoarticular tissues from sheep were transplanted into rats and the humoral antibody response of the recipients against the grafts was studied.

Recipients.—Female rats of the AS strain (3–5 months old) were used as recipients for the grafts. Grafts were placed in a subcutaneous pocket prepared in the back of the recipient animals by separating the dermis from the panniculus carnosus muscle. The incision was then closed by means of 3 Michel clips.

Grafts.—The femoral condyles were removed from the knees of 3 female Romney Marsh sheep aged about 6–7 years. Bone was removed by means of an air powered burr until a thin shell approximately 2 mm of bone remained beneath the articular cartilage. The shell was then cut into pieces measuring approximately 4 mm^2. Some of these blocks were used without further modification as whole composite grafts (WCG). Other blocks were washed in a stream of sterile distilled water at a pressure of 15 p.s.i. in order to remove as much red marrow as possible (marrow-free whole grafts, MFWG). In order to prepare grafts of articular cartilage alone (CG), slices of cartilage were removed which consisted of only half the complete cartilage depth. This is important, as in sheep bone marrow cavities can be seen to penetrate the subchondral bone and the articular cartilage at least as far as the calcified zone. In order that the amount of cartilage received by recipients of CGs should be comparable with that received by recipients of WCG or MFWGs, each rat received 2 CGs inserted into the same pocket. The bone remaining after the preparation of the CGs was then scraped with a scalpel until the remaining cartilage was removed and used as bone grafts (BG). Some BGs were washed with distilled water to form marrow-free bone grafts (MFBG).

Antibody studies.—Blood samples were obtained from the tail vein of recipient animals before operation, and at 2–3 day intervals until Day 18; thereafter blood samples were obtained at 4, 5, 6 and 7 weeks after grafting. Serum obtained from the blood was decomplemented by heating at 56° for 30 min. Cytotoxic antibodies against sheep lymphocytes were then sought for in each serum sample using a fluorochromatic cytotoxicity test (Elves. 1973). Each serum was tested on at least 3 occasions and if positive reactions were obtained in 2 out of 3 replicate tests the serum was considered to contain antibody. Most sera, however, gave consistent results in all replicate tests.

Histological techniques.—After the last blood sample had been taken the animals were killed and the grafts were recovered. These were then fixed in formol saline, embedded in paraffin and 6 μm sections cut, which were stained with alcian blue/haematoxylin.

RESULTS

The results of the examinations of serial serum samples for anti-sheep lymphocyte antibodies are summarized in the Table and Fig. 1.

TABLE.—*The Production of Antibodies against Sheep Lymphocytes by Rats Following Transplantation of Bone and Cartilage—Summary of Data*

Group	Graft type†	No. in group	% developing antibody	% developing antibody late (*i.e.* after 4 weeks)	% with antibody at 8 days
I	WCG	16	75	6·3	5/16 (31·3)
II	MFWG	11	81·8*	9·1	2/10 (20)
III	Cartilage	17	23·6*	11·8	0
IV	BG	14	100*	14·3	3/13 (23)
V	MFBG	11	72·7*	18·2	1/10 (10)

* Includes rats which had pre-existing antibody.
† See text for details.

Group I—Recipients of whole composite grafts

Sixteen rats received transplants of whole grafts in the course of 3 experiments. Although antibody was detected in the serum of one animal as early as 4 days

after grafting, it was not until 2 weeks later that the incidence of antibody in the animals reached its peak (62·5%). Four rats did not develop any detectable antibody during the course of the experiments, and in a further animal antibody did not appear until 7 weeks after grafting.

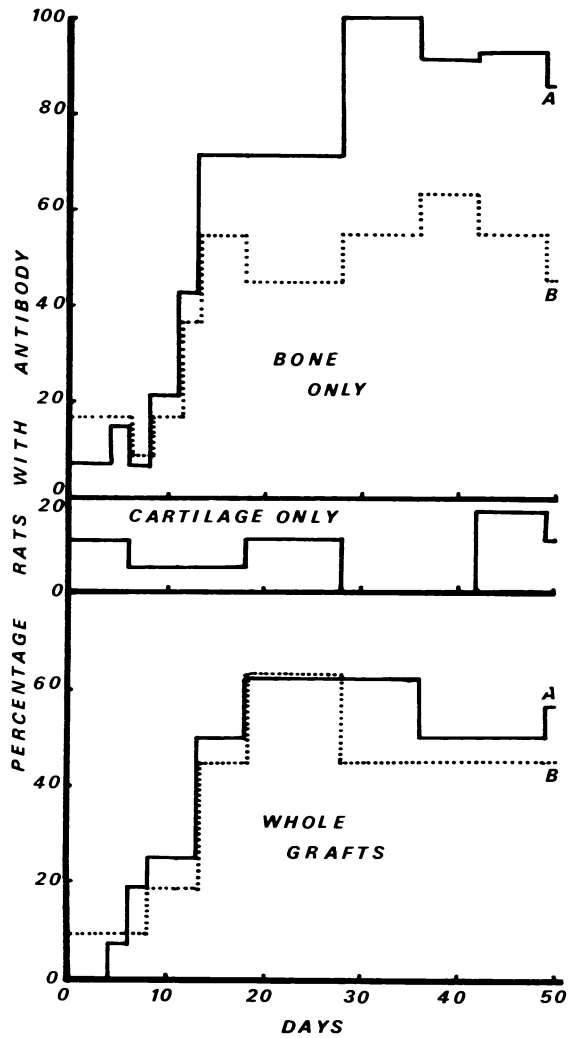


FIG. 1.—The development of anti-sheep antibodies in rats receiving osteoarticular tissue grafts or various components thereof. Dotted = grafts from which marrow has been washed out.

Group II—Recipients of marrow-free whole grafts

Eleven rats in 2 experiments received marrow-free whole grafts. One animal in this group was found to possess naturally occurring anti-sheep lymphocyte antibodies before grafting. These antibodies disappeared shortly after grafting, probably due to absorption by antigens in the graft, but antibodies reappeared

on the 18th day. Two rats in this group did not develop any antibody during the course of the experiment. Although antibody had appeared in one animal by the 6th day after operation, the peak incidence of antibody did not occur until the 18th day (63·6%). One animal showed a late response, having antibody in its serum by the 7th week.

Group III—Recipients of cartilage alone

In 3 experiments 17 rats received grafts of cartilage alone. Two rats showed the presence of anti-sheep antibodies before grafting. In one case this was a weak antibody and was not detectable by the 6th day after grafting. In the second case the antibody persisted up to the 18th day after grafting, then disappeared only to reappear again at the 7th week. Only one rat in this group showed antibody production against the sheep tissue during the first 5 weeks of the experiment; this response was, however, short-lived but reappeared at 6 weeks. A further 2 rats also developed antibody after 6 weeks and in both of these animals this was transitory.

Group IV—Recipients of bone grafts

In 3 experiments a total of 14 recipients of bone grafts were studied. One animal in this group possessed antibody against sheep lymphocytes before grafting. By the 6th day this antibody had been removed from the serum but reappeared a week later. No rat in this experiment failed to develop antibody against the graft, although in 2 instances the antibody did not appear until after the 4th week.

Group V—Recipients of washed bone grafts

In 2 experiments 11 rats received marrow-free bone grafts. Two rats possessed antibodies against sheep cells before grafting and in both instances these antibodies had disappeared transiently by the 6th day after operation. Three animals failed to develop antibodies against sheep leucocytes. In 2 rats antibody did not appear until the 4th week after grafting. In the remaining 6 rats antibody was present in the serum within 2 weeks of grafting.

Histological observations

Appearance of cartilage.—The histological appearance of the cartilage in those grafts which possessed this tissue was extremely variable, both from graft to graft and also between different areas of the same graft. There was no significant difference in appearance between those grafts in which the cartilage was associated with bone, and those which consisted entirely of cartilage. In most grafts there was some degree of surface fibrillation and loss of metachromasia from the matrix (Fig. 2a). Well-stained chondrocytes were seen in all grafts but to a variable extent. In some instances these cells were seen only in the deeper areas of the graft. Most grafts showed areas of dead, pale staining cells or empty lacunae and nesting, or clustering, of chondrocytes was a feature often seen (Fig. 2b). No distinction could be made between those grafts which had resided in animals possessing anti-sheep antibodies and those from animals without antibody.

All grafts were surrounded by inflammatory tissue. The tissue in contact with the cartilage consisted of large numbers of small lymphocytes with fewer larger, pale staining histiocytic cells and fibroblasts (Fig. 2c). Plasma cells were

not seen in this tissue. In some grafts lymphocyte-like cells were seen in the areas of cartilage fibrillation passing down the clefts in the matrix. There was no evidence to suggest the presence of lymphocytic cells within the cartilage matrix. Some cells with small dark nuclei could be seen beneath the cartilage surface but these were probably chondrocytes as they were always surrounded by the characteristic lacunae.

In one animal receiving whole cartilage, the graft was found to possess a thin layer of subchondral bone. This was one of the animals in this group which developed antibody.

Appearance of bone.—Of 52 animals receiving bone-containing grafts, new bone in small amounts was seen in only 2 cases (Fig. 3a). The bone marrow spaces in these grafts were filled with either a loose areolar tissue or inflammatory cells. The latter showed distinct differences from those cells seen in association with cartilage: whereas in the latter sites the small lymphocyte was prominent, the plasma cell was most often seen in the inflammatory tissue in contact with bone (Fig. 3b). Giant cells were also seen in relation to the bone grafts in most animals (Fig. 3c).

DISCUSSION

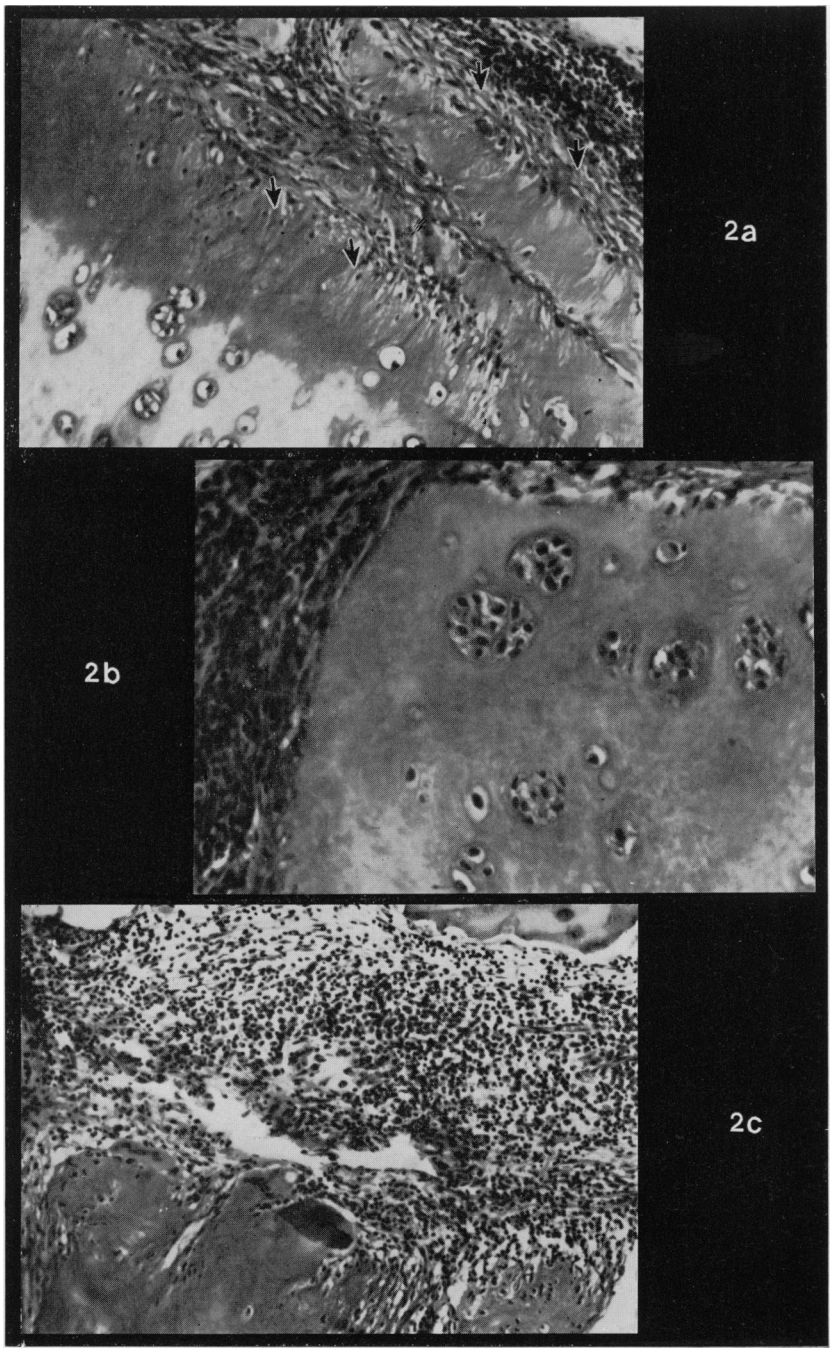
The results presented above clearly show that articular cartilage alone is very poorly immunogenic and does not arouse a humoral immune response. In only 3 out of 17 animals receiving cartilage grafts was there any evidence of a primary immune response. In the present experiments the cartilage graft in one of the 3 recipients which developed antibody did carry a thin layer of subchondral bone and so could not be considered as a purely cartilage graft. Its immunogenicity may have resided in this subchondral bone and not in the cartilage.

These findings are in agreement with those of some other workers. Peacock, Weeks and Petty (1960) implanted allogeneic xiphoid cartilage into mice and could not find evidence of sensitization of the host using test skin grafts. Stjernsward (1965) using similar grafts found evidence of sensitization, using subsequent skin grafts; he also was able to detect haemagglutining antibodies in the serum of host animals. However, xiphoid cartilage is not entirely hyaline cartilage and it possesses a substantial element of fibro-cartilage. Craigmyle (1958*a*) used diced costal cartilage as allografts in rabbits and found evidence of sensitization, again using skin grafts, in only 28% recipients. Lymph node changes characteristic of the cell mediated immune response were, however, not found in these animals (Craigmyle, 1958*b*). These changes occurred in nodes of rabbits receiving heterografts of costal cartilage or second-set cartilage allografts. There have been no

EXPLANATION OF PLATES

FIG. 2.—(a) Section through cartilage graft showing surface fibrillation (arrowed), lymphocyte invasion and loss of metachomasia. × 100. (b) Section through cartilaginous aspect of graft showing clustering of chondrocytes. × 165. (c) Section showing the nature of the inflammatory reaction tissue in contact with the cartilage of graft. The predominant cells are small lymphocytes. Haematoxylin, eosin and alcian blue. × 100.

FIG. 3.—(a) Section showing small amount of new bone formation in the bony aspect of a graft (arrowed). × 100. (b) Plasma cell reaction in an intertrabecular space of a bone graft. × 430. (c) Section showing the nature of the inflammatory reaction tissue associated with the bony aspects of a graft. Histiocytes, lymphocytes and giant cells (arrowed) are seen. Haematoxylin, eosin and alcian blue. × 165.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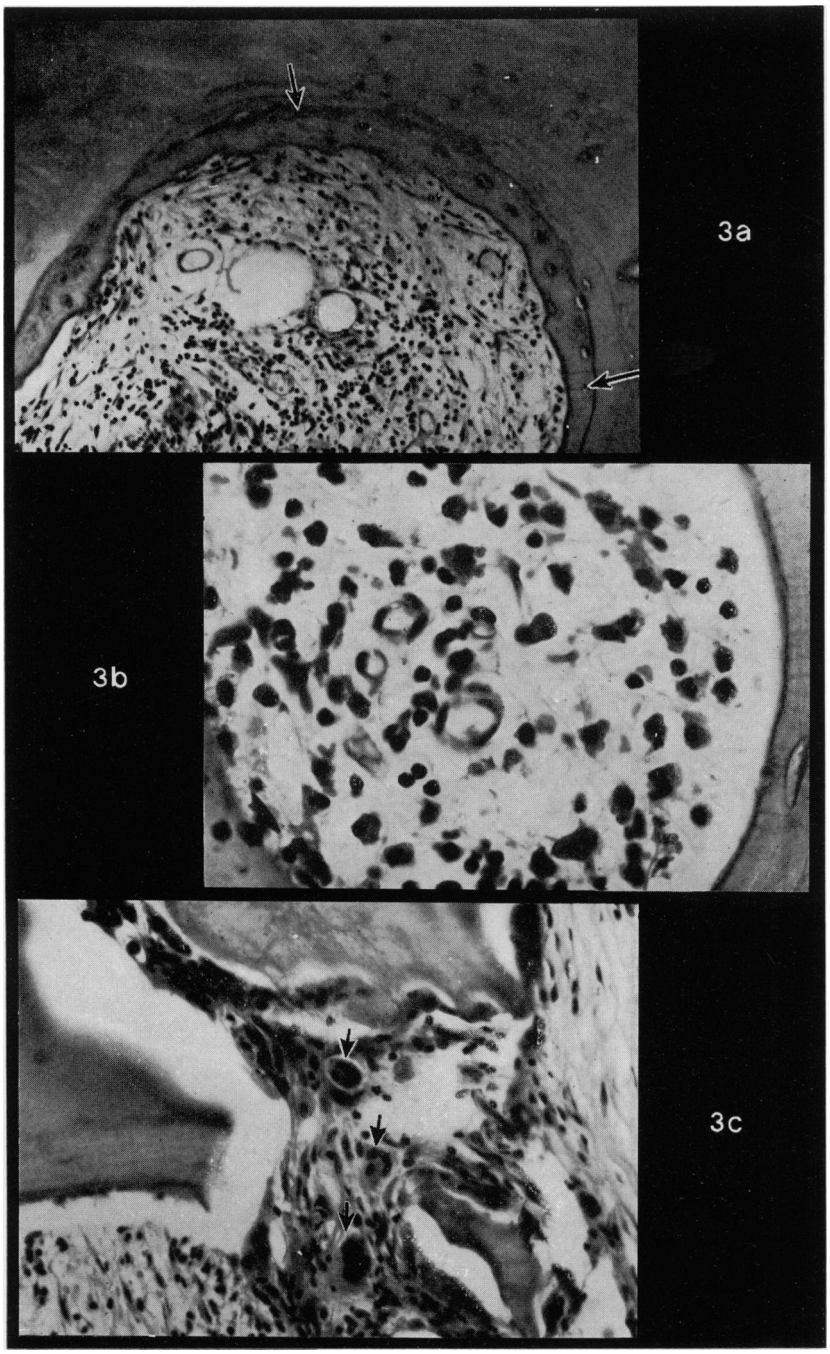
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previous studies, so far as the present authors are aware, of the immunogenicity of articular hyaline cartilage.

Chondrocytes possess major transplantation antigens in common with cells of other tissues, and hence the lack of immunogenicity of articular cartilage cannot be attributed to a lack of antigenicity (Elves, 1974*a*). The inability of hyaline cartilage to elicit an immunological reaction in the host is probably due to its possession of a dense protein and mucopolysaccharide matrix (Heyner, 1969; Bacsich and Wyburn, 1947). This material will prevent the antigens present on chondrocytes from reaching the lymphoid tissue, or the host lymphocytes from coming into contact with the antigen in the graft. An immune response cannot therefore be initiated. Extracts of articular cartilage would allow the release of antigenic material and such material will elicit the production of antibodies (Khvorostukhin, 1958). Two animals in the present experiment developed antibodies 6 weeks after grafting and it may be that this late response might have been initiated as a result of cells being liberated from the graft as the matrix was resorbed. Histological studies showed a variable degree of erosion of many grafts.

In all groups of recipients receiving bone-containing grafts the majority of animals developed anti-sheep antibodies. In each of the 4 groups, however, animals were found which either failed to develop antibody or else did not develop antibody until the 4th week after grafting. Again, the delayed response may be due to release of antigen as the graft was resorbed. In all groups a small number of recipients showed a very rapid response. Washing marrow out of the whole cartilage-containing grafts had relatively little effect on the immunogenicity. This procedure had slightly more effect in the case of the bone grafts (BG). These data are in agreement with results obtained in a previous study of bone heterografts in rats (Elves and Salama, 1974). These findings clearly point to the bony aspect of the graft as being the chief source of immunizing antigen. The relative ineffectiveness of reducing immunogenicity by removing the red marrow suggests that fixed endothelial cells and sinusoidal cells of the marrow may provide an antigenic stimulus in addition to the bone marrow cells.

The matrix of cartilage seems to form an effective block to the afferent (*i.e.* initiation) arm of the immune response. It may also act to block the effector arm of the response. It is well known, from the results of autoradiographic studies of ^{35}S uptake, that some cells in cartilage grafts, both from autologous and homologous sources, are able to survive for considerable periods of time (Wyburn and Bacsich, 1955; Gibson, Curran and Davis, 1957; Craigmyle, 1958*c*). It is not known, however, whether a state of immunity exists in the subjects of these studies. In the present experiments antibody was present in most recipients of whole osteoarticular grafts, and yet in all instances many chondrocytes in the grafts were still present. Lance and Fisher (1970), studying patella grafts in rabbits, found no difference in ^{35}S uptake between autografts and allografts. No immunological studies were carried out in these experiments but as these grafts contained a large bone component it is very probable that the animals became sensitized. In these laboratories autoradiographic studies of sulphur fixing ability of sheep osteoarticular grafts indicate survival of chondrocytes in most grafts 10 weeks after grafting. In all of these animals antibody was present not only in the serum but also in the synovial fluid (Elves, 1971; Elves and Ford, 1971).

It may therefore be suggested that the matrix of the cartilage also forms an

effective barrier to the effector mechanism of the immune response. Heyner (1969) has suggested that the small lymphocyte in presensitized rats can invade allografts of embryonic cartilage. The histological studies reported above confirm the predominantly lymphocytic population of the inflammatory reaction in contact with cartilage. This was true of both antibody producing and non-antibody producing animals. Lymphocytes were not seen, however, within the cartilage matrix but were confined to cracks and fibrillations. Peacock and his colleagues (1960) found that serum proteins do not diffuse readily through cartilage matrix. Preliminary studies by one of us have indicated that cytotoxic antibody has little effect upon the ^{35}S uptake by rat articular cartilage even when in contact with the cartilage for up to 24 hours (Elves, 1974*b*).

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