JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 3 (1992) 382–386

Evaluation of Elastin–Fibrin biomaterial in experimental pancreatic surgery

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Artificial connective soft tissue, so-called "Elastin–Fibrin" biomaterial, was investigated to reinforce a pancreato-jejunum anastomosis in the dog. The ambiguous results invite us, however, to improve the quality of the material, especially against proteolytic degradation: elastinolysis and fibrinolysis. Antibiotic was also added. The improved material was tested, first in rabbit then in dog, to repair a large loss of substance in the duodenum, just in front of the Wirsung duct. In view of the successful results, we are now attempting an evaluation in humans, for all indications throughout the digestive system.

1. Introduction

In spite of recent technical advances, in particular through the use of staple sutures, the problem of scarring processes in intestinal tissues has not been totally resolved. There are still conditions involving great risks of anastomosis leakage and requiring a contrivance such as a diverting enterostomy. The suture, however, should be reinforced rather than excluded.

Recently [1, 2] we developed a new biomaterial which acts as an artificial connective tissue and which could afford a solution. The material was prepared by adduct synthesis between fibrin monomers and elastin. When this adduct is formed, it may be rapidly separated from the batch, but if not immediately isolated, continuing formation of fibrin monomers makes their polymerization possible from the first monomer linked to elastin, leading to a crude matrix [3]. This matrix has been extensively improved by biophysical and chemical processes to constitute a true artificial connective matrix [4]. Eventually its biocompatibility in the rat [5] was demonstrated. Early investigations in the rat [6] had shown the capacity of the material to repair a large loss of substance in the caecum and to restore *ad integrum* the three layers of the intestinal wall. The same results were obtained when repairing experimental arteriotomies in the rabbit [7].

The original aim of this research was to test the capacity of "Elastin–Fibrin" material to reinforce suturing at the intestinal level. The pancreato-jejunum anastomosis described here was chosen as an experimental model, considering the high probability risk of leakage in humans [8–10], from 7 to 33%. In the course of this experiment it proved necessary to protect the material more efficiently against pancreatic secretions, with a view to reducing its biodegradability in that case [11]. The improved material was then experimented on in rabbit intestine, then in dog intestine, again to repair a large loss of substance just in front of the mouth of the Wirsung duct.

2. Materials

The biomaterial was made up of several components isolated from natural connective tissue: elastin from bovine ligament (Sigma, St Louis, Missouri, USA); human cryoglobulins, i.e. fibrinogen, fibronectin and F XIII from human plasma (Centre de Transfusion Sanguine, Bordeaux, France); and collagens (Institut Jacques Boy, Reims, France). Aprotinin was obtained as Iniprol from Choay, Paris, and thrombin as Thrombase from Houdé, Paris.

The biomaterial results from simultaneous reactions on elastin as described below. Briefly, to 100 mg elastin previously equilibrated in phosphate buffer (1 mM PO_4, 150 mM NaCl, 2 mM Ca^{2+} and 1 mM Mg^{2+}, pH 7.4; PB 7.4) and suspended in 0.6 ml of the same buffer, the following ingredients were added successively, with careful mixing (Vortex): 100 µl thiourea (1 mg ml^{-1}), 100 µl Aprotinin, 600 µl cryoglobulin solution in the buffer, 400 µl collagen then 20 µl thrombin (12.5 U (NIH)). The mixture was rapidly poured into the mould containing the polyglactine lattice of Vicryl (Ethnor, Ethicon, Neuilly, France), and was kept at 37 °C for 20 min. Excess buffer was removed up to a certain degree of desiccation, and the material was placed in small plastic bags under nitrogen, then sterilized by 25 kGy gamma-ray irradiation.

3. Methods

The first experiment was carried out on Beagle dogs, the dogs being operated on under anaesthesia (nembutal, 10 mg kg^{-1}, intravenously) with tracheal intubation. The pancreatic area was approached by medial

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laparotomy, the pancreas being cut transversely at the isthmus level. The proximal extremity was closed using a two-fold overcast seam with a biodegradable thread. The distal extremity was connected by anastomosis to a "Roux en Y" jejunal loop (Fig. 1). The pancreato-jejunal anastomosis was performed at two levels: a deep level, not tight, using nine stitches with prolene 5/0 (Fig. 1) and a superficial level through the biomaterial, attached with Tissucol (Immuno, Paris, France), all around the anastomosis. The setting was reinforced with a few stitches of biodegradable thread. Anastomosis imperviousness was achieved by the biomaterial itself.

During the first postoperative days, the dogs received 1.5 l day^{-1} Iono K perfusion (2 days) and prophylactic antibiotherapy (5 days). A computed tomography scan was carried out on the 19th and 30th postoperative days. The dogs were killed by an overdose of nembutal on the 60th day. A distal pancreas and an anastomosis loop were removed and processed immediately for macroscopic examination and histology.

In a second experiment, ten rabbits (Fauve de Bourgogne, aged 6 months, weight 2 kg) were investigated. In this animal a 1 cm^2 loss of substance was performed, in the duodenum, precisely in front of the biliary–pancreatic papilla, and a patch was sewn and attached with Tissucol.

4. Results

Six dogs were investigated according to this protocol. The first three dogs died early from an acute necrosing pancreatitis as seen at the autopsy and proven by histology in two dogs. Histological examination was not possible in the last dog. In these animals the biomaterial was more or less lysed.

The other three dogs survived and were killed as stated above. In the macroscopic examination the material had completely disappeared, without any visible trace (Fig. 2); there was a peri-anastomotic tissue with little inflammation, and the anastomosis was successful.

In histology, few agregates of material, stained with orcein (which is specific of elastin fibres) were observed inside the scarring connective tissue. The microscopic structure of the intestinal wall and of the pancreatic parenchyme were retained, as illustrated in Fig. 3.

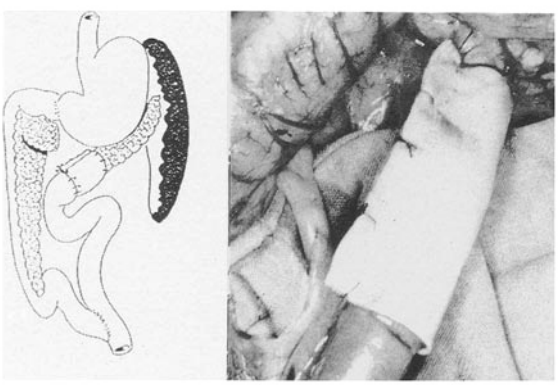

Figure 1 Pancreato-jejunal anastomosis in dog.

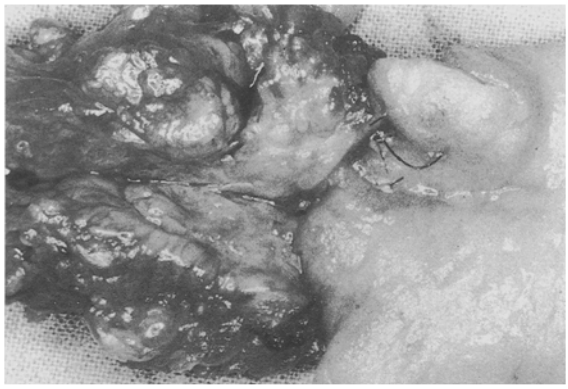

Figure 2 Internal macroscopic view of the anastomosis site at the 60th postoperative day.

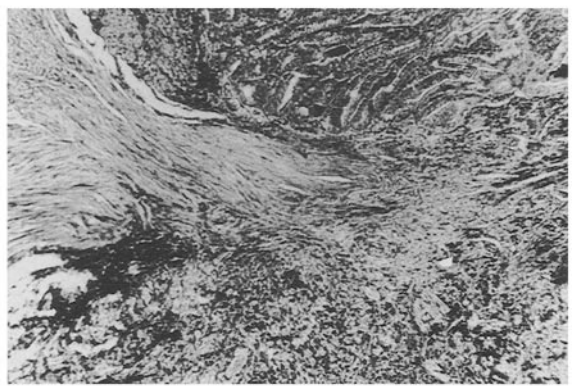

Figure 3 Histological feature of the anastomosis site at the 60th postoperative day (orcein staining).

The first experiment led us to the conclusion that the material was able to reinforce a pancreato-jejunal anastomosis but needed the incorporation of some substances with a view to reducing or delaying its degradation. Actually, the failures in three of the dogs was probably due to an early degradation leading to anastomosis leakage, which in turn caused the pancreatic secretion to diffuse in the peritoneal cavity.

As a consequence, we first investigated the addition of antiproteases to the material, taking great care not to degrade it; the basic reaction which yields the material is itself monitored by a protease (thrombin). Elastase and plasmin inhibitors were used, as they are instrumental in its conservation. Therefore, at the end of the reaction Aprotinin (Choay, Paris, France) and Eglin C (Ciba–Geigy, Basel, Switzerland) were incorporated into the desiccated material by means of rehydration with a solution containing both inhibitors. Naturally, it had previously been shown *in vitro* [11] that they were potent against trypin, plasmin, elastase and (obviously) against activated pancreatic secretion (Fig. 4). Also, in order to decrease local septicity, an antibiotic was incorporated into the material [11] (Céfuroxime, Curoxime, Glaxo, Paris, France), as was recently described with other materials [12, 13].

These *in vitro* results [11] had then to be confirmed with an *in vivo* study. The first model, the dog, was discarded as being too difficult owing to its complex

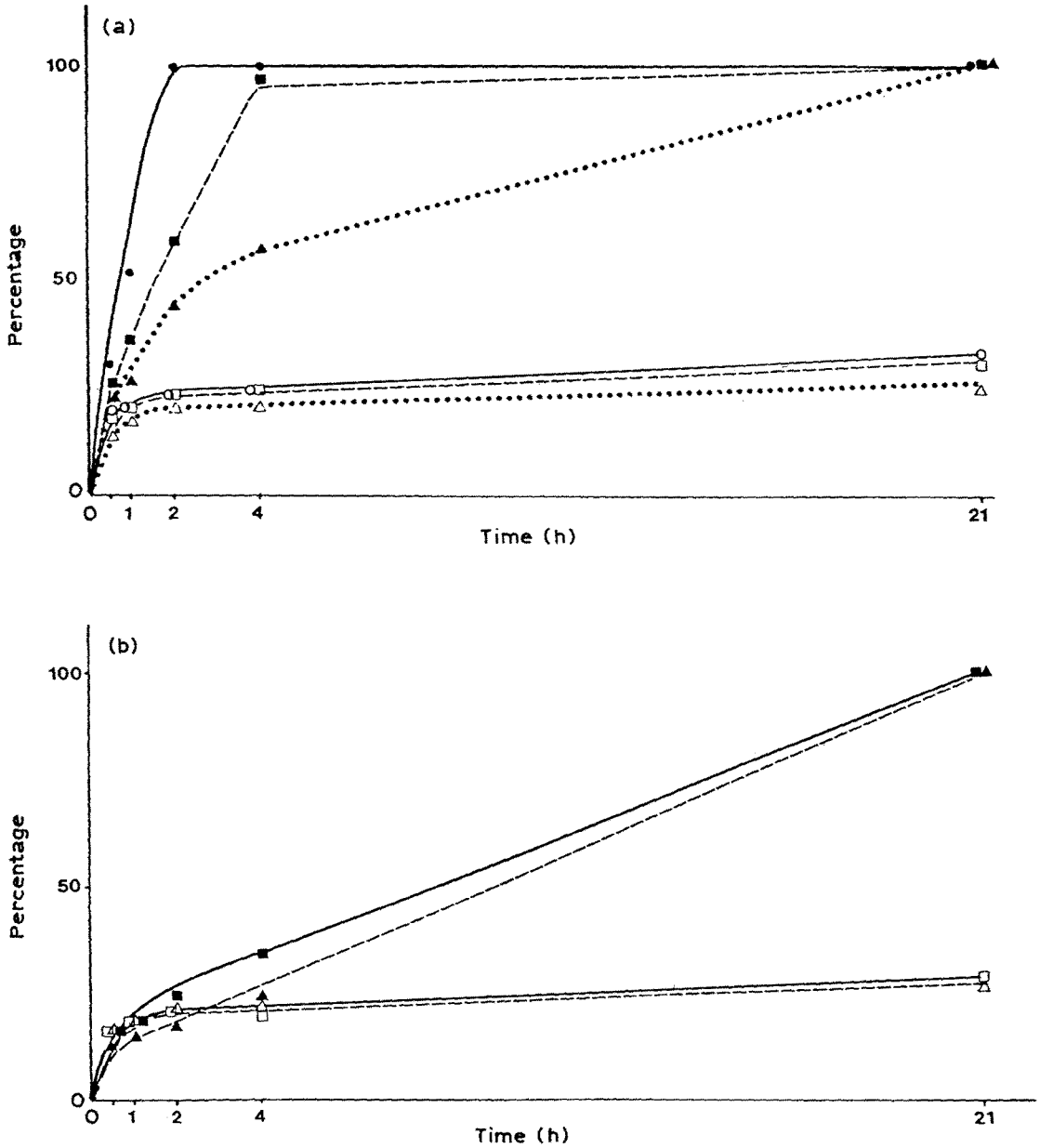

Figure 4 Conservative effects of antiproteases incorporated into the biomaterial. (a) With (open symbols) and without (closed symbols) Aprotinin (Choay) conservation of fibrin-like structure, labelled with ^{125}I-fibrin, against trypsin (circles), plasmin (squares) and crude pancreatic secretion (triangles). (b) With (open symbols) and without (closed symbols) Eglin C (Ciba–Geigy) conservation of elastin component, labelled with ^{125}I-elastin, against purified human pancreatic elastase (squares) and crude pancreatic secretion (triangles).

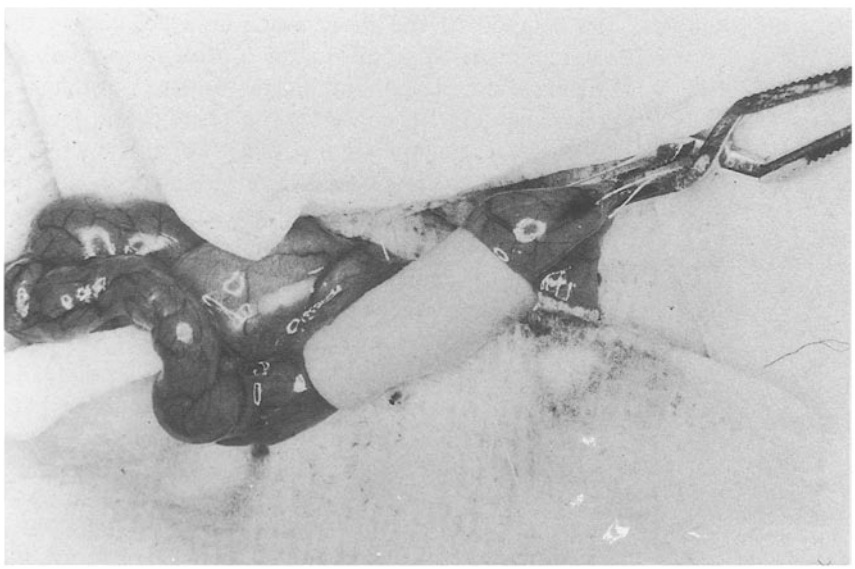

Figure 5 Macroscopic view of the patch placed on rabbit duodenum.

pancreatic structure and the rabbit was investigated (Fig. 5). No rabbit died before we killed them, after 8, 19, 30 and 80 days. As illustrated in Fig. 6, no macroscopic difference could be observed for the scarring process. The histological study was carried out on the rabbits killed after 80 days, where healing had not yet been totally achieved. Typical results are seen in Fig. 7, in order to show how the different structures were restored. The scarring process seems to start in the mucosal layer and the biomaterial is partly incorporated in the healing tissue; the remainder is eliminated in the serous layer, surrounded by a non-specific inflammatory tissue, as previously observed in the rat [3].

Furthermore, recent investigations were made in the dog with the same protocol as in the rabbit. It was proved that the improved material could be successfully used in the same model without any problem [14].

5. Discussion

Digestive stitching aims to join tightly, edge to edge, the two faces of the tissue with a view to achieving ideal conditions for scarring. The main element strengthening the scar is under the mucosal layer, owing to its fibre lattice of collagens and elastin. The scarring process follows three stages: the inflammat-

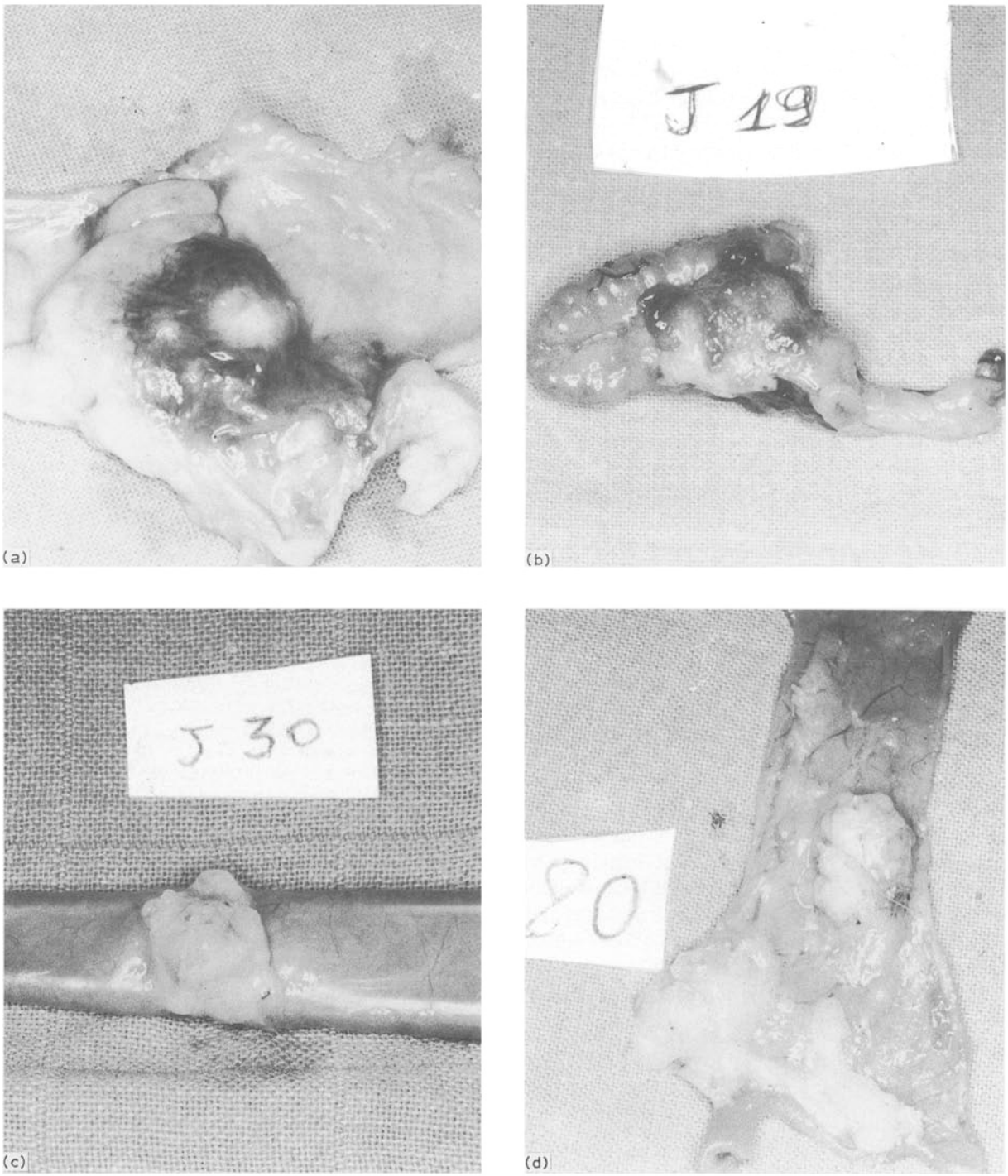

Figure 6 Macroscopic view of the scarring sites: progressive disappearance of the material in time at the (a) 8th, (b) 19th, (c) 30th and (d) 80th postoperative days.

Figure 7 Histological aspect of the scarring tissue at the 60th postoperative day.

ory phase, over the first 4 days, when the stitching is very weak; then the cellular phase to the end of the 15th day; eventually the restructuring phase, over 2–3 months. The use of the "Elastin–Fibrin" material on the scar area should accelerate the first two phases and reinforce the original tissue.

Because of the high septicity at intestinal level, a biodegradable material must be used; if not, continuous sepsis would be induced. The "Elastin–Fibrin" biomaterial, which acts as true soft artificial connective tissue, complies with these requirements. The material works as a bioactive scaffold, open to cell proliferation through blood cells and fibroblasts. It has already been assessed in the rat, with complete success, although this was performed away from the pancreas; this model did not take into account the enzymatic action which could noticeably alter the behaviour of the material. This is why in these experiments the patch was fitted quite close to the pancreas. Our purpose, however, was not to elaborate a material for pancreatic surgery alone, but for all digestive levels. Thus, as described above, we can add enzyme inhibitors and antibiotics in varying amounts, thus monitoring its biodegradability and making it suitable for every digestive site.

Consequently, we consider it appropriate to begin the investigations in humans, especially as inflammatory and immunological phenomena will certainly be of less importance; indeed, the main component is prepared from human blood entirely free from viruses such as acquired immune deficiency syndrome and Hepatitis. However, the most effective human pancreatic elastase inhibitor cannot be used because of its allergenic character. The present results show that an addition of Aprotinin and Céfuroxime affords sufficient protection to the material, and further investigations are in process in our laboratory to assess the most suitable protease inhibitor.

The Ethical Committee has authorized us to start human evaluation in severe situations of postoperative peritonitis. Following the first results (quite encouraging but not yet published), further use of the material in humans is to be extended to all cases of digestive surgery requiring a high-risk anastomosis.

Acknowledgements

The authors are deeply indebted to Marc Repellin, University of Bordeaux 3, for his helpful assistance in the preparation of the manuscript. We also express our gratitude to Claudie Daumy for her assistance during animal experiments. The excellent secretarial assistance of Véronique Silverio is gratefully acknowledged. The investigations were supported by INSERM, the University of Bordeaux 2 and the Conseil Régional d'Aquitaine.

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Received 26 June
and accepted 5 November 1991