Virchows Arch [Pathol Anat] (1981) 394:143–150

Virchows Archiv A

Evidence for the Presence of Pro-γ-Melanotropin, the NH_2-Terminal Fragment of the Corticotropin-β-Lipotropin Precursor, in Corticotropin-Producing Tumours

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Summary. Five corticotropin-producing tumours were examined for peptides related to the corticotropin-β-lipotropin precursor. Two were basophil pituitary adenomas and three were bronchial carcinoids. The cells of the two pituitary adenomas stained with antisera against β-endorphin and against pro-γ-melanotropin, the NH_2-terminal fragment of the corticotropin-β-lipotropin precursor, but not with antisera against α-melanotropin or β-lipotropin. The corticotropin-storing tumor cells of the bronchial carcinoids stained with antisera against β-endorphin, β-lipotropin or pro-γ-melanotropin. Only one of the three bronchial carcinoids contained cells reacting with the antiserum against α-melanotropin. Although the two types of corticotropin-storing tumours (pituitary adenoma and bronchial carcinoid) differed with respect to β-lipotropin content, the over-all picture indicates that the proteolytic processing of the corticotropin precursor proceeds along similar lines in tumour cells and in pituitary corticotrophs.

An acetic acid extract of one of the bronchial tumours was subjected to gel chromatography and immunochemical analysis of material related to pro-γ-melanotropin. The immunoreactive material displayed a considerable size heterogeneity, with the predominant components having a molecular weight larger than that of authentic pro-γ-melanotropin.

Key words: ACTH precursor – Pro-γ-melanotropin – Pituitary adenoma – Bronchial carcinoids.

Introduction

Corticotropin is produced by proteolytic processing of a large glycosylated precursor (Mains et al. 1977) which consists of 239 amino acid residues and has an approximate molecular weight of 26,000 (Nakanishi et al. 1979; Håkanson et al. 1980). The processing of this precursor molecule probably takes place in the secretory granules of the corticotropin cell and yields several biologically

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0340-1227/81/0394/0143/$01.60

important peptide fragments besides corticotropin, namely β-lipotropin, β-endorphin and the remaining NH_2-terminal part of the precursor. Recently, the NH_2-terminal fragment was isolated and partially sequenced. It consists of 103 amino acid residues, contains the γ-melanotropin sequence (Nakanishi et al. 1979), has an approximate molecular weight of 11,000 (amino acid backbone), and is glycosylated (Håkanson et al. 1980).

The present study attempts to demonstrate pro-γ-melanotropin, the NH_2-terminal fragment of the precursor, in corticotropin-producing tumours.

Materials and Methods

Five corticotropin-producing endocrine tumours were examined. Two were basophil pituitary adenomas and three were bronchial carcinoids. None of the five patients suffered from Cushing's syndrome. For immunohistochemistry, the material was fixed in 4% formalin or vapour-fixed with formaldehyde at 80° C for 1 h and embedded in paraffin. Deparaffinized sections (6 μm) were exposed to rabbit antisera against corticotropin, α-melanotropin, β-endorphin, β-lipotropin and pro-γ-melanotropin. The two corticotropin antisera stain pituitary melanotrophs as well as corticotrophs (Sundler et al. 1981). The α-melanotropin antiserum recognizes the free carboxyamide of α-melanotropin and does not stain the pituitary corticotrophs (Sundler et al. 1981). The β-endorphin antiserum does not cross-react with β-lipotropin and is probably directed against the NH_2-terminal portion of β-endorphin (Bramnert et al. 1981). The β-lipotropin antiserum does not cross-react with β-endorphin, neither at the radioimmunological (P. Schwandt, personal communication) nor at the immunocytochemical level (R. Håkanson, unpublished); hence, the antiserum is probably directed against the NH_2-terminal, non-β-endorphin-containing part of β-lipotropin. The antiserum against pro-γ-melanotropin, the NH_2-terminal fragment of the corticotropin-β-lipotropin precursor, was raised against a glutaraldehyde conjugate of the antigen. It does not cross-react with γ-melanotropin either at the immunohistochemical level or in a radioimmunoassay system (Ekman et al. 1981). Together these antisera cover the full corticotropin-β-lipotropin precursor sequence (Fig. 1). For further details on these antisera see Table 1. Several other antisera, against calcitonin (Alumets et al. 1980), leucine-enkephalin (Alumets et al. 1978), somatostatin (Alumets et al. 1977), substance P (Brodin et al. 1980) or vasoactive intestinal polypeptide (Larsson et al. 1976), were tested. The site of the antigen-antibody reaction was revealed by immunofluorescence (Coons et al. 1955), or by immunoperoxidase (peroxidase-antiperoxidase) staining (Sternberger 1974) as described in detail elsewhere (Alumets et al. 1977, and 1978). Sections exposed to antisera

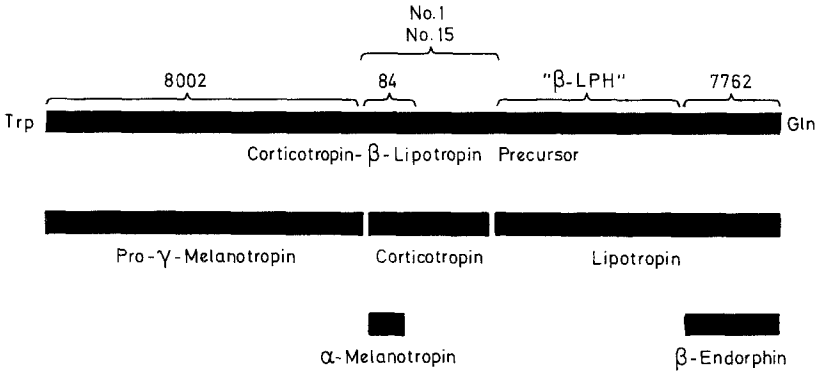

Fig. 1. The corticotropin-β-lipotropin precursor seems to consist of 239 amino acid residues; the NH_2-terminus is tryptophan and the CCOH-terminus is glutamine. The various antisera employed detect different regions in the precursor molecule. The code numbers are explained in Table 1

Table 1. Properties of antisera against corticotropin and related peptides

Antigen	Code no.	Working dilution		Source	Reference
		Immuno-fluorescence	PAP staining		
Corticotropin, porcine	No. 1	1:80	1:640	own	Sundler et al. (1981)
Corticotropin 1–24 synthetic human	No. 15	1:160	1:640	Tj. B. van Wimersma Greidanus, Utrecht, The Netherlands	Sundler et al. (1981)
α-Melanotropin, synthetic human	84	1:40	1:640	M.P. Dubois, Nouzilly, France	Sundler et al. (1981)
β-Endorphin, synthetic human	7762	1:40	1:640	own	Alumets et al. (1979)
β-Lipotropin, porcine	"β-LPH"	1:80	1:640	P. Schwandt, Munich, FRG	–
pro-γ-Melanotropin, porcine	8002	1:80	1:320	own	Lorén et al. (1981)

which had been inactivated by the addition of the respective antigen (10–100 μg per ml diluted serum) served as controls. In such controls the immunostaining had been abolished, leaving only non-specific or background staining.

One bronchial tumour was weighed (130 mg) and homogenized in 3.0 ml cold 1 M acetic acid, containing 28 mM HCl, 0.01% phenylmethylsulphonyl fluoride and 100 KIU Trasylol per ml. The homogenate was centrifuged at 5,000 × g at +4° C for 20 min. The supernatant was lyophilized and reconstituted in 0.5 ml 4 M guanidine-HCl for gel chromatography on a Bio-Gel P-100 column (0.9 × 60 cm) equilibrated with 4 M guanidine-HCl and eluted with the same solvent at room temperature. Fractions of 0.8 ml were collected. The column was calibrated using dextran blue (void volume = Vo), β-lipotropin (mw 11,700), β-endorphin (mw 3,500) and $Na^{125}I$ (salt peak = V_t). Aliquots of the fractions were analyzed for pro-γ-melanotropin by radioimmunoassay as described by Ekman et al. (1981).

Results

All five tumours contained numerous cells displaying corticotropin immunoreactivity. The cells of the two pituitary adenomas stained with antisera against β-endorphin and against pro-γ-melanotropin (Figs. 2 and 3) but not with antisera against α-melanotropin or β-lipotropin. It is not possible to say whether the immunostaining of the various peptides related to the corticotropin-β-lipotropin precursor reflects the presence of the various fragments per se or of larger precursor forms. The absence of β-lipotropin immunoreactivity was a notable finding, perhaps best explained by a further processing of β-lipotropin into smaller fragments of which only β-endorphin can be detected. Interestingly, the corticotropin-storing tumour cells of two of the three bronchial carcinoids stained with antisera against β-endorphin, β-lipotropin or pro-γ-melanotropin (Fig. 4) but not with the antiserum against α-melanotropin. The third bronchial carcinoid contained cells that stained with α-melanotropin antiserum; they were

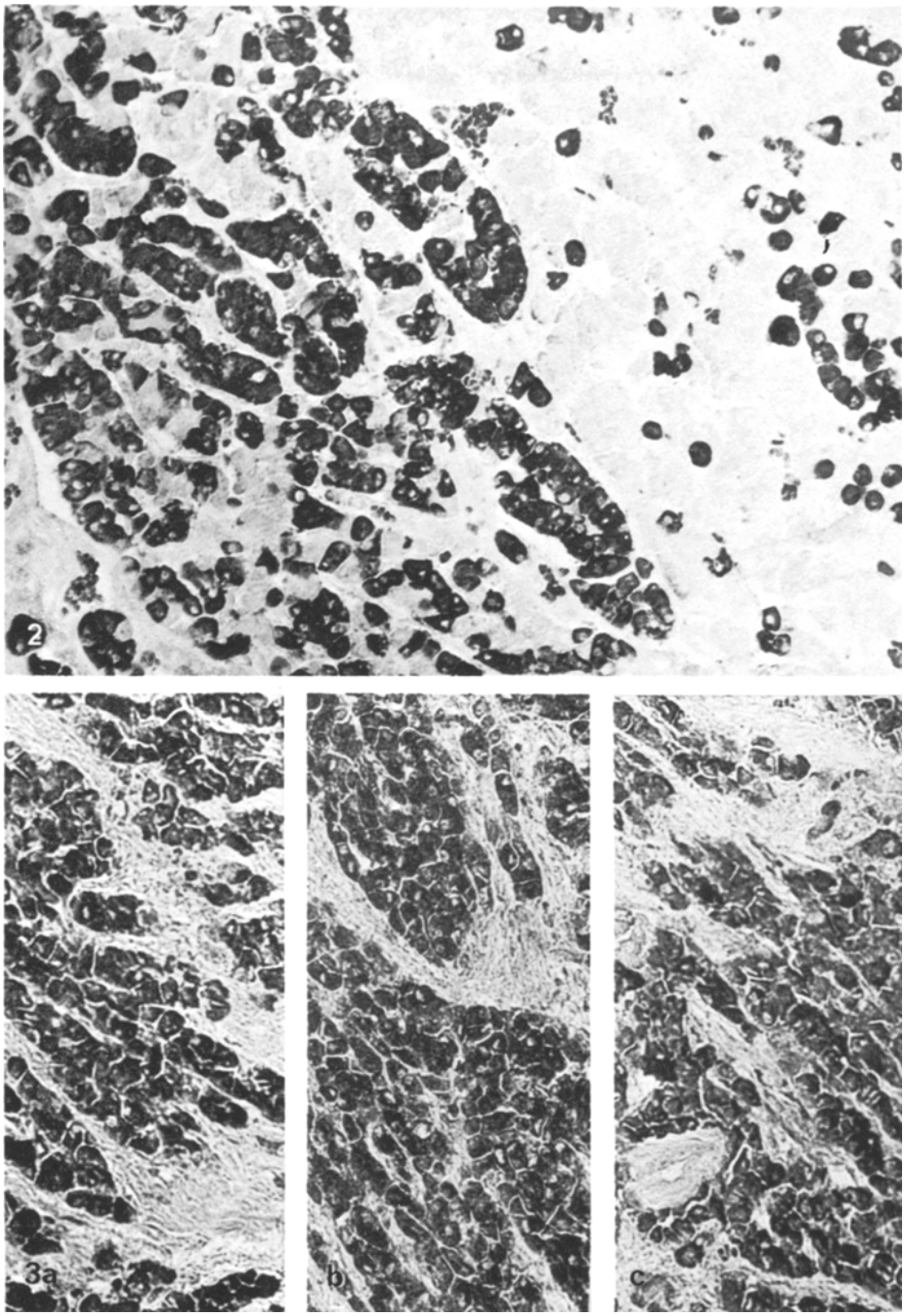

Fig. 2. Immunostaining of a pituitary adenoma with antiserum against pro-γ-melanotropin. Immunoperoxidase (PAP) staining. Part of the adenoma to the left and normal anterior pituitary to the right (× 250)

Fig. 3. Immunostaining of the second pituitary adenoma with antisera against pro-γ-melanotropin (a), β-endorphin (b), and corticotropin (c). Immunoperoxidase (PAP) staining (× 200)

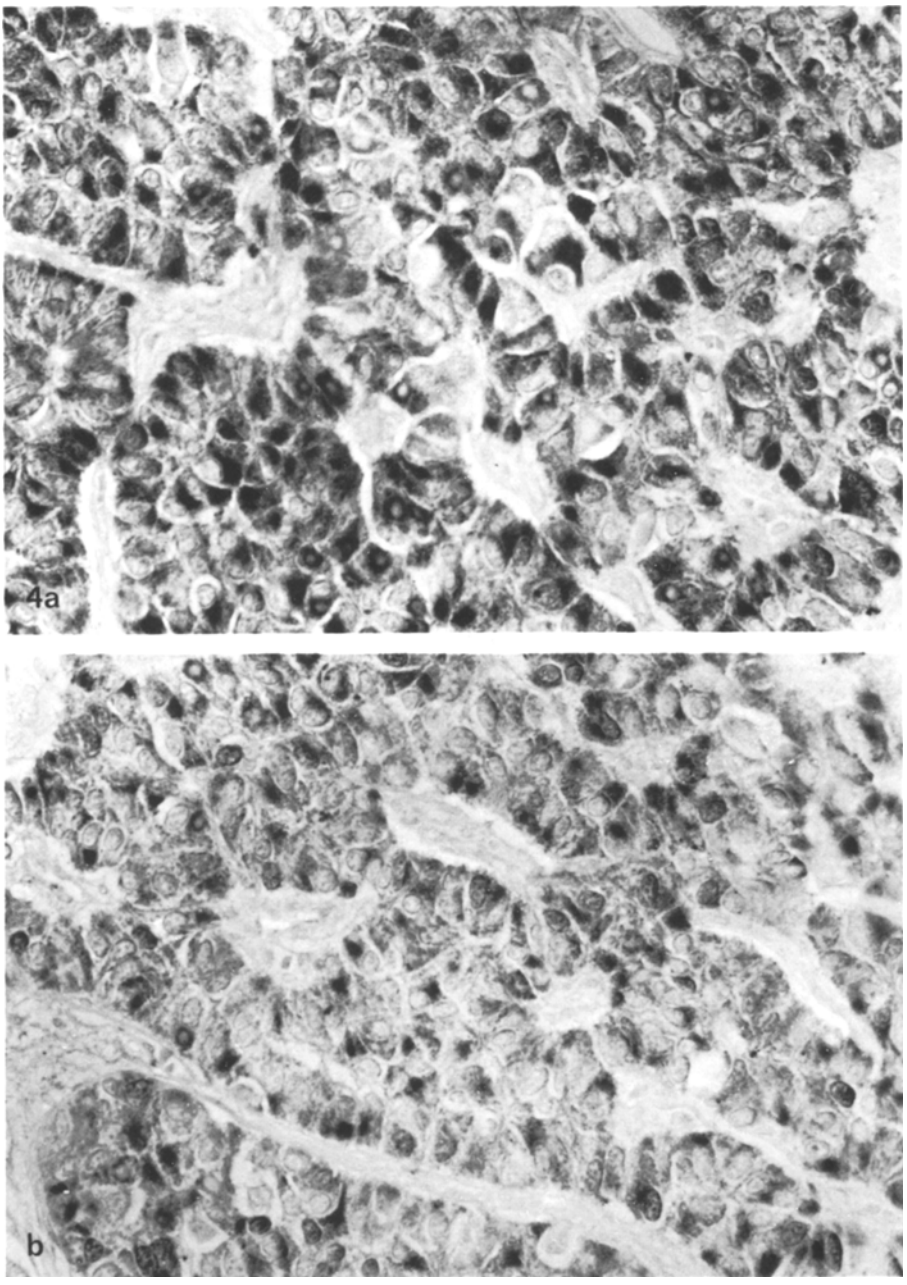

Fig. 4. Immunostaining of a bronchial carcinoid with antisera against pro-γ-melanotropin (a) and against corticotropin (b) (×350)

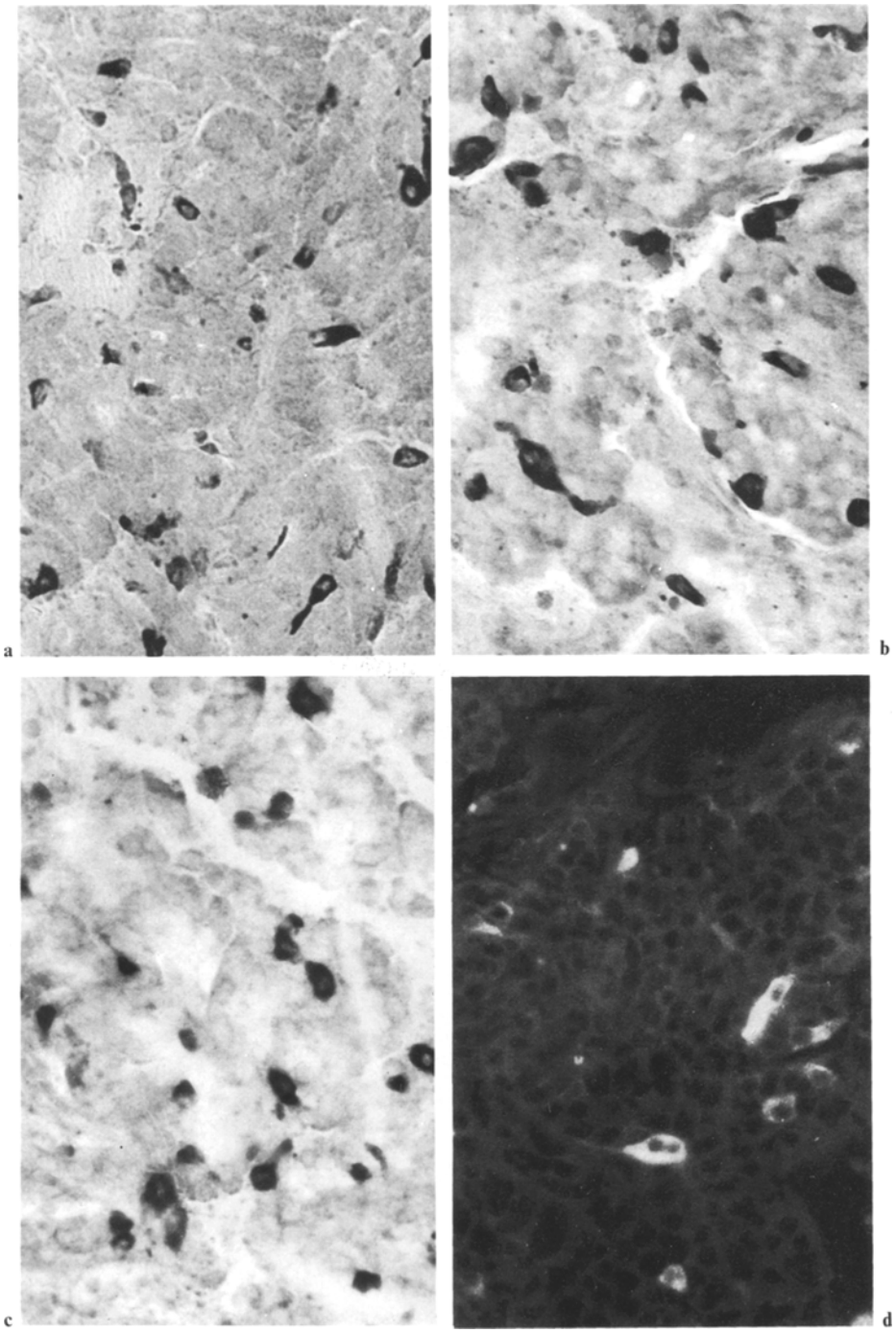

Fig. 5. Immunostaining of the third bronchial carcinoid with antisera against corticotropin (**a**), β-lipotropin (**b**), pro-γ-melanotropin (**c**), and α-melanotropin (**d**). Immunoreactive cells were scattered in the tumour parenchyma and the incidence of such cells varied greatly in the different parts of the tumour. α-Melanotropin-immunoreactive cells were generally fewer in number than the corticotropin cells (a $\times 300$, b–d $\times 400$)

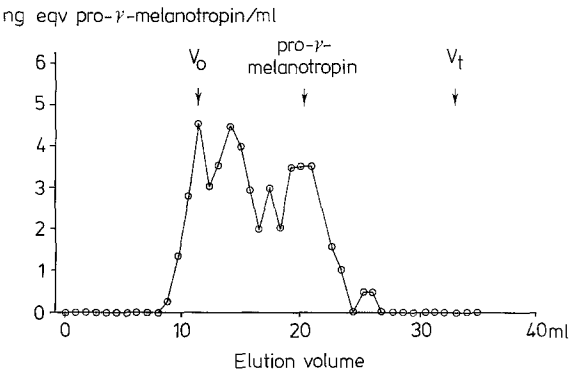

Fig. 6. Elution profile of pro-γ-melanotropin immunoreactive material on a Bio-Gel P-100 column. The elution position of authentic pro-γ-melanotropin isolated from porcine pituitaries (Håkanson et al. 1980) is indicated

fewer than the corticotropin cells (Fig. 5). A small number of cells in one of the first two bronchial carcinoids displayed calcitonin immunoreactivity. These cells were distinct from the corticotropin immunoreactive cells. The latter cells did not display any trace of calcitonin immunoreactivity despite the recent demonstration of a calcitonin-like sequence in the amino-terminal region of the precursor (Nakanishi et al. 1980). There was no staining with any of the other antisera tested.

An acetic acid extract of one of the bronchial carcinoids (same as shown in Fig. 4) was subjected to gel chromatography and the elution profile of immunoreactive pro-γ-melanotropin was established by radioimmunoassay. The most striking feature was the pronounced size heterogeneity of the immunoreactive material (Fig. 6) with the predominant immunoreactive components having a molecular weight larger than that of authentic pro-γ-melanotropin.

Discussion

Much of the pioneering studies on corticotropin biosynthesis from a large precursor has been carried out on a corticotropin-secreting mouse pituitary tumour cell line (Mains et al. 1977), and immunoreactive endorphins and lipotropins have previously been demonstrated in corticotropin-producing tumours (Orth et al. 1978; Yoshimi et al. 1980; Ueda et al. 1980). Moreover, Nakai et al. (1980) recently described γ-melanotropin-like immunoreactivity in ectopic corticotropin-producing tumours. Hence, available data suggest that the proteolytic processing of the corticotropin precursor molecule proceeds similarly in tumour cells and in pituitary corticotrophs although in the present study the two types of corticotropin-producing tumours (pituitary adenoma and bronchial carcinoid) differed somewhat in that the bronchial carcinoids contained immunoreactive β-liptropin whereas the pituitary adenomas did not.

The results of immunochemical analysis of the size heterogeneity of pro-γ-melanotropin-related material in one of the tumours suggested that pro-γ-melanotropin exists in larger forms than previously anticipated (see also Tsukada et al. 1981). Whether this represents a special feature of corticotropin-β-lipotropin-producing tumours or whether it is a property shared by pituitary corticotrophs and melanotrophs is unknown.

Acknowledgements. Grant support from the Swedish Medical Research Council (04X-4499, 04X-1007 and 03X-05917) and Riksföreningen mot Cancer (806-5XA).

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Accepted August 25, 1981