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# Demonstration of a tumour associated antigen in human oral cancers

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**A saline extract from pooled oral cancer biopsy specimens, termed as the crude oral cancer antigen (COCA) was used to produce xenogeneic antisera in rabbits. The COCA when reacted with unabsorbed antiserum (UAS) produced 4 precipitin lines in double diffusion plates. The UAS was completely absorbed with crude normal antigen extracted from normal oral biopsies. The absorbed antiserum (AAS) appears to be specific for COCA, as there was only one single line seen when the COCA was reacted with AAS. Thus in agar gel diffusion the COCA appears to contain an antigen unshared with the normal tissue antigens. Immunoelectrophoresis also confirmed these results. The COCA was fractionated into 4 protein peaks by gel filtration, and fraction 1 was found to be specifically reacting with the AAS. This fraction 1 was found to be absent in CNA, which contained only the other 3 peaks in Sephadex G-200 chromatography. The AAS was not cross reacting with CEA, stomodial antigen and CNA. Fraction I was shown to contain the oral cancer associated antigen (OCAA).**

Tumour associated antigens have been described in various types of neoplasma. Embryonal antigens like carcino-embryonic antigen[1], alpha fetoprotein[2] and oncofetal antigen[3] have been described. These antigens cross react in various other malignant and nonmalignant situations and hence are very nonspecific in nature. Moreover, the existence of tumour-associated antigens in many human neoplasms is now well-established[4,5]. Even though oral cancer constitutes about 30 per cent of the total cancer incidence in Kerala, no large scale study has been conducted so far to isolate the antigens present in such tissues. The present study was undertaken to detect the presence of a tumour associated antigen in oral carcioma.

## Material and Methods

*Tissue collection* : Patients with histopathologically proved epidermoid carcinoma having cauliflower-like growth in the oral cavity were included in this study. The patients were so selected that they were having normal mucosa on the opposite side of the tumour. Those having sub-mucous fibrosis and leukoplakia were excluded. By punch biopsy, a small tumour mass was removed after cleaning

the oral cavity by repeated gargling with hydrogen peroxide and swabbing with sterile normal saline. The biopsy specimens collected in the sterile normal saline were then washed three times in normal saline to remove the subcutaneous fat and the blood clots, if any, and stored at minus 70°C in sterile normal saline, till further processing. Similarly, normal tissues were also taken from the same subjects from the opposite side of the lesion and stored in the same manner as the tumour tissues.

*Preparation of tumour antigen* : All the sixty tumour biopsies were pooled in a dish and minced thoroughly. About 4 ml of 0·03*M* phosphate buffer of *p*H 7·4 was added to every gram of minced tissue. The cells were then ruptured by alternate freezing and thawing and then the material was thoroughly homogenised by grinding in a tissue grinder. The homogenate was then centrifuged at 4°C, at 10,000× *g* for 20 min. The supernatant was decanted, filtered and the protein content was estimated by Lowry's method. The protein was finally adjusted as 1 mg/ml and stored at minus 70°C till further use. This filtrate was termed as the crude oral cancer antigen (COCA). The crude normal antigen (CNA) was also prepared in exactly the same manner from the normal tissues collected, and stored at minus 70°C.

*Preparation of antisera* : Six adult healthy male rabbits were selected for raising antisera. The COCA was emulsified with equal quantity of Freund's complete adjuvant, and 0·25 ml of the emulsion was administered sc over the flank and 0·75 ml im, into the thigh muscles to each animal. This schedule was repeated every third week, for a total of 5 injections. Ten days after the last injection, the animals were bled, and the sera from all the six animals were pooled and stored at minus 20°C. This was termed the unabsorbed antiserum (UAS). Then the absorbed antiserum (AAS) was prepared by absorbing a part of the UAS with excess CNA for 3 days at 4°C. This AAS was also stored at minus 20°C.

*Gel filtration* : 0·75 ml of the COCA was passed through a Sephadex-G-200 column of 35 cm height and 1·5 cm diameter, and was eluted with 0·3*M* Tris-HCl buffer of *p*H 7·4, with a flow rate of 15 drops per minute. 2·5 ml fractions were collected and the protein content was monitored by spectrophotometer at 280 nm. The protein peaks were identified, pooled and lyophilised for further identification.

## Results

When the COCA was reacted with UAS, there were 4 precipitin lines observed in the immunodiffusion plate. The antiserum was completely absorbed by addition of CNA until the AAS thus produced gave only one precipitation line when reacted with COCA. This single line was found to be in continuation with one of the precipitation lines produced by the reaction of COCA with UAS. Immunoelectrophoresis using 1·4 per cent agar in barbitone buffer of *p*H 8·9 had shown 4 precipitation lines towards the trough containing UAS, but only one arc towards the AAS. This showed the presence of a specific tumour associated antigen in oral cancer tissues. In order to isolate the antigenic molecules, gel filtration technique using Sephadex-G-200 was utilised. The COCA could be fractionated by gel filtration into 4 peaks

(Fig. 1). But only 3 fractions were obtained when CNA was chromatographed and the peak corresponding to the fraction 1 of the COCA was absent in CNA (Fig. 2). The first peak of the COCA was found to be specifically reacting with AAS. All the fractions from COCA were separately tested against AAS in immunodiffusion plates and only fracton No. 1 gave a positive precipitation. This precipitation band was found to be completely confluent with that of COCA indicating immunological identity. There was no cross reactivity with embryonal antigens such as carcinoembryonic antigen (donated by the Chester Beatty Cancer Institute, London) and the stomodial antigen, prepared in our laboratory by preparing a saline extract from stomodia of fetuses of 16-20 wk size. The AAS was found to be reacting only with oral cancer tissues, but not with normal oral antigens.

## Discussion

Tumour associated antigens are considered to be very useful as biological

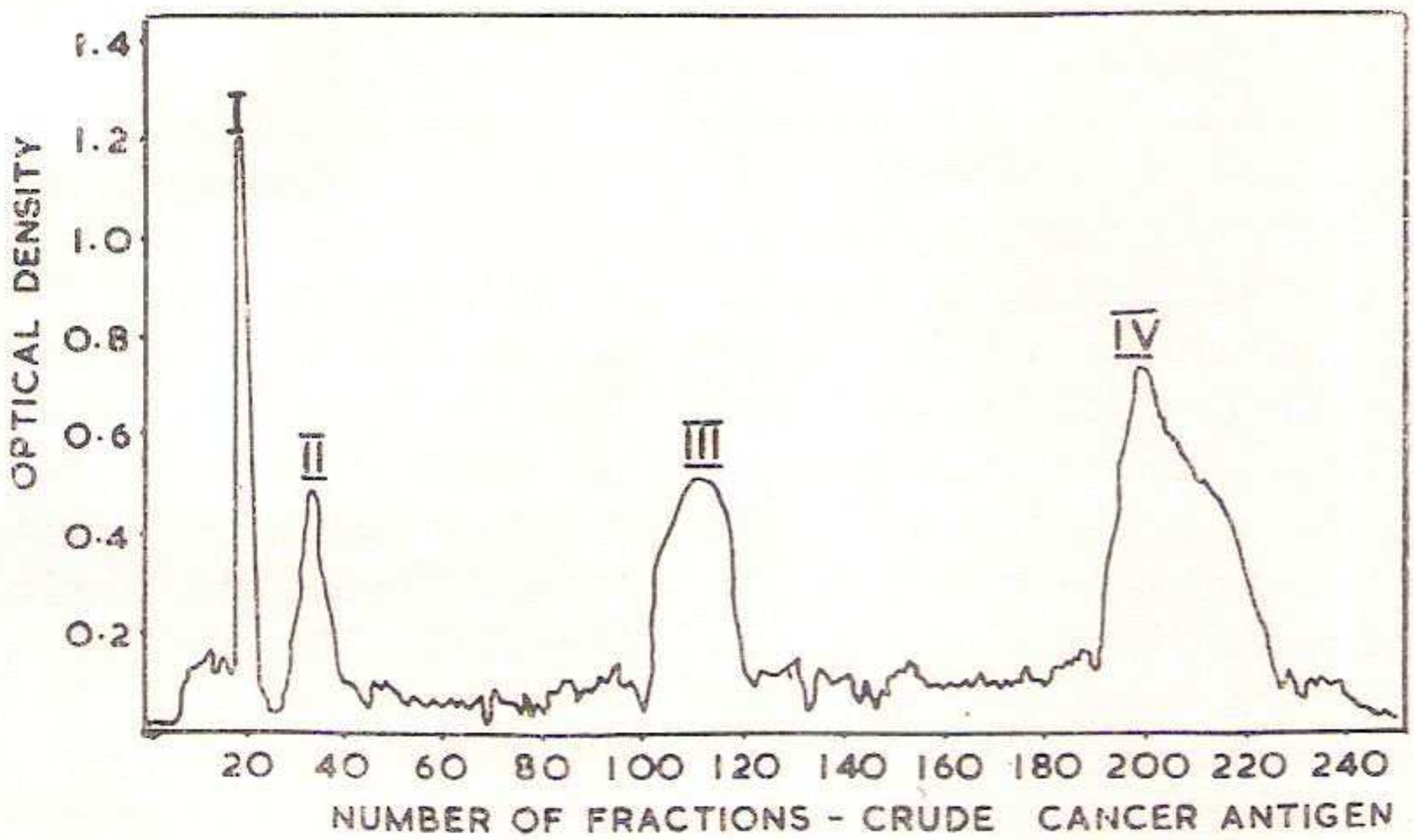


**Fig. 1.** Sephadex G-200 gel filtration of the COCA. Two ml fractions were collected and the optical density was monitored spectrophotometrically. Four protein peaks were identified.

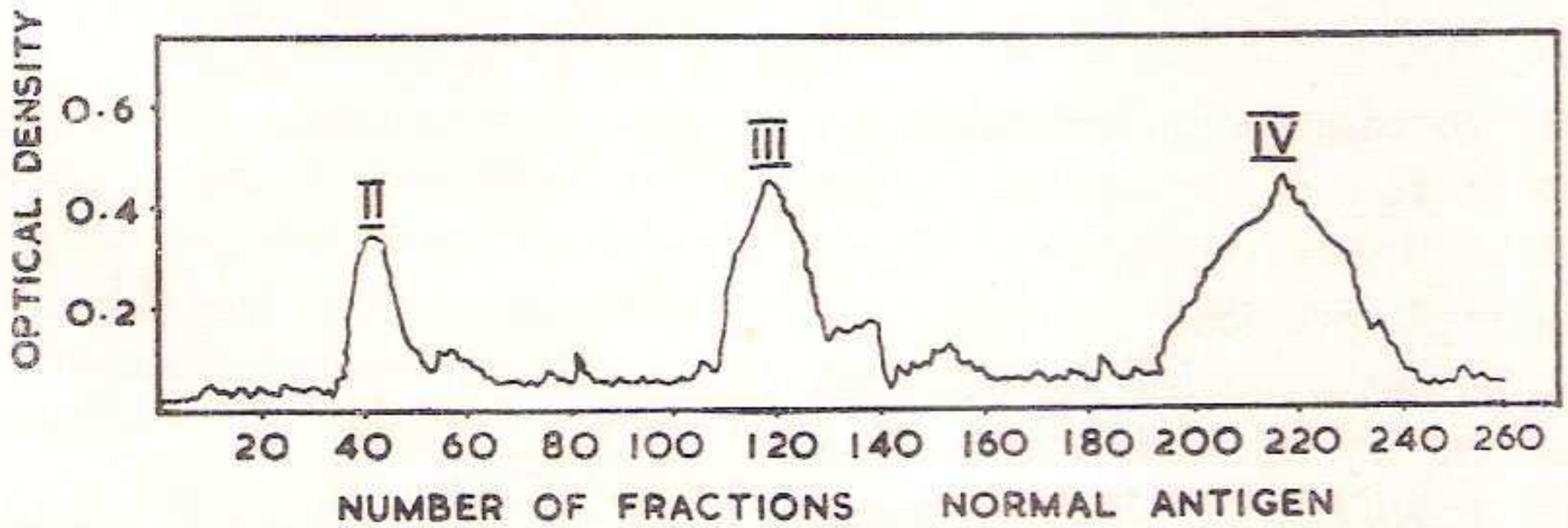


**Fig. 2.** Passing through Sephadex G-200 column, the CNA was fractionated into 3 protein peaks. The peak corresponding to the fraction I of the COCA was absent.

markers of neoplasia[6]. Many reports have appeared on the clinical assessment of colon carcinomas by the periodic estimation of carcino-embryonic antigen in the sera of patients. The CEA fluctuation has been proportionately related to the prognosis of the patients[7,8]. Attempts to induce the immunological mechanisms directed to the tumour specific antigen would, in the long run, prove to be the foundations for the definite immunotherapy.

We have described here the detection of a tumour associated antigen in oral cancers, by means of a saline extract of crude antigen prepared from pooled oral cancer biopsies. Pooling of the specimens would mask the individual specific antigens. Antiserum raised against this crude antigen was thoroughly absorbed by repeated absorption with the crude normal antigen prepared from the same patients. The possibility of HLA and individual specific antigens interfering with the experiment was eliminated by taking normal and cancer tissues from the same patients. Thus it is expected that after the absorption procedure, only the fraction reacting with tumour associated antigens will remain in the serum. The absorbed antiserum contained excess normal antigen as evidenced by the reaction between the UAS and AAS in immunodiffusion plate Therefore, the absorption procedure can be taken as complete with respect to gel diffusion test. The AAS thus produced was found to be reacting specifically with oral cancer tissues, but not with normal oral biopsy antigen by gel diffusion. The COCA when reacted with UAS produced 4 precipitation lines in immunodiffusion plates, and in immuno electrophoresis. The COCA was fractionated by Sephadex-G-200 into 4 separate protein peaks. When the different fractions were individually tested with AAS, it was found that only the fraction no. 1 reacted specifically. The AAS did not appear to react with embryonal antigens showing that fraction no. 1 contains a tumour associated antigen of oral cancer, which is not shared by the normal oral tissues. The possibility of cross-reactivity with embryonal antigens has been excluded by immuno diffusion. Further molecular charactrisation of this antigen by gel filtration, polyacrylamide electrophoresis and by ultra centrifugation is in progress.

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## References

1. Gold, P. and Freedam, S.O. Specific carcino embryonic antigens of human digestive system. *J Exp Med* **122** (1965) 467.
2. Abelev, G.I. Production of embryonal serum alpha globulin by hepatomas. Review of experimental and clinical data. *Cancer Res* **28** (1968) 344.
3. Fritsche, R. and Mach, J.P. Identification of a new oncofetal antigen associated with several types of human carcinomas. *Nature* **258** (1975) 734.
4. Thomson, D.M., Tataryn, D.N., O'Connor, R., Rauch, J., Friedlander, P., Gold P. and Schuster, J. Evidence for the expression of

human tumour specific antigen with associated beta-2-microglobulin in human cancer and in some colon adenomas and benign breast lesions. *Cancer Res* **39** (1979) 609.

5. Lopez, M.J. and Thomson, D.M.P. Isolation of breast cancer tumour antigen from serum and urine. *Int J Cancer* **20** (1977) 834.

6. Hollinshead, A.C., Stewart, T.H.M. and Takita, H. Tumour associated antigens; their usefulness as biological drugs in lung cancer. In : *Progress in therapeutic research.* F. Muggia and M. Rozeneweig, Eds. (Raven Press, New York) 1978, p 501.

7. Stewart, A.M., Nixon, D., Zamcheck N. and Aisenberg, A. Carcino embryonic antigen in breast cancer patients : Serum levels and disease progress. *Cancer* **33** (1974) 1246.

8. Cooper E.H., Turner, R., Steelel, Neville, A.M. and Mackay, A.M. The contribution of serum enzymes and carcinoembryonic antigen to the early diagnosis of metastati colorectal cancer. *Br J Cancer* **31** (1975) 111.

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