shown that the lymphocytes produce a slowing factor that then acts on the macrophages to produce a slowing effect in electrophoresis. Indeed the system is analogous with the mechanism of macrophage-migration inhibition (David, 1966; Bloom & Bennett, 1966), though the messenger protein need not necessarily be the same. Standard electrophoretic methods are not easily applicable to human cells for technical reasons, and a system was therefore devised whereby guinea-pig macrophages could be used as an indicator system for the specific interaction of human lymphocytes with antigen. The mixed-cell interaction of human and the contaminating guinea-pig lymphocytes (about 10–20%) was overcome by irradiating the exudate with 100rd of γ -radiation, which proved adequate for short-term experiments. Our final assay system was set up as follows: 10×10^6 irradiated guinea-pig macrophages, 0.5×10^6 human lymphocytes and antigen at concentrations up to $33 \mu\text{g/ml}$ made up to a final volume of 3 ml with medium TC199. The mixture was allowed to incubate at room temperature for 90 min before the electrophoretic mobility of the macrophages was measured in a Zeiss cytopherometer at 23°C . This instrument is extremely temperamental, large cells such as macrophages tend to cause turbulence, a multiplicity of joints tend to produce micro-leaks and electrode polarization restricts prolonged use, though with care, patience and experience many of these troubles may be overcome. Macrophages are timed in both directions of current, and we have found empirically that measurements differing by more than 10% must be discarded. For immune assays the results are expressed as a percentage slowing when the mobility in the presence of specific antigen is compared with the control mobility, i.e. no antigen.

This method was first used in the study of thyroid disease (Field *et al.*, 1970), later in nervous diseases (Caspary & Field, 1970) and cancer (Field & Caspary, 1970; Caspary & Field, 1971). Tests using encephalitogenic basic protein in neurological disease showed that the immune reaction was to the active determinant (P. R. Carnegie, E. A. Caspary, F. Westall & E. J. Field, unpublished work), an undecapeptide, and that this reactivity could be altered by substitution or deletion of amino acids. The immune interaction with encephalitogenic basic protein could also be inhibited by serotonin (Field *et al.*, 1971), but not by adrenaline or acetylcholine. Some hallucinogenic drugs proved almost equally effective.

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Identification of a Tumour Antigen

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Field & Caspary (1970) showed that lymphocytes from patients with malignant carcinoma reacted with the encephalitogenic basic protein from human brain and produced a factor that altered the surface charge of macrophages. When a variety of tumours were extracted by the procedure used to isolate encephalitogenic basic protein a mixture of proteins was obtained that was a more effective antigen than the encephalitogenic basic protein in the cytopherometer assay with these lymphocytes (Caspary & Field, 1971). The present report describes the identification of the protein that can react with lymphocytes from patients with malignant carcinoma.

Tumours of the vulva, cervix and breast were defatted with chloroform-methanol (2:1, v/v) and the residues were extracted with HCl between pH 3 and 2.5. The acid extracts were fractionated by gel filtration on Sephadex G-75 and the eluates were examined by electrophoresis in polyacrylamide gel (Adams & Fox, 1969). Pooled fractions were tested with lymphocytes from patients with cancer in the cytopherometer assay. Similar results were obtained with extracts of each tumour. The peak of antigenic activity was eluted with a volume 1.63 times the exclusion volume of the column. The major protein in this region, and the only component whose elution pattern paralleled the antigenic activity, had an electrophoretic mobility 0.85 times that of cytochrome *c*. The approximate molecular weight for this protein was calculated to be 16000. It contained one tryptophan residue.

Fractionation of a benign tumour (fibroid of uterus) and normal human liver yielded similar proteins, but their antigenic activity in the cytopherometer assay was significantly less ($P < 0.001$) than those of the protein from malignant tumours and of encephalitogenic basic protein.

The antigenic determinant in encephalitogenic

basic protein that reacts with lymphocytes from patients with cancer was shown to be identical with the encephalitogenic determinant Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg (Field *et al.*, 1971; Westall *et al.*, 1971; Lennon *et al.*, 1970; Carnegie, 1971). By treatment of the tumour antigen with 2-hydroxy-5-nitrobenzyl bromide it was shown that tryptophan was an essential residue in the tumour antigen. Studies with synthetic peptides (P. R. Carnegie, E. A. Caspary, F. C. Westall & E. J. Field, unpublished work) have shown that an insertion or substitution of a single amino acid residue can markedly alter the antigenic activity of the peptide.

We suggest that the difference between the normal protein and that from tumour which alters the antigenicity is the result of a slight change in the amino acid sequence around the sole tryptophan residue in the protein.

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Purification of Concanavalin A Receptor from Pig Lymphocyte Plasma Membrane

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It was proposed recently (Crumpton, 1971) that affinity chromatography is applicable to the purification of membrane receptors solubilized in 1% sodium deoxycholate. The validity of this proposal has been evaluated by using an insoluble form of the mitogenic plant agglutinin concanavalin A to separate the homologous receptor(s) from the pig lymphocyte plasma membrane.

The method described by Lloyd (1970), with minor modifications, was used to covalently attach concanavalin A (100mg of 3×crystallized in 10ml of 0.1M-NaHCO₃, pH8.5) to Sepharose 4B (settled volume 10ml) that had been activated with cyanogen

bromide (200mg). After being stirred at 2°C for 24h the product was packed into a column, washed successively with 0.1M-NaHCO₃, 1M-NaCl, water and 5% methyl α -D-glucopyranoside in 1% sodium deoxycholate until no further 280nm-absorbing material was eluted, and then equilibrated with 1% sodium deoxycholate. Approx. 90% of the concanavalin was bound as judged from the E_{280} value of the washings. Pig lymphocyte plasma membrane (20mg of protein) was extracted with 5ml of 1% sodium deoxycholate as described by Allan & Crumpton (1971); 85% of the membrane protein dissolved. The soluble fraction was added to the column and eluted with 1% sodium deoxycholate until the E_{280} value of the effluent returned to the base-line. The unretarded fraction contained approx. 75% of the added protein.

If interaction with concanavalin A persisted in 1% sodium deoxycholate then, in view of its known specificity (Poretz & Goldstein, 1970), membrane components with terminal α -D-glucopyranosyl, α -D-mannopyranosyl or sterically related sugar residues should have been retained. Elution of the column with 2% methyl α -D-glucopyranoside in 1% sodium deoxycholate gave a peak of 280nm-absorbing material. This material was precipitated by adding 0.1 vol. of 2% (v/v) acetic acid and was extracted three times with 10ml of ethanol to remove deoxycholate and methyl glucoside. The residue represented 5% of the protein added to the column. Examination by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate (Allan & Crumpton, 1971) revealed about six bands that stained for protein and carbohydrate; these bands corresponded to the most pronounced glycoprotein bands of the whole membrane. A comparison of the capacities of the lymphocyte membrane and of the residue to inhibit the transformation of pig lymphocytes by concanavalin A showed that the eluted glycoproteins were 20 times as effective as membrane.

The results indicate that membrane receptors can be purified by affinity chromatography and suggest that membrane glycoproteins may be fractionated by using insoluble plant agglutinins with different specificities.

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