receptors was investigated. IgG from Graves' sera with high thyroid-stimulating antibody activity were diluted in IgG from a pool of normal human serum (so that each sample contained the same amount of total IgG) and incubated overnight at 0°C with labelled thyrotropin and membranes solubilized with 0.5% Triton. The mixtures were then analysed by chromatography on Sepharose or by poly(ethylene glycol) precipitation.

The binding of labelled thyrotropin to the soluble-receptor preparations was inhibited in a dose-dependent manner by thyroid-stimulating antibodies (Fig. 2). Gel filtration of mixtures of antibodies, labelled thyrotropin and soluble thyrotropin-receptor preparations indicated that a trimolecular complex consisting of the three components was not formed. Further, analysis of mixtures of antibodies and labelled thyrotropin only by gel filtration and poly(ethylene glycol) precipitation showed that no direct interaction between them occurred, i.e. the antibodies did not demonstrate thyrotropin-binding activity. These data suggested that the binding of thyrotropin and thyroid-stimulating antibodies to the thyrotropin receptors was mutually exclusive, and that thyrotropin and the antibodies interacted with the same receptor molecule.


The Location of Prekallikrein in the Rat Pancreas

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Glandular kallikreins are peptidyl hydrolases (EC 3.4.21.8) that produce small peptide hormones called kinins. The kinins are believed to be involved in several pathological and physiological processes including regulation of local blood flow and vascular permeability (Reichgott & Melmon, 1973). Glandular kallikreins are found in tissue extracts and secretions of salivary glands, kidneys and pancreas (Webster et al., 1970). In the case of the latter organ the enzyme occurs as the inactive zymogen, prekallikrein. In previous studies kallikrein has been located in the striated duct cells of the rat sublingual gland, to the granular tubules of the rat submandibular gland (Ørstavik et al., 1975) and also to the distal tubular cells of the rat kidney (Ørstavik et al., 1976). The purpose of the present investigation was to locate prekallikrein in the rat pancreas by using a direct immunofluorescence technique.

Rat pancreas (6g), which had been extirpated immediately after death and stored at −80°C until use, was homogenized in 60ml of 0.01M-phosphate buffer, pH6.0, containing 0.1m-NaCl and 0.3mg of soya-bean trypsin inhibitor/ml in a motorized Potter-Elvehjem-type homogenizer at 2°C with 25 strokes at 1800rev./min. The homogenate was centrifuged for 5min at 500g (rev. 10.5cm) to sediment unbroken cells. The supernatant was filtered through gauze and stirred for 1h at 2°C after the addition of 0.5% (w/v) sodium deoxycholate. The preparation was centrifuged in an MSE High-Speed 25 centrifuge for 20min at 30000g (rev. 10.5cm). The supernatant was then filtered to remove fat and stored at 2°C. The sediments from the two centrifugations were resuspended in 20ml of the phosphate buffer and rehomogenized with 20 strokes at 1800rev./min. This homogenate was treated with sodium deoxycholate as before and centrifuged to produce a second supernatant. Finally, the sediment was re-treated to give a third supernatant. The three supernatants were then combined and the protein content of the preparation was determined by the method of Warburg & Christian (1942).
A preliminary experiment on ion-exchange chromatography was carried out essentially according to the details given in the legend to Fig. 1 except that fractions were pooled according to the absorbance profile. Those fractions were then tested for esterase activity against 20 mM-N-α-benzoyl-L-arginine ethyl ester hydrochloride by using a titrimetric assay at pH 8.0 and 25°C, both before and after suitable activation. Active fractions were then tested on immunodiffusion and immunoelctrophoresis against an antibody to rat submandibular kallikrein. Fractions corresponding to prekallikrein activity were shown to be electrophoretically different to rat submandibular kallikrein on immunoelctrophoresis, but behaved identically with the submandibular enzyme on immunodiffusion. Having shown this a radioimmunoassay was then used to monitor a further ion-exchange run.

A sample of pure rat submandibular kallikrein was labelled with $^{125}$I by the method of Hunter & Greenwood (1962) with chloramine-T as oxidant. The labelled enzyme was separated from free I$^-$ by gel filtration on Sephadex G-75, and shown to be pure by using ion-exchange chromatography.

Antibody to rat submandibular kallikrein was raised in two rabbits by injecting 150 µg of the pure enzyme at 2-week intervals until bleeding produced an antibody with sufficiently high titre and avidity for use. The antibody was normally used at a final dilution of 1:160000.

Pure rat submandibular kallikrein was used to construct standard curves with a range of concentrations from 0 to 10 ng/0.2 ml.

Samples were assayed as follows: 200 µl of standard or unknown, at suitable dilution, was incubated with 100 µl of labelled enzyme and 100 µl of antibody for 16–18 h at 2°C. The antibody–antigen complex was then separated from free antigen by immunoprecipitation. DASP (Organon) sheep anti-(rabbit γ-globulin) immunosorbent (0.5 ml) was added to each tube and incubated in a shaker bath at 25°C for 2 h. Then 3 ml of 0.07M-barbitone buffer, pH 8.5, was added to each mixture. The samples were then centrifuged on an MSE Mistral 4L centrifuge for 10 min at 2500 g (rav. 22.1 cm). The supernatant was removed by aspiration and the precipitated antigen–antibody

![Fig. 1. Ion-exchange chromatography of rat pancreatic homogenate](image-url)
The fraction that contained most of the antigenic activity was then used to locate the zymogen in the pancreas by using a direct immunofluorescence technique on tissue sections.

Antibody to rat submandibular kallikrein was labelled with tetramethyl-Rhodamine isothiocyanate. The conjugate was purified by gel filtration on Sephadex G-50 and by anionic-exchange chromatography by using the method of Brandtzaeg (1973a). The selected fractions had an absorbance ratio \( \frac{A_{280}}{A_{515}} \) of 2.4 and were used at an immunoglobulin concentration of 0.6 mg/ml as established by performance testing. Samples of the conjugate were adsorbed with mouse liver powder to decrease non-specific background staining. For the same purpose dilutions of the conjugate were made in 12% bovine serum albumin, and the tissue sections were preincubated with bovine serum albumin for 30 min (Brandtzaeg, 1973b). Conjugate incubation, washing and mounting of tissue sections were performed as described by Brandtzaeg (1973b). Immunological specificity of the fluorescence reaction was controlled by adsorption of the conjugate with purified rat submandibular kallikrein or the partially pure rat pancreatic prekallikrein.

Fluorescence microscopy was carried out with a Leitz Ortholux microscope equipped with a Ploem-type vertical illuminator. Conditions for narrow-band excitation and selective filtration of Rhodamine fluorescence (Brandtzaeg, 1973b) were used.

Prekallikrein was located in the acini of the rat pancreas. It may therefore be hypothesized that pancreatic kallikrein has its main function outside the gland and, upon secretion into the duodenum, may act upon the gut epithelium.

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**Effect of Glutathione on the Activity of Bilirubin-Binding Proteins from Rat Liver Cytosol**

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Liver cytosol of rats contains two protein fractions that bind bilirubin with high affinity. One, of mol.wt. 46000, is apparently identical with ligandin (Litwack et al., 1971) a protein that has also been known as aminoazodye-binding protein B (Ketterer et al., 1967) or Y-protein (Levi et al., 1969), and that is identical with glutathione \( S \)-transferase B (Habig et al., 1974). The other, of mol.wt. 14000, is probably identical with aminoazodye-binding protein A (protein A) (Ketterer et al., 1967, 1976) or Z-protein (Levi et al., 1969). Besides bilirubin (Meuwissen et al., 1972; Tipping et al., 1976a), a number of other substances both endogenous and exogenous have been shown to possess varying affinities for either one or both of these proteins (Tipping et al., 1976b, c; Ketterer et al., 1976). The binding proteins are of importance for the cellular transport of their ligands. In the case of bilirubin these proteins may be...