Albumin prepared from the plasma of patients with essential diabetes mellitus is highly antagonistic to insulin in vitro as well as in vivo at a concentration of 1.25 g/100 ml (synalbumin-positive), whereas the albumin obtained from healthy people without any family history of diabetes mellitus is non-antagonistic at this concentration. Studies with isolated rat liver perfusion experiments have shown that this biological phenomenon is due to the greater binding of B-chain of insulin by the diabetic albumin. The present study was undertaken to investigate the qualitative difference, if any, between the amino acid composition of synalbumin-positive and synalbumin-negative albumins.

METHODS

Albumins extracted from plasma by trichloroacetic acid/ethanol procedure of Debro et al., as modified by Vallance-Owen et al., were tested at a concentration of 1.25% for insulin antagonism by the rat hemidiaphragm assay method. The details of the method have been reported by us elsewhere. Three samples each of the antagonistic and non-

Key-words: Amino acid composition; Antagonism; Insulin; Synalbumin; Two-dimensional paper chromatography.

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antagonistic albumins were selected at random for the amino acid analysis. The albumins were subjected to acid and alkaline hydrolysis.

Acid hydrolysis was carried out with 6 N hydrochloric acid in sealed glass tubes at 110 °C for 22 hrs. The acid was then allowed to evaporate. The residue was dissolved in distilled water and again evaporated in order to remove any remaining acid. It was redisolved in distilled water and filtered. The filtrate was used for chromatographic analysis.

Alkaline hydrolysis was carried out with 0.38 N barium hydroxide in sealed glass tubes at 110 °C for 3 hrs. Ba(OH)\(_2\) was neutralised with 1 N sulphuric acid and the contents were centrifuged to remove the white precipitate thus formed. The supernatant was subjected to chromatographic analysis.

Two dimensional descending paper chromatography technique was employed by using solvent systems butanol/acetic acid/water (60 : 15 : 25) and phenol/water (80 : 20) for the first and second run respectively. The chromatograms were developed by spraying 0.5% solution of ninhydrin in acetone. The amino acids were identified from their Rf values as obtained from the standard chromatograms run and developed concurrently.

**RESULTS**

Figs 1 and 2 show the amino acid analysis of the synalbumin hydrolysates. Eighteen different amino acids were identifiable by this technique in the hydrolysates. Tryptophan, which was destroyed on acid hydrolysis, could be identified in alkaline hydrolysate. Methionine was identified in the alkaline hydrolysate only. There was no difference between the amino acid compositions of the synalbumin-positive and synalbumin-negative samples.

**DISCUSSION**

The amino acid composition of synalbumin-positive and synalbumin-negative albumins, as observed by us, is comparable to the already known composition of normal human albumin\(^7\). As no dif-

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**RISULTATI**

Le figg. 1 e 2 mostrano l’analisi degli aminoacidi degli idrolisati di sinalbumina. Con la tecnica descritta, è stato possibile identificare negli idrolisati 18 diversi aminoacidi. Il triptofano, che non resiste all’idrolisi acida, poteva essere identificato nell’idrolisato alcalino. La metionina fu identificata soltanto nell’idrolisato alcalino. Tra campioni sinalbumino-positivi e sinalbumino-negativi non è stata riscontrata alcuna differenza per quanto riguarda la composizione in aminoacidi.

**DISCUSSIONE**

La composizione in aminoacidi di albumine sinalbumino-positives e sinalbumino-negative, quale da noi osservata, è sovrapponibile a quella già nota per l’albumina umana normale\(^7\). Poiché non è