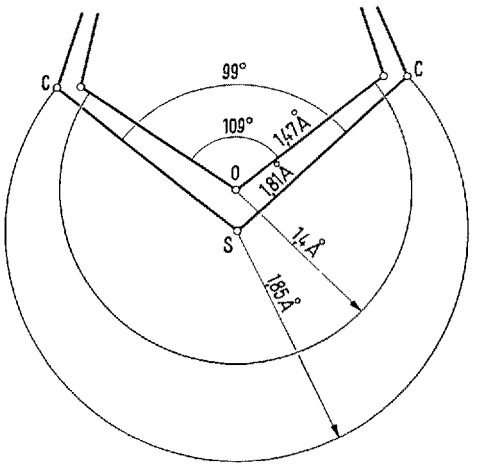
Furfurylverbindungen (5-Methylfurfuryltrimethylammonium und Furfuryltrimethylammonium) festgestellt wurde[10]. *Trans*-(4, 5)-Dehydro-Muscarin (XI) ist etwa gleich wirksam wie das vollständig hydrierte Muscarin, die *cis*-Verbindung analog dem Epi- oder Epiallo-Muscarin weniger aktiv. Die Stellung der beweglichen basischen Seitenkette hat auf die Wirksamkeit wenig Einfluss, sofern sie nicht in *cis*-Stellung zur Hydroxylgruppe steht. Ähnliche Überlegungen gelten für die Stellung der Methylseitenkette.



Struktur des Tetrahydrofuranringes im Muscarin verglichen mit der wahrscheinlichen Struktur des Tetrahydro-Thiophenhomologen.

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Summary

Replacing the tetrahydrofuran ring in muscarine by tetrahydrothiophene results in a markedly reduced biological activity. This may be explained by the inability of the sulfur atom to form hydrogen bonds with the cholinergic receptor group. The increase in ring size also affects the position and biological function of the neighbouring methyl group. Aromaticity of the furan ring has little effect on biological activity of muscarine, in contrast to the striking decrease which occurs when methyl or trimethylammonium groups are in *cis*-position with respect to the hydroxy-group.

Paper Electrophoresis of Soluble Proteins of Rat Liver Mitochondria

The presence of soluble proteins in mitochondria has been described recently by several authors. HOGEBOOM and SCHNEIDER[1] have succeeded in separating 4 protein components from rat liver mitochondria by ultra-centrifugation of sonically disrupted particles. DE LAMIRANDE *et al.*[2] have found by free electrophoresis 5 protein fractions in extracts of rat liver mitochondria obtained by incubating the particles for several hours in a barbiturate buffer. CONGIU[3,4] has recently succeeded in separating by free electrophoresis at least 7 protein components from mitochondria disrupted by a non-ionic tensioactive substance, Triton X-100.

The present paper deals with the paper electrophoresis of soluble proteins from rat liver mitochondria. The results obtained show the presence in these particles of at least 5 protein components, of 3 lipoproteins, and of 3 glycoproteins.

14 Wistar albino rats were used weighing 160 ± 15 g and fed on a semi-synthetic diet. They were killed by bleeding. The liver was immediately removed and transferred in the cold room at 2°C. Mitochondria were isolated from 5 g of the organ by centrifugation of 25% homogenates prepared in a Potter-Elvehjem type apparatus with 0·25 *M* sucrose. The centrifugation scheme was the same as that used by CONGIU[3]. No attempt was made to collect separately the mitochondrial fractions as suggested by DE DUVE *et al.*[5]. Mitochondria were washed once with 0·25 *M* sucrose and then suspended in 0·5 ml of the Michaelis buffer, prepared according to GRASSMANN *et al.*[6]. Its ionic strength was 0·1 μ and the pH was 8·6. 0·1% Triton X-100 was added to this buffer in order to disrupt mitochondria. The final volume was 1·8 ml. The mixture was stored at 2°C for 15 min and then centrifuged at 20000 g for 15 min. The supernatant fluid was used for paper electrophoresis, immediately after this time. In fact, incubation at room temperature for short time results in a rapid aggregation of the proteins. Whatman No. 1 paper strips 25 cm long and 4 cm wide were used. The extract was placed on previously moistened strips in the amount of 10 μl in the case of total proteins, of 40 μl in that of lipo- and glycoproteins. Electrophoresis was run in a plexiglass box with platinum wire electrodes. Michaelis barbiturate-acetate-HCl buffer, ionic strength 0·1 μ and pH 8·6, was the fluid phase. The applied current was 0·2 mA, at 125 V for 12 h at 2°C in the cold room. The strips were then removed and dried in an oven at 95°C for 15 min. Staining of the proteins was made by 1% bromophenol blue in ethanol saturated with $HgCl_2$. Differentiation of the spots was carried out with 0·1% acetic acid. Lipoproteins were stained by a saturated solution of Sudan Black B in 60% ethanol and differentiated by 60% ethanol. Glycoproteins were detected according to the method of ROMANI[7].

The optical density of the spots was then read in an Elphor apparatus after treatment of the strips with vaseline oil. Diagrams were constructed with the results obtained. The percentage distribution of single fractions was estimated by measuring on each diagram the area of the Gauss curves corresponding to each peak. The form of the patterns is given in the Figure, in which each of them represents the average of at least 5 measurements.

Paper electrophoresis pattern of total proteins consists of at least 5 components. Relative percentages, ± standard deviation, were: 15·3 ± 7·4 for peak No. 1, 16·3 ± 5·1 for No. 2, 19·5 ± 1·8 for No. 3, 45·2 ± 12·2 for No. 4, 3·8 ± 2·8 for No. 5. The form of the pattern is comparable with that obtained by CONGIU, who used free electrophoresis. The number of peaks is, however, less than that described by this author. It seems probable that peaks No. 2, 3, and 4 of paper electrophoresis correspond to those described as 'central group' by CONGIU. The form of

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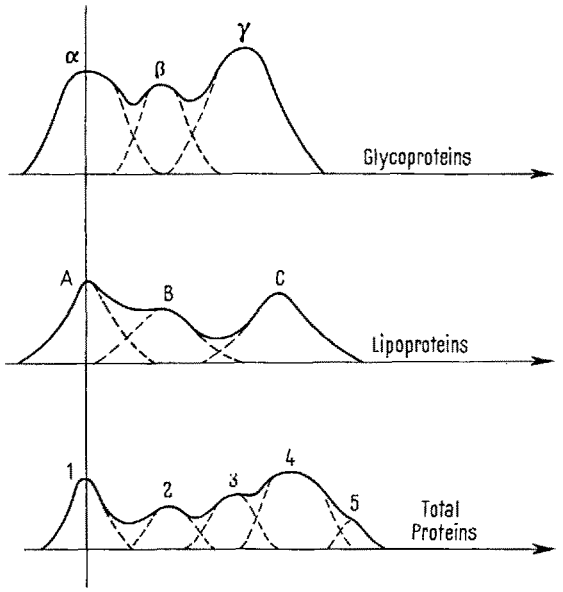
the pattern of the soluble proteins from rat liver mitochondria is, however, different from that of rat muscle sarcosomes as recently reported by DIANZANI MOR[8]. The lipoprotein pattern consists of at least 3 components. The first of them (A) occupies on the strip the same position as peak No. 1 of the total protein pattern. The second one (B) occupies the same position as peak No. 2; the position of the third one (C) corresponds to that of peaks No. 3 and 4. Relative percentages of lipoprotein components were respectively: 37·1 ± 7·9 for A, 32·2 ± 4·7 for B, and 30·7 ± 5·3 for C.



Total, lipo-, and glycoprotein diagrams of rat liver mitochondria extracts. The values on the abscissa represent the translation in mm; those on the ordinata represent the photometric absorption measurements. A factor 4 was applied in the case of glycoproteins.

The glycoprotein pattern consists of 3 peaks. Their position on the strip corresponds to that of lipoproteins peaks. Relative percentages were: 27·4 ± 5·7 for α, 26·3 ± 4·4 for β, and 46·2 ± 1·4 for γ.

In order to investigate the possible influence of sucrose on the concentration of glycoproteins in the mitochondrial extract, some experiments were run in which mitochondria were isolated from homogenates prepared with 0·125 *M* KCl instead of 0·25 *M* sucrose. No difference was found with respect to the mitochondria isolated from sucrose medium.

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Istituto di Patologia generale, Università di Cagliari (Italy), January 30, 1960.

Riassunto

Mediante elettroforesi su carta si dimostra la presenza nei lisati di mitocondri di fegato di ratto di almeno 5 componenti proteici. Le colorazioni specifiche dimostrano la presenza di 3 frazioni lipoproteiche e di altrettante glicoproteiche. I lisati dei mitocondri vennero ottenuti qer trattamento delle particelle con Triton X-100.

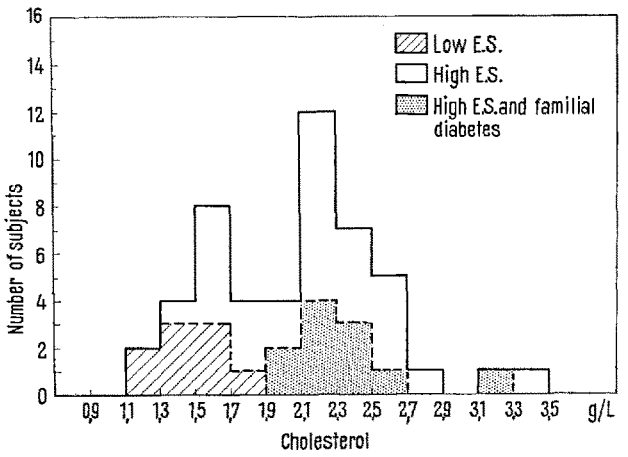
Normal Values of Plasma Potassium, Sodium, Cholesterol and Proteins and of Blood Glucose in South Indian People, in Reference to Western Standards

No systematic studies have been made so far to compare the normal blood composition of South Indian people with western standards, used as a reference. The present work is a preliminary investigation in this field.

Venous blood was taken before breakfast from 49 male Indians of Madras, all students or physicians, except for 9 who were servants of low economic status. Plasma, separated from heparinized blood by centrifugation, was analyzed for K and Na by flame photometry, for cholesterol by CRAWFORD's modification of the method of ZAK[1] and for protein by KJELDAHL. Blood glucose was determined by the FOLIN and WU method[2]. A few determinations were also made with 11 males of the white race (European or American) living in Madras.

Results are summarized in the Table. The individual values are spread symmetrically around their mean, and no significant difference is observed between Indian students or physicians, Indian servants, and white people, except for cholesterol. As shown in the Figure, these latter values are divided into two groups. The 9 Indians of low economic status are in the group of lower values, while eleven people with a familial diabetic background are all in the higher group. This distribution is statistically significant.

Our results fall in the normal range of western standards. Nevertheless, a group of 20 male Belgians of the same mean age as our Indian population show K values significantly lower (15.1 ± 1.59 mg %; HENROTTE[3]). The similarity of K values in Indians and Westerns in Madras



Distriubtion of cholesterol values (in g/l) in the Indian population. Low E. S. = Indian servants of low economic status. High E. S. = Indian students or physicians of high economic status. High E. S. and familial diabetes = same as the latters but revealing the occurrence of one diabetic or more in their family.

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