The heart volume/body weight ratio has been used as an index of relative heart size in man[7]. However, in man the body weight is an incomplete expression of the physical status of the individual, owing to the wide variation in nutritional state and body habitus. Therefore, in man, radiologically estimated heart volume expressed per square metre of the body surface bears a closer relationship to the heart size[1]. In rats, living in strictly standardized conditions, the deviations in the individual nutritional state and physical status are very slight, as shown by the close relation between body weight and heart volume (Figure 4). In healthy growing rats the correlation between the heart volume and the heart weight also was found to be significant at the $P < 0.001$ level[8]. This relationship could be expressed by the regression line $y = 347 + 2.44x$. The correlation factor was 0.880. Owing to these correlations, we found it unnecessary to correlate the heart volume with other parameters.

In preliminary experiments[8], we were able to follow the development and regression of various forms of experimental cardiomegaly in rats, using this method for determination of heart volume.

Zusammenfassung. Radiologische, unblutige Bestimmung des Herzvolumens bei der Ratte mit Modifikation der Methode zur Bestimmung des Herzvolumens beim Menschen und Nachweis einer Korrelation zwischen Körpergewicht und Herzvolumen.

J. EDGREN and J. VON KNORRING

Fourth Department of Medicine, Helsinki University Central Hospital, Unionsgatan 38, SF–00170 Helsinki 17 (Finland), 24 April 1973.

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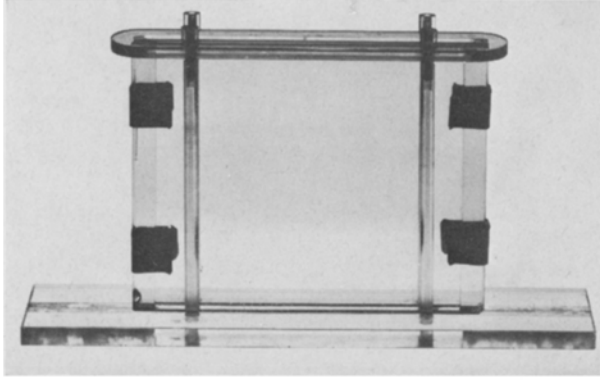
Two-Dimensional Electrophoresis of Rat Serum Esterases in Cellulose Acetate and Acrylamide Gradient Gel

Electrophoretic separation of serum esterases of different species has been demonstrated in a number of investigations. The separation and classification are mainly based on electrophoresis on cellulose columns[1–4] and on starch[5,6]. Two-dimensional technique, combining paper and starch gel electrophoresis, was used by HUNTER, DENUCÉ and STRACHAN[7]. They observed 8 new areas of esterase active protein in mouse sera. With polyacrylamide gel electrophoresis, it proved possible to demonstrate more proteins with esterase activity, because this system simultaneously exploits differences in molecular size and charge for purposes of fractionation[8,9]. Human serum proteins have been separated by a two-dimensional cellulose acetate, step-gradient polyacrylamide gel electrophoretic system[10], and the fine resolution made it possible to identify as many as 30 proteins.

In the present study, a similar two-dimensional electrophoresis system was used to separate non-specific esterases in the rat serum. As was expected, it was possible to obtain a more critical separation of the esterases and to reveal new enzyme spots which cannot be demonstrated with one-dimensional acrylamide gel electrophoresis.

Material and methods. Blood was collected by cardiac puncture from male Sprague-Dawley rats under ether anaesthesia. The serum was separated from the blood cells by centrifugation. Serum samples were studied with the Ortec high resolution electrophoresis unit using a step gradient, flat bed acrylamide gel system[11]. However, since difficulties were encountered with the removal of gels from the original cells, new cells were designed which were easy

a)



b)

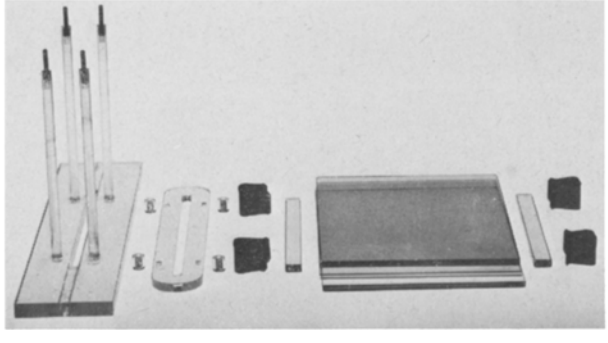


Fig. 1. a) A gel cell, assembled for casting in the cell holder. b) The same cell dismantled. The cell holder on the left. The gel 'sandwich' layers consist of an outer glass plate, 2 inner methacrylate plates separated by 2 glass spacer strips and another glass plate. The plates are clamped together with 4 steel clips.

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to dismantle after electrophoresis in order to remove the gel slabs for further handling. For casting procedures they were set up and fixed in the casting stand. Details of a cell are seen in Figures 1 a and b. As compared with closed cells, the simple cells, essentially consisting of 2 glass plates, 2 methacrylate plates and 2 glass spacers, proved convenient and the gel slabs could easily be removed without damage.

The electrophoretic separation in first dimension was carried out in cellulose acetate paper strips using 0.05 *M* *Tris*-barbituric acid buffer at pH 8.8 in a Sepratech tank (Gelman). The initial current was set at 2 mA and increased after $^1/_2$ hour to 4 mA. The voltage was 235 volts and the separation time about 1 h. Thereafter the cellulose acetate strips were cut longitudinally into 3 mm wide sections over the separation and placed on the surface of a step gel consisting of 4.5, 6, 8 and 12% of polyacrylamide. An 8% acrylamide gel cap was pressed against the upper surface of the strips. Other strips were stained with Amido black in order to identify the protein fractions. As a separating gel buffer, a 0.75 *M* *Tris*-sulphate buffer at pH 9.0 was utilized. Both the upper and the lower tanks were filled with 0.065 *M* pH 9.0 *Tris*-borate buffer. Constant power for the electrophoretic separation was provided by means of a pulsed constant power supply, in which the initial pulse rate was 75 pulses/sec. The pulse rate was increased by 75 pulses/sec at 5 min intervals until 300 pulses/sec was reached. The capacitance was set at 1.0 μ-farad and the voltage at 280 volts. The total time elapsed for the run was 45 min.

For protein staining, the gel was fixed in 12% trichloroacetic acid, stained with a 0.2% aqueous solution of Coomassie Blue and de-stained in 10% acetic acid. For demonstration of esterases, the gel was placed in a 0.1 *M* phosphate buffer (pH 7.4) solution for 10 min and then transferred to the incubation solution including a histochemical esterase substrate, α-naphthyl acetate (B.D.H. Laboratory chemicals, Poole) or α-naphthyl butyrate (Sigma chemical company, St. Louis). The enzyme activity was demonstrated principally according to the method of ALLEN, POPP and MOORE[8]. The incubation solution consisted of 100 ml of 0.1 *M* phosphate buffer (pH 7.4), 4 ml of a 1% acetone solution of the substrate and 100 mg of Fast blue RR (G.T. Gurr Ltd., London). The incubation time was 15 min at 37 °C. The reaction was stopped with an acid alcohol mixture consisting of 20 ml of 95% ethyl alcohol and 80 ml of 10% acetic acid.

For the study of specific esterases, the following inhibitors were used: eserine in concentrations of $10^{-3}M$ to $10^{-5}M$, tetraisopropylpyrophosphoramide (iso-OMPA) in concentrations of $10^{-3}M$ to $10^{-6}M$ and 1:5-*bis*-(4-allyl dimethylammoniumphenyl)pentan-3-one-diiodide (284 C 51). Each inhibitor was incorporated both in the preincubation fluid and the substrate-containing incubation solution. After demonstration of the esterase activity, some gels were stained with the protein stain. However, in this way it was possible to distinguish and correlate only the strongest esterase bands with serum proteins.

Results and discussion. A two-dimensional esterase electropherogram is illustrated in Figure 2 a and b, and a diagram of the esterase bands, based on several gels, is shown in Figure 3. A total of 16 bands was found in the unidimensional separation with α-naphthyl acetate as substrate. Two-dimensional separation revealed one additional band: the intensely reactive band 13 of albumin esterase appeared as 2 separate fractions. No other new fractions were found in the second dimension. The activity and resolution with α-naphthyl butyrate were not as high as with α-naphthyl acetate. α-Naphthyl butyrate did not reveal any new bands, nor did the longer incubation time with any of the two substrates.

A total of 29 protein bands could be identified in the two-dimensional separation. When the esterase-positive zones (Figure 2a) were compared with the protein bands (Figure 2b), it was found that the pattern of esterase activity considerably differed from that of the Coomassie-

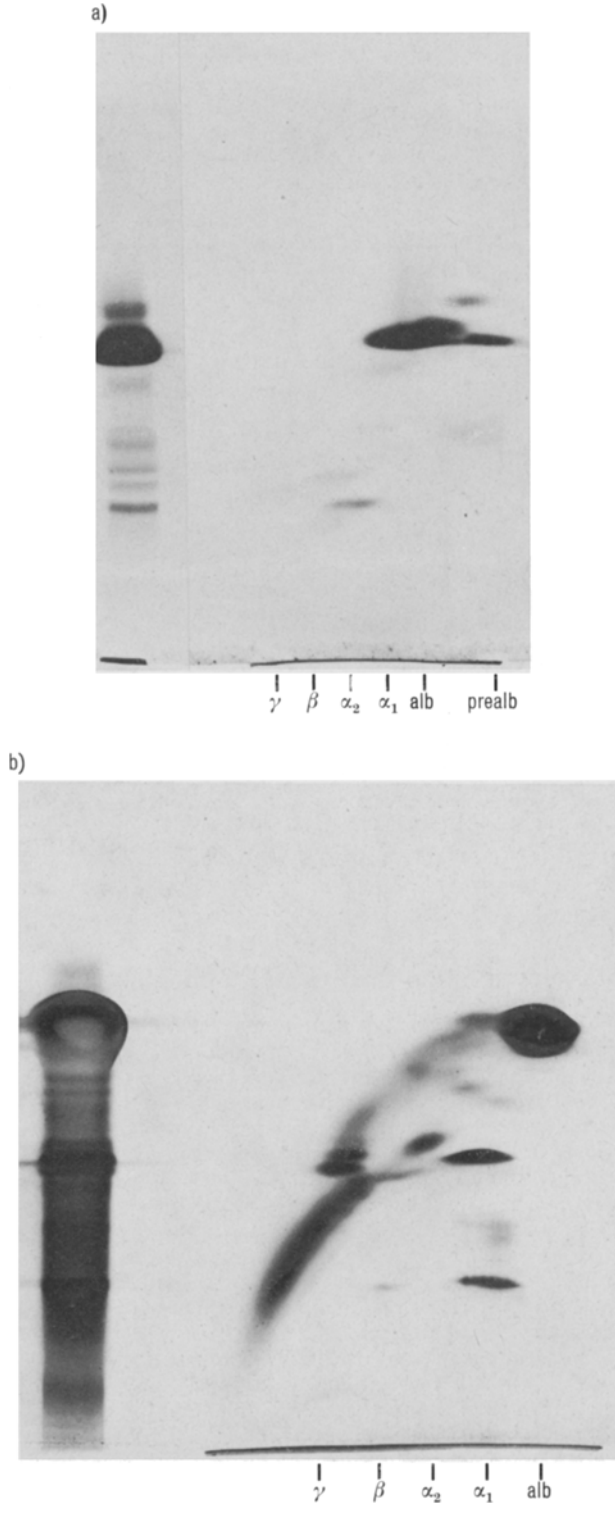

Fig. 2. a) One- and two-dimensional serum esterase patterns separated in the same acrylamide gel plate and demonstrated with α-naphthyl acetate as substrate. b) One- and two-dimensional separation of serum proteins, stained with Coomassie-Blue.

blue-stained protein bands. This also applied to the zones of the protein fractions separated in cellulose acetate. Esterase bands 7, 13, 15 and 16 were located in the prealbumin fraction, while Bands 10, 11 and 12 mostly in the α_1-globulin fraction, and Bands 2, 3, 4, 5 and 8 in the α_2-globulin fraction. Band 6 and, partly, Bands 2, 4 and 8 were in the β-globulin fraction and Band 1 mainly in the γ-globulin fraction.

In a concentration of $10^{-3}M$, which was found to be effective, eserine inhibited the activity in Bands 2, 3, 4, 5, 10, 11 and 12; with lower concentrations of eserine Bands 3 and 12 were only partially inhibited. In a concentration of $10^{-4}M$, iso-OMPA inhibited Bands 2, 3 and 11, presumably non-specific cholinesterases. In the concentration of $10^{-5}M$ only the band 11 was sensitive to the inhibitor. Even in the highest concentrations used ($10^{-3}M$) 284 C 51 failed to cause any inhibition of the esterase bands; this suggests that acetylcholinesterase is entirely absent from the rat serum.

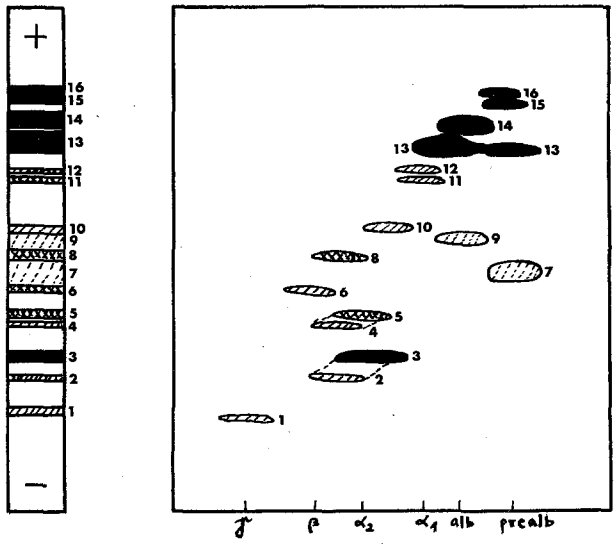

Fig. 3. Diagrammatic presentation of the esterase bands found. The same numbering is used in the text. The corresponding serum protein fractions are marked at the bottom of the two-dimensional separation.

With the two-dimensional step-gradient acrylamide electrophoresis, it was possible to correlate the individual serum proteins and esterase to the α-, β- and γ-globulin bands in the same way as was previously established by SMITHIES[12], using the less discriminating paper and starch system. One-dimensional gel electrophoresis gave very limited information of the distribution of esterases in the classical serum protein fractions. The observation that the esterase bands did not always correspond to the areas of stainable protein is in agreement with earlier observations by HUNTER et al.[7] on separations with paper and starch.

Zusammenfassung. Isoenzyme von Serumesterasen wurden zuerst mit Zelluloseazetat und dann mit einem Gradientgel von Polyakrylamid mit zweidimensionaler Elektrophorese bei der Ratte untersucht. Ein neues Band, das eine starke Albuminesteraseaktivität aufweist und unempfindlich gegen $10^{-3}M$ Physostigmin ist, wurde somit gefunden. Von 17 Esterasebändern waren 7 empfindlich auf Physostigmin, und 3 wurden auch durch Iso-OMPA gehemmt, während 284-C-51 gar keinen Effekt zeigte.

O. ERÄNKÖ[13], R. L. HUNTER[14] and A. I. KOSKIMIES[13]

Department of Anatomy, University of Helsinki, Siltavuorenpenger 20, Helsinki 17 (Finland),
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Mercury Binding to Human Haemoglobin

Mercury solutions, both as inorganic mercury salts and as organic mercury complexes, have found wide application in the estimation of sulphydryl groups in proteins[1–6]. These methods generally use either an amperometric back-titration of mercury after reaction with the -SH groups or a spectrophotometric determination. For these systems the organic mercury derivatives, such as '*p*-mercuribenzoate' have proved more successful then inorganic mercury which suffers from the disadvantage of its divalency since it can react with either one -SH group to form RSHgX or two to form $(RS)_2Hg$[1,2]. This has lead to conflict concerning the number of available sulphydryl groups present.

The degree of denaturation of the protein, especially with haemoglobin, markedly affects the estimated number of thiol groups. Strict adherence to the control of pH and composition is essential and even ammonia based buffers are reported to yield erroneous results due to the formation of metallic ammines[6]. However, the introduction of *tris* buffers has substantially improved the confidence placed in experimental results[7].

The work described here offers an alternative approach to the study of haemoglobin sulphydryl groups and their reaction with mercury using a technique analogous to that previously reported in connection with the interaction of lead with haemoglobin[8].

Method. Whole blood was collected by venepuncture, the cells separated and washed 3 times with physiological saline. The erythrocytes were then haemolysed by repeated freezing and thawing and the stroma removed by centrifugation[9]. The supernatant was removed, diluted $\times 2$ with *tris*-maleic acid buffer, pH 7.0 and samples taken for

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