Résumé. On a cherché à déterminer la presence d'un ARN bi-caténaire lors de la réplication du virus herpétique. La précipitation de LiCl 2 *M*, la filtration sur gel Sephadex, la chromatographie sur cellulose, la sédimentation en gradient de densité et l'induction d'interféron in vivo et in vitro semblent démontrer qu'une telle structure n'est pas indispensable à la réplication du virus herpétique.

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R. PEREZ-BERCOFF, G. CARRARA, A. DOLEI and G. RITA[19, 20]

Università di Roma, Istituto di Virologia, Viale di Porta Tiburtina 28, I-00185 Roma (Italy),
2 March 1973.

PRO EXPERIMENTIS

Radiological Determination of Heart Volume in Rats

In a preliminary study on experimentally induced cardiac hypertrophy and regression of the hypertrophy in rats, we found it necessary to develop a method for in vivo estimation of the heart volume. Our aim was to obtain information on changes in the heart volume of the same animal at different stages of the experiment. The method by JONSELL[1] for estimation of the heart volume in human beings was modified for use in rats. This paper is a report on the method and the changes of the heart volume relative to body weight in growing rats.

Animals and methods. Albino Wistar male rats aged 16 (group A) and 24 weeks (group B) were used. Each group consisted of 10 rats. The animals were reared on a commercial pelleted diet (Hankkija). The rats in group A were followed up to the age of 20 weeks, and in group B to the age of 35 weeks. After initial determination of the heart volume and body weight, these parameters were determined 5 times in group A and 4 times in group B at the time intervals indicated in the Table. During the study 2 rats in group A and 1 in group B died. The radiographs for determination of the heart volume were taken under light ether anaesthaesia with the rats fixed with adhesive tape to a plate especially designed for this purpose (Figure 1). At each determination 3 pairs of frontal and lateral films were made. The heart volume was expressed as the mean value of the volumes calculated from those films. The frontal and lateral films were exposed with the same values: 55 kV, 500 mA and 3 msec at a focus-film distance of 60 cm. The X-ray tube used had a focus size of 1.2 × 1.2 mm². The pictures were taken on an ordinary X-ray film for 90 s processing, and high resolution screens were used in the casette. Equipment for simultaneous exposure of the frontal and lateral projections was not available.

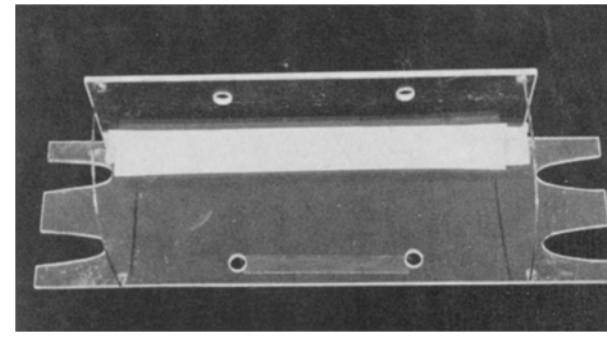

Fig. 1. Plate made of plexiglass on which the rats were fixed during picture-taking.

For determination of the heart volume, the rat heart was assumed to be an ellipsoid. Thus, the formula $V = l \times b \times d \times k$ generally used for determination of the human heart volume[1] could be used. In this formula l (length) and b (breadh) were measured from the frontal film and d (depth) from the lateral one (Figure 2). The constant $k = 0.47$ was calculated from the formula for the volume of an ellipsoid $3\ \pi/4\ {}^{l}/_{2} \times {}^{b}/_{2} \times {}^{d}_{2}$. Allowance was made for the geometrical enlargement due to the ratio of the focus-film and heart-film distances.

Results. The heart volumes of the rats in both groups are given in Figure 3. The deviations of the individual heart volumes from the growth curve increased with age. From 6 to 35 weeks the standard deviations of the heart volumes from the means increased from 245 to 655 mm³. Despite these deviations, the individual heart volume growth patterns were very similar, indicating that the deviations did not result from differences in volume determinations. Up to the age of 15 weeks, there was a statistically significant increase ($p < 0.001$) in the heart volumes between the given time intervals. After this age, a non-significant increase was noted up to 35 weeks of age.

Heart volume/body weight ratios of growing rats

Group	No. of animals	Age (weeks)	Heart volume/body weight ratio	
			mm³/g	*P*
A)	10	6	10.0 ± 0.9[a]	
	9	8	9.2 ± 0.9	NS[b]
	9	10	9.5 ± 1.1	NS
	8	15	8.4 ± 0.6	<0.005[b]
	8	20	7.7 ± 0.4	<0.05[b]
B)	10	24	8.6 ± 0.6	NS[c]
	9	27	9.0 ± 1.1	NS[c]
	9	31	8.8 ± 0.9	NS[c]
	9	35	8.7 ± 0.5	NS[c]

[a] Mean ± S.D. [b] Compared to group A, 6 weeks. [c] Compared to group A, 15 weeks. The same rats as in Figure 3.

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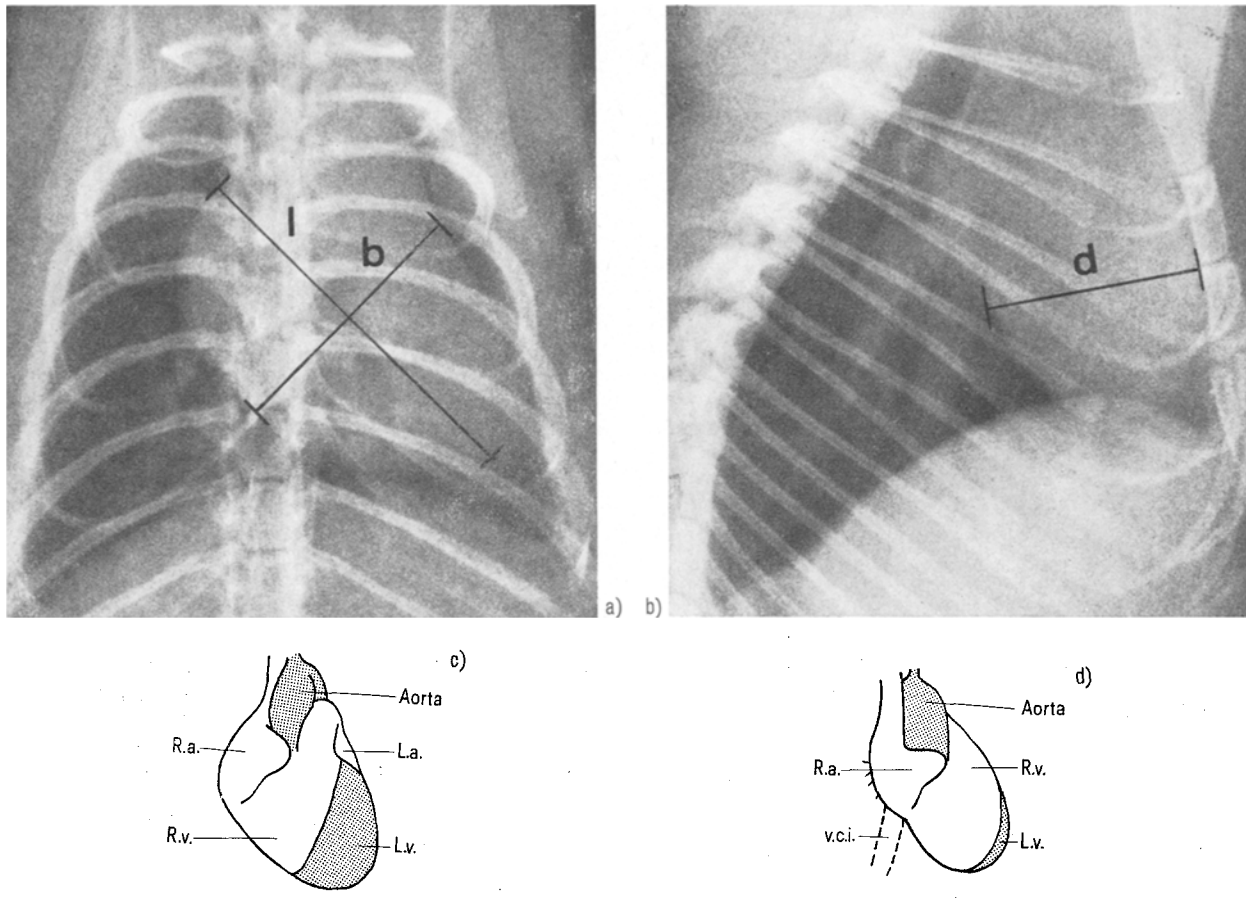

Fig. 2. Frontal and lateral pictures of a rat at 20 weeks of age. The measures marked on the pictures indicates l, length; b, breadth; and d, depth of the heart. R.a., right atrium; R.v., right ventricle; L.a., left atrium; L.v., left ventricle; v.c.i., inferior caval vein.

A close relationship was observed between body weight and heart volume (Figure 4). The correlation factor between these parameters was 0.930 and differed significantly from 0 at the level of $p < 0.001$. The heart volume/body weight ratio, expressed in mm^3/g decreased from 6 to 20 weeks of age, and thereafter remained at nearly the same level (Table).

Discussion. The heart weight/body weight ratio has been found to be a reliable index of the relative heart size in normally growing rats [2] and in rats in which an increase [3–6] or decrease [5] of heart size has been induced experimentally.

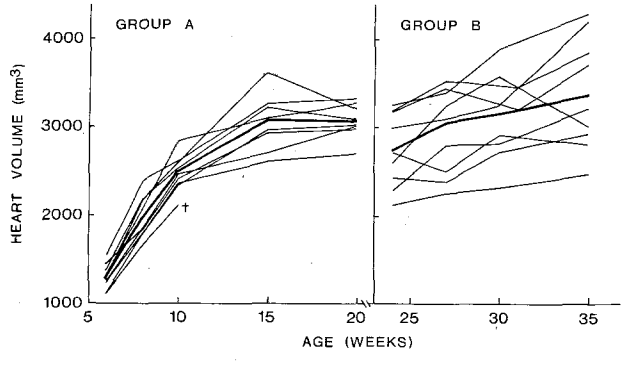

Fig. 3. Growth of heart volumes in 2 groups (A and B) of normal rats. The thick lines represent the mean heart volume in the 2 groups.

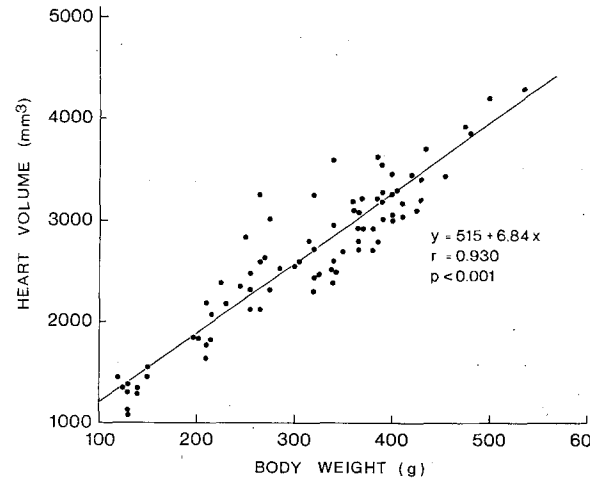

Fig. 4. Relation between body weight and heart volume in growing rats. Each point represents the heart volume of the animals at the time intervals indicated in the Table. y indicates the heart volume and x the body weight of the same animal.

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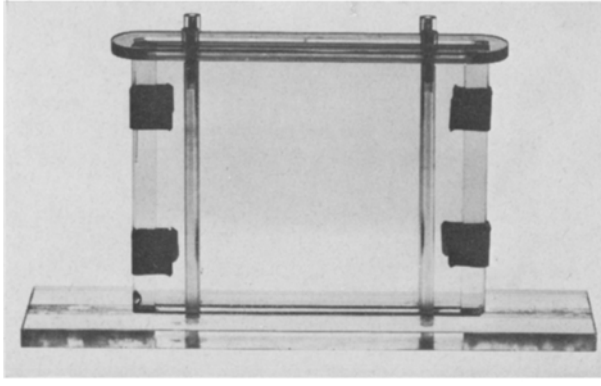
Two-Dimensional Electrophoresis of Rat Serum Esterases in Cellulose Acetate and Acrylamide Gradient Gel

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)

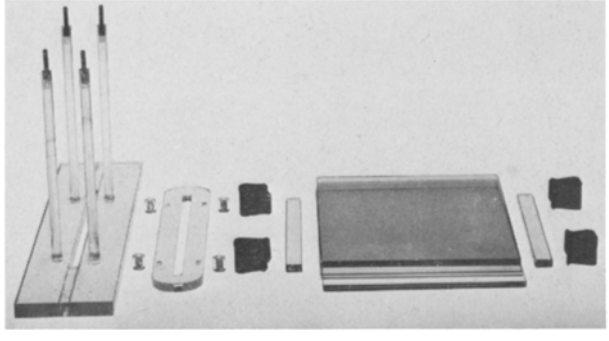


Fig. 1. a) A gel cell, assembled for casting in the cell holder. b) The same cell dismantled. The cell holder on the left. The gel 'sandwich' layers consist of an outer glass plate, 2 inner methacrylate plates separated by 2 glass spacer strips and another glass plate. The plates are clamped together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

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