observed in the optic nerve after ligation. Significant changes in GAD activity were clearly shown in the optic nerve on the 1st, 2nd, 4th and 7th days after ligation. GAD activity was higher in the optic nerve from the ligated rats than from the control rats, namely 36, 46, 25 and 30% higher on the 1st, 2nd, 4th and 7th day respectively after ligation (mean of 3–16 experiments each). On the other hand, no increase in GAD activity was found on the 3rd day after ligation. This observation may indicate that different mechanisms are responsible for the 1st (the 1st to 2nd day) and 2nd (the 4th to 7th day) periods of increased activity after ligation. However, the K_m values (100 mM) obtained graphically when studying the enzyme's properties were quite similar at various periods before and after ligation.

Discussion. GAD, a marker of GABAergic innervation, is mainly found in the nerve terminal fraction[15]. The ligation of the optic nerves induces an interruption of the axonal flow in the possible centrifugal neurons. The increased activity for the 1st period after ligation may correlate with the accumulation of GAD protein by the interruption of the axonal flow. Localization of GAD was also observed in the retina with GABA[16]. The accumulation of GAD protein may also be responsible for the 2nd period of increased activity after ligation, but the neurological characteristics are not yet clarified. Thus, further studies are necessary before it can be concluded that the possible centrifugal neuron to the retina is GABAergic.

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Quantitative lipoprotein analysis by direct cholesterol determination in the centrifugation medium

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Summary. The adaptation of the lipid extraction technique reported allows KBr to be eliminated and cholesterol to be assayed directly in the centrifugation medium. The recovery of cholesterol is about 96.1%, and the quantitative analysis of plasma lipoproteins is therefore improved.

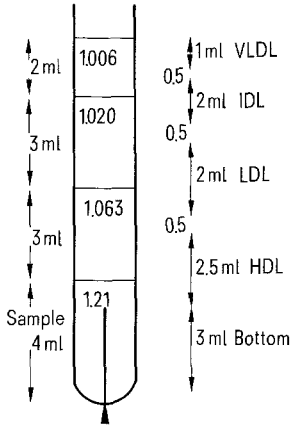
The methods used for the quantification of lipoproteins, either by weighing or by enzymatic assay of cholesterol, require dialysis of the fractions which causes large losses of material. The present paper reports an adaptation which allows cholesterol to be assayed directly in the KBr solution.

Techniques. The different classes of lipoprotein were separated by ultracentrifugation on a KBr density gradient, using Redgrave's technique[1] in a Beckman L65 centrifuge with an SW41 rotor.

When centrifuged the fractions were collected in the following way. First the VLDL (very low density lipoprotein) was removed by taking off the top 1 ml of the gradient with a syringe. The tube was then placed on a support and pierced with a syringe needle, the length of the needle being chosen to reach to 3 ml from the base of the tube. The fractions were collected in graduated tubes as shown in the figure. The purity of the fractions was checked by agarose gel electrophoresis[2] and the intermediary volumes were mixed with the corresponding fractions. In certain cases mixing occurs at the interfaces – this fraction is then discarded for analysis and cholesterol assay is carried out simply to calculate the yield.

An aliquot of the collected volume was taken for quantitative assay: 0.5 ml for the HDL (high density lipoprotein), the LDL (low density lipoprotein) and the IDL (intermediate density lipoprotein) and 0.1 ml for the VLDL. The remaining volume was dialyzed for 24 h against a 0.15 M NaCl solution containing 0.01% EDTA (pH 7.4) for qualitative analysis.

Total cholesterol was assayed in a non-dialyzed aliquot. In



Fractioning of the density gradient after centrifugation. The density composition of the discontinuous gradient is indicated on the left. After centrifugation the VLDL are removed from the top of the gradient and the other fractions are collected through the base of the tube. The volumes of the fractions are indicated on the right of the figure.

Plasma lipoprotein cholesterol (mg/100 ml)

	New Zealand rabbits	*Mesocricetus auratus*
VLDL	4.4± 0.74	16.9± 2.11
IDL	3.2± 0.47	9.1± 0.90
LDL	5.2± 0.85	39.4± 5.09
HDL	11.4± 1.28	89.5± 11.21

Mean± SEM of 12 rabbits and 11 hamsters.

order to eliminate the KBr, which masks coloration, the lipids were extracted with a 2/1 (v/v) chloroform/methanol mixture. After evaporation a 2nd extraction was carried out using petroleum ether. The KBr, which is insoluble in petroleum ether, was eliminated by washing with water, and the upper phase which contained the lipids was evaporated to dryness. This residue was used to assay the cholesterol[3].

Results and discussion. The presence of KBr hampers the assays but it can be easily eliminated during lipid extraction. It was noticed, however, that after extraction enzymatic assay was disturbed, so a colorimetric cholesterol assay was selected. Ultracentrifugation on a density gradient was chosen for lipoprotein separation since this method limits the losses incurred during each phase of successive centrifugation. Furthermore the absence of dialysis before cholesterol assay again reduces the errors due to loss - the yields obtained were therefore excellent. Out of 83 plasmas analyzed, an average of 96.1 ± 1.69% cholesterol was recovered. The table shows results obtained in 2 laboratory animals. In conclusion, this method allows quantitative and qualitative lipoprotein analysis, with high yield, after only 1 centrifugation, on 4 ml plasma.

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Bilinear correlation between tissue water content and diastolic stiffness of the ventricular myocardium

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Summary. In oedematous and dehydrated canine hearts a close bilinear correlation was demonstrated between myocardial water content and diastolic stiffness (characterized by the passive elastic modulus) with an optimal minimum of stiffness at normal myocardial water content.

Elevated diastolic stiffness of the ventricular wall plays an important role in the pathomechanism of a number of heart diseases[1-9]. Recently, we have found that left ventricular diastolic stiffness is equally elevated in myocardial oedema[10,11] and in myocardial dehydration[12-14]. The aim of the present study was, therefore, to demonstrate a universally valid correlation between the values of tissue water content and ventricular diastolic stiffness of the myocardium.

Methods. The calculations of the study were performed using the data from 45 mongrel dogs of both sexes, weighing 10-25 kg, subjected to our earlier investigations[10,11,13]. In 6 pancreatectomized animals myocardial dehydration was induced by hyperosmolality effected by 2 or 3 repeated i.v. bolus injections of 11 mmoles/kg glucose in 5 ml/kg water[13]. Insulin influences the dehydrating effect of glucose-induced hyperosmolality[13], therefore, pancreatectomy was performed in these animals. Myocardial oedema was induced partly by i.v. infusion of 1.0 µg/min/kg noradrenaline for 10 min, which was performed 2 or 48 h after the ligation of the left anterior descending coronary artery in 6 and 7 animals, respectively[10], and partly by the overloading of non-working hearts by hyposmotic solutions during cardiopulmonary bypass for 60 min in 18 animals[11]. The data from 8 intact animals served as controls. Tissue water content was determined by drying the myocardium to constant weight, and expressed as a percentage of the total wet weight. Left ventricular diastolic stiffness was characterized by the value of left ventricular passive elastic modulus, which was determined by the modified[15] method of Diamand and Forrester[2]. The determination of left ventricular passive elastic modulus was carried out in all instances on open chest and pericardiectomized animals. Stiffness of the left ventricle was characterized by the slope of the linear relationship between diastolic $\Delta P/\Delta V$ and mean intraventricular diastolic pressure at various segments of the exponential pressure-volume curve, where ΔP is the arithmetic difference between end-diastolic and end-systolic pressure and ΔV is the stroke volume. In order to measure $\Delta P/\Delta V$ as well as mean intraventricular diastolic pressure in a wide range, flow in the descending aorta was gradually blocked by means of step-by-step inflation of an embolectomy catheter introduced through the femoral artery. Since pressure-volume relationship is not exponential at low values of pressure, data obtained at pressure less than 333 Pa were excluded. The calculations were carried out using regression analysis. Bilinear correlation was calculated by the method of Kubinyi and Kehrhahn[16].

Results and discussion. Pooling data for animals with myocardial oedema of different origins, and in the control state, a close linear correlation ($y = 0.034\ x - 2.534$; $r = 0.625$) was found between the values for the left ventricular water content and the left ventricular passive elastic modulus. On the other hand, pooling the data obtained in myocardial dehydration and in the control state, an inverse linear correlation ($y = -0.010\ x + 0.865$; $r = 0.428$) was detectable between the above variables. Considering the diverse alterations of myocardial water content in synthesis, a close bilinear correlation ($y = 2.120 - 0.027\ x + 0.072 \cdot \log(\beta \cdot 10^x + 1)$ where $\log \beta = -77.76$; $r = 0.685$) was demonstrable between the values for tissue water content and those for the passive elastic modulus in the left ventricular myocardium. This finding demonstrates that the smallest (optimal) diastolic stiffness of the myocardial wall coincides with the normal level of tissue water content.

It is a common feature of biological phenomena that optimal function of a certain organ is assured solely in those cases where important characteristics do not deviate from their normal levels. The present observation demonstrates this kind of correlation between tissue water content and diastolic stiffness in myocardium. It is known that myocardial water content normally oscillates between extremly close limits, and 100.0 g of wet myocardium contains under normal circumstances 77.8 ± 0.1 g water (mean ± SEM)[10-14,17]. This fact indicates that the normal level of tissue water content is maintained in the myocardium by very sensitive regulatory mechanisms. This is not only of theoretical interest, but has clinical significance,