intestine is a quick process and that this system is perhaps an appropriate model for dietary regulation of cholesterol synthesis as suggested previously[7].

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The possibility of centrifugal projections to the retina in the rat

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Summary. In rats, glutamic acid decarboxylase activity increased in the proximal portion of the optic nerve after its ligation, whereas the activities of choline acetyltransferase and tyrosine hydroxylase remained constant. Possible centrifugal neurons to the retina are GABAergic.

In 1889 Ramón y Cajar speculated that the centrifugal axons through the optic nerve in pigeons terminated the amacrine cells in the retina[1], and thereafter the existence of centrifugal optic fibres in the mammal has been in dispute[2-10]. To analyze the possibility of centrifugal projections to the retina in the rat, I studied the activities of enzymes relating to the synthesis of putative transmitter substances, namely glutamic acid decarboxylase (GAD) for GABA, choline acetyltransferase (ChAc) for acetylcholine and tyrosine hydroxylase (TH) for dopamine.

Materials and methods. Adult male Wistar rats, weighing 180–220 g, were used. Ligation was performed on the optic nerves bilaterally and intraorbitally under ether narcosis. On the 1st, 2nd, 3rd, 4th and 7th days after the ligation, the ligated animals were decapitated under ether narcosis and the proximal portion of the optic nerves was taken; sheaths and blood vessels were eliminated. Tissue specimens thus dissected out were weighed and homogenized with 50 µl of 0.1% Triton X-100 solution. The activity of GAD in the tissues was analyzed by a radioactive $^{14}CO_2$ trapping method using [1-^{14}C] DL-glutamic acid[11] (New England Nuclear, sp. act. 47.25 mCi/mmole). ChAc activity was assayed by kalignost extraction of radioactive acetylcholine formed by a reaction with [acetyl-^{3}H] acetyl coenzyme A[12] (New England Nuclear, sp. act. 2.1 Ci/mmole). The activity of TH was determined by a radioactive $^{14}CO_2$ trapping method using [1-^{14}C] L-tyrosine[13,14] (New England Nuclear, sp. act. 53.6 mCi/mmole). The analysis of GAD, ChAc and TH on each piece of tissue was done in duplicate and the mean of the 2 trials used in subsequent calculation. If the results of the duplicate analyses were not reasonably close, within 5%, the results were discarded.

Results. Experimental animals with their optic nerves ligated showed practically no atrophy of their optic nerves by the 7th day after ligation. The putative transmitter-synthesizing enzyme activities in the optic nerve of the rats after ligation are shown compared to those of normal rats in the table. No significant change in ChAc and TH activities was

Effect of optic nerve ligation on GAD, ChAc and TH activities. Values are expressed as nmole product formed/mg wet wt/h. Number of experiments are shown in parentheses. Each value is the mean of duplicate determination ± SD

Days after ligation		GAD	ChAc	TH
1	Control	4.42±0.15 (3)	0.24±0.11 (3)	0.014±0.003 (3)
	Ligation	5.99±0.42 (8)*	0.27±0.10 (4)	0.012±0.005 (4)
2	Control	4.48±0.04 (3)	0.30±0.10 (3)	0.012±0.006 (3)
	Ligation	6.55±0.48 (6)*	0.32±0.09 (4)	0.010±0.006 (4)
3	Control	4.34±0.25 (3)	0.31±0.11 (3)	0.008±0.003 (3)
	Ligation	4.45±0.26 (8)	0.29±0.09 (4)	0.010±0.005 (4)
4	Control	4.49±0.22 (4)	0.33±0.07 (3)	0.009±0.005 (3)
	Ligation	5.63±0.51 (16)*	0.29±0.10 (4)	0.010±0.005 (4)
7	Control	4.66±0.47 (3)	0.26±0.13 (3)	0.011±0.005 (3)
	Ligation	6.06±0.47 (10)*	0.22±0.12 (4)	0.013±0.005 (4)

* Difference from the control value is significant at $p < 0.001$.

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.

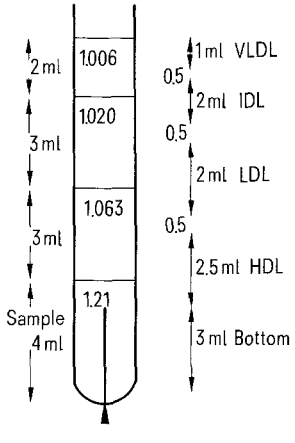
The methods used for the quantification of lipoproteins, either by weighing or by enzymatic assay of cholesterol, require dialysis of the fractions which causes large losses of material. The present paper reports an adaptation which allows cholesterol to be assayed directly in the KBr solution.

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.