(0.8×2 cm) and estimating the protein in the dialyzed lactose eluate. The binding capacity of arabinogalactan for these lectins was found to be 15 mg/ml of the gel.

Discussion. The binding capacity of *R. communis* lectins was very low for sepharose[4] or a ligand bound to sepharose[13]. Hence we investigated several β-galactosyl containing polysaccharides, and found arabinogalactan to be the most suitable as an affinity material. Arabinogalactan is a copolymer of D-galactose and L-arabinose in the ratio of 6:1. The main chain is β-(1→3) linked D-galactose with highly substituted 2-β-(1→6) linked D-galactose side chains and occasional side chains of 3-O-β-L-arabinopyranosyl-L-arabinofuranose[18]. The 2 proteins RCA_2 and RCA_1 have been separated effectively on this column utilizing their affinity for different sugars. Both the lectins have been obtained to electrophoretic purity. The capacity of arabinogalactan column to bind *R. communis* lectin in the present study was found to be 50 times higher than that of the conventional affinity matrices for these lectins. Studies reported here demonstrate that cross-linked arabinogalactan provides a high capacity affinity matrix for isolating *R. communis* lectins. It is hoped that cross-linked arabinogalactan will also find widespread applications in the purification of other galactose binding lectins.

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Inhibition of phosphodiesterase activity of human spermatozoa by spermine

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Summary. Sperm motility and metabolism are dependent upon the levels of cyclic AMP. Our studies have demonstrated that spermine by inhibiting sperm phosphodiesterase activity could regulate sperm physiology.

Cyclic AMP has been demonstrated to play an important role in the metabolism and motility of human spermatozoa[1-3]. Levels of cyclic AMP are regulated by 2 processes: a) Synthesis from adenosine triphosphate through the action of enzyme adenyl cyclase, and b) degradation of the cyclic AMP by phosphodiesterase. Studies carried out in our laboratory demonstrated that spermine stimulated the adenyl cyclase activity of human spermatozoa[4,5]. The present investigation examines the effect of this compound on the sperm phosphodiesterases.

Material and methods. The semen samples used for the study were obtained from fertile volunteers. Soon after liquefaction, the semen was centrifuged at 800×g for 30 min to sediment spermatozoa. The spermatozoa were then washed with the Krebs-Ringer bicarbonate buffer, recentrifuged and reconstituted to the original volume with Tris-HCl buffer (240 mM, pH 7.5). The spermatozoal suspension was sonicated at 0 °C every 30 sec for 10 sec during 4-5 min at 20 kc. The sonicate was then centrifuged at 2000×g for 10 min and the enzyme activity was determined in the supernate according to the method of Butcher and Sutherland[6]. The incubation mixtures consisted of cyclic AMP (1.0 mM), $MgSO_4$ (12.0 mM) Tris-HCl buffer (240 mM, pH 7.5) and the sonicated sperm supernate (40-60 µg protein per 0.1 ml). The total volume of the incubation mixture was 0.9 ml. Control tubes did not contain any spermine, whereas, in experimental tubes, different amounts of spermine were added. To 1 set of incubation tubes 100 µM theophylline was added to study its action on sperm phosphodiesterases under our experimental conditions. The enzyme activity is expressed in terms of ng of phosphate liberated per mg protein per 30 min. Determination of protein content of sperm supernate was carried out according to the method of Lowry et al.[8].

The data has been analyzed by the Student t-test and the levels of significance were read from 2-tailed table.

Effect of spermine on the cyclic AMP phosphodiesterase activity of human spermatozoa homogenate (ng of phosphate liberated per mg protein per 30 min)

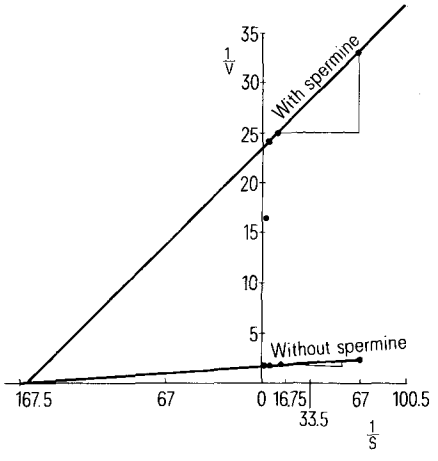
No.	Spermine (mM)	Number of observations	Mean ± SE
1	0	7	158 ± 16
2	1.9	6	107 ± 17
3	3.8	5	59 ± 6
4	7.6	3	18 ± 5
5	Theophylline (100 µM)	4	109 ± 4

Level of significance: between 1 and 2 $p<0.05$; 1 and 3 $p<0.002$; 1 and 4 $p<0.001$. The incubation mixture contained: Sperm homogenate (supernatant) in 240 mM-Tris, cyclic AMP (1.0 mM), $MgSO_4$ (12.0 mM) and spermine (0-7.6 mM).

Results and discussion. Results of our study demonstrate that spermine, like theophylline[3], inhibited the phosphodiesterase activity of human spermatozoa (table). Inhibition of the enzyme activity by spermine was dose-related. On studying the kinetics, this inhibition was found to be non-competitive (figure). The apparent K_m-value of the enzyme was 6.18 µM.

In our earlier studies, the effect of spermine on sperm adenylyl cyclase was determined in the presence of theophylline (a potent inhibitor of sperm phosphodiesterase[3]). Increased levels of cyclic AMP observed by us in these experiments were therefore due to the increased formation of cyclic AMP as a result of activation of adenyl cyclase by spermine. The results of the present studies suggest that spermine also decreased the degradation of cyclic AMP by virtue of its action on phosphodiesterase. The observed effects of spermine on accumulation of cyclic AMP by spermatozoa are therefore the net result of its effect on formation as well as on degradation of cyclic AMP. These effects of spermine on cyclic AMP levels may be of physiological importance as the cyclic AMP is known to be involved in all processes starting from the motility to the capacitation of the human spermatozoa prior to fertilization[1,3].

It has been reported that cyclic nucleotide phosphodiesterase inhibitors such as caffeine, theophylline, and papaverine markedly increased motility of bovine epididymal spermatozoa incubated with pyruvate, acetate, oxal-acetate or β-hydroxybutyrate[3]. Spermine, by virtue of its phosphodiesterase-inhibitory action may have an important role in the regulation of sperm motility and metabolism, especially when it is present in the unusually high concentrations found in human semen[9-11].



The Lineweaver-Burk plot showing the change in the phosphodiesterase activity of human spermatozoa sonicate (supernate) with variation in the substrate concentration (mM^{-1}) in presence or absence of 5.6 mM spermine in the reaction mixture.
The K_m-value was found to be 6.18 µM and the inhibition seems to be non-competitive.

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Cysteine oxidase and cysteine sulfinic acid decarboxylase in developing rat liver

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Summary. The patterns of development of cysteine oxidase (CO) and cysteine sulfinic acid decarboxylase (CSD) in rat liver are not similar. It was observed that CO is not under sex control as CSD is. The results obtained agree with the idea that, in liver, as well as in brain, CSD is the limiting factor for the regulation of taurine biosynthesis.

The physiological importance of taurine has been recognized since it was known that, in mammals, lipids are absorbed by the intestine as complexes with taurine and glycocolle bile salts (principally taurine conjugates in the rat). In addition, during recent years, taurine was shown to have other functions specially in the central nervous system[2]: in vision[3], in the heart[4], in skeletal muscles[5] and taurine is probably involved in endocrine[6] and reproductive[7] processes.

It is well accepted that the most significant biosynthetic pathway of taurine in the rat liver begins with the oxidation of cysteine to cysteine sulfinic acid. This acid is then decarboxylated into hypotaurine which is oxidized into taurine. Cysteine oxidase(CO) (EC 1.13.11.20) catalyses the 1st step, cysteine sulfinic acid decarboxylase (CSD) (EC 4.1.1.29) and hypotaurine oxidase catalyse respectively the 2nd and the 3rd steps. In rat liver, CO and CSD are well defined enzymes whereas little is known about hypotaurine oxidase.

The purpose of this study was to determine the developmental patterns of CO and CSD in liver of male and female rats.

Materials and methods. Albino rats of local breeding (from 2 days a.p. to 150 days p.p.) were used. From weaning they were maintained on a commercial stock diet (UAR 103). DL 3 ^{14}C cysteine and L 1 ^{14}C cysteine sulfinic acid (CSA) were purchased from CEA, Saclay, France. Rats were killed by decapitation and the livers quickly removed were homogeneized at 4 °C in water to a 20% (w/v) suspension. In experiments with fetuses, livers from several animals were pooled. All enzyme assays were performed immediately after the preparation of the tissue extracts. CO