(fig.), and this suggests there is another serum-derived sterol acceptor in the vicinity of 10^5 daltons. The uterus excludes proteins much above this molecular weight. From the optical density profiles (fig.), there is more protein in the uterine fluid of progesterone-treated rabbits.

The table presents the results of protein, cholesterol, and phospholipid assays[5] with these uterine fluids. They reveal a 15-fold increase with progesterone treatment in protein concentration, from 0.85 to 13.1 mg/uterine horn. Phospholipids and cholesterol were not detected in uterine fluid from ovulating does. By contrast, steroid-treated rabbits had 1.84 ± 0.56 and 0.047 ± 0.005 mg/uterine horn of these lipids, respectively. This yields a cholesterol/phospholipid mole ratio of 0.05. In addition, there were 4 mg cells/uterine horn in this fluid. The cell fraction was nondetectable in fluid from ovulating does. Subcellular membrane components of uterine fluid show a similar pattern of occurrence among rabbits treated with these hormones[16].

These results corroborate a report[17] that progesterone elevates uterine fluid lipid levels among rats. A progesterone-induced increase in uterine fluid cholesterol concentration (table) presumably helped suppress ^{14}C-cholesterol efflux from prelabeled sperm in utero (fig.), despite this fluid having a lower cholesterol/phospholipid mole ratio than ejaculated rabbit sperm cells (0.05 vs 0.88). A rabbit sperm cell contains an estimated 8.47×10^8 cholesterol molecules[2] and from present binding data (fig.) about 10% could be removed after 1.5 h during capacitation in utero. Hence, around 10^{15} cholesterol molecules are removed from 10^7 sperm, which is approximately the number that enter the uterus after mating. Results in the table indicate, however, there are an additional 10^{17} cholesterol molecules in uterine fluid from rabbits given progesterone. In agreement with present findings, the distribution of capacitation activity, assayed using hamster sperm, after elution of serum from a Sephadex G-150 column[18] suggests that both albumin and α-globulins act as capacitation factors. Cholesterol affinity in the latter has been experimentally established[19]. In this connection, analbuminemic rats with elevated α-globulins in their serum display normal fertility[20]. It would be interesting to further characterize the uterine fluid sterol-acceptor proteins and to establish the kinetics of sperm cholesterol efflux in capacitation.

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Binding of actinomycin D and divalent cations to lipopolysaccharides of *Agrobacterium tumefaciens* as studied by fluorescence spectroscopy

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Summary. The binding of actinomycin D and divalent cations to lipopolysaccharides of *A. tumefaciens* was studied. Fluorimetric titrations revealed 2 binding sites (low and high affinity sites) for divalent cations, and 1 high-affinity site for actinomycin D.

The lipopolysaccharides (LPS) of many gram negative bacteria have been studied earlier by various investigators[2]. Moreover, it has also been established that LPS, located in the external leaflet of the outer membrane of gram negative bacteria, are important for the barrier function of the membrane[3]. It has been postulated that stabilization of the outer membrane takes place as a result of the formation of ionic bridges between adjacent LPS phosphate groups, mediated by divalent cations[3]. However, it may also be that 2-keto 3-deoxy octulosonate (KDO) residues form a high affinity site for divalent cations, as has been established for sialic acid[4,5]. Schindler and Osborn[3] have shown that 3 KDO residues of the LPS molecule do indeed form the divalent cation binding sites which bind 1 mole of divalent cation per moles of LPS. Such divalent cation binding studies have not been carried out with the LPS isolated from *Agrobacterium tumefaciens*. Further, Walker and Durham[6] have shown that *Pseudomonas fluorescens* cells grown in glucose are much less sensitive to actinomycin D than succinate grown cells. They have established that the glucose grown cells have a higher concentration of LPS than the ones grown in succinate; this can influence the binding of actinomycin D to the cell surface, and thus exhibit its function in the cells in respect of different permeability conditions which are reflected in the sensitivity to the antibiotic. However, no reports are available regarding the LPS-actinomycin D binding in relation to the site and affinity of binding. In this communication the interactions

of *Agrobacterium* LPS with actinomycin D and also with Mg^{2+} as studied by fluorescence spectroscopy have been presented.

Materials and methods. Fluorescein isothiocyanate (isomer 1) was obtained from Sigma Chemical Co. St. Louis, Mo. Actinomycin D was obtained from Merck Sharpe and Dohme Research Lab. N.J. All other chemical reagents used were of the highest purity commercially available.

Bacterial cultures and LPS isolation procedures. The strain used in the investigation was *A. tumefaciens* TIP(Kerr 14) agrocin sensitive which was obtained from Prof. J. Schell, Laboratorium voor Genetika, Rijksuniversiteit, Gent, Belgium. Cells were grown under the conditions described by Das et al.[7]. LPS was isolated by the method of Leive and Morrison[8].

Fluorescence spectroscopy. FITC conjugation of LPS was carried out by the method described earlier[9]. Binding studies with $MgCl_2$, EDTA and actinomycin D were carried out in a MPF-44 B Perkin Elmer Spectrofluorimeter and the output signal was recorded on Perkin Elmer Chart paper using a single-pen recorder. The temperature was maintained throughout at 25 ± 0.3 °C. Excitations were carried out at 330 nm and emission spectra were scanned from 340 nm onwards. All the fluorescence filtrations and spectral (uncorrected) measurements were performed in buffers containing 50 mM Tris-HCl, pH 7.4 where the emission wavelength was set at 504 nm.

Results and discussion. Emission spectra of FITC-LPS in Tris buffer are shown in figure 1, A. Addition of 5 mM $MgCl_2$ to FITC-LPS caused an enhancement in the spectra, together with a blue shift from 504 to 495 nm of the peak. This enhancement was abolished on addition of 10 mM EDTA showing thereby that the enhancement was specifically due to binding of Mg^{2+} to LPS. Fluorescence titration of FITC-LPS with $MgCl_2$ in 50 mM Tris, pH 7.4 (fig. 2) revealed 2 saturable binding sites for Mg^{2+} with different K_D-values, viz. 5.75 μM and 1.0 mM for the high and low affinity sites respectively. K_D-values were determined by the

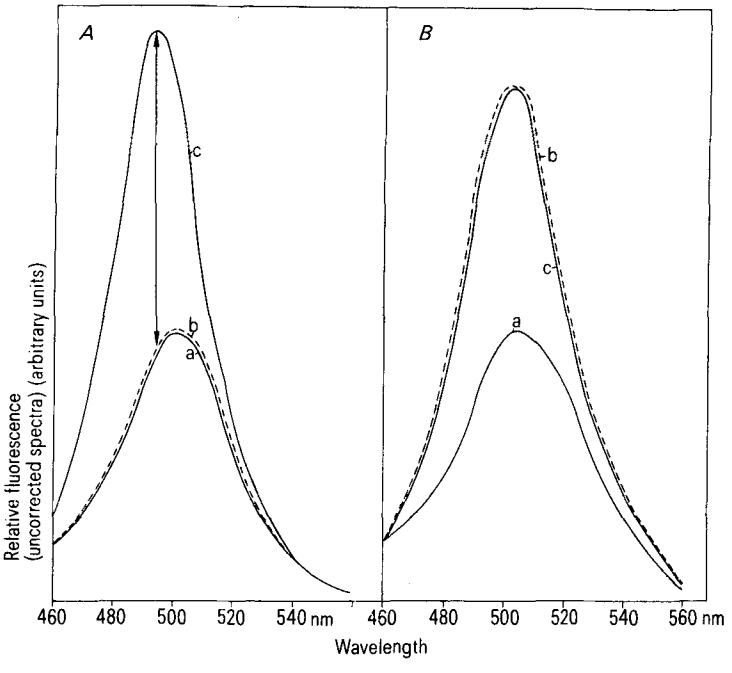

Figure 1. *A* Emission spectra of a) FITC-LPS; b) FITC-LPS + 5 mM $MgCl_2$ + 10 mM EDTA; c) FITC-LPS + 5 mM $MgCl_2$. Excitation at 330 nm, emission scanned from 340 nm onwards. *B* Emission spectra of a) FITC-LPS; b) FITC-LPS + 2.0 mM actinomycin D + 5 mM $MgCl_2$; c) FITC-LPS + 2.0 mM actinomycin D. Excitation at 330 nm, emission scanned from 340 nm onwards.

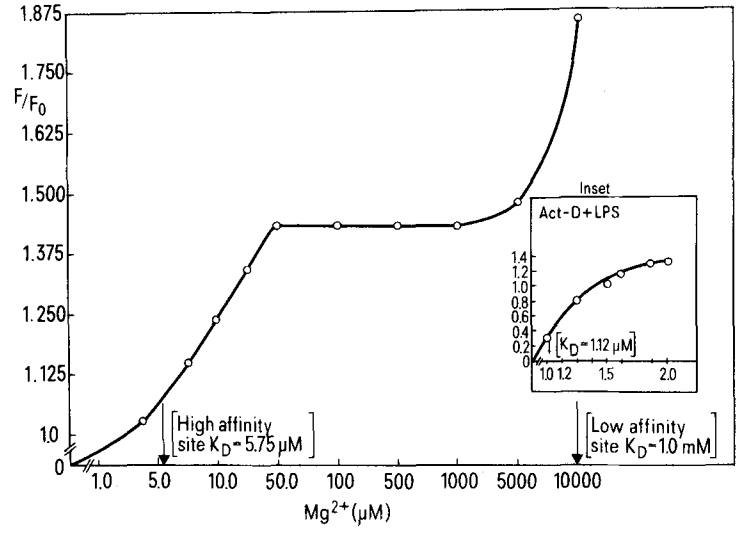

Figure 2. Fluorescence titration of $MgCl_2$ + FITC-LPS in 50 mM Tris JCl pH 7.4. Excitation set at 330 nm, emission set at 504 nm. Inset: Fluorescence titration of actinomycin D + FITC-LPS in 50 mM Tris HCl pH 7.4. Excitation set at 330 nm, emission set at 504 nm.

method of Schindler et al.[10]. The previous report of Schindler and Osborn[3] using dansylated LPS of *Salmonella* revealed K_D-values of Mg^{2+} binding to be 15 μM and 1.0 mM respectively for the high and low affinity sites. Our results indicate that LPS of *Agrobacterium* have greater affinity for Mg^{2+} than *Salmonella* LPS. Figure 1, B shows the emission spectra of FITC-LPS and the effect of addition of actinomycin D. The addition of actinomycin D causes an enhancement in the emission and a blue shift from 504 to 493 nm. However, unlike that of Mg^{2+}, the binding of actinomycin D did not exhibit 2 sites as seen from fluorescence titrations (fig. 2 inset). Divalent cations and EDTA were without effect on the actinomycin D-LPS binding. The K_D-value works out to be 1.12 μM. This value indicates a lower affinity for actinomycin D than for polymyxin B (0.3-0.5 μM) which can be accounted for by the much greater polarity of the polymyxin B molecule by virtue of the α, γ diaminobutyrate residues[3]. Like polymyxin B, however, it seems that actinomycin D binds rather nonselectively to both KDO and phosphate groups of LPS[3]. It is known that LPS isolated from gram negative bacteria can bind actinomycin D and thereby prevent its entry into the cell, but the mechanism of such interaction in relation to the nature of the binding sites, the affinity constants etc., which were not known so far, have been clarified to a certain extent by the present report.

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Effects of gonadal hormones on the lipid contents of the frog *Rana esculenta*

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Summary. The effects of exogenous gonadal hormones on the lipid contents of the liver and ovary and also on water content in the frog, *Rana esculenta,* were studied. Estrogen treatment significantly enhanced, whereas testosterone treatment reduced, the lipid and cholesterol contents. Water content of the frogs increased significantly after treatment by either hormone.

In recent years, a large volume of research, mainly in mammals, has shown the important role played by endocrine glands in general control of carbohydrate, protein and fat metabolism. In the non-mammalian vertebrates, estrogens have been shown to stimulate the synthesis and appearance in the plasma of the yolk precursor lipophosphoprotein, vitellogenin[2]. It has also been shown that estrogen increases plasma concentrations of lipids in teleosts. Specifically, it induces increases in total lipids and lipoprotein[3], cholesterol[4], lipid phosphorus in *Carassius auratus*[5], total and neutral lipids, free cholesterol and lipid phosphorus in *Oncorhynchus nerka*[6], lipoproteins in *Plecoglosus altivelis*[7] and a variety of lipids in *Salmo gairdnerii irideus*[8]. However, such extensive studies have not been made in the frogs. Seasonal variations in the glycogen of the liver, gonads and fat body of the common frog, *Rana temporaria* have been studied by Smith[9]. Pasanen and Koskela[10] investigated the seasonal and age variation in the metabolism of *R. temporaria* in Northern Finland. Sinha[11] studied the hematological changes on the prewintering and wintering frog, *R. esculenta.* Brehm[12] reported on the annual cycle of the parathyroid gland. Physiological activity and regulation of adrenal cortex of *R. temporaria* have been reported by Hanke and Webber[13]. Annual changes in the pars distalis of adenohypophysis of *R. temporaria* have been studied by Oordt[14], while Juszczyk[15] reported the development of reproductive organs of female frog in the early cycle. Brokelmann[16] investigated the changes in the interstitial cells of the testes during spermatogenetic cycle.

It is apparent from the literature cited above that not much work has been done on the effects of gonadal hormones on the lipemic actions in frogs in spite of the presumed importance of lipid as a source of energy for gonadal growth. The purpose of the present study was to examine the effects of exogenous gonadal hormones on the lipid contents of the liver, ovary and fat body of the female frog, *Rana esculenta.* During the course of the investigations it was observed that the body fluid accumulated in the frog after treatment with estrogen as well as testosterone and therefore it was considered worthwhile to quantify the water content of the frog.

Materials and methods. Healthy female frogs *(Rana esculenta)* weighing 25-30 g were obtained from the ponds in Basrah (30° 30′ N, 47° 50′ E) and brought to the laboratory. The frogs were divided into 3 groups of 5 animals each, and kept in separate aquaria. The aquaria were tilted slightly and contained enough water to form a pool at one end, while leaving the other end dry. They were kept in a well-ventilated room at 22±2 °C under natural photoperiod. The control group was injected with 0.1 ml cottonseed oil, one experimental group was injected with 100 μg estrogen and the other experimental group with 300 μg testosterone. The frogs were given 5 injections i.p. on alternate days (total 9 days) and on the 10th day lipid contents were estimated. Throughout the experiment the frogs were fed twice daily (normally at 09.00 and 16.00 h) except for a 24-h starvation period before the lipid estimations. A total of 90 animals (30 frogs per group) was investigated.