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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2 S.G. Wilkinson, Surface carbohydrates of the prokaryotic cell, p. 97. Ed. I.W. Sutherland. Academic Press, New York 1977.
3 M. Schindler and M.J. Osborn, Biochemistry *18*, 4425 (1979).
4 L.W. Jacques, E.B. Brown, J.M. Barrett, W.S. Brey, Jr, and W. Weltner, J. biol. Chem. *252*, 4533 (1977).
5 L.O. Sillerud, J.H. Prestégard, R.K. Yu, D.E. Schafer and W.H. Konigsberg, Biochemistry *17*, 2619 (1978).
6 C.A. Walker and N.N. Durham, Can. J. Microbiol. *21*, 69 (1975).
7 P.K. Das, M. Basu and G.C. Chatterjee, J. gen. appl. Microbiol. *24*, 121 (1978).
8 L. Leive and D.C. Morrison, Meth. Enzym. *28B*, 254 (1972).
9 D. Banerjee, M. Basu, I. Choudhury and G.C. Chatterjee, Biochem. biophys. Res. Commun. *100*, 1384 (1981).
10 M. Schindler, Y. Assaf, N. Sharon and D.M. Chipman, Biochemistry *16*, 423 (1977).

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

R.C. Sinha[1]

Physiology Laboratory, Department of Biology, College of Science, University of Basrah, Basrah (Iraq), 21 April 1981

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.

Concentrations of cholesterol and fat (mg/g wet weight) in the liver and ovary as well as water content (%) in untreated (control) and treated (experimental) frogs, *Rana esculenta*

Biochemical constituents	Control	Estrogen-treated	p-values (C vs E)	Testosterone-treated	p-values (C vs T)
Liver cholesterol	3.24±0.8	5.95±0.62	<0.001	2.83±0.81	<0.2 (NS)
Liver fat	26.8±6.2	36.7±8.4	<0.001	13.6±4.2	<0.001
Ovarian cholesterol	4.65±0.12	6.41±0.26	<0.001	2.55±0.43	<0.001
Ovarian fat	19.98±5.2	43.16±9.4	<0.001	14.62±4.8	<0.005
Lipid index	34.9±10.1	54.6±13.4	<0.001	25.8±8.1	<0.01
Water content (%)	76.4±5.6	88.2±8.1	<0.001	81.6±6.2	<0.025

Values are mean ± SD, n = 30.

A standard procedure was adopted for dealing with each frog in the sample. The frog was removed from the tank with a minimum of disturbance, care being taken to prevent the frog from struggling, and then immediately pithed. The frog was weighed on a Mettler balance (Model H-110), then dissected and the liver, ovary and fat body were taken out, placed on filter paper to soak up the moisture, and weighed on a Mettler balance.

The total lipids were extracted from the tissues following the method of Bligh and Dyer[17] using petroleum ether (boiling point 60–70 °C). The total cholesterol in the tissues was determined by Sackett's method as described by Varley[18]. The lipid index was determined by dividing the body fat by body weight and then multiplying by 100.

$$\text{Lipid index} = \frac{\text{body fat weight}}{\text{body weight}} \times 100$$

The water content of the frog was determined by taking the wet weight of the frog and then drying for 2 days at 105 °C in an oven and weighing until constant weight was obtained. The weights of the liver, ovary and fat body were deducted from the total body weight to compute water content. The data were analyzed statistically by Fisher's t-test[19].

Results and discussion. Comparison of the estrogen-treated frogs with those of the control group showed that the concentration of cholesterol and fat in the liver and ovary, as well as the lipid index, increased significantly after treatment with estrogen (table). In contrast, the above biochemical constituents (except for the cholesterol content of the liver) decreased significantly after treatment with testosterone. Water content increased significantly after treatment with either of the hormones, but the increase was greater in the estrogen-treated frogs (table).

Wiegand and Peter[2] reported that estrogen is involved in lipid mobilization in teleosts, but testosterone had no effect. Estrogens, acting on hepatic tissue, produce lipemia in pigeons and chickens[20,21]. Neutral fat, phospholipids and free cholesterol increase[22]. White crowned sparrows also became lipemic and hypercholesteremic when treated with estrogen but testosterone had no effect[23]. Esterase activity has been shown to increase the liver and fat body resulting in increased lipogenesis during autumn[10]. The results of the present investigations show that the estrogen-treated frogs have a higher liver fat, ovarian fat and lipid index than the controls, indicating lipemic action of estrogen. Similar reports have been made in fishes[2,3,8,24]. It is suggested that esterase is a hormone-sensitive enzyme and estrogen may be acting as inducer. The estrogen also has a hypercholesteremic action on the liver and ovary of the frog, *Rana esculenta*. This finding is similar to that of De Vlaming et al.[4], Ho and Vanstone[6] in fishes, and that of Kern et al.[23] in birds. Ovarian cholesterol in the frog is higher than ovarian cholesterol in the bird, *Calandrella acutirostris tibetana*[25]. It is suggested that the ovarian cholesterol accumulates in the ova in the form of fat globules to act as a potential source of lipoidal energy and in steroidogenesis, and also to help in flotation due to its lower weight, during the early stages of development.

The decrease in the fat of the liver and ovary, lipid index and ovarian cholesterol in the testosterone-treated frogs may be due to the accelerated lipid utilization owing to the catabolic effects of testosterone[26].

The increase in the water content of the frog after the treatment by estrogen or testosterone may be due to imbibition of water by the tissues[26,27].

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2 M.D. Wiegand and R.E. Peter, Can. J. Zool. *58*, 967 (1980).
3 V.L. De Vlaming, J. Shing, G. Paquette and R. Vuchs, J. Fish. Biol. *10*, 273 (1977).
4 V.L. De Vlaming, G. Delanunty, M. Prack and B. Bauer, Copeia 1979.
5 R.E. Bailey, J. exp. Zool. *136*, 455 (1957).
6 F.C.W. Ho and W.E. Vanstone, J. Fish. Res. Board Can. *18*, 859 (1961).
7 K. Aida, P.V. Ngan and T. Hibiza, Bull. jap. Soc. scient. Fish. *39*, 1091 (1973).
8 F. Takashima, T. Hibiza, P.V. Ngan and K. Aida, Bull. Jap. Soc. scient. Fish. *38*, 43 (1972).
9 C.L. Smith, J. exp. Biol. *26*, 412 (1950).
10 S. Pasanen and P. Koskela, Comp. Biochem. Physiol. *47A*, 635 (1974).
11 R.C. Sinha, unpublished data.
12 Von H. Brehm, Z. Zellforsch. mikrosk. Anat. *61*, 725 (1963).
13 W. Hanke and K. Webber, Gen. comp. Endocr. *4*, 662 (1964).
14 Van P.G.W.J. Oordt, Van W.J. Dongen and B. Lofts, Z. Zellforsch. mikrosk. Anat. *88*, 549 (1968).
15 W. Juszczyk, Annls UMCS, Lublin, *14*, 169 (1959).
16 J. Brokelmann, Z. Zellforsch. mikrosk. Anat. *64*, 429 (1964).
17 E.G. Bligh and W.F. Dyer, Can. J. Biochem. Physiol. *37*, 911 (1959).
18 H. Varley, in: Practical clinical biochemistry, pp. 309. Heinemann, London 1967.
19 G.W. Snedecor, in: Statistical methods applied to experiments in agriculture and biology, 5th edn. Iowa State Univ. Press, USA 1956.
20 O. Riddle, Endocrinology 31, 498 (1942).
21 R.H. Common, W.A. Rutledge and W. Bolton, J. Endocr. *5*, 121 (1947).
22 F.W. Lorenz, C. Entenman and I.L. Chaikoff, J. biol. Chem. *122*, 619 (1938).
23 M.D. Kern, W.A. DeGraw and J.R. King, Gen. comp. Endocr. *18*, 43 (1972).
24 S.N. Upadhyay, Thesis, Sciences naturelles, Université Pierre et Marie Curie, Paris, France 1977.
25 R.C. Sinha and S. Sinha, Experientia *35*, 1035 (1979).
26 C.D. Turner, in: General endocrinology, 3rd edn, p. 365. W.B. Saunders, Philadelphia/London, 1960.
27 C.L. Prosser, in: Comparative animal physiology, 3rd edn, p. 857. W.B. Saunders, Philadelphia/London, 1973.