The finding of LH in the serum of hypopituitary subjects is of interest and is similar to the situation seen with growth hormone levels in hypopituitarism. Only with suitable stimulative tests can one readily differentiate between the normal state and that of pituitary insufficiency(11). Even with surgical hypophysectomy one might expect to find circulating levels of LH, for LH-like material has been demonstrated by a fluorescent-antibody technique not only in the anterior pituitary but also in the pars tuberalis and the posterior lobe as well(12).

The results presented herein agree well with those of the previously published methods for the radioimmunoassay of LH(1,2) despite certain differences in methodology. These observations suggest that the radioimmunoassay for human LH is a reliable and specific method for quantitating this hormone in serum.

Summary. A sensitive and specific radioimmunoassay technique for quantitating human luteinizing hormone (LH) is described. Purified human pituitary LH is used for the development of antibodies, for radioiodination and for standards. A good correlation was seen between biological and immunological potency estimates of a number of pituitary extracts varying greatly in their LH and FSH contents. This method, when applied to serum, revealed differences in levels between normal adults and postmenopausal women. Cyclic variation was noted in the normally menstruating woman and the presence of LH in the serum of prepubertal children was also confirmed.

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ATP Content of Spermatozoa, Semen, and Seminal Plasma. (32296)

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The firefly assay has been employed for quantitative estimation of ATP in spermatozoa, whole semen, and seminal plasma. The intensity of the light reaction of the firefly *(Photinus pyralis* or *Photurus pennsylvanicus)* which is mediated by the enzyme luciferase depends on the amount of luciferin and ATP present(1,2). ATP can be quantitatively assayed by measuring the light intensity which follows addition of ATP to a luciferin-luciferase preparation(3,4).

Materials and methods. Six to twelve firefly lanterns (Sigma Chemical Co.) were used for each ml of crude luciferin plus luciferase desired. Luciferin and luciferase were ex-

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TABLE I. ATP Content of Washed Spermatozoa, Seminal Plasma and Whole Semen.

Species	Adenosine triphosphate content		
	Washed spermatozoa, $\mu g/10^8$ cells	Seminal plasma, μg/ml	Whole semen, μg/vol containing 10^8 cells
Boar	9.8 [a] (Range of 5 assays was 7.8 to 13.3)	—	—
Bull	4.4 [a]	.06, .15	5.4, 7.0
Cock	.5,[b] .6[b]	.5	1.2
Dog	1.3,[b] 3.9[b]	.01, .06	2.5, 6.6
Human	5.5 [b]	1.1	6.8
Rabbit	2.3,[a] 2.3[b]	.3	2.5 (Range of 11 assays was .9 to 3.7)
Stallion	5.5,[a] 10.1[a]	—	—

[a] Epididymal sperm; [b] ejaculated sperm.

tracted from lanterns by grinding in a mortar with 0.1 M sodium arsenate buffer at pH 7.4. The mixture was then centrifuged at 3,000 $\times$ *g* for 5 minutes. The supernatant liquid containing freshly prepared, crude luciferin plus luciferase was held in a stoppered test tube at 0°C.

Bull and rabbit semen was collected with an artificial vagina. Dog semen was collected by masturbation in the presence of an estrus bitch. Human semen was donated by volunteers 30 to 40 years of age. Semen was collected by a published procedure for the cock (5) as were epididymal spermatozoa of bull, boar and stallion(6). All semen, seminal plasma, and ejaculated sperm samples were from pools of contributions from at least 3 individuals. Spermatozoa were washed by described procedures(7) and counted in a hemocytometer. Seminal plasma in this work was the final supernatant fluid obtained after centrifugation of whole semen at 3,000 $\times$ *g* for 5 minutes, then followed by an identical centrifugation of the first supernatant fluid. No spermatozoa could be found in these preparations. All samples were extracted within 30 minutes after collection. ATP from semen, spermatozoa, or seminal plasma was extracted by boiling at 100°C for 5 minutes at neutral pH.

The assay reaction mixture consisted of (a) 0.1 ml crude luciferin plus luciferase, (b) 2.2 ml buffer (0.1 M Na_2HAsO_4 with pH adjusted to 7.4 with 5 N H_2SO_4 plus 10 mg/ml of $MgSO_4 \cdot 7H_2O$), and (c) 0.2 ml ATP standard solution or extract to be assayed. The constituents of the reaction mixture were added to an assay tube, mixed by inversion 3 times, and put into a light measuring instrument. Readings of light emitted 10 seconds after mixing were recorded. Light intensity values for each sample assayed were related to ATP content by reference to the curve resulting from reactions of aliquots of the same luciferin plus luciferase preparation with known amounts of the disodium salt of ATP (Sigma Chemical Co.). In this way samples containing 0.002 to 4.50 μg of ATP could be assayed for their ATP content directly. Light measuring instruments used were a Photovolt multiplier photometer model 520-M with a Varian G-10 graphic recorder, and a Keithley micromicroammeter with a Victoreen power supply.

Results. Boar epididymal spermatozoa were highest and ejaculated sperm samples of the cock were the lowest in ATP content. Likewise, cock semen contains less ATP than that of the other species assayed. Human semen and bull semen contain a comparable amount of ATP. The contribution of seminal plasma to the total ATP content of semen is much greater in the human than in the bull. ATP was found in the seminal plasma of all species assayed (Table I).

Higher assay values of rabbit semen were obtained when 0.1 ml of semen was diluted with water (1:10 or 1:15), and then added to 1.9 ml of boiling water for extraction. Assays of washed spermatozoa yielded higher values of ATP content when a number of spermatozoa comparable to that of diluted semen was extracted. When 0.5 ml of seminal plasma was extracted by addition to 1.5 ml of boiling water, repeatable assay values were obtained. ATP, in a concentration equal to that anti-

cipated for the sample being assayed, could be completely recovered when added to the boiling solution 2 minutes after the addition of the unknown.

Discussion. The assayed value for boar epididymal sperm ATP content is 53% higher than the highest value previously reported(8). The values for bull epididymal spermatozoa and semen reported here are lower than values reported for bull spermatozoa(9,10,11) of 13.9, 15.9, and 11.6 μg of ATP per 10^8 spermatozoa, respectively. In a nucleotide analysis of perchloric acid extracts eluted from a Dowex-1 column the mean ATP content of bull spermatozoa was reported as 6.5 μg ATP per 10^8 spermatozoa(12) which is in agreement with the value obtained for bull semen by the firefly assay (Table I). The latter workers(12) also found compounds in bull semen which were not nucleotides but which contained highly labile phosphate groups. The concentration of these compounds was about 250×10^{-18} moles per sperm cell, of which 50×10^{-18} moles was creatine phosphate. The remaining compounds were probably acetyl phosphate or 1,3 diphosphoglycerate. These compounds might have been responsible for some of the previous reports of higher values for ATP content of semen made by workers who measured labile phosphate groups released by 7-minute acid hydrolysis. Since the firefly light system is specific for ATP (2,13) this pitfall was avoided by the present method. The firefly assay allowed detection of ATP in seminal plasma for the first time.

Much variation in ATP content of comparable samples was observed throughout this survey. This may be accounted for by the observation that a decrease in ATP content coincides with impairment of activity(10). ATP exists in cells in a dynamic equilibrium, and the P : O ratio of spermatozoa has recently been shown to vary with different treatments of the cells(14,15,16). Using published data for endogenous respiration of bull epididymal spermatozoa, 3.4 μl O_2 taken up by 10^8 cells per hour, and the reported P : O ratio of 0.9(14) the value of 4.4 μg ATP per 10^8 bull epididymal spermatozoa represents 0.078% of the ATP synthesized per hour.

It has been suggested that ATP may leave the sperm cell as indicated by a stimulation of phosphorylation by added hexokinase(14). The low but consistent ATP content of seminal plasma even in the presence of powerful phosphatases also indicates passage of ATP out of the cell.

The ATP contents of the seminal plasmas and spermatozoa add up to the semen contents remarkably well with the cock, rabbit and human samples. The bull and dog seminal plasma contents appear low probably attributable to destruction by phosphatases during removal of the sperm.

Further investigation may reveal whether ATP content of sperm can be used as a criterion of their immediate state of viability and as a measure of their ability to fertilize ova.

Summary. Adenosine triphosphate (ATP) content of spermatozoa, semen and seminal plasma was obtained using a modification of the firefly bioluminescence assay method. Washed sperm of 6 mammalian species showed an ATP content ranging from 1.3 to 10.1 while cock sperm contain less than 1 μg ATP per 10^8 sperm. Separate assay of whole semen and seminal plasma indicated that the plasma and sperm contents add up approximately to the semen content with indication of some loss from bull and dog seminal plasma.

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Anti-Hypercholesterolemic Effect of Dehydroepiandrosterone in Rats. (32297)

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Interest in the use of steroid hormones as anti-hypercholesterolemic agents was first aroused by the report of Eilert(1), who showed that estrogens reduced the serum cholesterol level. The action of estrogens in man is naturally accompanied by undesirable feminizing effects(2), but their anti-hypercholesterolemic effect is superior to that of androgens.

Hellman *et al*(3) and other workers reported that androsterone, administered parenterally, was an effective anti-hypercholesterolemic agent, with the strongest effect occurring in hypothyroid patients with their typical increased serum cholesterol level. Hellman *et al*, therefore, imputed to androsterone a "thyromimetic" activity. On the other hand, Cohen *et al*(4) showed that androsterone had no effect on the serum protein-bound iodine, and that hence its anti-cholesterolemic effect is not mediated through a thyromimetic activity.

Because of the lack of activity of orally administered androsterone, synthetic and related androgens have been investigated. A new synthetic androgen, of the same order of potency as androsterone, *i.e.*, SC-12790, taken orally, was found to be active as an anti-hypercholesterolemic agent(5). However, its thyromimetic activity was not established. It is well known that in animals as well as in man hyperthyroidism or administration of thyroxine (T-4) or triiodothyronine (T-3) causes hypertrophy of many organs in the body, especially the heart(6-8), while a decrease in heart weight was noticed in hypothyroidism or after thiouracil administration (9). In the present study, the anti-hypercholesterolemic effect and the thyromimetic activity of dehydroepiandrosterone were studied and compared with that of SC-12790.

Methods and materials. Adult rats: 102 adult male rats of the "Sabra" strain of the Hebrew University were used. Experiments were carried out in normal as well as in hypercholesterolemic rats. Hypercholesterolemia was achieved by two methods: a) administration of 0.1% propylthiouracil (PTU) in the drinking water for 10 days; b) administration of both 0.1% PTU in the drinking water and 5% cholesterol in the food for 21 days. Daily oral doses of anti-cholesterolemic drugs were given together with the above hypercholesterolemic agents. Blood specimens were taken from the heart under ether anesthesia prior to and at the end of the experiment, without adding any anticoagulant. Serum cholesterol levels were measured by the method of Chiamori and Henry(10). The BMR of the hypothyroid rats was reduced by 10-20%.

SC-12790 (Fig. 1, I) and dehydroepiandrosterone (DHA) (Fig. 1, II), were suspended in 20% propylene glycol with some drops of 0.5% Tween-80 added and given orally. T-3 sodium was dissolved with a few drops of 0.1 N NaOH; H_2O was added to