replaced by potassium, although the concentration of potassium within the fibers did not appear to be greatly increased.

The administration of 160 mg of sodium amytal in divided doses during a period of an hour to another preparation nearly stopped the heart, and produced slight changes in the electrolyte concentrations in the heart that were very similar to those produced by the potassium.

It would appear, therefore, that with decreasing amounts of oxygen, or with increasing amounts of potassium or amytal, the general impermeability of the cardiac fibers to electrolytes is maintained at least as long as the heart continues to beat.

Summary. 1. The myocardium from a heart-lung preparation respiring air or a mixture of 5% CO_2 and air presents a nearly normal electrolyte pattern. 2. Heart failure from oxygen deficiency in such a preparation results in an increase in sodium, chloride, and water, and a decrease in potassium of the myocardium. 3. These changes are interpreted as indicating an extracellular edema without significant change in the fibers themselves. 4. Sufficient potassium or amytal to produce incipient heart failure produced only small changes in the cardiac electrolytes.

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Urinary Gonadotrophins in Normal Men.

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There is a dearth of information as to the gonadotrophins found in the urine of normal men. This is to some extent due to the difficulty of preparing non-toxic concentrates, for high doses are necessary to reveal the low titer of gonadotrophins characteristic of normal male urine. Recently investigators employing alcohol, tannic acid, or tungstic acid as precipitation agents, appear to be agreed that the level of excretion of gonadotrophins in normal male urine is approximately 5 to 25 mouse-uterus units[1] per liter.

Witschi[2] has pointed out that the ratio of follicle stimulating to luteinizing or interstitial cell stimulating activities in any given gonadotrophin may be characteristic of that gonadotrophin. Fraenk-

[1] Levin, L., and Tyndale, H. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **34**, 516.
[2] Witschi, E., *Endocrinol.*, 1940, **27**, 437.

el-Conrat, Li, Simpson and Evans,[3] using hypophysectomized rats have determined such ratios for various gonadotrophins having different hypophyseal and body-fluid sources, including one preparation from normal male urine. These workers found in an alcoholic precipitate approximately equal amounts of FSH and urinary ICSH. Leathem and Levin[4] in a similar assay, found that their tannic acid precipitate produced only follicular stimulation over a wide range of dosages, yet luteinization in the highest dosages.

The present study was undertaken to provide an accurate determination of the FSH:ICSH ratio in the urine of normal men, since the published data are in apparent disagreement. In addition the effort was made to estimate quantitatively the amount of FSH and ICSH in a unit volume of urine, since such data do not exist.

Procedure. Pooled samples of the urine of college men were precipitated in lots of 20 to 30 liters. All extracts were assayed for quantity of hormone in 25-day-old female rats, or in 21-23-day-old female Swiss white mice. The endpoint in rats was the production of large follicles in the ovary and/or estrous uteri. The 100% increase in uterine weight was considered the endpoint in mice. The extracts were assayed, in addition, in hypophysectomized female rats, 26 to 28 days old at operation, first injected 6 to 8 days after operation. In these animals the urine dosage levels at which interstitial tissue repair (M.E.D. for ICSH), follicle stimulation (M.E.D. for FSH) or luteinization occurred were determined by histological studies.

All dosages were administered subcutaneously on 4 successive days. Autopsy was performed on the fifth day. In working with urines containing only a few units of hormone per liter it is essential that a dependable procedure giving complete hormonal recovery be properly employed. We have used a modified alcoholic precipitation technic, which in our hands has proven more satisfactory than tannic acid or tungstic acid precipitation.

After adjusting the pH of the urine to 4.0 to 5.0, the precipitation was made by addition of 4 volumes of 95% ethyl alcohol. The precipitate was allowed to settle out in the cold room at 4°C. The clear supernatant fluid was siphoned off and the precipitate quantitatively transferred to centrifuge cups. After washing twice with 95% alcohol, and once with ether, the precipitate was dried. The dry powder was placed in a celloidin bag (Visking casing) and

[3] Fraenkel-Conrat, H., Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinol.*, 1940, **27**, 793.

[4] Leathem, J. H., and Levin, L., *Anat. Rec.*, 1941, **79**, suppl., 42.

the traces of powder remaining in the centrifuge cups were washed into the bag. After dialysis for 3 to 4 days at 4°C the supernatant in the bag was separated from any sediment by centrifugation. The supernatant was then frozen and desiccated under high vacuum while still in the frozen state. The powder so obtained was then weighed and aliquot parts were used for the assays. Levin[5] has pointed out that previous workers who have used the alcohol method tried to extract the hormone from the copious alcohol precipitate with small volumes of water. Since the water rapidly becomes saturated with urea and salts, this extraction is probably not complete. Solution of the hormone within the dialysis bag overcomes this objection. Secondly, drying of the extract while it is in the frozen state eliminates the loss of hormone which Levin indicates may occur during drying at room temperature.

Results. Quantitative. In 10 samples of pooled normal male urines the titer of hormone ranged from 1.25 to 4.5 rat units of gonadotropic activity per liter, or 5.0 to 20.0 mouse-uterus units (Table I). These values agree well with those obtained by Saethre,[6] Katzman,[7] and Levin.[5] In general, where both rat and mouse assays were made for the same urine, one rat unit was equal to 4 to 6 mouse units. This 5-fold greater sensitivity of the mouse over the rat has been found to hold in assays of hypophyseal gonadotrophin, menopause urinary gonadotrophin, or other gonadotrophins relatively rich in the follicle stimulating factor. In assays of the urinary interstitial cell stimulating factor (pregnancy, chorioepithelioma) the rat has been found from 2 to 5 times as sensitive as the mouse.

Results. Qualitative. In all instances in which it was possible to determine the minimum dosages for follicle stimulation (FSH) and interstitial cell repair (ICSH) in the hypophysectomized rat, it was found that these effects appeared at approximately equal dosage levels. Although ICSH activity at no time appeared at dosages lower than those required for follicle stimulation, it was never necessary to give more than 2 to 3 times the M.E.D. for FSH to produce repair of interstitial tissue. At high dosages luteinization of follicles and formation of corpora lutea were seen. In this respect our results are in accord with those of Leathem and Levin[4] who, however, do not mention the interstitial cell repair which must have occurred at lower dosage levels.

Discussion. Leathem and Levin[4] state that as far as effects on

[5] Levin, L., *J. Biol. Chem.*, 1940, **133**, suppl., lx.

[6] Saethre, H., *Klin. Wchnschr.*, 1935, **14**, 376.

[7] Katzman, P. A., *Endocrinol.*, 1937, **21**, 89.

TABLE I.
Summaries of Gonadotropic Assays of Concentrates from Normal Male Urine.

Pooled urine preparation Sample No.	Method of precipit.	MED in rats equiv. cc urine	MED in mice equiv. cc urine	MED for FSH in hyp. rat,* cc urine	MED for ICSH in hyp. rat,* cc urine	Ratio ICSH:FSH (approx.)
WN 9	alcohol	500	80	460	690	1:1.4
WN 28	"	400	80	1330	—	
WN 29	"	250	50	400	500	1:1.2
WN 30	.8 sat. $(NH_4)_2SO_4$	500	120			
WN 32	alcohol	570	140			
WN 33	tungstic acid	800	160	1000	—	
WN 34	tannic acid	—	200	2450	—	
WN 35	alcohol	225	—	350	930	1:2.7
WN 50	"	400	80	—		

*The MED for FSH is the minimal total dose which causes resumption of follicular development in rats hypophysectomized at 26-28 days of age, and first injected 6 to 8 days after operation. Injections are made subcutaneously for 4 days, once each day, and autopsy is performed on the fifth day. The MED for ICSH in similarly treated rats is that total dose which will cause repair of the ovarian interstitial tissue.

the ovaries of hypophysectomized rats are concerned, the urinary gonadotrophins of normal men resemble those found in normal women at or after the menopause. In the 3 samples of normal male urine, for which complete analyses are reported here, the ratio of urinary ICSH to FSH was 1:1.2, 1:1.4, and 1:2.7. It appears therefore, that it may not be strictly valid, at least when speaking of alcohol concentrates, to liken the normal male gonadotrophin to menopause gonadotrophin, where this ratio is 1:5 or 1:6.[3] The data of Fraenkel-Conrat, *et al.,* who reported the assay of one alcoholic precipitate of male urine in hypophysectomized rats and found a 1:1 ratio agree fairly closely with 2 of the samples reported here.

The slight difference in the 2 types of results described by Leathem and Levin on one hand and Fraenkel-Conrat, *et al.,* and this paper on the other hand, may be due to the difference in concentration technics. It is possible, since the difference may be referable to a loss of ICSH in the former case or a loss of FSH in the latter case, that one of the technics allows a selective loss of one of the hormones. That this is probably not true of the alcohol concentrates reported here is indicated by the normal quantitative titer obtained by assaying the same concentrates in normal rats or mice. Leathem and Levin report only the approximate ratio of FSH and LH in their tannic acid preparations but give no indication of the potency of these preparations expressed in terms of equivalent volumes of urine.

Summary. A modified alcoholic precipitation method is de-

scribed for the preparation of non-toxic gonadotrophic concentrates from normal male urine.

Assayed in normal rats, such concentrates of pooled samples of urine of healthy college men give a quantitative titer of 1.0 to 4.5 rat units per liter. In normal mice titers of 6.0 to 20.0 mouse-uterine units per liter were obtained.

Assayed in hypophysectomized rats it is found that follicle stimulation and interstitial cell repair are usually produced at the same dosage level, although in some instances follicle stimulation may be produced at a dosage level as low as one-third that necessary for interstitial cell repair.

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Qualitative Differences between Positive Streptococcal Antifibrinolysin Tests.*

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Positive streptococcal antifibrinolysin tests[1, 2, 3] are generally regarded as indicative of the presence of a specific "antibody." There are instances, however, when this seems unlikely.[4] Milstone[5] has recently demonstrated that the plasma-clots of rabbits are resistant to lysis by human strains of hemolytic streptococci because they lack a substance—"lytic factor"—which is present in normal human serum. The addition of "lytic factor" to rabbit plasma results in rapid liquefaction of the clot by fibrinolysin. This observation was of considerable interest to us because of its possible clinical application since it might explain some of the apparently non-specific positive antifibrinolysin tests (resistant clots) which we have observed in infants and children.[4]

Newborn blood frequently gives a positive test in spite of the

* Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

1 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

2 Tillett, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **13**, 47.

3 Tillett, W. S., *J. Clin. Invest.*, 1935, **14**, 276.

4 Boisvert, P. L., *J. Clin. Invest.*, 1940, **19**, 65.

5 Milstone, H., *J. Immunol.*, 1941, **42**, 109.