precision and accuracy by utilizing the gas dilution principle presented above. By varying the size of the chamber and the gas burette, it should be possible to perform repeated specific gravity determinations on intact animals of most species by this method. Knowledge of the empirical constants relating specific gravity to body fat and body water content should thus permit these latter quantities to be determined with ease and moderate precision.

Summary. A method of measuring specific gravity, and thereby estimating total body water and fat content in intact animals is presented. The dilution volume of helium, in a closed chamber of known capacity containing the animal, is measured, and subtracted from the volume of the empty chamber. This gives the volume of the animal. Weight/volume gives specific gravity. No correction for residual air is necessary. The results on 10 cats by this method corresponded closely to results obtained by underwater weighing of each carcass.

NOTE—Since this work was completed, an abstract has appeared(7) indicating that a helium dilution procedure has recently been applied to body volume measurement in man.

1. Behnke, A. R., *Harvey Lectures,* 1941-42, v37, 198.

2. Osserman, E. F., Pitts, G. C., Welham, W. C., and Behnke, A. R., *J. Appl. Physiol.,* 1949, v2, 633.

3. Pace, N., and Rathbun, E. N., *J. Biol. Chem.,* 1945, v158, 685.

4. Hastings, A. B., *Harvey Lectures,* 1940-41, v36, 91.

5. Miller, A. T., and Blyth, C. S., *J. Appl. Physiol.,* 1953, v5, 311.

6. Robertson, J. S., Siri, W. E., and Jones, H. B., *J. Clin. Invest.,* 1950, v29, 577.

7. Siri, W. E., *Fed. Proc.,* 1953, v12, 133.

Received March 30, 1953. P.S.E.B.M., 1953, v82.

Gonadotropins of the Pituitary Gland and Urine of the Adult Human Male. (20243)

ROBERT C. BAHN, NONA LORENZ, WARREN A. BENNETT, AND A. ALBERT.

From the Sections of Pathology and Physiology, Mayo Clinic, Rochester, Minn.

The repair of the gonadal atrophy occurring in hypophysectomized male and female rats provides a specific method for the characterization and assay of pituitary gonadotropins. Considerable information on the gonadotropins of the pituitary gland of the hog and the sheep has been derived by use of this method(1,2). However, only few definitive studies of the nature and amounts of gonadotropins of the pituitary gland of the human have been made, and much of what is known has been inferred from investigations on the gonadotropins of human urine(3-8). The purpose of this paper is to present comparative data on the gonadotropins of both the pituitary gland and the urine of the adult human male.

I. Materials and methods. a) Animals. Male and female Wistar rats (30 to 45 days old) were hypophysectomized by the parapharyngeal route. Postoperatively the animals received a solution containing 5% glucose and 0.9% sodium chloride as their drinking water and were allowed Friskie meal *ad libitum.* Beginning on the sixth postoperative day and extending for 4 days, each test animal received twice daily subcutaneous injections (1 cc) of the material to be tested. Groups of uninjected hypophysectomized and intact animals were included in each experiment. On the tenth postoperative day all the rats were killed by ether asphyxiation. A fairly complete gross and microscopic examination of the endocrine glands and other related organs was made. *b) Pituitary tissue.* The pituitary glands of 3 adult human males were obtained within 3 to 4 hours following sudden death.* None of the 3 had had any obvious endocrinopathy and in no case was there any clinical evidence of impairment of the pituitary-gonad system. The ages of the patients were 38, 55 and 61 years. The anterior lobe of each hypophysis was dissected free from the remainder of the

TABLE I. Effect of Human Pituitary Homogenate on Hypophysectomized Female Rats.

Animals			Ovaries		Uterus		Ovarian histology			
Condition	No.	Dose, mg	mg	mg/g	mg	mg/g	F.D.	C.L.	I.T.	V.O.
Hyp.	6	0	10.6	.13	36.6	.44	0	0	D	0
Intact	5	0	28.8	.25	159.3	1.39	+++	+	R	+
Hyp.	4	1	10.7	.10	75.4	.70	0	0	D	0
Hyp.	5	3	10.2	.13	29.7	.38	+	0	R	0
Hyp.	5	10	27.4	.40	136.3	2.01	++++	0	L	0
Hyp.	4	30	111.7	1.03	190.0	1.75	++++	+	L	+

Abbreviations: Hyp. = hypophysectomized; mg/g = organ wt/g body wt; D = deficient; R = repaired; I.T. = interstitial tissue; F.D. = follicular development; C.L. = corpora lutea; 0 = absent; + = present; L = luteinized; V.O. = vaginal opening.

gland, weighed, and frozen. The combined wet weight of the 3 anterior lobes was 1,051.4 mg. The glands were stored at −3°C for approximately 5 weeks. Immediately prior to injection, the 3 glands were homogenized, pooled, and suspended in 1% solution of sodium chloride. Groups of male and female hypophysectomized rats received individual total doses of pituitary homogenate equivalent to 1, 3, 10 and 30 mg of pituitary tissue (wet wt). *c) Urine.* Large volumes of urine were obtained from a group of healthy adult males. The pooled urine was worked up according to the method of Bradbury and associates(3). Groups of hypophysectomized male and female rats received a total dose per animal of urinary concentrate equivalent to 1,200 cc of pooled urine. This dose was selected because it was the minimal amount which produced a maximal increase in the uterine weight in the intact immature female rat. Each animal receiving the urinary concentrate was also given each day 0.5 mg of cortisone subcutaneously. In preliminary experiments cortisone therapy had substantially decreased the mortality rate due to the toxicity of large doses of urinary concentrate.†

II. Results. a) Pituitary homogenate in female animals (Table I). The presence of FSH is indicated by follicular stimulation in hypophysectomized rats. There was no effect at a dose of 1 mg. At 3 mg, follicular stimulation was produced without macroscopic enlargement of the ovaries. Ten milligrams of pituitary homogenate increased the weight of the ovaries to about 3 times that of the hypophysectomized controls, an increase associated with the presence of large cystic follicles. With 30 mg. the ovaries were 8- to 10-fold heavier than those of control rats, and contained many recent, completely luteinized follicles.

The *secretion of estrogen* is due to the synergistic action of FSH with small amounts of LH(10). Estrogen secretion, as judged from uterine weights, occurred at doses greater than 3 mg and increased with increasing dosage. The most copious estrogen secretion occurred at a dose of 30 mg since in addition to maximal uterine stimulation, vaginal opening also occurred.

The presence of LH is indicated by the repair of the ovarian interstitial "deficiency" cells(10-15). At 1 mg, the deficiency cells were unaltered. At 3 mg, repair was evident and with 10 mg, there was marked luteinization of the interstitial tissue. Complete luteinization of the granulosa cells occurred only in the 30 mg dose. At this dose, ovulation was presumed to have occurred, for no ova were

* This represented the earliest possible time following death that the pituitary tissue could be obtained. The glands appeared fresh. While some loss of gonadotropin may have occurred within the first few hours following pronouncement of death, we assume that the comparability of the results in this and subsequent papers is affected equally by this factor. In any case, by necessity, all of the studies on human pituitaries were done on glands obtained within three or four hours after death.

† Cortisone therapy did not impair the validity of the test, since (a) it did not influence the characteristic atrophy of the gonads and accessories following hypophysectomy, (b) it had no androgenic action on the accessories and (c) although it inhibits the growth of the accessories under androgenic stimulation(9), this effect is quantitative rather than qualitative and would make the positive results reported later even more significant.

TABLE II. Effect of Human Pituitary Homogenate on Hypophysectomized Male Rats.

Animals			Prostate		Seminal vesicles		Epididymis		Testicular histology				
Condition	No.	Dose, mg	mg	mg/g	mg	mg/g	mg	mg/g	M.T.D.	S.A.	E.St.	L.St.	I.T.
Hyp.	8	0	5.3	.09	6.0	.11	28.4	.50	.09	+	0	0	U
Intact	5	0	37.8	.41	22.0	.24	87.0	.94	.16	++++	+	+	Di.
Hyp.	3	1	20.9	.42	13.3	.26	57.0	1.13	.09	++	0	0	U
Hyp.	4	3	25.2	.35	29.0	.40	70.0	.98	.11	+++	0	0	Di.
Hyp.	5	10	24.2	.40	22.2	.36	72.8	1.19	.13	+++	+	0	Di.
Hyp.	5	30	27.2	.38	22.9	.32	83.2	1.17	.11	+++	+	0	Di.

Abbreviations: M.T.D. = mean tubular diameter (mm); S.A. = spermatocytic proliferation; E.St. = early spermatid; L.St. = late spermatid; I.T. = interstitial tissue; U = undifferentiated; Di = differentiated; others, see Table I.

TABLE III. Effect of Urinary Concentrate on Hypophysectomized Female Rats.

Animals				Ovaries		Uterus		Ovarian histology			
Condition	No.	Cortisone	Concentrate	mg	mg/g	mg	mg/g	F.D.	C.L.	I.T.	V.O.
Hyp.	2	0	0	9.8	.15	19.5	.30	0	0	D	0
Hyp.	4	+	0	7.9	.15	14.7	.28	0	0	D	0
Hyp.	11	+	+	20.3	.36	68.6	1.23	++++	0	D	+
Intact	5	0	0	21.6	.24	73.4	.81	+++	+	R	+

Abbreviations: See Table I.

observed within the luteinized follicles.

Survey of the data showed that distinct qualitative differences in response were obtained over the dose intervals, which were purposely widely separated to minimize overlap. At a dose of 3 mg, the first distinctive indication for the presence of FSH was seen. This response was designated as a unit response; therefore, 1 unit of FSH was present in 3 mg pituitary homogenate. At 3 mg also the first distinctive response in the interstitial tissue (repair of deficiency cells) occurred. This response was therefore designated as a unit response to LH; therefore 1 unit of LH was present in 3 mg homogenate. The average weight of the pituitary was about 300 mg; therefore, a total of 100 units of FSH and 100 units of LH, as defined, were present per pituitary. It is plain that this criterion of FSH activity is made in the presence of LH, and of LH in the presence of FSH, a situation which will be discussed and clarified later.

b) Pituitary homogenate in male animals (Table II). In the hypophysectomized male rat the testicular interstitial spaces are almost totally obliterated by closely packed seminiferous tubules(11-16). The cells in these spaces cannot be readily identified as Leydig cells, but resemble fibroblasts or capillary endothelial cells. LH activity is defined by the ability of a preparation to induce differentiation of these cells or to induce secretory activity of these cells as manifested by androgenic stimulation of the prostate and seminal vesicles(15). The first direct *evidence of LH* activity occurred at a dose of 3 mg. The interstitial spaces were better delineated, being apparently distended by fluid or some other amorphous intercellular material. Leydig cells were easily identified. With 10 and 30 mg, an epithelioid cell pattern of the Leydig cells was produced. Indirect evidence of LH activity was present at all dosages studied, as judged from the weight and from histologic examination of the accessory glands.

The repair of tubular atrophy and reappearance of spermatogenic activity denotes the presence of FSH(12,16). The first definite response of the tubular apparatus as measured by testicular weight and, more especially, by tubular diameter occurred at 3 mg. There was an increase in spermatogenic activity over the entire dose range, with greater mitotic activity occurring at higher dosages. At no dosage, however, did the mitotic activity of the tubules of the hypophysectomized rats receiving pituitary homogenate equal that of intact control rats.

The same assignment of units described previously was made. The smallest dose of

homogenate necessary to repair the Leydig cells was 3 mg. This was designated as a unit response for LH. The smallest dose causing definitive tubular stimulation was also 3 mg. This was designated as a unit response for FSH. Therefore, as measured in the hypophysectomized male rat, there were roughly 100 units of FSH and 100 units of LH in the pituitary gland of the adult male.

c) Urine in female animals (Table III). The presence of LH was shown by the copious secretion of estrogen, resulting in uterine enlargement and patent vaginae. However, since there was no repair of the interstitial tissue, considerably less than 1 unit of LH was present. The *presence of FSH* was shown by the increased ovarian weight and the marked follicular stimulation. The follicles were large cystic structures without signs of luteinization. The FSH response was about equal to that brought about by a dose of between 3 and 10 mg (perhaps 6 mg would be an interpolated value) of pituitary homogenate.

d) Urine in male animals (Table IV). There was no evidence of LH activity as revealed by histologic examination of the interstitial cells. The weight of the ventral prostate was not appreciably increased but microscopic examination of the ventral prostate showed a moderate increase in the height of the acinar epithelium and dilatation of the acini with secretion. This slight androgenic effect indicated the presence of traces of LH, considerably less than 1 unit of LH. The *testicular weight* was increased to 250% of the hypophysectomized control. Spermatogenesis had progressed universally to the late spermatid stage. The increase in testicular weight and especially in mean tubular diameter was roughly equivalent to that produced by 3 to 10 mg (perhaps 6 mg) of pituitary homogenate.

III. Discussion. Henderson and Rowlands (17) and Chance and associates(18) have shown that the human pituitary is an exceedingly rich source of gonadotropins as compared to the hypophyses of other mammals such as the horse, the pig, and the hog. That it contains FSH and LH has been shown by Noble and associates(19) who obtained luteinization in the ovary of the hypophysectomized female

TABLE IV. Effect of Urinary Concentrate on Hypophysectomized Male Rats.

Animals					Seminal vesicles		Epididymis		Testicular histology				
Condition	No.	Cortisone	Concentrate	Prostate mg	Prostate mg/g	mg	mg/g	mg	mg/g	M.T.D.	S.A.	E.St.	L.St.	Sz.	I.T.
Hyp.	4	0	0	8.2	.15	12.0	.21	43.0	.77	.10	+	0	0	0	U
Hyp.	3	+	0	8.9	.16	9.9	.17	32.7	.57	.11	++	0	0	0	U
Hyp.	9	+	+	9.9	.18	10.9	.20	51.2	.91	.12	+++	+	+	0	U
Intact	5	0	0	70.4	.60	53.6	.45	164.6	1.39	.21	++++	+	+	+	Di.

Abbreviations: See Table II. Sz. = spermatozoa.

rat with acetone-dried human pituitary. Bates and Schooley(20) concluded that the human pituitary contained more LH than that of the horse, the pig, or the sheep, but Witschi and Riley(21) and Witschi(22) reported that only traces of LH were present in the human pituitary.

It is difficult to compare these reports with one another and with the present data. Perhaps one difficulty is that different test animals were employed. The choice of units for FSH and LH may present another difficulty. For example, it would be entirely possible to choose an insensitive unit response for FSH activity, and a very sensitive unit response for LH. Under such circumstances, one could say that numerically, a great deal more LH than FSH was present. The reverse situation could also obtain. "Units" of FSH and LH are at present meaningless, since neither FSH nor LH is available in acceptable form with regard to either biological or chemical purity. Since biological purity is not assured, the effect of LH on the assay for FSH and vice versa in unfractionated pituitary tissue is largely unknown. Consequently, no absolute connotation is intended for our assay of 100 units of FSH per adult male pituitary. Rather this assay for FSH is defined as one carried out in the presence of, and perhaps excess of LH. The same consideration applied to the LH assay. Since chemical purity is not a characteristic property of existing gonadotropic preparations, and since no biologically pure but chemically impure reference standard preparations of LH and FSH are available, there can be no assay in terms of absolute or relative weights of the 2 hormones. It is impossible therefore to compare these 2 hormones in terms of the weights of each present in the pituitary, even if one were to dismiss the effects of synergism and interaction of these hormones on one another in the assay.

In order to circumnavigate partly the difficulties inherent in the present unsatisfactory situation, and to make some progress despite it, the following criteria were established: 1) Qualitative responses generally accepted as indicating the activity of FSH and LH were chosen as end points because of their distinctness and the ease with which they could be determined. 2) Doses of homogenate were chosen purposely separated by wide intervals (300%) so as to minimize or eliminate intragroup variation among animals, and to minimize minor variance in response due to differences in size and age of animals and to spontaneous statistical error. 3) Both male and female test animals were employed; in effect, 2 end points for FSH and 2 for LH were used. 4) The end points for FSH and LH were chosen because both appeared at the same dose level of homogenate in both male and female rats. By definition, the ratio of FSH and LH was made unity, so that gross departures from this ratio, as a standard for gonadotropin of the human pituitary, could be readily determined. 5) By determination of the amount of substance necessary to induce these end points, a rough calculation of the total gonadotropin present could be made. That this is a rough approximation and is not intended as a final meticulous assay is obvious from the use of a dose series separated by a log interval of 0.477, and by the existence of a factor of unknown quantitative significance, the factor of synergism.

Within the foregoing restrictions, the data show that about 100 units of FSH and 100 units of LH are present in the pituitary gland of the adult male. Comparative reports on similar material from females, children and infants will be made. At present, it is interesting to compare the gonadotropic content of the pituitary and urine of the adult man. In 1,200 g of pituitary tissue, some 400,000 units of FSH and LH are present (1,200 g/3 mg). In 1,200 cc of urine, perhaps 2 units of FSH and much less than 1 unit of LH were found.

These results have some bearing on a point which has been disputed for many years. This dispute concerns the duality of gonadotropins, which appears to have been settled, in a chemical sense, in the affirmative. However, Cole(23) in a recent review has suggested that LH may not be released from the pituitary and that, in fact, FSH is the only circulating gonadotropin. Studies on the blood of human beings are so fragmentary that no knowledge of the nature of circulating gonadotropin is at hand. Consequently, investigations of urinary concentrate, prepared by precipitation with

tannic acid or alcohol, by ultrafiltration, or by absorption methods, have been made to study the nature of excreted gonadotropins. Were the circulating gonadotropin only FSH, then one would expect no similarity between the activity of urinary concentrates and that of human pituitary tissue. On the contrary most of these studies on urine, including our own, have shown that both gonadotropic hormones are present. Our data, furthermore, show that proportionately much less LH relative to FSH is present and support Cole's suggestion to the extent that the ratio of the two gonadotropins in urine is not the same as that in the pituitary. The reason for such a disparity is not clear although a number of possibilities come to mind: differential secretion from the pituitary, differing routes of metabolism or utilization, or selective excretion or retention by the kidneys. It is also possible that the methods used to prepare urinary concentrates are much more effective for recovery of FSH than for LH. Which of these or other possibilities is the basis for the disparity must await further investigation.

Summary. A method is described whereby the ratio of FSH and LH present in human pituitary tissue can be ascertained in terms of well-known qualitative end points in the gonads of hypophysectomized rats of both sexes. Such end points, representing unit responses of FSH and LH, were chosen because they could be readily determined, and because they occurred at the same dosage of pituitary homogenate. It was thus determined that pooled homogenate of the pituitary glands of 3 adult men contained 100 units of FSH and 100 units of LH per pituitary, a ratio of 1:1. By comparison on an equal weight basis with pituitary tissue, the urine of adult men contained only minute amounts of gonadotropins, but proportionately, there was much more FSH relative to LH, in a ratio of much greater than 2:1.

1. van Dyke, H. B., P'an, S. Y., and Shedlovsky, T., *Endocrinol.*, 1950, v46, 563.

2. Greep, R. O., van Dyke, H. B., and Chow, B. F., *Endocrinol.*, 1942, v30, 635.

3. Bradbury, J. T., Brown, E. S., and Brown, W. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 228.

4. Evans, H. M., and Gorbman, Aubrey, PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 674.

5. Kassenaar, A. A. H., and Paesi, F. J. A., *Acta Endocrinol.*, 1951, v8, 125.

6. Leathem, J. H., and Levin, Louis, *Endocrinol.*, 1941, v29, 8.

7. Leathem, J. H., and Mills, E. J., Jr., *Endocrinol.*, 1942, v31, 318.

8. McArthur, Janet W., *Endocrinol.*, 1952, v50, 304.

9. Winter, C. A., Silber, R. H., and Stoerk, H. C., *Endocrinol.*, 1950, v47, 60.

10. Fevold, H. L., *Endocrinol.*, 1941, v28, 33.

11. Fraenkel-Conrat, Heinz, Li, C. H., Simpson, Miriam E., and Evans, H. M., *Endocrinol.*, 1940, v27, 793.

12. Fraenkel-Conrat, Heinz, Simpson, Miriam E, and Evans, H. M., *Endocrinol.*, 1940, v27, 809.

13. Li, C. H., Simpson, Miriam E., and Evans, H. M., *Endocrinol.*, 1940, v27, 803.

14. Simpson, Miriam E., Li, C. H., and Evans, H. M., *Endocrinol.*, 1942, v30, 969.

15. ———, *Endocrinol.*, 1942, v30, 977.

16. Greep, R. O., and Fevold, H. L., *Endocrinol.*, 1937, v21, 611.

17. Henderson, W. R., and Rowlands, I. W., *Brit. M. J.*, 1938, v1, 1094.

18. Chance, M. R. A., Rowlands, I. W., and Young, F. G., *J. Endocrinol.*, 1939, v1, 239.

19. Noble, R. L., Rowlands, I. W., Warwick, M. H., and Williams, P. C., *J. Endocrinol.*, 1939, v1, 22.

20. Bates, R. W., and Schooley, J. P., *Endocrinol.*, 1942, v31, 309.

21. Witschi, Emil, and Riley, G. M., *Endocrinol.*, 1940, v26, 565.

22. Witschi, Emil, *Endocrinol.*, 1940, v27, 437.

23. Cole, H. H., *Hormonal Control of Ovulation.* In Engle, E. T.: The Problem of Fertility, Proc. of Conf. on Fertility Held Under Auspices of Nat. Com. on Maternal Health, Princeton, N. J., Princeton University Press, 1946, pp. 74-90.

Received March 30, 1953. P.S.E.B.M., 1953, v82.