CCCII. ASSAY OF THE GONADOTROPIC HORMONE OF PREGNANCY URINE ON MALE RATS.

By VLADIMIR KORENCHEVSKY, MARJORIE DENNISON
AND SAMUEL LEVY SIMPSON.

From the Lister Institute, London, S.W. 1.

(Received September 30th, 1935.)

HITHERTO the assay of the gonadotropic hormones isolated from pregnancy urine or from the anterior lobe of the hypophysis has been made on immature female rats, thus measuring the female gonadotropic activity of these substances. For the unit of this activity we propose the term "female rat (or mouse) unit". It is equally important however to assay the male gonadotropic activity of these hormones, since it is clear that the results obtained from the female sexual organs cannot be applied to those of males. For the unit of this activity we propose the term "male rat (or mouse) unit".¹

It is now well established that the gonadotropic hormones produce hypertrophy of the secondary sexual organs (precocious sexual maturation) in immature male animals. Several authors therefore have suggested that it might be possible to use the sexual organs of immature rodents, in particular the seminal vesicles, for the assay of gonadotropic substances.

In our previous papers, chiefly for the purpose of the assay of testicular hormones, we studied the effects of some variables on the weights of different organs in normal [Korenchevsky *et al.*, 1933; Korenchevsky and Dennison, 1934] and castrated [Korenchevsky, 1932, 1, 2; Korenchevsky and Dennison, 1934] rats. From these results we concluded that the method of assay on castrated rats, which we had previously described "could be applied to normal rats for the study of physiological or pathological conditions or for the assay of substances which influence the weight of one or more organs" [Korenchevsky *et al.*, 1933, p. 1511]. In the present paper this method has been applied to the assay of the gonadotropic hormone of pregnancy urine using the normal immature male rat as the experimental animal.

Technique.

Experiments were performed on 173 rats belonging to 34 litters, the ages of which at the beginning of the injections were 22–25 days. The injections were made with a gonadotropic hormone extracted from pregnancy urine and prepared by Messrs Organon Ltd.² The strength of the preparation was given by the firm in female rat units (F.R.U.), one female rat unit being that minimum total amount of the hormone which when injected during 3 days would produce oestrus within 5 days in 50 % of the injected animals.

¹ We suggest the following symbols for these values: "Ml.R.U." ("Ml." in order to distinguish it from the already accepted symbol "M." for "mouse") and F.R.U.; or alternatively ♂ R.U. and ♀ R.U.

² We wish to express our thanks to Messrs Organon Ltd. for kindly supplying the hormone in generous quantities and without charge.

The first 19 litters (see Tables II to IV) were each divided into a control uninjected group, usually containing two rats, and one or two injected groups each containing 2 or 3 rats, though exceptionally only one rat. The percentage changes obtained in the weight of the organs in each injected group were averaged separately for each litter. The rats of litters 20 to 26 (Exp. No. 2), 27, 31 (Exp. No. 3) and 32 to 34 (Exp. No. 4) were arranged differently, each group in these experiments containing only one litter-mate (exceptionally two) from each litter. A general average was obtained for each group and included in the Tables II to IV. Therefore, in Exp. No. 4, for example, litters 32, 33 and 34 were chosen as each contained not less than 7 rats, since 7 groups were used in this experiment.

Table I. *Secondary sexual organs in normal uninjected rats.*

Average and maximum variations of body weight and of weight (actual and per 200 g. body weight) of sexual organs of all rats and in groups A and B separately. In brackets—maximum % deviations from average figures.

		Seminal vesicles mg.		Prostate (mg.)		Prostate with seminal vesicles mg.		Penis (mg.)	
		Final body weight g.	Per 200 g. body weight	Actual	Per 200 g. body weight	Actual	Per 200 g. body weight	Actual	Per 200 g. body weight
All rats 26-29 days old	Averages	60	15	50	74	250	88	300	65
	Maximum variation	41-79	10-23	40-62	54-100	211-325	67-120	257-378	48-85
	Maximum % deviation	(32)	(53)	(24)	(35)	(30)	(36)	(26)	(31)
A. Rats 26-27 days old	Averages	51	13	51	66	259	79	310	60
	Maximum variation	41-61	10-15	41-62	54-75	214-325	67-90	257-378	48-68
	Maximum % deviation	(20)	(23)	(22)	(20)	(25)	(15)	(22)	(20)
B. Rats 28-29 days old	Averages	68	17	49	81	241	98	290	69
	Maximum variation	58-79	13-23	40-60	65-100	211-267	78-120	259-322	56-85
	Maximum % deviation	(16)	(35)	(22)	(23)	(12)	(22)	(11)	(23)

Table II. *Weights of seminal vesicles.*

The average actual weights (mg.) of the seminal vesicles of the rats in each group of each litter.

No. of litter	Control rats	Rats injected with F.R.U.						
		0.5	1	2	3	4	6	10
1	23	—	—	—	—	—	—	65
2	19	—	—	—	—	46	54	—
3	16	—	—	23	—	33	—	—
4	15	—	—	25	—	—	40	—
5	13	—	—	—	—	—	—	42
6	13	—	—	—	29	—	—	—
7	12	—	—	24	—	30	40	—
8	15	—	—	—	33	—	40	—
9	20	—	—	—	36	—	—	—
10	15	—	—	—	—	—	38	—
11	13	—	—	26	—	—	—	—
12	10	—	—	22	—	27	—	—
13	13	—	—	—	29	—	—	—
14	14	—	—	—	29	—	46	—
15	14	—	—	—	27	—	—	40
16	14	—	—	—	29	37	33	—
17	12	—	—	—	—	—	—	31
18	13	—	—	—	—	23	—	21
19	19	—	—	32	—	—	—	—
20-26	13	17	17	29	—	—	—	—
27-31	14	17	21	—	—	—	—	—
32-34	18	—	25	30	30	42	45	51

Table III. *Weights of prostate.*

The average actual weights (mg.) of the prostate of the rats in each group of each litter.

No. of litter	Control rats	Rats injected with F.R.U.						
		0.5	1	2	3	4	6	10
1	95	—	—	—	—	—	—	155
2	88	—	—	—	—	144	—	174
3	70	—	—	113	—	127	—	—
4	75	—	—	100	—	—	118	—
5	59	—	—	—	—	—	—	116
6	55	—	—	—	104	—	—	—
7	73	—	—	94	—	110	114	—
8	72	—	—	—	109	—	130	—
9	100	—	—	—	140	—	—	—
10	75	—	—	—	—	—	129	—
11	66	—	—	100	—	—	—	—
12	57	—	—	94	—	103	—	—
13	70	—	—	—	119	—	—	—
14	89	—	—	—	153	—	155	—
15	75	—	—	—	123	—	—	159
16	77	—	—	—	118	128	117	—
17	73	—	—	—	—	—	—	112
18	54	—	—	—	—	92	—	96
19	84	—	—	110	—	—	—	—
20-26	65	76	79	101	—	—	—	—
27-31	72	72	88	—	—	—	—	—
32-34	76	—	99	111	112	136	133	158

Table IV. *Weights of penis.*

The average actual weights (mg.) of the penis of the rats in each group of each litter.

No. of litter	Control rats	Rats injected with F.R.U.						
		0.5	1	2	3	4	6	10
1	85	—	—	—	—	—	—	114
2	79	—	—	—	—	99	110	—
3	67	—	—	81	—	94	—	—
4	61	—	—	76	—	—	80	—
5	59	—	—	—	—	—	—	95
6	56	—	—	—	81	—	—	—
7	67	—	—	85	—	100	97	—
8	67	—	—	—	94	—	96	—
9	82	—	—	—	96	—	—	—
10	62	—	—	—	—	—	97	—
11	65	—	—	87	—	—	—	—
12	57	—	—	76	—	82	—	—
13	61	—	—	—	90	—	—	—
14	65	—	—	—	79	—	91	—
15	56	—	—	—	76	—	—	86
16	63	—	—	—	79	80	83	—
17	53	—	—	—	—	—	—	79
18	48	—	—	—	—	76	—	70
19	70	—	—	89	—	—	—	—
20-26	64	76	78	100	—	—	—	—
27-31	68	76	88	—	—	—	—	—
32-34	68	—	74	81	83	94	98	109

This arrangement permitted a more economic distribution of the rats, when a large number of groups were required, and allowed the use of a smaller number of controls. The accuracy of the results obtained in this way was not less than that given by the litters 1 to 19.

The number of rats used in each group was as follows:

Control rats	Rats injected with daily doses of F.R.U.						
	0.5	1	2	3	4	6	10
53	16	18	24	18	15	15	14

The rats were injected subcutaneously for three consecutive days, received no injection on the fourth day and were killed on the fifth day. The daily dose was injected in two half-doses, one in the morning and the second in the evening. The changes in the weights of the secondary sexual organs (prostate, seminal vesicles and penis) were used to indicate the effects of the injections. The general details of the technique used were the same as in our previous experiments (see references at the beginning of the paper). The seminal vesicles were cut from the prostate after fixation in Bouin's fixative. A tiny bridge of tissue, holding the two lobes of the seminal vesicles together, was left uncut. It was found to be unnecessary to separate the coagulating gland from the seminal vesicles. The term "weight of seminal vesicles" in these and also in our previous papers designates the weight of the seminal vesicles together with the coagulating gland. The urethral part of the prostate was cut off at the level of the upper part of the pubic bones.

*Variations in body weight and in the weight of the organs
of normal control uninjected rats.*

In the present assay immature rats were used within a narrow limit of age, namely, 22–25 days old. As young rats grow comparatively quickly, an investigation was made of the variations in the weights of the organs of the control rats used for the assay. The control uninjected rats were divided into two groups: A, 26 rats, 26–27 days old (final age), and B, 27 rats, 28–29 days old (final age). The results obtained are given in Table I and from these data the following conclusions may be drawn.

(1) In group B (the rats of which were only 2 days older than those of group A) the body weight and the actual weights of the sexual organs in spite of some individual variations were on the average definitely larger than those of Group A. In group B as compared with group A, the percentage increases in the body weight, seminal vesicles, prostate, prostate with seminal vesicles and penis were greater by 26, 31, 23, 24 and 15 % respectively.

(2) Per unit of body weight, on the other hand, there were either no definite changes in the weights of the sexual organs or in many cases even a slight decrease.

(3) The maximum variations within each of the two groups expressed as the percentage maximum deviation from the average figures (Table I) are comparatively small, exceeding 25 % only in the case of the seminal vesicles of one rat in group B for which the variation was 35 %.

(4) The maximum percentage deviations from the average figure are slightly higher when all the rats are considered together, but even then do not exceed 36 % except in the case of the seminal vesicles of one rat (deviation 53 %).

(5) The maximum percentage deviations of the weights of the sexual organs are smaller in most cases per unit of body weight than for the actual weights, thus showing that the body weight, as well as the age of the rats influences the weight of the sexual organs of normal rats.

The effect of injections of gonadotropic hormone.

In order to present the data within a reasonable space we give: (a) only the actual weights of the organs of the rats in each group of each litter (Tables II to IV); and (b) the general means of percentage increases of the weights of organs (actual and calculated per unit of body weight), omitting the individual changes for each group of each litter (Table V). The general averages given in

Table V. *Influence on seminal vesicles, prostate and penis.*

The percentage increase of (A) the average actual weights of the seminal vesicles of the rats in each group of each litter and of (B) general average weight per 200 g. of body weight. In brackets—maximum % deviations from general averages in individual cases.

	Rats injected with F.R.U.						
	0.5	1	2	3	4	6	10
Seminal vesicles:	A. Actual weights.						
General average	26	40	84	103	135	177	166
Maximum % deviation	(19)	(25)	(48)	(35)	(43)	(32)	(62)
	B. Weights per 200 g. body weight.						
General average	29	46	87	109	139	190	164
Maximum % deviation	(17)	(11)	(49)	(43)	(54)	(42)	(70)
Prostate:	A. Actual weights.						
General average	—	25	47	61	70	71	85
Maximum % deviation	—	(20)	(38)	(46)	(27)	(38)	(38)
	B. Weights per 200 g. body weight.						
General average	12	32	47	63	71	78	82
Maximum % deviation	(92)	(34)	(43)	(37)	(27)	(37)	(32)
Penis:	A. Actual weights.						
General average	16	20	30	32	40	41	51
Maximum % deviation	(44)	(55)	(87)	(50)	(45)	(37)	(33)
	B. Weights per 200 g. body weight.						
General average	19	28	31	33	41	47	48
Maximum % deviation	(26)	(18)	(90)	(48)	(39)	(74)	(35)

Note. The averages in the table represent the simple usual averages and they differ slightly from the statistical averages, from which the curves are constructed (see section on "Statistical description").

Table V were calculated in the usual way and are slightly different from those used for the statistical presentation of the results (Figs. 1 to 6), in which greater weight was given to the figures obtained from groups containing a larger number of rats.

In brackets, below the lines of general averages in Table V, are given the maximum percentage deviations of the individual cases from these averages. Although the weights of the organs (Tables II to IV) of the injected rats seem to show little variation, when expressed as the percentage increase (Table V) the maximum deviations from the average figures are in some cases considerable, being for the actual weights as much as 46 % for the prostate, 62 % for seminal vesicles and 87 % for the penis (excluding the small dose of 0.5 F.R.U. since this in some rats failed to produce a significant effect on the prostate). However, in spite of these individual variations, the average figures obtained give regular curves. As is shown in Tables I to V and Figs. 1 to 6, the following features are

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 1. Relation between dose of gonadotropic hormone and percentage increase in actual weight of seminal vesicles and of prostate.

Fig. 2. Relation between dose of gonadotropic hormone and percentage increase in weight of seminal vesicles and of prostate calculated per unit of body weight.

Fig. 3. Relation between dose of gonadotropic hormone and percentage increase in actual weight of seminal vesicles weighed together with prostate.

Fig. 4. Relation between dose of gonadotropic hormone and percentage increase of seminal vesicles weighed together with prostate per unit of body weight.

Fig. 5. Relation between dose of gonadotropic hormone and percentage increase in actual weight of penis.

Fig. 6. Relation between dose of gonadotropic hormone and percentage increase in weight of penis per unit of body weight.

characteristic of the effects of gonadotropic hormone on the secondary sexual organs of immature male rats.

(1) The effect on the seminal vesicles is greater than that on the prostate, and the effect on the prostate is greater than that on the penis.

(2) Flattening of the curves with the larger doses of the hormone is most pronounced in the case of the penis and least in the case of the seminal vesicles, the prostate taking an intermediate position.

Statistical description of the curves.

J. M. C. Scott, under the direction of Prof. E. S. Pearson, has represented the results of the assay graphically in the form of the curves given above. He writes: "Suppose some rats in a litter receive a dose x F.R.U. of the gonadotropic hormone and others are used as controls; and suppose the mean weight of a particular organ for the dosed rats exceeds the mean for the controls by y %; and let $z = \frac{y}{x}$, i.e. z represents the percentage increase in the weight of an organ per 1 F.R.U.

With these data the variation between litters in y , shows no appreciable increase with increasing x , except perhaps for $x=10$.

Within the range 1–6 F.R.U. the results plotted on a logarithmic scale can be represented by a straight line. This method was however not adopted because it fails for small doses. Since cubic curves did not seem quite satisfactory for large doses, another system of curves was evolved. These also contain three adjustable constants and pass through the origin, but approach a finite value for very large x . They were fitted to the data by the method of least squares. In Figs. 1–8 the average of y is plotted against x , for the actual weights or those per unit of body weight.

The data for $x=0.5$, 1 and 2 are consistent with the hypothesis that the response of seminal vesicles, prostate, and prostate with seminal vesicles to the hormone is linear: $y=kx$, or $z=k$ (k being constant); but for $x=3$, z becomes definitely smaller. Straight lines were therefore fitted to the data up to and including doses of 2 F.R.U. and the slopes k are given in Table VI.

Table VI. *Statistical summary of results of experiments (J. M. C. Scott).*

(1) Organ and basis of calculation	(2) Average z (all litters receiving 0.5 to 2 F.R.U.)	(3) (4) (5) Probable error of z if determined from		
		Values in column 2 (average z)	Single litter	Three litters
Prostate with seminal vesicles (actual)	27.8	± 1.4 (5.2%)*	± 5.4 (19%)	± 3.1 (11%)
Prostate with seminal vesicles (per 200 g.)	29.8	± 1.3 (4.2%)	± 4.7 (16%)	± 2.7 (9%)
Seminal vesicles (actual)	45.5	± 3.1 (6.8%)	± 11.5 (25%)	± 6.6 (15%)
Seminal vesicles (per 200 g.)	47.8	± 3.1 (6.4%)	± 11.5 (24%)	± 6.6 (14%)
Prostate (actual)	24.3	± 1.3 (5.4%)	± 4.9 (20%)	± 2.8 (12%)
Prostate (per 200 g.)	26.3	± 1.2 (4.4%)	± 4.4 (17%)	± 2.5 (10%)

* The figures in brackets are the values of the probable error expressed as a percentage of the average z of column 2.

In Table VI the following data are given. In column 1, organ and basis for calculation; column 2, average of z for all doses not exceeding 2 F.R.U.; in column 3, probable error of the estimates given in column 2; column 4, probable error of a value of z , determined from one litter; column 5, probable error of a value of z determined from three litters.

For columns 4 and 5 a "litter" was taken as consisting of two rats, each injected with 2 F.R.U. and two control rats. In reducing Dr Korenchevsky's results, a comparison between m rats and n controls was assigned a "weight $\frac{m \times n}{m + n}$ ".

The proposed method of assay of the gonadotropic hormones and definition of the male rat unit.

(1) *The age of rats used for assay.* The rats in these experiments were used not earlier than the second day after weaning (22 days old) in order to allow the rats at least one day to acclimatise themselves to the change of feeding and surroundings and not older than 25 days so that the initial weights of the sexual organs used in the assay should not differ too much. It has already been shown (Table I) that when the control uninjected rats were divided into two groups (A and B), there being an average difference of 2 days between the ages of the rats of the two groups, the sexual organs of the older rats were found to be slightly heavier.

In order to ascertain if this difference influenced the results of assay in any way the hormone-injected rats used in these experiments were also divided into similar A and B groups according to age. These results are summarised in Table VII. Although the percentage increase in the weights of the organs varies in the two groups, this variation does not show a definite or constant effect of the difference in age of the two groups, nor in most cases was the variation considerable. It may therefore be concluded that a suitable choice had been made of the age (22–25 days old at the beginning of the assay), at which the immature rats were to be used for the assay experiments.

(2) *The use of the curves.* For the purpose of the assay of the minimum dose representing one rat unit the part of the curves approximating to a straight line which lies within the range 0 to 2 F.R.U. may be considered to be the most convenient, since the responses of the prostate and of the seminal vesicles are here approximately proportional to the dose of the hormone. There is little difference between the curves based on changes in the actual weights (Figs. 1, 3, 5) and those based on the changes per unit of body weight (Figs. 2, 4, 6) or between the respective maximum deviations (Table V), or the respective probable errors (Table VI). Therefore, for purposes of assay, the curves based on changes in the actual weights appear to be satisfactory and sufficiently accurate.

In order to keep within the range selected for these curves (upper limit about 2 F.R.U.), the value of the male rat unit should be represented by a percentage increase in the weight of the prostate not exceeding 50% and in that of the seminal vesicles not exceeding 90% (see Fig. 1).

At the same time, taking into consideration the fact that the maximum percentage deviations from the average may be considerable (see above), the percentage increase chosen to represent a rat unit should not be too small. It would seem that the same round figure of 40% increase in the weight of the prostate that was chosen in Korenchevsky's method of assay of testicular hormone, would be suitable for the assay of gonadotropic hormone. The corresponding increase in the weight of the seminal vesicles would be about 70% (Fig. 1), in that of the prostate weighed together with seminal vesicles 45% (Fig. 3) and in that of the penis approximately 30% (Fig. 5).

It would be necessary to work out similar curves of assay in each laboratory, since the technique of dissection of the organs, as well as the strain and breeding of the animals might be sufficiently different to affect the slopes of the curves.

Since the logarithmic curves for doses 1–6 F.R.U. approximate to straight lines, these could also be used for assay, but are not given here in order to economise space.

The test.

According to the statistical interpretation, if 3 litters of rats (containing not less than 2 animals in each group) are used for assay, the probable error of the results obtained should not exceed $\pm 12\%$ for the prostate and $\pm 15\%$ for the seminal vesicles. However, taking into consideration the possibility of an occasional considerable maximum deviation from the average, we would suggest the following procedure. The assay should consist of two satisfactory experiments, one preliminary and one final experiment, the former, with the help of the whole range of the curves, giving an approximate orientation of the strength of the preparation. In this preliminary experiment, say, four different doses would be injected into each litter of which there should be at least 4, each containing not less than 5 rats (otherwise more litters must be used). Such a distribution, in which one rat in each litter is left as the control and each of the remaining rats of the litter receives a different dose (as was used in the present experiment with litters 20 to 34), is to be preferred for the reasons given above. By fitting the results obtained in this preliminary experiment to the curves, a suitable dose will be found for the final experiment of the assay which is to determine the value in M.L.R.U. of the preparation assayed. This dose should fit into the part of the curves between 1 and 2 F.R.U. and for this experiment also 4 litters of rats are required. Significant discrepancies between the preliminary and final experiments and the curves will necessitate the carrying out of a third experiment.

If the results from which the curves are drawn are obtained from a large number of rats, the preliminary experiment alone will give a sufficiently accurate estimate of the strength of the preparation, provided that (a) the results of this preliminary experiment lie within the range of the curve before the occurrence of sharp flattening, and (b) the number of rats used in this preliminary experiment is sufficient.

The male rat unit.

We suggest that one male rat unit of gonadotropic hormone be that minimum total dose, which, in the injected rats, will produce on the fifth day an increase of 40% in the actual weight of the prostate and of about 70% in the weight of the seminal vesicles as compared with the average weight of the respective organs of the uninjected control litter-mates in at least 4 litters of immature males. For the injection the total rat-dose must be divided into six equal portions, one portion being injected in the morning and evening of each of 3 consecutive days, no injections being made on the fourth day. The corresponding percentage increase in weight of prostate weighed together with seminal vesicles will be about 45% and of the penis about 30%.

Advantages of the method.

The method of assay which is worked out in the present investigation and the proposed male rat unit of gonadotropic hormones present the following advantages:

- (1) A measure of the male activity of the hormone can thus be ascertained.
- (2) The curves of "dose-response" permit a comparatively easy orientation in the preliminary experiment for choosing the correct dose for the final assay experiment.
- (3) In addition to the response of the prostate, which, in our opinion, is the more reliable for the definition of the rat unit, the method proposed provides a

confirmation of the results in the response of the seminal vesicles and, with a smaller degree of reliability, of the penis.

Disadvantages of the method.

As compared with the assay on females, the method of assay suggested necessitates a rather more complicated technique involving accurate dissection of the sexual organs, their fixation in Bouin's solution (1 day) and weighing.

Accuracy of the method.

Statistical investigation showed that the method was sufficiently accurate. This is further illustrated by the figures for groups A and B in Table VII. The division of the rats into the two groups A and B, though approximately halving

Table VII.

Average % increase in weight of organs obtained after injections of the hormone in rats of group A (age 26–27 days) as compared with that of group B (age 28–29 days).

Dose injected F.R.U.	Group	Seminal vesicles		Prostate		Penis	
		Actual	Per 200 g. body weight	Actual	Per 200 g. body weight	Actual	Per 200 g. body weight
1	A	50	49	22	21	29	27
	B	35	45	26	37	16	28
2	A	92	97	45	45	30	29
	B	76	77	48	50	31	32
3	A	121	124	70	65	44	41
	B	91	93	55	61	24	28
4	A	132	132	67	62	50	47
	B	136	145	73	79	33	37
6	A	189	197	65	66	40	41
	B	170	186	74	85	42	51
10	A	148	148	76	72	52	48
	B	184	180	94	92	49	48

the number of rats in each average, leaves the results obtained without considerable change. The values for groups A and B, especially in the case of the prostate (Table VII), differed little from the respective general averages given in Table V, especially if it is remembered that one rat unit is represented by 40% increase in the weight of prostate and 70% increase in that of the seminal vesicles. The ultimate proof of both the accuracy of the method and its suitability will depend upon the similarity of the results obtained in different laboratories. The accuracy of the assay made in different laboratories will be much improved when a standard preparation is available for comparison with the tested preparations.

The comparison of the male and female rat units of gonadotropic hormones.

We did not make a special assay on female rats. The standardisation of the hormone in F.R.U. as used by Messrs Organon was performed in their laboratories. Therefore this value of the standardisation on females is not necessarily the same as would be obtained by the somewhat different methods of assay used in other laboratories.

However, in our assay 40% increase in weight of the prostate with a corresponding increase of 70% in weight of the seminal vesicles, 45% in weight of the seminal vesicles with prostate weighed together, and approximately 30%

increase in the weight of the penis, was produced by a daily dose of 1.65 F.R.U. or a total dose of 5.0 F.R.U. (1.65 F.R.U. $\times$ 3 days). Thus a comparison of the results of our assay on males with the results of the assay on females obtained in the laboratories of Messrs Organon shows that one male rat unit of gonadotropic hormone was equal to about 5.0 female rat units.

The comparison of the effects produced by gonadotropic hormone with those produced by androsterone and androsterone-diol.

In our previous papers [Korenchevsky and Dennison, 1935, 1, 2; Korenchevsky *et al.*, 1935] we made an assay of fat- and water-soluble preparations of androsterone and androsterone-diol. It is not possible of course to compare the dosages and relative strengths, *i.e.* the quantitative effects of these preparations with those of gonadotropic hormone. It is however possible to compare the qualitative effects.

In the papers mentioned above we found that the chief difference between androsterone and its diol is that the former stimulates the development of the prostate to about the same or to a greater degree than that of the seminal vesicles, whilst the diol stimulates the development of the seminal vesicles much more than that of the prostate.

In Table VIII we have compared the average ratio ($\times 100$) of the percentage increase in the actual weight of the prostate (1) with that of the seminal vesicles and (2) with that of the penis, after the injection of gonadotropic hormone, androsterone and androsterone-diol.

Table VIII.

Average ratio ($\times 100$) of percentage increase in actual weight of prostate to that of seminal vesicles and of prostate to penis after injection of gonadotropic hormone as compared with those after injection of androsterone and androsterone-diol.

Ratio	Gonadotropic hormone	Androsterone	Androsterone-diol
1. Prostate/ seminal vesicles	54	118	64
2. Prostate/ penis	165	230	f.s. 158 w.s. 242

The effects of water-soluble (w.s.) and fat-soluble (f.s.) preparations of androsterone and the diol, as represented by the ratio prostate/sem.ves. were the same or nearly the same [Korenchevsky *et al.*, 1935, p. 2141, Table VIII], and they are, therefore, averaged in the present Table VIII.

The same similarity was observed in the ratio prostate/penis between the fat-soluble (228) and the water-soluble androsterone (232, excluding from this average as accidental the effect on the penis of the dose 7971 γ). A general mean of 230 was therefore tabulated in the present Table VIII. The ratio prostate/penis in the case of fat-soluble diol (158) was however different from that of water-soluble diol (242), owing to the fact that whilst the effect on the prostate of water-soluble diol was much greater than that of fat-soluble diol, the effects of these two substances were much more nearly equal in the case of the penis. These ratios are therefore given separately in Table VIII.

It is clear from the Table that qualitatively the effect of gonadotropic hormone on the secondary sexual organs is very similar to that of diol and very different from that of androsterone. In the ratio prostate/penis however the similarity is only shown between gonadotropic hormone and fat-soluble diol and not by water-soluble diol.

Gonadotropic hormone acts on the secondary sexual organs by stimulating

the testes to a normal secretion of the testicular hormones in the normal ratio. The comparison made above shows that this normal testicular endocrine activity is very similar to the effect produced on the sexual organs by the diol but not to that produced by androsterone.

This supports the statement which we made in the previous paper [Korenchevsky *et al.*, 1935, p. 2141] that the diol produces a return towards the normal ratio in the development of the secondary sexual organs, whilst androsterone fails to do so.

SUMMARY.

1. On the basis of experiments performed on 173 normal immature male rats belonging to 34 litters a method of assay of male activity of gonadotropic hormone preparations is suggested.

2. A regular relation has been found to exist between the dose of the gonadotropic hormone preparation isolated from pregnancy urine and the effect on the prostate, seminal vesicles and penis. This relation has been investigated statistically and represented in the form of curves.

3. In contradistinction to the female rat unit (F.R.U. or ♀ R.U.) of the gonadotropic hormones, a male rat unit (M.R.U. or ♂ R.U.) is suggested.

4. We suggest that one male rat unit of gonadotropic hormone be that minimum total dose which in the injected rats will produce on the fifth day an increase of 40 % in the actual weight of the prostate and of about 70 % in the weight of the seminal vesicles as compared with the average weight of the respective organs of the uninjected control litter-mates in at least 4 litters of immature male rats. For the injections the total rat-dose must be divided into 6 equal portions, a portion being injected on the morning and evening of each of 3 consecutive days, no injections being made on the fourth day. The rats are to be killed on the fifth day.

5. The corresponding increase in weight of prostate weighed together with seminal vesicles will be about 45 % and of the penis approximately 30 %.

6. One M.R.U. of the gonadotropic hormone tested was found to be equal to about 5 F.R.U. (F.R.U. being assayed in the laboratories of Messrs Organon).

7. The qualitative effect on the sexual organs of the gonadotropic hormone from pregnancy urine was found to be very similar to that of androsterone-diol and different from that of androsterone.

8. This supports our previous statement that the diol had more of the qualities of normal testicular endocrine activity than androsterone.

9. The effect of some variables and the advantages and disadvantages of the method of assay suggested are discussed.

Grants from the Medical Research Council and from the Lister Institute have enabled us to carry out this work and to them our thanks are due. We are much indebted to Prof. E. S. Pearson, to Mr J. M. C. Scott and to the Department of Applied Statistics of University College for so kindly helping us in the statistical interpretation of the results obtained.

REFERENCES.

- Korenchevsky (1932, 1). *Biochem. J.* **26**, 413.
— (1932, 2). *Biochem. J.* **26**, 1300.
— and Dennison (1934). *J. Path. Bact.* **38**, 231.
— (1935, 1). *Biochem. J.* **29**, 1720.
— (1935, 2). *Biochem. J.* **29**, 2122.
— and Kohn-Speyer (1933). *Biochem. J.* **27**, 1506.
— and Levy Simpson (1935). *Biochem. J.* **29**, 2131.