Differences between Duplicate Assays of Human Urinary Gonadotrophins

Sheps and Munson[1] have shown how the internal statistics of a single hormone assay may underestimate the variation actually observed on replication of that assay. Inter-assay errors may therefore be important when demonstrating qualitative differences between two hormone preparations by the simultaneous use of two different methods of assay. Such differences have been shown in human urinary gonadotrophins[2], and the errors between duplicates of the assays used have therefore been investigated.

The assays were for (*a*) total gonadotrophin, using the uterine response in immature mice, and (*b*) follicle-stimulating hormone, using the ovarian response in similar mice treated with excess human chorionic gonadotrophin[3]. Pairs of extracts obtained from human urine by the kaolin–acetone method were selected only on the grounds of availability and the fact that they had previously been assayed against one another. They were then re-assayed under the conditions described below, which were arranged so that inter-assay errors were likely to be maximal.

The coded solutions were made up by the same person but were assayed by different persons on the two occasions. In the second assay the dose-levels were designed to be as different from the original ones as was practicable. In five pairs of assays for total gonadotrophin the initial assay was calculated from quantal data and the second from the graded response. From one to seventeen months elapsed between the initial and second assays to allow for effects of storing the extracts. In assaying follicle-stimulating hormone a different batch of chorionic gonadotrophin was used in each assay of a pair.

The results of the assays using graded responses were calculated using log organ weight as the response metameter. Examination of the untransformed results pooled for each method of assay showed an obvious correlation of variance with response in both. The use of log-response stabilized variance satisfactorily in both assays, and such a transformation seems desirable in calculating the results of these assays.

Tables 1 and 2 show respectively the results of ten pairs of assays for total gonadotrophin and ten pairs for follicle-stimulating hormone. In the majority of the duplicate assays the results agree well. The fiducial limits ($P = 0{\cdot}95$) are similar in range to those which have to be used in practice[2], and in two of the twenty cases the limits of the two estimates do not overlap. These cases show significant inter-assay errors and suggest that the failure of the limits to overlap should not be used alone as evidence of significant differences between the results of two assays of a gonadotrophin. The degree of difference of the best estimates must also be taken into account and in only one case in these twenty duplicates does the ratio of the two estimates reach a value of two.

Table 1. Duplicate Assays of Total Gonadotrophin

Pair	Months between assays	Number of dose-levels 1st	Number of dose-levels 2nd	Percentage potency (95 per cent fiducial limits) 1st	Percentage potency (95 per cent fiducial limits) 2nd	Ratio of potency estimates
1	1	2+2	2+3	72 (48–96)	92 (65–125)	1·28
2	1	2+2	2+2	348 (296–408)	238 (208–274)	1·46
3	4	2+2*	3+3	80 (49–115)	72 (53–119)	1·11
4	2	2+2	3+3	84 (56–117)	79 (43–116)	1·06
5	2	2+2	2+3	119 (70–232)	96 (74–124)	1·24
6	15	3+3*	3+3	82 (63–112)	71 (54–90)	1·15
7	15	3+3*	2+3	23 (17–31)	11 (7–15)	2·09
8	1	2+2	3+3	51 (34–72)	57 (51–69)	1·12
9	13	3+3*	2+2	114 (86–167)	100 (85–133)	1·14
10	13	3+3*	2+3	75 (52–114)	59 (35–76)	1·27

* Calculation from quantal data.

Table 2. Duplicate Assays of Follicle-Stimulating Hormone

Pair	Months between assays	Number of dose-levels 1st	Number of dose-levels 2nd	Percentage potency (95 per cent fiducial limits) 1st	Percentage potency (95 per cent fiducial limits) 2nd	Ratio of potency estimates
11	2	2+2	2+2	292 (176–440)	154 (32–270)	1·90
12	11	2+3	3+3	27 (20–38)	29 (17–49)	1·07
13	11	2+3	2+3	17 (12–23)	9 (4–16)	1·89
14	10	2+3	2+3	986 (694–1,370)	735 (508–971)	1·34
15	12	2+3	2+2	848 (633–1,140)	877 (763–1,040)	1·03
16	1	2+3	2+3	264 (168–413)	266 (140–440)	1·01
17	4	2+2	3+3	1,620 (952–3,920)	1,810 (1,260–2,740)	1·12
18	17	2+2	2+3	67 (34–114)	95 (68–113)	1·42
19	2	3+3	3+3	15 (10–22)	15 (6–27)	1·00
20	8	2+2	3+3	2,600 (1,780–4,880)	4,800 (2,790–11,700)	1·85

The magnitude of the inter-assay error is roughly similar for the two methods. It is therefore likely that when a gonadotrophin is assayed by each method a similar error will occur between the two estimates of potency. The conditions of the present experiment were designed to accentuate inter-assay errors and a two-fold difference in estimates occurred only once in twenty pairs. If, therefore, the two methods of assay under normal conditions give estimates which show a more than two-fold difference, and the fiducial limits of which do not overlap, it is very likely that these results demonstrate a genuine qualitative difference. This is being used as a rough working principle until more precise analyses of these and similar data are available.

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Concentration in the Human Ovarian Follicular Fluid of Radioactive Tracers and Drugs circulating in the Blood

The potential hazard of genetic changes induced by radioactive substances and of mutagenic and radiomimetic action of non-radioactive compounds is of current interest. Information, however, as to the intimacy of contact of these substances, when introduced into the human body, with the reproductive cells, particularly the ovum, is scant.

It has been well established that many radioactive tracers, compounds and drugs are transferred from the vascular into other compartments of the human body. However, it is not known whether in the human these substances are transferred into the ovarian follicular fluid. Since the follicular fluid surrounds the ovum and corona radiata and the egg might depend for its nutrition on this material, a transfer of exogenous substances into the ovarian