A SIMPLIFIED PROCEDURE FOR THE ASSAY OF PROGESTERONE

By

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Abstract

During one year 8000 determinations of plasma progesterone have been made, using a simple petroleum ether extraction of the plasma followed by competitive protein binding analysis. The selection of the petroleum ether is crucial for the specificity, which is acceptable for the determination of progesterone during the luteal phase of the menstrual cycle and during pregnancy. The limit of sensitivity is 0.1 ng. Only 0.25 ml of plasma is needed for the determination during the luteal phase and 0.05 to 0.10 ml during pregnancy. One technician can assay 20 samples in one day with good accuracy and a precision of 7.9 per cent in the most favourable range of measurements. In research projects involving drugs the influence of these drugs on the competitive protein binding system has to be tested. Some of the samples should be further purified by thin layer chromatography as a constant check on the specificity for progesterone.

When it was realized that the competitive protein binding (CPB) technique was applicable to the measurement of progesterone (Murphy 1967) and specific methods were constructed on this basis (Neill et al. 1967; Yoshimi & Lipsett 1968), this was greeted with enthusiasm by gynaecologists. The sensitivity and speed of the methods promised to make possible progesterone determinations for clinical purposes. However, CPB methods involving thin layer chromatographic steps were soon found to be frequently plagued by interfering factors derived from the thin layer sheets (Thompson et al. 1969).

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Murphy (1967) originally pointed out that a carefully selected petroleum ether would extract practically only progesterone out of the possible steroids with high affinity for transcortin present in plasma. Work along this line yielded a method that requires only one extraction with petroleum ether followed by CPB quantitation. The method is specific enough for clinical purposes and the determination can be completed within a day (Johansson 1969a). Since the report of Johansson (1969a) one year has passed, during which (1969) around 8000 plasma progesterone determinations have been carried out in this laboratory. In this communication some small changes of the method will be reported and the applicability and limitation of the method will be discussed.

METHOD AND EVALUATION

A general outline of the method as used for routine work is shown in Fig. 1. The method consists of an extraction of plasma with a selected petroleum ether and thereafter quantitation by CPB (Johansson 1969a). This method stands and falls with the selection of the petroleum ether. As petroleum ether is a mixture of hydrocarbons within a given boiling range, in our case 30–60°C, its ability to extract steroids will vary between distillers and also between batches from the same distiller. The batch of petroleum ether selected for this progesterone

![Diagram of Rapid Progesterone Method](https://example.com/diagram.png)

Fig. 1.
A general outline of the rapid progesterone method.
method should extract less than 0.5 per cent of the adrenal steroids, no more
than 20 per cent of 17α-hydroxyprogesterone and more than 80 per cent of
progesterone.

The batch used during 1969 was Mallinckrodt 4980 lot THT. It extracted
85.3 ± 2.4 (s) per cent of progesterone, when the sample was 0.25 ml of female
plasma and this plasma was extracted with 10 volumes of the petroleum ether
by hand shaking for one minute. Under the same conditions less than 0.3 per
cent of the corticosteroids were extracted. Out of 17α-hydroxyprogesterone
and 20α-dihydroprogesterone 13 and 62 per cent were recovered, respectively.
Thus, lot THT has almost identical extracting properties as those petroleum
ether lots reported in the previous paper (Johansson 1969a).

In order to avoid the use of an internal standard the extraction is rigidly
standardized. After one minute of shaking the extraction tubes are centrifuged
at 2000 rpm for 5 min. The petroleum ether is withdrawn by means of Pasteur
pipettes. It is very important to avoid any traces of plasma as this will
drastically influence the CPB.

The percentage recovery of progesterone is checked every other week by all
the technicians using the method. As shown in the table some individual dif¬
fferences can be found. In fact, each technician should have her own correction
factor, which they seem to be able to keep fairly constant. To further decrease
the error of the extraction, the glass stoppers are sealed with water and the
used tubes are regularly being replaced.

Competitive protein binding

The protein binding solution (CBC-B3H) is prepared as follows: To a 100 ml
measuring cylinder is added 17.5 ng of corticosterone-1,2-3H (specific activity

Table 1.
Recovery of 11.0 ng of progesterone-14C from 0.25 ml of female plasma extracted with
10 volumes of petroleum ether (Mallinckrodt THT) tested every second week during
one year.

<table>
<thead>
<tr>
<th>Technician</th>
<th>Recovery percentage</th>
<th>s</th>
<th>Recovery range percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. L.</td>
<td>88.0</td>
<td>1.5</td>
<td>84.5-91.5</td>
</tr>
<tr>
<td>K. S.</td>
<td>83.7</td>
<td>2.2</td>
<td>78.0-88.0</td>
</tr>
<tr>
<td>K. D.</td>
<td>87.1</td>
<td>1.8</td>
<td>83.0-91.0</td>
</tr>
<tr>
<td>K. G.</td>
<td>85.5</td>
<td>2.0</td>
<td>79.0-90.0</td>
</tr>
<tr>
<td>L. L.</td>
<td>82.6</td>
<td>1.6</td>
<td>78.0-91.5</td>
</tr>
<tr>
<td>all</td>
<td>85.3</td>
<td>2.4</td>
<td>78.0-91.5</td>
</tr>
</tbody>
</table>
50 Ci/mmol obtained from New England Nuclear Corporation) in ethanol solution (250 μl). This is evaporated to dryness under nitrogen and then dissolved in 100 ml of glass-distilled water. To this solution is added 75 μl of plasma from a pool obtained from women on combined contraceptive pills. Compared to the previous description (Johansson 1969a) the specific activity of the tritiated steroid is somewhat lower and we have to use 50 per cent more plasma than before in order to achieve a binding of 60–70 per cent of corticosterone-1,2-3H. The present plasma pool may therefore contain either a smaller amount of transcortin or a higher amount of endogenous steroids bound to transcortin.

The separation of the free steroids from the protein bound steroids is the most difficult step in the CPB methods. We are still using Florisil for this separation even if we had some difficulties with it. As pointed out by Strott & Lipsett (1968), variation in the affinity for steroids can occur between batches of Florisil. Every new batch has to be tested. This is done by determining the binding ability of 80 mg of Florisil for corticosterone-1,2-3H in a water solution. The test is performed under the same conditions as the assay. If more than 80 per cent of the steroids is bound the batch is accepted for use in the assay. We have found batches that bind as little as 30 per cent. Unfortunately we have also found one batch that drastically reduced its binding ability for steroids only one day after it had been taken out from the oven. This rapid decline of affinity for steroids might be due to hydration as renewed drying restored the original affinity.

As the equilibrium is disturbed as soon as the Florisil is poured into the tubes, the timing has to be rigidly controlled for each tube (Johansson et al. 1968).

The CBG-B3H solution is generally not used for more than 24 hours after preparation. In the majority of the solutions some decline in performance is seen after 48 hours. Fig. 2 shows the effect of storage at 5°C for 5 days. The sensitivity and useful reading range are reduced. However, the aging of the solution does not seem to be a constant phenomenon as we also have had one solution that performed equally well after 14 days.

Standard curves

For routine clinical samples and for research projects with many samples we use the standard curve in Fig. 2. A standard curve is included in each run. If more precise measurements are required at levels below 1 ng, the standard curve of Fig. 3 is used. The CBC-B3H solution contains, in this case, only 50 μl of plasma, which improves the sensitivity but decreases the range of reading. The first point containing progesterone is 0.1 ng, which is clearly different from zero. The standard curve is always run in duplicate. The difference between duplicates shown in Figs. 2 and 3 is typical of that we usually get.
A typical standard curve (number 1) used for clinical samples. Curve 1 is made from fresh CBG-B³H solution, while curve 2 is made from the same solution after storage in the refrigerator for 5 days. The points are means of duplicates. CPM bound is counts per min of corticosterone-1,2-³H remaining bound to proteins. The efficiency for tritium is 25 per cent in this system.

A standard curve and CBG-B³H solution used when low levels of progesterone are measured. See also legend to Fig. 2.
Petroleum ether (2.5 ml) is added to each tube in the standard curve as a precaution. We have not been able to demonstrate any influence of petroleum ether except at the zero point, where a depression corresponding to 0.1 ng progesterone was found on several occasions.

_Sensitivity_

When crystalline progesterone is added to the reaction tubes and assayed like the standard curve tubes, 0.05 ng can be distinguished from zero using the CBG-B4H system of Fig. 3 (P < 0.01) and 0.1 ng in the system of Fig. 2 (P < 0.01). When the sample in the assay is 0.25 ml, it should be possible to detect 0.5 ng of progesterone per ml. However, the coefficient of variation was 24 per cent at this level. Using 0.5 ml of plasma and the more sensitive CBG-B3H system, 0.3 ng per ml could be detected with a coefficient of variation of 29 per cent.

_Specificity_

The specificity of the method is a function of the petroleum ether and of the binding system. The specificity has been carefully investigated for the normal luteal phase (Johansson 1969a) and normal pregnancy (Johansson 1969b). As expected from the extraction pattern of the petroleum ether, 17α-hydroxyprogesterone and 20α-dihydropregesterone interfere with the readings resulting in somewhat elevated values. However, when several steroids are competing for the binding site on transcortin, the total effect is less than expected from the sum of measurements of the individual steroids.

In our research projects the values of the rapid method are constantly checked by parallel determinations in some samples using the method of Neill et al. (1967). The only difference from the rapid method is that a thin layer chromatographic step is inserted between the petroleum ether extraction and the CBP. It is pointed out that one thin layer step can only be used when small amounts of plasma are extracted with petroleum ether, e.g. when a partial purification is made before the sample is applied to the thin layer sheets. We use precoated thin layer sheets (Silica gel with fluorescent indicator Eastman chromatogram sheet 6060). The sheets were until recently washed by immersion successively into four baths containing 1) 50 per cent methylene chloride/95 per cent methanol containing 0.005 mol sodium EDTA; 2) 50 per cent methylene chloride/methanol; 3) acetone and 4) methanol (Rosenfield et al. 1969). After the washings the sheets were dried at 80°C for 20 min and kept in a desiccator until used. Unfortunately we have had more trouble with the thin layer method used for checking the rapid method than with the latter method itself due to interfering factors from the thin layer sheets (Thompson et al. 1969). As found for other reagents, the interaction of the thin layer
Interference from the thin layer chromatogram sheets. Curve 1 is the ordinary standard curve. Curve 2 resulted from eluting thin layer pieces without pretreatment into the tubes and curve 3 after the thin layer sheets had been washed in four baths (see text). The points are per cent counts per min of the zero point (= 100 per cent) and the mean of 6 different runs.

sheets with the binding system differ from batch to batch. During most of 1969 we used series 1243 of the thin layer sheets. Fig. 4 shows how points of the standard curve are depressed by eluting directly into the reaction tubes from empty pieces of the thin layer sheets without any pretreatment (curve 2, Fig. 4) and after the washing described above (curve 3, Fig. 4). The washing of the sheets improved the sensitive part of the standard curve. During the writing of this report we started to use series number 1262. Using this new batch of thin layer sheets we have not been able to detect any influence from the sheets on the binding system.

The immediate preovulatory period of the normal menstrual cycle creates special problems in the rapid method as the plasma progesterone levels are low (Johansson & Wide 1969a) and the levels of 17α-hydroxyprogesterone are relatively high (Johansson & Wide 1969b; Strott et al. 1969). With our petroleum ether the overestimation by the rapid method compared to thin layer purified samples has never been found to exceed 20 per cent in the preovulatory period.

Precision

The precision of the extraction step is shown in the table. The precision of the CPB step was calculated from the duplicate determinations of the points on the standard curve (Snedecor 1956). The lowest coefficient of variation was
found for 1 and 2.5 ng points (3.5 per cent), while the error increased at lower and higher values but never exceeded 5 per cent. One technician came below 3 per cent on the 1 and 2.5 ng levels. The overall precision for the complete assay has improved very little since the first report (Johansson 1969a). The coefficient of variation was 7.9 per cent between the 1.0 to 2.5 ng readings of the standard curve, but increased with readings above and below this range. Once again differences could be found between technicians.

Recovery studies of added unlabelled progesterone to female plasma from the follicular phase yield values very close to the expected values (Johansson 1969a) but as expected the errors were greater than those found when the precision between duplicate analyses was studied.

RESULTS AND DISCUSSION

There are few menstrual cycles with daily progesterone levels reported. A comparison between authors is often made difficult due to lack of clinical data about the subjects and how they were selected. In Fig. 5 is shown a composite graph of plasma progesterone levels determined in 20 normal menstrual cycles in healthy women, in which the normality of the luteal phase was determined by the pattern of the oestrogen excretion and the constant length of the cycle (Johansson 1969a). During the follicular phase, defined as the period preceding the first rise of LH, extraction of 0.25 ml of plasma mostly gave values below or at the limit of sensitivity (Fig. 5). When 1.0 ml of plasma was extracted, a mean of 0.34 ng per ml (range 0.1–0.8, n = 25) was found. Parallel determinations from a plasma pool made up from the same samples as above using thin layer purification gave a mean of 0.40 ng per ml (range 0.2–0.6, n = 6). Five ml of plasma was extracted. These levels found during the follicular phase agree well with those found by van der Molen & Groen (1965), Neill et al. (1967) and Yoshimi & Lipsett (1968), but are lower than those found by Hagerman & Williams (1969) and Martin et al. (1970). Even if the rapid method performs well during the follicular phase, the precision in this range is poor and a single value should not be trusted.

During the luteal phase better agreement on the range of plasma progesterone levels are found among the reports. Good agreement is found between the levels of Fig. 5 and the normal cycles reported by Neill et al. (1967) and Strott et al. (1969). The normal cycle reported by Hagerman & Williams (1969) is of special interest as the samples were determined both by a double isotope dilution method (Woolever & Goldfien 1963) and a CPB method (Neill et al. 1967) and showed excellent agreement between the two methods.

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Fig. 5.

A composite graph of the daily plasma progesterone levels from the luteal phase of 20 normal menstrual cycles arranged from the onset of the next menstrual bleeding. The dashed line is the limit of sensitivity. From Johansson (1969a).

With a rapid method for progesterone it should be possible to evaluate more satisfactorily the variation of the luteal pattern in the normal menstrual cycles and to relate this to gonadotrophin and oestrogen values (Johansson et al. 1970a). However, the question of changes in the luteal activity during the fertile period of a woman can not be answered yet. On the other hand, it is fairly easy to screen out some abnormal menstrual cycles by hormone analyses. One large group consists of short menstrual cycles of less than 25 days’ duration. In these cycles we usually find a normal mid-cycle peak of total oestrogens, a normal to low LH peak and very little increase of plasma progesterone, at most up to 2–3 ng per ml. The bleeding starts less than 10 days after the LH peak (Johansson 1969a; Johansson et al. 1970b). Women with long cycles (> 35 days) or oligomenorrhoea were usually found to have normal progesterone pattern when they eventually ovulated (Johansson et al. 1970b). In clinical work with infertile women the method has been useful, specially during induction of ovulation (Johansson & Gemzell 1969; Yuzpe et al. 1969; Gemzell et al. 1970) and during artificial insemination (Carlberg et al. 1969). It is of special value that the results are available within 24 hours, which is in time to influence clinical decisions.

During pregnancy the rapid method has been applied mainly to the first half of pregnancy (Johansson 1969b). Fig. 6 shows weekly levels during the first half of pregnancy. The composite curve is derived from 440 determina-
tions in 321 women. The levels found by the rapid method are within the range of those found by Yannone et al. (1968) using gas chromatography, but somewhat lower than the values of van der Molen (1963).

A recent contribution to the controversy around the question of progesterone withdrawal during hypertonic saline-induced abortions (Csapo et al. 1969) is of interests also from a methodological point of view, as each plasma sample was assayed by four different methods. The method of van der Molen & Groen (1965, gas chromatography) gave the highest values, while the methods of Wiest (1967, double isotope technique) and Neill et al. (1967, CPB) gave results close to the mean of all four methods. The lowest values were found by the method of Wyman & Sommerville (1968, gas chromatography). However, the values from all four methods followed the same trend. Approaching the same problem using the rapid method the levels found agree well with those reported by Csapo et al. (1969), although our conclusion concerning the role of progesterone is different (Holmdahl et al. 1970).

The importance of progesterone determinations for clinical purposes during pregnancy is uncertain. Some prognostic help might be gained in cases of threatening abortions, insufficient function of placenta (Johansson 1969b) and when labour could easily be initiated with oxytocin (Johansson 1968), but these matters must be studied further.

Our greatest effort has been devoted to the study of the effect of contra-
ceptive substances on ovarian function. As small a dose as 0.3 mg of nor-ethisterone given daily will result in a partial depression of plasma progesterone levels during the luteal phase (Larsson-Cohn et al. 1970a; Larsson-Cohn et al. 1970c). Chlormadinone acetate 0.5 mg daily seems to disturb the normal ovulatory pattern in a more limited way (Larsson-Cohn et al. 1970c).

Recently, the effect of several substances administered after ovulation has been studied. In Fig. 7 is shown how 5 mg of norethisterone daily for six days will depress the peripheral plasma levels of progesterone and reduce the length of the luteal phase when the treatment is started on the third postovulatory day. These studies are quite exciting since they might lead to the development of a new contraceptive technique that will interfere with normal hormonal functions less than is the case with the existing hormonal contraceptives.

In conclusion it must be emphasized that the measurements of progesterone by means of one petroleum ether extraction followed by competitive protein binding analysis without further purification can easily give erroneous values. Success is dependent on completely controlled conditions. The reagents have to be tested before use. It is important to note that the performance of the reagents does not follow the ordinary criteria of purity, e.g. distillation may not be enough to improve a reagent that gives interference in the method. Last, but not least, it is mandatory to have facilities for thin layer chromatographic purification of the petroleum ether extract in order to check the validity of the results from time to time.

\[ \text{Fig. 7.} \]

Plasma progesterone levels during one normal menstrual cycle and another cycle from the same woman during treatment with 5 mg of norethisterone (norethindrone) daily for six days starting on the third postovulatory day.
ACKNOWLEDGMENTS

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REFERENCES

DISCUSSION

Lipsett: Dr. Johansson, you have convinced me that your method is quite adequate for plasma progesterone during the luteal phase and during pregnancy. Can you give me some idea of the 95% confidence limits of a plasma progesterone level during the luteal phase?

Johansson: The method is very imprecise below one ng; at one ng we have a coefficient of variation of 20%, but between 2 and 20 ng the coefficient of variation is around 10%. In pregnancy, in order to use the same binding solution, we have to extract very small amounts of plasma. We extract 50 microliters, sometimes down to 25 µl, and when you then multiply these values up to 1 ml of plasma, the confidence limits are sometimes about 20%.

Reeves: Dr. Johansson's comment that one technician can do up to 100 samples of progesterone a week brings up the problem of making a valid comparison among methods and laboratories in the very practical sense. Dr. Lipsett mentioned earlier that in his laboratory one technician can do 40 progesterone samples per week (plus other samples); Dr. de Souza mentioned that in Stockholm one technician could do 18. These comparisons assume, of course, that all equipment is equally efficient, which I doubt is the case.

Secondly, I would like to ask the experts in nomenclature or semantics if there is some way to distinguish between reports of sensitivity (in the Ekins' sense) of the standard curve and sensitivity of measured samples, assuming that many methods may not resolve the interference problem totally, as apparently Dr. Lipsett has done.

Johansson: I am afraid I cannot answer your question, Dr. Reeves. We have found in Uppsala that it is wise for us to measure plasma samples from the menstrual cycle in one room and plasma samples from pregnant subjects in another room, because in pregnancy the levels are very high and when we try to get follicular phase samples measured, there is an enormous difference, and just small droplets can contaminate the room. So we do not only have contamination with »hot« material, but also with »cold« material. One technician we cannot use during her luteal phase with this method. She gets high values. I wonder if anyone else has similar experience? Does progesterone appear in the saliva, for instance?

Korenman: It is well established that saliva concentrates cortisol and I wonder whether anyone knows about concentration of oestrogens or progestins in salivary secretions?

Southam: Dr. Jorge Rosner in Argentina has shown that the parotid gland can make steroid hormones. Gerald Oster, a physical chemist at the Mount Sinai Hospital has
demonstrated that secretions from salivary glands in humans show the typical ferning at the time of the oestrogen surge in the menstrual cycle.

Abraham: Interfering materials in the competitive protein binding assays of steroids could be the steroids themselves. We had problems of this kind when oestrogens in gram amounts were used in the same room where the assay was performed. Our problems were solved when we moved the assay performance into another room. This problem is even more important with oestrogens, since they sublime at room temperature and atmospheric pressure, and therefore could accumulate at the ceiling of the laboratory and slowly fall down into the assay tubes.

Williamson: Dr. Johansson, might your coefficient of variation be reduced by washing your disposable tubes? We have found that variation between replicate determinations was significantly reduced by washing all these tubes with distilled water, ethanol and freshly distilled ether.

Johansson: I have tried this for my disposable tubes, but I don’t get any better precision. On the standard curve I have beautiful precision. I have a coefficient of variation of 3–4% in the optimal reading part, which includes all my reagents, which are really just binding solution and petroleum ether. If I find a petroleum ether that in some way displaces the curve, then I don’t use that petroleum ether batch.

Midgley: Can you redistill any batch of petroleum ether and make it useful?

Johansson: Redistillation reduces, or eliminates the blank, but it does not change the extraction performance of the batch. I also found that Mallinckrodt’s petroleum ethers usually work best if they come from the Middle East. Petroleum ether is just a mixture of hydrocarbons, and I am presently trying to make out which mixture will be best for this type of extraction.

Korenman: I don’t wash the tubes. Why bother if they work well without washing?

Corker: Dr. Williamson, what is the advantage in using disposable tubes if you have to wash them before use?

Williamson: We still use disposable tubes because this facilitates the disposal of steroids and Florisil and hence reduces the possibility of laboratory contamination.

Pearlman: You clearly indicated that administration of synthetic progestins results in a depression of progesterone levels in plasma. What was the effect on plasma LH and FSH?

Johansson: During the luteal phase we cannot find any difference either in the urinary excretion of LH or in the plasma levels of LH.

Lunenfeld: Does medroxyprogesterone acetate interfere in your assay system for progesterone?

Johansson: I do not know.

Southam: Some primate centers are determining plasma progesterone and mating animals on the first day of the rise. When do you think ovulation occurs in relation¬ship to the LH and plasma progesterone rise?

Johansson: I think ovulation occurs 24–36 hours after the LH rise. In humans we have studied sperm penetration related to this event, and there is a very distinct peak of
sperm penetration at this time, 24–48 hours before this period. When you come to
insemination in humans, it really improves the results.

**Southam:** When does ovulation occur in relationship to the plasma progesterone rise?

**Johansson:** In the human I think ovulation occurs on the second day after the pro-
gestosterone rise. In the monkey, maybe on the second day, but it depends on how good
your method is.

**Lipsett:** Since plasma progesterone rises very slowly at the time of the LH peak and
not at all in some subjects, the very early rise of progesterone would not be a good
index of ovulation. Since ovulation in the human occurs 24–36 hours after the LH
peak, the plasma progesterone is increasing, but there is considerable variation from
subject to subject. Plasma progesterone levels thus would be a poor index of ovulation.

**Southam:** It is just an observation. It doesn't matter, you get as good a breeding record
in rhesus monkeys by just picking the 13th day of the cycle. You also get a good
breeding record in humans by just picking the 15th day before the next expected
period.

**Ryan:** I think we need more exact measurements than this and I am reminded of the
experiment that Roger Short did in terms of canulating the ovarian vessel and showing
an almost instantaneous rise in progesterone as soon as the ovulation had occurred. It
may make no difference from a fertility point of view, but it would be of interest to
try to understand what is going on.

**Borth:** Since there exists, at present, no method which detects ovulation at the very
moment when it occurs, I feel that the question raised by Dr. Southam concerning
progesterone increase and time of ovulation cannot really be answered as yet. We have
started work on the development of such a method, and if Dr. Southam still asks the
question in 2–3 years, we might be able to answer it.

**Lunenfeld:** Can we relate the rise in plasma progesterone to the LH peak? The value
we saw from the graph of Dr. Johansson for progesterone is rather low in that area,
and I wonder whether the precision of the assay actually permits to conclude that
there is a real rise in progesterone at the time prior to the LH peak.

**Johansson:** I am not able to detect any rise of progesterone prior to the increase of
plasma LH, while at the same time as LH goes up, or possibly, a little bit later, there
is a significant rise of the material that I measure.

**Lipsett:** The plasma progesterone rises in some patients and not in others on the day
of the LH peak. The average shows a small rise. Twenty-four hours later there is a
clear rise in progesterone, presumably with luteinization. My interpretation of this
very early rise at the time of the LH peak is that this progesterone is being released
in the sense that Roger Short would call from a »leaky gland«, i.e. the follicle at
this time is busy synthesizing oestrogen. Large amounts of 17α-hydroxyprogesterone
are released, but only small amounts of progesterone. Then, 24 hours later, comes
ovulation, rapid luteinization, and then the major increase in progesterone.

**Corker:** Since it is possible to get luteinization of the follicle prior to rupture, is it
necessary to postulate a »leaking gland« to explain a preovulatory rise in progesterone?

**Lipsett:** There may be many hypotheses. I picked this one because we know from
studies of adrenal glands stimulated with ACTH, or Leydig cells stimulated with
HCG, that whenever you stimulate a steroid synthesizing gland and increase the secretion of the important product of that gland, the biosynthetic intermediates are also secreted in increased amounts.

Mikhail: I think the data of Zander many years ago have proven that progesterone is present in high concentration in the follicular tissue itself. We have shown at least one case in which the concentration of progesterone in the vein blood from the ovary containing the ripe follicle was at least ten times that of the peripheral value in a case that had not ovulated yet, by all available criteria.

Concerning the »leaking gland«: secretion of hormone by a gland is a function of the difference in concentration of gland and peripheral blood. But how do we distinguish between leakage and secretion?