Respiratory and Electrolyte Effects Induced by Estrogen and Progesterone

Ruth L. Goodland, M.S., John G. Reynolds, A.B.,
Augusta B. McCoord, Ph.D., and W. T. Pommerenke, Ph.D., M.D.

Cyclic changes in the reproductive system of the normally ovulating human female have long been attributable to the periodic production of estrogen and progesterone. Discovery of the elaboration of these substances by the ovaries and of their rather dramatic and apparently specific effects upon the reproductive system understandably led to their designation as "female sex hormones"—a term, in light of subsequent studies, perhaps ill chosen because these internally secreted substances not only influence accessory organs as well, but also systemic bodily functions and mechanisms. Moreover, estrogenic substances, instead of being localized in origin to the ovaries, occur widely in nature, notably in certain plants quite as innocent of eroticism as the humble yam. Furthermore, synthetic preparations whose actions closely simulate those of the natural estrogens have established their worth in replacement therapy. The hormones, or their synthetic homologues, which cause profound changes in the reproductive tract will also, directly or indirectly, produce functional changes elsewhere in the organism. Thus, variations in basal temperature and metabolism and in the composition of respiratory gases in man, and the obvious expression of sexuality associated with the complicated patterns of behavior at estrus in domestic animals,

---

From the Departments of Obstetrics and Gynecology, and of Pediatrics, The University of Rochester and Strong Memorial Hospital, Rochester, New York.

Supported by a grant from the Ortho Research Foundation.

Presented at the First World Congress on Fertility and Sterility, New York, May 25, 1953, and in part before the American Physiological Society, Chicago, April 7, 1953.

are illustrative of the extragenital responses attributable to the gonadal hormones.

While some of the somatic reactions produced by estrogens and progesterone may not as yet have fixed significance, it is easy to speculate that they contribute to a total economy and environment favorable to the reproductive function. Thus, the thermogenic effect of progesterone illustrates a mechanism which helps control the manner in which the body burns fuel by causing it to use more oxygen. The manifestation of increased energy needs in pregnancy may be compared to the mass response by which adrenaline enables an animal in distress to deal comprehensively with the multiple aspects of an emergency. Seemingly of significance is the observation that some of the collateral manifestations, or side plays, resulting from administration of the sex hormones are as capable of objective measurement as are their effects on the organs of generation. This paper is concerned with some such measurements.

Basal body temperature graphs of normally menstruating women present a characteristic cyclic pattern. In a typical cycle the basal body temperature fluctuates around a comparatively low level during the postmenstrual phase of the cycle. Approximately at the fourteenth day of the usual 28-day cycle, and sometimes preceded by a further transient fall, the temperature rises, either abruptly or slowly, and is maintained at a higher level until the occurrence of the next menstrual period. The shift in basal temperature is often regarded as a criterion of ovulation, and observance of the temperature records suggests the optimal time for attempting an impregnation. This maintenance of the temperature at a higher level is believed to be due to the influence of progesterone.[1, 7, 12]

Rubenstein, in 1938, found that the basal metabolic patterns during the menstrual cycle closely parallel the basal body temperature patterns. Both values reach their lowest levels just before the mid-period of the cycle and fluctuate about a higher level during the postovulatory phase. It is possible, therefore, that this increase in basal metabolism may also be attributable to the action of progesterone.

Another cyclic fluctuation demonstrable in normally menstruating women is the change in alveolar carbon dioxide tension. In 1929 Griffith and coworkers reported periodic variations in alveolar pCO_2 referable to different phases of the menstrual cycle. Using the Haldane-Priestley method of col-

lection, they observed the lowest values shortly preceding the onset of menstruation and the highest values during the second or third weeks of the cycle. In general, these observations have been confirmed.[4, 8, 11]

Hormonal influence on serum electrolytes by the adrenal cortical steroids has long been recognized, and the similarity in chemical structure to these substances of estrogen and progesterone has led to the speculation that they also might effect electrolyte balance. Although Danforth *et al.* and Greisheimer *et al.* concluded that there are no consistent serum electrolyte changes during the menstrual cycle, recent investigation by this laboratory[14] would seem to confirm Eckstein *et al.'s* findings that quite constant cyclic variations do occur in certain serum electrolytes. In general, it has been concluded that serum sodium levels increase during the pre-ovulatory phase of the menstrual cycle, reach a peak at about the time of ovulation, and decrease during the progestational phase of the cycle. Eckstein found that the serum chloride followed approximately the same pattern as the serum sodium, while this laboratory[14] observed that the serum chloride increases from the end of menstruation until the twenty-fourth day of the average 28-day cycle.

The data presented herewith are intended to show the effects of estrogen and progesterone, parenterally administered, singly and in various combinations, on basal body temperature and metabolism, alveolar carbon dioxide tension, and serum sodium and chloride. To obviate the cyclic features due to the periodicity of elaboration of estrogen and progesterone in the female with its concomitant changes in the reproductive tract, male subjects were employed, attention being given only to the effects of these preparations on the systemic responses.

EXPERIMENTAL PROCEDURES

Subjects

The subjects were 20 healthy male medical students ranging in age from 21 to 31 years and exhibiting no signs of endocrine dysfunction.

Series

The investigation consisted of several series with the various subjects of each series receiving similar injections. Each single series consisted of intramuscular injections of one of the following:

A. 1 ml. of sesame oil,* the vehicle for the steroids, as control.
B. 50 mg. (1 ml.) of progesterone.*
C. 10 mg. (1 ml.) of estradiol benzoate.*
D. 30 mg. (3 ml.) of estradiol benzoate administered as 10 mg. (1 ml.) per day on each of 3 successive days.
E. 10 mg. (1 ml.) of estradiol benzoate plus 50 mg. (1 ml.) of progesterone.
F. Same as D plus 50 mg. (1 ml.) of progesterone concomitantly with the third estrogen injection.

A total of 38 complete sets of experiments were carried out on these subjects, some of whom were used in more than one series with a minimum lapse of 14 days between successive courses of injections. Each individual was initially instructed in the use and operation of the apparatus and became familiar with the equipment through several practice sessions prior to the actual determinations. The tests were made at 24-hour intervals under basal conditions, the subjects having been instructed to fast for at least 10 hours and to obtain a night's sleep preceding the analyses. Oral basal body temperatures were taken each morning by the individuals before arising. Each subject rested in a recumbent position for at least 30 minutes before the tests were made. The injections were given following the determinations on the second day after base line values had been established. In all instances but one observations were continued on each individual for 72 hours after the last injection.

METHODS

Alveolar carbon dioxide was collected by the Rahn and Otis method[16] without interruption during normal breathing and analyzed using the Cambridge CO_2 Analyser.[15] This method has an advantage over those generally employed heretofore in that it permits one to make continuous analyses, thus determining the establishment of a steady and reproducible respiratory state. The machine was calibrated before use with a gas mixture containing a known percentage of CO_2. Readings were repeated at 1-minute intervals until 5 consecutive values agreed to within 0.15 per cent. Per cent CO_2 was then converted to pCO_2 (mm. of Hg). The waterless Sanborn apparatus served for the determination of basal metabolism. The metabolic rate was calculated, taking into consideration the oxygen consumption, age,

* The sesame oil, progesterone, and estradiol benzoate were generously supplied by Schering Corporation.

sex, weight, and height of the subject and is here reported in terms of per cent on the basis of the Mayo Foundation standards. Determinations were repeated until there was good agreement.

Blood for serum electrolyte determinations was drawn by venipuncture without stasis immediately following the alveolar pCO_2 and basal metabolism determinations, that is, under basal conditions. Serum was separated by allowing the blood to clot, rimming the clot, and centrifuging at 2500 r.p.m.

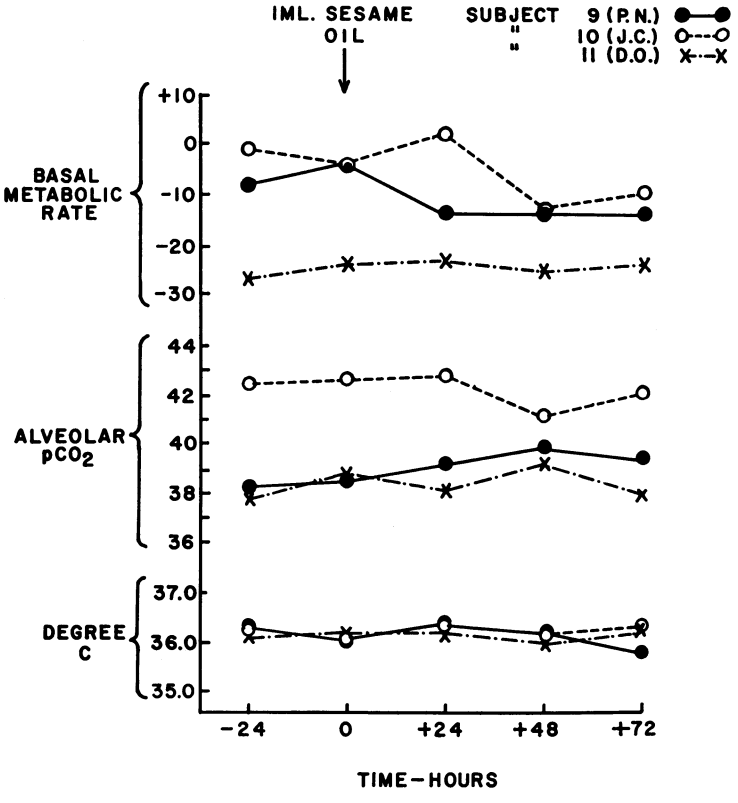

Fig. 1. Characteristic responses to the control injection of sesame oil, the vehicle for the steroids. The arrow indicates the time of injection.

for 5 minutes. Serum sodium values were determined on the Perkin Elmer flame photometer (model 52A), using lithium as the internal standard. Serum chloride concentrations were determined according to the method of Van Slyke, as modified by Wilson and Ball, using 1 ml. of serum. All

determinations were made in duplicate or triplicate depending upon agreement. Average values are reported in terms of mEq./L.

RESULTS

Basal Metabolic Rate, Alveolar Carbon Dioxide Tension, and Basal Body Temperature

Series A. In this series 9 subjects received single injections of 1 ml. of sesame oil, the vehicle for the steroids. In addition, 1 of these subjects received a second injection after a 2-month interval. Figure 1 presents three typical patterns of basal metabolic rate, alveolar pCO_2, and basal body temperature in response to these injections. The injections were given immediately after the second day's values had been obtained, this moment being labeled zero time. As is indicated in the figure, fluctuations were noted in all three categories, but these showed no consistent trend. Table 1 pre-

TABLE 1. Average Changes from Zero Time in 10 Subjects, Each Receiving 1 ml. of Sesame Oil

Hours before (−) or after (+) injection	*Basal metabolic rate* (%)	*Alveolar* pCO_2 (mm.Hg)	*Basal body temperature* (°C.)
− 24	− 1.23	− 0.24	0.00
+ 24	− 2.24	+ 0.63	+ 0.05
+ 48	− 1.59	+ 0.56	0.00
+ 72	− 1.44	− 0.07	+ 0.11

sents the average changes for all subjects in this series. The greatest change occurred in the lowering of the basal metabolic rate, but this amounted to only 2.24 per cent. This effect was noticed early in the series and necessitated a larger number of control subjects than originally planned. Handled statistically by the method of paired differences, even this effect was not significant. The statistics presented in the tables were all computed by this method of paired differences.

Series B. This series consisted of a single injection of 50 mg. of progesterone in sesame oil. A total of 7 injections using 6 subjects was made. Figure 2 illustrates the results obtained for basal metabolic rate, alveolar pCO_2, and basal body temperature with 3 subjects. In all cases the basal body temperature rose and the alveolar pCO_2 fell after the progesterone administration. After 48 hours the values began to approach preinjection

(zero time) levels. The changes in basal metabolic rate were not consistent, increasing in 2 individuals and decreasing in 1. Table 2 presents the average changes for all subjects. The basal metabolic rate showed an increase after progesterone administration, then a lowering and finally another increase. None of these values has statistical significance. This hormone was found to

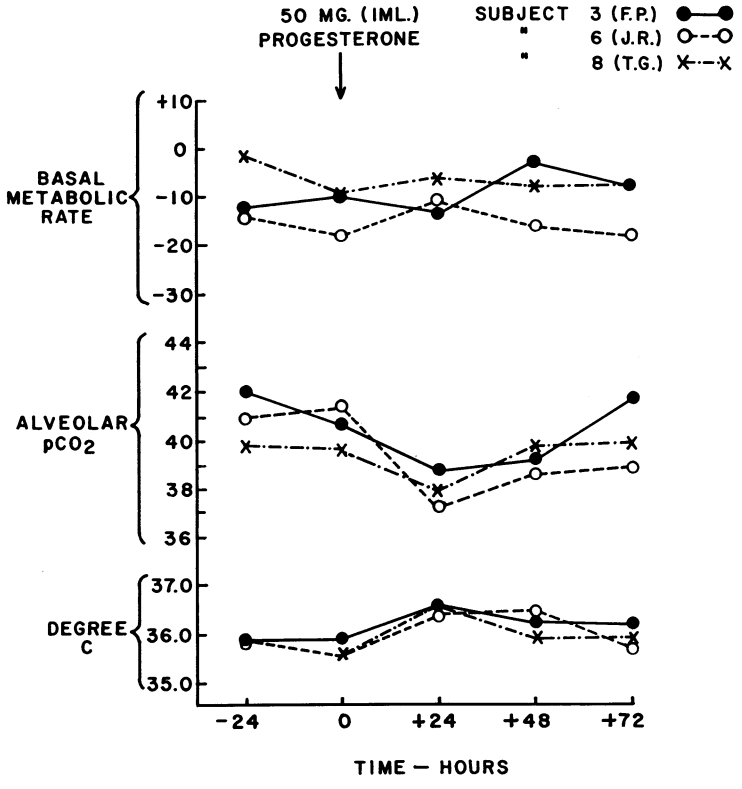

Fig. 2. Characteristic responses to the injection of 50 mg. of progesterone. The arrow indicates the time of injection. Note the increase in basal temperature and the decrease in alveolar pCO_2 in all cases.

have a definite effect upon basal body temperature and alveolar pCO_2. Twenty-four hours after the injection the basal temperature increased an average of 0.67° C., and the alveolar pCO_2 decreased an average of 2.54 mm. of Hg. Both of these changes have statistical significance, with a probability of less than 1.0 per cent.

Forty-eight hours and 72 hours after the progesterone injection, the temperatures were still significantly elevated but were approaching the zero

time values. In most cases they were down to their original levels after 96 hours. The 48- and 72-hour values for the alveolar pCO_2 were also approaching those of the zero time.

One postmenopausal woman was also given a 50 mg. injection of progesterone. The response in basal metabolic rate, alveolar pCO_2, and basal

TABLE 2. Average Changes in 7 Male Subjects Before and After Single Injections of 50 mg. of Progesterone

Hours before (−) or after (+) injection	*Basal metabolic rate* (%)	*Alveolar* pCO_2 (mm.Hg)	*Basal body temperature* (°C.)
− 24	+ 1.07	− 0.04	+ 0.06
+ 24	+ 2.90	− 2.54[a]	+ 0.67[a]
+ 48	+ 1.90	− 1.93[b]	+ 0.50[a]
+ 72	+ 2.29	− 0.89	+ 0.26[a]

[a] Probability of less than 1.0 per cent.
[b] Probability of less than 4.0 per cent.

body temperature followed the pattern characteristic of the men subjects who had received the same material.

Series C and D. In series C the subjects received a single injection of 10 mg. of estradiol benzoate in sesame oil. A total of 6 injections using 6 subjects was given. Figure 3 illustrates the results obtained on basal

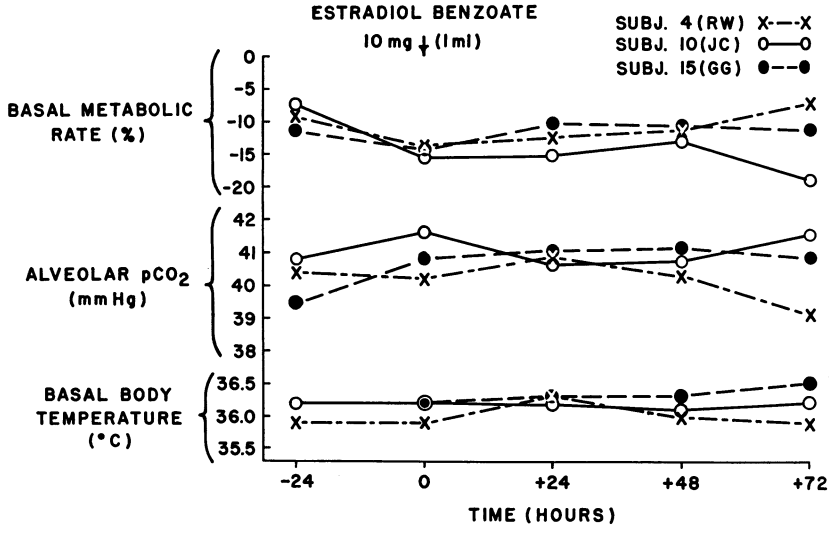

Fig. 3. Characteristic responses to single injections of 10 mg. of estradiol benzoate. The arrow indicates the time of injection.

metabolic rate, alveolar pCO_2, and basal body temperature with 3 subjects. Twenty-four hours after the estrogen the basal temperature of 2 individuals increased slightly, while it remained constant in the third person. The changes in alveolar pCO_2 also varied while the basal metabolic rates all increased slightly.

In series D 4 subjects received 3 injections of 10 mg. each of estradiol benzoate on three consecutive days, each subject thus receiving a total of 30 mg. Through this program it was intended to learn the effects of repeated injections on the same individuals. Table 3 presents the average changes for

TABLE 3. Average Changes Before and After Injections of Estradiol Benzoate

Hours before (—) or after (+) first injection	*Basal metabolic rate* (%)	*Alveolar* pCO_2 (mm.Hg)	*Basal body temperature* (°C.)
	10 MG. AT 0 TIME, 6 SUBJECTS (SERIES C)		
— 24	+ 5.30	— 0.75	— 0.03
+ 24	+ 2.87	— 0.23	+ 0.20
+ 48	+ 1.67	+ 0.10	+ 0.07
+ 72	+ 1.82	— 0.38	0.00
	30 MG.,[a] 4 SUBJECTS (SERIES D)		
— 24	— 2.58	— 1.13	— 0.13
+ 24	— 0.55	— 0.28	+ 0.03
+ 48	— 2.58	— 0.83	— 0.03
+ 72	— 2.45	+ 0.23	+ 0.08
+ 96	+ 0.53	0.00	+ 0.05
+ 120	— 2.43	— 0.20	+ 0.10

[a] 10 mg. (1 ml.) estradiol benzoate per day for 3 successive days—at 0 time, + 24, and + 48 hours.

all subjects in these two series. With a single injection of 10 mg. of estrogen the basal body temperature was found to increase slightly, but this increase was not significant. With three successive injections of estrogen, making a total of 30 mg., this thermogenic effect is not distinct. The increase at 24 hours (after 10 mg.) is negligible, while at 48 hours (after 20 mg. total) the average change shows a decrease. These changes are small enough to come within the range of daily variation. At 24 hours after the estrogen, alveolar pCO_2 decreased in both series by about the same amount, although in neither case was this significant. Subsequent changes after the single injection point to this decrease as again being within daily variation. After two injections of estrogen (20 mg. total) the alveolar pCO_2

was still decreasing. At this point it had the appearance of the start of a true effect. After the third injection the direction of change was reversed. In both series there were such wide variations in basal metabolic effects that no true tendency could be established.

Series E and F. In series E both estradiol benzoate and progesterone were given. Six subjects received 10 mg. of estrogen and, at the same time, 50 mg. of progesterone as separate injections. Figure 4 illustrates the results

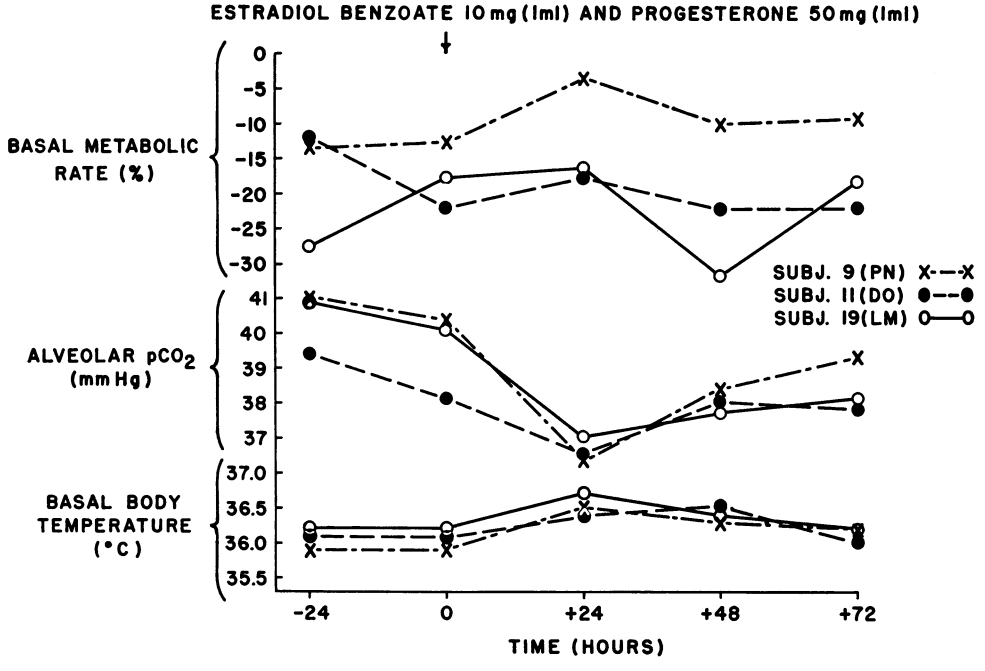

Fig. 4. Effect of simultaneous injections of estradiol benzoate and progesterone. The arrow indicates the time of injection.

obtained with 3 subjects. Here again the increase in basal temperature and decrease in alveolar pCO_2 were consistently and clearly produced. There appeared to be a slight increase in basal metabolic rate, but this effect was not nearly as pronounced as the other responses.

In series F 3 subjects received 3 injections, each of 10 mg. of estradiol benzoate, on consecutive days with 50 mg. of progesterone being given in addition at the time of the third estrogen injection. Table 4 presents the average changes for all subjects in these two series. In series E the increase in basal body temperature and the decrease in alveolar pCO_2 are again

statistically significant, as was the case when progesterone alone was injected (Table 2). In series F the 72-hour values are those obtained 24 hours after the final injection. Since only 3 subjects were used in this series, the data were not handled statistically, but the trend for temperature increase was apparent. It was a much smaller increase than that obtained in either series B or E. The alveolar pCO_2 decrease was comparable to that obtained in both series B and E. The changes in basal metabolism in these series again showed such variations that no conclusions appear warranted.

TABLE 4. Average Changes Before and After Injections of Estradiol Benzoate (E) plus Progesterone (P)

Hours before (−) or after (+) first injection	*Basal metabolic rate* (%)	*Alveolar* pCO_2 (mm.Hg)	*Basal body temperature* (°C.)
	10 MG. E + 50 MG. P AT 0 TIME, 6 SUBJECTS (SERIES E)		
− 24	+ 0.05	+ 0.88	0.00
+ 24	+ 2.02	− 2.20[a]	+ 0.48[a]
+ 48	− 1.27	− 1.60[b]	+ 0.37[a]
+ 72	− 0.50	− 1.00[b]	+ 0.10
	30 MG. E[c] + 50 MG. P, 3 SUBJECTS (SERIES F)		
− 24	+ 1.15	+ 0.10	+ 0.10
+ 24	+ 0.17	− 0.23	− 0.07
+ 48	+ 0.23	− 0.87	+ 0.10
+ 72	+ 1.07	− 2.47	+ 0.27
+ 96	+ 3.20	− 2.43	+ 0.23
+ 120	− 0.20	− 1.97	+ 0.10

[a] Probability of less than 1.0 per cent.
[b] Probability of less than 4.0 per cent.
[c] 10 mg. (1 ml.) estradiol benzoate per day for 3 successive days—at 0 time, + 24, and + 48 hours with 50 mg. (1 ml.) progesterone in addition at + 48 hours.

When the data obtained 24 hours after injection were pooled for all subjects receiving 10 mg. of estradiol benzoate alone at any one time, the basal body temperature was found to increase 0.08° C., the alveolar pCO_2 to decrease 0.25 mm. of Hg, and the basal metabolic rate to increase 1.19 per cent. None of these changes was found to have statistical significance. When the results obtained 24 hours after two injections on consecutive days (20 mg. estrogen total) were pooled, it was noted that the changes in basal temperature and basal metabolism appeared to fall within the range of normal variation. However, the resultant decrease in alveolar pCO_2, a drop of 0.84 mm. of Hg, was regarded as significant (probability of 0.03 per cent).

Serum Electrolytes

Table 5 summarizes the mean changes in serum sodium and chloride concentrations in the various experimental series. It will be noted in A that the control injection of sesame oil alone did not produce any marked or significant changes.

In series B—the one in which single injections of 50 mg. of progesterone were given—serum sodium increased an average of 1.29 mEq./L. 24 hours after injection. After a subsequent dip, the mean change at 72 hours rose to

TABLE 5. Mean Changes in Serum Sodium and Chloride Concentrations in mEq./L.

Series		Time (hours) from first injection					
		− 24	+ 24	+ 48	+ 72	+ 96	+ 120
A. 1 ml. sesame oil	Na	+ 0.63	+ 0.74	− 0.56	− 0.87		
	Cl	− 0.04	+ 0.12	− 0.48	− 0.35		
B. 50 mg. progesterone	Na	− 1.16	+ 1.29	+ 0.39	+ 1.91		
	Cl	− 0.33	+ 0.69	− 0.37	− 0.36		
C. 10 mg. estradiol benzoate	Na	+ 1.03	+ 0.60	− 0.87	+ 1.00		
	Cl	− 0.73	+ 0.08	+ 0.18	+ 0.03		
D. 30 mg. estradiol benzoate[c]	Na	+ 1.08	+ 5.08[a]	+ 2.20	+ 1.00	+ 2.35	+ 1.90
	Cl	− 0.92	− 1.60[b]	− 0.85	− 0.55	− 0.25	− 0.27
E. 10 mg. estradiol benzoate plus 50 mg. progesterone	Na	+ 0.60	− 2.13	− 0.63	− 1.65		
	Cl	− 0.39	− 0.60	− 0.42	− 0.20		
F. 30 mg. estradiol benzoate plus 50 mg. progesterone[d]	Na	...	...	...	...	...	...
	Cl	+ 0.25	+ 0.13	− 0.83	+ 0.30	+ 0.50	+ 1.17

[a] Denotes probability of less than 1.0 per cent.
[b] Denotes probability of less than 5.0 per cent.
[c] 10 mg. estradiol benzoate per day for 3 successive days—at 0 time, + 24, and + 48 hours.
[d] 10 mg. estradiol benzoate per day for 3 successive days—at 0 time, + 24, and + 48 hours, with 50 mg. progesterone in addition at + 48 hours.

1.91 mEq./L., this rise being observed in 6 of the 7 subjects. Serum chloride showed an average increase of 0.69 mEq./L. 24 hours after injection, pre-injection levels being reached again at 48 hours.

In series C, in which single injections of estradiol benzoate were given, the serum sodium showed an increase at 24 hours after injection followed by a drop at 48 hours and a subsequent rise at 72 hours. The serum chloride values remained relatively constant up to 72 hours following injection.

In series D, in which three successive injections of 10 mg. of estradiol benzoate were given on consecutive days for a total of 30 mg., serum sodium values had risen in all instances by 24 hours after the first injection. Despite further injections this increase was not maintained. Twenty-four hours after the first injection, serum chloride dropped in all subjects, the average decrease being 1.60 mEq./L. Subsequent injections did not augment this observed decrease in serum chloride concentration, for the later values gradually approached the pre-injection level. This initial fall had not been observed in series C.

In series E—the one in which 10 mg. of estradiol benzoate plus 50 mg. of progesterone were administered simultaneously—a marked fall in serum sodium concentration was observed at 24 hours. The chloride decreased and then rose to approach pre-injection levels at 72 hours.

In series F, in which three successive injections of 10 mg. of estradiol benzoate were given on consecutive days for a total of 30 mg., plus 50 mg. of progesterone concomitantly with the third estrogen injection, serum chloride showed little change at 24 hours, dropped 0.83 mEq./L. below the zero level at 48 hours, and then increased steadily until it was 1.17 mEq./L. above the zero value at 72 hours after the last injection. Serum sodium values were not obtained for this series.

By pooling the data from the 13 subjects who had received 10 mg. of estradiol benzoate alone, it was observed that an average decrease of 0.42 mEq./L. in serum chloride occurred after 24 hours. After 48 hours, that is, after the subjects had received 20 mg. of estrogen, this value had further decreased to 0.84 mEq./L. This fall was observed in 5 of 7 subjects. Serum sodium values in this same over-all compilation of data showed average increases of 2.01 mEq./L. 24 hours after 10 mg. of estrogen and 2.08 mEq./L. after 20 mg. of estrogen.

DISCUSSION

Of the two steroids tested progesterone readily produced decided changes in the basal body temperature and alveolar carbon dioxide tension. These changes are similar to those reported by Döring *et al.* in 1950[5] with the exception that our data showed a much larger temperature increase following progesterone. This observation adds credence to the explanation that the temperature elevation which becomes manifest in the second half of the menstrual cycle is attributable to the corpus luteum. No definite conclusion

seemed justified from our data on the effect of the injection of progesterone or estrogen, either alone or in combination, on the basal metabolic rate. The parallelism which Rubenstein described between the basal metabolic rate and the basal body temperature was not demonstrated statistically in these experiments, but our data suggest this parallelism. The over-all tendency of the data would appear to indicate an increased basal metabolic rate resulting from these injections. One may speculate then that the increased oxygen consumption characteristic of the postovulatory phase bears a relationship to the increased energy requirements of early pregnancy, and that one of the functions of progesterone is to stimulate metabolism.

No significant changes were observed when 10 mg. of estradiol benzoate alone was injected, the subsequent fluctuations being similar to those found after injection of sesame oil alone. After 20 mg. of estrogen, that is, after the second 10 mg. injection, the alveolar pCO_2 was found to decrease, this being the only significant change observed. Döring *et al.*[5] had also found a significant lowering with a single injection of 25 mg. of estradiol benzoate. The third 10 mg. injection of estrogen in our series then reversed this effect. One might hypothecate that with a series of daily injections in the male, intermediate or end products in the metabolism of the steroid could inhibit or actually bring about this reversal of effect.

The literature abounds in discussions of the interactions between estrogen and progesterone. Courrier's review gives an excellent picture of the antagonism or synergism which may be obtained using various proportions of the two steroids. Döring *et al.*[5] found synergism in the action of estradiol benzoate (25 mg.) and progesterone (50 mg.) on the increase of temperature and decrease of alveolar pCO_2. Rothchild and Barnes could find no effect of estrogen upon the temperature increase produced by progesterone. The results obtained by this laboratory suggest antagonistic effects between estradiol benzoate and progesterone upon temperature. When 50 mg. of progesterone was given with 10 mg. of estrogen, the increase was apparent, but this was not as great as that produced with progesterone alone. When progesterone was administered concurrently with the third of three 10-mg. injections of estrogen, the rise was about 40 per cent that obtained with progesterone alone. The alveolar pCO_2 data, after both hormones were administered in the amounts given, point to a prolongation of the effect rather than an actual synergism. Although two injections of estrogen alone caused a decided decrease in alveolar pCO_2, the third injection was fol-

lowed by a reversal of this effect. This reversal did not occur when progesterone was administered simultaneously with the third injection of estrogen. On the contrary, the effect was approximately equal to that observed with progesterone alone and did not diminish as rapidly.

It might be expected, in view of the work of previous investigators,[19] that the injection of progesterone would lead to an increase in serum sodium and chloride levels. Our data tend to bear out this hypothesis, although statistical significance could not be attached to these findings. Variability of human response to administered progesterone has been noted by Perlman, and inconstancy of effect is perhaps responsible for our inability to attach definite significance to these observations.

Administered estrogen in experimental animals has been shown to result in decreased renal excretion of sodium and chloride.[19] Retention of sodium and chloride in women, reaching a maximum at ovulation, when estrogen production is at a high level, has also been demonstrated.[6, 14] Our results showed that estradiol benzoate, in the quantities administered, induced increased serum sodium following injection. This new level was not maintained by subsequent injections. Thorn[20] demonstrated in dogs that continued estrogen administration failed to prevent a return of sodium excretion to pre-injection levels after the initial sodium retention. The serum chloride values in our series appeared initially to be lowered by estrogen administration. Trends toward reduction in both serum sodium and chloride concentrations following combined injections of 10 mg. of estradiol benzoate and 50 mg. of progesterone are suggested.

Reference is again made to the provocative discussion of Courrier, who emphasized that the results following administration of estrogen and progesterone in different quantities and combinations are variable. Individual compensatory mechanisms doubtless contribute to the measured responses. A considerable fluidity and lability must exist in the various reciprocal relationships that apply in endocrine systems. Any effect of the administration of these hormones must therefore be considered individually. Variability in sensitivity of the several responses may therefore explain seemingly divergent data. Hemodilution, a characteristic response of women to the injection of estrogen,[22] was also noted in the 2 subjects in whom this factor was followed. Such hemodilution might, in fact, explain the observed electrolyte concentrations.

It is offered as a thesis that a study of the electrolytes sodium and chloride

in the serum, and of the respiratory gases will provide still other methods for appraising or measuring the effects of estrogen and progesterone. While the foregoing experiments were performed on men, by conjecture the conclusions drawn may also apply to women. Since characteristic extragenital changes are induced by progesterone and/or estrogen, another method becomes available for estimating the occurrence of ovulation.

SUMMARY

The effects of injections of progesterone and estradiol benzoate alone and in various combinations upon the basal metabolic rate, alveolar carbon dioxide tension, basal body temperature, and serum sodium and chloride concentrations were investigated using adult men as subjects. Control injections of sesame oil, the vehicle for the steroids, produced no significant physiologic effects. Progesterone caused a significant increase in basal body temperature and a decrease in alveolar pCO_2. Its effect upon basal metabolic rate was variable but seemingly produced a rise. No statistically significant changes were observed in the electrolytes; however, it appeared that progesterone tended to increase serum concentrations of both sodium and chloride. Lowering of alveolar pCO_2 occurred following two injections of estradiol benzoate on successive days. A significant increase in serum sodium was seen and a marked decreased in serum chloride concentration was noted following estrogen administration.

The thermogenic effect of progesterone was diminished when estradiol benzoate was simultaneously administered. The suppression of alveolar pCO_2 by progesterone was prolonged when estrogen was also injected. Under these conditions both serum sodium and chloride levels appeared to be lowered.

It is proposed that a study of electrolytes and respiratory gases may be employed as substitutes or supplements to morphologic criteria of estrogen and/or progesterone activity.

REFERENCES

1. Buxton, C. L., and Atkinson, W. B. *J. Clin. Endocrinol. 8:*544, 1948.
2. Courrier, R. *Vitamins and Hormones VIII:*179, 1950.
3. Danforth, D. N., Boyer, P. K., and Graff, S. *Endocrinology 39:*188, 1946.
4. Döring, G. K., and Loeschcke, H. H. *Arch. ges. Physiol. 249:*437, 1947.
5. Döring, G. K., Loeschcke, H. H., and Ochwadt, B. *Arch. ges. Physiol. 252:* 216, 1950.

6. Eckstein, F. M. P., Lubran, M., and McKeown, T. *Guy's Hosp. Reports 90:*28, 1940-41.
7. Forbes, T. R. *Am. J. Obst. & Gynec. 60:*180, 1950.
8. Goodland, R. L., and Pommerenke, W. T. *Fertil. and Steril. 3:*394, 1952.
9. Greisheimer, E. M., Oppenheimer, M. J., and Ellis, D. *Endocrinology 38:*258, 1946.
10. Griffith, F. R., Jr., Pucher, G. W., Brownell, K. A., Klein, J. D., and Carmer, M. E. *Am. J. Physiol. 89:*449, 1929.
11. Loeschcke, H. H. *Arch. ges. Physiol. 252:*301, 1950.
12. Palmer, A. *Obst. & Gynec. Surv. 4:*1, 1949.
13. Perlman, R. M. *J. Clin. Endocrinol. 8:*586, 1948.
14. Phillips, R. S., McCoord, A. B., and Pommerenke, W. T. *Fertil. and Steril. 3:*402, 1952.
15. Rahn, H., Mohney, J., Otis, A. B., and Fenn, W. O. *J. Aviation Med. 17:*173, 1946.
16. Rahn, H., and Otis, A. B. *J. Appl. Physiol. 1:*717, 1949.
17. Rothchild, I., and Barnes, A. C. *Endocrinology 50:*485, 1952.
18. Rubenstein, B. B. *Endocrinology 22:*41, 1938.
19. Thorn, G. W., and Engel, L. L. *J. Exper. Med. 68:*299, 1938.
20. Thorn, G. W., and Harrop, G. A. *Science 86:*40, 1937.
21. Wilson, D. W., and Ball, E. G. *J. Biol. Chem. 79:*221, 1928.
22. Witten, C. L., and Bradbury, J. T. *Proc. Soc. Exper. Biol. and Med. 78:*626, 1951.

DISCUSSION

Dr. S. H. Sturgis, *Boston, Massachusetts:* Those familiar with research reported from Dr. Pommerenke's department can always be sure the project will be beautifully planned and executed with meticulous attention to detail. Blood is drawn by venipuncture "without stains," duplicate and triplicate analyses are made, controls are carefully established. When, therefore, these workers state—for instance—that a significant increase in serum sodium concentration and a decrease in serum chloride was seen following estrogen in their male subject, one can feel confident that these results need no further confirmation and will stand as established facts. I, for one, doubt if the proposal to substitute such electrolyte studies for morphologic criteria of steroid activity will be widely accepted. The value of these studies, it seems to me is much more fundamental. I am interested in the figures suggesting an increased metabolic rate after progesterone that would correlate with the known thermogenic action of this steroid. In the female it has been tempting to associate these two systemic effects with the specific growth and differentiation of the sensitive target organs of the reproductive tract. Increased temperature and oxygen consumption due to sex steroids in the male is obviously devoid of such an association.

We know very little about the mechanisms whereby these steroids cause specific actions. Are the target organ responses a reflection in sensitive tissues to systemic, biochemical alterations? Or are they specific to the tissues that alone show such response? This study presents one approach; by selecting males, any sex specificity or target organ sensitivity to estrogen and progesterone is essentially eliminated. Significant changes in circulating electrolytes and alveolar carbon dioxide concentration have been demonstrated. Yet, in appraising the

total response, these workers have set themselves a difficult interpretive task. The gross effects here reported may reflect only the net result of a confusing tangle of enzyme system responses in liver, kidney, and other organs. Another approach is that of studying the response of isolated organs where the results are a bit more definitive—and the ultimate approach in specific response is derived from work on the single cell.

In our laboratory, we have been interested in some effects of the sex steroids—estrogen, progesterone, and testosterone—on a single, isolated organ, such as the uterus of the rat. It is known, for instance, that estrogen and testosterone together increase the growth of the castrate uterus more than either hormone separately. It has also been reported, surprisingly enough, that this increased growth rate was not balanced by an equal increase in oxygen consumption of the isolated uterus. We have made the added observation that the high metabolic rate of this organ induced by estrogen not only is depressed by testosterone given before sacrificing the animal, but an equally significant depression results from addition of testosterone after sacrifice to the flask containing the isolated uterus in the respirometer.

Dr. Pommerenke has studied the intact animal—but so to speak, has thrown away the specifically sensitive target organs, the female reproductive tract—by choosing males. We have studied the uteri by preparations in vitro after throwing away the rest of the animal. By accumulation of such data we may hope some day for a better understanding of how and why these steroids cause gross morphologic change. Dr. Pommerenke and his group may be congratulated on a most stimulating piece of work.