GESTATIONAL EFFECT OF ALLYLESTRENOL

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In a previous papers (Allen and Wu, 1959a and b; Wu, 1961), we suggested the method of evaluating gestational effect of progestins by checking the effect on the endometrium and the development of the blastocysts, the effect on implantation and the maintenance of further pregnancy in castrated rabbits.

Agents only are able to produce good progestational effect on endometrium, or bring about good deciduoma or even able to produce the apparently normal development of the blastocysts are not always able to secure normal implantation and maintaining further pregnancy (Allen and Wu, 1959a). Agents which have all these 4 effects might be regarded as the actually really good gestagens. We evaluate the new orally active agent allylestrenol* by this method. As the abortion rate of rabbits is highest in experimental castrated animals available, the rabbits are used in these experiments.

MATERIALS AND METHODS

For the study, mature rabbits, each weighing 7~9 lbs, were used. All rabbits were isolated in separate cages before the use. Successful mating with 1 or 2 males was confirmed by the finding of the spermatozoa in the vagina. The technique of operation followed that described by Corner and Allen (Corner and Allen, 1929). The operation were done under intravenous penthotal anesthesia. The allylestrenol was dissolved in sesame oil with proper dilution so that 0.2cc of the solution provided the desired daily dose for subcutaneous injection. For the experiment in which the allylestrenol was given orally, the desired daily dose was dissolved in 1.0 cc of sesame oil. The methods of experiment followed that described by Wu and Allen (Allen and Wu, 1959a and b; Wu, 1961).

RESULTS

Endometrium and blastocysts

Agent under investigation is examined for its power to produce complete progestational proliferation and the normally developed blastocysts. The result of this study are given in Table 1. Complete progestational proliferation was produced in all cases and the blastocysts were of normal size when the daily parenteral dose was 4.0 mg or more. Equally good results were obtained when the daily oral dose was 20.0 mg or more.

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* The Allylestrenol used in these experiments was kindly supplied by N.V. Organon Co. through the courtesy of Dr. Pijpekamp. The trade name of this compound is "Gestanon".

Table 1. Comparison of effects of progesterone and gestanon on endometrium and blastocysts in rabbits castrated 20~24 hrs. after mating

Daily dose (mg)*	Average no. blastocysts recovered	Average diameter blastocysts†	No. blastocysts recovered No. ruptured follicles (%)	Progestational proliferation
Progesterone subcutaneously				
2.0	6.3	2.59	82.6	4 ⁺
1.0	5.7	1.88	68.0	4 ⁺
0.5	5.3	1.57	60.9	4 ⁺
0.25	3.7	1.27	57.9	3 ⁺
0.125	1.0	0.8	16.7	1~2 ⁺
Gestanon subcutaneously				
10.0	4.8	2.20	78.5	4 ⁺
5.0	6.3	1.72	66.0	4 ⁺
2.5	7.0	0.98	57.2	2 ⁺ ~3 ⁺
1.25	3.7	0.65	18.3	1 ⁺ ~2 ⁺
Gestanon orally				
20.0	5.6	2.11	88.0	4 ⁺
10.0	6.8	1.58	86.9	4 ⁺
5.0	6.2	0.99	54.6	2 ⁺ ~3 ⁺
2.5	5.0	0.60	14.2	1 ⁺ ~2 ⁺

* Three animals were used for each dosage level.

† Measurement made when animals were killed 144 hrs. after mating. Albumen coat is not included.

Effect on implantation

The rabbits were castrated 20~24 hrs. after mating. A constant daily dose of allylestrenol was given thereafter. Details of the experiment followed that described by Wu and Allen (Wu and Allen, 1959b; Wu, 1961). The result of this study are given in Table 2. When the daily subcutaneous dose was 5.0 mg or more, implantation appeared to be normal. The implantations of normal appearance were subsequently grow to be normal fetuses. No implantations were observed when the daily dose was 2.5 mg or less. The minimum daily oral dose necessary to secure normal implantation was 20.0 mg. No implantations were observed when the daily dose was 5.0 mg. However, with the marginal dose of 10.0 mg, only 20% of the cases have successful implantation and further maintenance of pregnancy.

Effect on maintenance of pregnancy in rabbits castrated on the fourteenth day

In the previous paper we reported that the absorption of 17-*a*-hydroxyprogesterone capronate was too slow to compensate for the acute deprivation of progesterone caused by castration unless 8 times the minimal dose was given or unless the injection of the capronate was begun 2 days before castration (Wu and Allen, 1959b). In this study, therefore, medication of allylestrenol was begun on the 11th day in one series of experiments and on the 14th day (the day of castration) in another. Same kind of slow compensation for acute deprivation of progesterone was noted in allylestrenol also. No pregnancy maintained in every cases in each group up to 40.0 mg daily, when the medication begun on

Table 2. Comparison of effects of norlutin and gestanon on implantation in rabbits castrated 20~24 hrs. after mating

Daily dose (mg)	No. ruptured follicles	Total no. implantations	No. normal implantations No ruptured follicles (%)
Norlutin subcutaneously			
4.0	19	0	0
2.0	35	7Ra 1Nb	0
1.0	33	3R 6Nc	0
0.5	26	0	0
0.25	30	0	0
Gestanon subcutaneously			
20.0	24	22	92
10.0	22	21	95
5.0	26	21	81
2.5	20	0	0
1.25	23	0	0
Gestanon orally			
20.0	32	23	72
10.0	20	4	20
5.0	27	0	0
2.5	32	0	0
1.25	29	0	0

a: R=resorbing implantations N=normal appearing implantations

b: Injections continued, one normal implantation resorbed by 16th day

c: Injections continued, six normal implantations resorbed by 14th day

14th day of pregnancy, the day of castration. Short time of development noticed when 20.0 mg or more daily are given. However, when the injection was started on 11th day, 3 days before castration, maintenance of pregnancy are successful. The minimum effective daily dose for successful implantation is 20.0 mg subcutaneously as well as 40.0 mg perorally. When medications were given every 12 hrs. a daily dose of 20.0 mg or more subcutaneously maintained the pregnancy normally to term in each case, as did a daily oral dose of 40.0 mg. When injection or per os medications were given every 8 hrs., minimum requirement for maintaining pregnancy drop to 10.5 mg daily subcutaneously or 21 mg daily perorally.

No masculinizing effect on the female fetuses are noted judging from the length of the perineum of female young. No toxic effects noted even with the high dose of 40.0 mg daily for more than 2 weeks.

Allylestrenol is effective in maintaining later half of pregnancy parenterally and perorally. However due to slow compensation and relatively short acting effect, medication should be started earlier or should be given more than twice a day.

Table 3. Effect of gestanon in rabbits castrated on the 14th day of pregnancy, medication begun 14th day

Daily dose (mg)*	Average no. normal implantation on day 14	Average no. normal implantation on day 20	Average no. living fetuses delivered by Caesarean section
Gestanon subcutaneously q. D.			
40	6.8	6.8	6.8R
20	6.0	5.2+0.8R	5.0R
10	8.2	8.2R	—
5	8.6	8.6R	—
Gestanon orally q. D.			
40	5.6	4.8+0.8R	4.8R
20	7.3	0.5+6.8R	0.5R
10	7.0	7.0R	—
5	6.4	6.4R	—
Gestanon subcutaneously B. I. D.			
40	6.2	6.2	6.2
20	7.8	7.8	7.8
10	5.0	3.0+2.0R	2.0R
5	4.8	4.8R	—
Gestanon orally B. I. D.			
40	7.2	7.2	7.2
20	8.2	2.0+6.2R	2.0R
10	6.4	6.4R	—
5	7.0	7.0R	—
Gestanon subcutaneously T. I. D.			
42	7.4	7.4	7.4
21	6.8	6.8	6.8
10.5	7.0	7.0	7.0
5.25	5.8	5.8R	—
Gestanon orally T. I. D.			
42	7.0	7.0	7.0
21	8.0	8.0	6.0+2.0R
10.5	5.5	5.5R	—
5.25	6.3	6.3R	—

* Five animals were used for each dosage levels.

** R=resorbing implantations

Table 4. Effect of gestanon in rabbits castrated on the 14th day of pregnancy, medication begun 11th day

Daily dose (mg)*	Average no. normal implantation on day 14	Average no. normal implantation on day 20	Average no. living fetuses delivered by Caesarean section
Gestanon subcutaneously			
40	6.4	5.8+0.6R	5.8
20	7.2	6.0+1.2R	5.2
10	8.0	3.0+5.0R	2.5
5	6.6	6.6R	—
Gestanon orally			
40	5.2	4.6+0.6R	4.6
20	6.6	5.2+1.4R	5.0R
10	7.0	7.0R	—
5	6.8	6.8R	—

* Five animals were used for each dosage level.

** R=resorbing implantations

DISCUSSION

Comparison with 17-alpha-ethinyl-19-nor-testosterone is of special interest. The chemical structure of this compound is very similar to the structure of 17-alpha-ethinyl-19-nor-testosterone. Both are derivatives of 19-nor-testosterone. The two compounds differ in that Norlutin has ethinyl group and allylestrenol has allyl group on the position C-17. This slight difference, can scarcely be responsible for the remarkable difference in activity. (Allylestrenol is able to secure normal implantation and bring further maintenance of pregnancy, whereas Norlutin does not (Allen and Wu, 1959).) In another respect, however, the two compounds are quite different chemically. Allylestrenol are devoid of =O at position 3 and Norlutin has the one. This might contribute to the successful gestational effects.

Relative potency of allylestrenol judging from Wu and Allen's bioassay method (Effect on the Endometrium and the size of the blastocysts), is about half time as active as progesterone when both are given subcutaneously, and about 1/8 as active orally as progesterone is subcutaneously.

The daily dosage required to maintain pregnancy in rabbits castrated on the 14th day seems to be somewhat more than the dosage required to produce normal blastocysts on the 6th day and normal implantation on the 10th day.

It is also important to note that allylestrenol is rather short acting as shown by the effect on maintaining later half of pregnancy. Medication of more than twice a day brings about the successful results in maintaining pregnancy.

It is worthwhile to mention the result that even large dose (40.0 mg daily for first 3 days) does not inhibit the normal ovum tubal transportation. All ovum transported to the uterus within 82 hrs. after mating even when the large dose of allylestrenol are given daily, for first 3 days.

SUMMARY AND CONCLUSION

1. Allylestrenol is complete oral and parenteral gestagen. It has all of the gestational effects of progesterone in the rabbits. This agent produces normal gestational proliferation of the endometrium, normal blastocysts, secure normal implantation and maintain the pregnancy in a normal manner to term.

2. Relative potency of the compound is about half time as active as progesterone when both are given parenterally and about 1/8 as active orally as progesterone is parenterally.

3. As allylestrenol is rather short acting, it is necessary to give it more than twice daily in order to bring about the successful result in maintaining pregnancy.

4. The compound is free from toxic effect and virilizing action on the mother and female fetuses.

5. Even large dose of allylestrenol does not inhibit the normal ovum tubal transportation.

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