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PREVENTION OF IMPLANTATION BY ANTIPROGESTERONE

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SUMMARY

If progesterone (P) is indispensable in the protection of normal pregnancy during the pre-implantation period, then treatment with antiP (AP), should prevent implantation. The validity of this premise has been examined in the rat model, using the synthetic steroid: Isoxazol. This AP inhibits the conversion of pregnenolone to P, by inhibiting the enzyme 3β-HSDH. In an earlier study, a single oral dose of 2.5 mg Isoxazol invariably interrupted pregnancy shortly after implantation and 20 mg predictably provoked parturition preterm. In the present study, conducted between days 0 and 4 of the pre-implantation period, a single oral dose of 20 to 100 mg Isoxazol has been administered to sperm-positive rats. The controls were treated with vehicle only. At day 4, when the blastocysts are already loosely attached to the endometrium, 40 mg Isoxazol compromised normal implantation, as a rule, and the conceptuses became resorbed. At day 3, when the morulas enter the uterus, this dose had a moderate effect only while 60 mg increased efficacy and 100 mg prevented the implantation of most blastocysts. As in the previous studies a single dose of AP provoked a drastic P withdrawal (Pw). The premise that it was Pw and not some side effect of Isoxazol which intercepted normal implantation was verified by the demonstration that the prevention of the AP-induced Pw, by P treatment, prevented the antifertility action of Isoxazol.

INTRODUCTION

Once conception has triggered the sequence of events leading to unwanted pregnancy, legal abortion can be prevented only by preventing implantation. Contraception effectively controls fertility. However, to protect the physiological condition of non-pregnancy, during those few hours of the menstrual cycle when it is threatened by ovulation and conception, contraception requires *chronic* therapy. It demands the use, often for years, of biologically potent steroids which profoundly alter the endocrine conditions of the patient, or the permanent presence of a foreign body in the uterine cavity. In sharp contrast, post-conceptional therapy has the potential to control fertility during the pre-implantation period through an *acute* intervention, which only tips off the regulatory balance for a few hours, before restoring the normal menstrual cycle.

An understanding of the nature of the regulatory mechanism, controlling the normal cycle and pregnancy, has challenged researchers for decades. Regardless of the purpose of post-conceptional therapy, *i.e.* whether it aims at the protection or termination of pregnancy, this understanding is the key to rational and effective treatment. A decisive step in basic research directed toward the exposure of this controlling mechanism was the discovery of progesterone (P), the progestational component of the regulatory system and the demonstration in the rabbit model that, regardless of its stage, normal pregnancy cannot withstand a P withdrawal (Pw) of critical degree [1]. However, the clinical relevance of this discovery was documented only recently [2] by evidence from luteoctomy studies in early pregnancy. This extension of the basic animal studies to the human has been reinforced [2] (again by luteoctomy evidence) by showing that the normal pregnant human uterus cannot release its maximum cyclic activity, despite massive stimulation with prostaglandin (PG) or oxytocin unless a Pw of critical degree preceded the attempted activation of the myometrium. Thus, it became apparent that a regulatory *balance* between PG and P is *indispensable* for the maintenance of normal pregnancy, and only when this balance is tipped off by Pw, converting the refractory uterus into a reactive organ, can PGs exert their oxytocic action and terminate pregnancy [2].

Recognition of the indispensability of P in pregnancy maintenance has prompted broad and extensive research. An important component of this effort was directed toward the understanding of the normal sequence of events preceding implantation (for review see [3]) and of the consequences on pregnancy of a short-lived Pw [4]. Considerable efforts were also invested in the identification of luteolytic agents which, by creating a regulatory imbalance, could prevent implantation. However, the hope that PGs could be developed into luteolytic drugs for therapeutic use in patients has greatly diminished. It was shown in the human [5] and the rat [6] that the primary post-conceptional target of PGs is the *conceptus*, rather

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than the corpus luteum, and that their luteolytic action is indirect, mediated by the disruption of the endocrine function of this product of conception. During the luteal phase of the cycle in patients [7] and the pre-implantation period in the rat [6] PGs exerted no luteolytic action. Indeed, even the reduction of biologically available P during its transport from the corpus luteum to the uterus, by treatment with antiP (AP) antiserum which binds P with immunological specificity, was less effective in the rat during early pregnancy than midpregnancy [8]. These findings fully exposed the difficulties in provoking biologically effective Pw during the pre-implantation stage of pregnancy.

A possible approach to fertility control has been exposed recently by the observation that the synthetic steroids: andrastano [2,4-d]-Isoxazols induce Pw and terminate pregnancy (for review see [9]). By inhibiting the enzyme 3β-hydroxysteroid dehydrogenase, these APs reduce the conversion of pregnenolone to P. A single oral dose of Isoxazol [(17β)-4,4,17-trimethylandrosta-2,5-dieno [2,3-d]-isoxazol-17-ol] predictably reduced P and increased estradiol (E_2) and PGF levels in the uterine tissue of 10-day pregnant rats and terminated pregnancy [9]. Qualitatively the same regulatory imbalance, provoked by this steroidal AP at day 19 of pregnancy, also induced pre-term labor [10]. These changes in regulatory profile at days 10 and 19 are similar to those which precede the spontaneous onset of labor in this species [10], as well as in others such as the sheep and the goat [11]. Since these actions of Isoxazol were readily prevented by P treatment, the specificity of action (at the dose levels used) has been demonstrated [9, 10]. Encouraged by these findings, the studies with Isoxazol in the rat model have been now extended to the pre-implantation period. The present article describes the results of these experiments.

EXPERIMENTAL

The conduct of the studies was the same as before [9, 10], except that the biological action of a single oral dose of Isoxazol was examined during the *pre-* rather than the post-implantation period. A total of 230 Sprague–Dawley (Holtzman) rats were selected when sperm positive at day 0. They were kept in individual cages at constant temperature (22°C), exposed to 14 h of light and 10 h of darkness and were fed a Purina diet. Three studies were performed as described in Tables 1 and 2 and Figs 1 and 2.

In the first study 149 rats were used. Of these 138 were treated before implantation at days 0, 3 or 4 with a single oral dose of vehicle (1% aqueous gum tragacanth), or Isoxazol (Table 1) and 11 animals were given Isoxazol after implantation at day 10. In this first study the ovaries, the uterus and the conceptuses were examined once only at autopsy, performed at days 7, 14 or 21 of pregnancy. This protocol has been used, since in the rat the trophoblastic invasion

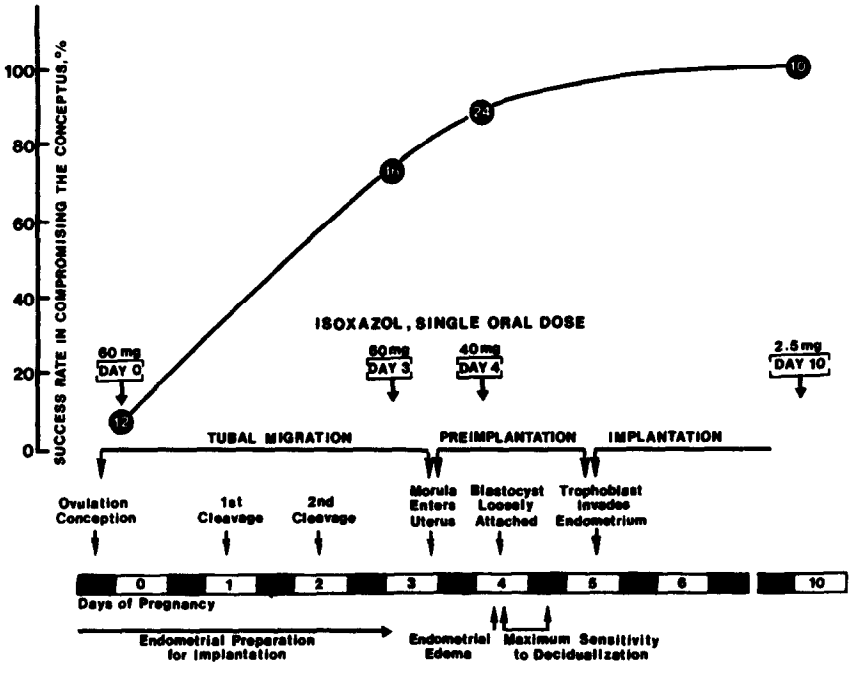

Fig. 1. The antifertility effect of Isoxazol during the pre-implantation period. The sequence of events preceding implantation are illustrated schematically, according to Psychoyos[3]. Superimposed on this schema is the treatment schedule of the present study and the success rate in compromising the conceptuses. Each arrow represents a single oral treatment at different times and dose levels. Repeated doses were not applied in the present study. The conceptus was considered compromised when at day 14 it only left behind an empty implantation site or a resorbing mass. For detailed description see text.

of the endometrium begins at day 5 of pregnancy [3] and the shift in the luteotrophic support of the corpus luteum, from the pituitary to the conceptus, occurs at day 10 of pregnancy [11, 12].

On completion of this study it became apparent, however, that in comparison with the controls the number of "non-pregnant" animals in the Isoxazol group was excessive when treatment was effective. This diagnosis of "non-pregnancy" was based on failure to identify living or resorbing conceptuses, or empty implantation sites in the uteri of these animals. At autopsy it could not be determined with certainty whether these rats with empty uteri had failed to conceive, or conceived but failed to implant. The rare incidence of non-pregnancy in the controls suggested that the second alternative is the likely one. Nevertheless, as a measure of caution in calculating the success of Isoxazol treatment these animals with empty uteri were not included. Thus, the "success rate" was calculated as the percentage of total implantation sites being empty or occupied by resorbing or dead conceptuses. The completely empty uteri, which would have had about 10 empty implantation sites each, had conception left behind visible marks, were ignored in the calculation of "success rate". However, the number of these animals with empty uteri are shown in Table 1, to indicate that the success rate might have been considerably higher than calculated.

In order to clarify this uncertainty in the biological meaning of empty uteri in the Isoxazol-treated rats, a second study was performed using 30 rats mated on the *same* day (Table 2). At day 3, 8 animals were treated with 100 mg and at day 4 a group of 10 animals were given 40 mg Isoxazol while 12 received 40 mg Isoxazol + 5 mg progesterone [(P) i.m. in oil]. The Isoxazol + P rats were included in the study, both to re-examine the earlier findings that P prevents the post-conceptional action of Isoxazol [9, 10] and to determine the incidence of conception versus implantation failure in this group of 30 rats. Since the groups were formed by random selection at days 3 and 4, when it is impossible to determine whether or not the animal had conceived, the 12 rats protected by P truly reflect the frequency of conception in all the 30 animals. In addition in this second study, the uteri of all animals were examined during laparotomy on day 7, then at autopsy on day 14, to compare in the 3 groups the number and size of their implantation swellings and thus further substantiate the premise that the frequent incidence of empty uteri in the Isoxazol rats (Table 1) represents implantation rather than conception failures.

In the third study 28 rats were used (Fig. 2). In this experiment 6 rats were treated with 100 mg Isoxazol at day 3 and 4 rats with 40 mg Isoxazol at day 4 and 18 animals served as controls. In the Isoxazol rats 1 ml tail blood was collected before treatment and subsequently at 6, 12, 24, 48 and 96 h after treatment, to determine the Isoxazol-induced changes in the circulating plasma P levels by radioimmunoassay (RIA). In the 18 controls blood was collected before and at 48 and 96 h after vehicle treatment. The techniques used for the collection and processing of blood samples and for RIA have been described earlier [9]. This study was complemented by another experiment in which tail blood was collected at day 14 from 6 rats after Isoxazol treatment at day 4. Furthermore, 12 controls were treated with vehicle and 5 rats with 40 mg Isoxazol at day 4 and autopsied at day 10 to collect uterine tissue for the measurements of P, E_2 and PGF by RIAs, as described earlier [9]. The purpose of these experiments was a comparison of the action of Isoxazol on the regulatory balance of pregnancy during the pre-implantation and post implantation periods [9, 10]. The data collected were tabulated and subjected to statistical analysis, using Student's *t*-test.

RESULTS

Figure 1 illustrates the treatment schedule of the first study in relation to the migrating morula and implanting blastocist. Table 1 describes the litter size and treatment of the various groups of animals, the success rate of post-conceptional Isoxazol treatment and the weight of living and resorbing conceptuses at different days of autopsy. In a total of 22 vehicle controls (Table 1) the resorption rates were low, only 5% at day 14 when determination is easiest. Since a 2–8% resorption rate is a frequent finding in a group of normal pregnant (untreated) rats, a "success rate" of this magnitude in the present study in the Isoxazol animals was considered a negative finding. The mean weights of the living conceptuses (fetus + placenta) in the normal controls describe the character of normal intrauterine growth from days 7 through 14 to 21 of pregnancy.

Table 1 shows that post-implantational treatment at day 10 with a single oral dose of only 2.5 mg Isoxazol resulted in the resorption of all conceptuses. Due to this 100% "success rate", no living conceptus was found at day 14 and the resorbing conceptuses had 29 ± 2 mg, rather than the normal 418 ± 3 mg, weights ($P < 0.001$). This degree of resorption at day 14 suggests that the conceptuses were compromised within a few hours after Isoxazol treatment at day 10. This conception is consistent with the earlier observation [9] that the post-conceptional action of Isoxazol is prevented with a single dose of 5 mg P, when given within 6 h after the AP but not if delayed until after 6 h.

In sharp contrast to the 100% efficacy of 2.5 mg Isoxazol at day 10, a single oral dose of 20 mg Isoxazol at day 4 [when the blastocists are only loosely attached to the endometrium (Fig. 1)] only achieved 51% success rate in compromising the conceptuses. Furthermore, the weight of the resorbing conceptuses at day 14 was high (46 ± 7 mg), demonstrating that despite this early treatment, the blastocists still implanted and developed to some degree before resorp-

Table 1. The success rate in the Isoxazol-induced compromise of the conceptus during the pre-implantation period in the rat

(*n*) Pregnant (non-pregnant)	Litter size	Dose of Isoxazol (mg)	autopsy	Success rate (%)	Conceptus weight (mg)	
					Dead	Alive
			Vehicle at days 0, 3 or 4			
4(+0)	10 ± 1	Vehicle	7	?	?	14 ± 1
12(+1)	10 ± 1	Vehicle	14	5	59 ± 6	418 ± 3
6(+1)	9 ± 1	Vehicle	21	4	25 ± 8	6459 ± 22
			Isoxazol at day 10			
10(+1)	10 ± 1	2.5	14	100	29 ± 2	None
			Isoxazol at day 4			
6(+1)	10 ± 2	20	14	51	46 ± 7	358 ± 6***
20(+7)	9 ± 1	40	14	86	18 ± 2	402 ± 7*
4(+3)	8 ± 3	40	21	88	10 ± 1	6310 ± 90
			Isoxazol at day 3			
15(+2)	11 ± 1	20	7	?	?	13 ± 1
5(+1)	9 ± 1	40	7	?	?	8 ± 1***
4(+0)	11 ± 1	40	14	45	100 ± 14	328 ± 7***
13(+12)	9 ± 1	60	14	71	85 ± 9	198 ± 7***
3(+5)	8 ± 1	60	21	73	46 ± 36	4584 ± 84***
			Isoxazol at day 0			
12(+3)	11 ± 1	60	21	6	17 ± 7	6250 ± 51

All values are means ± S.E. Numbers in ( ) are the number of animals with empty uteri at autopsy, diagnosed "non-pregnant". The symbol ? denotes uncertainty about success rate at day 7, because the uncertainty regarding the compromise of the conceptus. Numbers marked by * are significantly different from the control values at the levels of *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$. The weight of dead conceptuses is significantly different ($P < 0.001$) from the control values. For description see text.

tion. Increasing the Isoxazol dose from 20 to 40 mg increased the succcess rate to 86% (at day 14) and 88% (at day 21) and significantly reduced the weight of the surviving conceptuses. At 40 rather than the 20 mg dose level the weight of the resorbing conceptuses was significantly less (18 ± 2 mg, at day 14), indicating that the greater Isoxazol dose shortened the time needed to compromise the conceptus. However, this 40 mg dose, administered *before* implantation (at day 4), is 16 times greater than the 2.5 mg dose which achieved 100% success rate *after* implantation (at day 10).

At day 3 of pregnancy [when having completed tubal migration the morula just enters the uterine cavity (Fig. 1)] the efficacy of Isoxazol was further reduced. A single oral dose of 20 mg of this AP, which achieved 51% success rate at day 4, seemed ineffective, as indicated by the similar conceptus weights at day 7 of the Isoxazol (13 ± 1 mg) and control (14 ± 1 mg) rats. The increase of the Isoxazol dose to 40 mg, however, had an apparent effect, indicated at day 7 by the significant difference in conceptus weight of the control (14 ± 1 mg) and Isoxazol (8 ± 1 mg) rats. Since this effect could be explained by either delayed implantation or growth retardation, the uncertainty has been clarified in another group of rats by delaying autopsy to day 14. In this instance the 40 mg dose at day 3 achieved 45% success rate, which was definitely established at day 14 by the difference in the conceptus weights of the control and Isoxazol rats. As before (day 4 treatment) the weights of the compromised conceptuses (100 ± 14 mg) showed that implantation did occur, but development was arrested in the resorbing and suppressed in the salvaged conceptuses (328 ± 7 mg). A further increase of the Isoxazol dose to 60 mg increased the success rate to 71% and further reduced the weights (at day 14) of both the resorbing (85 ± 9 mg) and living (198 ± 7 mg) conceptuses. Apparently, while higher doses of Isoxazol were needed for compromising the conceptus at day 3 (when the morula just enters the uterine cavity) than at day 4 (when the blastocist is already loosely attached to the endometrium) the success rate at high level treatments was similar, specifically if the empty uteri are considered as representing implantation, rather than conception, failures. The highly significant differences in weight of the living conceptuses in the Isoxazol and control rats at day 14 (198 ± 7 and 418 ± 3 mg) and at day 21 (4584 ± 84 and 6459 ± 22 mg) remain to be explained by delayed implantation or growth retardation. The present experiments offer no choice between these two alternatives.

The distinct antifertility action of Isoxazol at day 3 or 4 could not be reproduced even with 60 mg Isoxazol at day 0, when tubal migration of the fertilized ovum just begins (Fig. 1). Apparently, whether the fertilized ovum is still migrating or it already entered the uterine cavity and whether its attachment to the

Table 2. The preventive action of progesterone on the post-conceptional action of Isoxazol and the estimation of conception rather than implantation failure

(*n*) Pregnant (non-pregnant)	Litter size	Dose of Isoxazol (mg)	Day of autopsy	Success rate (%)	Conceptus weight (mg)	
					Dead	Alive
			Isoxazol + P at day 4			
11(+1)	12 ± 1	40	14	8	94 ± 10	402 ± 3
			Isoxazol at day 4			
9(+1)	10 ± 1	40	14	86	15 ± 2	417 ± 10
			Isoxazol at day 3			
5(+3)	5 ± 1	100	14	96	15 ± 3	250 ± 0

All values are means ± S.E. The mating date of all animals is the same and the grouping of the animals is random. Numbers in ( ) are the number of animals with empty uteri at autopsy. Dose of progesterone (P) = 5 mg, i.m. For description see text.

endometrium is loose or invasive, critically alters the efficacy of treatment. However, the post-conceptional efficacy of Isoxazol between days 0 and 3 at higher dose levels deserves extensive study, to further explore the therapeutic potential of this AP.

Since the biological meaning of empty uteri (without implantation sites) remained uncertain (Table 1), an additional study was conducted in 30 rats of identical mating date. The purpose of this study was to clarify whether in addition to compromising the conceptus and thus effect its normal implantation, Isoxazol can *prevent* implantation leaving behind empty uteri. This study was expected to exclude the alternative that the empty uteri represent conception rather than implantation failures. Table 2 shows that 11 of the 12 animals, treated at day 4 with 40 mg Isoxazol + 5 mg P, were pregnant at day 14, with normal conceptus weights of 402 ± 3 mg and an 8% resorption rate. This finding illustrates that when P treatment prevents the Isoxazol action, conception, implantation and "success rate" are the same as in the vehicle controls (Table 1). Treatment with Isoxazol resulted in similar conception and implantation rates, but a high success rate (86%) in the compromise of the conceptuses. Despite this high success rate in the antifertility action of Isoxazol, however, the 14% surviving conceptuses had normal weight (417 ± 10 mg). This observation confirms the earlier finding [9] that even if only 1 conceptus survives in the whole litter, the growth of that single conceptus can be normal.

Of special importance here is the finding that the 8 rats which received 100 mg Isoxazol at day 3 had an excessive incidence (40%) of empty uteri (without implantation marks), in contrast to the 22 remaining rats which had only 10% empty uteri. This finding strongly suggests that the 3 rats in the group of 8 animals did conceive but failed to implant. This contention is further supported by an additional observation. At day 7, when the uteri of all 30 animals were examined, the number of implantation swellings in the day 4 rats was the same as at autopsy (day 14), while in the day 3 rats the number was higher than at day 14, although the swellings were considerably smaller). Apparently, some of the blastocists in the day 3 rats failed to implant. This conclusion is supported by the finding that litter size in the day 3 animals was only 5 ± 1, a definite implantation failure when compared to 12 ± 1 and 10 ± 1 in the day 4 animals (Table 2). In view of these results it would appear that the success rate described in Table 1 for effective Isoxazol treatment was actually higher than indicated, because several animals (with empty uteri) failed in implantation but not in conception. Table 2 illustrates that Isoxazol can both interfere with normal implantation and *prevent* implantation.

Figure 2 illustrates that in the present preimplantation study as in the earlier post-implantation studies

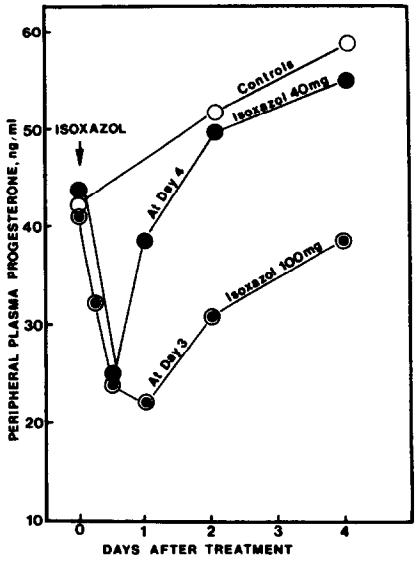

Fig. 2. The effect of Isoxazol on peripheral plasma progesterone levels. Tail vein blood was sequentially collected during 4 days in 2 groups of rats, after Isoxazol treatment at day 3 (n = 6) and 4 (n = 4) of pregnancy, while 18 rats served as controls. In the collected plasmas progesterone (P) was measured by radioimmunoassay. While the P levels increased in the controls ( ) there was a significant but transient decrease in the Isoxazol rats. For detailed description see text.

Table 3. The effect of Isoxazol on the uterine tissue levels of progesterone, prostaglandin F and oestradiol

	Progesterone (ng/g)	Prostaglandin F (ng/g)	Oestradiol (pg/g)
Vehicle control (*n* 12)	82 ± 4	94 ± 7	288 ± 15
Isoxazol 40 mg (*n* 5)	85 ± 11	105 ± 14	283 ± 41

All values are means ± S.E. Treatment at day 4, autopsy at day 10 of pregnancy. For further description see text.

[9, 10] the circulating plasma P levels were significantly ($P < 0.001$) reduced at 12 h after Isoxazol treatment on days 3 or 4. A single oral dose of 100 mg Isoxazol suppressed P levels for 24 h, while 40 mg was effective only over 12 h. However, while the initial Pw was similar, regardless of whether Isoxazol was administered before (Fig. 2) or after implantation [9, 10], the regulatory imbalances generated by Pw were different. To appreciate this difference it has to be recalled that in the rat the trophic support of luteal P-synthesis shifts at day 10 of pregnancy from the pituitary to the conceptus [11, 12]. Accordingly, after implantation [9, 10] Isoxazol irreversibly reduced P levels because by compromising the conceptuses it intercepted their luteotrophic support. In contrast, before implantation (at day 3 or 4, Fig. 2) Isoxazol only provoked reversible Pw because, despite its compromise of the conceptuses, it did not effect the luteotrophic support of the pituitary. However, the Isoxazol action on the conceptuses at day 4 manifested in the P levels at day 14, when the lack of luteotrophic support was revealed by the low (31 ± 4 ng/ml) P levels. Apparently, whether the Isoxazol-induced Pw is reversible or irreversible is determined by the timing of treatment in relation to the "pituitary–placental shift" in luteotrophic support. The finding that despite a short-lived Pw (Fig. 2) 70–80% of the conceptuses were compromised (Table 1), indicates that the conceptus is exceedingly sensitive to Pw of even a few hours' duration and that recovery from Pw does not modify its compromising action.

In the earlier post-implantation studies [9, 10] the Isoxazol-induced irreversible Pw provoked increases in the levels of both E_2 and PGF in the uterine tissue. In contrast, in the present pre-implantation study (Table 3) the transient Pw left behind no regulatory imbalance of this type. This finding indicates that a Pw of critical degree is sufficient to compromise the conceptus even if it only lasts for 12 h (Fig. 2) and the regulatory imbalance in the uterine tissue provoked by lasting Pw is aimed at the evacuation of the uterine contents.

DISCUSSION

Our studies have shown that Isoxazol is an effective post-conceptional agent in the rat not only during the post-implantation period [9, 10], but also during the pre-implantation period. However, in contrast to its efficacy at day 10 of pregnancy, when a single oral dose of 2.5 mg Isoxazol had 100% success rate in irreversibly compromising the conceptuses [9], at day 4 only 86% (uncorrected) success rate has been achieved by 40 mg Isoxazol, at day 3 only 71% by 60 mg Isoxazol, and at day 0 the 60 mg dose was ineffective (Table 1). If these success rates are adjusted, by considering that each empty uterus in a given group of rats represents about 10 blastocists which failed to implant and therefore left no implantation marks, the corrected success rate closely approximates 100%, but the differences in the effective dose levels of Isoxazol, between the pre- and post-implantation periods, remain. The 100 mg Isoxazol dose at day 3 achieved 96% uncorrected success rate and the frequent incidence of empty uteri and the reduced number of implantation sites clearly revealed that Isoxazol at day 3 not only provokes the resorption of the conceptuses but also *prevents* the implantation of most blastocists. Indeed, in this group of rats (Table 2), out of an estimated number of 80 conceptuses, only one single conceptus survived between day 3 and 14 with a drastically reduced weight.

The mechanism of Isoxazol action was found to be the same at days 3 and 4 as at day 10 [9]. Within 12 h after treatment, Pw in the Isoxazol rats reached about 50%, followed by a rapid recovery in the case of the 40 mg and a slower recovery in the case of the 100 mg dose (Fig. 2). In contrast, the controls showed increasing P levels during the study period. However, there was a significant difference in the recovery of P levels between the post- and pre-implantation treatment groups. At day 10, when the conceptus provides luteographic support, Isoxazol treatment and the Pw induced compromise of the conceptuses suspended this signal and thus there was no recovery in P-synthesis and P levels [9]. In contrast, at days 3 and 4, when the pituitary provides the luteotrophic stimulus, the Isoxazol-induced compromise of the conceptuses did not affect luteotrophic support and thus the P levels recovered soon after the rapid Pw. It was most probably this difference in luteotrophic support and in the recovery of P levels which prevented in the days 3 and 4 animals the Pw-induced oestradiol and prostaglandin F surges, previously observed in the uterine tissue of the day 10 animals [9]. The contention that recovery from Pw of the pre-implantation rats was due to undisturbed pituitary support of luteal P-synthesis was verified by the observation that this recovery was transient and

after day 10 the P levels again decreased if the conceptuses were compromised.

As expected, a significant but only short-lived Pw, when provoked by Isoxazol during the pre-implantation days of 3 and 4 of pregnancy, prevents implantation or intercepts its normal progress and interferes with the normal development of the blastocists. This finding exposes the therapeutic potential of APs in preventing or interfering with the normal progress of implantation. However, since the endocrine conditions are constantly changing during the pre-implantation period as does the environment of the migrating morula and blastocist, only more extensive work can determine the ideal timing and formulation of effective treatment. Thus, further work in animal models is needed in preparation for clinical trials, to clarify the potential of APs in fertility control.

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REFERENCES

1. Corner G. W. and Alleff W. M.: Physiology of corpus luteum—VII. Maintenance of pregnancy in rabbit after very early castration, by corpus luteum extracts. *Proc. Soc. Exp. Biol. Med.* **27** (1930) 403–405.
2. Csapo A. I. and Pulkkinen M.: Indispensability of the human corpus luteum in the maintenance of early pregnancy. Luteoctomy evidence. *Obstet. Gynecol. Surv.* **33** (1978) 69–81.
3. Psychoyos A.: Hormonal control of ovoimplantation. *Vitams Horm.* **31** (1973) 201.
4. Dickman Z.: Implantation in the rat; progesterone priming requirements. *J. Endocr.* **53** (1972) 327.
5. Csapo A. I.: "Prostaglandinimpact" for menstrual induction. *Population Report, Series G* 4 (1974).
6. Csapo A. I.: Work in progress. Pregnancy termination in the rat "model" by a synthetic prostaglandin analogue ICI 81008. *Prostaglandins* **7** (1974) 141–148.
7. Wentz A. C.: Menstrual regulation and interception of pregnancy. In *The Prostaglandins, Brook Lodge Symposium* (Edited by E. M. Southern). Futura, New York (1972) pp. 505–510.
8. Csapo A. I. and Erdos T.: The critical control of progesterone levels and pregnancy by anti-progesterone. *Am. J. Obstet. Gynecol.* **126** (1976) 589–601.
9. Csapo A. I., Resch B. A., Csapo E. F. and Salau G.: Anti-progesterone effects on pregnancy—Part I. Mid-pregnancy. *Am. J. Obstet. Gynecol.*, **133** (1979) 176.
10. Csapo A. I. and Resch B. A.: Induction of preterm labor in the rat by antiprogesterone. *Am. J. Obstet. Gynacol.*, in press.
11. Takayama M. and Greenwald G. S.: Direct luteotropic action of estrogen in the hypophysectomized-hysterectomized rat. *Endocrinology* **92** (1973) 1405.
12. Pepe G. J. and Rothchild I.: A comparative study of serum progesterone levels in pregnancy and in various types of pseudopregnancy in the rat. *Endocrinology* **95** (1974) 275–279.
13. Warnock D. H. and Csapo A. I.: Progesterone withdrawal induced by ICI 81009 in pregnant rats. *Prostaglandins* **10** (1975) 715.