METABOLISM OF [U-¹⁴C]GLUCOSE IN THE RAT UTERUS IN THE PRESENCE OF AN INTRA-UTERINE CONTRACEPTIVE DEVICE

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Summary. This paper reports studies on the incorporation of [U-¹⁴C]-glucose into lipid, RNA and proteins of the rat uterus in the presence of an IUD after treatment with oestrogen and progesterone and on Day 5 of pregnancy at the time of implantation. The results show that there is an increase in the incorporation of [U-¹⁴C]glucose into lipid, RNA and proteins in the IUD horn compared with that of the control horn of ovariectomized and oestrogen-treated animals. The increase in weight of the IUD horn compared with the control horn can be interpreted as due to an increased oestrogen-like response by the uterus in the presence of an IUD. This response may be caused by oestrogens, histamines or other amines.

A significant decrease in the incorporation of [U-¹⁴C]glucose into lipid, RNA and proteins of the IUD horn compared with the control horn was found on treatment of the animals with oestrogen+progesterone and progesterone alone for 6 hr. Treatment of the animals for 9 days with oestrogen+progesterone also significantly depressed the rate of protein synthesis in the IUD horn compared with the control horn. On Day 5 of pregnancy at the time of implantation, there was a statistically significant decrease in the incorporation of [U-¹⁴C]glucose into lipid, RNA and proteins of the IUD horn compared with the control horn. These experiments indicated an increased sensitivity of the uterus to progesterone in the presence of an IUD.

The uterus shows an increased oestrogen-like response in the presence of an IUD. The increased sensitivity of the uterus to progesterone at the time of implantation produces asynchronous changes in the uterus and results in an anti-implantation effect of the IUD. The mode of action of an IUD in the light of these observations is discussed.

INTRODUCTION

Intra-uterine contraceptive devices (IUDs) are being extensively used for fertility control, but there are a large number of unsolved problems associated with the use of these devices. Knowledge of their mode of action would aid considerably in overcoming some of the side effects associated with their use

and also give information about the optimum time for insertion. Investigations on the mode of action of IUDs have revealed that these devices may be acting by different mechanisms in different species and, since the material from which these devices are made varies, this may also affect their mode of action. In general, it appears that an IUD prevents conception by disturbing the implantation processes in some manner in women (Jessen, Lane & Greene, 1963), rats (Doyle & Margolis, 1963), rabbits (Adams & Eckstein, 1965) and cows (Hawk, Conley, Brinsfield & Righter, 1965). There is a lack of information on the biochemical changes produced by IUDs. Kar, Goswami, Kamboj & Chowdhury (1964) found that IUDs altered the biochemical composition of the luminal fluid and inferred that this might cause destruction of the pre-implantation blastocyst. Kar, Kamboj, Goswami & Chowdhury (1965) also found that the oxygen uptake rate was doubled in the IUD-treated horn, but other biochemical constituents did not undergo any noteworthy change.

This paper reports the metabolism of uniformly labelled [$U-^{14}C$]glucose in the rat uterus in the presence of an IUD and after treatment with oestrogen and progesterone. Glucose metabolism in the rat uterus on Day 5 of pregnancy at the time of implantation in the presence of an IUD has also been studied. Preliminary reports have been presented (Yadava & Laumas, 1968; Laumas, 1969).

MATERIALS AND METHODS

Animals

Three-month-old adult female rats of the Institute animal house were used in this study and were kept on the usual diet with unrestricted access to water. The animals were ovariectomized and a surgical silk suture was inserted through the anti-mesometrial wall of the right uterine horn according to the procedure of Doyle & Margolis (1963). In another group, the silk suture was inserted in intact animals. Experiments were begun 4 to 6 weeks after insertion of the suture.

Six-hour hormone treatment

Forty female ovariectomized animals with a silk suture inserted in one of the horns were divided into four groups. Group I (C group) animals were used as controls and received no treatment. Group II (E group) animals were injected with 5 μ g oestradiol-17 β in 0.1 ml propylene glycol. Group III (E+P group) animals received 5 μ g oestradiol-17 β in 0.1 ml propylene glycol and another injection of 2.0 mg progesterone in 0.2 ml propylene glycol. Group IV (P group) animals were injected with 2.0 mg progesterone in 0.2 ml propylene glycol. All injections were given intraperitoneally. Six hours after the hormone injections, the animals were killed by cervical dislocation. The control horn and IUD horn were freed from adhering fat, weighed and incubated separately with [$U-^{14}C$]glucose as mentioned under the incubation procedure.

Nine-day hormone treatment

Two groups of ovariectomized rats with an IUD in one horn were used in this

experiment. Group I animals were injected with 1.0 µg oestrone/0.1 ml olive oil daily for 9 days. Groups II animals were injected with 1.0 µg oestrone/0.1 ml olive oil daily for 9 days, but from Day 6 to Day 9, these animals were also injected with 3.0 mg progesterone/0.1 ml olive oil. All injections were given subcutaneously and the animals were killed 24 hr after the last injection. Control and IUD-containing uteri were removed and incubated with [U-¹⁴C]glucose as described below.

Experiment on Day 5 of pregnancy

Intact adult female rats with a silk suture in one horn were used. The animals were mated with males of proven fertility 15 days after insertion of the silk suture. The day on which spermatozoa were found in vaginal smears was designated Day 1 of pregnancy. The animals were killed on the morning of Day 5 of pregnancy at 10.00 hours and the control and IUD horns were separately incubated with [U-¹⁴C]glucose.

Incubation procedure

Each horn used for incubation was cut into three pieces. Control and IUD horns from each animal were incubated separately in 2.0 ml Robinson's medium (Umbreit, Burris & Stauffer, 1959) containing 1.0 µCi [U-¹⁴C]glucose (specific activity 22 mCi/m-mole), at 37° C for 2 hr in the presence of carbogen gas (95% O₂ and 5% CO₂). After the termination of incubation, the tissues were removed and twice washed with cold Robinson's medium. The washed tissues were homogenized in 2.0 ml of 5% perchloric acid.

Separation of lipids, RNA, DNA and proteins

The homogenate was centrifuged and the supernatant removed. The residue was twice washed with 0.6 N-perchloric acid in order to remove acid soluble nucleotides. The residue left after the removal of acid-soluble nucleotides was extracted once with absolute alcohol, once with chloroform-ether mixture (2:1), and twice with dry ether. Each extraction was carried out with 2.0 ml of the solvents. The solvent extracts were pooled and constituted the lipid fraction.

The residue left after the removal of lipids was hydrolysed with 3.0 ml of 0.2 N-sodium hydroxide for 16 hr at 37° C (Scott, Fraccastoro & Taft, 1956). The reaction was stopped with 1.0 ml of cold 0.6 N-perchloric acid. After centrifugation, the supernatant was collected and RNA estimated spectrophotometrically by reading the optical density at 260 mµ. The residue left after the removal of RNA was prepared for DNA estimation by hydrolysing the residue with 3.0 ml of 1.6 N-perchloric acid at 70° C for 10 min. The DNA in the hydrolysate was estimated by the diphenylamine reaction (Burton, 1956). The final residue obtained after removing DNA contained proteins and these were estimated by Lowry's method (Lowry, Rosenbrough, Farr & Randall, 1951).

Counting procedure

The lipid fraction containing total lipids was reduced to about 0.5 ml under

nitrogen gas and 15 ml of diatol scintillation liquid (3.2 g PPO, 65.0 mg dimethyl POPOP, 52 g naphthalene dissolved in 150 ml methanol, 250 ml toluene and 250 ml of distilled dioxane) was added. The samples were counted in a liquid scintillation counter (Model 3314, Packard Instrument Company, Downers Grove, Illinois, U.S.A.). Aliquots (0.6 ml) for RNA were taken up in 15 ml of diatol scintillation liquid and counted as above. The protein residues were dissolved in 0.5 ml hyamine hydroxide (Packard Instrument Company) at 55° C overnight and 10 ml of scintillation liquid (4 g PPO, 100 mg dimethyl POPOP in 1 litre of re-distilled toluene) were added. Sample counts were carried out in a liquid scintillation counter. The lipid, RNA and protein samples counted as above were subjected to quenching correction with the use of an internal ^{14}C standard.

TABLE 1
WET WEIGHT (MG/100 G BODY WT) OF RAT UTERUS IN THE PRESENCE OF
AN IUD (6 HR HORMONE TREATMENT)*

Group	Control horn (C) (Means $\pm$ S.E.)	IUD horn (T) (Means $\pm$ S.E.)	P (C versus T horn)
1. C (8)†	17.2 $\pm$ 0.09	34.9 $\pm$ 1.7	< 0.001
2. E (9)	18.7 $\pm$ 0.09	32.4 $\pm$ 2.3	< 0.01
3. E + P (8)	18.3 $\pm$ 0.07	32.6 $\pm$ 1.8	< 0.01
4. P (8)	14.3 $\pm$ 0.06	24.6 $\pm$ 1.1	< 0.01
<i>Statistical analysis</i>			
C versus E group	P < 0.01	P > 0.5	
C versus E + P group	P < 0.01	P > 0.6	
C versus P group	P < 0.001	P < 0.001	
E versus E + P group	P > 0.7	P > 0.8	

* Ovariectomized animals with an IUD in one horn belonging to the C group received no treatment. E group animals received 5 μg oestradiol-17 β in 0.1 ml propylene glycol. E + P group animals were given 5 μg of oestradiol-17 β /0.1 ml propylene glycol along with 2.0 mg progesterone/0.2 ml propylene glycol. P group animals received only 2.0 mg progesterone in 0.2 ml propylene glycol. All animals were killed 6 hr after injections and the control horn (C) and IUD horn (T) incubated separately as described in the text.

The control and IUD horns in the same group were compared by applying Student's *t* test to the difference of the control and IUD horn. The *t* test was also applied to compare the means of two different groups of animals.

† Number of animals shown in parentheses.

RESULTS

The results of experiments involving 6-hr hormone treatment on the response of rat uterus to exogenous oestrogen and progesterone in the presence of an IUD are presented in Tables 1 to 4.

Wet weight of the uterus

Table 1 gives the wet weight of rat uterus in the presence of an IUD. The weight of the IUD horn is significantly higher in the C, E, E + P and P group animals compared with the corresponding control horn. The increase in weight of the IUD horn was nearly of the same proportion in all the IUD-treated groups. When between-group weights were compared, there was no significant

TABLE 2
INCORPORATION OF [U-¹⁴C]GLUCOSE INTO RAT UTERINE LIPIDS IN THE
PRESENCE OF AN IUD (6 HR HORMONE TREATMENT)*

Group	Control horn (C) (Means $\pm$ S.E.)	IUD horn (T) (Means $\pm$ S.E.)	P (C versus T horn)
C (8)	6265 $\pm$ 2088	13013 $\pm$ 2212	< 0.05
E (9)	18828 $\pm$ 3290	23791 $\pm$ 4338	< 0.02
E+P (8)	16262 $\pm$ 2998	8992 $\pm$ 1901	< 0.001
P (9)	16238 $\pm$ 2816	11764 $\pm$ 2828	< 0.001
<i>Statistical analysis</i>			
C versus E group	P < 0.01	P < 0.01	
C versus E+P group	P < 0.01	P < 0.05	
C versus P group	P < 0.01	P > 0.9	
E versus E+P group	P > 0.05	P < 0.001	

* Same as under Table 1.

Results expressed as disintegrations/min/mg RNA $\pm$ S.E.

difference except that the weight of the control uterus in the P group was significantly lower ($P < 0.001$) compared with that in the C group. In the case of the IUD-treated uterus also there was a depression in weight in the P group compared with the C group. However, the decrease in the weight of the IUD horn of P group of animals was more than the decrease noted in the control horn of the same group.

[U-¹⁴C]Glucose incorporation into lipids

An interesting feature of the effect of various hormone treatments on [U-¹⁴C]glucose incorporation was that they markedly enhanced or decreased lipid synthesis in the IUD horn compared with the control horn. There was a significant increase in the incorporation of [U-¹⁴C]glucose into lipids of the IUD horn compared with the control horn in the C group ($P < 0.05$) and E

TABLE 3
INCORPORATION OF [U-¹⁴C]GLUCOSE INTO RNA OF RAT UTERUS IN
PRESENCE OF IUD (6 HR HORMONE TREATMENT)*

Group	Control horn (C) (Means $\pm$ S.E.)	IUD horn (T) (Means $\pm$ S.E.)	P (C versus T horn)
C (8)	1362 $\pm$ 287	11224 $\pm$ 947	< 0.001
E (9)	8818 $\pm$ 994	14544 $\pm$ 1288	< 0.001
E+P (8)	7804 $\pm$ 1116	4766 $\pm$ 454	< 0.001
P (9)	7940 $\pm$ 842	5138 $\pm$ 454	< 0.001
<i>Statistical analysis</i>			
C versus E group	P < 0.001	P < 0.05	
C versus E+P group	P < 0.001	P < 0.001	
C versus P group	P < 0.001	P < 0.001	
E versus E+P group	P > 0.8	P < 0.001	

* Same as in Table 1.

Results expressed as disintegrations/min/mg RNA $\pm$ S.E.

group ($P < 0.02$). However, there was significant decrease in the incorporation of [$U-^{14}C$]glucose into lipids of the IUD-treated horn of the E+P ($P < 0.001$) and P ($P < 0.001$) group of animals. Six-hour oestrogen treatment of animals increased [$U-^{14}C$]glucose incorporation into lipids in the control and the IUD horn. E+P treatment of animals did not markedly decrease the incorporation of [$U-^{14}C$]glucose into lipids of the control horn while, in the case of the IUD horn, the incorporation became much less compared with the control horn.

[$U-^{14}C$]Glucose incorporation into RNA

The results of [$U-^{14}C$]glucose incorporation into RNA in the various groups of animals is given in Table 3. As in the case of lipids, there was an enhanced incorporation of [$U-^{14}C$]glucose into the IUD horn of the C and E group ($P < 0.001$) when compared with the corresponding control horns. There was a marked decrease in the incorporation of [$U-^{14}C$]glucose into RNA of the IUD horn of the E+P group ($P < 0.001$) and the P group ($P < 0.001$) when

TABLE 4
INCORPORATION OF [$U-^{14}C$]GLUCOSE INTO RAT UTERINE PROTEINS IN
THE PRESENCE OF AN IUD*

Group	Control horn (C) (Means $\pm$ S.E.)	IUD horn (T) (Means $\pm$ S.E.)	P (C versus T horn)
C (8)	52 $\pm$ 11.8	1046 $\pm$ 69.8	< 0.001
E (9)	952 $\pm$ 109	1994 $\pm$ 205	< 0.001
E+P (8)	1198 $\pm$ 241	464 $\pm$ 52.6	< 0.01
P (9)	1174 $\pm$ 237	383 $\pm$ 36.2	< 0.01
<i>Statistical analysis</i>			
C versus E group	$P < 0.001$	$P < 0.01$	
C versus E + P group	$P < 0.001$	$P < 0.01$	
C versus P group	$P < 0.01$	$P < 0.01$	
E versus E + P group	$P > 0.3$	$P < 0.001$	

Results expressed as disintegrations/min/mg protein $\pm$ S.E.

compared with the corresponding control horns. Between groups there was a slight decrease in the incorporation of [$U-^{14}C$]glucose into RNA of the control horn in the E + P group compared with the same horn in the E group. However, in the IUD horn of the E + P group there was a highly significant ($P < 0.001$) decrease in the incorporation of [$U-^{14}C$]glucose into RNA compared with the same horn in the E group. Progesterone treatment of animals increased [$U-^{14}C$]glucose incorporation into RNA in the control horn of P group of animals but in the case of IUD horn there was a significant decrease ($P < 0.001$) when compared with the same horns of the C group.

[$U-^{14}C$]glucose incorporation into proteins

As with lipid and RNA synthesis, there was a significant increase in the incorporation of [$U-^{14}C$]glucose into proteins of the IUD horn compared with the control horn of C group ($P < 0.001$) and E group ($P < 0.001$) of animals. However, in the case of E + P and P groups, there was a statistically significant

TABLE 5

INCORPORATION OF [$U\text{-}^{14}\text{C}$]GLUCOSE INTO UTERINE PROTEINS IN THE PRESENCE OF AN IUD (9 DAY HORMONE TREATMENT)*

Group	Uterus wt (mg/100 g body wt) C T	Proteins (d.p.m./mg protein) C T
E (11)† E+P (10)	57.3±7.5‡ 72.6±6.3 52.8±11.5 102.6±8.0	272±25 386±32 70±12 42±6.0
Statistical analysis C (group E) versus T (group E) C (group E+P) versus T (group E+P) E versus E+P	P<0.01 P<0.01 P>0.8 P<0.01	P<0.01 P<0.01 P<0.001 P<0.001

* Ovariectomized rats of E group were subcutaneously injected with 1.0 µg oestrone/0.1 ml olive oil daily for 9 days. The ovariectomized rats of the E+P group were similarly injected with 1.0 µg oestrone/0.1 olive oil daily for 9 days and 3.0 mg progesterone/0.1 ml olive oil daily from Day 6 to Day 9.

† Number of animals shown in parentheses.

‡ Mean±S.E.

§ d.p.m. = disintegrations/min.

decrease in the incorporation of [$U\text{-}^{14}\text{C}$]glucose into proteins of IUD horn compared with the control horn (Table 4). The incorporation of [$U\text{-}^{14}\text{C}$]glucose into proteins of the E+P group was markedly decreased ($P<0.01$) in the IUD horn compared with the incorporation in the IUD horn of the E group.

Incorporation of [$U\text{-}^{14}\text{C}$]glucose into proteins (9-day hormone treatment)

The results of this experiment are presented in Table 6. After prolonged treatment of the animals with oestrone for 9 days, the incorporation of [$U\text{-}^{14}\text{C}$]glucose into the IUD horn was significantly higher than in the control horn. Administration of 1.0 µg oestrone for 9 days along with 3.0 mg progesterone from Day 5 to Day 9, depressed the rate of protein synthesis from [$U\text{-}^{14}\text{C}$]glucose in both the control and IUD horns. A significantly greater depression of protein synthesis was noted in the IUD horn (89.1%) compared with that in the control horn (74.3%).

TABLE 6

IN VITRO INCORPORATION OF [$U\text{-}^{14}\text{C}$]GLUCOSE INTO RAT UTERINE LIPID, RNA AND PROTEINS ON 5TH DAY OF PREGNANCY AT 10.00 HOURS

Group	No. of animals	Uterus wt (mg/100 g body wt)	Lipid (d.p.m./mg DNA)	RNA (d.p.m./mg RNA)	Proteins (d.p.m./mg protein)
Control horn (C)	7	74±6.1	58801±3176	4824±1312	129.7±15.8
IUD horn (T)	7	123±5.2	27245±2546	2748±545	47.2±8.6
Statistical analysis Control versus IUD horn		P<0.01	P<0.001	P<0.001	P<0.01

Incorporation of [U-¹⁴C]glucose into lipids, RNA and proteins on Day 5 of pregnancy

The results of these experiments are given in Table 6. The weight of the IUD horn was significantly higher ($P < 0.01$) than the control horn, the increase being 66.2%. The incorporation of [U-¹⁴C]glucose into lipids, RNA and proteins in the IUD horn was 46.3%, 57.0% and 36.4%, respectively, of the incorporation that occurred in the control horn.

DISCUSSION

The present results show that [U-¹⁴C]glucose metabolism is markedly altered in the presence of an IUD. An increase in the weight of the IUD horn was observed in ovariectomized animals and after oestrogen treatment. Increase in weight of the uterus in the presence of an IUD has also been noted by others (Kar *et al.*, 1964; Parr & Segal, 1966). Parr & Segal (1966) showed that there is uniform growth of the uterus in the IUD horn. It has been suggested that the growth of the IUD horn is due to inflammation (Greenwald, 1965), but this is unlikely since the increase in weight of the IUD horn cannot be prevented by hydrocortisone (Parr & Segal, 1966). The present investigation shows that there is an increased synthesis of cellular constituents like lipids, RNA and proteins in the IUD horn compared with the control horn and accounts for the greater weight of the IUD horn. This is not only observed in ovariectomized animals but also in ovariectomized animals treated with oestrogen and on Day 5 of pregnancy. The effect of oestrogens in increasing the weight of the uterus is well known. Nicolette & Gorski (1964) found that oestradiol increases [U-¹⁴C]-glucose metabolism and its incorporation into lipids, RNA and proteins of the rat uterus. Aziawa & Mueller (1961) reported that oestradiol increased phospholipid synthesis in the rat uterus and this is a sensitive indicator of oestrogen action. The present finding of an increased synthesis of lipids, RNA and proteins in the IUD horn of an ovariectomized animal could, therefore, be interpreted as due to an oestrogenic type of effect. The IUD may thus be producing an increased oestrogen-like effect in the IUD horn and increased synthesis of lipids, RNA and proteins. Pincus (1965) has suggested the possibility of an IUD producing a state of hyperoestrogenism in the uterus. Vorys, DeNeef, Boutselis, Dettman, Scott, Stevens & Besch (1965), in their preliminary studies, found elevated levels of urinary FSH excretion in women using an IUD. It suggests, but by no means proves, an increased synthesis of oestrogen by the ovary. The report of Bonney, Glasser, Clewe, Noyes & Copper (1966) on the higher recovery rate of tubal ova from women with an IUD compared with controls could also be explained on the basis of the increased sensitivity of the genital tract to oestrogens. However, Szego & Lawson (1964) have shown that histamine is able to mimic many of the actions of oestrogens and the demonstration by Parr (1967) of increased levels of histamine in the IUD horn compared with the control horn indicates that histamine or other amines may also be factors in producing an oestrogen-like effect in the presence of an IUD. The effect of an IUD on uterine cell permeability also needs elucidation.

Psychoyos (1966) has ruled out the suggestion of the device having a systemic effect involving the pituitary-ovarian or ovarian-uterine axis. If that were so,

both horns should be refractory to the blastocysts. Furthermore, if both horns are subjected to trauma when the uterus is in the receptive phase, the intact horn responds normally whereas the horn with the thread shows a reduced vascular response. It is possible that the small amounts of oestrogen produced by the adrenal cortex, may be acting on the IUD horn while not affecting the control horn. This would mean an increased sensitivity of the IUD horn to 'oestrogens' and is supported by the increased incorporation of [$U\text{-}^{14}\text{C}$]glucose into lipids, RNA and proteins in the IUD horn of ovariectomized animals, and by the work of Kar *et al.* (1964).

It was of interest that the administration of 2.0 mg progesterone alone with 5.0 μg oestradiol produced different effects on glucose metabolism in the control and IUD horns. The marked decrease in [$U\text{-}^{14}\text{C}$]glucose incorporation into lipids, RNA and proteins of the IUD horn compared with the control horn indicated an increased sensitivity of the IUD horn to progesterone. An increased response to progesterone in the IUD horn was also found when progesterone alone was administered to the animals. The observation of uterine sensitivity to progesterone received additional support from studies on the [$U\text{-}^{14}\text{C}$]glucose incorporation into proteins after 9 days of hormone treatment, when a marked decrease in the incorporation was seen in the IUD horn compared with the control horn. Thus, the effect of the same amount of progesterone in suppressing the incorporation in the IUD horn was more marked compared with that in the control horn. It is known that progesterone suppresses the oestrogen-stimulated protein synthesis in the uterus and vagina (Little & Lincoln, 1964; Laumas & Farooq, 1966) at certain dose levels. The markedly enhanced decrease in protein synthesis in the IUD horn compared with the control horn in the presence of the same amount of progesterone indicates a marked sensitivity of the uterus to progesterone in the presence of an IUD.

Greenwald (1965) recovered normal embryos from the oviduct in the presence of an IUD until Day 4 of pregnancy, but the embryos were missing from the tract on Day 5. If the IUD horn was ligated at the cervix on Day 4, degenerating embryos were recovered on the next day. Therefore, at the time of implantation on Day 5, the IUD somehow exerted an anti-implantation effect and the ova were expelled from the uterus. Extensive evidence has accumulated (DeFeo, 1963; Psychoyos, 1966) which shows that implantation takes place between 02.00 hours and noon of the 5th day of pregnancy in the rat. It is clearly important to know how the IUD produces the anti-implantation effect at this critical time of pregnancy. The results on the incorporation of [$U\text{-}^{14}\text{C}$]glucose into lipid, RNA and proteins showed a significantly decreased incorporation in the IUD horn compared with the control horn between 10.00 hours and noon on the 5th day of pregnancy. These results can be explained on the basis of the hypothesis of uterine sensitivity to progesterone in the presence of an IUD. Abundant evidence is available which shows that the blastocyst requires a proper preparation of the endometrium for its implantation on Day 5 of pregnancy. Marcus & Shelesnyak (1967) have considered the various phases of nidation in which progesterone plays an important role in preparing the endometrium. If the uterus is sensitive to progesterone it would exhibit an increased progestational effect in the presence of small amounts of progesterone.

The decreased incorporation of [U- ^{14}C]glucose into lipid, RNA and proteins on Day 5 of pregnancy in the IUD horn, compared with the control horn, shows that there was an increased progestational response producing asynchronous changes in the uterus, and this disturbed the delicate balance of oestrogen and progesterone action in the uterus necessary for implantation. Glasser (1967) has reported that an IUD produces retardation of the increase in the non-phospholipid to phospholipid ratio that occurs at the time of ovulation. This suggests some impairment in the biochemical preparation of the endometrium. Electron microscopy (Wynn, 1967) has also shown asynchronous changes in the endometrium in the presence of an IUD which supports the present conclusions.

The present findings on sensitivity of the uterus to progesterone in the presence of an IUD as a mechanism for the anti-implantation action of the device draws support from the work of Psychoyos (1966) in rats on the decidual response to trauma in the presence of an IUD, but the mechanism responsible for this sensitivity is not known. It was previously found (Laumas & Farooq, 1967) that an IUD did not alter the uptake and disappearance of [1,2- ^3H]progesterone from rat uterus. However, in that study, the uptake of progesterone in the total uterus was studied and it is not known whether the uptake of progesterone is selectively altered in the endometrium in the presence of an IUD. Psychoyos (1966) showed that trauma caused an increase in uptake of [^{14}C]progesterone by the rat uterus and suggested that an IUD has the same effect as trauma with regard to progesterone uptake by the uterus. According to Psychoyos (1966), this may contribute to increased sensitivity of the uterus to progesterone in the presence of an IUD. Elucidation of the mechanism for such an increased sensitivity must await actual demonstration of higher levels of progesterone in the endometrium when an IUD is present.

Inhibition of decidua formation in the presence of an IUD has been suggested as the mechanism for the anti-implantation effect of an IUD in the rat (Doyle & Margolis, 1965; Marston & Chang, 1965), but Segal (1965) feels that the critical event of disintegration of the blastocyst takes place before decidua formation. The presence of an IUD causes endometrial accumulation of glycogen (Parr, 1966), mobilization of mast cells (Mathur & Chaudhury, 1968), increase in total uterine content of histamine (Parr, 1967), transient increase in oxygen consumption (Kar *et al.*, 1965), and an increase in mucopolysaccharide (Cooper & Hawk, 1968) in the uterus. The IUD also causes the uterine fluid to become viscous with increased albumin and globulin concentrations. Many possible explanations for the antifertility action of an IUD based upon the above and on other observations have been proposed and recently summarized (Corfman & Segal, 1968; Kar, 1968). In addition to the changes in uterine environment and uterus mentioned above, the present results indicate that the IUD produces an oestrogen-like response and sensitivity to progesterone in the rat uterus. This sensitivity of uterus to progesterone in the presence of an IUD produces asynchronous changes and makes the uterus non-receptive to blastocysts at the critical time of implantation and hence there is no implantation. Once the blastocyst is unable to implant it disintegrates in a uterine milieu which is viscous and has a dehydrating influence on the blastocyst as suggested

by Kar *et al.* (1964). This result may be compared with the observations by Doyle & Margolis (1965), Greenwald (1965) and Segal (1965) in the rat and, more recently, by Marston & Kelly (1968) in the monkey. This explanation for the mode of action of an IUD in the rat has considerable experimental support, but the primary event which is responsible for increased oestrogen-like effect and increasing the sensitivity of the uterus to progesterone in the presence of an IUD is not yet known.

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REFERENCES

- ADAMS, C. E. & ECKSTEIN, P. (1965) The effect of intrauterine foreign bodies on pregnancy in the rabbit. *Fert. Steril.* **16**, 508.
- AZIAWA, Y. & MUELLER, G. C. (1961) The effect *in vivo* and *in vitro* of oestrogen on lipid synthesis in the rat uterus. *J. biol. Chem.* **236**, 381.
- BONNEY, W. A., JR, GLASSER, S. R., CLEWE, T. R., NOYES, R. W. & COPPER, C. (1966) Endometrial response to the intrauterine devices. *Am. J. Obstet. Gynec.* **96**, 101.
- BURTON, K. (1956) A study of the conditions and mechanism of diphenylamine reaction for colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**, 315.
- COOPER, B. S. & HAWK, H. W. (1968) Effects of intrauterine devices on endometrial mucopolysaccharides in cow. *Fert. Steril.* **19**, 310.
- CORFMAN, P. A. & SEGAL, S. J. (1968) Biologic effects of intrauterine devices. *Am. J. Obstet. Gynec.* **100**, 448.
- DEFEO, V. J. (1963) Determination of the sensitive period for the induction of deciduomata in the rat by different induction procedures. *Endocrinology*, **73**, 488.
- DOYLE, L. L. & MARGOLIS, A. J. (1963) Intrauterine foreign body: effect on pregnancy in rat. *Science, N.Y.* **139**, 833.
- DOYLE, L. L. & MARGOLIS, A. J. (1965) Intrauterine foreign body studies in rodents. *Proc. IInd int. Conf. Intrauterine Contraception. Excerpta med. Fdn. Int. Congr. Series*, No. **86**, 185.
- GLASSER, S. (1967) Response of human endometrial lipids to the intrauterine devices. *Fedn Proc. Fedn Am. Soc. exp. Biol.* **26**, 536.
- GREENWALD, G. S. (1965) Interruption of pregnancy in the rat by a uterine suture. *J. Reprod. Fert.* **9**, 9.
- HAWK, H. W., CONLEY, H. H., BRINSFIELD, T. H. & RIGHTER, H. F. (1965) Contraceptive effect of plastic devices in cattle uteri. *Proc. IInd int. Conf. Intrauterine Contraception. Excerpta med. Fdn. Int. Congr. Series*, No. **86**, 189.
- JESSEN, D. A., LANE, R. E. & GREENE, R. R. (1963) Intrauterine foreign body. A clinical and histopathological study on the use of Grafenberg ring. *Am. J. Obstet. Gynec.* **85**, 1023.
- KAR, A. B. (1968) Mechanism of action of intrauterine contraceptive devices. *J. scient. ind. Res.* **27**, 70.
- KAR, A. B., GOSWAMI, A., KAMBOJ, V. P. & CHOWDHURY, S. R. (1964) Effect of foreign body on the response of the uterus of ovariectomised rats to oestrogen. *Steroids*, **4**, 159.
- KAR, A. B., KAMBOJ, V. P., GOSWAMI, A. & CHOWDHURY, S. R. (1965) Effect of an intra-uterine contraceptive suture on the uterus and fertility of rats. *J. Reprod. Fert.* **9**, 317.
- LAUMAS, K. R. (1969) *Biochemical changes in rat uterus in the presence of an IUCD*. In: Review of Research Work in India on Intrauterine Contraceptive Devices, Ed. K. R. Laumas. Indian Council of Medical Research, New Delhi (in press).
- LAUMAS, K. R. & FAROOQ, A. (1966) Progesterone inhibition of oestrogen stimulated protein synthesis in rat vagina. *Excerpta med. Int. Congr. Series*, **111**, 324.
- LAUMAS, K. R. & FAROOQ, A. (1967) Uptake and disappearance of $1,2\text{-H}^3$ -progesterone from rat uterus in the presence of an intrauterine contraceptive device. *Indian J. exp. Biol.* **5**, 12.
- LITTLE, B. & LINCOLN, E. (1964) Effect of oestrogen, progesterone and testosterone on the incorporation of L-valine- 1-C^{14} into protein of rat liver and uterus. *Endocrinology*, **74**, 1.

- LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951) Protein measurement with Folin-phenol reagent. *J. biol. Chem.* **193**, 265.
- MARCUS, G. J. & SHELESNYAK, M. C. (1967) Studies on the mechanism of nidation. XXVI. Proestrous estrogen as a hormonal parameter of nidation. *Endocrinology*, **80**, 1038.
- MARSTON, J. H. & CHANG, M. C. (1965) Action of intrauterine foreign bodies in the rat and rabbit, *Proc. IInd Int. Conf. Intrauterine Contraception*, 1964, New York, Excerpta med. Fdn. Int. Congr. Series, No. **86**, 242.
- MARSTON, J. H. & KELLY, W. A. (1968) Effect of an intrauterine device on uterine motility in rhesus monkey. *Nature, Lond.* **217**, 774.
- MATHUR, V. S. & CHAUDHURY, R. R. (1968) The effect of an intra-uterine plastic device on mast cell count in rat uterus. *J. Reprod. Fert.* **15**, 135.
- NICOLETTE, J. A. & GORSKI, J. (1964) Effect of oestradiol on glucose-U-C¹⁴ metabolism in rat uterus. *Archs Biochem. Biophys.* **107**, 279.
- PARR, E. L. (1966) Glycogen accumulation in the rat uterus containing an intrauterine contraceptive device. *Fert. Steril.* **17**, 797.
- PARR, E. L. (1967) Histamine content of rat uterus containing an IUCD. *J. Reprod. Fert.* **14**, 321.
- PARR, E. L. & SEGAL, S. J. (1966) The effect of an intrauterine contraceptive device on the weight of the rat uterus. *Fert. Steril.* **17**, 648.
- PINCUS, G. (1965) In Discussion, chapter on mechanism of contraceptive action of IUCD. *Proc. IInd Int. Conf. Intrauterine Contraception*, 1964, Excerpta med. Fdn. Int. Congr. Series, No. **86**, 211.
- PSYCHOYOS, A. (1966) *Recent researches on egg implantation*. In: Egg Implantation, Ciba Foundation Study Group No. 23, p. 4. Eds. G. E. W. Wolstenholm and M. O'Connor. Churchill, London.
- SCOTT, J. F., FRACCASTRO, A. P. & TAFT, E. B. (1956) Studies in histochemistry: determination of nucleic acid in microgram amount of tissue. *J. Histochem. Cytochem.* **4**, 1.
- SEGAL, S. J. (1965) In Discussion, chapter on mechanism of contraceptive action of IUCD. *Proc. IInd Int. Conf. Intrauterine Contraception* 1964, New York, Excerpta med. Fdn. Int. Congr. Series, No. **86**, 211.
- SZEGO, C. M. & LAWSON, D. A. (1964) Influence of histamine on uterine metabolism. Stimulation of incorporation of radioactivity from amino acids into protein, lipid and purines. *Endocrinology*, **74**, 372.
- UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1959) *Manometric techniques*, p. 149. Burgess Publishing Co., Minneapolis.
- VORYS, N., DENEEF, J., BOUTSELIS, J. G., DETTMAN, F. G., SCOTT, W. P., STEVENS, V. C. & BESCH, P. K. (1965) Effect of intrauterine devices on the normal menstrual cycle. *Proc. IInd Int. Congr. Intrauterine Contraception*, 1964, New York, Excerpta med. Fdn. Int. Congr. Series, No. **86**, 147.
- WYNN, R. (1967) Intrauterine devices: Effect on ultrastructure of human endometrium. *Science, N.Y.* **156**, 1508.
- YADAVA, H. S. & LAUMAS, K. R. (1968) Incorporation of glucose-U-C¹⁴ into lipid, RNA and proteins of the rat uterus in the presence of an intrauterine foreign body. *Indian J. Physiol. Pharmac.* **12**, P. 7.