FROM INSTITUTO DE INVESTIGACIONES HEMATOLOGICAS, ACADEMIA NACIONAL DE MEDICINA, BUENOS AIRES, AND INSTITUTO DE INVESTIGACIONES MEDICAS, ROSARIO, SANTA FE, ARGENTINA.

STERILITY DUE TO RADIOACTIVE PHOSPHORUS IN MICE

by

E. A. D. HOLMBERG, C. DOSNE DE PASQUALINI, E. ARINI, A. PAVLOVSKY and S. L. RABASA

Radioactive phosphorus, ^{32}P, induces sterility in female BALB mice at a dose of 40 μC while males remain fertile following any dose compatible with life (up to 205 μC Ld_{60}) (ref. 4). Since LD_{50} has been calculated as 168 μC, it follows that the reproductive system of females is sensitive to doses that are well sublethal while that of males is resistant even to doses producing a high rate of mortality. Pregnancy affords some protection since females injected in this condition remain fertile after doses of 40 μC and 60μC, 90 μC being required to effect sterility (ref. 5). Two hypotheses have been advanced to explain this protection: (1) the radiophosphorus uptake is decreased in the ovary due to a shift of the concentration of the isotope towards the pregnant uterus (fetuses), and (2) the ovaries of pregnancy (through the action of the corpora lutea) are in some way refractory to the effect of ^{32}P.

The authors have injected progesterone in order to see whether it affords the same protection as pregnancy; this would mean, in turn, that pregnancy may act through this hormone. In addition, the uptake of ^{32}P has been studied in normal, pregnant and progesterone-treated mice. The investigation also covered the effect of the beta rays both in fetal and adult life.

Submitted for publication 20 January 1964.

Table 1

Fertility of non-pregnant BALB mice treated with ^{32}P

Dose μC	Females treated	Pregnant within 30 days	Pregnant beyond 30 days	Pregnant beyond 90 days
40	22	7	4	2
60	10	8	0	0
90	9	4	0	0
120	8	1	0	0

Material and Methods. Mice belonging to the BALB strain were used throughout the experiments. Their fertility in the laboratory is high since no male and only 9 out of 600 females tested have been found to be naturally sterile. The animals were included in the experiments when 2 1/2 to 4 months old, having been previously mated, that is, checked for fertility. After treatment, the injected females and males were mated with normal mice and observed twice a week for a period up to eight months.

Radioactive phosphorus was administered by the intraperitoneal route at doses ranging from 32 to 205 μC. In the case of the animals exposed to this isotope during fetal life, the pregnant females were injected from the 11th to the 15th day of pregnancy. Here again, the fetally-exposed mice were mated with normals when 2 months old for a period of two months.

Progesterone in the form of microcrystals was injected subcutaneously in a dose of one milligram twice a week during 4 weeks prior to and two weeks after the administration of the radioactive agent.

The uptake of ^{32}P by the ovaries, uterus and fetuses, was determined as follows: 24 hours after the injection the animals were killed and the organs were weighed and heated at 60° C for one hour in nitric acid. The solutions were homogenized and 0.1 ml samples of each were dried in an oven for 24 hours. The counting was carried out with a gas flow counter, using a ^{32}P solid standard (2.76×10^{-5} mC). The mice were placed in metabolism cages after the administration of the isotope in order to determine the elimination rate. The urine, feces and alimentary residues were collected in glass containers during 24 and 48 hours, and after the careful washing of all the material used the suspension was dried by evaporation and reduced to dust in a muffle at 800° C for one hour. The final dust was weighed and the counting was performed in a Geiger-Müller counter with a mica window using a 1 μC standard of ^{32}P prepared from the same batch used to inject the animals.

Histologic studies of the ovaries and testes were performed at the time of natural death or when the animals were deliberately sacrificed.

Table 2

Fertility of pregnant BALB mice treated with ^{32}P

Doses μC	Females treated	Fertile beyond 30 days	Fertile beyond 90 days
40	8*	8	4
60	8	6	2
90	6	0	0

* Four were killed before 90 days.

Results

^{32}P-induced sterility is established approximately 30 days after the injection (Table 1). A varying proportion of animals receiving from 40 to 120 μC remain fertile within 30 days after the injection but only some (4 out of 22) of those receiving 40 μC, and none in the higher-dose groups, were still fertile from 30 to 90 days after the administration of ^{32}P. Out of those receiving 40 μC, two remained fertile for 110 and 162 days, respectively.

When the agent was administered during pregnancy (11th to 15th day) a definite protection was achieved, since all 8 females receiving 40 μC, and 6 out of 8 injected with 60 μC, were fertile when tested beyond 30 days after treatment (Table 2). This result is significant at the 0.01 level (x^2 : 8.36; x^2 : 0.01 : 6.635). Moreover, of the 4 mice receiving 40 μC and kept alive beyond 90 days, all were fertile, as well as 2 out of 8 treated with 60 μC. The protective effect of pregnancy vanished completely when the dose was increased to 90 μC.

It will be seen from Table 3 that progesterone affords a protection similar to that of pregnancy. Almost all the animals mated within 30 days remained fertile irrespective of the dose of ^{32}P (40 to 90 μC) (x^2 : 14.69; x^2 : 0.001 : : 16.827) and beyond 90 days, 8 out of 29 treated with 40 μC and 2 out of 7 receiving 60 μC were still fertile. These results when compared with the untreated controls are significant at the 0.05 level (x^2 : 4.14; $p < 0.05$; x^2 : 0.05 : 3.841).

The comparative effect of ^{32}P in fetal and adult males and females is shown in Table 4. Two out of 6 females (33 %) in the 40 μC group exposed to the agent during fetal life remain fertile when mated 90 days after the treatment; on the other hand only 2 out of 22 (9 %) receiving ^{32}P as adults became pregnant during the same period. Of the males, only 37 % exposed to the same dose as fetuses were fertile 90 days after exposure, while 100 % of males surviving doses even as high as 205 μC administered during adult life were fertile. This table also shows that the ovaries and testes during

Table 3

Fertility of progesterone-treated BALB mice injected with ^{32}P

Doses μC	Females treated	Fertile within 30 days	Fertile beyond 30 days	Fertile beyond 60 days	Fertile beyond 90 days
40	14	13	6	5	3
40	15	(not mated)	9	2	5
60	7	7	0	0	2
90	4	4	0	0	0

Table 4

Comparison of fertility of female and male BALB mice exposed to ^{32}P during adult and fetal life — all animals were mated 90 days after exposure

Doses μC	Adult-treated females Fertile/treated	Fetally-treated females Fertile/treated	Adult-treated males Fertile/treated	Fetally-treated males Fertile/treated
40	2/22	2/6	1/1	3/8
60	0/10	0/8	2/2	0/6
90	0/9	0/9	1/1	0/1
120	0/8	—	—	—
205	—	—	3/3	—

fetal life have about the same sensitivity to the β-rays of ^{32}P, since 33 % of the females and 37 % of the males were fertile after 40 μC. When larger doses were administered, animals of both sexes became completely sterile. This phenomenon is not observed when the treatment is administered during adult life.

The histologic examination of the ovaries from sterile females of the fetally-exposed group revealed complete atrophy of follicular tissue, with hypertrophy and luteinization of the stroma, appearances very similar to those described in sterile females injected during adult life (ref. 4). The testes of the sterile males exposed during fetal life appeared completely atrophied upon macroscopic examination, and microscopy revealed total atrophy of the germinal epithelium with an increased number of Leydig cells.

Table 5 contains the results of the ^{32}P uptake by different organs in the three experimental groups. The amount picked up seems fairly constant throughout the groups and no definite trend is evident, excepting the effect of dosage. The ovaries of progesterone-treated mice injected with 60 μC have an uptake lower than that of normals injected with the same dose but no similar difference appears with the other doses administered to the same hormone-treated groups while the pregnant mice exhibit no decreased uptake if compared with normals.

Table 5

^{32}P activity, mC × 10^{-7} per milligram of tissue, in mice killed 24 hrs after the administration of the isotope — numbers of mice studied are indicated by the numerals within parentheses

Doses μC	Controls		Progesterone treated		Pregnant				
	Ovaries	Uterus	Ovaries	Uterus	Ovaries	Uterus plus content	Uterus	Fetuses	Placenta
32	5.72 (3)	9.24 (3)	5.75 (7)	6.00 (7)	7.51 (3)	8.24 (2)	7.62 (1)	7.99 (1)	7.59 (1)
60	13.42 (5)	11.06 (3)	10.33 (3)	9.96 (8)	10.76 (4)	8.59 (1)	—	—	—
90	15.17 (3)	17.65 (3)	18.59 (10)	19.34 (10)	30.94 (2)	20.84 (1)	21.59 (1)	27.85 (1)	15.54 (1)

Table 6

Elimination, in μC, of ^{32}P — the number of animals studied are indicated by numerals within parentheses

Doses μC	Controls		Progesterone-treated	
	24 hrs	48 hrs	24 hrs	48 hrs
32	6.52 (2)	1.77 (1)	3.18 (3)	1.29 (3)
60	10.13 (4)	3.54 (3)	6.10 (5)	2.50 (4)
90	22.33 (2)	4.49 (1)	9.88 (2)	4.10 (2)

The elimination studies summarized in Table 6 were carried out in controls and in progesterone-treated animals. The amount eliminated in the first 24 hours by the latter group is significantly decreased. Even in 48 hours, the quantity eliminated by the control mice is definitely higher than in the hormone-treated animals. As a consequence of the decreased elimination, the blood levels of ^{32}P are lower in normals than in the progesterone-treated mice both one and 24 hours after the injection (Table 7).

Discussion

Pregnancy affords some protection against the sterilizing effect of ^{32}P, an effect not attributable to a lower uptake of the agent by the ovary. Progesterone-treated females had developed a similar degree of protection, and again no decreased uptake by the ovary was detected. It follows that the action of both pregnancy and progesterone is not mediated by a change of uptake of ^{32}P detectable by the method used. The progesterone-treated group had

Table 7

Blood levels, given in μC, of ^{32}P — the number of mice studied are indicated by numerals within parentheses

Doses μC	Controls		Progesterone-treated	
	1 hr	24 hrs	1 hr	24 hrs
60	15.31 (3)	11.19 (3)	17.09 (3)	16.25 (3)

paradoxically a higher blood level of the isotope and a decreased elimination when compared with the controls. It may be that pregnancy acts either through progesterone itself or through a hormonal state that makes the ovary more resistant to ^{32}P. It may therefore be concluded that neither a dilution due to the increased weight during pregnancy nor a shift of radioactive phosphorus towards the fetuses can explain the facts observed.

Disappearance of the follicles and permanent (up to 9 months) luteinization occurred in the ovaries of adult mice treated with sterilizing doses of radio-phosphorus as previously described (ref. 4, 5). This is in agreement with the changes observed in the vaginal smears of those animals; the changes at first consisted of cycle irregularities, followed by permanent diestrus. It may be noted that the animals accepted mating in spite of the absence of estrus. The ovaries of females exposed to the radioactive agent during fetal life also presented similar appearances, while males displayed the masculine counterpart of the same pattern, that is, absence of spermatogenesis and hypertrophy of the Leydig cells.

The presence of such a gonadal lesion might be attributed to a defect in the feed-back mechanism from target organ to hypothalamus brought about by the gonadal alteration itself; it could also be explained by hypothalamic lesions of the median eminence (ref. 9) induced by ^{32}P. Proof is however lacking and, moreover, the difference in sensitivity between the two sexes in the adult groups lends little support to this hypothesis. Experiments now in progress are directed toward clarifying this point.

The difference in sensitivity in the gonads between the two sexes according to the stage of development is interesting. The ovary appears to be almost equally resistant during fetal and adult life; as for the testes, the greater sensitivity during intra-uterine life is obvious. It may therefore be concluded that the testes in mice decrease in sensitivity to ^{32}P during the process of maturation while the ovaries do not.

These responses to radioactive phosphorus are similar to those reported in rats treated with roentgen irradiation (ref. 1, 2). As opposed to roentgen

radiation, ^{32}P has however more than an immediate effect, and its action should be compared to that of other isotopes.

Suckling mice have been submitted to the action of ^{131}I injected in the mother (ref. 7). It was observed that females were more sensitive than males to the sterilizing effect of this isotope. When ^{137}Ce was administered on the 18th day of pregnancy (ref. 3), and its effect compared to that of 300 r roentgen irradiation it was observed that the ^{137}Ce radiation induced atrophy of the ovaries and testes of the offsprings, while the roentgen radiation affected only the testes. It would therefore appear that roentgen irradiation applied during prenatal life is more deleterious to the male than to the female gonad (ref. 6, 8). It may be concluded that the radiation of ^{32}P has the same effect as the gamma radiation of ^{137}Ce on the gonads during prenatal life, and that the difference between these two isotopes and roentgen radiation may reside in the continuous rather than the immediate action of the former.

Acknowledgement

The authors are indebted to the Comisión Nacional de Energia Atomica for supplying the radioactive phosphorus. The investigation was supported in part by Fundaleu Fundación para combatir la leucemia.

SUMMARY

The uptake of ^{32}P has been studied in a material of normal, pregnant and progesterone-treated mice. Pregnancy affords some protection against the sterilizing effect of radiophosphorus and progesterone lends a significant protection against the isotope. It is suggested that pregnancy may act through the production of progesterone or through a hormonal state that makes the ovary more resistant to radiophosphorus.

ZUSAMMENFASSUNG

Die Aufnahme von ^{32}P wurde an normalen Mäusen, schwangeren Mäusen und mit Progesteron behandelten Mäusen untersucht. Schwangerschaft schützt in gewissem Grade vor dem sterilisierenden Effekt des Radiophosphors und Progesteron gibt beträchtlichen Schutzt gegen das Isotop. Es wird angenommen, dass Schwangerschaft entweder durch die Produktion von mehr Progesteron oder durch hormonale Einflüsse die Ovarien mehr widerstandsfähig gegen den Radiophosphor macht.

RÉSUMÉ

La fixation de ^{32}P a été étudiée sur des souris normales, gravides et traitées par la progestérone. La gravidité confère une certaine protection contre l'effet stérilisant du radiophosphore et la progestérone donne une protection importante contre cet isotope. Les auteurs pensent que la gravidité peut agir par production de progestérone ou par un état hormonal qui rend l'ovaire résistant au radiophosphore.

REFERENCES

1. BEAUMONT H. M.: Changes in the radiosensitivity of the testis during foetal development. Inter. J. Rad. Biol. 2 (1960), 247.
2. — Radiosensitivity of oogania and oocytes in the foetal rat. Inter. J. Rad. Biol. 3 (1961), 59.
3. ERSHOFF B. J., and BRADT V.: Comparative effect of prenatal gamma radiation and x-irradiation on the reproductive system of the rat. Amer. J. Physiol. 198 (1960), 1119.
4. HOLMBERG E. A. D., PAVLOVSKY A., DE PASQUALINI C. D., and RABASA S. L.: Fertility of mice treated with phosphorus-32. Nature 187 (1960), 876.
5. — — PEDACE E. et coll.: Estudio experimental de un posible efecto secundario del fosforo radioactivo. Medicina 21 (1961), 84.
6. RUGH R.: Fetal x-irradiation and fertility. Proc. Soc. exp. Biol. 80 (1952), 388.
7. — Selective sterilization of young female mice by radioiodine transmitted through the mother's milk. Proc. Soc. exp. Biol. 83 (1953), 762.
8. — and JACKSON S.: Effect of fetal-x-irradiation upon the subsequent fertility of the offsprings. J. exp. Zool. 138 (1958), 209.
9. TALEISNIK S., and MCCANN S. M.: Effects of hypothalamic lesions on the secretion and storage of hypophyseal luteinizing hormone. Endocrinology 68 (1961), 263.