Table 2. UTERINE WEIGHTS OF MICE INJECTED WITH EXTRACTS OF THE MILK OF WOMEN TREATED WITH RADIOACTIVE STEROIDS

Subject	Steroid administered	Day	Uterine wt. mg mean ± S.E.	Uterine wt. of controls mean ± S.E.
M.P.	Norethynodrel	1	8·2 ± 0·39	8·2 ± 0·82
M.M.	Norethynodrel	1	7·9 ± 0·22	
		2	9·2 ± 0·99	
		3	8·4 ± 0·83	
		4	7·5 ± 0·29	7·1 ± 0·63
M.E.A.	Ethynodiol diacetate	1	8·3 ± 1·35	
		2	8·0 ± 0·27	
		3	8·2 ± 0·64	
		4	8·8 ± 0·40	
V.F.T.	Ethynodiol diacetate	1	7·9 ± 0·51	
		2	7·0 ± 0·55	10·5 ± 1·01
		3	8·0 ± 1·34	
		4	7·4 ± 0·51	

percentage of the radioactive dose present in milk collected from each subject over 24 h periods.

Half the total extract from each milk sample was suspended in sesame oil and then injected subcutaneously into Swiss albino female mice 21 days old. The volume of sesame oil was such as to provide sufficient material for injection into five mice over a three day period. Uterine weights of the animals killed 72 h after the first injection are an indication of oestrogen action. In this type of assay, a significant increase in uterine weight is obtained with 0·03 μg of oestrone or 0·1 μg of ethynyloestradiol-3-methyl ether. Table 2 shows the results of the biological assays of milk. None of the values obtained was statistically different from the controls treated with oil alone, which indicates that the oestrogenic activity of the extracts was too low to be detected by this assay.

The results show that in both the rabbits and the women very little of the radioactive dose was transferred to the milk. The biological assays showed no significant oestrogenicity in the human milk extracts, results similar to those of previous workers, who showed that when either 17β-oestradiol[3] or progesterone[4] is administered to cattle, very little of the steroid can be found in the milk. The conditions of the experiment and the absence of suckling, however, drastically reduced milk flow, as is shown by the volumes of milk per day listed in Table 1. The proportion of the steroid administered which might appear in the milk if suckling was maintained might therefore be larger than that found in these experiments.

We thank the Department of Social Services and the staff of the Department of Obstetrics and Gynecology of the Rio Piedras Municipal Hospital, Rio Piedras, Puerto Rico, for help in collecting the human milk samples. The expenses of the work were in part provided by a U.S. Public Health Service grant.

G. PINCUS
G. BIALY
D. S. LAYNE
M. PANIAGUA
K. I. H. WILLIAMS

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts.

[1] Layne, D. S., Golab, T., Arai, K., and Pincus, G., *Biochem. Pharmacol.*, **12**, 905 (1963).
[2] Flood, C., Layne, D. S., Ramcharan, S., Rossipal, E., Tait, J. F., and Tait, S. A. S., *Acta Endocrinol.*, **36**, 237 (1961).
[3] Lunaas, T., *Nature*, **198**, 288 (1963).
[4] Williams, W. F., *J. Dairy Science*, **45**, 1541 (1962).

Incorporation of Subcutaneously Administered Iron-59 by Mouse Erythrocytes

IN experiments on the uptake of iron-59 by mouse erythrocytes the isotope is usually administered intravenously (jugular or tail vein), or intraperitoneally. Anaesthesia and incision are required for jugular venipuncture, and part of the iron-59 may be lost by extravasation. Damage to the abdominal organs or accidental injection of iron-59 into the intestine can occur during intraperitoneal injection. The subcutaneous injection of iron-59 is more rapid and is free from the problems that may be encountered during intravenous or intraperitoneal administration.

Female Swiss–Webster mice, *ICR* strain, 7–9 weeks old, 22·5–31·5 g in weight, were used. One microcurie of iron-59 labelled citrate in saline was administered subcutaneously in the region behind the neck. The incorporation of iron into the circulating red blood cells was determined by measuring the radioactivity in 0·2 c.c. of blood obtained by cardiac puncture 24 h later. Calculations of the percentage incorporation were based on an assumed blood volume of 7·5 per cent. The haematocrit was determined on the sample of cardiac blood, which was mixed with dried heparin to prevent coagulation.

Table 1. PERCENTAGE UPTAKE OF IRON-59 BY MOUSE ERYTHROCYTES AT 24 HOURS

Ref.	No. of mice	Age (weeks)	Strain	Administration of iron-59	Uptake of iron-59* (per cent)	Haematocrit*
1	156	6	Swiss *ICR/HA*	Jugular venipuncture	27·2	
2	9	9–12	*CF*-1	Tail vein	28·8 ± 5·03	
3	10		Swiss	Intraperitoneal	26·9 ± 2·1	
Present work	33	7–9	Swiss–Webster *ICR*	Subcutaneous	27·2 ± 1·42	45·4 ± 0·41

* Average ± standard error.

Table 1 shows the percentage incorporation of iron-59 in circulating erythrocytes 24 h after the subcutaneous administration of iron-59. The value obtained compares favourably with the results of studies using intravenous or intraperitoneal techniques.

The mean uptake of iron-59 by mouse erythrocytes 24 h after injection was 27·21 per cent of the subcutaneously administered dose, with a standard error of the mean of ± 1·42. This is comparable with results obtained when the isotope was administered by the intravenous and intraperitoneal route.

This work was supported by contracts with the U.S. Air Force and the U.S. Atomic Energy Commission.

R. A. E. THOMSON

Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York.

[1] Mirand, E. A., Hoffman, J. G., and Prentice, T. C., *Proc. Soc. Exp. Biol. and Med.*, **104**, 457 (1960).
[2] Mendel, G. A., *Blood*, **18**, 727 (1961).
[3] Schooley, J. C., and Garcia, J. F., *Proc. Soc. Exp. Biol. and Med.*, **110**, 636 (1962).

Influence of Polyphosphates on Retention of Radioactive Strontium in Rat and Mouse

PLASMA ultrafiltrate is thought to contain polyphosphates which prevent the nucleation of calcium salts on collagen. Under physiological conditions, these substances are thought to be inactivated by alkaline phosphatase, and so do not interfere with nucleation in bone tissue. We have tested the efficiency of several polyphosphates in preventing the skeletal deposition of strontium-85 in experimental animals. In a series of experiments, groups of ten albino Sprague–Dawley rats (200 g) or *ASL* mice (20 g) were injected intraperitoneally with 1 μc. of strontium-85 free of carrier.

Initially, polyphosphates were injected intraperitoneally into rats in doses such as to produce extracellular fluid concentrations equivalent to those required to inhibit nucleation on collagen *in vitro*[1]. Sodium dihydrogen phosphate (3·75 μmoles/kg), sodium tripolyphosphate (3·75 μmoles/kg), sodium hexametaphosphate (3·75 μmoles/kg) and sodium decapolyphosphate (0·375 μmoles/kg) were each tried by injection simultaneously with the radiostrontium, followed by a double dose of the phosphate 7 days later. In no case did the retention curve of the treated groups differ from the mean curve for controls by more than one standard deviation, and we concluded