sponse elicited by the strong erythropoietic stimulus of $CoCl_2$ is affected by gonadal hormones. Since the values obtained for the castrated males and females fall between those obtained for the intact males and females, the data support the conclusion of previous investigators that testosterone stimulates and estrogen inhibits erythropoiesis.

However, when the probabilities of the differences found between intact and castrated males and between intact and castrated females were calculated, p was greater than .05 but less than 0.1 for the former and less than .01 for the latter, suggesting a greater inhibitory effect of estrogen than a stimulatory effect of testosterone.

It is of further interest that an estimate of the survival time of the erythrocyte formed in response to $CoCl_2$ stimulation may be made from the data presented. It is our interpretation that maximal hematocrit level is reached when rate of erythrocyte destruction equals rate of formation; further, that this level is attained when the cells formed in response to the initial $CoCl_2$ injections begin to be removed. Accordingly, from the curves in Fig. 1 and 2 it is estimated that survival time of the erythrocytes formed in response to $CoCl_2$ is 42-49 days for the female rat and 49-56 days for the male. Burwell, *et al.* (3) employing radioiron-tagged erythrocytes established survival time of normal red cells of the rat to be 45-50 days. Therefore, estimates of survival time of erythrocytes formed in response to $CoCl_2$ stimulation, obtained from the hematocrit curves, compare quite favorably with survival times of normal cells established by more elaborate procedures.

Summary. Following injection of $CoCl_2$ for 63 days, intact male and female rats attained significantly different maximal hematocrit values of 66.7% at the end of the seventh week for the former and 59.5% at the end of the sixth week for the latter. Intermediate levels of 64.0% and 63.7% were reached by castrated males and females respectively under the same stimulus, both at the end of the seventh week. Survival time of erythrocytes formed in response to $CoCl_2$ stimulation appears to be normal.

1. Finkelstein, G., Gordon, A. S., Charipper, H. R., *Endocrin.*, 1944, v35, 267.

2. Mirand, E. A., Prentice, T. C., Slaunwhite, W. R., *Ann. N. Y. Acad. Sci.*, 1959, v77, 677.

3. Burwell, E. L., Brickley, B. A., Finch, C. A., *Am. J. Physiol.*, 1953, v172, 720.

Received June 14, 1961. P.S.E.B.M., 1961, v108.

Fluoride Metabolism in Pregnant Rats.* (26933)

A. BRZEZINSKI, I. GEDALIA, A. DANON AND F. G. SULMAN

Departments of Gynecology and Obstetrics, Preventive Medicine, and Applied Pharmacology, Hebrew University-Hadassah Medical School and School of Pharmacy, Jerusalem, Israel

With increasing use of fluoride salts for prevention of dental caries, metabolic and storage studies of this element (F) in humans and animals have become ever more important. Such studies in pregnant women have shown that an accumulation of F takes place in the placenta(1,2,3,4,5) and that it is also transferred to the fetus(6). At a concentration of 0.5-0.6 ppm F in the drinking water this accumulation is reflected in decreased excretion of F in urine and saliva(7). Investigations carried out in pregnant rats fed diets of varying F levels indicate that about 10 ppm of F must be ingested by the mother rat before any appreciable increase in carcass F is found in the pups(8,9,10,11,12). The present study was initiated to investigate whether F changes in the diet during pregnancy of rats would noticeably affect maternal blood, urine, osseous structure and F storage in the fetuses.

Materials and methods. One hundred female white laboratory rats of the Hebrew

* Supported by a grant from Joint Research Fund, Hebrew Univ.-Hadassah Medical School.

TABLE I. Fluoride Quantities Given 6 Groups of Normal and Pregnant Rats.

Group	F in food	F in water	F total
	ppm		
I	.2	0	.2
II	.2	5	5.2
III	4.55	.65	5.2
IV	4.55	10	14.55
V	4.55	50	54.55
VI	4.55	100	104.55

University strain Sabra, weighing 170 ± 20 g S. D., were divided into 6 groups receiving different F diets:

Group I was given a F-poor diet of 0.2 ppm F, consisting of 73% starch, 18% casein, 5% soya oil, 4% salt mixture, and vitamins, added as powder (thiamine HCl 0.5 mg, riboflavin 0.7 mg, pyridoxine HCl 1.0 mg, calcium pantothenate 140.0 mg, nicotinamide 7.0 mg, choline chloride 25.0 mg per 100 g of diet). Aqueous suspensions of the following vitamins were also added: Vit. A—120 u/rat/day, Vit. D—25 u/rat/day and Vit. E—0.8 mg/rat/day. Lower vitamin contents resulted in a marked degree of barrenness. The drinking water was distilled.

The rats in Group II received the same F-poor food as Group I (0.2 ppm F) with a further 5 ppm F (as NaF) in (distilled) drinking water.

In Group III, the food (mixed grain) contained 4.55 ppm F and the drinking (tap) water 0.65 ppm F.

Group IV received the same mixed grain diet (4.55 ppm F) with a further 10 ppm F in the drinking (tap) water.

Group V received the same food containing 4.55 ppm F and 50 ppm F in drinking (distilled) water.

The rats in Group VI received the same diet containing 4.55 ppm F together with a 100 ppm F level in their drinking (distilled) water.

Food and water were given to all groups *ad libitum*. The total F supply of the 6 groups is summarized in Table I.

The female rats were housed 5 to a large cage and nourished on the respective F diets for one week before the experiment, to allow the animals to adapt themselves. The male rats were introduced one into each cage and left there for 2 weeks, during which time about half the female rats could be considered to have been successfully mated. Then the female rats were put in metabolic cages thrice weekly and urine was collected during 24 hours for F examination.

The pregnant rats were separated a few days before delivery and sacrificed, together with their pups, immediately after delivery. The fetuses were weighed, homogenized, alkalinized to pH 9 and the mash dried. The femur bones, the pooled blood as well as the fetal bone tissue were examined for their F contents.

The rats which had not become pregnant during the 2 weeks' mating period served as controls.

Measuring the F content. Bones were ashed, pulverized, weighed and placed in F stills for distillation. The urine and blood samples were alkalinized with F-free $Ca(OH)_2$ in portions of 25 ml; 2 ml of a 2% solution of $Ca(OH)_2$ were added as F fixative and the whole evaporated to dryness. After ashing, the samples of urine, blood and fetus bone-mash were transferred to the F stills. F was steam-distilled at 135°C in the presence of 20 ml sulfuric acid (50%), and determined colorimetrically in the distillate, as previously described by us(5,13). The accuracy of the F determination in urine, blood and fetus bonemash was ± 0.03 ppm and in the bones ± 5%.

Results. Table II (a & b) indicates that F concentration in the femur ash of pregnant rats receiving F diets ranging from 0.2 to 104.55 ppm does not differ from F concentration in the femur ash of non-pregnant rats receiving the same F diet. By increasing F intake above a 14.55 ppm level, the F concentration in the femurs of both groups increased, but not in the same proportion as the F concentration in the diets. F concentration in the offspring of mothers receiving a low F concentration in the diet, ranging from 0.2 up to 5.2 ppm F, was almost identical. At higher F concentration in the diet, F concentration in the fetuses increased accordingly.

In fetal bone-mash we found F values

TABLE IIa. Fluoride Metabolism in Non-pregnant Rats Receiving Different Amounts of F.

Group	F supply, ppm	Non-pregnant rats: Femur, ppm F Range	Femur, ppm F Mean	Blood, ppm F	Urine, ppm F Range	Urine, ppm F Mean
I	.2	280-300	297	.09; .12	.02- .19	.10
II	5.2	340-360	350	—	.11- .30	.17
III	5.2	300-330	315	.10; .13	.10- .22	.14
IV	14.55	320-340	330	.12; .13	.13- .32	.23
V	54.55	530-560	540	.17	.14-1.6	.54
VI	104.55	710-740	725	.15	2.5 -3.7	3.0

TABLE IIb. Fluoride Metabolism in Pregnant Rats and Their Fetuses Receiving Different Amounts of F.

Group	F supply, ppm	Pregnant rats: Femur, ppm F Range	Femur, ppm F Mean	Blood, ppm F	Urine, ppm F Range	Urine, ppm F Mean	Fetuses: Fetus bones, ppm F Range	Fetus bones, ppm F Mean
I	.2	270-300	289	.10	.04- .27	.11	.300- .330	.317
II	5.2	300-350	329	—	.08- .26	.19	.300- .360	.330
III	5.2	305-345	319	.09	.10- .28	.16	.300- .335	.318
IV	14.55	290-320	309	—	.13- .32	.23	.425- .550	.487
V	54.55	540-550	546	—	.20-1.6	.62	2.700-3.335	3.062
VI	104.55	720-770	746	.14	2.0 -3.0	2.5	3.600-4.000	3.833

ranging from 0.3 to 4.000 ppm for the 6 F levels studied.

Mean F concentrations in the urine of pregnant and non-pregnant rats increased with F concentration in the diet. F concentration in blood increased only in rats receiving at least 54.55 ppm F in the diet. No difference was observed between F values in urine and blood of pregnant and non-pregnant rats.

Discussion. The main finding of these studies is that in rats an increase of fluoride in the diet up to values of at least 10-15 ppm is necessary in order to pass the placenta and to increase appreciably the total fluoride content in the pups. This agrees with earlier reports(9,10,11,12); Buttner(14) found that the teeth of the pups born of rats receiving 50 ppm F in drinking water during pregnancy were more acid resistant. In pregnant women, however, even small amounts pass to the fetus, which may produce anticariogenic effects in the primary teeth of their offspring (15).

The experiments with Group IV demonstrate the facility of increased F transfer through the rat placenta to the fetuses at a level of 14.55 ppm F in the food. This occurs at a level where the F content of the rat mother's blood and bones is stable (Group I-III) and only the surplus (left over after covering the special needs of the fetuses) is excreted with the urine of the mother animal. On the other hand, in pregnant women (shortly before delivery) a significant increase in blood F has been established when the F content of their drinking water was increased to 1 ppm(1,2,4,16). This points to a significant difference between the F metabolism of pregnant women and pregnant rats. In women the "non-exchangeable bone" reserve amounts to 99% and the "exchangeable bone" to 1% only(17). In the rat, which grows during most of its life span, the proportion between the 2 bone reserves is about 50:50. This is reflected in Table II (Groups I-VI) where F content of the bones increased in normal and pregnant rats at similar rates and attained very high levels with increased F supply. The F content of fetal bones reached much higher levels than that of the rat mothers, because their "exchangeable bone" amounts to 100% (cf. Group VI).

Summary. The F metabolism was compared in pregnant and non-pregnant rats of 170 g weight. In the bones, F content was similar in both groups, and when F supply

exceeded 14.55 ppm, there was a pronounced increase in bone F content. In fetal bones, the increase appeared at lower levels of supply, *i.e.* 5.2-14.55 ppm. In the blood, F levels were similar in pregnant and non-pregnant rats. At intakes exceeding 14.55 ppm, there was only a slight increase in blood F in both groups. In the urine, F levels increased in both groups similarly, and rather steeply with increased F intake, thus indicating immediate removal of any F surplus with the urine in pregnant and non-pregnant rats. It is stressed that this metabolism is different from that observed in pregnant women, who tend to retain an F surplus during pregnancy.

Authors thank Mr. N. Portuguese for devoted help.

1. Gardner, D. F., Smith, F. A., Hodge, H. C., Overton, D. E., Feltman, R., *Science,* 1952, v115, 208.

2. Held, H. R., *Schweiz. med. Wschr.,* 1954, v84, 251.

3. Feltman, R., Kosel, J., *Science,* 1955, v122, 560.

4. Ziegler, E., *Mittlg. naturwiss. Gesellsch., Winterthur,* 1956, v28, 39.

5. Gedalia, I., Brzezinski, A., Bercovici, B., Lazarov, E., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 147.

6. Brzezinski, A., Bercovici, B., Gedalia, I., *Obst. and Gynec.,* 1960, v15, 329.

7. Gedalia, I., Brzezinski, A., Bercovici, B., *J. Dent. Res.,* 1959, v38, 548; Lerman, D., Gedalia, I., Rosenzweig, K., Brzezinski, A., *Proc. 6th Congr. ORCA,* 1959, in press.

8. Murray, M. M., *J. Physiol.,* 1936, v87, 388.

9. Lehman, D., Muhler, J. C., *J. Dent. Res.,* 1954, v33, 669.

10. Buttner, G., Muhler, J. C., *ibid.,* 1958, v37, 326.

11. Koenig, K. G., Marthaler, T. M., Schait, A., Muehlemann, H. R., *Schweiz. Mschr. Zahnheilk.,* 1960, v70, 279.

12. Maplesden, D. C., Motzok, I., Oliver, W. T., Branion, H. D., *J. Nutrition,* 1960, v71, 70.

13. Gedalia, I., Yardeni, J., *J. Dent. Res.,* 1957, v36, 203.

14. Buttner, W., Muhler, J. C., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 822.

15. Hartenstein, H. J., *Int. J. Prophyl. Med. and Sozialhyg.,* (Vienna), 1960, v4, 1.

16. Brzezinski, A., Bercovici, B., Gedalia, I., 1961, in press.

17. Rasmussen, H., *Scient. Am.,* 1961, v204(4), 56.

Received June 15, 1961, P.S.E.B.M., 1961, v108.

Effects of Insulin on Adrenaline and Noradrenaline Content of Some Rat Tissues.* (26934)

B. C. R. STRÖMBLAD† (Introduced by Mark Nickerson)

Department of Pharmacology and Therapeutics, University of Manitoba Faculty of Medicine, Winnipeg

It is well known that injection of insulin can deplete the suprarenals of adrenaline(1, 2,3). In contrast to these results, administration of insulin has been found to increase considerably the adrenaline content of the heart and liver(3).

Recently we have found that the adrenaline content of several organs is markedly increased in rats after injection of large doses of adrenaline(4). It therefore seemed possible that the increased amounts of adrenaline found in the heart and liver following insulin administration were due to accumulation of adrenaline released from the suprarenal glands. To test this hypothesis, the effects of insulin on adrenaline and noradrenaline contents of hearts and salivary glands were studied in animals from which the suprarenal medullae had been removed. Demedullation does not itself alter the catecholamine content of the organs studied(4).

Methods. Male albino rats of the Sprague-Dawley strain weighing 300 to 400 g were

* Supported by grants-in-aid from Nat. Research Council of Canada and Manitoba Heart Foundation.

† Postdoctoral Fellow of Nat. Research Council of Canada.