6. Cohn, G. L., Bondy, P. K., *Clin. Res.,* 1958, v6, 300.

7. Barniville, H. T. F., Misk, R., *Brit. M. J.,* 1959, v1, 337.

8. Kunin, C. M., Glazko, A. J., Finland, M., *J. Clin. Invest.,* 1959, v38, 1498.

9. Streeten, D. H. P., *Gastroenterology,* 1959, v37, 643.

10. Schneider, W. C., Hogeboom, G. H., *J. Biol. Chem.,* 1950, v183, 120.

11. Stotz, E., in Colowick, S. P., Kaplan, N. O., *Methods in Enzymology,* Academic Press, N. Y., 1955, v2, 743.

12. Strominger, J. L., Maxwell, E. S., Axelrod, J., Kalckar, H. M., *J. Biol. Chem.,* 1957, v224, 79.

13. Myron, J., *Acta Path. et Microbiol. Scandinav.,* Suppl., 1956, v116.

14. Schmid, R., Axelrod, J., Hammaker, L., Swarm, R., *J. Clin. Invest.,* 1958, v37, 1123.

15. Arias, I. M., *Bull. N. Y. Acad. Med.,* 1959, v35, 450.

16. Chojecki, Z., Kern, F., Jr., *Clin. Res.,* 1959, v7, 391.

17. Isselbacher, K. J., McCarthy, E. A., *J. Clin. Invest.,* 1959, v38, 645.

18. Bollman, J. L., *Gastroenterology,* 1959, v36, 169.

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Placenta and Salivary Gland Fluoride Transfer in the Rat.* (25683)

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Concentration of fluoride in drinking water of a pregnant rat must be approximately 10 ppm before an appreciable placenta transfer of fluoride occurs(1). In these studies fluoride transfer occurred by comparing fluoride concentration in pups whose mothers received varying levels of dietary fluoride with comparable controls receiving no fluoride. Such a technic provides for no biological estimation of effect of fluoride. It is possible that even micro-quantities of fluoride transferred through the placenta could result in some anticariogenic effects. For example, Jenkins(2) demonstrated that concentrations of fluoride as low as 1 ppm have a detectable inhibitory effect on salivary acid production. Therefore, to estimate one effect of fluoride, namely, its antisolubility, pregnant rats were given fluoride at different gestation and lactation periods and solubility of pups' molar teeth evaluated for acid solubility.

Materials and methods. Eight pregnant Sprague-Dawley strain rats divided into 4 groups were housed in pairs in raised screen cages in air-conditioned room. Fluoride-free filter paper was placed in each cage for the mother to make nests for her pups. Group I was composed of 2 pregnant rats which served as controls and received fluoride-free drinking water. Pregnant rats in Group II received 50 ppm F (as NaF) in drinking water during pregnancy. One day before delivery she was placed in separate cage, and upon delivery she and her pups were placed on fluoride-free drinking water. Group III was composed of pregnant rats which received the same concentration of fluoridated drinking water as animals in Group II, except that they received fluoride both during pregnancy and lactation, while rats in Group IV received same concentration of fluoride during lactation. All animals received the same stock non-cariogenic diet (F = 0.01 ppm). All pups were sacrificed at 30 days of age and their mandibular teeth prepared for estimation of acid solubility by methods previously described(3). Due to high concentration of fluoride used, it was possible that some fluoride could pass into the oral cavity from salivary tissues and cause a reduction in acid solubility to molar teeth. Since this effect could not be divorced from the antisolubility effect resulting from conversion by blood fluoride of a portion of the hydroxyapatite in developing rat molars to fluorapatite, a further study was planned to investigate the effect of fluoride availability in

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TABLE I. Acid Solubility of Mandibular Teeth of Rats Whose Mothers Received 50 ppm F/Day in Drinking Water during Various Periods of Pregnancy and Lactation.

Group	No. of pups	Mean P dissolved in 20 min. (μg)
Control	9	63 ± 3*
F during pregnancy	8	57 ± 2
F during pregnancy and lactation	8	56 ± 3
F during lactation	6	61 ± 2

* Stand. dev.

TABLE II. Comparison of Acid Solubility of Rat Molar Teeth Placed in Various Solutions.

Group	pH of solution	μg P removed	"P"*
Distilled water	5.8	54 ± 4†	
Saliva only	8.0	59 ± 7	
Saliva from rats receiving 2 mg F/day	8.0	55 ± 5	
Saliva to which 400 ppm F was added	8.0	41 ± 3	.02
Aqueous fluoride (400 ppm)	5.5	39 ± 3	.01

* Calculated by means of Student "t" test.
† Stand. dev.

saliva. Intact embedded molar rat teeth were placed in various aqueous fluoride solutions or saliva solutions to which fluoride had been added, and their acid solubility subsequently evaluated. In this study there were 5 groups. Group I evaluated effect of rat molar solubility in aqueous solution of distilled water and served as controls. Group II received distilled water and Group III received 2 mg F/day (as NaF) for 2 weeks. Group IV consisted of evaluation of the effect of rat saliva to which 400 ppm of F (as NaF) was added and Group V compared the effect of 400 ppm F (as sodium fluoride) as an aqueous solution. In Groups II and III, 2 ml of saliva were collected according to technics previously described(4) into which molar rat teeth were placed. Estimations of enamel solubility were made by placing hemi-jaws prepared for acid solubility determination in respective solutions for 16 hours according to methods previously described(3).

Results are found in Tables I and II. The data in Table I show that solubility of mandibular teeth of rats whose mothers received fluoride during entire pregnancy, at a level known to pass the placenta (Group II) was 57 μg P. Similar rats whose mothers received fluoride both during pregnancy and lactation had an acid solubility of 56 μg P, while rats whose mothers received fluoride only during lactation had an acid solubility of 61 μg P. Only the solubility of molar teeth of animals in Groups II and III was significantly different from control group ($P = 0.05$).

The data in Table II show that rat molar teeth placed in distilled water had an acid solubility of 54 μg P. When similar rat molars were placed in saliva 59 μg of P was removed. When saliva from rats which received 2 mg F/day for 14 days (containing approximately 1 ppm F) 55 μg P was removed. If 400 ppm F were added to saliva from control animals, and rat molar solubility evaluated, 41 μg P was removed. This is significantly different from the control group at the 0.02 level of confidence. If an aqueous solution of fluoride at the same concentration was used as in Group IV, 39 μg P was removed. This is also significantly different from the control group ($P = .01$).

Discussion. Micro-concentrations of fluoride passing the placenta contribute significantly to the increased acid solubility of developing molar teeth of rats. These data are of interest to human nutrition since they suggest that mothers residing in a fluoride area may transfer sufficient fluoride to produce a measurable degree of resistance to dental caries in deciduous dentition. Of course, it is not possible to translate reductions in acid solubility in rat molar teeth unequivocally to reductions in human dental caries, but there is sufficient evidence to suggest that increased resistance to acid solubility is one mechanism by which fluoride reduces dental caries. When rats nursed from mothers receiving fluoride both during pregnancy and lactation added antisolubility benefits were not shown. This suggests that availability and biological activity of fluoride may be reduced when present in milk, as shown previously by other technics (5,6). Saliva also either inactivates the biological antisolubility effect of fluoride or under conditions of this study, sufficient fluoride did not pass the salivary barrier to affect solubility of the teeth. It is of considerable interest to learn the reason for these later find-

ings and to determine if salivary glands are selective in their failure to transfer (excrete or secrete) fluoride in contrast to the placenta. as well as to learn more about the biological availability of fluoride in saliva.

Conclusions. When pregnant rats receive 50 ppm F/day during entire pregnancy, their pups' molar teeth show a significant reduction in acid solubility. If fluoride administration is continued during lactation, no added anti-solubility effect is shown. Saliva either inactivates fluoride or salivary tissue is selective against fluoride secretion.

1. Buttner, G., Muhler, J. C., *J. Dent. Res.,* 1958, v37, 326.
2. Jenkins, G. N., *Arch. Oral Biol.,* 1959, v1, 33.
3. Buttner, W., Muhler, J. C., *J. Dent. Res.,* 1957, v36, 897.
4. ———, *ibid.,* 1958, v37, 860.
5. Muhler, J. C., Weddle, D. A., *J. Nutrition,* 1955, v55, 347.
6. Ericsson, Y., *Acta, Odont. Scand.,* 1958, v16, 51.

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A Hypoglycemic Factor in Leukemic Tumors. (25684)

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The syndrome of fibrogenic tumors and hypoglycemia is becoming more frequently recognized(1). Hypoglycemia was observed in rabbits inoculated with a homogenate of spindle cell sarcoma from rats(2); in mice with leukemic tumors(3); and in mice bearing transplantable pituitary tumors(4). We observed earlier considerable amelioration of alloxan diabetes in AKR mice inoculated with lymphatic leukemia tumor BW5147. Comparison of progression of growth of tumor with onset and magnitude of decrease in blood sugar in normal and diabetic animals suggested that increase in the mass of tumor alone did not explain hypoglycemia in these mice. Further studies employing C^{14} glucose revealed no excessive avidity of these tumors for glucose. The pancreas and adrenal glands from normal animals with tumor and from diabetic animals with and without tumor were studied. No islet cell hypertrophy or hyperplasia in normal animals with tumor was observed. The pancreas of tumor-bearing animals and of normal animals revealed equally severe damage to islet cells after administration of alloxan. Furthermore, as diabetes in tumor-bearing animals was ameliorated the pancreas did not show regeneration of islet cells. Adrenal glands of diabetic animals with and without tumor were similar. These findings suggested possible presence of a hypoglycemic factor in this tumor.

Methods. AKR mice and the tumor BW5147 of lymphatic leukemia were obtained from Jackson Memorial Lab., Bar Harbor, Me. Three types of extracts were prepared from 15-day-old tumors. At this age of tumors, mice exhibited profound hypoglycemia and tumors were large with little gross evidence of necrosis. All animals were initially inoculated subcutaneously into the right thigh with 50 mg of tumor extract.† The tumor was homogenized in Waring blendor with 4 ml of 0.9% solution of sodium chloride/g of tumor. The homogenate was refrigerated 48 hours, centrifuged and the supernatant decanted. The fluid was evaporated in a watch glass in air at room temperature to concentration of 1 g of tumor/0.5 ml of saline solution. A second extract was made as described by Scott and Fisher(5) for extraction of insulin. The final material for assay was reconstituted in saline at pH 2.5 so that the material extracted by insulin procedure from 1 g of tumor would be present in 0.5 ml of the acid saline solution. For the third extract the

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