castration, primarily hypertrophy of the reticularis(3). This area does elaborate sex hormones. However, histologic preparations from adrenal glands harvested from our infected and uninfected mice 2 to 3 weeks after castration revealed no hypertrophy. Furthermore, administration of testosterone following orchiectomy did not increase the susceptibility of experimental male animals to the test virus (Table III). The dose of the hormone preparation used for this experiment was adequate for replacement therapy. It is unlikely, therefore, that in the male the withdrawal of testicular androgen initially or the compensatory secretion of male sex hormone by the adrenal played an important part in the protective effect observed or in the delayed return to preoperative susceptibility.

Lastly, since sham-operated animals remained normally susceptible to Coxsackie B1 virus infection (Table I), it does not appear that the stress of operation alone influenced resistance. However, one cannot state unequivocally that the sham procedure was as debilitating to the experimental animal as true gonadectomy.

The following conclusions are consistent with our present knowledge: (1) gonadectomy decreased the susceptibility of the adult mouse to Coxsackie B1 virus infection and (2) the altered resistance was short lived and unrelated to the sex of the experimental animal. These findings make it unlikely that the sudden withdrawal of sex hormones following castration or the compensatory secretion of similar materials by the adrenal played an important part in the response observed.

Summary. The Coxsackie B1 virus used in this experiment was unusually virulent for adult "Swiss albino" mice; approximately 15% of the infected animals died and the survivors were severely ill. However, following gonadectomy the death rate fell significantly. This protective effect of castration was not sex-linked and was no longer present 30 days after surgery. Possible hormonal mechanisms responsible for this altered susceptibility are discussed.

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Calcium and Phosphorus Content of Ash of Bones and Teeth of Human Fetuses in Relation to Fluoride Content of Drinking Water.* (30274)

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(Introduced by F. G. Sulman)

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We have presented data(1,2) on the fluoride content of ash of bones and teeth from 4-9-month-old fetuses obtained from areas in which the concentration of fluoride in the drinking water was low (about 0.1 ppm), medium (0.5-0.6 ppm) or higher (about 1 ppm). The results revealed that the fluoride content of fetal bones and teeth increased with increasing age of the fetus when the fluoride concentration in the drinking water was medium or high, but did not increase with age when the fluoride concentration in

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TABLE I. Calcium and Phosphorus Contents of Ashed Fetal Skeletal Tissues. Drinking water contained about 0.1 ppm F.

Fetal age, mo	No. of cases	Mean calcium value of ash (%)						Mean phosphorus value of ash (%)					
		Femur	SD	Mandible	SD	Teeth	SD	Femur	SD	Mandible	SD	Teeth	SD
5	9	33.22	.87	33.86	.62	—	—	18.26	.46	18.60	.42	—	—
6	14	33.63	1.34	34.04	1.22	31.50	.87	18.57	.56	18.55	.45	18.23	.40
7	20	34.36	1.36	34.44	1.22	33.92	1.36	18.73	.63	18.60	.40	18.83	.35
8	13	34.15	1.25	34.30	1.38	33.81	.88	18.60	.50	18.50	.55	18.10	.50
9	17	34.00	1.16	34.18	1.10	34.35	1.01	18.44	.55	18.31	.60	18.42	.43

TABLE II. Calcium and Phosphorus Contents of Ashed Fetal Skeletal Tissues. Drinking water contained about 0.5-0.6 ppm F.

Fetal age, mo	No. of cases	Mean calcium value of ash (%)						Mean phosphorus value of ash (%)					
		Femur	SD	Mandible	SD	Teeth	SD	Femur	SD	Mandible	SD	Teeth	SD
5	13	35.29	.98	34.93	.98	—	—	18.80	.50	18.70	.41	—	—
6	13	34.54	.83	34.54	.72	36.00	—	18.60	.45	18.65	.48	18.55	—
7	17	34.94	.77	35.12	.87	36.25	—	18.45	.42	18.65	.43	18.35	—
8	7	34.93	.45	35.29	.95	35.05	.74	18.90	.55	18.80	.55	18.30	.41
9	27	35.19	1.11	35.13	.73	35.06	.91	18.15	.48	18.50	.50	18.45	.35

the drinking water was low.

This investigation was undertaken to study the effect of different concentrations of fluoride in the drinking water on the calcium and phosphorus contents of the ash of fetal bones and teeth.

Materials and methods. Estimations of calcium and phosphorus were carried out on the ash of the femoral bone, mandibular bone and teeth of the same stillborn fetuses, aged 5-9 months, that were used in the investigations of fluoride metabolism(1,2). The fetuses were from women living during pregnancy in areas supplied by drinking water containing about 0.1 ppm F (83 fetuses), 0.5-0.6 ppm F (77 fetuses) and about 1 ppm F (70 fetuses). Fetal age determinations and preparation of bone and tooth ash have been described(1). Calcium was estimated by a modification of the procedure designed for blood serum(3) in which the calcium is precipitated as oxalate from a 0.1 N HCl solution of a weighed ash sample. The calcium oxalate was then dissolved in a measured excess of buffered EDTA and the excess chelating agent was determined by back titration with standard $MgCl_2$ solution using eriochrome black as indicator. The experimental error of this method is not more than ± 5%. Phosphorus was determined according to the colorimetric method of Fiske and Subbarow(4). The experimental error of this method was within ± 5%. In fetuses 5-7 months of age, tooth germs of several fetuses had to be pooled for the determinations. Pooling was carried out in fetuses from the same area, of the same age and with similar fluoride content in the femora and mandibles(1).

Results. The results are summarized in Tables I-IV. The mean calcium content of the bone and tooth ash of fetuses from low-fluoride areas was lower than the mean calcium content of the bone and tooth ash of fetuses from medium-fluoride areas. Mean calcium content of the bone and tooth ash of fetuses from medium-fluoride areas was lower than mean calcium content of the bone and tooth ash of fetuses from high-fluoride areas. The difference between the mean calcium content of the ash of bones and teeth of 5- or 9-month-old fetuses from low- and medium-, or medium- and high-fluoride areas, was significant ($p<0.01$). In the low-fluoride area the mean calcium content of the bone and tooth ash of the younger fetuses was significantly lower than was found in the older fetuses ($p<0.05$). No difference was found between the mean phosphorus contents of the bone and tooth ash of fetuses from the different drinking water fluoride areas. The

TABLE III. Calcium and Phosphorus Contents of Ashed Fetal Skeletal Tissues. Drinking water contained about 1 ppm F.

Fetal age, mo	No. of cases	Mean calcium value of ash (%)						Mean phosphorus value of ash (%)					
		Femur	SD	Mandible	SD	Teeth	SD	Femur	SD	Mandible	SD	Teeth	SD
4	4	37.25	.96	36.50	.58	—	—	18.82	.45	18.60	.48	—	—
5	9	36.72	1.00	37.00	.83	—	—	18.80	.30	18.67	.37	—	—
6	15	36.94	.91	36.45	.89	35.12	.48	18.60	.50	18.50	.40	18.50	.45
7	8	37.00	.89	36.45	1.08	37.25	.88	18.77	.55	18.50	.45	18.70	.30
8	13	36.95	1.07	36.74	1.27	36.42	.63	18.50	.40	18.40	.46	18.56	.32
9	21	36.26	1.16	36.27	1.58	36.56	1.28	18.66	.35	18.71	.35	18.60	.48

TABLE IV. Calcium and Phosphorus Weight Ratios.

Fluoride content in drinking water	Ca/P ratio in femur ash		Ca/P ratio in mandible ash		Ca/P ratio in tooth ash	
	5 mo old fetuses	9 mo old fetuses	5 mo old fetuses	9 mo old fetuses	6 mo old fetuses	9 mo old fetuses
Low (about .1 ppm)	1.85	1.84	1.82	1.86	1.73	1.86
Medium (.5-.6 ppm)	1.88	1.94	1.87	1.90	1.96	1.90
High (about 1 ppm)	1.98	1.94	1.90	1.93	1.90	1.90

results presented in Table IV indicate that the Ca/P ratios in fetal bones and teeth increase with increasing fluoride concentration in the drinking water.

Discussion. Our results revealed significant differences in calcium content of bone and tooth ash of human fetuses, dependent upon fluoride intake of the mother from the drinking water during pregnancy without any difference in the phosphorus content (Tables I-III). Higher contents of fluoride in cord blood in regions of high-fluoride concentration in the drinking water probably increase the proportion of the precipitated fluorapatite of higher Ca/P ratios in the hard tissues of the fetus (Table IV). In regions where the fluoride concentration in the drinking water is low, Ca/P ratios in the precipitated hydroxyapatite are probably lower. According to Brudevold *et al*(5), traces of fluoride (of the order of 0.2 ppm) when present in a calcifying solution of pH 6.1-7.4 greatly accelerate hydroxyapatite crystallization. In experiments carried out by Kuyper and Kutnerian(6), addition of about 3 ppm of fluoride or even less to a suspension of bone salts in physiological saline precipitated the calcium and phosphate from the solution as fluorapatite.

According to Likins *et al*(7), bone and tooth mineral with low Ca/P ratios is more soluble in acid. A higher content of fluorapatite, which is known to be less soluble than hydroxyapatite, may decrease bone resorption. Prolonged use of drinking water low in fluoride has been associated with a higher incidence of osteoporosis in humans(8). An increase in the calcium content of the bone ash of young rats drinking water of 50 ppm F for 4 weeks as compared to controls on distilled water was reported previously(9). Menczel *et al*(10) found no difference in the calcium and phosphorus contents of the bone ash of rats which had drunk distilled or fluoridated water (50 ppm) for 320 days, and Zipkin *et al*(11) did not observe differences in calcium and phosphorus contents of the bone ash of human beings who had drunk water containing up to 4.0 ppm fluoride. The differences between the results obtained by Menczel *et al*(10) and Zipkin *et al*(11), on the one hand, and those reported by us in this and the previous study(9) may possibly be due to the much younger age of the human beings and rats in our experiments. Dallemagne(12) and MacGregor and Brown(13) are of the opinion that the mineral part of newly deposited bone and tooth mineral is not exclusively hydroxyapatite, but that it contains also octocalcium phosphate with a lower Ca/P ratio. Thus the lower calcium values in bones and teeth of the younger fetuses from the low fluoride areas may be explained (Table I).

Summary. The calcium and phosphorus contents of the ash of femora, mandibles and teeth were determined in 230 fetuses, aged 5-9 months, in areas supplied by drinking water containing about 0.1 ppm F (low), 0.5-0.6 ppm F (medium) and about 1 ppm F (high). Significant differences in the mean calcium content of bone and tooth ash, depending upon fluoride intake of the mother during pregnancy from the drinking water, without any change in the mean phosphorus content, were demonstrated. In the low-fluoride area the mean calcium content of the bone and tooth ash of the younger fetuses was lower than was found in the older fetuses, whereas in medium- and high-fluoride areas no such difference was found. The observed differences in Ca/P ratios of the hard tissues of the fetus may possibly be due to the different constitution of the precipitated apatitic calcium phosphates.

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Ingestion of Soluble Ferritin-Antiferritin Complexes by Phagocytic Cells.* (30275)

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The ingestion of soluble antigen-antibody complexes by spleen cells in an *in vitro* system has been demonstrated by isotope tracer and immunofluorescent techniques(1). The previous investigation utilized albumin-antialbumin complexes prepared in varying degrees of antigen excess and the uptake of the complexes by spleen cells *in vitro* was related to the degree of antigen excess used in preparation of the soluble complexes.

Ferritin has been used similarly here to study the uptake of soluble ferritin-antiferritin complexes in an *in vitro* system. Ferritin, which can be identified readily in electron micrographs, is antigenic(2), readily purified by dextran gel chromatography(3) and can be trace labelled with I^{131}(3).

Materials and methods. Antigen and antibody. Horse spleen ferritin (HSF) was obtained commercially (Pentex Corp., Kankakee, Ill.) and further purified by chromatography through Sephadex G-200(3). Nitro-

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