were tested in immature hypophysectomized recipients. The growth-stimulating potency of normal rat plasma was equivalent to that recently reported by Contopoulos *et al.*(9).

Summary. Follicle-stimulating activity was detected in blood plasma of adult female castrate rats as early as 7 days post-operative by injection of plasma into immature hypophysectomized female rats for a 4-day period. Follicle-stimulating activity continued to increase slowly but progressively as the post-operative interval increased to a 4 months' period. With this bioassay method no direct evidence for circulating interstitial cell-stimulating activity was found at the highest dosage level tested.

were split mid-sagitally, fixed in formol, and stained with $AgNO_3$(8). The uncalcified portion of proximal epiphyseal cartilage was measured with micrometer eyepiece and average widths of 200 μ or greater (i.e., approximately 50% increase over uninjected control widths) were considered evidence for growth-stimulating activity.

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Relationship of Fluoride and Dietary Fats to Serum Cholesterol in Rats.* (24126)

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It was shown(1) that doses of 0.5, 1 or 2 mg fluoride as aqueous solutions of sodium fluoride daily did not alter normal serum cholesterol level in the rat. Since Miller and Phillips(2) have found an enhancement of fluoride toxicity in rats on a diet containing 15% cottonseed oil, further work pertaining both to fluoride toxicity and serum cholesterol in which different sources of dietary fat are used seemed indicated.

Methods. A total of 150 weanling male Sprague-Dawley strain rats were divided equally into 5 series. Series A was divided into 3 groups, all receiving a stock sucrose diet. Group I animals received no fluoride in their drinking water, Group II received fluoride as sodium fluoride at concentration of 30 μg F/ml in the drinking water, and Group III received 2 mg of fluoride daily (as sodium fluoride) by stomach tube. Each of the 4 remaining series was divided similarly into 3 groups and received the same amount of fluoride as Series A animals either in drinking water or by stomach tube, but the type of dietary fat was changed. The animals in Series B received cottonseed oil, those in Series C corn oil, Series D Crisco, and Series E lard. Fat was added at a level of 20%. Animals in Group I and III of each series received a fluoride low (F = 0.05 μg/ml) drinking water *ad libitum*. Composition of the experimental diets which were also available *ad libitum* are summarized in Table I. All animals were housed individually in raised screen cages in air-conditioned room and were weighed twice each week throughout experiment of 10 weeks. Serum cholesterol was determined at beginning of experiment and at weekly intervals (except for 6 and 9 week period) by methods previously described(1).

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TABLE I. Composition of Experimental Diets (g %).

Component of diet	Animal series A	B	C	D	E
Sucrose	60	45	45	45	45
Vit. test casein	30	30	30	30	30
Cottonseed oil	5	20			
Corn "			20		
Crisco				20	
Lard					20
Salt mixture*	4	4	4	4	4
Vit. " *	1	1	1	1	1
Oleum percomorphum†					

* For composition of salt and vitamin mixture, see *J. Nutr.*, 1954, v54, 481.
† 15 drops/kilo diet.

Results. Body weight gains for animals receiving various experimental diets (Table II) indicate that all diets produced relatively good weight gains.

Serum cholesterol values (Table III) indicate that there is a steady increase in serum cholesterol with age of animals. Type of fat or amount of fluoride has little effect on the serum cholesterol level.

In Table IV the weekly mean serum cholesterol data are presented. Again the steady increase in serum cholesterol with the age of animals is evident.

Table V shows mean serum cholesterol level obtained by averaging the 10 weekly analysis in each dietary fat series, in the control group, and in the fluoride groups I and III. Vertical averages in this Table indicate the effect of varying fluoride levels on serum cholesterol; horizontal averages show the effect of fat on serum cholesterol level. The similarity of values in any direction demonstrates that neither fluoride nor any of the diet variables caused significant changes in serum cholesterol level.

TABLE II. Weekly Weight Gain of Animals Receiving Various Experimental Diets. (In each series 30 animals equally divided into 3 groups.)

Dietary group	Wt (g) Initial*	5 wk	10 wk
A Sucrose	92	208	244
+ 30 μg F/ml	98	194	252
+ 2 mg F/da	90	196	238
B Cottonseed oil	96	195	252
+ 30 μg F/ml	96	191	250
+ 2 mg F/da	103	215	272
C Corn oil	118	240	320
+ 30 μg F/ml	107	217	261
+ 2 mg F/da	110	232	281
D Crisco	120	228	293
+ 30 μg F/ml	112	203	254
+ 2 mg F/da	104	204	226
E Lard	117	212	231
+ 30 μg F/ml	126	227	260
+ 2 mg F/da	114	200	230

* Data were obtained at weekly intervals and will be provided upon request.

TABLE III. Effect of Various Experimental Diets on Serum Cholesterol Level in the Rat. (In each series 30 animals equally divided into 3 groups.)

Series	Weekly serum cholesterol levels (mg %) 1 wk*	5 wk	10 wk
A Sucrose	98 ± 4†	117 ± 3	124 ± 7
+ 30 μg F/ml	114 ± 2	121 ± 3	114 ± 5
+ 2 mg F/da	97 ± 3	110 ± 5	108 ± 6
B Cottonseed oil	100 ± 2	111 ± 4	116 ± 5
+ 30 μg F/ml	98 ± 4	113 ± 4	150 ± 5
+ 2 mg F/da	92 ± 5	100 ± 4	137 ± 4
C Corn oil	100 ± 4	121 ± 8	138 ± 2
+ 30 μg F/ml	94 ± 4	104 ± 6	140 ± 7
+ 2 mg F/da	94 ± 4	103 ± 5	130 ± 5
D Crisco	84 ± 1	119 ± 2	129 ± 7
+ 30 μg F/ml	95 ± 4	125 ± 4	150 ±11
+ 2 mg F/da	78 ± 5	134 ± 6	135 ± 5
E Lard	110 ± 5	109 ± 5	124 ± 8
+ 30 μg F/ml	81 ± 4	108 ± 4	115 ± 2
+ 2 mg F/da	98 ± 6	92 ± 3	110 ± 5

* Determinations were made at weekly intervals and will be provided upon request.
† Stand. dev.

TABLE IV. Increase in Serum Cholesterol as a Function of Age (mg %, 30 Rats/Series).

Series	Weeks 1	2	3	4	5	7	8	10
A Sucrose	103	109	113	112	116	105	118	115
B Cotton-seed oil	97	105	108	105	108	108	166	134
C Corn oil	96	98	102	109	109	138	114	136
D Crisco	86	90	105	111	126	123	108	138
E Lard	96	115	124	98	103	155	138	116
Avg	96	103	110	107	112	126	129	128

Discussion. As judged by body weight increases, the effect of 20% fat in the diet does not seem to increase toxicity of fluoride when compared to a similar diet containing only 5% dietary fat. Furthermore, in all cases the animals which received fluoride weighed as much if not more than their respective controls not receiving fluorides, except in Series C and D where corn oil and Crisco, respectively, were the sources of

TABLE V. Effect of Fat and Fluoride Level on Serum Cholesterol (mg %, 30 Rats in Each Series, Equally Divided into 3 Groups).

	Series A	B	C	D	E	
Group	Sucrose	Cottonseed oil	Corn oil	Crisco	Lard	Avg
I No fluoride	110	113	115	112	123	115
II 30 μg F/ml	116	120	115	112	115	115
III 2 mg F/da	109	116	108	109	117	112
Avg	112	116	113	111	118	

dietary fat. Even in animals receiving the latter 2 diets the final weight difference of the groups receiving fluoride as compared to their controls does not suggest an increased fluoride toxicity since, unfortunately, the initial weight of the fluoride animals was less than the respective controls, thus making actual final weight comparison less meaningful. For animals receiving 2 mg of fluoride a day, this is approximately 600 times as much fluoride as would be received by a 170 lb man receiving 1 mg fluoride a day, an amount considered optimal for dental health.

It is evident that at times during experimental period there are what appear to be pronounced increases in cholesterol level, but upon subsequent measurement the high values return to approximately the mean for the non-fluoride groups. Fluorides, whether administered in drinking water or by stomach tube are without significant effect.

These data suggest that the animals receiving diets containing 20% fat have a tendency to higher serum cholesterol levels than animals receiving 5% fat-containing control diet. However, in animals receiving 20% cottonseed oil, the increase occurred only during the eighth week, and by the end of the tenth week the serum cholesterol in the control 20% cottonseed oil group was less than the control sucrose group (containing 5% cottonseed oil). Similarly, the increase in serum cholesterol in the corn oil and Crisco group occurred during the tenth week. The final serum cholesterol levels for the lard groups were quite similar to the sucrose groups. Thus, these data serve to illustrate the large, but presumably normal, variation which occurs between groups during an experiment. When a statistical analysis was carried out, no significant differences were found between any of the groups in this study.

Obviously, one is concerned about the role of cholesterol metabolism and its suggested relationship to cardio-vascular disease(3). Since fluoridation is an accepted anticaries public health procedure one must be doubly concerned not to increase cholesterol level by use of fluorides. These data, as well as those obtained in a previous study(1) would suggest that the use of fluorides, even at very high levels as in this study, is not associated with changes in cholesterol metabolism. Some caution is needed in relating these data to those obtained in the human since the rat is considered by some workers to be moderately resistant to slight changes in serum cholesterol level.

Summary. Serum cholesterol level was determined in 5 series of rats receiving either different fats in their diets or different fats plus fluoride to determine whether the use of fluoride is associated with an increased serum cholesterol level. After administration of 2 mg of fluoride daily throughout a 10-week period there was no significant change in serum cholesterol level of rats regardless of their dietary fat source or the presence of fluoride. Comparison of body weight gains of the various groups fails to suggest that any of the fats used resulted in increased fluoride toxicity.

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