carbonic acid with subsequent ionization into a hydrogen ion and a bicarbonate ion. The assumption that hydrogen ions so formed are transferred to blood or extravascular fluid while sodium ions from blood are transferred to cells, to become associated with bicarbonate ions and be secreted in the saliva, would account for the rise in pH, sodium, and bicarbonate levels produced by increasing secretion rates.

Summary. Stimulated parotid saliva was collected from 50 individuals using flavored chicle as the stimulatory agent. Analysis of the flow rate and composition indicated a significant positive correlation between the flow rate and the pH, sodium, calcium, and bicarbonate contents. Intercorrelations between the various salivary constituents were also noted. No differences were found in the composition of saliva samples collected in the fasting and postprandial state. The composition of the parotid secretion was found to vary not only from person to person but between the two glands in the same person.

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Effect of Glucagon on Growth of Chick Embryo. (23799)

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It has been reported that glucagon causes a significant increase in the weight of Leghorn chick embryos and this finding has been used as evidence that glucagon is a growth-stimulating factor(1). In these experiments a single dose of glucagon (5 or 25 μg) was injected "in" the chorioallantoic membrane on the ninth day of incubation, and the embryos weighed 4, 7 or 9 days later. Since relatively crude preparations of glucagon* were used in these studies, it was felt that the experiments should be repeated using highly purified, crystalline glucagon.

Methods. Five experiments were done using a total of 113 control and 111 experimental White Leghorn x Delaware (Hyline strain) chick embryos, incubated at 37°C in a forced-draft incubator. The doses of crystalline glucagon† per embryo ranged from 5 to 80 μg administered in 1 to 5 injections over the course of 1 to 9 days. The injections were done according to the following technics: After puncturing the air space with a sharp needle, a hole was carefully drilled through the shell over the embryo. The shell membrane was moistened with sterile saline and carefully torn with a needle. After ascertaining that the chorioallantoic membrane had dropped, the tip of a hypodermic needle was inserted into the opening and the appropriate volume of solution delivered. The openings in the shell were sealed with paraffin, after

* Lot No. 208-108B-234: contains 20% of the activity of crystalline glucagon.

† Prepared by Dr. A. Staub according to the method of Staub *et al.*(3).

TABLE I. Effect of Glucagon on Chick Embryo Weight.

Exp.	No. of embryos	Dose of glucagon (μg)	Age of embryos (days) at Onset of exp.	Age of embryos (days) at End of exp.	Mean wt of embryos (g)	Diff. between control and exp. (g)	Significance of diff. (p)	Mortality, No. (%)
1	Cont. 12	0	9	½: 16 ½: 18*	16.54	+ .67	.54	0
	Exp. 11	5	9	" "	17.22			1 (8.3)
	Cont. 15	0	9	" "	16.50	+ .25	.82	1 (5.5)
	Exp. 16	10	9	" "	16.75			0
2	Cont. 19	0	9	19	18.63	+1.38	.07	2 (10)
	Exp. 21	10 × 4	9	19	20.01			0
3	Cont. 24	0	9	17	15.06	- .20	.70	0
	Exp. 24	20 × 4	9	17	14.86			0
4	Cont. 20	0	11	19	21.00	- .67	.30	4 (16.6)
	Exp. 24	5 × 5	11	19	20.33			0
5	Cont. 23	0	11	19	21.81	-1.17	.22	0
	Exp. 15	2.5 × 4	11	19	20.63			8 (34.7)

* In view of the small No. of cases in this exp., the means for 16- and 18-day embryos in each of the 2 groups (Cont. and Exp.) were averaged.

the injection, and the egg returned to the incubator. The glucagon solutions were made up freshly for each experiment using sterile Pannett-Compton's solution(2) after dissolving the glucagon in 2 drops of 0.2% NaOH. The glucagon concentration was adjusted so that each dose was administered in a volume of 0.2 ml. Control embryos were injected with 0.2 ml of the Pannett-Compton-NaOH solution. At the end of each experiment the embryos were removed from their shell and extra-embryonic membranes. They were then carefully dried with filter paper and weighed within 2 minutes to avoid weighing errors due to desiccation.

Results. Table I gives a summary of the data from the 5 experiments. It is apparent that highly purified, crystalline glucagon administered in a wide range of doses has no significant effect on chick embryo weight.

The present findings do not confirm the report of Cavallero that glucagon increases chick embryo weight(1). The experimental conditions used were similar to those used by Cavallero except for the difference in the purity of the glucagon used. Furthermore, there was no dose-response relationship in Cavallero's experiments, a 25 μg dose of glucagon did not elicit a greater response than that achieved with a 5 μg dose. These considerations suggest that Cavallero's findings may have been due to a contaminant(s) in his relatively impure glucagon preparation. It is of interest that one of us reported(4) a growth stimulating effect in the young, hypophysectomized rat with crude glucagon preparations and that this effect could not be confirmed by subsequent workers(5,6) who used much purer glucagon preparations.

By the last third of the incubation period the growth of the chick embryo is probably under the control of its own endocrine system. Inhibition of thyroid function by goitrogenic compounds(7,8,9) or removal of the developing hypophysis by "partial decapitation"(10, 11,12) results in failure to attain normal body weight.

Growth stimulation in the chick embryo has been reported from thyroxine(13) or pituitary growth hormone(14) administration. Insulin, although causing a hypoglycemic and a teratogenic effect when administered to the young embryo(15) does not alter body weight when given at later stages(1).

Summary. The effect of highly purified, crystalline glucagon on the weight of chick embryos was studied in a series of 5 experiments and using a wide range of doses. No significant effect on weight of embryos was observed. It is suggested that a previous report of significant increases in embryo weight induced by glucagon may have been due to contaminants in the relatively crude glucagon preparation used.

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Hypercholesteremia and Atherosclerosis Induced in Rabbits by Purified High Fat Rations Devoid of Cholesterol.* (23800)

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Investigations in humans(1-6) have recently established that a difference in cholesterol levels can be induced by dietary use of certain vegetable oils and some fish oils as compared to hard animal fats and hydrogenated fats. A large dietary intake of certain hard animal fats or hydrogenated fat is capable of elevating blood cholesterol levels. Conversely highly unsaturated vegetable oils either have no such effect or will actually lower plasma cholesterol levels. Speculation as to agents in fats or oils which affect cholesteremia has included: saturated or unsaturated fatty acids(1), essential or polyunsaturated fatty acids(7), beta-sitosterol(8) and short chain fatty acids(1). Following the classic studies of Anitschkow(9) numerous investigators have produced hypercholesteremia and aortic atheroma in rabbits by adding cholesterol to commercial rabbit feeds. Kritchevsky *et al.*(10) showed that type of fat was important in determining extent of this effect. They found that, in the presence of exogenous cholesterol, partially hydrogenated vegetable shortening produced higher blood cholesterol values and more severe aortic lesions than did corn oil. In the absence of dietary cholesterol, Kritchevsky found negligible atheroma production with either vegetable shortening (Primex) or corn oil when both were fed at 9% of the diet. This latter finding led Deuel and Rieser(11) to the conclusion that, "These data likewise refute the concept that fat *per se* has an atherogenic effect." Steiner and Dayton(12) more recently reported that blood cholesterol levels could be elevated 4-5 times in rabbits without dietary cholesterol by prolonged feeding of diets containing 50-75% ground peanuts. They also reported small areas of gross aortic atherosclerosis in 2 of 33 rabbits after 5 to 12 months, thus suggesting that atherosclerosis could be induced by diets low in cholesterol. Use of diets of known controlled chemical composition makes possible more accurate control of all dietary components than can be attained with the usual rabbit diet. In this paper we describe 2 rabbit experiments using the purified diet of Thacker(13).

Methods. Male New Zealand albino rabbits averaging 1.8 kg in weight were housed in individual cages. They were divided into groups of comparable weight. Diet consumption was estimated daily and body weight weekly. Rabbits were bled from the marginal ear vein before initial feeding and at suitable

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