min mixture not containing tocopherol (Diet D) had no significant lesions even though they did not appear to ingest more spaghetti than the rats fed the same diet without vitamins. The substitution of glucose for wine, without the addition of vitamins (Diet C) somewhat prolonged the time of survival, and at the same time modified the nature of the lesion so that fatty infiltration and fibrosis developed rather than necrosis. It is noteworthy that the glucose-fed rats obtained relatively more of their calories from glucose and less from spaghetti, when compared to wine-fed rats. The spaghetti was the sole source of choline and methionine.

Summary. Significant hepatic lesions, consisting of diffuse parenchymal necrosis and marked fatty infiltration, have been produced in rats fed diets of spaghetti, olive oil and wine or glucose solution. Both lesions were associated with a moderate degree of fibrosis. The specific dietary factors responsible for the changes were not ascertained.

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Lipides Distribution and Phospholipide Turnover in Tissues of Rabbit Shortly After Growth Hormone. (22117)

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The concept that arteriosclerosis is a disease of metabolism has focused attention on endocrine control of lipide metabolism and their relationship to development of arterial degenerative lesions. It is increasingly evident that for elucidation of this relationship more information is required of the modes of action and nature of effects of hormones on lipide metabolism in different tissues. Certain adrenocortical steroids(1-3) and epinephrine (3-5) were reported to exert an influence on lipide distribution in tissues and development of aortic degenerative changes. There is ample evidence that growth hormone causes accelerated mobilization and catabolism of endogenous fat(6-10), but there is little information concerning the effect of growth hormone on lipide partition in tissues. Recently, Greenbaum and MacLean(7) studied distribution of lipides in plasma and liver of intact rats at intervals after treatment with growth hormone. Greatest changes occurred 6 hours after growth hormone when plasma and liver neutral fat and plasma phospholipide were significantly increased. The authors suggested that increased plasma phospholipide could be explained by accelerated synthesis of this lipide since Geschwind *et al.*(11) reported increased turnover of liver phospholipide in normal and hypophysectomized rats after 14 days or more of treatment with growth hormone. Mayer and Jones(12) reported a significant, but slight, increase in blood cholesterol of mice after 2 weeks treatment with growth hormone.

The present study was undertaken to determine concurrently lipide partition and phospholipide turnover in plasma, liver and aorta of intact rabbits a few hours after treatment with growth hormone. Knowledge of phospholipide-cholesterol-neutral fat relationships and phospholipide metabolism in the above tissues was a necessary preliminary to a study of the influence of growth hormone on lipide

TABLE I. Summarized Data of the Effect of Growth Hormone on Body and Organ Weights, Plasma Glucose, Liver Glycogen.

	Plasma glucose, mg %	Liver glycogen, g %	Liver wt, g/kg	Aorta wt, g	Body wt, kg
Controls (9)	118 ± 1.7*	3.44 ± .35	29.0 ± 1.2	.409 (.300-.472) †	2.4 (1.9-2.7)
G.H. (9)	132 ± 2.3	4.43 ± .19	35.5 ± 1.2	.404 (.346-.466)	2.4 (2.2-2.7)
P =	<.001	.02	.004	ns‡	ns

* Mean ± S.E. † Mean and range of values in group. ‡ ns = not significant.
"P" = Probability of difference between means occurring by chance alone.

metabolism in experimental atherosclerotic rabbits.

Materials and methods. New Zealand white female rabbits of pedigreed stock were given a rabbit pellet diet and tap water *ad libitum* for several weeks prior to day of experiment. Food and water were not available during experimental period. Control animals received a single subcutaneous injection of physiological saline (0.5 ml/kg body weight). Another group of rabbits received a single subcutaneous injection of 5 mg/kg of anterior pituitary purified growth hormone (Armour Co., Lot #M308)* in sterile saline 10 mg/ml. Immediately after injection of the placebo or growth hormone, 0.5 mc of radioactive phosphate (P-32)† was given intravenously. Sample of heart blood (4-5 ml) was obtained 3 hours after P-32 injection for determination of radioactive and chemical plasma acid-soluble phosphates(13). Six hours after initial hormone or saline injection a large sample (30-40 ml) of cardiac blood was obtained, the animal then killed with intravenously administered overdose of EVIPAL (n-methyl-cyclo-hexenyl-methylbarbituric acid). Whole liver and aorta were removed. A section of liver (approx. 0.5 g) was taken for determination of liver glycogen(14,15). Plasma glucose was determined by the method of Nelson(16). A section of liver (about 2 g) was placed in ice-cold 10% trichloracetic acid and used for determination of inorganic and organic acid-soluble phosphates(17). Following homogenization of the liver sample, neutralization of filtrate to phenol red, and addition of magnesia mixture the samples were kept in the refrigerator overnight to permit magnesium ammonium phosphate to settle (18). After centrifugation determinations were done for P-31 and P-32 on the supernatant (organic acid-soluble phosphates) and the acid-dissolved precipitate (inorganic acid-soluble phosphates). A large specimen of liver, the whole aorta, and a plasma aliquot of terminal blood sample were submitted to hot alcohol-ethyl ether extractions. Combined alcohol-ether extractions were evaporated under reduced pressure, and residue redissolved in petroleum ether (b.p. 30-60°C). Suitable aliquots of petroleum ether were used for chemical and radioactive determinations as follows: Total lipid was calculated from weight of residue after evaporation of petroleum ether and drying to constant weight. Lipide phosphorus was determined(13,19), and phospholipides were calculated from lipid-P values x 26. Total and free cholesterol were determined by the method of Schoenheimer and Sperry(20). Neutral fat was calculated by subtraction of phospholipide, free cholesterol, and cholesterol oleate concentrations from total fat value. For radioactivity determinations of phospholipide and acid-soluble phosphorus suitable aliquots were mounted on Whatman ash-free filter paper backed by aluminum foil, and samples allowed to evaporate. After covering samples with Scotch tape, they were counted with a Victoreen No. 1B85 Geiger tube for a minimum of 4000 counts.

Results. Inspection of the data in Table I shows that plasma glucose and liver glycogen concentrations, and total liver weight were moderately, but significantly, increased a few hours after growth hormone.

With the exception of liver ester choles-

* The growth hormone used was supplied through the generosity of Dr. Irby Bunding of Armour and Co.

† Obtained from Abbott Laboratories, Oak Ridge, Tenn. as sodium radio-phosphate, sterile solution.

TABLE II. Effect of Growth Hormone on Concentration of Lipide Fractions of Plasma, Liver and Aorta 6 Hours after Administration in Rabbits.

Groups	Total lipide	Phospho-lipide	Cholesterol			Neutral fat
			Total	Free	Ester	
			Liver lipide partition†			
Controls (9)	5.01 ± .15*	3.76 ± .07	.27 ± .01	.24 ± .008	.03 ± .008	.96 ± .13
G.H. (9)	6.24 ± .26	3.27 ± .09	.24 ± .01	.20 ± .008	.04 ± .006	2.54 ± .28
P =	.001	<.001	.05	.002	ns	<.001
			Plasma lipide partition			
Controls	.323 ± .031	.093 ± .012	.071 ± .007	.025 ± .002	.046 ± .005	.125 ± .015
G.H.	.599 ± .034	.154 ± .007	.114 ± .011	.043 ± .004	.072 ± .008	.245 ± .019
P =	<.001	<.001	.003	.001	.015	<.001
			Aorta lipide partition			
Controls	8.68 ± .61	.99 ± .03	.18 ± .003	.15 ± .004	.03 ± .004	7.49 ± .61
G.H.	6.61 ± .61	.95 ± .05	.19 ± .008	.16 ± .01	.03 ± .007	5.70 ± .58
P =	.025	ns	ns	ns	ns	.045

* Mean ± S.E. † All values expressed as g/100 g wet tissue wt or per 100 ml plasma.
"P" = Probability of difference between means occurring by chance alone.

terol, concentrations of the lipide fractions in plasma and liver were significantly changed following administration of growth hormone (Table II). Concentrations of liver phospholipide and free cholesterol were significantly decreased, while neutral fat was significantly elevated. Concentrations of all plasma lipide fractions were significantly increased after treatment with growth hormone. With regard to the aorta lipide partition, only a significantly decreased neutral fat was found in the treated group which was mainly responsible for depression of the aorta total lipide level.

In Table III are presented the specific activities of phospholipide of plasma, liver and aorta, and specific activities of acid-soluble phosphates of liver and plasma. Liver phospholipide specific activity was significantly increased in growth hormone-treated animals. Plasma phospholipide specific activity in the experimental group was not different from controls. However, this value, *per se*, is not a valid index of plasma phospholipide turnover since there had occurred a significant increase in chemical concentration of this lipide fraction. Calculation of the amount of radioactive phospholipide per ccm of plasma (specific activity × concentration) showed that the actual amount of plasma phospholipide synthesized during experimental period was 250% greater in

TABLE III. Effect of Growth Hormone on Turnover of Lipide and Acid-Soluble Phosphorus of Liver, Plasma, and Aorta.

Groups	Liver phospholipide-P		Plasma phospholipide-P		Aorta phospholipide-P	
	mg/100 g	S.A. × 10^3	mg/100 ml	S.A. × 10^3	mg/100 g	S.A. × 10^3
Controls (9)	148.7 ± 4.2*	9.5 ± .9	2.52 ± .5	3.5 ± .6	37.18 ± 2.7	5.6 ± .4
G.H. (9)	130.9 ± 2.7	13.1 ± 1.4	5.87 ± .6	3.8 ± .7	38.93 ± 2.2	6.9 ± .7
P =	ns	.05	<.01	ns†	ns	ns
	Plasma		Liver		Liver	
	Acid-soluble phosphate		Inorganic phosphate		Organic acid-soluble phosphate	
	mg/100 ml	S.A. × 10^3	mg/100 g	S.A. × 10^3	mg/100 g	S.A. × 10^3
Controls	5.75 ± .3	87.8 ± 8.5	25.0 ± .9	143.2 ± 15.0	105.0 ± 2.4	46.0 ± 5.3
G.H.	5.67 ± .2	93.1 ± 11.6	23.5 ± 1.5	136.3 ± 12.4	107.0 ± 4.7	41.9 ± 3.5
P =	ns	ns	ns	ns	ns	ns

* Mean ± S.E. † Amount of radioactive phosphorus (S.A. × conc.) incorporated in plasma phospholipides of rabbits injected with growth hormone was significantly greater than controls (P = < 0.001).
S.A. is specific activity expressed as percentage of injected dose/mg of phosphorus.
"P" = Probability of difference between means occurring by chance alone.

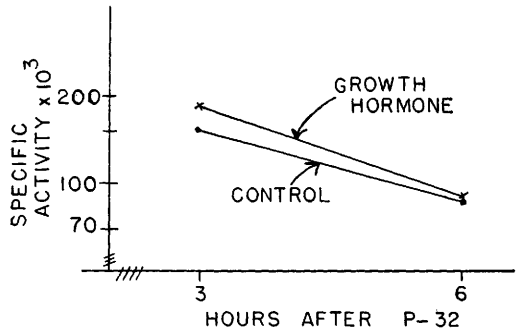

FIG. 1. Semi-logarithmic plot of the specific activities of plasma acid-soluble phosphate against time.

rabbits which received growth hormone than in controls. Inspection of terminal specific activities of acid-soluble phosphates of plasma and liver (Table III) and specific activity curves of plasma acid-soluble phosphate (Fig. 1) show that neither chemical concentration nor turnover of precursors of liver and plasma phospholipides were altered after growth hormone. It, therefore, may be concluded from the radioactivity data that accelerated formation of plasma and liver phospholipides occurred in treated rabbits. In contrast, the data show that specific activity of aortic phospholipide was not affected by growth hormone administration.

The magnitude of alterations (expressed as percentage change from controls) in various lipide fractions of plasma, and the entire liver and aorta of rabbits treated with growth hormone is shown in Fig. 2. Also shown for comparison are data recalculated from a recent experiment(5) illustrating changes in lipide fractions after a single subcutaneous injection of epinephrine in oil (1 mg/kg). Except for the different hormones administered, the conditions and experimental procedures in the two studies were exactly the same. It may be noted that in the group which received growth hormone, the greatest changes occurred in the neutral fat fraction of tissues analyzed: liver (+245%), plasma (+95%), and aorta (−25%). Also, liver ester cholesterol was changed +200%, while liver free cholesterol and phospholipide were considerably less (+25%). The plasma phospholipide, free and ester cholesterol were about 50% greater than control levels. On the other hand, 6 hours after epinephrine injection the changes determined in lipide fractions of plasma, liver and aorta were: liver neutral fat +217%, liver ester cholesterol +100%, and plasma phospholipide +25%. The extent of increases in the two liver lipide fractions after epinephrine was comparable to that found with growth hormone. In contrast with the alterations in lipide distribution produced by the latter hormone, it is apparent that the effects of epinephrine on lipide composition in these tissues was comparatively small.

Discussion. Our data showed that marked alterations in lipide distribution of plasma, liver and aorta, and significantly increased turnover of liver and plasma phospholipides occurred 6 hours after injection of growth hormone.

Changes in amounts and rate of synthesis of phospholipides of plasma and/or liver have been reported after exogenous administration of a variety of hormones and after destruction of function of certain endocrine gland(s). In studies in which blood cholesterol also was determined, a parallelism was noted in incre-

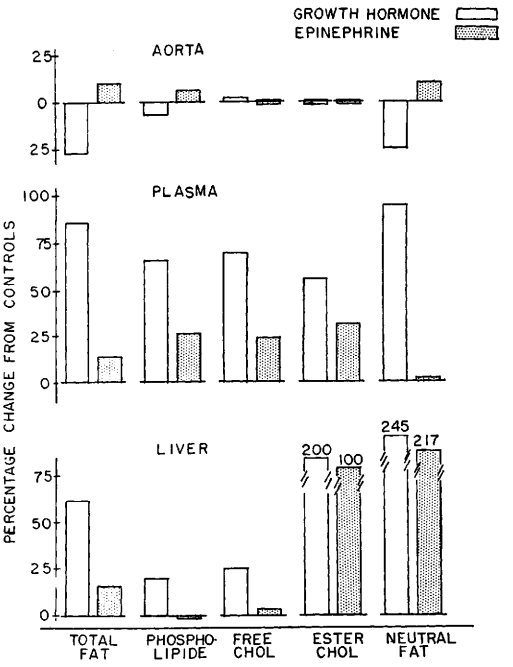

FIG. 2. Effect of growth hormone and epinephrine (see text for doses) on total lipide and lipide fractions in plasma and entire liver and aorta 6 hr after injection. Data are expressed as percentage change, increase or decrease, from respective control values.

ment or decrement of phospholipide and cholesterol levels(1-3,21). In this investigation also blood phospholipide and cholesterol were found elevated to a similar extent and accompanied by increases in the entire liver content of these two lipides. It is possible that related metabolic mechanism(s) are responsible for the regulation of blood levels of phospholipide and cholesterol; this control probably is hormonal. The radioactivity data indicated that accelerated synthesis of liver and plasma phospholipides occurred in animals administered growth hormone. Further, since practically all plasma phospholipide is believed to be produced in the liver the increased content of plasma phospholipide during the period of the experiment suggests an increased exchange of this lipide between liver and plasma had occurred. It does not seem feasible, however, at this time to deduce that alteration in rate of formation or amounts of phospholipides resulted from a specific action of growth hormone on this parameter of metabolism. This appears warranted in view of the variety of individual hormones and endocrine situations with which a change in phospholipide metabolism has been associated (22). Moreover, there is evidence(23) that changes in amount of phospholipide and their rate of formation in a given tissue are related to metabolic needs of the tissue, as an increased rate of fat catabolism. In considering this, Lotspeich and Petersen(9) and Campbell and Davidson(6) recently reported increased acetoacetate formation and oxygen uptake in liver slices obtained from rats a few hours after treatment with growth hormone. They interpreted these findings as indicating an accelerated catabolism of endogenous fat. Our data suggest that fat mobilization (accumulation of liver neutral fat and elevated plasma levels of this lipide) occurred concurrently with alterations in chemical concentrations and turnover of phospholipides of liver and blood. It, therefore, appears more likely, at least from the data now available, that increased amounts of phospholipide and their increased rate of formation a few hours after growth hormone, were not the resultant of a direct effect of this hormone on phospholipide metabolism but rather alterations in the latter concomitant but subsidiary to the primary action of growth hormone of accelerated mobilization and catabolism of endogenous fat. It is noteworthy in this regard that Dury(5) recently reported evidence of markedly increased turnover of phospholipide of plasma, liver, and aorta in intact rabbits shortly after a single injection of epinephrine while there occurred only a moderate increase in liver neutral fat, and none in the plasma.

Change in aorta lipide distribution after growth hormone has not to our knowledge been reported. The depressed level of aortic neutral fat is especially intriguing since in other studies done at this laboratory(3-5,24) it was found that aorta lipide distribution was not altered after acute or chronic exhibition of cortisone or epinephrine. Speculation on this finding is rendered difficult in view of the paucity of information on arterial lipide metabolism, and especially the influence of hormones on the latter. Two points, however, are noteworthy as possibly related to this subject: 1) data in this and other studies have demonstrated that the aorta is particularly rich in neutral fat; and 2) aorta lipide composition was affected, at least qualitatively, as adipose tissue following administration of growth hormone.

The magnitude of alterations of lipide distribution in plasma and liver and rapidity of occurrence were remarkable. Calculation of the relative contribution of different fractions to increased total lipide in the entire liver after growth hormone revealed that neutral fat contributed 47%, phospholipide 15%, free and ester cholesterol each contributed 1.5%. With regard to total lipide increment in plasma, the relative contribution of neutral fat was 53%, phospholipide 19%, free cholesterol 6%, and ester cholesterol contributed 8%. The decreased level of aorta neutral fat was responsible entirely for the fall in aorta total lipide content. Lotspeich and Petersen (9) suggested that the liver fat mobilizing action of growth hormone might be a secondary phenomenon which occurred as a result of a primary depletion of glycogen. We did not find such a change in the intact rabbit. Indeed, in this study the liver glycogen and blood glucose concentrations were moderately,

but significantly, greater than the respective control values. Since the experiment of above authors and the present one are not exactly comparable (differences of species, alimentation, and hormone dosage) a feasible explanation cannot be made of the different effects observed on carbohydrate metabolism.

We are inclined to believe that alterations in lipides distribution and accelerated phospholipide metabolism, in this study, are best explained by the concept proposed by Weil (10) of a primary adipokinetic action of growth hormone. Since this hormone apparently modifies lipide metabolism without mediation of other target glands, further studies concerning the role of growth hormone, if any, in the patho-physiology of arteriosclerosis seem warranted.

Summary. 1. A study was made of the effects of a single injection of anterior pituitary purified growth hormone on distribution of lipides and turnover of phospholipides in plasma, liver and aorta a few hours after administration in intact rabbits. 2. Plasma glucose and liver glycogen concentration, and total liver weight were moderately, but significantly, increased in animals which received growth hormone. 3. Contents of all lipide fractions of plasma and entire liver were significantly increased in experimental animals. In the aorta, the only change was a significantly decreased neutral fat content. 4. Liver total lipide content was increased 64%, plasma total lipide was increased 85%, and aorta total lipide was decreased 25%. The relative contribution of each lipide fraction to percentage increased or decreased total lipide determined in these tissues was as follows: in liver, neutral fat contributed 47%, phospholipide 15%, and free and ester cholesterol 1.5% each; in plasma, neutral fat contributed 53%, phospholipide 19%, free cholesterol 6%, and esterified cholesterol 8%; in the aorta, neutral fat contributed 100%. 5. Radioactivity data showed that accelerated synthesis of liver and plasma phospholipides occurred after treatment with growth hormone; but aortic phospholipide turnover was not different from controls. 6. It is suggested that increased rate of formation of phospholipides of liver and plasma need not necessarily be a specific effect of growth hormone but a change in this parameter of metabolism subsidiary to enhanced mobilization of endogenous fat. It is further suggested that all elements of altered distribution of lipides in tissues analyzed could be explained in accordance with the concept of a primary adipokinetic action of growth hormone.

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