dicate that marked sodium depletion does not alter the resistance of intact rats to a potassium load. Early but transitory urinary electrolyte changes were observed during chronic hydrocortisone treatment. The decreased resistance to potassium stress in animals treated with hydrocortisone is probably due to hypofunction of the adrenal-pituitary axis rather than to steroid-induced alterations of electrolyte balance.

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Effect of Choline, Heparin and Aureomycin on Fatty Livers of Dogs.* (22256)

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Gyorgy, *et al.*(1,2) demonstrated that Aureomycin exerts a lipotropic and anti-cirrhotic effect in rats fed a high fat, low protein, choline deficient diet. Baxter and Campbell (3) noted prevention of severe renal injury and some diminution of liver fat after Aureomycin therapy. More recently the amount of fat in livers has been reduced in cholesterol-fed rabbits(4) and rats(5) by the daily administration of heparin. It therefore became of interest to compare the effectiveness of choline, Aureomycin and heparin in a different species, the dog, and to ascertain whether the mechanism of lipotropic action of Aureomycin was related to the choline sparing effect of this drug(3,6).

Methods. Healthy mongrel dogs, previously maintained on Purina laboratory chow were fed 14 g per kg body weight of a high-fat, low-protein, choline-deficient diet‡(7) for 21 days. Eight of these animals received 0.5 g of Aureomycin§ (Chlortetracycline) in capsule form in the morning and late afternoon simultaneously with the feeding of the diet. Seven animals received daily oral supplements of 1% choline chloride.‖ Nine other animals were injected twice a day with heparin§ 2 mg/kg body weight intramuscularly. On the 21st day, some animals were killed by rapid intracardiac air injections and liver slices were made by a Martin slicer(8). Liver slices were incubated with radioactive phosphate for one hour, with and without the *in vitro* addition of choline chloride (1 mg/cc of medium). Composition of medium and isolation of phospholipides have been described(6). From the specific activity of the inorganic phosphate of the medium and the total radioactivity in the phospholipide, the *in vitro* rate of phosphatide synthesis was calculated. The remaining dogs were sacrificed after the 3 week experimental period. Phospholipide phosphorus was determined on an

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‡ The diet was composed of 38% lard, 44% sucrose, 8% vitamin test casein, 3% cod liver oil and 5% Cowgill's salt mixture. Any animal that did not consume entire diet upon feeding was force-fed.

§ Kindly donated by Lederle Co., Pearl River, N. Y.

‖ Kindly donated by Merck and Co.

TABLE I. Effect of Choline, Heparin and Aureomycin on Liver Lipide Partition.*

Group	No. animals	Phosphatide	Total cholesterol	Total lipide	Triglyceride + cholesterol ester fatty acid
Normal	7	29.4 ± 1.9	2.43 ± .13	43.1 ± 2.1	12.4 ± 1.7
HF†	11	20.1 ± 1.2	2.68 ± .14	64.7 ± 5.2	42.0 ± 4.0
" + choline	7	21.5 ± 1.6	2.32 ± .22	35.9 ± 2.4	12.2 ± 1.1
" + aureomycin	8	17.1 ± 1.2	2.92 ± .22	94.5 ±14.1	73.3 ±13.9
" + heparin	9	16.9 ± 1.1	2.46 ± .30	47.6 ± 3.5	28.3 ± 4.1

* Lipide values expressed as mg/g fresh tissue wt (mean ± stand. error).
† HF = High fat.

alcohol-ether, petroleum ether extract of liver (9). Total cholesterol was analyzed by the method of Sperry and Webb(10) and total lipides were gravimetrically determined by evaporating an aliquot of the petroleum ether extract. The difference between the total lipide on the one hand and the amount of phospholipide (lipide P x 25) plus cholesterol on the other was designated as triglyceride plus cholesterol-ester fatty acid fraction.

Results. The lipide partition in the livers of dogs on the various regimens is presented in Table I. The liver phosphatide concentration of animals on laboratory chow was significantly greater than that in any of the other groups. Neither treatment with heparin or Aureomycin, nor addition of daily adequate choline supplements had any effect on the depressed phosphatide levels of dogs maintained on the high-fat diets. Thus it appears that the decrease in liver phosphatides of dogs on the basic high-fat, low-protein diet was not the result of a simple choline deficiency but may be related to absence of adequate amino acids or other factors from the diet.

In contrast to the decrease in liver phosphatides, the cholesterol concentration was remarkably constant in all animals. The greatest alterations occurred in the "triglyceride" fraction (last column of Table I). Since the total cholesterol did not differ greatly from one group to another and constituted only a small fraction of total liver lipides, it is reasonable to assume that the different values in the last column of Table I primarily reflect alterations in triglyceride concentrations. Thus we may conclude that supplementing the high fat diet with 1% choline during the 3 week experimental period maintained triglycerides at normal concentrations. Administration of heparin to dogs on the high-fat diet appeared to exert some lipotropic effect since lowering of liver triglycerides was statistically significant at the 3% level of confidence. Aureomycin, on the other hand, did not exert any lipotropic action. Quite to the contrary, it appeared that triglyceride levels in Aureomycin treated animals exceeded those of the untreated controls on the same diet ($P = 2.5\%$).

From these experiments one might conclude that Aureomycin aggravated the degree of choline deficiency, but the next set of experiments throws additional light on these results. Table II summarizes data on the rate of phosphatide synthesis by liver slices incubated with radioactive phosphate in the presence and absence of choline in the medium. The first 3 rows confirm our earlier findings (6) that *in vitro* addition of choline markedly stimulates formation of P^{32} labeled phospholipides in choline-deficient liver slices, but has practically no effect on liver slices from dogs maintained on Purina laboratory chow or an high-fat, low protein diet supplemented with 1% choline. If the high triglyceride content of livers of Aureomycin-treated dogs was the result of a choline deficiency similar to that of the animals on unsupplemented high-fat diet (second row of Table II) one would expect a marked increase in phosphatide synthesis of slices in choline supplemented baths. That this is not the case may easily be seen from the figures in the bottom row of Table II. Slices incubated in choline supplemented medium showed a slightly greater synthesis of radioactive phospholipides than slices incubated without choline, but the difference did not compare to the

TABLE II. Phospholipide Concentrations* and Rates of Synthesis in Liver Slices† with and without Added Choline.

Group	mg phospholipide P/g		γ P converted to phospholipide/hr/g	
	Without choline	Choline added	Without choline	Choline added
Normal	.93 ± .052	.99 ± .044	11.8 ± 2.5	12.9 ± 1.2
HF‡	.60 ± .049	.68 ± .063	9.5 ± .7	21.9 ± 2.3
" + choline	.73 ± .060	.75 ± .056	8.6 ± .3	9.7 ± .9
" + aureomycin	.57 ± .073	.58 ± .053	10.8 ± 2.0	14.2 ± 2.1

* Concentrations expressed as mg of phospholipide phosphorus/g fresh liver. Values are given as mean of 4 dogs ± stand. error.

† Each animal liver furnished 6 slices; 3 incubated with choline, 3 without; one slice/flask; each slice was analyzed separately.

‡ HF = High fat.

nearly 2½ fold increase observed in animals with known choline deficiency. According to this test, therefore, Aureomycin decreased the severity of choline deficiency. The inability of Aureomycin to influence the rate of phospholipide synthesis of liver slices agrees with the report of Cornatzer and Gallo(11) that this antibiotic had no effect on phospholipide turnover in the liver of intact rats.

Discussion. It might be well to emphasize that our results were obtained on adult dogs maintained on a high fat, high carbohydrate diet, deficient in choline and methionine, and possibly also in other unknown factors. The fact that addition of choline to our basal ration completely restored the normal appearance and normal triglyceride content of livers should not obscure the fact that the choline supplement failed to restore normal liver phospholipide levels. As a matter of fact, this finding constitutes perhaps one of the most interesting aspects of our study, namely, that the frequently observed parallelism between the lipotropic effect of choline and its effect on liver phospholipide metabolism may not be a cause and effect relationship, but may be more of a coincidental phenomenon. In the present study, for example, the *daily additions* of choline to high-fat diet exerted a marked lipotropic effect but failed to raise the liver phospholipide concentration or the rate of phospholipide synthesis in the liver slice (8.6 γ/g/hr as compared to 9.5 γ/g/hr). This finding is difficult to reconcile with the well known phospholipide-turnover-stimulating action of a single dose of choline given to choline-deficient intact animals(12), or added to their liver slices(6,12).

Gyorgy(2) employing a diet similar to ours observed that in rats Aureomycin markedly decreased liver lipide concentrations. Kaplan *et al.*(13) employing blood lipide alterations as his criterion concluded that in pancreatic duct-ligated dogs, Aureomycin exerted some lipotropic activity. Some choline sparing action appeared to occur in our animals as demonstrated by the failure of the liver slice to respond to added choline. However, this choline made available by Aureomycin was not effective in lowering liver lipides. Since large doses of Aureomycin have been reported to cause fatty livers in mice(14), we fed 1 g of Aureomycin per day for 3 weeks to 2 dogs maintained on Purina laboratory chow without any visible or chemical effects on liver lipides. Similar results were reported by Kaplan *et al.*(13) and Sutherland *et al.* (15). Thus, administration of this amount of Aureomycin to dogs on a nutritionally adequate diet was not associated with fatty liver development. Under the conditions of our experiments Aureomycin apparently exerted some choline sparing action without a concomitant lipotropic effect on the liver, but such an hypothesis deserves further studies.

Summary. The triglyceride concentration in livers of dogs maintained for 3 weeks on a high-fat, low-protein, choline-deficient diet was lowered by daily injections of heparin and by supplementation of the diet with choline. Oral administration of Aureomycin increased liver triglyceride concentrations. Choline, heparin or Aureomycin did not alter liver phosphatide or cholesterol concentra-

tions of animals receiving the high-fat diet. Aureomycin, *per os,* did not affect *in vitro* synthesis of liver phosphatides, but modified the response of liver slices to the *in vitro* addition of choline.

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Rapid Procedure for Erythrocyte Packed Cell Volume and Sedimentation Rate Determinations. (22257)

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During laboratory investigations involving large numbers of human blood samples, erythrocyte sedimentation rates and packed red cell hematocrit values were required for correlation with other observations. This need stimulated efforts to develop time saving modifications of the conventional procedures primarily to devise a rapid preliminary screening operation for selection of abnormal samples for further study by standard methods. However, in actual practice the rapid procedure yielded accuracies sufficiently comparable to standard methods that its routine substitution for the more time-consuming technics appeared possible. The procedure was put on a quantitative basis and appropriate comparison studies with accepted methods(1,2) were carried out. Results here presented indicate that the values are satisfactory for routine clinical use and that they may be obtained with substantial time saving and other economies. The method has been used in testing over 10,000 patient blood samples. The essence of the modification consists in determining sedimentation rate and hematocrit values directly in the original blood collection tube without necessity of transfer to a secondary, calibrated hematocrit tube. The recent introduction of rubber stoppered, evacuated, blood collection tubes containing appropriate anti-coagulants contributes to the speed and convenience of this technic. However, any standard test-tube or collection tube may be substituted or adapted to this procedure. A basic problem of this modification, the variable amount of blood sample in each collection tube, has been overcome by use of a proportional volume chart accurately indicating percentage of relative parts of a test tube blood sample irrespective of its total volume (Figs. 1 and 2). The

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