vivo formation of choline, it is unwise to advance reasons for the inconsistent findings. In the present curative study, the lipotropic effect of vitamin B₁₂ was very distinct, at least on the glyceride fraction; the effect on cholesteryl esters was less definite.

SUMMARY

1. Crystalline cholesterol was added at different concentrations (0.2, 0.4, 0.8 and 1.6 %) to a hypolipotropic diet. The livers of rats fed these rations and of other rats fed similar rations containing various lipotropic supplements were analysed for lipid content.

2. At moderate intakes of cholesterol (0.2 %) sufficient dietary choline or betaine were able to prevent the excessive deposition of both glycerides and cholesteryl esters.

3. In rats consuming diets rich in cholesterol, even considerable concentrations of choline (1.28 %) in the diet failed to prevent an excessive accumulation of cholesteryl esters in the livers.

4. In a curative experiment, betaine hydrochloride at a concentration of 0.42 % in the diet was about as active as an equimolecular amount of choline in hastening the removal of glycerides and cholesteryl esters from 'cholesterol fatty livers'. The limited data suggest that had the effect of lower dosages been compared, betaine would have been found to be less effective than choline.

5. Vitamin B₁₂ exerted a considerable curative lipotropic action on the glycerides but much less on the cholesteryl ester fraction of liver lipids.

6. Inositol was without any appreciable lipotropic effect on either fraction in either type of study.

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Effects of Lipotropic Substances on the Cholesterol Content of the Serum of Rats

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Some investigators believe that there is a causal relationship between atherosclerosis and the level of serum cholesterol. The well-known effect of lipotropic agents upon lipid deposits in the liver has suggested to many workers that these substances might be useful in treating or preventing the lesions in the blood vessels. The literature concerning the effects of lipotropic substances on serum cholesterol is very inconsistent. This prompted us to determine free and total cholesterol in the sera of certain rats

used in the lipotropic study reported in the preceding paper (Ridout, Lucas, Patterson & Best, 1954).

Some of the discrepancies in the literature may be related to our observation that under certain conditions, ingestion of diets rich in cholesterol leads to a marked postprandial elevation in the bound serum cholesterol when the food contains choline. Our findings indicate that the same substances that prevent or cure fatty livers are also concerned in the

control of serum cholesterol but they do not provide any support for the claims that lipotropic substances reduce the concentration of cholesteryl esters in the serum.

EXPERIMENTAL

Groups of ten or more rats were fed purified diets (containing casein 8%, gelatin 6% and soybean protein 6%) for periods of from 1 day to 10 weeks, as described in the preceding paper (Ridout *et al.* 1954). Supplements of choline were added as chloride in amounts 1.15 times the amounts mentioned in the text and of betaine as hydrochloride in amounts molecularly equivalent to the choline. At intervals from 2 to 18 hr. after removal of the food containers from the cages, the animals were anaesthetized by intra-peritoneal injection of sodium amytal and blood was collected from the carotid artery in 15 ml. conical centrifuge tubes. The blood stood at room temperature for several hours and then remained in a refrigerator overnight. The tubes were centrifuged; as much serum as possible was removed with a pipette and if necessary the serum was again centrifuged. Serum from haemolysed blood was rejected.

The lipids were extracted from the serum with ethanol-ether (3:1, v/v), essentially as outlined by Bloor, Pelkan & Allen (1922). Cholesterol was precipitated as digitonide and determined colorimetrically, as described by Sperry & Webb (1950).

RESULTS

Natural rations. Free cholesterol in the serum of young adult rats consuming the stock diet (Master Fox Chow, Toronto Elevators, Ltd.) supplied in our

laboratory varied from 8 to 18 mg./100 ml. The average for 18 female rats was 12.0 mg. with a standard deviation of ± 1.7 and for 29 male rats was 12.6 mg. ± 2.5 . Bound cholesterol varied from 40 to 75 mg./100 ml., two-thirds of all values falling between 48 and 66. These limits are indicated by a vertical arrow in Fig. 1 and by dotted horizontal lines in Figs. 2 and 3. The average bound cholesterol in the serum of females was 56 ± 9 and in males was 57 ± 11 .

Preventive studies

Purified diets without added cholesterol. Blood samples were collected 2 to 4 hr. after removal of food dishes from the cages. The bound serum cholesterol of rats fed for 3 weeks on the basal (choline-free, cholesterol-free) purified ration was 32 ± 9 mg./100 ml. In rats fed the same diet containing 0.08% choline the bound cholesterol was 61 ± 6 mg. and doubling the amount of choline did not cause any further increase (57 ± 6 mg.), i.e. the presence of choline appears necessary in the diet to maintain the serum cholesterol within the normal range.

It may be mentioned that we have observed values for bound cholesterol as low as 10–12 mg./100 ml. serum in rats maintained for longer periods (6 months or more) on severely hypolipotropic, low protein diets. In these animals, liver damage was pronounced and in many of them cirrhosis of moderate severity was present.

Purified diets with added crystalline cholesterol. The data shown in Fig. 1 were obtained in a preventive experiment after the rats had been on the cholesterol-containing diets for 3 weeks. Blood samples from rats of all groups but one were collected in mid-morning 2–4 hr. after the food dishes had been removed from the cages. In one group (cholesterol 0.8%, choline 0.48%) blood was collected 18 hr. after removal of food. In the animals given choline a distinct relationship may be seen (during the post-prandial period) between the percentage of cholesterol in the diet and the average concentration of bound cholesterol in the serum. Because of the superposition of the points, it is impossible to show in Fig. 1 all the values determined, e.g. with 0.8% of cholesterol in the diet, the average bound serum cholesterol with different supplements of choline (0.32, 0.64 and 1.28%, respectively) was the same, viz. about 100 mg./100 ml. Thus, under these conditions considerable variation in the choline content of the diet, above the undetermined minimum, does not affect the cholesterol content of serum.

Data for the effects of betaine are not shown in Fig. 1, since the points would be superimposed on those already present. With 0.2% cholesterol in the diet and betaine equivalent to 0.16% choline the range of bound serum cholesterol was 51–79,

Fig. 1. Effect of dietary cholesterol on bound cholesterol in blood serum during the absorptive state. Groups of ten rats fed test diets for 3 weeks. All blood samples taken 2–4 hr. after food dishes removed, with the exception of one group (⊙) (choline 0.48%, no food available for 18 hr.). No choline (●); choline supplements (given as chloride in amounts 1.15 times values mentioned): 0.04% (▽), 0.08% (○), 0.16% (□), 1.28% (×). Vertical arrows represent limits within which two-thirds of all values normally fall in rats of our colony.

mean 62, s.d. ± 8 ; twice as much betaine gave similar values (61–77, mean 67 ± 7). At this dietary level of cholesterol supplementary inositol (0.32%) had no effect on bound serum cholesterol, values found ranging from 23 to 38 (mean 30 ± 6).

Data in the previous paper (Ridout *et al.* 1954) showed that when excessive amounts of cholesterol are ingested, even large doses of choline are unable to prevent considerable deposition of esters in the liver: when 0.8% cholesterol was in the diet, the amounts of cholesteryl esters that accumulated in the liver during 3 weeks with different amounts of dietary choline (0.08, 0.16 and 1.28%) were, as percentage dry, fat-free liver, 10.3, 7.3 and 4.6, respectively, yet the sera from these different groups of animals contained essentially the same average concentrations of bound cholesterol. The levels in these sera are not related to the amounts of esters present in the liver. Obviously, the variations in the concentration of bound serum cholesterol shown in Fig. 1 must have another explanation. In all groups but one, blood was taken while some food may have still been present in the digestive tract. When the blood was not collected until 18 hr. after removal of food, the concentration in the serum was found to have returned practically to the normal range (mean value 68 mg./100 ml. as compared with about 100 mg. at 2–4 hr. after removal of the food). This observation seems to relate the concentration of cholesterol in the serum to the amount of cholesterol being absorbed from the gut, when choline is present in the food.

The influence of dietary choline on serum cholesterol was therefore examined further under two different conditions: (1) during absorption of cholesterol, with a much smaller deposition of cholesteryl esters in the liver; and (2) in a curative

study, when there was no cholesterol in the diet but a large accumulation of esters was present in the liver.

The first experiment involved two groups of rats fed the basal diet supplemented with 0.8% cholesterol; those in one group got the hypolipotropic diet, the others were given the same food containing 0.3% choline. The animals were offered these diets for 48 hr. and then ten rats from each group were killed 2, 4, 8 and 16 hr., respectively, after removal of food. When the diet contained choline, the bound serum cholesterol increased considerably between 2 and 4 hr. after a meal containing this excessive amount of cholesterol (Fig. 2); the level in the serum returned to normal in 16–24 hr. In the absence of choline there was a suggestion of a postprandial increase but the values scarcely left the normal range (69 mg./100 ml. at 2 hr. and 53 mg./100 ml. at 16 hr.). Analyses of the livers of the animals given choline revealed that a small accumulation of esters (0.4–0.6% fresh liver) had occurred (this concentration was about 5 times normal compared with about 20 times normal in the previous experiment). Unfortunately, enough bound cholesterol was present in these livers so that mobilization rather than absorption might account for the peak. However, in the curative experiments now to be discussed the absence of any peak when the diet lacks cholesterol and the liver contains a large deposit of cholesteryl esters would seem to exclude mobilization as an important factor in the postprandial elevation of serum cholesterol.

Curative studies

Blood was collected from rats during two curative experiments after a cholesterol-rich diet had been fed for a preparatory period of 3 weeks to produce 'cholesterol fatty livers'. Cholesterol was determined in the serum of ten rats at this time. Blood was taken 2–4 hr. after removal of food dishes. The bound serum cholesterol varied from 23 to 62 mg./100 ml. (average 41). Cholesterol was removed from all diets during the curative phase; lipotropic supplements were incorporated in some of the rations.

In the first experiment half the rats were continued on the basal ration and half were given the diet supplemented with choline (0.48%); groups of animals were killed after 1, 2, 4, 7 and 14 days, respectively, on the test diets. Within 24 hr. after transfer to the (cholesterol-free) ration containing choline, the bound cholesterol in the serum changed to a higher level (41–77 mg./100 ml., average 57) and remained there (on the 2nd day at 64, on the 4th day at 58, on the 7th day at 61 and on the 14th day at 68 mg./100 ml.). The corresponding average values were distinctly lower (43, 48, 44 and 41 mg./100 ml., respectively) in rats maintained on the

Fig. 2. Effect of absorptive state on bound cholesterol in serum. Dotted horizontal lines show limits within which two-thirds of values normally fall in our colony. Groups of ten rats fed diets containing 0.8% cholesterol for 48 hr.; food dishes removed and blood samples collected after intervals shown. No choline (●), choline 0.32% (○).

cholesterol-free basal diet for the same periods. The prompt restoration of the bound serum cholesterol to normal by choline is noteworthy. In these rats there was no dietary cholesterol in the chyme but there was an excessive accumulation of cholesteryl esters in the liver. No postprandial increase in serum cholesterol was observed in this experiment. Indeed, in this short-term curative study the bound cholesterol in the serum of rats not getting choline was on the average slightly below normal (41–48 mg./100 ml.) while in those given choline the values were in the upper portion of the normal range (57–68 mg./100 ml.). These findings suggest that choline may have a limited mobilizing effect, viz. that it acts to maintain the bound serum cholesterol within the normal range. Thus the large postprandial increases seen in the previous experiment were more likely due to some effect of choline on absorption or utilization, rather than on mobilization.

In subsequent experiments blood samples were not collected until 18 hr. after the removal of food, although the absence of cholesterol from the food may make the long interval unnecessary in curative studies; this procedure was followed, however, for the sake of uniformity and to minimize the effects of absorption.

During the long-term curative experiment, in which rats were fed the cholesterol-free test diets for periods of 14, 28, 42 and 70 days, respectively, the bound serum cholesterol fell progressively (Fig. 3) in the animals fed the choline-free basal ration. In other rats, when choline or betaine was restored to the diet, the concentration of bound cholesterol in the serum returned to the upper region of the normal range and remained there throughout

the test periods. Inositol had no such effect. Vitamin B₁₂ had a distinct effect in restoring the serum cholesterol; in the single set of data available (at 42 days) the value was within the normal range.

To give some idea of the variability of the concentration of bound cholesterol in the sera under these conditions, the ranges and means with standard deviations are given for the animals that had been fed the cholesterol-free test diets for 42 days: hypolipotropic basal diet, 24–44, mean 35 ± 8 ; plus choline, 55–70, mean 66 ± 6 ; plus betaine, 53–74, mean 63 ± 8 ; vitamin B₁₂, 46–60, mean 51 ± 5 ; inositol, 36–45, mean 40 ± 4 ; choline plus inositol, 46–73, mean 57 ± 14 mg./100 ml.

Mention may be made of the fact that a striking parallelism was observed between the behaviour of bound cholesterol and of serum phospholipids in the few cases where the latter were determined.

DISCUSSION

The concentration of cholesterol in the serum at any instant is the resultant of a number of interacting processes: (1) absorption, (2) mobilization, (3) synthesis, (4) degradation, and (5) excretion. Some factors which affect the regulation of blood cholesterol have been mentioned by Byers, Friedman & Rosenman (1952). In a review of lipid metabolism and atherosclerosis, Gould (1951) concluded that the normal plasma cholesterol content appears to be regulated chiefly by the liver. It is well known to clinicians that in patients with fatty infiltration of the liver or with cirrhosis, cholesteryl esters in the serum are low (Peters & Van Slyke, 1946).

The widely recognized value of the lipotropic agents in maintaining normal liver function may explain why in preventive experiments choline checks the fall in serum cholesterol which would otherwise occur. In the curative studies choline or betaine brought the serum cholesterol up to the normal value. In these curative studies the serum cholesterol was restored to normal within 24 hr. by choline, although as long as 10 weeks or more may be required to restore the cholesteryl esters in liver lipids to normal. The significance of this is not yet understood.

The experimental conditions adopted in other laboratories for the investigation of factors affecting serum cholesterol have differed in various ways from those followed in the present work, yet there is an essential agreement that supplementary choline increases the serum cholesterol in omnivorous animals fed cholesterol-containing hypolipotropic diets (Castro-Mendoza, Jiménez Díaz & Vivanco, 1947; Handler, 1948). We prefer to express this idea by saying that choline prevents the fall in serum cholesterol which is observed in rats fed hypolipotropic diets. In a series of experiments in which

Fig. 3. Effect of lipotropic substances on bound serum cholesterol at different stages of a curative experiment. Groups of ten rats with 'cholesterol fatty livers' fed test diets for periods shown. No lipotropic supplement (●), choline 0.32% (○), betaine HCl 0.42% (□), vitamin B₁₂ 10 μg./100 g. (△), inositol 0.32% (▽), choline 0.32% plus inositol 0.32% (+). Horizontal dotted lines represent normal values.

cholesterol absorption was enhanced by incorporating bile salts in the diet, Weiss, Marx & Marx (1952) showed that supplementary choline plus inositol more than doubled the concentration of serum cholesterol. These high values may be postprandial peak values rather than sustained levels.

Others have noted that in dogs maintained on hypolipotropic diets the cholesteryl esters of the plasma were decreased, and that restoration of choline to the rations brought the ester concentration back towards normal (McKibbin, Thayer & Stare, 1944; McKibbin, Ferry, Thayer, Patterson & Stare, 1945). Burns & McKibbin (1951) also noted a progressive fall in plasma cholesterol in dogs during prolonged feeding of a hypolipotropic diet and reported that vitamin B₁₂ ameliorated the condition. In the present study, a similar observation was noted with vitamin B₁₂ in rats.

The failure of inositol to affect the serum cholesterol may be related to its inability to prevent the accumulation of cholesteryl esters in the liver or to hasten their removal from it. This is another example of a difference between the effects of inositol and of the substances (choline, betaine, methionine and vitamin B₁₂) referred to collectively as the lipotropic agents. It may be recalled that inositol also fails to protect weanling rats against the haemorrhagic kidney lesions induced by a deficiency of the lipotropic agents (Best, Lucas, Patterson & Ridout, 1946; Handler, 1946).

One point which our studies have emphasized is the importance of the time interval between the last meal and the collection of the blood sample. Under our experimental conditions, it was only when both an excessive amount of cholesterol and some choline were present in the diet that the concentration of bound serum cholesterol was significantly elevated in the first few hours after ingestion of the food. The magnitude of this elevation was related to the amount of cholesterol ingested and was apparently independent of the quantity of choline (or betaine) above some minimum. It takes much longer (18–24 hr.) for the postprandial increase in serum cholesterol to return to normal after a meal than it does for that of some of the other blood components, such as glucose or amino acids. This postprandial increase in the presence of choline may be brought about by a direct effect on the absorption process, by a secondary one on this process exerted perhaps through an effect on bile formation, or by some more obscure effect on utilization or storage.

Some workers have contended that an increase occurs in serum cholesterol after cholesterol feeding; others have insisted that dietary cholesterol has at most only a moderate influence on the level of cholesterol in the blood. The presence or absence of lipotropic agents in diets containing cholesterol and the state of absorption at which the blood samples

were taken may partly explain the contradictions. The extent to which differences in the response of different species may also be a factor has not been fully explored. The failure of many investigators to record the interval of time between the last meal and collection of the blood samples is unfortunate since variations in this interval could give results leading to quite different impressions as to the influence of choline on the serum cholesterol. In order to establish more precisely these time relationships we plan to repeat parts of the study, and to feed the final meal by stomach tube.

Much of the experimental work in this field has been done under extreme conditions in which excessive amounts of cholesterol (1 or 2%) have been added to natural diets which would contain variable amounts of the ubiquitous lipotropic substances. Diets containing 1 or 2% of cholesterol (as percentage of dry, total solids) are probably never ingested under natural conditions. Actually, values exceeding 0.3% cholesterol are rarely encountered in rations ordinarily consumed. Keys (1949) has reported the habitual cholesterol intakes of 312 American men in a comfortable economic situation. The average weekly intake of the most extreme group in his series was 4.8 g. of cholesterol. Pihl (1952) has calculated the average daily intake of cholesterol in an average Norwegian diet to be about 550 mg. These values are in good agreement. Assuming that the dry solids in the diets would average between 500 and 700 g. per day, cholesterol would rarely exceed 0.1% of the total dry solids. The much more limited data reported for the cholesterol intake of nurses in a Canadian hospital (Ridout, Lucas, Patterson & Best, 1952) agree moderately well with this percentage of cholesterol in an average diet. Thus, many of the experimental diets used in studies of atherosclerosis have contained at least 10 to 20 times the amounts of cholesterol usually found in rations eaten by man.

In rats consuming diets containing lipotropic factors, it was only with excessively high intakes of dietary cholesterol (greater than 0.4%) that a marked postprandial elevation in bound serum cholesterol occurred. At reasonable intakes, corresponding to those in average rations consumed by man, the increase after meals was so small that the peak value did not go beyond the upper limits of the normal range.

Hypercholesterolaemia was observed in dogs fed a high fat diet containing cholesterol to which lecithin of adrenal origin had been added (Corwin, 1938) and also in human subjects and dogs given diets containing egg-yolk powder, which is rich in cholesterol and lecithin (Okey & Stewart, 1933; Steiner & Domanski, 1941; Glomset & Bollman, 1943; Messinger, Porosowska & Steele, 1950). Messinger *et al.* (1950) comment that the amount of

egg-yolk necessary to produce this effect is well above that consumed in any ordinary diet. Comparable effects were not produced by feeding diets rich in fat to which purified cholesterol had been added (Turner & Steiner, 1939), a fact which led Steiner & Domanski (1941) to postulate that the hypercholesterolaemia might be related to the lecithin contained in the egg-yolk. To test this hypothesis Steiner & Domanski (1944) added 'soya lecithin' (25 g. daily) to the hospital diet of eight patients; in every case a decrease occurred in the level of serum cholesterol. Others have noted a similar decrease when 'soya lecithin' was added to the diet (in patients with xanthomatosis, Gross & Kesten (1943) and Adlersberg & Sobotka (1943); Kesten & Silbowitz (1942) found that the feeding of 'soya lecithin' inhibited the hypercholesterolaemia usually observed in rabbits fed diets rich in cholesterol). The soybean product sold under the trade name of 'soya lecithin' contains only about 20 % of lecithin; its residual composition in approximate percentage, is 'cephalins' 20, oily glycerides 30, carbohydrates 10, inositol-containing complexes 15, and phytosterols 2, which is obviously a very different mixture from the egg-yolk and adrenal lecithin fed by the workers previously mentioned, who observed the opposite effect. To add to the confusion in this field Delevett & Bruger (1948) reported that 'soya lecithin' (either crude or oil-free) failed to reduce the serum cholesterol in four patients with xanthomatosis.

Because of the frequency with which atherosclerosis occurs spontaneously in birds, the chicken has often been used in the study of factors concerned with the development and control of vascular lesions. Using this species Stamler, Bolene, Harris & Katz (1950) observed that choline plus inositol failed to exert a prophylactic effect on the hypercholesterolaemia produced by feeding diets enriched with cholesterol. Indeed, their data show that during the first weeks of the experiment, addition of lipotropic factors actually aggravated the hypercholesterolaemia. It should be noted that the basal ration they adopted was made by adding cholesterol to a commercial chick starter mash containing mixed grains, dried buttermilk, soybean meal, meat and bone scraps, etc. Since these commercial rations are designed to be adequate with respect to all nutriment required by the growing chick, it may be assumed that essentially optimum amounts of choline and inositol were present in the basal ration. In the light of this and of our findings in rats, it is difficult to interpret their observation that adding more of the lipotropic agents caused such a remarkable elevation of the cholesterol in plasma.

Hypercholesterolaemia and atherosclerosis have been produced in Cebus monkeys by Mann, Andrus, McNally & Stare (1953) by feeding purified diets

containing 5 % cholesterol. The hypercholesterolaemic response appeared to depend on a diet low in methionine and upon the presence of choline. In these experiments the hypercholesterolaemia was not merely postprandial but was sustained. The difference in response of different species to dietary cholesterol and choline may be much greater than is generally recognized.

During recent years there has been widespread use in North America of lipotropic substances in patients with atherosclerosis. There are, however, very few carefully controlled clinical studies. Many experimenters and clinicians have complicated the situation and rendered the interpretation of their results difficult by adding choline to natural diets of variable but unknown choline content. Sometimes this has been done deliberately. Firstbrook (1950), for example, reported that even large doses of supplementary choline had no effect on the total cholesterol of the blood of rabbits fed a commercial ration supplemented daily with 1 g. of crystalline cholesterol. It would have been clearer to the uninitiated if it had been emphasized that the effect of very large doses of supplementary choline were being tested and that no study of the effect of basal diets low in lipotropic factors was made. Davidson (1951) has reviewed this field and concluded that despite individual positive reports, consideration of all the available data indicates that there is no general agreement that choline or inositol has any specific influence upon atherosclerosis or upon the serum cholesterol in man or in experimental animals.

Katz & Stamler (1953) have pointed out that practitioners are being persuaded to prescribe expensive lipotropic preparations for the prophylaxis and therapy of human arteriosclerosis despite the inconsistency of the results and the paucity of controlled clinical data: 'From both scientific and socio-economic considerations, therefore, it is essential that the actual effects of lipotropic factors on atherosclerosis be clarified.' One of us (Best, 1950), evaluating the evidence for the therapeutic use of the lipotropic agents in atherosclerosis stated: 'This claim for choline has no firm basis in fact and may serve as a false pathway which diverts energy from both the profitable study of atherosclerosis and the real realms of choline action'. It is, of course, possible that *new* approaches may reveal a relationship of the lipotropic agents to atherosclerosis. Some suggestive leads are now being explored in our laboratory (e.g. Hartroft, Ridout, Sellers & Best, 1952).

SUMMARY

1. Free and total cholesterol have been determined in the blood sera of young adult rats maintained on natural and on purified diets. In rats of

our colony fed the stock ration, the mean total cholesterol is 70 mg./100 ml. (s.d. ± 11) of which 12 mg./100 ml. are in the free form. The values found in males and in females did not differ significantly.

2. The time, with respect to the last meal, at which the blood sample is taken is important when the diet contains lipotropic agents and excessive amounts of cholesterol. The postprandial elevation in bound serum cholesterol was related to the concentration of cholesterol in the ration. Increasing the lipotropic agents (choline) above an undetermined minimal amount produced no further effect. The elevated serum cholesterol values returned slowly to normal after about 18–24 hr. In the absence of choline the postprandial increase (if any) was very slight even in the presence of large amounts of cholesterol in the diet.

3. In rats fed purified hypolipotropic diets, the bound serum cholesterol fell progressively as the experiment continued.

4. In preventive studies choline and betaine maintained the serum cholesterol within the normal range but inositol failed to prevent the characteristic fall.

5. In curative studies replacement of choline in the diet promptly restored (within 24 hr.) the concentration of serum cholesterol to normal; betaine also was able to restore and maintain the serum cholesterol; vitamin B₁₂ exerted a similar effect; inositol did not alter the values observed in the rats continued on the basal ration.

6. Our experimental findings do not support the claims that lipotropic substances reduce the concentration of bound cholesterol in the serum. They do indicate that the same substances which prevent or cure fatty livers are also involved in the control of the cholesterol level in the serum.

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