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Lipotropic Factors

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Volume XVIII | Friday, May 16, 1947 | Number 27

INDEX

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William A. O'Brien, M.D.

I.

UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
CALENDAR OF EVENTS

May 19 - May 24, 1947

No. 158

Monday, May 19

9:00 - 9:50 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 10:50 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; Interns' Quarters, U. H.

10:00 - 12:00 Neurology Ward Rounds; A. B. Baker and Staff; Station 50, U. H.

11:00 - 11:50 Roentgenology-Medicine Conference; Staff; Veterans' Hospital.

11:00 - 11:50 Physical Medicine Conference; Physical Therapy in peripheral vascular disease; Ernest C. Christensen; E-101, U.H.

12:15 - 1:20 Obstetrics and Gynecology Journal Club; M-435, U. H.

12:30 - 1:20 Pathology Seminar; Relationship of lymphocytes to antibody production; Karl Karlson; 104 I. A.

12:15 - 1:20 Pediatrics Seminar; Clinical Pathological Conference; 6th Floor Seminar Room; U. H.

12:30 - 1:20 Physiology Seminar; No meeting; 214 M. H.

12:30 - 1:50 Surgery Grand Rounds; A. A. Zierold, Clarence Dennis and Staff; Minneapolis General Hospital.

4:00 - 5:20 School of Public Health Seminar; Subject to be announced; 113 MeS.

8:00 - Joint meeting - Hennepin and Ramsey County Medical Societies; Edgar Gordon and James D. Bifgard; Museum of Natural History.

Tuesday, May 20

9:00 - 9:50 Roentgenology-Pediatrics Conference; L. G. Rigler, I. McQuarrie and Staff; Eustis Amphitheater, U. H.

8:30 - 10:20 Surgery Reading Conference; John R. Paine; Small Conference Room, Bldg. I, Veterans' Hospital.

10:30 - 11:50 Surgical-Pathological Conference; John R. Paine and Nathaniel Lufkin; Veterans' Hospital.

12:30 - 1:20 Pathology Conference; Autopsies; Pathology Staff; 102 I. A.

2:00 - 2:50 Dermatology and Syphilology Conference; H. E. Michelson and Staff; Bldg. III, Veterans' Hospital.

3:15 - 4:20 Gynecology Chart Conference; J. L. McKelvey and Staff; Station 54, U. H.

3:30 - 4:20 Clinical Pathological Conference; Staff; Veterans' Hospital.

3:45 - 4:50 Pediatrics Staff Rounds; I. McQuarrie and Staff; W-205, U. H.

4:00 - 4:50 Surgery-Physiology Conference; Surgical Significance of Biliary Sphincters; George S. Bergh and Robert W. Utendorfer; Eustis Amphitheater, U. H.

5:00 - 5:50 Roentgenology Diagnosis Conference; Staff; University Hospitals.

Wednesday, May 21

8:00 - 8:50 Surgery Journal Club; O. H. Wangensteen and Staff; M-515, U. H.

8:30 - 9:50 Psychiatry and Neurology Seminar; Staff; Veterans' Hospital.

11:00 - 11:50 Pathology-Medicine-Surgery Conference; Hypernephroma; E. T. Bell, C. J. Watson, O. H. Wangensteen and Staff; Todd Amphitheater, U. H.

12:00 - 12:50 Physiological Chemistry Journal Club; Staff; 113 MeS.

7:30 - 8:50 Histopathology of the Skin; Dermatology Staff; Todd Amphitheater, U. H.

Thursday, May 22

8:30 - 9:20 Surgery Grand Rounds; John R. Paine and Staff; Veterans' Hospital.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; Todd Amphitheater, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:50 Surgery-Radiology Conference; Daniel Fink and John R. Paine, Veterans' Hospital.

12:00 - 12:50 Physiological Chemistry Seminar; Lipid Metabolism; Walter O. Lundberg; 214 M. H.

1:00 - 1:50 Fracture Conference; A. A. Zierold and Staff; Minneapolis General Hospital.

4:00 - 4:50 Bacteriology Seminar; The spreading factor, Abraham I. Braude; 214 M. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling W. Hansen and Staff; E-534, U. H.

5:00 - 5:50 Roentgenology Seminar; Case Reports; Staff; M-515, U. H.

7:30 - 8:50 Physical Medicine Seminar; William G. Kubicek; 111 MeS.

Friday, May 23

9:00 - 9:50 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U.H.

9:00 - 9:50 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amphitheater, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:20 Medicine Grand Rounds; Staff; Veterans' Hospital.

10:30 - 11:50 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Department; U. H.

11:30 - 12:50 University of Minnesota Hospitals General Staff Meeting; Electrokymography; Herbert M. Stauffer; New Powell Hall Amphitheater.

1:00 - 1:50 Dermatology and Syphilology; Presentation of Selected Cases of the Week; H. E. Michelson and Staff; W-312, U. H.

1:00 - 2:50 Neurosurgery-Roentgenology Conference; W. T. Peyson, Harold O. Peterson, and Staff; Todd Amphitheater, U. H.

5:30 - 6:20 Surgery Literature Conference; Clarence Dennis and Staff; Mpls. General Hospital.

Saturday, May 24

7:45 - 8:50 Orthopedics Conference; Wallace H. Cole and Staff; Station 21, U. H.

9:00 - 9:50 Neurology Grand Rounds; A. B. Baker and Staff; Station 50, U. H.

9:00 - 9:50 Surgery-Roentgenology Conference; O. H. Wangensteen, L. G. Rigler, and Staff; Todd Amphitheater, U. H.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; M-515, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; M-515, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff; Station 44, U. H.

11:00 - 12:20 Anatomy Seminar; Multiple myeloma; Dorothy Sundberg; and The Effect of X-rays upon Inflammation; W. A. Townsend; 226 I. A.

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Note: Special Course - May 19 - 24 - in Dermatology; at Center for Continuation Study; Drs. Jessner, Mayer and Pinkus.

II. LIPOTROPIC FACTORS

Elizabeth G. Frame

The present report consists of a review of some of the recent literature on factors which prevent, or remove, an accumulation of fat from the liver. The application to human disease of the findings which are discussed, is not as yet entirely clear, but the experimental results clarify to some extent our knowledge of fat metabolism.

Two "types" of fatty liver will be discussed: (1) that which is produced by dietary means, and (2) that which develops in depancreatized dogs maintained with insulin. By definition, a lipotropic factor is a substance which will prevent the accumulation of,or accelerate the removal of, fat from the liver.

DIETARY FATTY LIVERS

To review briefly the history of the discovery of lipotropic factors it may be recalled that purely dietary fatty livers were first produced in normal rats by Best and his associates[1] in 1932 by feeding a diet containing a large amount of fat with a small amount of protein, and that these fatty livers failed to develop if lecithin was included in the diet. Fractionation of the lecithin revealed that choline was the component of the lecithin molecule which was active in preventing the development of fatty livers. It was soon found that choline was also able to remove the excess liver fat which had accumulated.

Lipotropic Effect of Proteins

It soon became evident that choline was not the only factor which possessed lipotropic activity. Channon and Wilkinson[2] observed that rats on a low-protein high-fat diet developed fatty livers, but that if the protein content of the diet was increased, with the high fat content remaining unchanged, excess fat failed to accumulate in the liver. If the protein content was low, regardless of the amount of fat in the diet, fatty livers developed. To Tucker and Eckstein[3] belongs the credit for the discovery that the lipotropic action of protein was due to its content of methionine. Subsequent investigations, especially by Du Vigneaud and his group, have made it quite certain that the lipotropic action of methionine is attributable to its ability to transfer methyl groups to choline. The animal body appears to have unlimited ability to manufacture non-methylated choline (ethanolamine), but it requires provision in the diet of "labile" methyl groups, such as methionine is able to furnish. In the absence of choline there is an accumulation of fat in the liver, a condition which can be prevented or cured by the inclusion in the diet of choline or by an adequate supply of "labile" methyl groups such as can be contributed by methionine.

That cystine, the other sulfur-containing amino acid which occurs in proteins, participates in the fatty liver picture has been recognized for some time, but its exact rôle has been a difficult one to elucidate. Its first implication came with the observation by Beeston and Channon[4] that the addition of cystine to a low-protein diet caused a marked increase in the fat content of the livers of rats, even over the high values of the control animals. That is, cystine had an effect directly opposite to that of methionine. The most likely explanation of thie effect of cystine can perhaps be best understood if viewed in the light of some recently beautifully-controlled experiments by Beveridge and his associates.[5]

A question which was raised early and which still is being investigated is whether the lipotropic action of protein can be accounted for exclusively by its methionine and cystine content, or whether some of the other amino acids are involved. On paper it would seem to be a simple experiment to feed one group of animals a protein- and choline-free diet containing added known amounts of methionine and cystine, and to a comparable group a diet containing the same amounts of methionine and cystine in protein. The experiment has been done in at least three laboratories, with widely varying results. Best and Ridout[6] and Channon[7] reported that dietary casein exerted a distinctly stronger lipotropic

effect than did the methionine and cystine contained in it, whereas Eckstein[8,9] found the free amino acids to be more effective. Examination of the data of the different experiments revealed differences in the content of the basal diets, in the age of the animals, et cetera, and indicated the necessity for more adequately controlled experiments.

The required controlled experiments were those of Beveridge[5] referred to above. Their rats received a constant nutritional background with respect to total protein and vitamins and the diets were free from choline. Some of their results are simplified in Table I. A is the control diet, with adequate methionine present in the 25% casein to prevent liver fat accumulation. When the casein content was reduced to 10%, the amount of methionine was not sufficient to allow adequate choline formation, with the result that fat accumulated in the liver. In diet C, which contained the same amount of protein and amino acid nitrogen as did diet B, but where casein was replaced by gelatin, and methionine and cystine were added, the livers were normal as to fat content. The significant difference between diets B and C lay in the content of essential amino acids. In Diet D essential amino acids were added to diet C in amounts similar to that of diet B.

TABLE I

Diet	% Cystine	% Meth.	% Essential Am. Acids	Liver Lipids
A. 25% casein	0.113	0.484	7.1	normal
B. 10% casein	0.045	0.194	2.84	high
C. Gelatin + cystine + meth.	0.045	0.194	low	normal
D. Gelatin + cyst. + meth. + essential a. a.	0.045	0.194	2.84	high

B, C, and D identical total N content

Here liver lipids were elevated. The probable explanation of these experiments is as follows: On diet C, where the supply of the essential amino acids is low, there is little possibility for protein synthesis, for growth, or even for tissue maintenance to be carried on. This leaves all the methionine available for lipotropic activity, with a consequent prevention of fat accumulation in the liver. On the other hand, when the essential amino acids are provided, as in diets B and D, the available methionine is utilized by other metabolic processes of apparently higher priority, such as growth and maintenance, with the result that not enough remains for lipotropic action, and fat accumulates. In this sense, then, the lipotropic activity of protein is determined not only by its methionine and cystine contents, but also by the content of essential amino acids. The practical conclusion to be drawn from this experiment is not that diet C is superior to B or D as far as liver fat is concerned, but rather that diet A is superior to them all.

Results which can be explained in a similar manner were obtained by Eckstein and his associates[10]. They found that in adult rats, liver lipids could be prevented from accumulating equally well whether the known amount of methionine was present in protein (casein) or whether it was added as a supplement to a low-protein diet. In young rats, on the other hand, the addition of methio-

nine to the low-protein diet caused no fat accumulation, whereas the same amount of methionine incorporated in the casein allowed fat to accumulate. The probable explanation is similar to that above, viz., that the young rats used the available methionine for purposes of growth when receiving the higher casein diet, thus allowing little for lipotropic action. In the adult rat the amount of methionine required for tissue protein synthesis was less, so that more was available for lipotropic action.

Returning again to the consideration of the specific effect cystine on liver lipids, an explanation similar to the one outlined above may account for the finding that the administration of cystine causes the development of fatty livers. It may be recalled that this effect was observed when cystine was added to diets which were low in protein. Griffith[11] was the first to point out that the low-protein diet was very inadequate, and that the effect of the added cystine was to improve the nutritional state of the animal, with the diversion of the small amounts of available methionine to tissue synthesis, with a consequent decrease in the amount available for lipotropic activity, and an ensuing accumulation of fat in the liver. The so-called cystine effect, then, would be merely a manifestation of choline deficiency. Whether this is the complete explanation for the action of cystine on liver lipids is not yet entirely clear, but it does account for a large number of otherwise unexplainable experimental results.

Without wishing to reiterate the above concept too often, it may be pointed out that such an explanation accounts for other experimental observations. For example, it is well known that the mere restriction in consumption of a diet which, if given ad libitum, causes fat to accumulate in the liver, allows normal liver fat concentration. It is also an important consideration in a discussion of the effects of vitamins on the liver lipid picture.

Relation of Vitamins to Liver Lipids

McHenry[12] in 1937 advanced the idea that the administration of thiamin to animals caused the development of fatty livers, and the term "thiamin fatty liver" came into use. His evidence rested on his observation that rats on a high-carbohydrate low-choline diet did not develop fatty livers unless thiamin was provided, and that when thiamin was added to the previously thiamin-deficient diet there was an increase in both liver and body fats. McHenry proposed, therefore, that thiamin was concerned in the synthesis of fat in the body. This work has not been confirmed by other investigators. The experimental results of McHenry probably can be explained by the fact that when thiamin is lacking from the diet anorexia ensues. The consequent decreased food intake alone can prevent liver fat accumulation, as was pointed out above. The broader conclusion by McHenry, that that thiamin is concerned with fat synthesis in general by the body, also has recently been challenged by Boxer and Stetten[13]. Their results are summarized in Table II. After 16 days on the three different diets, the

TABLE II

Diet	Newly synthesized fatty acids in 5 days
A. Complete, ad libitum	2.25 gms.
B. Thiamin - deficient, ad lib.	0.18
C. Complete, pair-fed with B.	0.27

body fluids of rats were enriched with heavy water and kept at a constant isotope level for 5 days. It had previously been shown by Schoenheimer that such deuterium became incorporated in newly synthesized fatty acids. The results in Table II indicate that the failure of the thiamin-deficient animals to synthesize fatty acids was attributable to the decreased food intake rather than to any specific action of thiamin.

In addition to thiamin, almost all of the other members of the vitamin B com-

plex have at some time been implicated as causative or curative agents of fatty livers. Most of the claims have not been substantiated and with one exception will not be considered here.

The member of the vitamin B complex which appears to be directly associated with liver lipid physiology is inositol. This vitamin was shown by Gavin and McHenry[14] to possess lipotropic activity, an effect which has been confirmed by other investigators. Table III contains illustrative data from a paper by Best and his collaborators[15]. It may be observed that inositol is a less effective lipotropic agent than is choline but it does possess considerable activity. Best considers that there is a synergistic effect between choline and inositol, that is, that the two together are more effective than either alone.

TABLE III

Diet	Total liver lipids % wet wt.
A. Stock	5.5
B. Low-protein, low-choline	15.2
C. B + choline (20 mg/day)	8.0
D. B + inositol (20 mg/day)	10.1
E. B + choline (20 mg/day) + inositol (20 mg/day)	5.8

"Cholesterol" fatty livers

It was first observed by Blatherwick and his collaborators[16] in 1933 that fatty livers can be produced in animals by the inclusion of cholesterol in a diet which otherwise was without such an effect. Okey[17] analyzed the livers of rats treated in such a way, and found that the increased lipid content of the liver was partly due to an increase in the neutral fat fraction and partly to an increase in the cholesteryl ester fraction, with no effect on the free cholesterol content. The "cholesterol" type of fatty liver has been studied extensively. Some of the results are illustrated in Table IV which presents in a condensed form some data from a recent paper from Best's laboratory[18]. It may be observed from Table IV that when rats are maintained on a diet which is low in protein and in choline, but which does not contain added cholesterol, there is a marked increase in the total lipid content of the liver, most of which can be accounted for in the triglyceride fraction. There is also a definite though not great increase in the cholesteryl ester fraction. The slight increase in free cholesterol is not considered to be significant. It should be mentioned that other investigators have not observed any departure from normal in the total cholesterol content of livers from rats on a diet low in protein and choline. On diet C, which in addition to being low in protein and choline contains 0.5% cholesterol, there occurs a still greater increase in the total lipid fraction, about half of which is due to the neutral fat fraction and half to the cholesteryl ester increase, accompanied by a small increase in free cholesterol. This effect is in agreement with a number of earlier investigations. A possible explanation for this action of cholesterol in increasing the neutral fat and cholesteryl ester fractions of the liver will be offered later in this paper.

If choline is added to diet C there occurs a very marked reduction in the neutral fat fraction, to nearly normal levels, and also a marked decrease in the cholesteryl ester fraction, though proportionately less than in the neutral fat. Free cholesterol is reduced to normal. The relatively greater resistance of cholesteryl esters, as compared with neutral fat, towards the lipotropic action of choline has been observed by a number of workers. If large enough amounts of choline are given the cholesteryl ester can likewise be reduced to normal values. A striking feature in Table IV is the remarkable constancy in the phospholipid fraction considering the large changes in the other lipid fractions. This is in agreement with the idea that phospholipids are concerned with fat transport rather than with fat storage, a point which will be elaborated later.

It may be mentioned that McHenry has considered that inositol has a specific lipotropic action against cholesteryl esters, but Best and his group have not found this to be true. The further postulation by McHenry that the administration of biotin causes the production of fatty livers also lacks confirmation by others.

Mechanism of Choline Action

A large body of evidence has accumulated to indicate that choline possesses lipotropic activity by virtue of the fact that it forms a part of the lecithin molecule. Such a concept is in agreement with the widely-accepted idea that the phospholipids are important in fat transport. Experimental evidence has been greatly extended by the use of isotopic tracers. Perlman and Chaikoff[19] used radioactive phosphorus as an indicator of phospholipid metabolism, and observed that choline administration caused a marked increase in the rate of turnover of phospholipid in the liver of rats. Later Chaikoff

TABLE IV

Diet	Composition of liver lipids (mg/liver) Total Lipids	P Lipids	Free Chol.	Chol. Esters	Neutral Fat
A. Control	415	252	15.0	5	142
B. Low-protein, lowpcholine	4973	236	20.4	97	4619
C. B + 0.5% chol.	6089	280	37.7	631	5141
D. C + 0.3% choline	627	236	16.8	126	248

and his associates[20,21] made similar observations in dogs on a high-fat low-choline diet. With the administration of radioactive phosphorus and choline they observed an increased turnover of phospholipid phosphorus in liver and in plasma. They consider that the primary action of choline is on the liver phospholipids. Boxer and Stetten[22] have reached a similar conclusion from experiments in which they administered to rats choline containing isotopic nitrogen. They maintained rats on two types of diets: (1) low-protein low-choline high-fat, and (2) similar to (1) with the addition of choline. The animals in group (1) developed fatty livers while those in group (2) had a normal liver fat content. A small amount of tagged choline was then administered, and its concentration in the liver phospholipids at varying intervals of time allowed them to calculate that the half-life of phosphatide choline in the animals in group (1) was 18 days as compared with 6 days in group (2). In agreement with other investigators, Boxer and Stetten did not find any differences in the total amount of phospholipid choline in either the liver or the carcass in the two groups of animals. It appears, then, that the development of fatty liver is connected with the rate at which the choline-containing phosphatides are turned over in the body while their quantity may remain constant.

One of the striking features of choline deficiency is that even though the diet is sufficiently poor in choline and its precursors to produce a severe fatty liver, the analytical content of choline in the animal need not fall below normal levels. Also, even when there are signs of extreme choline deficiency as shown by large accumulations of fat in the liver, there seems to be no depletion of methyl

groups as evidenced by the fact that methylated products in the urine are not reduced in amount. It appears that the choline already present in the phosphatides of the body may not be effectively utilized in the prevention of the pathological lesions. The animal seems to require "new" choline, arising either from the diet or by synthesis from suitable precursors in order to maintain a normal level of liver lipids.

Stetten and Salcedo[23] have presented direct evidence that fat accumulates in choline deficiency because of interference with the normal process by which liver fatty acids are transported to the depots. In experiments similar to those described earlier where newly synthesized fatty acids can be detected by enriching the body fluids with heavy water, they found that in normal animals the depot fats contained 12-18 times as much newly synthesized fatty acids as did the liver, whereas in choline deficiency only 3 - 7 times as much was present in the depots as in the liver.

To summarize the mechanism of choline action in fat metabolism, it appears that "new" choline is required for the formation of phospholipid, and that this phospholipid is constantly and rapidly being removed from the liver and transported to the peripheral tissues. It should be added that a recent paper by Chaikoff[24] casts some doubt on the adequacy of the above explanation.

An insight into the mechanism by which cholesterol administration causes the development of fatty livers is gained by the observation of Perlman and Chaikoff[25] that cholesterol decreases the rate of phospholipid turnover in the liver, an effect which was evident before there were any signs of fat accumulation, and which could be prevented by choline administration. This can account for the accumulation of neutral fat in the liver after cholesterol administration. Since cholesteryl esters also accumulate it appears that the metabolism of cholesterol, as well as of fat, is blocked in the liver if the turnover of phospholipids is retarded. It is not known why cholesterol slows the rate of turnover of phospholipids. It is possible that since both cholesterol and phospholipids preferentially form esters with unsaturated fatty acids, the additional cholesterol may incorporate a large share of the available unsaturated acids into its esters, thus leaving a depleted supply for new phospholipid synthesis.

The mechanism of the lipotropic action of inositol is unknown. A possible clue is the isolation by Folch and Woolley[26] of a phosphatide from brain and spinal cord which contains inositol. Conceivably inositol owes its activity, like choline, to its participation in the formation of certain phospholipids.

Liver Function and Experimental Choline Deficiency

There have been few attempts to correlate liver function with fatty infiltration. One such investigation is that of McKibbin and his associates[27]. They observed that puppies which were given diets low in choline and methionine, and which developed fatty livers, showed a rise in plasma phosphatase, an impairment in bromsulfalein elimination, and a fall in plasma cholesterol, especially in the ester fraction. In severe deficiency there occurred also an increase in prothrombin time. The only liver function test which they performed and which did not indicate impaired liver function was the colloidal gold test, which yielded normal results. Li and Freeman[28] obtained similar results in adult dogs, which on low-protein diets showed impaired hepatic dye clearance and elevated serum phosphatase. McKibbin[29] observed also that the addition of choline or methionine to the diet of the puppies described above caused withdrawal of excess lipid from the liver, and restoration of liver function to normal in 5 to 10 days.

On microscopical examination of the fatty livers of the pupies described above, McKibbin and Dutra[30] observed that the irregular distribution of fat was unlike that of pure fat infiltration, and they concluded that the lesion in the liver was a combination of both fatty degeneration and fat infiltration. This concept was strengthened by the correlation of liver function tests with

the anatomical evidence. A group of pantothenic acid-deficient puppies showed a liver fat content equal to that of the choline-deficient animals, yet showed little functional abnormality of the liver. It seems imporbable, therefore, that the mere presence of fat is responsible for diminution of liver function.

Diet and Hepatic Pathology Other than Fat Infiltration

There have been a number of reports in the literature of the regular production in rats of hepatic injury, including necrosis and cirrhosis, on diets which included a minimum of all lipotropic factors. One of the most detailed accounts is that of Gyorgy and Goldblatt[31], who found that the feeding of a low-protein low-choline diet to rats caused fat infiltration of the liver in 14 days, but if the experimental period was extended to 150 days, hepatic injury resulted. In a study of the pathology of the livers of these animals Gyorgy[32] found a variety of pathological changes. Fat infiltration, in some degree, was almost invariably present. The most significant changes were diffuse or focal necrosis, with or without accompanying hemorrhage, and varying degree of cirrhosis, with fibrotic changes. The conditions of cirrhosis and necrosis were often found combined in the same liver. He found that the inclusion of choline or methionine in the diet prevented the development of the liver lesions; that methionine was more effective than choline alone, and that choline plus cystine was as active as methionine. He considers that the combined administration of choline and cystine is necessary for the synthesis of a third substance (methionine?) which is concerned in the prevention of hepatic injury. This latter hypothesis is not in line with the biochemical data which has already been considered as far as fatty infiltration of the liver is concerned, where choline appears to be of primary importance. It is possible that when liver pathology has gone beyond the stage of fatty infiltration to necrosis and cirrhosis other mechanisms may be involved.

The question arises as to whether a deficiency of the same factors which cause fatty infiltration of the liver are responsible also for necrosis and fibrosis. Before a definite reply to this question could be furnished the situation has become more complicated by reports that necrosis and cirrhosis were different from each other in dietary etiology. The most extensive experimental work on this latter distinction is that of Himsworth and Glynn[33]. Working with rats they have claimed the production of two distinct hepatic lesions by dietary means:(1)Massive hepatic necrosis, which they consider to resemble closely "acute yellow atrophy" in man. Its development is attributable to a lack of the one specific substance, methionine, and not to choline or any other lipotropic agent. It develops completely independently of fatty infiltration. (2) Diffuse hepatic fibrosis, which is considered to be the counterpart of human portal cirrhosis. This condition is produced by the lack of all lipotropic factors, and is always preceded by fatty infiltration. According to Himsworth, then, methionine has a doubly protective action against dietary liver injury. It appears, therefore, that in the prevention of dietary liver lesions methionine is the agent of choice.

The literature on the more practical problem of treatment of necrosis and fibrosis is unfortunately quite limited. The only published report directly in line with the subject as developed above appears to be that of Lowry, Ashburn and Sebrell[34]. These investigators fed to rats low-protein diets for 60 - 80 days, at which time the livers had become cirrhotic. Administration now of choline or of a diet containing large amounts of casein, caused a striking improvement in the gross and microscopic appearance of the liver. The therapy had no recognizable effect on the fibrous tissue present, but it did prevent further progression of the cirrhotic process, and caused improvement in the histological appearance of the parenchyma.

There are a number of reports of beneficial effects of treatment of human liver cirrhosis with high-caloric diets which are high also in protein and in the

vitamin B-Complex, and which contain also supplements of choline or methionine. It is impossible to evaluate such results as far as the specific activity of the lipotropic factors is concerned. From a survey of the literature the author has drawn 3 tentative conclusions as to the therapeutic value of lipotropic factors: (1) Pure fat infiltration can be successfully treated with choline or methionine; (2) Where the protein intake is adequate, additional choline or methionine cannot be expected to have beneficial effects on abnormal hepatic function; (3) Where fibrotic changes are present, a reversal cannot be expected to occur by choline or methionine administration, but the cirrhotic process may be arrested.

ANTI-FATTY LIVER FACTOR OF PANCREAS

Shortly after the discovery of insulin Allan, Bowie, Macleod and Robinson[35] observed that depancreatized dogs receiving adequate amounts of insulin, and on diets containing large amounts of lean meat, did not survive for periods longer than a few months. The animals showed signs of impaired liver function, and the livers were found to be infiltrated with fat. Later, Hershey[36] and Hershey and Soskin[37] found that lecithin could prevent the fat infiltration, and this observation led to the discovery by Best and Huntsman[38] that choline was the component of lecithin which was responsible for the prevention of fatty liver development.

In 1936 Dragstedt and his associates[39, 40] began investigations on the control of liver fat in depancreatized dogs, and postulated the existence of a new hormone in the pancreas which they termed "lipocaic", which prevented and relieved the fatty infiltration of the livers of depancreatized dogs maintained with insulin. Therewith began a polemic which has continued up to the present time, but which now appears close to a settlement. The controversy settled about the nature of the anti-fatty liver factor of the pancreas, with Dragstedt the leading proponent of the idea that "lipocaic" was a hormone, that is, an internal secretion, and Chaikoff the chief contender that the lipotropic pancreatic agent was an external secretion, present in pancreatic juice. The following summaries list the points of agreement and disagreement of the opposing groups.

Points of Agreement

1. Preventable by feeding raw pancreas.
2. Preventable by feeding choline.
3. Preventable by feeding extracts of pancreas, not accountable for by choline or methionine content.

Points of Disagreement

1. Effect of ligation of pancreatic ducts of normal dogs.
2. Effect of feeding pancreatic juice to depancreatized dogs.

Dragstedt and his associates[39] obtained completely opposite experimental results on the last two mentioned points from those of Chaikoff and his collaborators[41,42,43]. Dragstedt found that if the pancreatic ducts of normal dogs were ligated, fatty livers did not develop, and that the feeding of pancreatic juice to depancreatized dogs was without effect on the liver fat. These results he interpreted as evidence for his contention that "lipocaic" was a hormone, unaffected by the presence or absence of the external secretion of the pancreas. In contrast, Chaikoff and his group have advanced very convincing evidence for locating the anti-fatty liver factor of the pancreas in the external secretion. They have found recently[44] that the administration of as little as 10 cc. a day of normal pancreatic juice is sufficient to prevent fatty livers in depancreatized dogs maintained with insulin, indicating that pancreatic juice contains an active factor. In addition, they have advanced experimental evidence which elucidates the mechanism by which pancreatic juice exerts its action.

Chaikoff and his associates[45] routinely fed their depancreatized animals a diet which allowed each dog 500 gms. of lean meat a day, an amount which was found to contain 3 gms. of methionine

and 0.5 gm. choline. Fat infiltration of the liver occurred. If they added to this diet 2 - 3 gms. of free methionine daily the fat content of the liver was normal.. This suggested to them that the depancreatized dog lacked the proteolytic enzyme necessary to liberate methionine from the protein, so that the animal actually suffered a methionine, and in turn a choline deficiency, which accounted for the fatty livers. Further evidence for the correctness of this explanation has recently been offered by Chaikoff[46]. The inclusion in the diet of 38% casein caused the livers to become infiltrated with fat, with a total liver fatty acid concentration of 15.1%. If, on the other hand, the diet contained 38% hydrolyzed casein, fat failed to accumulate, and the concentration of total liver fatty acids was reduced to 1.8%. Further experimental evidence indicates quite clearly that methionine is the component of the hydrolyzed casein which is the responsible agent.

The conclusion appears justified that in the gastro-intestinal tract of the completely depancreatized dog maintained with insulin there is an interference with the mechanism whereby the methionine of the protein is made available for lipotropic purposes. This defect, as far as the fatty liver is concerned, can be overcome by feeding choline, or its precursor methionine, or by fractions from raw pancreas presumably containing the proteolytic enzyme. It remains to be determined whether the anti-fatty liver factor of pancreas is identical with trypsin. If Chaikoff's concept proves to be correct, our original distinction between dietary fatty livers and fatty livers of depancreatized dogs maintained with insulin becomes an artificial differentiation.

References

1. Best, C. H., Hershey, J M., and Huntaman, M. E. J.Physiol.75:76, '32.
2. Channon, H. J. and Wilkinson, H. Biochem.J., 29:350, '35.
3. Tucker, H. F. and Eckstein, H. C. J.Biol.Chem., 121:479, '37.
4. Beeston, A. W. and.Channon, H. J. Biochem.J.30: 280, '36.
5. Beveridge, J. M. R., Lucas, C. C., and O'Grady, M. K. J.Biol.Chem., 160:505, '45.
6. Best, C. H. and Ridout, J. H. J.Physiol., 97:489,'39.
7. Channon, H. J., Manifold, M. C., and Platt, A. P. Biochem.J. 34:866, '40.
8. Tucker, H. F., Treadwell, C. R., Eckstein, H. C. J.Biol.Chem.135:85, '40.
9. Treadwell, C. R., Groothias, M., Eckstein, H. C. J.Biol.Chem. 142:653, '42.
10. Treadwell, C. R., Tidwell, and Gast. J.Biol.Chem.156: 237, '44.
11. Griffith, W. H. J.Nutrition, 21:291, '41.
12. McHenry, E. W. J.Physiol.,89:287, '37.
13. Boxer, G. E., and Stetten, DeW. J.Biol.Chem. 153:607,'44.
14. Gavin, G. and McHenry, E. W. J.Biol.Chem.139:485, '41.
15. Best, C. H., Lucas, C. C., and Patterson, J.M., and Ridout, J. H. Biochem.J. 40:368, '46.
16. Blatherwick, N. R., Medlar, E. M., Bradshaw, P. J., Post, A. L., and Sawyer, S. D. J.Biol.Chem.97:xxxiii, '32.
17. Okey, R. J.Biol.Chem.,100:lxxv, '33.
18. Ridout, J. H., Lucas, C. C., Patterson, J.M. and Best, C. H. Biochem.J.40:494, '46.
19. Perlman, I. and Chaikoff, I. L. J.Biol.Chem.,127:211, '39.
20. Friedlander, H. D., Chaikoff, I. L., and Entenman, C. J.Biol.Chem.158:231, '45.
21. Entenman, C., Chaikoff, I. L., and Friedlander, H. D. J.Biol.Chem.162:111, '46.
22. Boxer, G. E. and Stettin, De W. J.Biol.Chem.,153:617, '44.
23. Stetten, De W. and Salcedo, J. J.Biol.Chem., 156: 27, '44.
24. Entenman, C., Chaikoff, I. L., and Zilversmit, D. B. J.Biol.Chem.166: 15, '46.
25. Perlman, I., and Chaikoff, I. L. J.Biol.Chem.,128:735, '39.
26. Folch, J. and Woolley, D. W. J.Biol.Chem.,142:963, '42.
27. McKibbin, J. N., Thayer, S., and Stare, F. J. J.Lab.and Clin.Med. 29:1109, '44.

28. Li, T. and Freeman, S.
Am.J.Physiol., 145:646, '46.
29. McKibbin, J. M., Ferry, R. M., Thayer, S., Patterson, E. G., and Stare, F. J.
J.Lab.and Clin.Med., 30:422, '45.
30. Dutra, F. R. and McKibbin, J. M.
J.Lab. & Clin.Med., 30:301, '45.
31. György, P. and Goldblatt, H.
J.Exp.Med.75:355, '42.
32. György, P.
Am.J.Clin.Path., 14:67, '44.
33. Himsworth, H. P. and Glynn, L. E.
Clin.Sci.5:93,133, '44.
34. Lowry, J. V., Ashburn, L. L., and Selnell, W. H.
Quart.J.Studies Alc., 6:271, '45.
35. Allan, F. N., Bowie, D. J., Macleod, J. J. R., and Robinson, W. L.
Brit.J.Exper.Path., 5:75, '24.
36. Hershey, J. M.
Am.J.Physiol,, 93:657, '30.
37. Hershey, J. M. and Soskin, S.
Am.J.Physiol., 98:74, '31.
38. Best, C. H. and Huntsman, M. E.
J.Physiol.75:409, '32.
39. Dragstedt, L. R., Van Prohoska, J., and Harnes, H. P.
Am.J.Physiol., 117:175, '36.
40. Van Prohoska, J., Dragstedt, L. R., and Harnes, H. P.
Am.J.Physiol.117:166, '36.
41. Chaikoff, I. L., and Kaplan, A.
J.Biol.Chem.112:155, '35.
42. Kaplan, A. and Chaikoff, I. L.
J.Biol.Chem.119:435, '37.
43. Kaplan, A. and Chaikoff, I. L.
J.Biol.Chem. 120:647, '37.
44. Montgomery, M. L., Entenman, C. and Chaikoff, I. L.
Am.J.Physiol. 148:239, '47.
45. Chaikoff, I. L., Entemna, C., and Montgomery, M. L.
J.Biol.Chem. 160:489, '45.
46. Chaikoff, I. L., Entemna, C., and Montgomery, M. L.
J.Biol.Chem., 168:177, '47.

III. GOSSIP

The following editorial on "Our Social Obligations" by Richard H. Young, M.D., appeared in The Journal of the Omaha Mid-West Clinical Society April 1947. "Because of the political activities of certain groups of socially oriented individuals, the rank and file of the medical profession have shown an uneasiness toward social attitudes. Once aroused to a social need, the physician has always acted with distinction. This has been evidenced by leadership in preventive programs, suoh as the immunization of children, and more recently, by inaugurating plans for voluntary health insurance. It would be a fair criticism to state, however, that the medical profession, like other groups, exhibits a "social lag". In other words, we are slow to discharge our social responsibilities.

What sort of a social attitude should the physician harbor? The present-day attitude of resistance to things social is fundamentally human. It is society that makes us social, and then only under protest. The result is that groups advocate social reforms which are to their benefit, the old story of private gain in disguise. Unfortunately, the medical profession has been so busy defending itself from the aggressive attacks of those who wish for a passive, dependent existence that it has had little time to investigate social problems which need medical leadership.

There are many social problems that need medical direction. A good examplo is Alcoholism, a condition treated as a delinquency rather than a sickness, and treated punitively rather than medically. About one case in five that comes to a welfare agency has alcoholism as its main problem, and there is a similar ratio in cases admitted to the State Hospitals. This constitutes an opportunity for medical leadership in developing plans for prevention and treatment, and seeking the help of interested groups for support. In this and similar ways the physician can aggressively discharge his social responsibility and bring credit to the profession."....Carl Buck, a member of the public health team which visited us this week, has had an interesting and varied career. His vocation is public health surveys, but he has many avocations. He was born of academic parents but a gypsy strain cropped out in him. As a young man he was an outstanding athlete. Later he joined a circus where he did a high dive in a tank of shallow water. Now he often tours with his wife and whenever the spirit moves him, they camp right there. He sleeps well anywhere at any time..... Dr. Theodore Althausen of the University of California (another visitor) was born in Russia. He left his ship in World War I and stayed here. After working his way through medical school he was awarded a Guggenheim Fellowship for foreign travel and study. Today he is an outstanding authority on the liver and its diseases.....Edith L. Potter, who is visiting us today, is a Minnesota graduate and has gone far in her studies on the pathology of the newborn. She was the sparkplug for the group which set an all-time record in Chicago by doing a higher percentage of postmortem examinations on infants than has ever been done in any other city. As a result of this investigation, Chicago set up an infant and premature care program which has gotten results. Her latest book is on the Rh factor.....The students and faculty of the medical group at the Center for Continuation Study are planning a picnic at Interstate Park near St. Croix Falls, Wisconsin, on May 24. Feature will be a barbecue done by an expert. Adults, $2, but bring all your children (free). Transportation can be secured by telling us of your needs.. .....Ephraim Shorr has delighted everyone with his excellent presentations and pleasing personality.....W. J. Kolff, of Kampen, Holland, will tell of his experiments with the artificial kidney, May 16 at 4 p.m. (today), in the auditorium of the Museum of Natural History. His investigations were written up recently in Life and they are all the more remarkable because they were done under trying conditions.....The University gardeners have acquired a new plot and yours truly has again taken up the hoe. Early in the war the newspapers photographed me leaning on my hoe as my wife worked on the ground. This provided entertainment and amusement for G.I.'s everywhere. The most touching tribute came from my good friend, Colonel Murphy, who thought it resembled "The Angelus"...