1947

Lipotrophic substances

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LIPOTROPIC SUBSTANCES

by
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Senior Thesis
Presented to University of Nebraska
College of Medicine
January, 1947

INTRODUCTION

The importance of fat metabolism in the body economy has been recognized since before the advent of Modern Medicine. The relationship of fat metabolism to carbohydrate and protein metabolism has long been studied and little understood until during recent times animal experimental work has greatly accelerated the study of the absorption, assimilation and functions of fats within the animal body. Since the middle of the last century the action of cholesterol in altering the constituency of tissue fat components in the body has been recognized but not understood until animal experimentation opened the field to research work on the mysteries of fat metabolism.

The disease Diabetes Mellitus was the inciting factor to this energetic search for the understanding of the function lipid containing substances within the body. Dogs were depancreatized to study the cause of diabetes. The discovery of insulin and its use in the studies of depancreatized dogs demonstrated that there were functions of the pancreas in addition to the production of insulin. Raw pancreas was required in the diet to maintain the life of depancreatized dogs.

This experimental evidence showed that fat metabolism is dependent upon some substance other than insulin or in addition to insulin produced by the pancreas. Since 1924 animal experimental work has been carried continuously in attempt to find a

substitute for the pancreatic product which is essential to fat metabolism.

In 1936 Dragstedt found a pancreatic extract called lipocaic which appeared to fill the breach in the absence of the pancreas in keeping depancreatized dogs alive when maintained with insulin. This finding stimulated the research work to wider considerations of biological substances an processes involved in lipid metabolism. The entire solution is not yet provided but great progress has been made which shows evidence of possible clinical application in the treatment of human pathologic conditions resultant from the failure of physiological metabolism of fats in the body economy.

HISTORICAL

In his report on the relationship of choline and other methylated compounds du Vigneaud noted that in 1849 Staecker isolated choline from bile lecithin. (62) Hershey and Soskin report that Mehring and Minkowski produced experimental diabetes in dogs in 1889 and raw pancreas was shown by Sandmeyer in 1895 to be an effective aid to digestion in the depancreatized dog. (97) In 1916 Mc Means studied fat deposition in relation to vital organs. (121) Also in 1916 Bailey studied fat metabolism feeding cholesterol to rabbits. (6)

The discovery of insulin as a pancreatic hormone by Best and Banting in 1921 opened up new avenues for conjecture and was followed by new approaches to the problem of substituting substances in the diet to replace the function of the pancreas in the body economy. In 1924, Fisher, reporting on results of experimental work with a depancreatized dogs maintained with insulin, stated that insulin probably does not represent the entire pancreatic hormone since administration of insulin alone in these dogs does not maintain life, nor control all the diabetic symptoms such as fatty liver, atheromatous changes and weight loss. (73) In the same year independent from Fisher's work, Allan, Bowie, Macleod and Robinson, using depancreatized dogs adequately maintained with insulin, found that insulin could not prevent the ultimate breakdown of hepatic function nor maintain life for long periods. (1)

Hershey in 1930 considering the theory advanced by Leathes and Raper in 1925 that phospholipids were involved in fat transportation from the liver tried to substitute lecithin for raw pancreas in the diets of depancreatized dogs adequately maintained with insulin. He found that the dogs survived longer than with insulin alone but lived only a few months. At autopsy the livers showed fat content up to thirty-five per cent and a low iodine number of about sixty-five showing increased saturation of the fats. (96) Expanding on this work Hershey and Soskin in 1931 feeding a diet of glucose and lean beef muscle found that the dogs survived for longer periods without pancreatic juice when adequate lecithin was included in the diet. Hershey's idea that pancreatic juices were involved only in absorption of fats from the intestine and not involved in the metabolism of fat led him to try substituting lecithin for raw pancreas. (97)

This work pointed the way and other investigators began the search for the substitute that would perform the function of the pancreas other than that which could be accounted for in pancreatic juice and insulin. Inositol was found to be active in the metabolism of some lipid fractions.

In 1936 Dragstedt reported that he had discovered a second pancreatic hormone, which he calls lipocaic that is essential to fat metabolism. Phospholipid involvement in fat metabolism has been proven.

EXPERIMENTAL

Hershey in 1930 demonstrated that substituting lecithin for raw pancreas in the diet of depancreatized dogs maintained with insulin would not keep the dogs alive but would extend their lives longer than if administered insulin alone. At autopsy the livers contained as high as 35% fat which had an iodine number of about 65, indicating increased saturation of the liver fats.(96) This work stimulated an interest in the study of lipotropic substances in other laboratories. The term "lipotropic" is defined as a substance which prevents or removes an accumlation of excess fat from the liver. Expanding on Hershey's work, Hershey and Soskin in 1931 found that the dogs could survive for long periods without pancreatic juice when fed a diet of glucose and lean beef muscle if sufficient lecithin was given.(97)

Using normal rats instead of the dogs, Best, Hershey and Huntsman in 1932 demonstrated that choline could be used effectively in place of lecithin with a high fat, low protein diet and prevent the development of fatty livers, requiring only 10 mg. of choline per day to accomplish this. (19) During the same year Best et al. studied the lipotropic effects of lecithin and found sodium oleate, sodium glycerophosphate and ethanolamine to be non-effective, but choline was definitely lipotropic though it had no effect upon the excretion of fat, (20) and (21). Best, Ferguson and Hershey (17) found that choline was effective in preventing

development of fatty liver in the diabetic dog concluding that choline was the active principle of raw pancreas. In 1933 Best and Ridout reported that the deposition of fat in the rats' livers produced by feeding cholesterol could be prevented by feeding either choline or betaine (24) but choline is not so effective when cholesterol is fed as when it is eliminated from the high fat diet. (29) Also in 1933 Blatherwick et al. (32) producing dietary fatty livers in rats found that feeding whole liver caused the development of fatty livers with large amounts of fat and cholesterol esters. Chanutin and Ludewig (47) found that cholesterol feeding when continued as long as three weeks increased the free cholesterol content of the rats' livers.

Various proteins have been shown to effect lipotropic functions in varying amounts. Channon in 1935 reported experimental proof that protein exerts on inhibitory action against the production of fatty liver. (46) During 1936 several laboratories worked on the problem of determining the constituent in protein which gave protein its lipotropic potency. Best (27) found that when rats are fed cholesterol the lipotropic effect of protein is on theglyceride fractions of the liver fats. Best et al. (18) found also that deposition of liver fat was affected by the amount of casein in the diet. Baernstein (5) in studying the lipotropic action of various proteins reported that the lipotropic potency of protein is proportional to its methionine content. Beeston (11)

found that only 5 to 8 mgm. of choline supplied as much preventative action as one gram of casein against production of fatty liver in rats on high fat, low protein diet. Continuing his investigation on sulphur containing proteins Beeston (10) found that though methionine prevents fatty liver, cystine augments fatty infiltration.

Tucker (142) in 1937 collaborated Beeston's report by substituting methionine and cystine in the diet of rats. Further work in 1938 by Tucker et al. found by feeding high fat diets c̄ gliadin that adding lysine to this diet made no difference in relation to development of fatty liver; adding cystine demonstrated that the lipogenic action of cystine is nullified by gliadin; adding methione showed that gliadin enhanced the lipotropic action of methione. These same authors demonstrated that the lipotropic effect of casein is more than that of edestin and gliadin is less potent than edestin. (143 and 144)

Singal and Eckstein (135) in 1939 presented evidence proving that adding cysteine and homocystine as well as cystine produce an increase in the deposition of liver fat in rats. Further confirmation of the opposing actions of the two sulfur containing amino acids is contained in the report by Channon et al. in 1940 that cystine augments the deposition of liver fat and that methionine inhibits this process in rats when fed a high fat, low protein diet. (42) Earle and Victor in their reports (64 and 65) showed that it

is the proportional amount of cystine in the diet rather than the total amount that influences the severity of the liver lesions in rats. They further show that continued excess of cystine causes liver hemorrhage and necrosis. Again in 1942 Treadwell et al. (141) reported that cystine incorporated in the rats' diet increased the liver lipid content; methionine decreased the liver lipid content, and that casein produced a less potent lipotropic action than methionine. They also found that free methione was more effective than an equal amount of methionine contained in protein complexes. Best (28) in 1940 reported free methionine to be a potent lipotropic substance.

In 1945 after continued experimental work with methionine Treadwell et al. (140) found evidence of preferential utilization of methionine for growth of the animals. Methionine in excess of that used for growth is available for lipotropic function when other essential amino acids are provided in the diet. Taurog, Entenman and Chaikoff (139) in 1944 and Handler (93) in 1943 found similar results in their work. Draft, Sebrell and Ridout in 1942 reported that neither high fat diet nor cystine are essential to the production of liver cirrhosis in rats. They show that choline and methionine exert a preventative action against liver cirrhosis, while cystine and methionine exert preventative action against hepatic hemorrhage and necrosis. (54) Work by Horning and Eckstein in 1944 reaffirms the evidence that methionine inhibits and cystine augments the deposition of liver fats in rats fed a high fat, low

protein diet. (98)

Channon et al. (40) reported in 1938 that the marked lipotropic action of certain proteins was correlated with the methionine content of the protein. Beveridge (31) in 1944 confirmed this work of Channon's group. Channon (40) suggested the possibility that lipocaic may owe its lipotropic function to methionine. These authors further reported (43) the lipotropic potency of methionine is only about one-twelfth that of choline; later work revised this percentage to about one-fifth. They also report that large doses of methionine prevent "fat" fatty livers in rats but is less active on the "cholesterol" fatty livers.

Singal and Eckstein confirm the report of Channon's group with the statement that proteins with low methionine content lack lipotropic action. (135)

At this point it is appropriate to explain the nature of choline, its derivation and its part in the efficacy of protein substances which demonstrate lipotropic properties.

Stetton proposed in 1941 that choline was synthesized in vivo. Working with rats he found that betaine is demethylated to glycine and enthanolamine with little or none going directly into choline synthesis. The lipotropic effect of betaine is mainly attributable to its function as a donor of methyl groups in the synthesis of choline. (137)

du Vigneaud using white rats to do research on methionine

to determine the interrelationship between choline and other methylated compounds within the animal body. He found metabolic interrelationships between methionine and sulfur metabolism with choline and fat metabolism on the one hand; and between mthionine and choline metabolism with creatine metabolism on the other. The body is incapable of generating the methyl group for the purpose of certain methylations. The methyl groups for these methylations must be present in the diet in a certain utilizable or labile form; and in these particular methylations the methyl group has been found to be transferred as a unit. (62) In another report the same year using methionine with deuterium labeled methyl groups in the diets of white rats, du Vigneaud et al. traced the methyl groups through the methylation processes in vivo. During a period of fourteen weeks feeding the deuteromethionine it was found that 85% of the deuterium was contained in the methyl groups of choline and creatine in the body tissues and creatinine in the urine. The choline, creatine and creatinine each contained about the same percentage of the deuteromethyl groups at equal lengths of time of feeding the deuteromethionine and progressed the same as the time lengthened. By feeding deuterocholine and homocystine the transfer of methyl groups from choline to creatine was demonstrated by isolation of the deuterocreatinine from the urine. The ability of the white rat to transfer methyl groups to synthesize choline and creatine of the body tissues is experimentally demonstrated. (63)

The lipotropic action of methionine is demonstrated to be due to transfer of its methyl groups in the synthesis of choline. Qualitatively methionine and choline have the same lipotropic action. They differ quantitatively because of the part played by methionine in the synthesis of choline. (63)

McKibbin et al. state that methionine is the precursor of methyl groups in the synthesis of choline. This was demonstrated by the feeding of methionine to prove its lipotropic effect in weanling puppies. (120)

There are various types of fatty livers developed in experimental animals when the feeding of the animals is not standardized. The efficacy of some lipotropic substances is altered by the type of fatty liver. McHenry in 1937 described the "thiamin" fatty liver as being characterized by containing large amounts of glycerides and small amounts of cholesteral, either free or esterified. This type of liver includes the "fat" fatty liver and "dietary" fatty liver and is produced in rats by including thiamin in a high carbohydrate diet, either with or without fat, and in the absence of choline. Feeding a diet of over 40% fat in the absence of thiamin also produces this type of fatty liver. The "thiamin" fatty liver can be prevented by small amounts of choline in the diet. (115)

In his review in 1945 McHenry found that many conflicting

reports have occurred as a result of the authors not being cognizant of the variation in the fat constituents in the livers of the experimental animals. The other major type is the "biotin" fatty liver which is characterized by a high content of free cholesteral or its estêrs. The "biotin" fatty liver is produced by feeding rats a liver fraction or by feeding biotin with riboflavin, pantothenic acid, pyridoxine and choline. This type of fatty liver is similar to the liver produced in the depancreatized dog and both ar resistant to large doses of choline but are prevented or cured by lipocaic. (116) Confirmed by Dragstedt in 1945.(48)

One of the earliest reports on the effect of high cholesterol diets is that of McMeans in 1916 in which he reports the increase in lipid content of the liver and other organs in experimental animals caused by feeding high cholesterol diet. (121) In 1934 Channon and Wilkinson (45) reported that choline was not effective on what they called "cholesterol" fa ty liver. Best in 1934 reported that rat experiments demonstrated that choline prevents neutral fatty livers, but is not effective on cholesterol fatty livers and exerts little effect on the cholesterol esters of fatty livers. (16) and (26) In 1935 Best et al. confirmed the above report (25), adding that choline is effective on the glyceride fraction in cholesterol fatty livers and up to 60% of cholesterol esters when cholesterol is included in the diet.(15)

They found that a high carbohydrate diet fed without choline causes fatty liver in rats.(22) Best et al. reported that fatty liver resulting from phosphorus and carbon tetrachloride poisoning was not prevented by choline but choline did increase the disappearance of the fat during the recovery period.(18),(19),(23),(7).

Aylward's(3) report in the same year confirms Best's reports. Becston reported that including large amounts of casein in the diet would inhibit production of fatty livers.(12) He also found that feeding liver to rats caused an increase of cholesterol esters in the liver lipids.(13) Loizides in 1938 reported that in the "cholesterol" fatty liver in rats there is also present a larger amount of glyceride fraction lipids in the livers than there is when "thiamin" liver is produced in the absence of cholesterol. Choline inhibits the production of cholesterol esters if given in large doses and prevents the accumulation of glycerides.(106)

Channon and Smith (44) found choline related compounds to possess lipotropic action. In studying the vitamin B-1-sparing action of fats McHenry (114) found that the effects of choline, vitamin B-1 and fat are interrelated in the body weight gain in young rats. The effect of fat is augmented by choline and the body weight is maintained on a diet deficient in vitamin B-1 by increasing dietary fat to 40%. Thiamin in the absence of choline will maintain the body weight on a diet containing only 10 to 26% fat.(114). Further work disclosed that vitamin B-1 is complimentary to choline as just described, but is antagonistic

to the lipotropic action of choline.(115)

In 1939 Perlman and Chaikoff using radioactive phosphorus as an indicator found that ingestion of cholesterol depressed the phospholipid metabolism by decreasing the liver content of newly formed phospholipids even before the liver became fatty. Betaine was effective in stimulating phospholipid turnover in the liver, but less active than choline.(127) and (128)

Study of dietary liver fat deposition in the rat was carried out by several investigators separately; Blumberg and McCollum(33), Webster(147), Lillie et al.(105), Connor(49) and Gyorgy and Goldblatt(91); all making similar reports to the effect that choline acts lipotropically on glyceride fractions of fat; that fatty infiltration of the liver is prerequisite to cirrhosis and that conditions causing fatty infiltration early will, if continued, result in hepatic cirrhosis. Blatherwick et al. conducted a series of experiments in 1931-33 feeding liver extracts to rats demonstrating that beef liver contains a factor causing fatty liver in rats.(32) McHenry and Gavin (117) and (118) confirmed this report in 1940 by feeding rats an alcoholic extract of beef liver in small amounts with a fat-free diet and the vitamin B complex which caused acutely fatty livers within seven days. The body fat also increased and the authors concluded the increased

amount of fat was due to fat synthesis. The basal diet and supplements were free of cholesterol but the fatty livers contained large amounts of cholesterol esters. Large doses of choline had no beneficial effect but administration of lipocaic proved effective.

Further work using rats was carried out by several groups producing fatty livers using various diets. Halliday(92) feeding liver extracts reported that pyridoxine deficiency caused fatty liver. Forbes (75) fed nicotinic acid and reported increased liver cholesterol level. McHenry and Gavin(77) fed pure pyridoxine but found no evidence of lipotropic action. They substituted biotin for the liver extract in the diet used in previous experiments.(117) and (118) Fatty liver developed which was resistant to choline as before but prevented by lipocaic or inosital.(79) Their further diet experimental work demonstrated that administration of pantothenic acid and pyridoxine altered the liver lipids to the extent that choline was ineffective.(80)

Handler(93) and (94) found that thiamin deficiency does not retard liver regeneration and that thiamin and other B vitamins ten to prevent development of fatty liver with choline deficiency. He reported that administration of nicatinomide during choline deficiency caused marked loss in body weight, but no increase in liver lipid content. Gyorgy and Goldblatt feeding rats a diet of

18% casein, 68% sucrose, 10% fat and 4% salt mixture supplemented with thiamin, riboflavin and pyridoxine developed acute, diffuse necrosis of the liver, which developed into hepatic cirrhosis when the casein in the diet was cut to 18%.(90). Draft (53) concluded that diet composition is the essential factor in dietary liver cirrhosis in rats.

Longenecker presents experimental evidence disproving the formerly accepted theory that fatty reserve tissue is laid down only when the body caloric intake surpasses its caloric requirement. He shows that there is a constant state of flux of depot fat with deposition, withdrawal and replacement of constituent fatty acids. There is a continual source availabe either from food or by syntheis in vivo. The liver is the probable site of fat synthesis from carbohydrate which requires thiamine and is augmented by the presence of riboflavin and other B vitamins.(107)

Choline is essential in fat metabolism in addition to its lipotropic action in preventing fatty infiltration of the liver. Cox (50) in 1929 found that dietary increase of cystive was related to production of necrotic kidney lesions. In the light of present knowledge there probably coexisted a choline deficiency. Gyorgy and Goldblatt (91) demonstrated that choline deficiency causes lesions of the kidney tubules. Choline dosage of about one-fifth that required to benefit fatty liver will benefit kidney lesions

of this nature. Griffith, Wade and Mulford in research work from 1939 to 1941 report that methionine is the source of methyl groups for in vivo synthesis of choline which is essential in rats for normal metabolic maintenance of tissue structure and survival. Renal hemorrhagic degeneration is one of the principle effects of choline deficiency in young rats. Increase of cystine, fat or cholesterol in the absence of choline augments the condition, and the administration of choline inhibits the renal damage. The relative proportions in the diet of cystine, choline and methonine rather than total amounts is the determining factor in renal lesions rats.(84),(85),(86),(87),(88) and (89), and (125).

Chaikoff and Kaplan in 1934 verified the reports of other groups that there is a definite increase in the liver lipid content and a decrease in the blood lipids in depancreatized dogs maintained with insulin and fed a diet containing no lecithin, choline or raw pancreas.(39) Fletcher, Best and Solondt in 1935 compared the effects of raw pancreas with the effects of choline added to the dogs' diets and reported that 2 grams of choline in addition to that in the basal diet to exert the benefit of 100 grams of raw pancreas which contains only about 250mgm. of choline.(74) Ralli et al. (130) found raw pancreas to be more effective in preventing the deposition of low unsaturation lipids in the dog liver than could be accounted for on its lecithin

content. Chaikoff and Kaplan (38) observed the decrease in the plasma phospholipid levels coincident with the increase in the liver lipids and found that feeding raw pancrease caused an increase in the blood lipid levels not observed when feeding choline.

Dragstedt et al. presented evidence in 1936 indicating that the effect of feeding raw pancreas to depancreatized dogs could not be accounted for on the basis of its content of insulin and pancreatic enzymes alone. They reported the isolation of a specific substance in the alcoholic extraction of beef pancreas, which when given orally permits survival of the dogs and relieves fatty infiltration and degeneration of the liver. This substance extracted in alcohol and purified with ether to remove all lipid materials including lecithin, was named lipocaic. The authors believe lipocaic is a pancreatic hormone, defending this belief by presenting evidence that is is fifteen times as effective as the choline contained in an equal amount of raw pancreas. Brain and liver fed in the diet proved ineffective as lipotropic agents though each contains as much choline as raw pancreas.(59), (145).

In 1937 Kaplan and Chaikoff in a series of experiments found that there are two phases of fatty liver appearing in depancreatized dogs. The first phase appears soon after pancreatectomy and is relieved by administration of insulin. The second phase follows even with continuous control of the blood liver

levels with insulin. They showed that raw pancreas prevents both phases of the fatty liver, but autoclaved pancreas and choline control only the first phase. Raw pancreas raises the blood lipids back to normal but choline and autoclaved pancreas do not. This evidence is against the theory that the effect of raw pancreas is enzymatic.(102),(103) and (104).

Aylward and Holt(4)report that raw pancreas prevents fatty infiltration of the liver in rats on a high fat,low protein diet but disputed the claim of MacKay(109) that raw pancreas is more effective than choline. Best and Ridout.claimed that MacKay's results were due to the choline and protein content in raw pancreas.(30) Best et al. reported in 1938 that fatty liver in rats produced by the anterior pituitary "ketogenic" fraction causes a marked increase in liver fat at the expense of body fat.(8) and (14) MacKay and Barnes (110) and (111) reported that anterior pituitary fatty liver in rats is not prevented by choline, although the ketonuria is reduced. Channon et al. reported that pancreatic extract is of greater lipotropic potency than the amount of choline contained in the same amount of pancreas and this effect is not accounted for by the protein and choline content of the pancreas. They concluded that pancreatic extract contains some lipotropic substance other than choline.(41)

In 1938 Goodpasture et al. reported that the Bromsulphalein

liver function test proved the effectiveness of lipocaic in reducing liver damage by doing biopsies of the damaged livers before and after lipocaic therapy and comparing the liver tissue changes with the successive bromsulphalein tests results. In depancreatized dogs the liver function returned to normal in 10 to 16 days.(81) Dragstedt et al. demonstrated that lipocaic caused the blood lipid levels to raise to normal after the drop accompanying fatty infiltration of the liver.(58) In 1939 Dragstedt et al. defended their claim that lipocaic is a hormone by painting out the experimental results which showed that the depancreatized dog is not restored to health by administration of insulin and pancreatic juice, or by the addition of choline nor other organs containing as much lecithin as equal amounts of pancreas. The deficiency is corrected by the administration of insulin plus either raw pancreas or lipocaic. They refer to the two types of fatty liver mentioned above. Both types appear in diabetes mellitus and the pancreatic diabetes in depancreatized dogs. The first phase of the fatty liver is due to poor dietary control of the diabetes and inadequate insulin therapy. This is characterized by normal or high blood lipid levels and is relieved by insulin alone. The second phase is due to lipocaic deficiency and is characterized by low blood lipid levels, impaired liver function, decreased dextrose

excretion and insulin sensitivity. This phase of the liver pathology is not improved by giving insulin and pancreatic juice but responds readily to insulin and lipocaic.(60)

Engel (67) in 1942 fed rats large amounts of vitamin B complex with 10 mgm. of choline per day which just failed to control deposition of liver fat. To this regime 3 mgm. of inositol per day was added, which resulted in the control of the liver fat. This work confirmed the earlier claim by Gavin and McHenry (78) that inositol acted like lipocaic in control cholesterol deposition in biotin type fatty liver. Julian et al.(101) definitely demonstrated in 1946 that lipocaic prevents the fatty liver in rats produced by the administration of anterior pituitary extract.

In 1943 Allen at al.(2) reported that the elimination of pancreatic juice, from an otherwise normal dog by resecting the portion of duodenum which received the external pancreatic secretion and attaching it to the outside body surface, did produce permanent fatty livers. Entenman et al.(72) did not believe this procedure satisfactorily eliminated all the pancreatic juice and continue with their contention that pancreatic juice with insulin does prevent the development of fatty livers in depancreatized dogs.

Ralli et al. (131) are in accord with the views expressed

by Chaikoff and associates based on results of their experimental work with depancreatized dogs carried on through 1938 to 1944. They claim that the absence of pancreatic juice does contribute to development of fatty liver. The external pancreatic secretion may supply lipocaic directly or may cause the liberation and consequent absorption of a lipotropic from injested foods. They agree that lipocaic possesses lipotropic effectiveness which can not be attributed to its content of choline or other protein. They do not believe the experimental evidence has demonstrated lipocaic to be a hormone.(70),(71),(72),(122),(123),(126) and (128). Reaffirmed by same group in 1945.(37)

Cancodo in his article on lipocaic in the Brazil-medico makes reference to the two types of granular cells in the Isles of Langerhons in the pancreas. The beta cells have no specific function identified with them. Concodo remarks that the hypothesis that the pancreas furnishes more than one hormone was offerred with the discovery of two types of island cells.(35)

Dragstedt in 1940 recounts the sequence of developments in the depancreatized dog: 1.- Immediate hyperglycemia and glycosuria develops. 2.- The animal becomes rapidly emaciated and dies from pancreatic diabetes in from 1 to 4 weeks. 3.- A marked fatty infiltration immediately occurs. 4.- If the dog is given a diet of protein, carbohydrate and fat plus active pan-

creatic juice and adequate insulin, either protamine zinc or regular, life is prolonged and the lipocaic deficiency becomes manifest. The early fatty liver disappears, acidosis is relieved and the blood lipid levels approach normal. 5.- The passage of time brings a gradual decrease in dextrose excretion which the decrease in insulin dosage does not reverse. In 6 to 8 weeks the daily insulin requirement is down to 2 or 3 units, and the bromsulphalein test shows impaired liver function. 6.- The blood lipid levels decrease. 7.- Progressive weakness, anorexia and emaciation are followed by death. 8.- At autopsy the liver is three to four times normal architecture is obscured by fat deposition. 9.- The administration of lipocaic at the time of lowest sugar excretion and the insulin requirements below five units per day brings about a striking change in the animal. Immediately dextrose excretion increases and the insulin requirements goes back up to 20 to 25 units a day. The blood lipid level rises within a short time and the bromsulphalein test show marked improvement of liver function. Liver biopsies show rapid return to normal morphology.(55)

The function of the phospholipids is not a completely settled problem at this time. Sinclair (134) states that the relatively small volume of plasma phospholipid would probably make it

insufficient for transportation of all the plasma fatty acids. Chaikoff et al. states that in the depancreatized dog maintained with insulin the blood lipid level, especially cholesterol, drops materially,(37), but one gram per day of pancreatic extract prevents the decrease in plasma choline.(68) These authors demonstrated that most of plasma choline contained in the plasma phospholipids.(69) The same authors demonstrated that the administration of pancreas fraction increased plasma choline level in dogs fed on a low choline diet.(36) McHenry and Patterson attribute the lipotropic action of choline to its action in the formation of phospholipids.(119)

Dragstedt, basing his idea on experimental evidence, suggests that the large excretion of dextrose following the administration of lipocaic to the depancreatized dog with proven fatty infiltration of the liver may be due to conversion of fat in the liver to dextrose (55). Drury (61) concluded from evidence obtained with depancreatized dogs and mice that in diabetic animals the large amounts of carbohydrate stored can only partially be accounted for in glycogen, therefore it must be changed to fats. Crandall, Ivy and Clini (51) found the mgm. of energy output of glucose plus acetone bodies available from their oxidation equal to the energy output of glucose alone before the onset of ketosis in the fasting dog. Longenecker (107) believes

the liver is the probable site of synthesis of fat from carbohydrate. Stetton (138) states that in rats about 3% of the glucose ingested is converted to glycogen and 30% is consumed in the production of fatty acids. Greene found in human patients with controlled diabetes mellitus that an increased carbohydrate intake did not produce an increase in calories corresponding with the increase in oxidation of dextrose. (83) Soskin and Levine (136) point out that it is now well recognized that ketosis occurs under conditions in which large amounts of carbohydrate are being oxidized. It has been impossible to demonstrate any relationship between the degree of ketosis and the rate of carbohydrate oxidation. The relatively recent advances in knowledge acquired from animal experimentation explains a great deal about fat and carbohydrate metabolism and their interlocking relationships. Further work with the fatty acids may bring the solution of many metabolic problems in diabetes and other diseases affecting the metabolic phenomena of the body.

CLINICAL

The great advancement in the knowledge of lipotropic substances acquired through animal experimental work appears to extend some promise of benefit to human beings. Presenting a few case histories illustrating the clinical application of this new knowledge seems to be indicated here.

Grayzel and Radwin used lipocoic in three juvenile diabetics with hepotomegaly.(82) The complete report is presented.

> "Hepatic enlargement is not a common complication of diabetes mellitus. Its incidence is much higher during the first two decades of like than later. Excluding cirrhosis of the liver, the most frequent pathologic change in cases of this type consists of fatty infiltration and degeneration of the liver.
>
> As a rule, the condition is associated with severe and uncontolled diabetic involvement. In most cases, adequate management of the diabetes with diet and control and administration of insulin and, more recently, of protamine since insulin results in hepatic recession. However, there are instances in which careful dietary management coupled with adequate insulin therapy fails to accomplish a return of the liver to its normal size." The writers have in their own clinic four patients in whom the liver has enlarged progressively in spite of such treatment.
>
> "In animal experimentation, similar experiences have been noted." Various authors have observed that completely depancreatized dogs, even though they were treated with insulin, showed extensive fatty infiltration and degeneration of the liver. The changes did not occur, however, if the diatetic and insulin therapy was supplemented by the feeding of raw whole pancreas."
>
> On the basis of results in animals, the writers undertook a determination of the efficacy of a pancreatic extract in three of their diabetic patients with hepatomegaly.

"Method. -- The three children had suffered for a number of years from extensive enlargement of the liver. Previous efforts to cause a reduction in the size of the liver had been unsuccessful. All three patients had been under fairly good control and under close supervision.

The pancreatic extract was prepared according to the method of Dragstedt and others. This solution was then divided into 21 equal portions, each constituting the equivalent of approximately 100 Gm. of raw beef pancreas. One portion was given daily in two equal doses.

This type of preparation proved highly unpalatable, however, and in addition it frequently caused gastrointestinal disturbances, so that after several months of treatment the patients rebelled at further therapy. As a result, no extract was administered for varying periods, during which retrogressive changes in the liver were carefully watched for. Eventually the extract, prepared as before, was evaporated in a large flat dish to a dry brown paste by means of a fan and was put up in gelatin capsules coated with shenyl salicylate. Each capsule contained the equivalent of 50 Gm. of the raw beef pancreas. Two capsules were given daily. This medicament was well tolerated. In this manner were provided two separate periods of treatment, with an effective control period whereby the efficacy of the tested material could be better studied.

The management of each patient's diabetic condition was not changed as regards insulin dosage or diet during the treatment with pancreatic extract. In one case protamine zinc insulin was given; during this therapy no pancreatic extract was administered. No apparent effect on the hepatomegaly was noted.

During the course of the investigation blood lipid studies were performed at intervals, . . . in order that one might note any changes in the blood occasioned by administration of the extract.

Case 1. -- S.C. first came to the outpatient department . . . On June 19, 1926, at the age of 6 years. Diabetes mellitus had developed when he was 4 years of age, and he had been treated elsewhere for two years with strict

dietary regulation and insulin. His diabetic condition was kept well under control during the 11 years that he was under care in the pediatric diabetic clinic. His urinary excretion of dextrose, although occasionally excessive when he exceeded his diet, usually averaged approximately 13 Gm. in 24 hours. His average insulin requirement during the past four years had been 20 units twice a day.

From the onset of diabetes, there was noted a significant retardation in growth. The average annual increment in height had been 1 9/16 inches (4 cm.). Although he had recently made a greater fain in height than at any previous time, he was only 61 1/2 inches (156.2 cm.) tall on entry. He weighed 105 pounds (47.6 Kg.).

In January 1933 the patient was admitted to the hospital because of intermittent abdominal pain localized about the umbilicus. A thorough investigation failed to disclose a satisfactory cause for the pain. About one year later acute, low grade catarrhal jaundice set in, but it cleared within two or three weeks. On Dec. 9, 1935, after a period of three weeks during which there had been an infection of the upper respiatory tract, with chills and fever, the patient complained of similar abdominal pain accompanied by nausea but no vomiting. Jaundice appeared the next day, with tenderness and enlargement of the liver, the edge extending 3 inches (7.6 cm.) below the right costal margin in the midclavicular line. All the symptoms except hepatomegaly disappeared within two or three weeks. The liver receded to a level 1 inch (2.5cm.) below the right costal border. However, shortly thereafter a slow but progressive enlargement of the liver recurred in spite of the fact that the patient's urinary excretion of dextrose was only 3 to 10 Gm. He was given a diet which was rich in lecithin and choline as well as a commercial pancreatic extract daily. The liver remained large in spite of treatment. On May 16, 1936, the lower margin of the liver was 3 3/4 inches (9.5 cm.) below the right costal margin in the midclavicular line. Liver function tests all gave normal results.

At this time the saline solution of the pancreatic extract was administered. This was continued until October 28, when the patient protested against taking

the medicament. Since the liver had receded so that it was just palpable while the patient was under this medication, the extract was discontinued in order that one might determine the effect of the cessation of treatment. By December 26, after two months without therapy, the liver was again enlarged, the border being 2 1/4 inches (5.7 cm.) below the costal margin. On that date, pancreatic extract was again given in capsules coated with phenyl salicylate, which were well tolerated. By April 17, 1937, about four months later, the liver was no longer palpable.

Case 2. -- A.E. was first admitted . . . on Sept. 23, 1925, in diabetic coma. He was then 8 years old. Diabetes had developed at the age of 3 1/2 years. During the first year of his illness the child had not received insulin. He was subsequently given dietary and insulin therapy. On 15 different occasions he was admitted to the hospital because of acidosis or for regulation of his diabetic condition. His diabetes had always been severe, and he required as much as 100 units of insulin daily. His average urinary excretion of sugar, aside from occasions of dietary indiscretion, had been 18 Gm. in 24 hours, while his average insulin requirement had been 42 units twice a day.

He had always been short, his average yearly growth being only 1 5/16 inches (3.4 cm.). He was 61 1/4 inches (155.6 cm.) in height at the beginning of the present study.

Aside from occasional episodes of acidosis, his progress was uneventful until Feb. 16, 1935, when there were noted progressive enlargement of the abdomen and enlargement of the liver, which soon reached the level of the unbilicus. There was tenderness in the right upper quadrant, and soleral evidence of jaundice was observed. On February 19 the boy was admitted to the hospital, where he stayed until March 8. The diagnosis was hepatomegaly resulting from fatty infiltration secondary to diabetes mellitus. The jaundice cleared spontaneously, but the liver remained enlarged. All tests for liver function gave normal results during the patient's stay in the hospital.

In spite of the adequate control of the diabetes and administration of a diet rich in lecithin and choline to-

gether with a commercial pancreatic extract, the hepatic enlargement continued." On July 11, 1936, the pancreatic extract prepared by the writers was given in saline solution. "The liver at the time was 3 1/2 inches (8.9cm.) below the right costal margin in the midclavicular line. Because of the unpleasant taste and other unfavorable effects of the preparation, the patient did not take it regularly, and the liver failed to decrease in size. On December 19 the therapy was discontinued. The boy was then given protamine zinc insulin (100 units daily), that one might determine whether a reduction in the size of the liver could be effected. with this substance alone. However, by Jan. 30, 1937, his liver was 3 9/16 inches (9.1 cm.) below the costal margin, in spit of the favorable effect of this new insulin preparation on his diabetic status.

"On this date the patient was given the pancreatic extract in capsules coated with phenyl salicylate. He took these regularly, with the result that in two weeks the edge of the liver was only from 3/4 inch (1.9 to 2.5 cm.) below the costal margin and by April 24 was barely palpable. It could not be felt on June 1."

Case 3. -- W.S. was admitted . . . for the first time on December 8, 1926, at the age of 3 years, several days after the discovery of sugar in his urine. At the age of 8 years a benign type of pulmonary tuberculosis developed. There was a history of exposure to tuberculosis at home. The pulmonary condition cleared spontaneously and never recurred. The diabetes was of moderate severity and was controlled fairly easily, except occasionally when he exceeded his diet. He required an average of 18 units of insulin twice a day. Because he never rigidly adhered to his diet, there had been glycosuria, an average of 75 Gm. of sugar being excreted in 24 hours. He was readmitted to the hospital three times for acidosis, which was usually secondary to an infection or a disease of childhood. He had had no attacks since 1928. He was short, measuring only 55 3/4 inches (141.6 cm.). For the past 10 years his average annual increase in height had been 1 9/16 inches (4.2 cm.).

On April 2, 1932, it was noted that his liver was 2 inches (5.1 cm.) below the right costal margin in the midclavicular line, but no symptoms were observed. On Sept. 6, 1935, jaundice developed and the liver was found to extend 2 1/2 inches (6.3 cm.) below the costal margin.

Two weeks later the liver had receded spontaneously to a level 1 1/2 inches (3.8 cm.) below the costal margin, and the jaundice had cleared. However, shortly thereafter the liver again increased in size, by July 25, 1936, it extended 5 inches (12.7 cm.) below the costal margin.

On August 19 the saline solution of the pancreatic extract was given for the first time. The liver slowly receded; by November 28 it was only 1 1/2 inches below the costal margin. As disagreeable gastric symptoms had again been caused by the medication, on this date the use of the pancreatic extract was discontinued. On Jan. 2, 1937, the liver had enlarged again to a level 2 1/2 inches below the costal margin. At this time, administration of the partially desiccated extract in capsules coated with phenyl salicylate was started. Under this therapy the liver decreased in size; by May 1 it was no longer palpable.

The studies of the blood lipid revealed certain findings common to all three cases. The initially high level of total lipid tended to become much lower with administration of the extract. A similar tendency was observed in the concentrations of the lactic acid and lipid phosphorus of the blood. These low values were noted even during the second period (the period of no medication), although the liver in each instance again became enlarged. The values of total free and unbound cholesterol, which throughout the studies were within normal range, remained materially unchanged with or without pancreatic therapy."

Concluding, the dramatic recession of hepatomegaly and the marked lowering of the level of the blood lipids following administration of the extract justify the tentative assumption that the enlargement of the liver in these cases was due to fatty infiltration and that the therapeutic agent is a lipotropic substance.

Dragstedt, who discovered lipocaic commented in his paper in 1940 on the results obtained by Grayzel and Radwin in their clinical trial of lipocaic, stating that these children suffered from the second type of fatty infiltration of the liver associated with

diabetes mellitus and characterized by impaired liver function and low blood lipid levels and does not respond to insulin and dietary therapy. He remarked that the fatty infiltration of the livers described by Marble, et al. in the following report were of the first type which result from inadequate insulin and dietary management, and are characterized by normal or high blood lipid levels and acidosis and is corrected by adequate insulin therapy. (55)

The following abstract of the report of Marble, White, Bogan and Smith (112) does not reveal any benefit derived from the use of lipocaic. They had 60 children in their study averaging 14.9 years of age, with an average duration of diabetes mellitus of 11.9 years. From the onset of the disease until onset of hepatomegaly was an average of 5.7 years for the group and after development of the hepatomegaly the group was under observation for an average of 3.6 years. The directors of the study found considerable resistance on the part of the patients against taking the solution containing the pancreatic extract because of its nature making it very unpalatable. For this reason, very few of the patients received what would probably be considered adequate dosage over a sufficient length of time. The directors of the study concluded that the primary hepatomegaly was due to fat deposition. They state that hepatomegaly seems to go along with complications of diabetes mellitus, such as arteriselerosis, abdominal pain, dwarfism and skin and urinary diseases.

Further study of the same group of children, (113) holding them under better dietary and insulin control, brought out the conclusion of Marble, et al. that hepatomegaly in diabetic children is due to poor control of the diabetes, rather than to the lack of choline or some other agent derived from raw pancreas.

Dr. Joslin (100) has recognized for many years the pronicity of diabetics to atheromatous vascular changes, greater sensitivity to infection, hepatic and renal degenerative changes and gangrene.

Six patients were treated with methionine for toxic hepatitis after exposure to high concentration of carbon tetrachloride gas. Two other people received the same exposure and died within two weeks without medical care. Post-mortem examination of the livers of these patients revealed marked hepatomegaly and nearly total necrosis of the liver cells with no evidence of regeneration in the first patient. The second one showed less degree of hepatomegaly but identical microscopic finding only less widespread throughout the liver.

The six survivors went under the care of Dr. J. H. Eddy, Jr. after the deaths of the two. On admission to the hospital all the patients had very high icteric index and marked hepatomegaly with tenderness. Each patient was tube-fed with a high protein, low fat diet and given 2 grams of methionine per day. The course of

treatment extended up to a month. All the six patients survived.

Methionine has been used successfully for toinitrotoluene toxic hepatitis also.(66)

Drs. Watson and Castle treated a patient, who was sixty years of age, suffering from chronic hepatitis with evidence of hepatic insufficiency. The patient received one gram of choline chloride per day for seventy days. During this time the patients hemoglobin increased and a preexisting condition of macrocytic anemia improved with the general improvement of the patient.(146)

Dr. A. J. Beams had a group of 20 patients with cirrhosis of the liver and ascites. These patients received high protein, low fat diet supplemented with yeast and a combination of choline and cystine.

Twelve of these people did not have hepatomegaly and showed no benefit from the treatment. Of the eight with hepatomegaly seven patients made good recovery from liver decompensation.

The record of these seven patients was compared with that of fifteen similar patients who had received the same treatment except they received no choline and cystine. The results indicate that the combination of cystine and choline is effective in treating hepatic cirrhosis.(9)

Drs. Browne and Thomas treated a patient suffering from

fatty hepatomegaly and pancreatic fibrosis. The patient was a white woman complaining of upper abdominal pain, nausea and vomiting, night sweats, nocturia, frequency, insomnia, nervousness, thirst, anorexia and weight loss. Until three years previously the patient had been in good health. The first symptoms were a feeling of tiredness which continuously got worse, and the abdominal pain which became acute about a week before admission to the hospital. Examination revealed marked emaciation, blood pressure of 98/70, palpable, tender liver, non-functioning gall bladder.

Laporatomy disclosed large liver lobes with pinkish yellow mottled color and round margins. The pancreas was very small and hard. Recovery was uneventful. A week post-operative the blood cholesterol was 280 mgm.% and the Hanger liver function test showed evident improvement of function.

During the next two and a half months the patient received 2 grams of lipocaic a day. She gained weight, lost the hepatomegaly. The patient discontinued using lipocaic and dropped her diet. The symptoms soon recurred and the patient returned to the hospital in less than a year with all the former symptoms. Lipocaic was again given and the result was successful a second time.

The authors believe the small fibrosed pancreas caused a

deficiency which lipocaic replaced.(34)

Dr. Rosenberg had a patient who recovered from fatty metamorphasis of the liver. The 59 year old female patient entered the hospital with the liver margin 10 cm. below the costal margin. The surgace was smooth, the edge sharp, increased consistency and moderate tenderness to pressure. The blood cholesterol level was 176 mgm. and the blood pressure 185/95.

Treatment was began with complete bed rest, 800 cal. diet and digitalis. In two and a half weeks the blood pressure was 150/80, but no improvement in the hepatomegaly occurred. At the end of two months the Bromsulphalein test showed 20% retention after 30 minutes, and the blood sugar was 173 mgm %.

A laparotomy revealed a pathologic ovary which was removed, the gall bladder was grossly enlarged. Biopsy of the liver was taken and revealed liver architecture barely recognizabel, the cells were badly swollen but the cords were more normal, and the central veins normal.

The patient left the hospital with the diabetes controlled, but no improvement of the liver. Five months after the operation administration of lipocaic, 76 gr.(5 grams) a day orally. Weekly check-ups showed steady general improvement of the patient. The liver showed no noticeable change till after 6 weeks. At ten weeks the liver was down only 4 cm., not tender and of normal consistency.

During the twelfth week the patient suffered a gall bladder attack. Operation revealed the liver as normal in color, consistency and size. The gall bladder was removed. Liver biopsy showed recognizabel architecture and very marked improvement of the organ morphologically. The post-operative course was uneventful and the fasting blood sugar level remained within normal limits.

Lipocaic was responsible for the improvement of the fatty metamorphosis of the liver and the final return to normal size and consistency. The blood sugar level was easy to control also. (133)

Since the continued success of lipocaic treatment in experimental diabetes in dogs and the favorable reports from clinical use. Dr. Dragstedt believes that total pancreedtomy is now a feasible surgical procedure. Between insulin and lipocaic the pancreatic functions can be substituted for and maintain physiological metabolism of the carbohydrates and fats.(57)

Drs. Priestley, Comfort and Radcliffe performed a total pancreetectomy for hyperinsulinism due to islet cell adenoma. The patient, a 49 year old Jewish woman had suffered hypoglycemic reactions for about three years.

The post-pperative course was uneventful and the patient's

diabetes mellitus as controlled with moderate insulin therapy. The patient ate a high protein diet rich in choline and was still in good health sixteen months after the operation. The authors state that the patient never showed any evidence of lipocaic deficiency.(129)

Dr. Rosenak treated a case xanthomia tuberosum with 30 grains a day of lipocaic for a period of two months, then discontinued for one month and then gave 45 grains a day for two months. There was no change during the course of treatment in either the serum cholesterol level or the skin lesions.(132)

Dragstedt has shown that lipocaic influences the blood lipid levels in dogs and has suggested that lipocaic may play a part in the conversion of fats to sugar in the metabolic processes. In experimental animals the blood cholesterol level in influenced by lipocaic.(56)

Lipocaic has been used clinically with benefit in regulating fat deposition in the liver and influencing the blood cholesterol level.(34)

Hueper (99) speaking on the relation between etiology and morphology in degenerative and sclerosing vascular diseases stated that hypercholesterolemia is the main factor in producing experimental atheromatosis in animals. Well developed atherosclerotic lesions are noted in arteries of small children and

young adults suffering from lipoid nephrosis, a disease characterized by hypercholesteremia and lipemia. Frequent and precocious appearance of severe atherosclerosis in diabetes mellitus in which appreciable hypercholestermia is often present is a well established fact. Atheromatous lesions are found exclusively in colloidal disturbances of the lipoids and carbohydrates in the plasma. Mechanical and other factors also play an appreciable part, especially as to the locations of the atheromatous lesions.(99)

Page (124) restates the fact that many elderly people have inelastic vessels but no atherosclerosis and in young people atherosclerosis may be present without any significant loss of vessel elasticity. Evidence is against the view that the blood lipid level is controlled by dietary intake except under special circumstances. The post-absorption blood lipid concentration is almost independent of lipid content of the diet. The blood lipid levels are regulated by the lipotropic factors, independent from the diet.

Dock is in agreement that one of the important factors in the etiology of atherosclerosis is the blood cholesterol level. Altered cholesterol metabolism and arterial hypertension are important factors in the pathogenesis of coronary occlusion.(52)

In comparing results obtained in animal experiments work to those found in human diseases Hansen and Burr note the low

iodine numbers of plasma lipids present in rats suffering from fat deficiency and find the same condition in two infants and one adult on low fat diet. In animals and man the greatest drop in iodine number is in the cholesterol ester fatty acids. Normally the cholesterol ester fatty acids are the most highly unsaturated of the fatty acids in the blood serum.

In both animals and man on low fat diets the total fatty acid concentration in the various fractions has been within normal limits. This finding indicates that the organism can synthesize sufficient fat to maintain a normal blood lipid level, but is unable to synthesize certain highly unsaturated fatty acids.(95) This idea was quoted from du Vigneaud in the section on experimental results.

The importance of lipids and lipotropic substances cannot be overemphasized in the physiological functioning of the body economy.

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