Abstracts of Current Literature

EXPERIMENTAL LIVER DISORDERS

The genesis of fatty livers in animals fed diets high in fat, cholesterol or sucrose was studied nearly thirty years ago by Best and his colleagues. Choline, betaine and then methionine were demonstrated to be curative of hepatic lipid infiltration in animals fed protein-deficient diets. Subsequently the importance of the labile methyl group derived from a number of compounds or, perhaps, arising de novo for the synthesis of choline through transmethylation reactions, was recognized as the mode of action of lipotropic factors.

Production of Periportal Fatty Infiltration of the Liver in the Rhesus Monkey by a Protein Deficient Diet. M. G. Deo and Ramalingaswami. *Lab. Invest.* 9: 319, 1960.

Rhesus monkeys weighing 2.15 to 3.6 kg. were fed as follows: (1) A diet free of protein for three weeks; (2) a diet free of protein supplemented with 30 per cent fat for two weeks; (3) a diet containing 15 per cent casein, but no fat for three to four weeks. Tapioca was used as a source for carbohydrate, coconut oil for fat and purified casein with traces of choline (2.5 mg. per 100 gm.) for protein. The diets thus varied only in their fat and protein contents, and were adequate in all other nutrients. The monkeys were tube-fed and each of them received 100 cal. per kg.per day. In all protein-deficient animals severe periportal fatty change of the liver was found. The cells near the efferent veins were unaffected or showed only slight fatty change. The periportal lipoidosis developed also in the absence of excessive dietary fat, but the high fat diet accentuated the fat deposition. M. SILBERBERG

Studies with a Poor Vegetarian Rice Diet. III. Chemical and Morphological Changes Induced in the Livers of Mature Albino Rats Fed the Basal Diet for Long Periods. K. Dakshinamurti. *Ann. Biochem. & Exper. Med.*, 19: 75, 1959.

A poor vegetarian rice diet was fed to albino rats. Even short-term feeding for seven months produced septal fibrosis. The first change was an increase in the level of chemically determinable fat. After the peak was reached there was a gradual lowering of fat in the liver and a corresponding increase in fibrous tissue. These findings, considered in the light of the supplementation experiments reported, suggest the existence of threonine imbalance or deficiency in the basal diet. This diet is similar to that consumed by a considerable portion of the population of India, and it is believed that this investigation may throw some light on the role of malnutrition in the etiology of liver injury. It is probable that the poor South Indian diet could be improved by supplementation of threonine. S. O. WAIFE

Nuclear and Nucleolar Alterations in Hepatic Parenchymal Cells of the Choline-Deficient Rat. I. Volume Changes. J. W. Grisham, B. B. Banson and W. S. Hartroft. *Arch. Path.*, 70: 50, 1960.

Male albino rats weighing 90 to 100 gm. at the beginning of the experiment were kept on a choline-deficient diet for periods up to six months. Nuclear volume, number and volume of nucleoli, total nucleolar volume per nucleus and the nuclear: nucleolar ratio were determined. As early as after one week of observation, there was increase in total nucleolar volume and in the nucleolar: nuclear ratio. This became even more prominent and reached a peak after three weeks. After one month and later, depending on the progress of regeneration, the findings reverted to a more normal pattern. However the increase in nucleolar size persisted in both the nodular regenerating and non-nodular areas. Ac-

cording to these findings early changes in cell volume are indicative of liver regeneration.

M. SILBERBERG

Recovery from Dietary Cirrhosis of the Liver in the Rat. Changes in Hepatic Collagen and in Microscopic Appearance. A. J. Patek, Jr., D. E. Oken, A. Sakamoto, N. de Fritsch and M. Bevans. *Arch. Path.*, 69: 168, 1960.

Male albino rats of the Sprague-Dawley strain six weeks of age and weighing 100 to 150 gm. were kept on a diet containing 4 gm. of vitamin-free casein as the only protein. Within six months, this diet produced liver damage, especially fatty metamorphosis and/or cirrhosis in 70 per cent of the animals, as shown by liver biopsies. Subsequently groups of these rats were put on a complete diet for ten months in order to study processes of repair that might occur. Recovery in the animals fed the complete diet was indicated by disappearance of fat from the liver cells, decrease or disappearance of both mononuclear cell infiltration and connective tissue overgrowth. Complete return to normal occurred in rats in which liver damage had been moderate at the age of six months. In animals in which liver injury had been severe, strands of connective tissue and slight mononuclear cell infiltration were still recognizable. Determinations of collagen as measured by the values of hydroxyproline were also made. The liver in rats with cirrhosis contained twice as much hydroxyproline as in rats which had recovered.

M. SILBERBERG

Protection of the liver from damage induced by alcohol ingestion employing vitamin supplementation has not been adequately demonstrated.

Vitamins as Agents for Protection of the Liver from Necrosis. W. Eger and H. Nassr-Esfahani. *Ztschr. Vitamin- Hormon- u. Fermentforsch.*, 11: 1, 1960.

Vitamin A, vitamin B_1, vitamin B complex, vitamin B_{12}, ascorbic acid and tocopherol were investigated in the allyl alcohol test on rats in order to determine whether they exerted any protective action on the liver.

Vitamin B_1 in the form of thiamine has no necrotropic properties whatever. On the contrary, in response to high dosages, liver damage due to allyl alcohol tends to increase. Only when given in the form of thiamine disulfide does the substance exercise a necrotropic action roughly equivalent to that of cocarboxylase.

Nicotinic acid amide and folic acid have a clear-cut necrotropic effect. Riboflavin, pantothenic acid, nicotinic acid, vitamin B_6 in the form of pyridoxal and pyridoxine, and vitamin B_{12} show no significant influence on liver damage due to allyl alcohol, and cannot therefore be classified as necrotropic substances.

Neither vitamin A nor vitamin C can be regarded as liver-protecting agents. On the contrary, vitamin C definitely increases the toxic effect of allyl alcohol.

AUTHORS

While an increase in hepatic iron content develops in choline-deficient animals with cirrhosis, no such correlation has been established in clinical cirrhosis in studies conducted on the Bantu and others.

Experimental Pigment Cirrhosis. Its Production in Rats by Feeding a Choline-Deficient Diet with Excess of Iron. R. A. MacDonald. *Am. J. Path.*, 36: 499, 1960.

The aim of this investigation was to study the pathogenesis of pigment cirrhosis experimentally. Male rats of the Sprague-Dawley strain about three months old and weighing 200 to 300 gm. were fed a choline-deficient diet or a complete stock diet *ad libitum*. The diets were supplemented with 3 per cent of powdered ferric ammonium citrate containing 17 per cent iron and were fed for twenty-one days or for three months. Choline-deficient rats of the latter series were fed subsequently a complete diet for three weeks. At that time liver biopsy specimens were obtained from both control and experimental animals, and both series of rats were fed the complete stock diet supplemented with 3 per cent ferric citrate for forty more days. The liver changes were studied microscopically; iron determinations were made, and autoradiographic studies of the synthesis of DNA were undertaken by using tritiated thymidine. The latter studies failed to show increased regeneration of liver cells in the choline-deficient rats. Liver weights and the total nonhemic hepatic iron content (6 mg.) were much higher in the choline-deficient rats than in the control animals (1.8 mg.) after twenty-one days of observation. In the prolonged experiments, the liver weights and the total nonhemic iron content was likewise higher (3.8 mg.) in the choline-deficient rats than in the control animals (2.8 mg.). Thus choline-deficiency not only produced cirrhosis but also caused excessive absorption of iron, and iron deposition in liver, kidneys and stomach. However, in contrast to human hemochromatosis, bile ducts, pancreas and adrenals were not involved. It is concluded that hemosiderosis and hemochromatosis are not identical diseases but variants of the same disorder.

M. SILBERBERG

Investigations Concerning Experimental Hemochromatosis. C. Hedinger. *Path. Microbiol.*, 23: 222, 1960.

These investigations deal with the role of liver injury, especially cirrhosis, in the pathogenesis of hemochromatosis. The effect of increased ingestion of iron—administered in the diet or in drinking water—was studied in rats in which cirrhosis of the liver was produced by feeding a choline-deficient diet. Many animals died within three months of advanced liver cirrho-

sis with fatty change. The livers contained less iron than in control animals fed the complete diet supplemented with iron. Hemosiderosis was not observed.

In order to study the changes occurring after prolonged ingestion of increased amounts of iron in rats with cirrhotic livers, the animals were exposed to temporary choline deficiency and subsequently kept on a complete diet. The observations could thus be extended up to a period of sixteen months. In completely fed rats having received supplements of iron, there was advanced hemosiderosis of liver, spleen and lymph nodes, but no increase of connective tissue in the liver. In the choline-deficient animals having received iron, a marked cirrhosis of the liver and hemosiderosis had developed. The iron was deposited preferably in the connective tissue, while liver parenchyma and Kupffer cells were essentially free of the mineral. The iron content in these livers was again lower than in completely fed animals having received the supplement of iron, but it was higher than in control animals that had received no additional iron. The diagnosis of hemochromatosis could not be made. Attempts to produce hemochromatosis in rats in which the bile ducts had been severed or liver injury was produced by carbon tetrachloride poisoning and to which additional iron was given were likewise unsuccessful. According to these experiments it seems doubtful that liver injury plays a primary role in the pathogenesis of hemochromatosis.

M. SILBERBERG

The cytologic effects of hepatotoxins permit histochemical investigation of regenerative processes involved in recovery from exposure to these agents.

The Morphogenesis of Liver Cirrhosis Produced by Thioacetamid in the Rat. J. R. Rüttner and R. Rondez. *Path. Microbiol.* 23: 113, 1960.

In order to study the toxic hepatic effects of thioacetamid which is used for food preservation, male and female rats of the Wistar strain weighing about 250 gm. were fed a stock diet to which 50 mg. per cent of thioacetamid had been added. The first group of experiments deals with the early changes seen in the liver within the first four weeks of feeding the toxic substance, the second with the prolonged changes observed for periods up to 300 days. Early liver alterations consisting of cell proliferation, especially of Kupffer cells with increased periodic acid-Schiff stainability and pyroninophilia, originated about the central areas. This early reaction was followed by proliferation of tubular structures resembling bile ducts. After six weeks some of the latter underwent atrophy and lost their lumen, others continued to grow forming cholangiomatous areas with marked formation of collagen fibrils. True regenerated liver cells were not seen before three months; they originated from lobules which had sustained only minor injury. Subsequently small nodules were found but even as late as after 300 days no noticeable new formation of collagen fibers was observed. M. SILBERBERG

DIETARY FATS AND THROMBOGENESIS

Numerous studies have suggested that hyperlipemia alters the blood coagulation mechanism and depresses fibrinolytic activity. The enhanced coagulability of blood following the ingestion of meals containing fats may have serious implications for persons with coronary artery disease for obvious reasons. It is significant that anginal pain may be induced or aggravated during the lipemic phase. The lipid factors responsible for increased blood coagulability have not been conclusively demonstrated.

The Effect of Fatty Acids on the Formation of Thrombi. W. E. Connor and J. C. F. Poole. *Quart. J. Exper. Physiol.*, 46: 1, 1961.

This investigation deals with the effect of fatty acids on thrombus formation *in vitro*, as studied in blood circulating in a plastic tube according to the method of Chandler and modified by Poole. These artificial thrombi closely resemble those noted in human subjects with thromboembolic disease.

The effects of a variety of saturated and nonsaturated fatty acids were investigated. The findings disclosed that straight saturated fatty acids with 16 to 26 carbon atoms markedly increased the size of thrombi and accelerated their formation. Short-chain fatty acids were ineffective. Unsaturated long-chain fatty acids, such as oleic, elaidic, ricinoleic and arachidonic acid, likewise failed to show noteworthy thromboplastic effects. The thrombus-enhancing action of long-chain saturated fatty acids was reduced by supplements of substances rich in methyl groups.

M. SILBERBERG

Egg-Containing Meals and Blood Coagulation. E, Orma, D. N. Rhodes and A. Keys. *Lancet*, 1: 388. 1959.

Some phospholipids produce activation of coagulation *in vitro*, especially when Russell-viper venom is present. The authors' objective was to determine whether either of the two main phospholipids in egg is the causal factor of recent claimed activity of ingested egg fat on blood coagulation. Normal men (nine middle aged patients with chronic schizophrenia) were divided into three groups and test meals were rotated over six experiments conducted at weekly intervals. The results of the study did not support recent reports that shortening of "stypven-time" after eating eggs is due to the phospholipids in them. Total lipid was no more effective than equal quantities of other food fat. Furthermore, any effect that it does have is not dependent alone upon total phospholipid or phosphatidyl ethanolamine content. For example, a meal with 120 gm. of butterfat produced double the shortening of plasma stypven-time as that reported for 13.5 gm. of fat in the yolks of three eggs. Whole blood coagulation time (in siliconized tubes) was not significantly shortened

by ingesting that amount of egg lipids. However, it is considered to be possible for changes in the amount or physical state of the phospholipids of the blood lipoproteins to accelerate stypven-clotting-time after a fat meal. The changes may occur by ingestion of neutral fats or phospholipids by formation of chylomicrons containing newly elaborated triglyceride. Under such conditions endogenous phospholipid, but not exogenous phospholipid, is required for the acceleration effects.

E. COHEN

Particle Size as a Factor in Thromboplastin Formation. P. W. Boyles. *Blood*, 14: 1063, 1959.

Some workers reported that phospholipids and fatty meals decreased blood clotting time. Others found that phospholipids could be substituted for platelets in the *in vitro* thromboplastin generation test. As far back as 1863, Lister observed that foreign surfaces accelerated blood coagulation. How phospholipids influence coagulation is not known. The author attempted to differentiate whether chemical composition of phospholipids or physical characteristics, as particle size, were responsible. Latex particles of nine different sizes were substituted for platelets in the thromboplastin generation test. The particles with a diameter of 0.14 μ were effective. However, none of the different latex particles significantly affected clotting time, prothrombin time and prothrombin consumption of normal blood. The latex particles did not give "complete" thromboplastin formation, when compared with either platelets or phospholipid. There was also an inhibitor action observed with mixtures of platelets and latex particles, possibly due to surface absorption of proteins by the latex particles. It is possible the observations may have some bearing on recent studies of effects of purified phospholipid materials or fatty meals on coagulation time. E. COHEN

Mobilization and Utilization of Body Fat as an Aetiological Factor in Occlusive Vascular Disease in Diabetes Mellitus. A. G. Beckett and J. G. Lewis. *Lancet*, 2: 14, 1960.

This study originated from the clinical observation that in certain subjects with diabetes serious vascular complications developed during periods of weight loss, often with well controlled diabetes. Six representative patients are described from a total of twenty-four whose vascular symptoms either first appeared or markedly increased during periods of weight loss.

It is suggested that these events followed fat mobilization, producing a relative deficiency of essential unsaturated fats, so disturbing the normal equilibrium of blood fats and coagulation to produce thrombosis or acceleration of atherosclerosis.

The authors recommend that weight reduction should be slow in overweight subjects with diabetes and it is further suggested, on theoretic grounds, that the addition of essential unsaturated fatty acids to the diet might be of value. F. E. HYTTEN

A puzzling feature of the reported investigations is that not all laboratories have demonstrated an effect of fat ingestion upon blood coagulation. The test which is most uniformly affected is the Russell-viper venom time.

Effects of a High Fat Meal on Coagulation in Hemophilia. J. H. Lewis. *J. Lab. & Clin. Med.*, 55: 245, 1960.

A basic breakfast of eggs, bacon, butter and cream, totaling 114 gm. of fat was given to seven normal subjects, ten patients with AHF-deficient hemophilia A, and six patients with PTC-deficient Christmas disease. The battery of coagulation tests was impressive: clotting time (in glass and silicone-treated glass), serum prothrombin time, thromboplastin from human brain and commercial source, Russell-viper venom time, thrombin time, prothrombin (per cent standard), proconvertin (per cent standard), PTC (per cent standard). In addition, turbidity of serum specimens was determined. However, no correlation was obtained between coagulation changes and turbidity. There was no clot-accelerating activity of fat ingestion on blood from normal or hemophilic subjects. *In vitro* experiments, on the other hand, showed a marked acceleration of the Russell-viper venom time on addition of different quantities of fat, but not the other coagulation tests. The authors even went as far as to have the coagulation tests performed by competent technicians "unfamiliar with the controversial literature." Nevertheless, no satisfactory methods are available for measuring coagulation *in vivo*. E. COHEN

In addition to decreasing the clotting time of blood, dietary fats have been shown to inhibit the fibrinolysis of blood clots.

Plasma Fibrinolysis in Man: The Effect of Chylmicrons Derived from Different Dietary Fats. J. W. Farguhar, T. C. Merigan and M. Sokolow. *J. Exper. Med.*, 113: 587, 1961.

The effect of various dietary lipids, such as butter fat, safflower oil or egg yolk, on fibrinolysis was studied in seventeen healthy males; furthermore the fatty acids of the triglycerides and phospholipids of the chylomicrons were analyzed by gas-liquid chromatography in order to investigate some factors that might be responsible for the inhibitions of fibrinolysis subsequent to the ingestion of fat. There were no differences in the inhibiting action of either butter fat or safflower oil. Chromatographic studies of purified chylomicrons derived from butter fat, egg yolk or safflower oil disclosed that the inhibition was quantitatively similar irrespective of the lipid ingested despite marked differences in the fatty acid composition of the lipids. It is believed that the protein and nonfatty acid portions of the phospholipids in the chylomicrons may determine the inhibiting effect on fibrinolysis exerted by the ingested lipids. M. SILBERBERG