Vagotomy produced a marked decrease in secretion to histamine stimulation.

In 3 of 4 animals tested, secretion was high in volume and acidity in the fasting state. Within 15 minutes after feeding, there was a pronounced augmentation in secretion, followed in 15 to 30 minutes by a profound inhibition in volume, free acidity, and peptic power. After 3 to 7 hours, secretion returned to the fasting level. Following vagotomy, there was no augmentation with feeding, and the period of inhibition appeared as before.

The ineffectiveness of partial vagotomy was well demonstrated in 2 animals. In one the left, in the other the right vagus nerve was crushed in the cervical region. The secretory response to insulin hypoglycemia was unaffected, and there was no diminution in the 24-hour secretion either in volume or acidity.

Three dogs died of peptic ulcer. Two died of perforation 4 and 7 days after operation; the third died of hemorrhage 5 days after vagotomy.

Conclusions. 1. The totally isolated stomach with intact blood and nerve supply secretes large amounts of gastric juice even in the absence of food-taking.

2. On the ingestion of food there occurs an immediate augmentation of secretion followed by a period of inhibition lasting 3 to 7 hours and then a period of profuse secretion.

3. Section of the vagus nerves above the diaphragm reduces the secretion of gastric juice in the isolated stomach by an average of 56% and the output of hydrochloric acid by 77%. Nervous factors are thus more important than other mechanisms in determining gastric secretion in these animals.

4. Chronic progressive peptic ulcers occur frequently in these isolated stomachs and cause death by hemorrhage or perforation. They rarely develop in such preparations that have been denervated, and following vagotomy, they tend to heal.

5. Partial vagotomy has little or no effect on gastric secretion.

6. After complete vagotomy, the secretory response to a standard dose of histamine is markedly reduced.

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Scarring and Precirrhosis of the Liver in Chronic Phosphorus Poisoning of Guinea Pigs.

L. L. ASHBURN, A. J. MCQUEENEY, AND R. R. FAULKNER. (Introduced by K. M. Endicott.)

From the Pathology Laboratory, Experimental Biology and Medicine Institute, National Institute of Health, Bethesda, Md.

In a previous report,[1] we described the fibrous tissue in dietary cirrhosis of rats and carbon tetrachloride cirrhosis of rats and guinea pigs as forming primarily about the hepatic veins. It was suggested that at least in certain experimental cirrhoses the proliferation and condensation of fibrous tissue occur in the same part of the liver lobule as the parenchymal damage. It seemed desirable to test this concept further by repeatedly injuring the peripheral (portal) part of the liver lobules to determine whether or not fibrous tissue would form about and connect the portal areas.

White phosphorus was selected for use in this study since it has been stated that this substance produces periportal fatty degeneration and necrosis of the liver, and since Mallory,[2] among others, has produced cirrhosis in rabbits with phosphorus.

We were not successful in producing frank liver cirrhosis in the animal used (guinea pig). However, other striking extensive

[1] Ashburn, L. L., Endicott, K. M., Daft, F. S., and Lillie, R. D., *Am. J. Path.*, 1947, **23**, 159.

[2] Mallory, F. B., *Am. J. Path.*, 1933, **9**, 557.

asymmetrical lesions were observed; a description of these changes is the main purpose of this paper.

Experimental Methods. Fifty-one guinea pigs weighing between 300 and 500 g were divided into 2 groups and fed a diet of rabbit pellets and leafy vegetables. The animals in one group received 0.75 mg/kg of phosphorus 4 days each week, while those in the second group were given 1.5 mg/kg twice weekly. The phosphorus was made up as a 0.1% solution in olive oil. This solution was administered orally by the use of a 1 cc tuberculin syringe and a 15-gauge needle one inch long; the end was made slightly bulbous by a small amount of solder. The guinea pigs were held in a vertical position and the proper dose was expelled slowly into the back of the throat. The dose of phosphorus used in the study was, for certain periods, above the tolerance limit of some animals; so, based on the appearance of the animal and the weight curve, it was sometimes necessary to reduce or omit single doses. Occasionally 2 or more successive doses were reduced or omitted to obtain a more satisfactory weight curve. The experiment was continued for a period of 35 weeks. In order to study the anticipated changes as they progressed, from 2 to 4 animals were killed at irregular intervals beginning in the first week.

At autopsy most of the livers were injected as previously described[1] with a charcoal gelatin mass to mark the hepatic or portal veins in the microscopic section. Livers were fixed either in 10% formalin or Helly's fluid, paraffin sectioned, and stained by the Van Gieson technic and with azure eosinate. Some were also stained with hematoxylin and eosin, and by Foot's modification of Rio Hortega's silver method for demonstrating reticulum. In addition frozen sections were stained with oil red O.

Results. An analysis of the results showed no difference in incidence or type of lesions found in the livers of the animals given phosphorus four days a week and those given this substance twice a week; hence all animals will be considered as one group.

Gross Findings. The first gross lesions were seen in an animal killed 9 weeks after the beginning of the experiment. This liver showed, on its ventral aspect, dark reddish brown areas at the hilar portion of most lobes with extension for short distances toward the free borders. The lesions were sharply marginated, depressed, and suggested a loss of parenchymal substance. As the time on the experimental regimen increased, lesions were seen with greater frequency and extensiveness, and involving a greater number of lobes. These lesions varied considerably in size and shape. They were triangular, oval, stellate, or linear, and when isolated they measured up to 2 cm in their greatest dimension. Although they were commonly seen in the hilar region, they were by no means limited to this area. Various lobes of the same or different livers showed variously deep notches along their margins, extreme atrophy of the proximal segment, punched-out areas on dorsal or ventral surfaces, or were incompletely traversed by fissures. When these changes occurred in a single lobe, there was great distortion. Occasionally atrophy and shrinkage of one or more lobes were extreme. When the accessory lobes were involved in this fashion, the gall bladder bed was thus lost and this organ was found in abnormal locations. In one liver the left main and right and left accessory lobes were shrunken to the point where their combined mass measured only 2 x 1 x 0.2 cm (Fig. 1E). The right main and caudate lobes of this liver showed no focal lesions but were considerably enlarged. Hypertrophy was generally present in uninvolved lobes when others showed marked atrophy. The distal third of lobes was rarely involved. This portion was sometimes hypertrophied and connected with the remainder of the liver by a shrunken band of tissue representing the proximal two-thirds of the lobe. Nodular hyperplasia was not seen.

Examination of the cut surface occasionally showed small lesions which did not reach the surface. The externally evident lesions penetrated the liver for varying depths, usually less than half of the thickness of the lobe. At times the entire thickness of the lobe was involved.

The incidence of involvement of the various

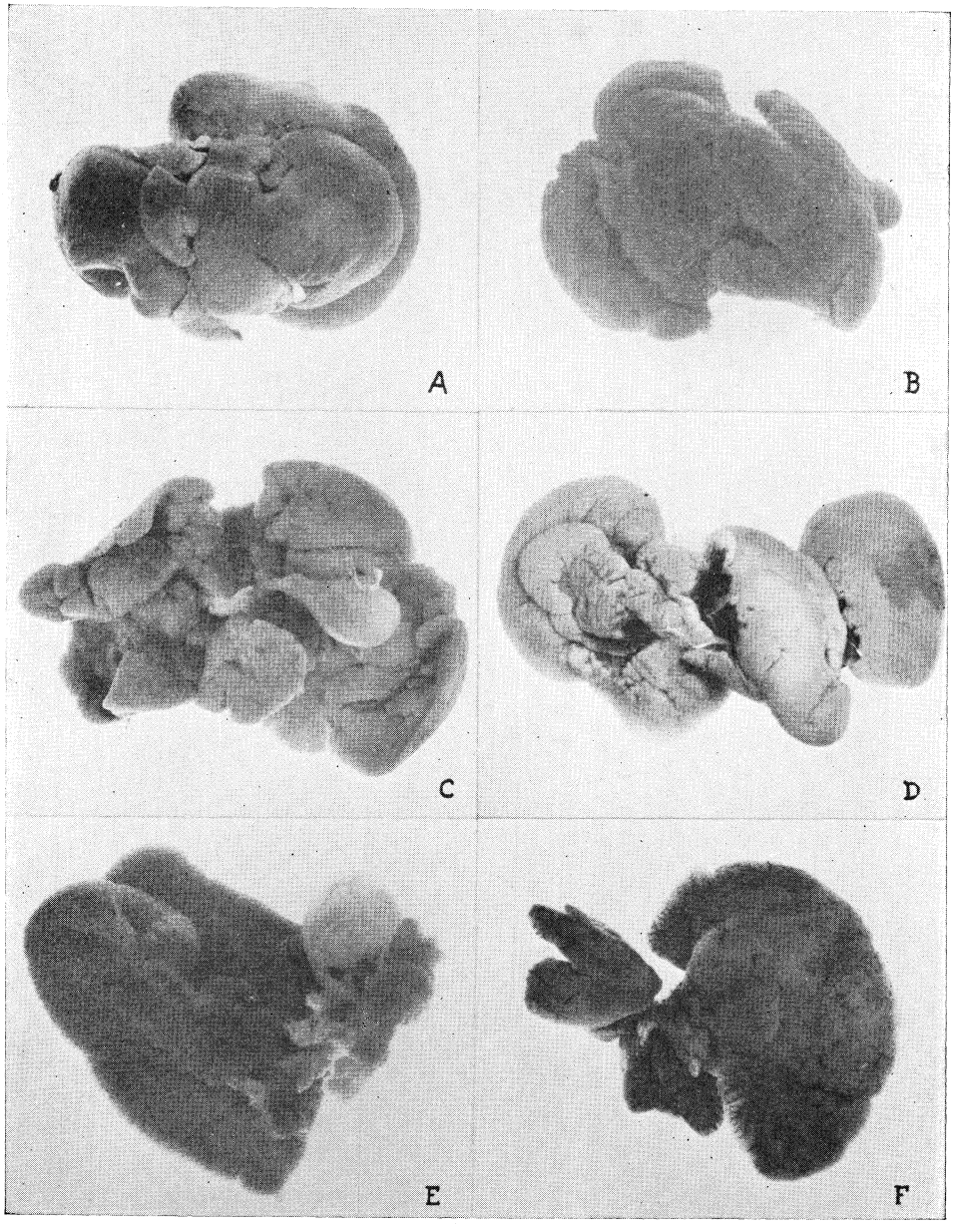

FIG. 1.

Gross photographs of guinea pig livers showing various degrees of deformity following chronic phosphorus poisoning.

A. Diaphragmatic surface—shows defects along margins of lobes.

B. Diaphragmatic surface—similar to A, but more advanced changes.

C. Inferior surface—numerous deep notches and fissures give appearance of nodular hypertrophy. The gall bladder has become detached from its bed (enlarged notch at top) and lies in a horizontal position.

D. Diaphragmatic surface. There is marked atrophy of the right accessory lobe (dark area in center of photograph), fore-shortening of right main, and atrophy of proximal portion of caudate lobe. The atrophic portion of caudate is largely covered by the right main lobe.

E. Inferior surface. The small mass of tissue to the right of, and below the gall bladder is all that remains of the left main and left and right accessory lobe.

F. Diaphragmatic surface. The large mass of tissue is the left main lobe. All other lobes are atrophic.

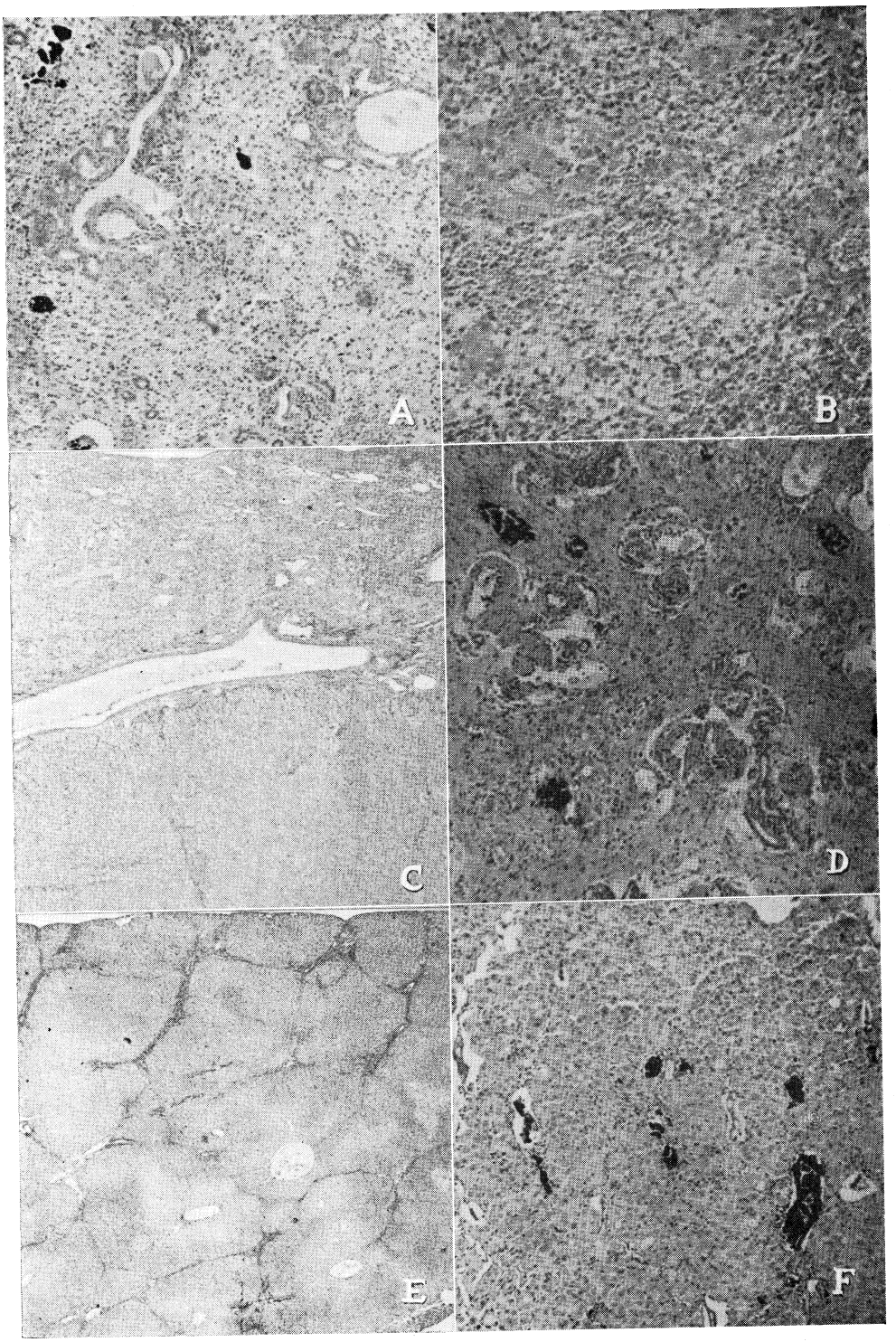

FIG. 2.

Photomicrographs of lesions of guinea pig livers in chronic phosphorus poisoning. Three of the livers were injected with a carbon-gelatin mass.

A. Histologically early lesion showing almost complete disappearance of parenchymal cells,

blood in some sinusoids and no appreciable fibrosis. Hepatic veins were injected. Hematoxylin and eosin stain, × 120.

B. Similar to A but from a different liver. Area was selected to show a few small groups of remaining liver cells and more blood in the non-collapsed sinusoids. Van Gieson stain, × 160.

C. The liver below the hepatic artery is normal. Superficial to it there is subtotal disappearance of liver cells with collapse of sinusoids. Hematoxylin and eosin stain, × 25.

D. Late lesion. Liver cells are replaced by dense fibrous tissue. Note sections of the tortuous hepatic artery. Hepatic veins were injected. Van Gieson stain, × 100.

E. Thin partially fibrous trabeculae connect many portal areas. Prominent hepatic veins are seen within some of the enclosed lobules. Van Gieson stain, × 30.

F. Carbon-gelatin mass was injected in the portal vein. Four portal and three hepatic veins are shown. There is marked reduction in size of lobules but no increase in connective tissue. Azure eosinate stain, × 90.

lobes was irregular, with no particular lobe showing outstanding susceptibility to injury. The right accessory lobe was involved in 22 livers, the left accessory in 19, the right main in 19, the left main in 15, and the caudate in 10. Although the caudate showed the lowest incidence of gross lesions, it revealed the greatest relative incidence of extensive damage. Of the 10 instances of caudate involvement, the lesions were considered extensive in five. This contrasts with the findings in the left main lobe in which only 3 of 15 showed extensive lesions. The other lobes fell between these extremes.

Microscopic Findings. The lesions noted grossly varied in microscopic appearance. This variation was considered as a function of age of the lesions and this interpretation is used to facilitate the description of microscopic appearance. The early lesions were of variable size and showed from moderate to subtotal disappearance of liver cells (Fig. 2, A and B). Sinusoids in most lesions were generally collapsed. In some they were open and blood-filled. In areas of collapse, there was both an apparent and an actual increase in cellularity since large mononuclear cells and occasionally lymphocytes were present in variable numbers. The liver cells remaining in such areas occasionally showed hydropic, fatty or other degenerative changes. Hyperplasia of the remaining cells was not seen. In these early lesions connective tissue fibers were not numerous and reticulum fibers predominated.

In most lesions bile ducts appeared to be present in greater number than could be accounted for by a collapse of lobules. This belief was supported by the presence of biliary epithelium arranged in cords without lumens and by the presence of atypical cords. Bile duct proliferation was never more than moderate and often was only of slight degree.

As the lesions became older they showed an increased amount of fibrous tissue, a decrease in cellularity, somewhat fewer bile ducts and fewer liver cells. What appeared to be the end stage of these lesions was seen in a few livers after the twenty-third week. They showed moderate to marked fibrosis, small to moderate numbers of spindle cells, scattered normal bile ducts and, in some cases, numerous sections of the convoluted or undulating hepatic arteries (Fig. 2D). Occasionally a lesion was seen in which only patchy areas showed fibrosis.

Extensive necrosis was seen in only one animal. This occurred in a liver in which the portal areas were markedly widened due to disappearance of adjacent parenchymal cells, cellular infiltration and proliferation of bile ducts. The majority of the liver cells between such areas were in various stages of karyorrhectic necrosis. Some lobules in this area of involvement showed no liver cells; only blood-filled sinusoids separated the thickened portal areas.

In the vicinity of a few lesions and occasionally elsewhere, liver lobules showed considerable reduction in size without other striking alteration (Fig. 2F). Central veins were approximately centrally located and collagen and reticulum stains showed no appreciable increase in connective tissue fibrils either within the lobules or about central veins or portal areas. There was, however, some disarrangement of the usual radial distribution of cell cords; only rarely was a necrotic liver cell seen in such areas.

In addition to the lesions just described, there were changes which were more widespread. The livers from guinea pigs killed

during the early part of the experiment showed fine droplet fat deposits in the parenchymal cells. In most, it was of moderate degree and distributed diffusely throughout the lobule. In a few, the fat was sharply limited to a zone around the portal areas. This was seen most often when the fat was present in only small amounts. Although fatty change was seen throughout the experimental period (35 weeks), it was occasionally absent and not infrequently was of slight degree, particularly in the last half of the experimental period.

Incomplete hyalinization of liver cell cytoplasm was often present but frank necrosis was limited to a few isolated cells or to very small groups of cells. In a few animals the small number of necrotic liver cells present bordered the portal area but in most livers the necrotic cells were irregularly distributed. In some livers, necrosis was not seen.

Portal cellular infiltrate, composed of lymphocytes and large mononuclear cells, was seen in most animals. When present it usually was of slight to moderate degree and in general was less frequent and less prominent in the latter part of the experimental period.

Proliferation of bile ducts or biliary epithelium was quite irregular, both as to occurrence and prominence. It was evident in less than one-half of the animals and usually was of slight to moderate degree. It was seen most frequently in the first part of the study.

The presence of increased amount of collagen in or about portal areas, although noted earlier in an occasional animal, was seen regularly after the sixteenth week. The collagen deposition was usually slight. In a number of livers after the sixteenth week, the portal areas were broader than normal and projected toward each other between the liver lobules. This tendency was in general progressive and in 4 animals thin trabeculae connected portal areas and surrounded liver lobules (Fig. 2E). Three of these occurred after the thirty-third week of the experiment. This trabeculation did not occur in all lobes and lobule involvement was variable in degree.

The portal changes described did not appear to bear any specific or consistent relationship to the lesions seen grossly. The portal involvement occurred in some lobes showing no gross lesion and gross lesions were present in some lobes in which portal changes were absent or inconspicuous. Occasionally the portal alteration was somewhat more prominent in areas bordering the large lesions.

Discussion. The main purpose of this experiment, the production of clear-cut portal cirrhosis, was not achieved. In 4 of the guinea pigs, certain lobes or parts of lobes showed lobules surrounded by thin trabeculae formed partly of collagen. In a number of other livers, narrow cellular and fibrous portal extensions partly surrounded lobules in an irregular fashion. However, the process was not diffuse, and there was no striking disorganization of lobule architecture and arrangement. Since these latter features were missing, we do not feel justified in claiming the production of portal cirrhosis of the liver by the feeding of phosphorus. Many of these livers were thought of as showing precirrhotic changes. It is possible that frank cirrhosis might have resulted if the experiment had been continued for another six months.

This experiment throws little light on the working hypothesis that repeated zonal damage to the liver parenchyma will be followed by collagen deposition in the involved areas. This is true first because the necrosis was slight, inconstant in occurrence with only a tendency to be portal in location and second because intracellular accumulation of histologically demonstrable fat was often of slight degree and was periportal in location in only about one-half the livers studied.

This study cannot be compared to that of Mallory since in his experiment, in which cirrhosis was produced, rabbits were used almost exclusively. Our choice of the guinea pig as the experimental animal was made in order to avoid difficulties of interpretation which might arise in the rabbit due to the common presence of coccidial fibrosis.

The principal purpose of this paper is to report the occurrence of the focal lesions. We have not previously observed this type of injury following the chronic administration of a hepatotoxic agent.

The early pathogenesis of the focal lesions is not clearly evident. Localized acute necrosis of the parenchyma seems to us the most likely explanation. However, this was seen only in one liver. In evaluating this suggestion it should be borne in mind that cell debris resulting from acute necrosis is often removed from the site in 3 to 4 days. Also it would seem pertinent to recall here that the early necrosis phase of epidemic hepatitis was not observed in the cases reported by Lucké.[3] An alternate explanation of the formation of these localized lesions would be the gradual loss of single or small groups of cells from an area. Against this theory is the fact that generally the entire lesion seemed to be of the same histologic age. Central fibrosis with the peripheral part of the lesion showing only collapsed sinusoids was not observed. It is true that the largest lesions occurred in the latter part of the study but these were considered as probably resulting from the development of adjacent rather than growth of individual lesions. It seems that the very gradual loss of cells from the lobule without replacement would lead simply to a reduction in size of the lobule, without sinusoidal collapse and condensation or proliferative fibrosis; this type of lesion was observed focally in a few livers.

Since histologically "early" lesions were seen throughout the experiment, it cannot be concluded that all lesions would progress to the end stage described above. The "early" lesions seen in the livers of animals killed in the latter part of the experimental period could be chronologically old lesions which had failed to undergo fibrosis. Against this alternative concept is the fact that in general the number of lesions per liver was greater in the second half of the experimental period and that lesions with extensive fibrosis were not seen before the twenty-third week.

A question raised by this experiment concerns the factor responsible for the localized nature of the lesions. It seems quite illogical to assume that the parenchymal cells in one part of the liver are inherently more susceptible to injury by phosphorus than another. It would appear that a more reasonable explanation could be based on some physiologic alteration of blood supply to the area. In an occasional superficial lesion sectioned vertically to the surface, the deep margin of the area of collapse was sharply limited by a fairly large hepatic artery (Fig. 2C). Primary vascular lesions were not observed.

Inasmuch as the hepatotoxic agent was administered by mouth, it is interesting that the gross lesions were neither limited to nor strikingly concentrated in the right or left half of the liver. From this observation, and reasoning from the theory that portal blood from different parts of the gastrointestinal tract reaches different lobes of the liver,[4] it might be suggested that absorption of phosphorus in olive oil is not limited to one segment of the intestines. If the oil phosphorus mixture passed by the lacteals into the general circulation before reaching the liver, this suggestion would be invalid.

It is often difficult to determine whether fibrous tissue, which is seen in some areas where liver cells have disappeared and the sinusoids are collapsed, represents condensation fibrosis or newly proliferated collagen. In the present study we feel that some of the lesions showed amounts of fibrous tissue far beyond that which could be accounted for on a condensation basis. This belief is supported by the fact that the early lesions resembled the microscopic appearance of "acute yellow atrophy" with little or no increase in collagen, even though the sinusoids often were collapsed, whereas the few densely fibrotic lesions were seen only in the latter part of the study. In all likelihood the fibrosis occurred through both mechanisms.

Summary. In an attempt to produce portal cirrhosis, guinea pigs were fed white phosphorus in olive oil by mouth for periods up to 35 weeks. Microscopic examination of livers from animals killed after one week showed inconstant, slight to moderate cytoplasmic hyalinization and occasional to few necrotic liver cells. There was also moderate

[3] Lucké, Balduin, and Mallory, Tracy, *Am. J. Path.*, 1946, **22**, 867.

[4] Bartlett, F. K., Corper, H. J., and Long, E. R., *Am. J. Physiol.*, 1914, **35**, 36.

cellular infiltration of portal areas and parenchymal fatty metamorphosis; the latter was sometimes periportal in location. These changes continued to be observed in most animals throughout the experimental period with increasing amounts of collagen in portal areas appearing as the experiment progressed. The increase in collagen was definite but never great. In 4 animals thin collagenous trabeculae connected portal areas and surrounded lobules. In a number of other animals fibrous extensions of portal areas were present but incomplete. There was neither prominent lobule distortion nor parenchymal hyperplasia. The failure to produce clear-cut portal cirrhosis is considered to be due to the inconstant and minimal degree of periportal necrosis.

After 9 weeks, the livers showed an increasing incidence of depressed gross lesions of irregular shape and size. The lesions in some livers were of such extent and severity as to produce marked deformity and shrinkage of various lobes. Microscopically, the lesions showed moderate to marked loss of parenchymal cells with collapse of sinusoids and reticulum stroma. In the latter part of the experiment a few such lesions showed marked fibrosis. The probable pathogenesis of these lesions is discussed and the incidence by lobes is given.

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Hemagglutination by Bacterial Suspensions with Special Reference to *Shigella alkalescens.*

JAMES J. GRIFFITTS. (Introduced by M. V. Veldee.)

From the Biologics Control Laboratory, National Institute of Health, Bethesda, Md.

Agglutination of red blood cells by various viruses has interested many workers, and has been found useful in immunological investigations of virus host relationships. Recently, Keogh, *et al.*[1] have reported agglutination of red blood cells by saline extracts of *Hemophilus pertussis, Hemophilus parapertussis,* and *Hemophilus bronchisepticus.* These authors suggest that this property of *Hemophilus pertussis* is related to the virulence of the organism and its ability to produce protective antibodies in mice.

In the course of an incomplete and unsuccessful survey of bacteria for Rh-like antigens using anti-Rh_0 blood typing serum, it was noted that saline suspensions of certain bacteria agglutinated human red cells in the absence of serum. It is the purpose of this report to describe some aspects of this phenomenon.

Experimental Observations. Hemagglutination by various bacterial species. Saline suspensions of bacteria grown for 24 hours on horsemeat infusion agar (and in a few instances on 5% rabbit blood agar) were prepared to have a density equal to 500 p.p.m. silica standard.[2] This type of suspension was used throughout these studies unless otherwise stated. Equal parts of these suspensions and 2% saline suspensions of once washed human Group O Rh-negative red cells were placed in small tubes. Agglutination was looked for after one hour's incubation at 35°C.

No agglutination of human red cells was observed using suspensions of the following bacteria (wherever more than one strain of organism was examined, the number of strains is given in parenthesis): *Aerobacter aerogenes, Alcaligenes fecalis, Bacillus anthracis, Brucella abortus, B. melitensis, Corynebacterium*

[1] Keogh, E. V., North, E. A., and Warburton, M. F., *Nature,* 1947, **160**, 63.

[2] *Standard Methods for Examination of Water and Sewage,* 6th Edition, American Public Health Association, New York, 1925, p. 4.