REGENERATION OF LIVER AND KIDNEY FOLLOWING YELLOW FEVER *

OSKAR KLOTZ, M.D., AND T. H. BELT, M.D.

(From the Department of Pathology and Bacteriology, University of Toronto, Toronto, Canada)

The present report is concerned with the study of liver and kidney tissues taken from six rhesus monkeys which had been experimentally infected with yellow fever at the laboratories of the West African Yellow Fever Commission of the Rockefeller Foundation at Yaba, Nigeria, in 1928. Each animal suffered a short but typical attack of the disease, manifesting symptoms of prostration, anorexia, fever, albuminuria and slight jaundice. Each recovered its usual health shortly after the cessation of fever, and in each case an active immunity was proved before the monkey was finally killed. The accompanying table indicates the individual histories, briefly.

Case Histories of Rhesus Monkeys Experimentally Infected with Yellow Fever

Monkey	Strain of virus	Incubation period	Fever	Post febrile period	Killed by
		days	*days*	*days*	
1	Asibi	2	4	16	Blow on head
2	A. S.	3	2	72	Ether
3	Asibi	5½	2	51	Blow on head
4	P.	5	3+	66	Blow on head
5	A. S.	7	2	54	Blow on head
6	L	5	3	55	Blow on head

There is every reason to believe that these animals suffered appreciable damage to the liver and kidney during their attacks of yellow fever. Eighteen other monkeys, infected at approximately the same time, with the same viruses, and under the same conditions, died of the disease in periods ranging from two to eight days after the onset of fever. They showed, without exception, marked necrosis of the liver, sometimes amounting to total destruction of the parenchyma;

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

Received for publication June 10, 1930.

and the kidney in each case presented a severe tubular degeneration. The monkeys that recovered and those that died manifested similar clinical symptoms, except that in the latter the rise in temperature was of a higher and more constant character and the symptoms in general were of greater severity.

Assuming, then, that the six monkeys that recovered had sustained liver and kidney injuries comparable to those found in the fatal cases, the organs were examined with the express purpose of determining the final result of these injuries.

The autopsies revealed no unusual gross findings. Without exception, the livers and kidneys presented a healthy appearance, and showed no signs of antecedent injury. Microscopically there was an absence in all the preparations of the distinctive lesions associated with the acute phase of the disease, and scarring was not observed.

None of the livers showed increase in fibrous tissue or proliferation of biliary channels, such as is commonly seen following other types of liver injury. The lobules were of the usual size and structure, and the vascular channels were practically free from blood.

The liver cells possessed a more or less normal appearance. The nuclei were uniform, of the usual size, and well filled with chromatin, and they stained in a normal fashion. There was no indication of mitosis in any part.

In five of the six cases there was some degree of zonal differentiation. Thus, in three cases the cells of the portal zones were slightly swollen and more eosinophilic than those of other parts; the cytoplasm was finely granular and the adjacent sinusoids compressed. In the other two livers this same change was observed in the central vein areas, and the portal zones were occupied by a well marked fatty infiltration. It is possible that these zonal alterations may represent some functional readjustment to an antecedent zonal necrosis. On the other hand they may be related to an entirely irrelevant condition, such as, for instance, helminthiasis, which was present in practically all of the animals.

The liver of *M. rhesus* 6 was quite different in many respects from the livers of the other five monkeys of the series. There was no zonal differentiation. All parts of the sections showed a uniform rarefaction of the parenchymal cytoplasm and a marked edema of the cells. The sinusoids were compressed and the trabecular arrangement could not be made out. The swollen, hydropic cells were unusu-

ally clear in outline, and their closely packed arrangement lent a mosaic appearance to the sections.

Some minor changes of an indefinite character were found in the livers of all the monkeys of the series. The Kupffer cells were slightly more prominent than usual, both by virtue of their numbers, and because of the deep staining qualities of their nuclei. In several instances there appeared to be some distortion and irregular enlargement of the liver cords in the midzonal regions of the lobules. Sometimes there were four or five cells abreast in a single cord, suggesting an alteration in the original orderly arrangement of the tissue. The distortion was, however, unaccompanied by signs of karyokinetic activity or alterations in staining qualities, and it cannot be regarded as definite evidence of regeneration. The same appearance of distortion may be produced in normal liver tissue, if the microtome knife sections the lobule at an unfavorable angle. There was also in most instances slight lymphocytic infiltration of the portal sheath, a relatively insignificant lesion which occurs very commonly in monkeys that have not had yellow fever. Thus there is no obvious association between the present findings and the initial injury.

The kidneys of all the monkeys in the series presented a more or less uniform appearance, which did not deviate appreciably from the normal histological picture. The acute renal lesions associated with the fastigium of the attack and recently fully described by Magalhães, were absent. No casts were seen, no scarring nor increase in fibrous tissue and, with two exceptions, no evidence of inflammation. M. *Rhesus* 5 and M. *rhesus* 6 each presented an acute non-suppurative nephritis of a focal character, confined to the interstitial tissue. The process in both instances was of very recent origin, and apparently quite unrelated to the attack of yellow fever.

The glomeruli were not congested and the capillary tufts showed no cellular increase. The straight tubules possessed empty lumina and their epithelium was not altered.

The convoluted tubules were examined carefully, because these structures are most seriously affected in yellow fever. They contrasted in a normal fashion with the rest of the tissue, possessing the usual deep eosinophilic staining reaction. The cytoplasm was of the usual density, but finely granular. The cells were somewhat swollen and the margins of the lumina were often slightly frayed. There was no vacuolation nor desquamation of the epithelium, however, and

there were no collections of cellular débris in the tubules. Moreover, the nuclei were healthy in appearance and indicated that the apparent cloudy change present in the cytoplasm was not associated with a lowered vitality of the cells. On the other hand, there was no definite evidence of an increased vitality or vegetative activity such as one might interpret as a sign of recent regeneration. The nuclei in cross-sections of the tubules were not increased,* nor were there any of the epithelial giant cell formations which Oertel interprets as evidence of repair.

Discussion

It has been emphasized elsewhere (De Lamar Lectures 1927–1928), that cirrhosis of the liver does not follow yellow fever in man, and that a contracted kidney is likewise not a sequel of the disease. To this statement may now be added the weight of experimental evidence which shows that in the stage of repair, restitution of the affected organs is accomplished by complete regeneration of the functional tissues. The animals under discussion presented a practically normal histological picture of the organs except for certain irrelevant lesions. In liver and kidney alike, the restoration of the *status ante quo* was such that no sign remained to indicate the extent of regeneration or the manner in which it was accomplished.

The typical condition of the kidney in the acute stages of yellow fever corresponds in the main to a well known type of nephropathy, namely, the nephrosis of Volhard and Fahr, the "nephropathia degenerativa" of Aschoff, or more particularly the "bichloride kidney" of Elwyn. A distinction has long been made between this purely degenerative type and the inflammatory lesions of the kidney. Nephrosis is a condition met with commonly enough in acute toxemias, and it is generally recognized as a lesion which clears up completely when the toxemia passes off. The yellow fever kidney affords a very good example of a pure nephrosis, and subsequent complete regeneration is, therefore, not to be viewed as unusual or materially different from the sequence of events following nephrosis in other diseases.

The acute liver injury, on the other hand, while having several features in common with that of the kidney in yellow fever, is of a

* According to Ribbert the average normal number of nuclei is seven.

distinctive character, as we have been at some pains to show in a previous communication; and in consideration of the severe and extensive necrosis it is noteworthy that complete and scarless restitution of the liver takes place in cases of recovery. Clinical experience with yellow fever shows that it differs from similar pathological processes of equal intensity in the matter of freedom from sequelae in the liver. Various kinds of hepatorenal poisonings, as for instance, arsenic, phosphorus and carbon tetrachloride poisonings, and some toxemias of pregnancy, have a tendency to produce permanent liver damage, which not infrequently progresses into the fatal stages of acute yellow atrophy (Roman, 1927). Similarly, infective processes such as catarrhal jaundice and some obscure forms of hepatitis lead to marked scarring of the liver. In yellow fever, on the contrary, both clinical and experimental evidences tend to show that normal hepatic function is quickly restored in individuals that recover, and that these individuals never suffer cirrhosis of the liver or acute yellow atrophy as a consequence of their attack.

What are the factors which favor this peculiar power of regeneration? Undoubtedly the self-limiting character of the infection is of importance. Not only is the toxemia of relatively short duration, but there is no recurrence after the initial attack, no repetition of injury such as is commonly held to account for the sclerosing diseases. Yet in yellow fever the single injury is probably often of greater magnitude than the sum total of repeated injuries which in other diseases produce sclerosis. Thus the fact of a short period of injury is not a sufficient explanation. We must look to some peculiar features of the pathological process in yellow fever for an interpretation of the end results.

Inquiry into the absence of fibrous response in the yellow fever liver leads to a consideration of the character of the initial lesion. As we have pointed out elsewhere, yellow fever *per se* does not induce an inflammatory response. There is no exudative response to the injury and no appreciable migration of leucocytes to the damaged tissues. The Councilman necrosis of the liver, peculiar to this disease, is coagulative rather than autolytic, and since leucocytes are not attracted to the foci of necrosis, there is, presumably, no pouring out of proteolytic substances. The vascular channels, both extra- and intralobular, remain free from thrombi so that the circulation is not interrupted, and no digestion of tissue arises through infarction. It

is probably in consequence of these facts that rapid autolysis of the destroyed cells does not occur; no dissolution of tissue is to be found during the acute and fatal stages of the disease. The injury is thus qualitatively and quantitatively limited, and as a result only the parenchymal cells suffer from the destructive process. Not only is the stroma of the portal sheaths unaffected, but the slender intralobular connective tissue framework, which supports the trabeculae, remains intact and can be so demonstrated by Van Gieson staining technique even when necrosis of liver cells is most intense. The absence of scarring in the yellow fever liver is to be attributed, therefore, to an absence of injury or irritation upon the stroma, which in turn must be related in part to the non-inflammatory, non-autolytic character of the degenerative process, and in part to the maintenance of a normal blood supply.

The yellow fever liver then, provides a striking illustration of the point which Mallory emphasized (1911), that destruction of the parenchymal cells alone does not, of itself, stimulate connective tissue proliferation. Whipple and Sperry (1909), and Schultz, Hall and Baker (1923) have made the same observation in connection with chloroform poisoning. Before the process of fibrosis is set in motion, the injury must involve tissue elements other than parenchymal cells, and this is true of the kidney as well as of the liver. This view is somewhat at variance with that of Kretz (1905), MacCallum (1904), and Milne (1909), all of whom consider that cirrhosis arises in consequence of a primary destruction of liver cells.

We have indicated the conditions associated with the absence of fibrosis in the yellow fever liver. For a discussion of the factors which incite fibrosis in other diseases, the reader is referred to Mallory (1911), Pearce (1904, 1906), Muir (1908), Milne (1909), Opie (1910), Rolleston (1912), Herxheimer and Gerlach (1921), Schultz, Hall and Baker (1923), Hall and Ophüls (1925), Roman (1927), MacMahon and Mallory (1929), and MacMahon, Lawrence and Maddock (1929).

Our investigations of a large series of fatal cases of yellow fever in man and monkey show that active regeneration of liver and kidney tissues is practically at a standstill during the toxic phase of the disease. The series of animals that recovered, which we report here, shows however, that regeneration is complete within sixteen to seventy-two days after the cessation of fever. Therefore, the rep-

arative process must go on to completion within the ordinary period of convalescence.

In the case of the liver, it is not difficult to imagine how breaches of continuity in the parenchymal cords are bridged over by proliferation of liver cells which have survived the toxemia. The slender cylindrical stroma encasements of the trabeculae are preserved and the growing cells spread by direct extension inside of these limiting membranes to replace the cells which have been destroyed and absorbed. Thus, the original pattern of the tissue is restored. All investigators of repair in the liver agree that the chief rôle in the regeneration of the parenchyma is played by old, undamaged liver cells. MacCallum (1902) observed that "where well-differentiated liver cells still persist the new liver tissue is very simply produced by their mere multiplication by division, and the less highly differentiated gall-ducts take no part in the process, but remain quiescent in their subordinate position as conductors of the secretion of the liver cells." The much debated question as to whether liver cords may take their origin from biliary epithelium does not arise in connection with the present study, for, as we have seen, there is no proliferation of the bile ducts in the tissues under discussion. It is only when the supporting stroma has been damaged and stimulated to proliferation, as in the case of acute yellow atrophy and in the cirrhoses, that attempts at regeneration take the peculiar distorted form of pseudobile ducts or pseudotubules and nodular hyperplasia. This type of regeneration, fully reviewed by Hess (1913), Blum (1923), Roman (1927), and Fishback (1929), does not concern us in connection with yellow fever.

In the kidney, regeneration of the convoluted epithelium is no doubt accomplished largely by proliferation of cells which survive the attack. Islands of living cells always remain from which a new lining may originate. In certain cases of extensive necrosis, it is probable also that the cells of the straight tubules may grow in to replace the destroyed secretory epithelium, assuming its morphology and its specialized function. As in the case of the liver, the intact basement membranes which remain unaltered in the disease orientate the new cells into the old pattern, as proliferation and extension take place. The functionless regeneration phenomena described by Oertel (1909) in chronic nephritis, and by Podwyssozki (1887), Ribbert and Peipers (1895), Thorel (1907), and Pearce (1909)

in partial extirpation experiments, as might be expected, are not to be found in the yellow fever kidney.

Summary

Six rhesus monkeys which had recovered from experimental yellow fever showed complete and scarless regeneration of the liver and kidney. This bears out clinical evidence that neither cirrhosis of the liver, nor contracted kidney follows yellow fever in man.

Special attention is directed to the sequence of events taking place in the liver. Except in cases of chloroform poisoning, liver damage of equal magnitude rarely occurs without producing some scar formation. The yellow fever liver proves that destruction of parenchymal cells alone is not a sufficient stimulus to induce replacement fibrosis.

The absence of fibrosis in the liver and kidney is due to a peculiar immunity which the stroma structures manifest toward the yellow fever injury; there is no stimulation of connective tissue elements during the acute stage of the disease. The reasons for this are, we believe, related to the non-inflammatory, non-autolytic character of the acute pathological process, and to the absence of thrombosis in the small parenchymal vessels.

Regeneration originates in islands of parenchymal cells which have survived the attack, and quickly restores the tissues to their original state.

BIBLIOGRAPHY

Blum, R. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1923, **72**, 95.

Elwyn, H. Nephritis. Macmillan Co., New York, 1926.

Fahr, T. *Ergebn. d. allg. Pathol. u. path. Anat.*, 1919, **19**, 1

Fishback, F. C. *Arch. Path.*, 1929, **7**, 955.

Hall, E. M., and Ophüls, W. *Am. J. Path.*, 925, **1**, 477.

Herxheimer, G., and Gerlach, W. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1921, **58**, 93.

Hess, O. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1913, **56**, 22.

Hurley, L. E., and Cameron, G. R. *Quart. J. Med.*, 1927, **20**, 449.

Klotz, Oskar. De Lamar Lectures, 1927–28. Williams & Wilkins Co., Baltimore.

Klotz, Oskar, and Belt, T. H. *Am. J. Trop. Med.*, in press.

Kretz, R. *Internat. Clin.*, 1905, **3**, 289.

MacCallum, W. G., *Johns Hopkins Hosp. Rep.*, 1902, 10, 375.
MacCallum, W. G. *J. A. M. A.*, 1904, 43, 649.
MacMahon, H. E., Lawrence, J. S., and Maddock, S. J. *Am. J. Path.*, 1929, 5, 631.
MacMahon, H. E., and Mallory, F. B. *Am. J. Path.*, 1929, 5, 645.
Magalhães, A. *Jornal dos Clinicos*, 1930.
Mallory, F. B. *Bull. Johns Hopkins Hosp.*, 1911, 22, 69.
McDonald, S., and Milne, L. S. *J. Path. & Bact.*, 1908–09, 13, 161.
Milne, L. S. *J. Path. & Bact.*, 1908–09, 13, 127.
Muir, R. *J. Path. & Bact.*, 1908, 12, 287.
Oertel, H. *Publications of the Russell Sage Institute of Pathology*, 1909, No. 1.
Opie, E. L. *J. Exper. Med.*, 1910, 12, 367.
Pearce, R. M. *J. Med. Res.*, 1904, 12, 329.
Pearce, R. M. *J. Exper. Med.*, 1906, 8, 64.
Pearce, R. M. *J. Med. Res.*, 1909, 20, 53.
Pearce, R. M. *Arch. Int. Med.*, 1910, 5, 133.
Podwyssozki, W. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1887, 2, 1.
Ribbert, H. *Virchows Arch. f. path. Anat.*, 1882, 88, 11.
Ribbert and Peipers. *Arch. f. Entwicklungsmechn. d. Organ.*, 1895, 1, 69.
Rolleston, H. D. Diseases of the Liver, Gall-Bladder and Bile Ducts. Macmillan, London, 1912, 194.
Roman, B. *Arch. Path. & Lab. Med.*, 1927, 4, 399.
Schultz, E. W., Hall, E. M., and Baker, H. V. *J. Med. Res.*, 1923, 44, 207.
Stengel, A., and Kern, R. A. *Nelson's System of Medicine*, 1920, 5, 512.
Thorel, C. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1907, 18, 115.
Volhard, F., and Fahr, T. Die Brightsche Nierenkrankheit. Julius Springer, Berlin, 1914.
Whipple, G. H., and Sperry, J. A. *Bull. Johns Hopkins Hosp.*, 1909, 20, 278.