THE EXPERIMENTAL PRODUCTION OF NECROSIS OF THE LIVER IN THE GUINEA PIG.*

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The starting point for our experiments was the observation that intravenous injections of various substances, as leech extract, colloidal solutions of metals, casein, and nucleoproteid caused an inhibition in the growth of mouse tumors.[1] In order to determine the mechanism through which these substances act, we studied their actions on the organs of guinea pigs, and we noticed that a single intravenous injection of solutions of various proteids and of hirudin apparently caused necrosis in the liver of the injected animals. In the mouse, on the other hand, we did not observe this effect on the liver. Observations concerning the experimental conditions under which necrosis of the liver is produced in the guinea pig are complicated through the apparently spontaneous occurrence of necrosis in the liver of these animals.

Mallory,[2] studying necrosis in the human liver, reports on some experiments in the guinea pig in which he found that various experimental procedures, as massage of the spleen, pressure exerted on the liver, subcutaneous injection of diphtheria toxin, or the passing of a galvanic current through the abdomen of an animal, apparently caused necrosis in the liver. He appears doubtful, however, as to the interpretation of these observations. He noticed this necrosis two hours as well as twenty-four hours after the experimental interference, and furthermore found necrosis in untreated control animals. He comes, therefore, to the conclusion that the guinea pig is entirely unsuited for the study of necrosis of the liver. Inasmuch as no data were given in Mallory's work which make possible a comparison between the number and extent of necroses of the

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[1] Loeb, L., and Fleisher, M. S., *Jour. Am. Med. Assn.*, 1913, lx, 1857.

[2] Mallory, F. B., *Jour. Med. Research*, 1901, vi, 264.

TABLE I.

	Injected with 10 c.c. of 1½% solution of casein.	Injected with 2½ c.c. of 15% solution of serum globulin.	Injected with 2 c.c. of 15% solution of nucleoproteid.	Injected with 4–8 c.c. of ¼% solution of hirudin.	Injected with 10 c.c. of colloidal copper.	Injected with 10 c.c. of distilled water.	Injected with 5 c.c. of 15% solution of ovalbumin.	Injected with 3 c.c. of 5% solution of protamin.	Injected with 10 c.c. of 5% solution of starch.	Injected with strophanthin 0.039 mgm. in 10 c.c. of water.	Controls uninjected.
No. of animals	87	27	51	43	53	24	16	20	21	20	297
No lesion	15 (17%)	4 (15%)	8 (16%)	5 (11%)	9 (17%)	3 (12%)	6 (38%)	6 (30%)	9 (43%)	4 (20%)	192 (64%)
1 very small lesion	8 (9%)	6 (22%)	3 (6%)	2 (4%)	4 (7%)	1 (4%)	1 (6%)	2 (10%)	2 (10%)	2 (10%)	30 (10%)
2 or 3 very small lesions	8 (9%)	1 (4%)	9 (18%)	4 (9%)	3 (6%)	7 (29%)	2 (12%)	.....	4 (19%)	1 (5%)	22 (7%)
Few small or larger lesions	18 (20%)	3 (11%)	4 (8%)	10 (23%)	11 (21%)	4 (17%)	3 (19%)	5 (25%)	3 (14%)	5 (25%)	27 (9%)
Multiple lesions	38 (47%)	13 (48%)	27 (52%)	24 (53%)	26 (49%)	9 (38%)	4 (25%)	7 (35%)	3 (14%)	8 (40%)	26 (9%)
Typical lesions (included among multiple lesions)	14 (1 %	7 (26%)	14 (28%)	9 (21%)	10 (19%)	2 (8%)	.....	2 (10%)	1 (5%)	1 (5%)	4 (1¼%)

liver in control animals and in animals subjected to various experimental procedures, this conclusion of Mallory's appears to be justified.

In order to decide definitely how far the necrosis which we found in the livers of guinea pigs was caused by our experimental procedures, and how far it was apparently due to other spontaneous factors not analyzed, it was necessary to determine first the frequency and extent of necroses of the liver in a large number of control animals.

For this purpose we examined the livers of 297 guinea pigs, some of which served also as controls for the animals used for intravenous injections of various substances. They were usually animals obtained from the same breeder and at the same time as the animals which were injected. We added as controls a number of animals which had been used in other experiments, especially animals in which incisions had previously been made in the uterus and ovaries.

The last columns of tables I and II show the frequency and extent of the necroses in the liver of these control animals.

TABLE II.

	Group A.	Group B.	Controls.
No. of animals	261	101	297
No lesion	41 (16%)	28 (28%)	192 (64%)
1 very small lesion	23 (8%)	8 (8%)	30 (10%)
2 or 3 very small lesions	25 (10%)	14 (13%)	22 (7%)
Few small or large lesions	46 (17%)	20 (20%)	27 (9%)
Multiple lesions	128 (49%)	31 (31%)	26 (9%)
Typical lesions (included among multiple lesions)	54 (21%)	6 (6%)	4 (1⅓%)

We see that 64 per cent. of the control guinea pigs were free from lesions, while 17 per cent. of the remaining animals had only very slight necrosis. 9 per cent. had a few small or larger necrotic areas, and only 9 per cent. had marked multiple lesions. Of these latter animals 1⅓ per cent. had the typical multiple necrosis in which certain more or less extensive parts of the livers were dotted with small necrotic spots between which at some places larger conglomerate necrotic areas could be found.

If we analyze further the occurrence of necrosis in the liver of

the control animals, all but one of the cases in which multiple lesions were found occurred in animals which had been examined either within five days following an operation (usually affecting the ovaries or uterus), or one or two days after arrival from a railway journey lasting more than twenty-four hours, and during the hot summer months. In these two classes of control animals exclusively the severe (typical) lesions were found. Many control animals were examined twelve to fifteen days after an operation; these were in most cases free from macroscopical lesions of the liver (table III).

TABLE III.

	Total.	Examination within 5 dys. of operation.	Sick preceding death.	Day after arrival on which examined.	Bled.
No lesion	4	2	1	1	–
1 very small lesion	6	5	–	–	1
2 or 3 very small lesions	4	1	1	2	–
Few small or larger lesions	8	3	1	4	–
Multiple lesions	17	7	1	9	–
Typical lesions (included among multiple lesions)	4	1	–	3	–

If we compare with these control animals, guinea pigs which received, usually twenty to twenty-eight hours before examination, an intravenous injection of a solution of casein, nucleoproteid, serum globulin, hirudin, or of colloidal copper, we find very much more marked necrosis. The difference between these groups and the control animals becomes especially clear if we compare the frequency of severe lesions in both classes of animals. In table II under the heading of group A, the average frequency of 261 animals is given. Marked multiple lesions were observed in almost one half of the animals, and in not quite one half of the animals showing multiple lesions the liver showed over certain areas the typical dotted appearance. On the other hand, in animals injected with distilled water, solutions of egg albumin, protamin, starch, and strophanthin (group B), the lesions, although much more marked than in control animals, were distinctly less than in those animals united under group A. In group B especially the severe lesions are less marked than in group A. Altogether we examined 659 guinea pigs. Considering the large number of the animals examined our

results are sufficiently concordant to present the following conclusions: (1) In a certain percentage of guinea pigs there occurs apparently spontaneous necrosis of the liver. This necrosis is very much more severe and frequent in animals subjected to injurious influences, as to a long journey in hot weather, or to an abdominal operation. Within a few weeks after the exposure to the injurious influence, the majority of the necroses seem to disappear. In healthy animals not subject to these injurious influences, necrosis of the liver is on the whole not frequent, although small lesions may occasionally occur. (2) Intravenous injections of a great variety of substances dissolved in water, and even of distilled water cause a marked increase in the necrotic lesions found in guinea pigs. It seems, however, that different substances show an unequal tendency to produce these lesions.

Microscopical Examination of the Lesions.—Most lesions were examined approximately one day after the injection of the various substances. Some, however, were examined at an earlier period; others, from six to ten days after the injection. Many lesions in control animals were also examined. We found the lesions in the beginning situated somewhere between the portal and central areas. If the lesions were larger they sometimes extended to the portal or central veins. At first the lesions were isolated and well defined. If they became more extensive neighboring lesions could join and form conglomerate lesions. Small lesions consisted of a single cell or of a few cells with somewhat shrunken, dense nuclei and hyaline cytoplasm which stained markedly with eosin. In larger lesions and at a later period, at first the center and later the greater part of the necrotic area showed signs of autolysis, the nuclei disappeared, the cytoplasm became vacuolar, and in the end a network of fibers remained. Very soon polynuclear leucocytes collected in the necrotic areas. At first they were situated in the capillaries, later they penetrated into the cells and helped to dissolve them. There were also visible occasionally some cells which probably represented swollen endothelial cells of the capillaries of the liver or a few isolated immigrated connective tissue cells. Thrombi occluding the vessels were absent, if we do not regard collections of polynuclear leucocytes in the capillaries, the number of which, however, varied

exceedingly at different places and in different specimens, as representing cell thrombi. It is probable that the collection of the polynuclear leucocytes is a secondary phenomenon, the necrosis of some liver cells being the primary change which caused the leucocytes to collect around the diseased cells. Connective tissue cells begin to grow into the necrotic areas, and in pieces taken out six days after the injection the nuclei in the necrotic areas have faded; connective tissue has supplanted a considerable part of the material. The ingrowing connective tissue cells may assume the shape of epithelioid cells. Proliferating bile ducts may be visible in the growing connective tissue. Ten days after the injection areas of newly formed connective tissue with dilated blood vessels and proliferated bile ducts and with some polynuclear leucocytes are visible. The necrotic material has entirely disappeared at that period. Hemorrhages are frequently visible in and around the necrotic areas; even ten days after the injection there may still be present some signs of hemorrhage. They are perhaps especially marked after injection of hirudin. At other times, however, hemorrhages may be absent.

We may conclude from the microscopical studies (1) that thrombi are not responsible for the origin of the necrosis, (2) that the lesions have usually an intermediate position, and (3) that they become organized through connective tissue.

Notwithstanding their substitution by connective tissue and the relative frequency of these lesions, they evidently do not lead to cirrhosis of the liver. Cirrhotic changes in the liver of the guinea pig must be extremely rare. It seems that after a few weeks these lesions disappear entirely or almost entirely, without causing retractions at the surface of the liver, notwithstanding the fact that they are situated preferably near the surface of the liver. In our investigations into experimental myocarditis we also had indications of the apparent gradual disappearance of the lesions. For the production of cirrhosis there must perhaps occur extensive and repeated necrotic changes in a certain area.

The fact that so many different conditions have the same effect, causing focal necrosis of the liver, suggested the possibility that one common factor may be present in all of them. The idea suggested itself that the presence of bacteria in the circulation and their

subsequent retention in the liver might be responsible for the lesions. We searched, therefore, a certain number of animals showing necrosis of the liver for the presence of bacteria either in the blood or in the liver. We examined three control guinea pigs which showed necrotic spots in the liver. A platinum wire was pushed into the necrotic areas, withdrawn, and the adhering material distributed into bouillon tubes. In one case blood was withdrawn from the heart and mixed with sterile bouillon. The results were negative. In four animals in which through intravenous injection of colloidal copper necrosis of the liver had been produced, a similar bacteriological examination of the necrotic areas also proved negative. The colloidal solution of copper used for injection was found to be sterile. The results obtained after injection of a solution of hirudin were, however, positive. In five animals we examined the heart blood bacteriologically approximately one day after the injection of the hirudin, and we found bacteria in one animal. In nine animals we examined the necrotic spots in the liver for bacteria by means of cultural methods, and found them present in four animals. An examination of the hirudin solution used for injection showed the presence of apparently similar organisms to those found in the blood or necrotic spots of the liver. It is probable that in the case of hirudin bacteria were introduced into the circulation of the animals with the solution and that the bacteria afterwards lodged in the necrotic areas. We may, however, on the basis of our negative results in control animals and in animals injected with colloidal copper, conclude that in general bacteria are not responsible for the liver necrosis produced through the various experimental interferences.

An analysis of the shape and distribution of the necrotic area as it reveals itself to macroscopic examination aids us somewhat in the etiologic interpretation of these lesions. The edges and the superficial parts of the lobes of the liver are favored,—an observation made also by previous investigators in the case of other necroses of the liver. At these places the circulation is most easily interfered with, and interference with the circulation may, therefore, be suggested as one of the factors of importance in the causation of this necrosis. Frequently the necrosis has the shape of straight lines, sometimes

running almost parallel to the suspensory ligament of the liver. Occasionally they seem to be located at places where the ribs exert pressure on the liver. It is not uncommon for a lesion to be continued from one lobe to an adjoining one, as if the two lobes formed one connected whole. This is in some cases probably due to the fact that pressure is simultaneously exerted on two overlapping or adjoining lobes; in other cases, where bacteria cause the lesions, it may be due to a transmission of the organisms from one lobe to the neighboring one through contact.

The readiness with which this necrosis is produced seems to vary greatly in different species of animals. While in the guinea pig it is evidently easy to produce necrosis of the liver, it is much more difficult to do so in the mouse. In the mouse we did not observe necrosis produced by one injection of either colloidal copper or hirudin, as in the guinea pig. In one mouse we found necrotic spots in the liver after repeated injections of hirudin.

At present it is not possible to give a definite analysis of the factors causing necrosis in the liver of guinea pigs. Some are in all probability caused by certain bacteria lodging in the liver. But this explanation cannot apply to the majority of the necroses produced in our experiments. The various methods which we use have in all probability one or two factors in common, and in every case these cause the necrosis. We have indications that mechanical factors that weaken the circulation at certain places in the liver are at least partly responsible for the origin of the necrosis. It may be that injection of the various substances contributes to the production of the necrosis by reducing still further the pressure with which the circulation is maintained in the liver, or that these substances reduce the resistance of the liver cells to unfavorable conditions of the circulation by altering the metabolism of the liver cells. We are tempted to analogize the necrosis of the liver produced experimentally through these injections with the ulcer of the stomach produced by Rehfuss[3] in the guinea pig through the injection of venom of Heloderma and of various other substances. All that these substances have in common is a marked influence on the general circulation and vitality of the animal. It is probable that

[3] Rehfuss, M. E., *Carnegie Institution of Washington Publications,* 1913, No. 177, 125.

as a result of the weakening of the circulation the epithelial cells of the gastric mucosa become injured, lose their power of resistance to the action of the gastric juice, and are, therefore, digested by the latter. In both cases thrombi are not the primary cause of the lesions; hemorrhages are common to both lesions; in both cases very diverse substances can produce the same result and the common factor underlying the action of all of them is probably a reduction of the vitality of the cells either through lowering the blood pressure or through toxic interference with the metabolism of the cells. In both cases secondary factors are added,—mechanical factors (pressure) in one case, digestive action of the gastric juice in the other. We may add that the intravenous injection of hirudin causes in a certain number of cases not only necrosis of the liver but also gastric ulcers. Hemorrhage is probably the cause of these ulcers.

Necrosis of the liver has been produced experimentally in the liver of animals (Flexner,[4] Theobald Smith,[5] Boxmeyer,[6] Pearce,[7] Joannovics,[8] and Opie[9]). The necrosis observed by Flexner after injection of ricin showed much similarity to the necrosis observed by us in the liver of the guinea pig. Flexner found no definite relation between the necrosis and thrombi in the blood vessels supplying the necrotic area. He therefore doubts an etiological significance of thrombi which may occasionally be found in the affected areas; he thinks it possible, however, that lesions in the capillaries lead to an increased transudation of toxin-containing lymph and may thus be responsible for the focal necrosis in the liver. Pearce laid special stress on the occurrence of agglutinative thrombi in the vessels of the liver accompanying necrosis produced through intravenous injection of hemolytic serum, and he referred the necrosis in the main to interference with the circulation caused by these thrombi, although he admitted the possibility of other factors playing a part

[4] Flexner, S., *Johns Hopkins Hosp. Rep.*, 1897, vi, 359; *Jour. Med. Research,* 1902, viii, 316; *Med. News,* 1894, lxv, 116.

[5] Smith, T., and Moore, V. A., *U. S. Department of Agriculture, Bureau of Animal Industry,* 1894, *Bulletin No.* 6.

[6] Boxmeyer, C. H., *Jour. Med. Research,* 1903, ix, 146.

[7] Pearce, R. M., *Jour. Exper. Med.,* 1906, viii, 64.

[8] Joannovics, G., *Ztschr. f. Heilk.,* 1904, xxv, 25.

[9] Opie, E. L., *Jour. Exper. Med.,* 1910, xii, 367.

in their origin. One of us has shown[10] that the necrosis produced through intravenous injection of ether is due to two factors: (1) a direct injurious action of the ether on liver tissue, and (2) autolytic changes induced through clotting in the larger blood vessels. The presence of the first factor could be demonstrated in an exact manner through observation of the development of the necrosis *in vitro* under conditions where interference with the circulation no longer could determine the origin of localized, focal necrosis at the same places where the necrotic areas would have arisen in the living body. At a later date the extension of the necrosis took place in the area supplied by blood vessels thrombosed as a result of the primary action of the ether and of the primary necrosis. The clotted blood vessels at this period are no longer open to the passage of carmin particles injected into the vessels of the liver. Karsner and Aub[11] were not able to produce, through injection of hemolytic serum, the same effects as those obtained by us through injection of ether. This was not to be expected inasmuch as hemolytic serum acts very much less strongly than does ether introduced into the circulation. Karsner and Aub were, therefore, unable to determine the manner in which the injection of hemolytic serum produces necrosis in the liver. It may be suggested that possibly some light may be thrown on the relative significance of direct toxic action on the blood vessels and liver tissue and of secondary effects due to thromboses by an exact determination of the time relations in the development of the necrosis. It is to be expected that effects due to a direct toxic action appear earlier than the effects due to thrombosis, the latter proceeding approximately at the same rate as the autolytic processes due to complete ligation of a lobe of the liver.

SUMMARY.

Through the intravenous injection of various substances differing very much in character, multiple necrosis can be produced in the liver of the guinea pig. In the mouse the effect of these substances is absent or much less marked. Different substances seem to differ,

[10] Loeb, L., and Meyers, M. K., *Virchows Arch. f. path. Anat.*, 1910, cci, 78.
[11] Karsner, H. T., and Aub, J. C., *Jour. Med. Research*, 1913, xxviii, 377.

however, in their power to produce necrosis. In control animals necrosis in the liver is much more rare. It is found especially in animals subjected to various injurious influences. The necrotic areas are usually situated between the portal and central areas of the liver acini. Their development is not due to thromboses interfering with the circulation in certain areas of the liver. They are probably due to a weakening of the circulation in the liver or to interference with the metabolism of the cells as a result of the injection of foreign substances. Mechanical factors (pressure on the liver cells) may have an additional effect. This necrosis may be compared etiologically to the acute gastric ulcers which can be produced through a great variety of toxic substances in the guinea pig.