MORPHOLOGY OF CALCULOUS CHOLECYSTITIS

S. F. Yushkov

From the Chair of Pathological Morbid Anatomy, I. P. Pavlov First Leningrad Medical Institute
(Director – Prof. M. A. Zakharevskaya, Dr. Med. Sci.)

(Received March 7, 1957. Presented by N. N. Anichkov, Member AMS USSR)

In most of the researches devoted to the study of the morphology of calculous cholecystitis attention has been focussed chiefly on changes in the epithelium and subjacent tissues of the gall bladder; insufficient attention has been paid to changes in the intramural nervous apparatus, although quite detailed studies have been made of the normal structure of the intramural nervous system, and of its relations with other tissues elements of this organ [13].

There are also numerous published papers in which the outstanding importance of functional changes in the nervous system for the pathogenesis of extrahepatic bile duct disease has been indicated [15].

We could, however, find only two published papers describing pathological changes of the intramural nervous apparatus of the gall-bladder in chronic cholecystitis. The neurohistological studies described in these papers were, however, conducted without sufficient regard to the state of other tissues of this organ [13, 17]. Apart from this, the material on which these studies were based was derived from cholecystectomy operations, and could not throw much light on the dynamics of the changes in the intramural nervous apparatus under inflammatory conditions, particularly in the early stages of the inflammatory process. The elucidation of the interrelations between changes in the intramural nervous system and in other tissue elements involved in the inflammatory process, as well as of the sequence of these changes, is of practical and theoretical importance, and can be achieved only by an experimental approach. With this object, experiments were performed on 90 guinea pigs, in which a model condition of cholelithiasis had been induced, by the method of N. N. Petrov and N. A. Krotkina [11], with some minor modifications.

EXPERIMENTAL METHODS

The animals were operated under morphine-ether anesthesia, under conditions of strict asepsis. After laparotomy, the gall-bladder was withdrawn from the incision, an opening was made in the fundus, and a sterilized mass of small glass beads, held together by thin copper wire, was introduced into the lumen of the gall-bladder. The gall-bladder and anterior abdominal wall wounds were than closed. The animals were killed by exsanguination at various times after the operation: 30 minutes, 6 hours, 12 hours, 2, 5, 8, 9, 12, 15, 25, 33, 45, 60, 80, 107, 140, 172, 210, 265, 317, 381, 420, 453, 510, 578, 612, and 680 days. The gall-bladder was immediately removed, and immersed in 12% neutral formalin. After fixing for 5 days, half of the organ was impregnated with silver, by Bielschowsky's method as modified by Gross, and the other half was dehydrated and embedded in celloidin-paraffin. The histological sections were stained with hematoxylin-eosin, picrofuchsin, and mucicarmine. Some sections were stained with iron hematoxylin, according to Heidenhain.

EXPERIMENTAL RESULTS

The nervous elements of gall-bladder did not show any morphological changes during the early stages (up to 2 days) of the acute inflammatory process.

The epithelium was desquamated, remaining intact only in the mucosal folds. The mucosa and muscle coats were edematous, and the vessels were engorged with blood, with marked marginal stasis of leucocytes. A fair number of leucocytes was seen in the edema fluid between the tissue elements. By the 5-8th postoperational day the epithelium surviving in the depths of the mucosal folds had begun to regenerate rapidly, and to cover the rugae. During this regenerative process, and in particular in its early stages, dedifferentiation of the epithelial cells was evident; they lost their cylindrical form, and became flattened. The cell boundaries were obliterated in the growth zone and adjacent parts, and the plates closing the epithelial cells were not apparent. Instead of being located at the base of the cells, as is usual for endodermal epithelium, the nuclei were situated at the most diverse levels. As the process continued, the edematous mucosal rugae became covered with regenerating epithelium, the height of which increased; the cells were at first cuboidal, and later on columnar, and mucinogen droplets appeared. Such secondary differentiation of regenerated epithelium was seen only in those areas of the mucosa which were not in direct contact with the foreign body; where such contact existed, the epithelial cells remained squamous, and mucinogen droplets were not seen in them.

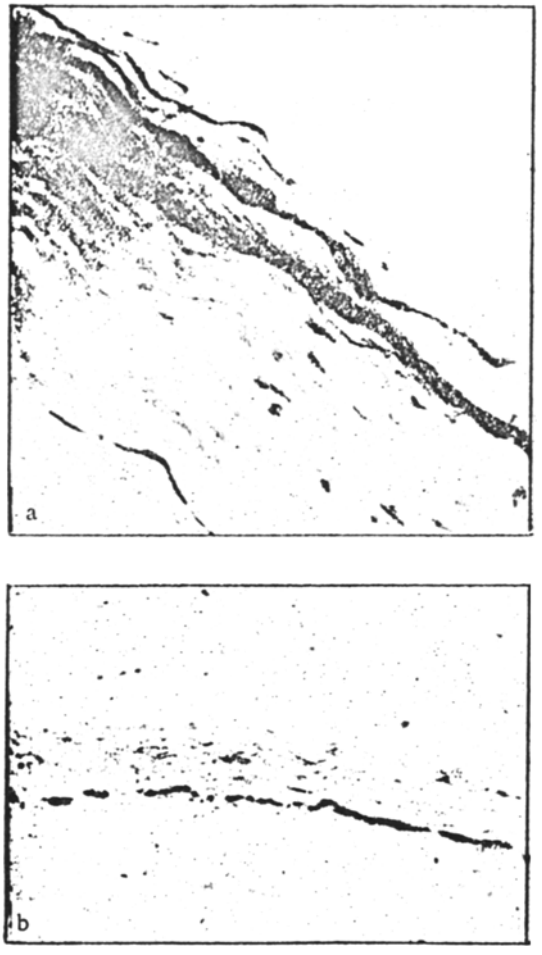

Fig. 1. Changes in nerve fibers of the muscle coat of the gall-bladder in experimental cholecystitis. a) varicose swelling and enhanced argentophilia of nerve fibers. Eight days after operation. b) disintegration of a nerve fiber, 60 lays after operation. Impregnated according to Bielschowsky-Gross. Magnification: a) oc. 8 ×, obj. 40 ×; b) oc. 8 ×, obj. 8 ×.

By this time the first morphological changes had appeared in the intramural nervous apparatus, in the form of heightened argentophilia of the nerve fibers, demyelination of the areolar nerve fibers, and thickening of the receptors (Fig. 1, a). There were no visible morphological changes in the nerve cells of the intramural ganglions.

After the 8th day we could not distinguish receptor formations in the mucosa, although they were still evident in the muscle and subserosal layers of the gall-bladder.

By the 12th day changes in the structure of the mucosa had become evident. The great majority of the epithelial cells of the rugae and of the crypts were overloaded with mucinogen. Intensified new formation of mucous glands was seen at the same time.

In the normal guinea pig gall-bladder the mucous glands are isolated, and are located in the depths of the crypts, mostly in the vicinity of the neck. They are rarely found in the body and the fundus of the bladder. In their structure, they resemble the alveolar type of gland [5].

We were able to trace the new formation of mucous glands in both the body and the fundus of the gall-bladder not only in the depressed parts, but also higher up, on the sides of the rugae. Pre-existing glands, of primitive structure, also underwent development, to the more complex tubuloalveolar type.

As the inflammatory condtion progressed from the acute to the chronic state, we observed, apart from proliferation of connective tissue and glands, a hypertrophy of the muscle coat, as a result of which the wall of the gall-bladder had attained a thickness of 3,5 mm by the 60th day. By this time the changes in the intramural nervous apparatus were more pronounced. Receptors had disappeared not only from the mucosal, but also from the deeper layers of the walls of the gall-bladder. irreversible changes were evident in the majority of the nerve fibers, in the form of fragmentation and

disintegration (Fig. 1b). At the same time as dystrophic changes were seen in most of the nerve fibers, we observed a weak tendency towards regeneration of unmyelinated nerve fibers in the muscular coat of the gall-bladder. These regenerated fibers are, however, unstable, and we observed varicose swelling, fragmentation, and disintegration of such fibers. This coincided with intensification of the inflammatory proliferation of the epithelium with formation of glands. These glands were formed not only in the proliferating connective tissue of the mucosa, but also within the muscular layer, down to the serous coat of the gall-bladder; glandular and cystic structures appeared, with a pronounced tendency towards differentiation into mucous glands. By the 80th day, the whole wall of the gall-bladder was penetrated by a mass of intricately ramified tubulo-alveolar glands. The ducts leading from these glands are lined with epithelial cells from the surface musoca, and are known as Luschka ducts, the intensive formation of which was seen on the 30th day after the operation. Retention cysts very often formed, owing to hindered outflow of secretion. The trapped secretion undergoes condensation, and adsorbs bile pigments and calcium salts, giving rise to formation of intramural bile pigment and calcium stones (Fig. 2).

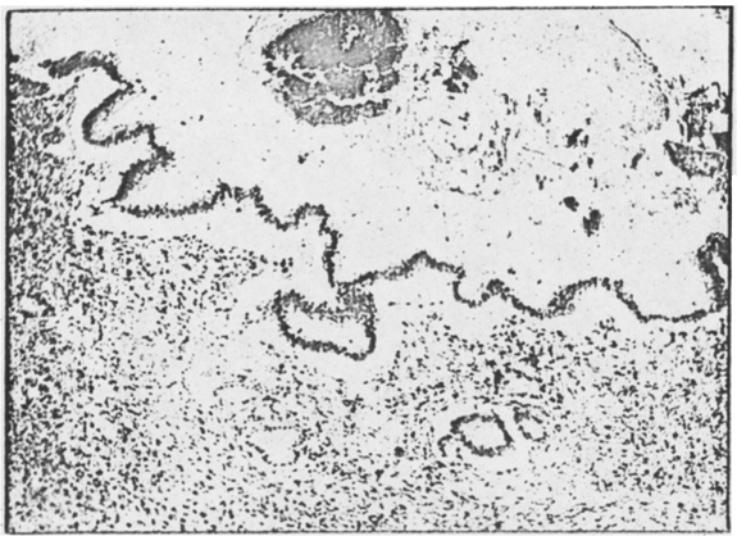

Fig. 2. Wall of cyst in a newly formed mucous gland. The lumen contains condensed secretion, with deposits of calcium salts and bile pigments. Specimen taken 107 days after operation. Stained hematoxylineosin. Magnification: oc. 8×, obj. 8 ×.

Dystrophic changes were evident by the 107th day, consequent to the continuing irreversible degenerative changes in the intramural nervous apparatus and to the hypertrophy of the muscular coat. Vacuoles of various sizes were seen in the muscle fibers, which showed homogenization of parts of the protoplasm, with granular disintegration and pyknotic nuclei. In such locations we observed proliferation of connective tissue between the degenerate muscle fibers, initially rich in cellular elements, but eventually maturing into cicatricial tissue. By the 172nd day, the whole of the wall of the gall-bladder had undergone transformation into scar tissue, with sporadic inclusions of muscle fibers and of cystic formations distended with secretion, being mucous gland ducts. It was only at this stage that we observed changes in the intramural ganglion nerve cells. Large vacuoles appeared in some of them, and masses of different sizes, staining intensively with silver, in others. Nerve cells not showing these changes were smaller than normal. We were not able, in all such cases, to demonstrate the presence of cell processes by silvering techniques. Activation of satellite cells was apparent around the most degenerate nerve cells. Unmyelinated nerve fibers entering the adventitia of arteries supplying the gall-bladder survived longer than other nervous elements.

Thus the inflammatory process brings about a total reconstruction of the wall of the gall-bladder, involving profound changes affecting its basic elements. The changes seen in the intramural nervous apparatus are of secondary origin, i.e., they appear as the inflammatory process progresses. In the early stages, when the

inflammatory reaction was of acute nature, we were unable to find any morphological changes in the nervous system of the gall-bladder. In the later stages these changes, being of secondary origin, could undoubtedly affect the development of irreversible changes in other tissues, during the reconstruction of the walls of the gall-bladder resulting from the intercurrent low-grade inflammatory process.

The resistance of nervous formations to acute inflammatory conditions has been noted by a number of authors [1, 2, 3, 8, 14]. Low-grade inflammatory processes, however, appear to involve profound changes in the nervous system [1]. It is however, recognized that inflammatory processes in denervated tissues are characterized by their extreme indolence [4, 9, 10, 17 and others). Attention has of recent years been directed to the role of the sensory neuron in the development of tissue reactions. Thus, according to T. A. Grigoryeva [4] organs deprived of their sensory connections resemble foreign bodies, of which the organism tries to rid itself by means of development of inflammatory processes within them. According to E. I. Zlotnik [7] and to E. A. Rudnik (1952), denervation of glandular organs leads to a profound reconstruction of the mucosal coats. Formation of mucous glands producing large amounts of mucous secretion takes place during this reconstruction process. Motor denervation of smooth muscle has been shown by I. P. Lukyanova [10] to lead to acute necrobiotic changes.

I. D. Khlopina [16] has found that denervation causes a disturbance in the mutual relations between the epithelium and the underlying tissues, thus interfering with achievement of regeneration, owing to which the healing of neurotrophic ulcers proceeds sluggishly, and in an irregular manner.

In our experiments, reconstruction of the gall-bladder mucosa was observed to coincide with disappearance of receptor formations and nerve fibers. The indolent course of the inflammatory process observed by us in the late stages of our experiment is evidently to a large extent due to denervation proceeding during the inflammatory process. It follows that the intense inflammatory proliferation of the epithelium is due rather to the profound dystrophic changes taking place in the underlying tissues of the gall-bladder epithelium as a result of denervation, than to injury of the epithelium by the foreign body introduced into the gall-bladder. This is because the foreign body loses its mobility as the inflammatory process passes into the chronic stage, becoming fixed in position by epithelial outgrowths. This fixation reduces its harmful action to a minimum. This is in agreement with the views of N. N. Petrov and N. A. Krotkina [11], who found pronounced epithelial proliferation even after the foreign body introduced into the gall-bladder had been ejected into the intestine.

According to G. N. Zabusov and I. F. Ivanov [6], the possibility of regeneration of the intramural nervous apparatus of the gall-bladder after its destruction in the course of an inflammatory process is inconsiderable. We, too, noted the limited extent of regeneration of nerve fibers in the walls of the gall-bladder, and the instability of the regenerated fibers. It is possible that the denervation occurring during the process of chronic inflammation of the gall-bladder removes tissue processes taking place in this organ from central nervous control, and may to some extent prepare the ground for the development of cancer.

SUMMARY

Experimentally induced cholecystitis was studied in guinea pigs at periods varying from several minutes to 680 days. It was established that the intramural nervous apparatus of the gall bladder does not show any morphological changes in acute stage of the inflammatory process. Degenerative changes develop at first in the receptor apparatus and later when the inflammatory process changes into subacute and chronic phase they appear in the nerve fibers. This process terminates by disintegration of the latter. In the late stage of this experiment the nerve cells of intramural ganglia under dystrophic changes.

LITERATURE CITED

[1] S. S. Vail, Vegetative Nervous System, and Localized Tissue Lesions,* Ogiz, 1935.

[2] S. S. Vail, Arkhiv Patol. Anat. i Patol. Fiziol., 3, No. 1, pp. 38-42 (1937).

[3] S. S. Girgolav, Morphological Changes in the Terminal Nervous System in Septic Inflammation. Vestnik. Khirug. i Pogran. Oblastei, 1, No. 1, 15 (1922).

[4] T. A. Grigoryeva, Doklady Akad. Nauk SSSR, 78, No. 2, pp. 387-390 (1951).

[5] N. I. Grigoryev, Reactivity of the Epithelium of the Small Intestine, Gall-Bladder, and Liver of Vertebrates and Humans,* Thesis, Leningrad, 1955.

* In Russian.

[6] G. N. Zabusov and I. F. Ivanov, Works of the Kazan State Medical Inst.,* No. 2, pp. 69-71 (1938).

[7] E. I. Zlotnik, Khirurgiya, No. 5, pp. 20-29 (1950).

[8] M. F. Kirik, Normal and Pathological Histology of Nervous Elements of the Vermiform Appendix, Collection of Papers edited by B. I. Lavrentyev,* pp. 224-244 (1946).

[9] M. A. Kotlyarevskaya, Doklady Akad. Nauk SSSR, 80, No. 1, 129 (1951).

[10] I. P. Lukyanova, Changes in Smooth Muscle Tissues following Motor Denervation,* Thesis, 1953, Moscow.

[11] N. N. Petrov and N. A. Krotkina, Voprosy onkol. 1, No. 2, pp. 89-100 (1928).

[12] N. N. Petrov and N. A. Krotkina, Ztschr. f. Krebsforsch., 38, pp. 249-264 (1933).

[13] M. K. Radionov, Innervation of Extrahepatic Bile Channels,* Thesis, Stalingrad, 1953.

[14] P. V. Sipovsky, Role of the Neurogenic Factor in the Etiology and Pathogenesis of Gastric Ulcer,* Leningrad, 1947.

[15] F. Ya. Sokolovskaya, Contributions to the Study of Certain Aspects of the Pathogenesis of Angiocholecystitis in Children,* Thesis, Tomsk, 1953.

[16] I. D. Khlopina, Arkh. Anat., Gistol., i Embriol., 29, No. 2, pp. 46-53 (1952).

[17] G. Szanto, Deutsche Zeitschrift für Chirurgie, 1936, Bd. 246, H. 5-6. S. 326-342.

* In Russian.