Some Observations on the Innervation of the Extrahepatic Biliary System in Man *

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THERE ARE STILL some unsettled points concerning the innervation and the physiology of the human extrahepatic biliary system. In 1895, 1896 and 1899, Dogiel in a series of brief comments, referred to the presence of ganglion cells in the wall of the gallbladder in man and in various mammals.[19-21] Later, Greving,[33] on the basis of an exhaustive study, could not verify the existence of nerve cells either in the gallbladder or in the bile ducts. His findings were tentatively accepted as correct by Kuntz.[42] More recently, German, Japanese, and other workers substantiated the correctness of Dogiel's findings, and provided many useful details concerning the finer morphology of the intramural ganglionated plexuses of the extrahepatic biliary passages.[1, 14, 32, 34, 35, 38, 51, 57, 64]

Unfortunately, even many of the more recent workers have used drawings rather than photomicrograms to illustrate their findings concerning the presence and distribution of nerve cells and the finer details of the autonomic nerve supply to the extrahepatic biliary system. Because of this, and also because of the dramatic advances during the last few years in knowledge of the histology of the intramural divisions of the autonomic nervous system in the gut, we considered that the whole problem needed re-investigation and clarification.

Our own work is directed principally towards the verification of the presence of intramural ganglia, their mode of distribution in relation to the intramural nervous plexuses, and the course and termination of the nerve fibres in the various effector and receptor areas. We shall also make an attempt, as many previous workers did, to correlate our morphological findings with existing physiological theories concerning the function of the extrahepatic biliary system, and try to find some harmony between our own observations and the often contradictory views expressed by earlier workers. Finally we hope to find an answer to the question, to the best of our knowledge unasked so far by anyone, "why are the extrinsic biliary passages the only glandular duct system, which had to be provided with an intrinsic ganglionated nervous reflex apparatus?"

Material and Methods

Our investigations covered the entire extrahepatic portion of the human biliary tree, but, for understandable reasons, a much fuller histological examination of the gallbladder and the cystic duct could be carried out. Specimens for these two areas were obtained principally at operations, and only some of the preliminary studies were made on postmortem material. Only routine methods (e.g., hemalum and eosin, and Masson's trichrome stain) were used for the study of postmortem material, as it is well known that bile has a macerating effect on the cells and tissues with which it

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comes into contact after death, and this fact, coupled with postmortem autolytic changes, renders this material well nigh useless for neurohistological studies. On the other hand, our investigations concerning the hepatic and common bile ducts, for obvious reasons, had to be conducted mainly on fresh postmortem material, though even a few hours after death the mucosal surface already showed signs of serious histological deterioration. On rare occasions, we were able to obtain fresh portions of the common bile duct at operation (e.g., pancreatoduodenectomy for carcinoma of the head of the pancreas).

Specimens obtained at operation were fixed without delay in twelve per cent neutral formalin. Several blocks were cut from representative areas of the fundus, body and neck of the gallbladder and from the proximal, mid, and distal portions of the cystic duct. As a general rule in every case the histological qualities were assessed by routine treatment of some of the blocks by hemalum and eosin, and by Masson's trichrome stain, while selected pieces were put aside for a special study by neurohistological methods. Towards this end two technics were employed, both of which gave one of us, (F. W. G.), in the past, consistently good results on a variety of tissues and organs.[23, 29]

The first procedure was a modified Bielschowsky-Gros silver diammine ion method.[30] For this purpose, the tissues were fixed in 12 per cent neutral formalin for a minimum period of four days. Thereafter the pieces were washed in running water for a few minutes only, and cut by the freezing microtome at 15 to 20 microns. The sections were collected in distilled water and treated by the special silver solution at room temperature. When the impregnation reached the desired intensity and selectivity, the subsequent stages of the procedure were carried out and the sections were washed thoroughly in distilled water and mounted in a thick levulose syrup. This has not only the advantage of considerable saving in time, but also avoids shrinkage, which is inevitable with the customary dehydrating and clearing processes.

The second special method used was one of the many modifications of the supra-vital methylene blue staining.[66] After the gallbladder and cystic duct had been removed at operation, they were perfused through the cystic artery with a warm solution of 0.02 per cent methylene blue at a minimum pressure, over a period of half an hour. From time to time, selected pieces were excised, spread out on a slide and examined under the microscope with the low power. When staining became optimal, it was made permanent by fixation in an 8.0 per cent aqueous solution of ammonium molybdate in the refrigerator. The pieces were then washed, dehydrated, cleared and finally enclosed as whole mounts in Canada Balsam.

It was found that sections impregnated with silver were the most suitable for the study of the finer morphology of nerve cells and fibers, while the whole mounts stained with methylene blue gave more useful information on the general topography and distribution of the nerve plexus.

Results

Examination of the silver impregnated and the methylene blue stained material shows beyond doubt that the entire extrahepatic portion of the biliary tree has a rich nerve supply. Many of the nerve fibers are myelinated of large or medium diameter, but the majority are of small diameter with beading. Nerves can be observed in all three main layers, and are, as a rule, in their final distributions in close, if not intimate, relationship with the functionally important structural elements, blood vessels, glands, smooth muscle cells, and probably also the lining epithelium.

Associated with the intramural nerve plexuses are numerous small and large

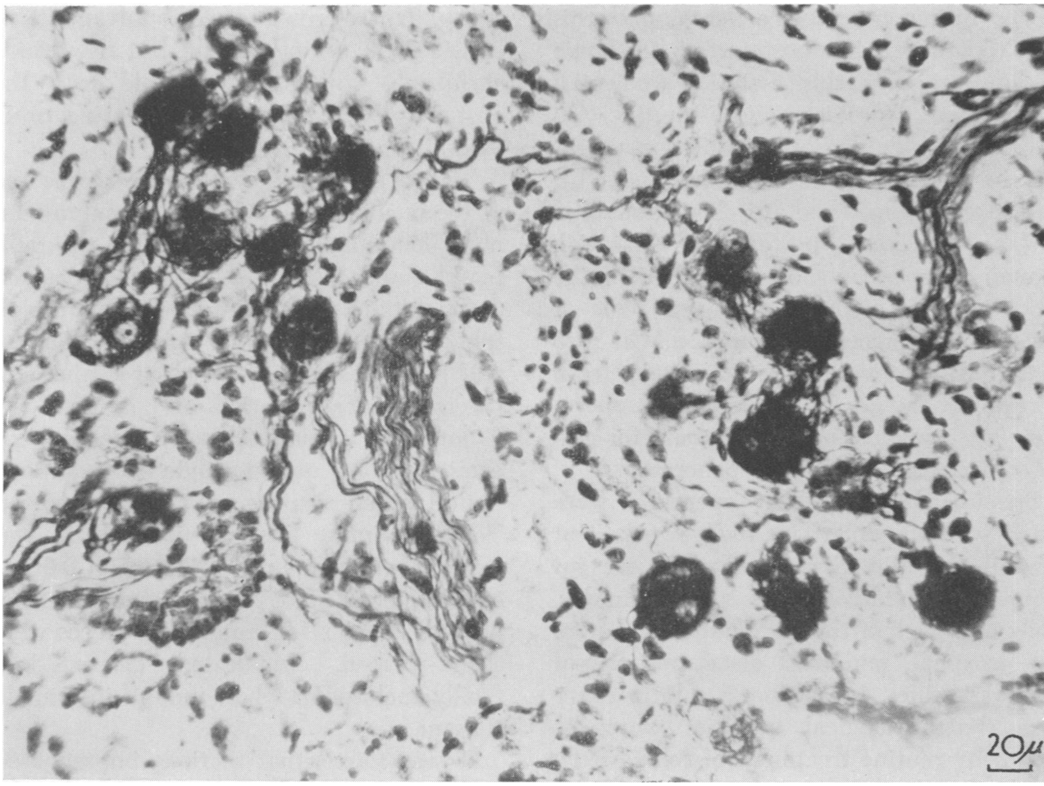

FIG. 1. Cystic duct. Two small ganglia in the muscular plexus. Modified Bielschowsky-Gros, × 300.

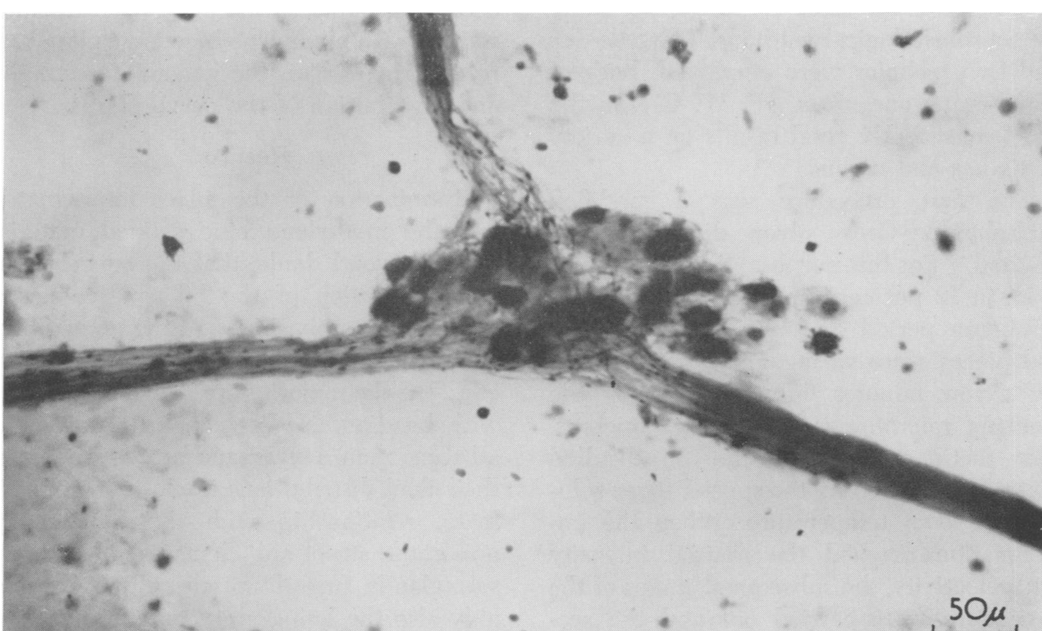

FIG. 2. Gallbladder. Ganglion in the subserous plexus. Methylene blue. × 160.

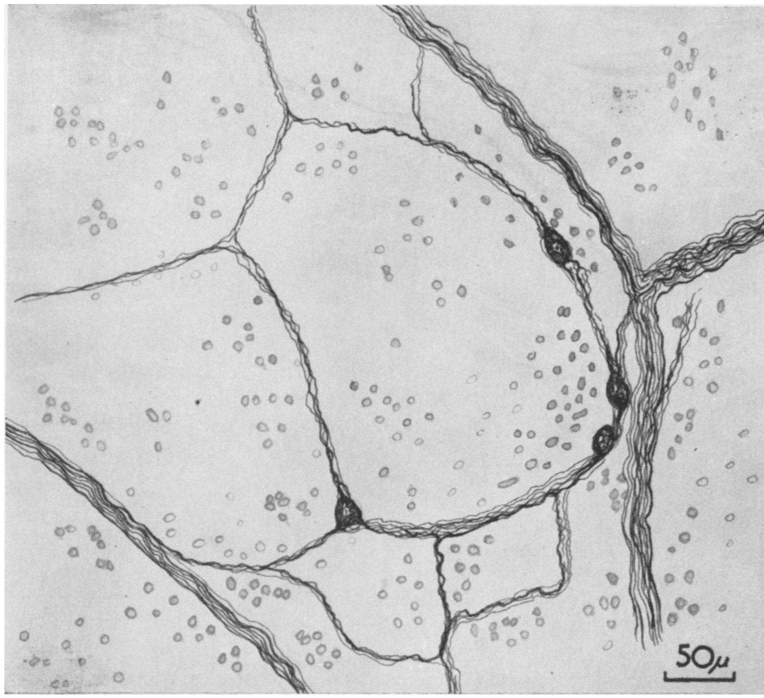

FIG. 3. Gallbladder. Occasional single nerve cells in the tertiary plexus of the subserous coat. Drawing. Modified Bielschowsky-Gros. × 100.

groups of nerve cells (Fig. 1), which give the groups a distinctly ganglionated appearance. In the adventitial or serous coat, these ganglia can be of considerable size and may, on occasion, contain up to 20 to 30 cells (Fig. 2). These ganglia are situated at somewhat irregular, and not infrequently, at considerable distances from each other and show little similarity to the *close mesh* and more regular arrangement of the ganglia of the myenteric plexus of the gut. The larger ganglia are usually found at the meeting points of the stronger primary divisions of the subserous plexus; somewhat smaller ones can be seen in association with the secondary divisions, and occasionally, single nerve cells are present along the course of the very much finer tertiary bundles (Fig. 3). The nervous plexus, situated on the outer surface of the muscle layer and also among its bundles, is more closely woven, and is much nearer in its character to the myenteric plexus of the gut. It is connected with, and fed by, branches of the subserous plexus.

Both in the plexuses of the subserous and of the muscular coats, the nerve cells are of two types. Cells of Dogiel Type 1 (Fig. 4abc) possess numerous short and usually one much longer axonal processes. The short processes, as already observed by Hermann (1952), frequently form in the vicinity of the parent cell several neurofibrillar pedicles. The axonal processes of

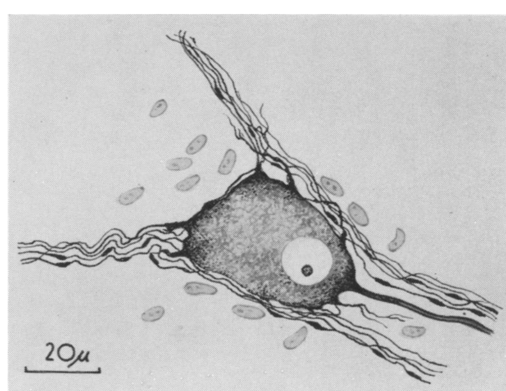

FIG. 4A. Cystic duct. Type 1 nerve cells in the muscular plexus. Modified Bielschowsky-Gros. a = drawing, × 500; b and c = photomicrograms, × 450.

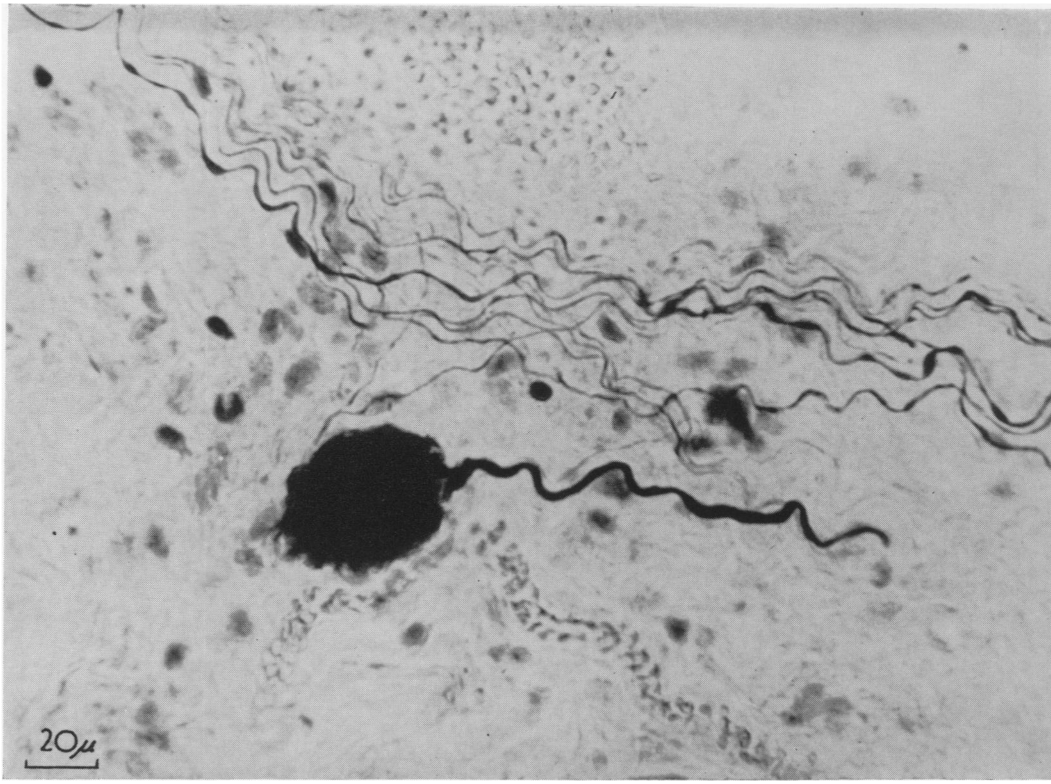

FIG. 4B.

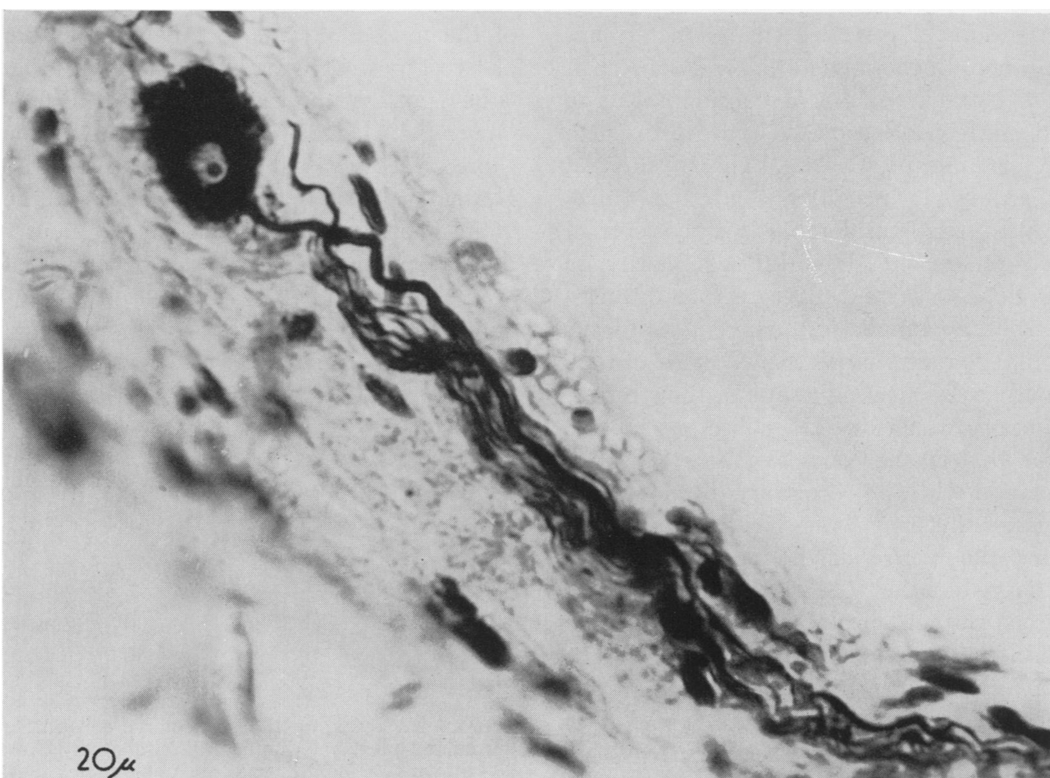

FIG. 4C.

these cells travel for considerable distances away from the cell body, but their exact mode of termination is not certain. These cells consistently take up silver salts more readily than those of Type 2, and the term *argentophil* sometimes applied to them, is justifiable.

Cells of Type 2, (Fig. 5abc) have relatively fewer, but usually strong, dendritic processes, which, after a short course, undergo repeated dichotomous divisions and produce around the cell body a densely woven network (Fig. 6). Their axons can be followed frequently into the muscle bundles of the muscle layer, where they or their branches run in intimate association with individual smooth muscle cells (Fig. 7). In association with the secondary and tertiary plexuses the cells are mostly of type 2. Most of the nerve fibers in these smaller plexuses are of narrow diameter showing beading.

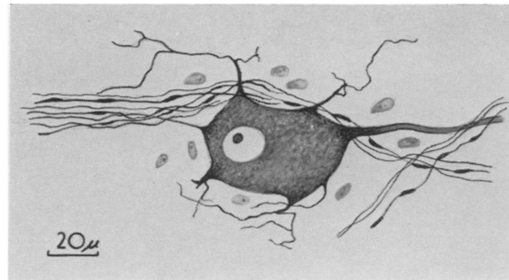

FIG. 5A. Cystic duct. Type 2 nerve cells in the muscular plexus. Modified Bielschowsky-Gros. a = drawing, × 500; b and c = photomicrograms, × 450.

In the mucosa there is a more delicate nervous network, which reminds one of the submucous plexus of the intestine. This mucosal plexus is fed by branches from the plexuses of the subserous and muscle layers and has, similarly to them, a three-dimensional arrangement with several layers. Mostly at the meeting points, but elsewhere in the plexus too, there are occa-

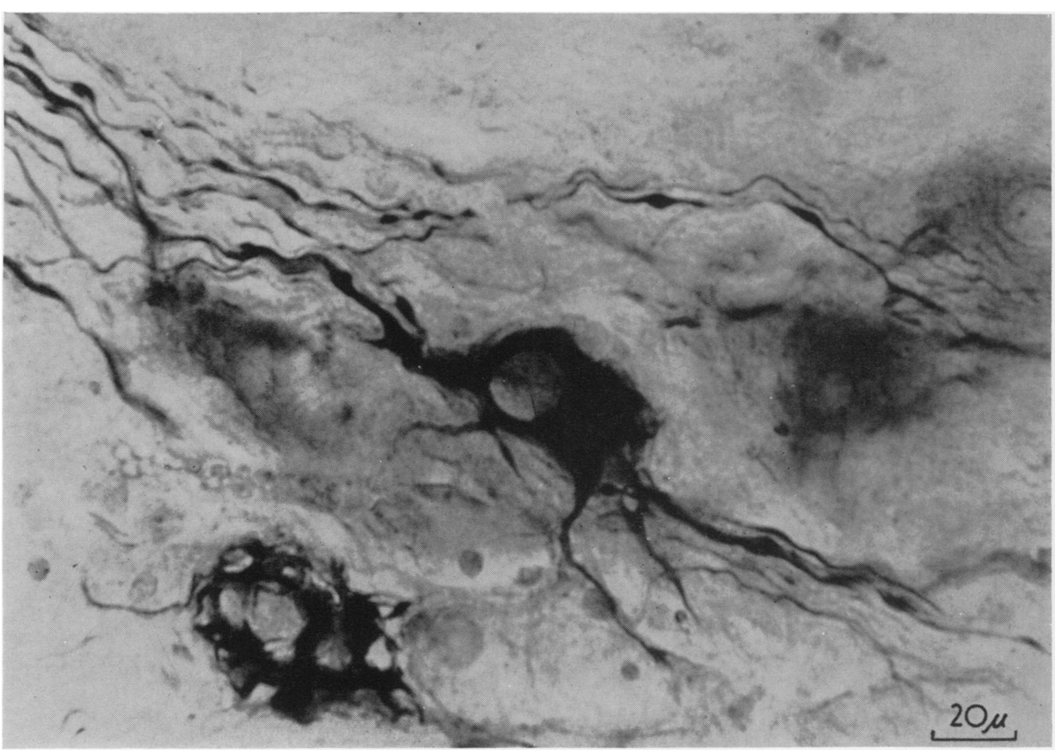

FIG. 5B.

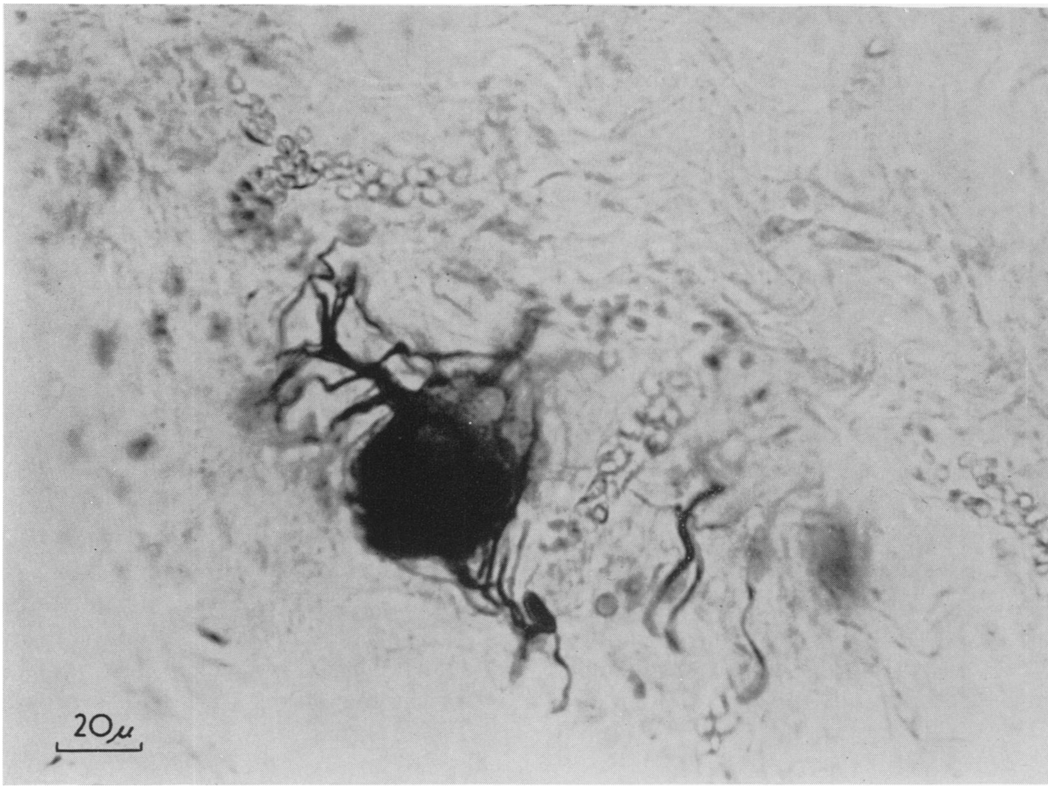

Fig. 5C.

sional single nerve cells or groups of never more than two to three cells. These nerve cells are mostly of Type 2, but generally somewhat smaller in size; in fact, many of the smaller ones cannot be typed at all. As we near to the mucosal surface the nerve cells become progressively fewer in number, and finally are completely absent from

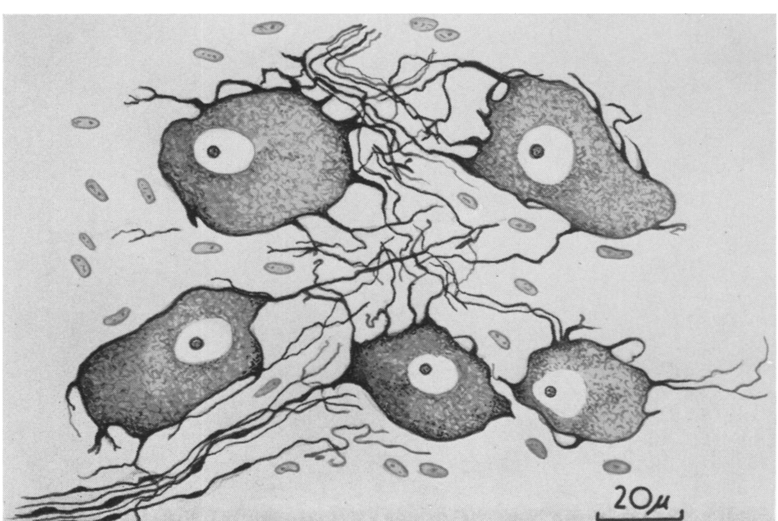

Fig. 6. Gallbladder. Small group of Type 2 cells in the muscular layer. Drawing. Modified Bielschowsky-Gros, × 500.

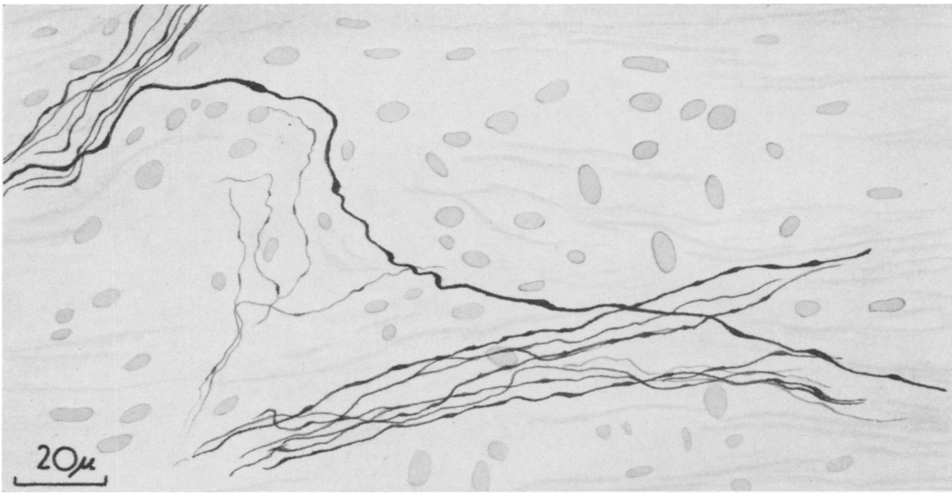

FIG. 7. Cystic duct. Small diameter, beaded fibers in the tertiary plexuses of the muscle layer. Drawing. Modified Bielschowsky-Gros, × 700.

the innermost parts of the mucosal plexus. In addition to nerve cells within the nerve plexuses, the best silver-stained specimens show a smaller type of cell already identified in the gut by many authors as the "autonomic interstitial cells of Cajal" (A.I.C.). The processes of these cells appear to form a syncytial network in close

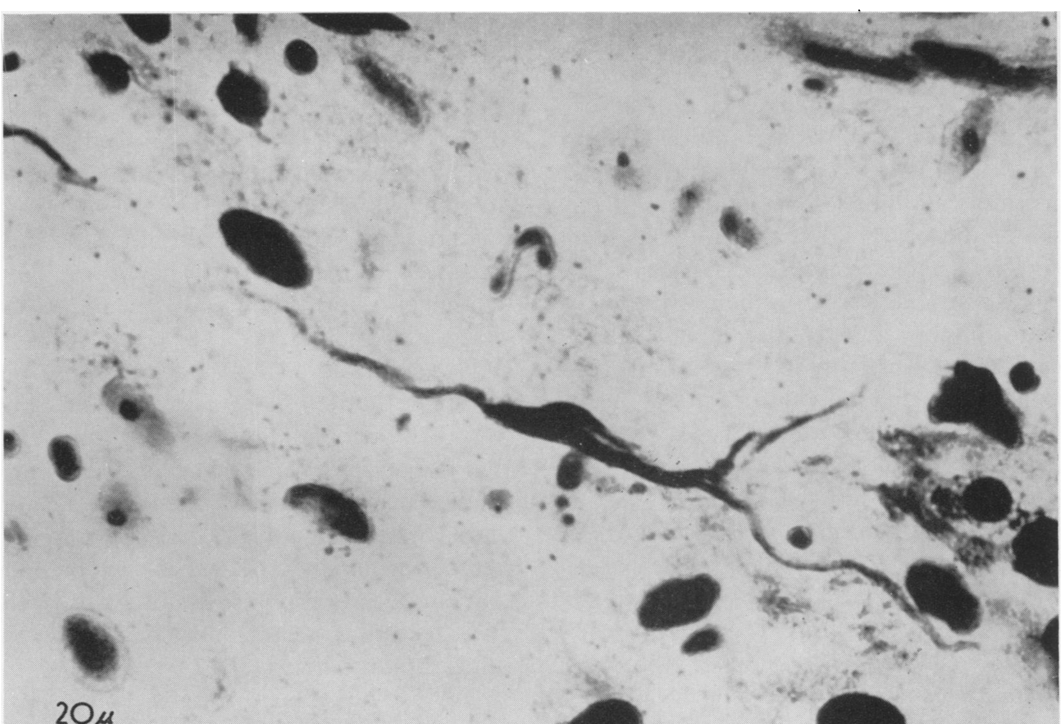

FIG. 8. Cystic duct. Interstitial cell of Cajal in the mucosal layer. Modified Bielschowsky-Gros, × 900.

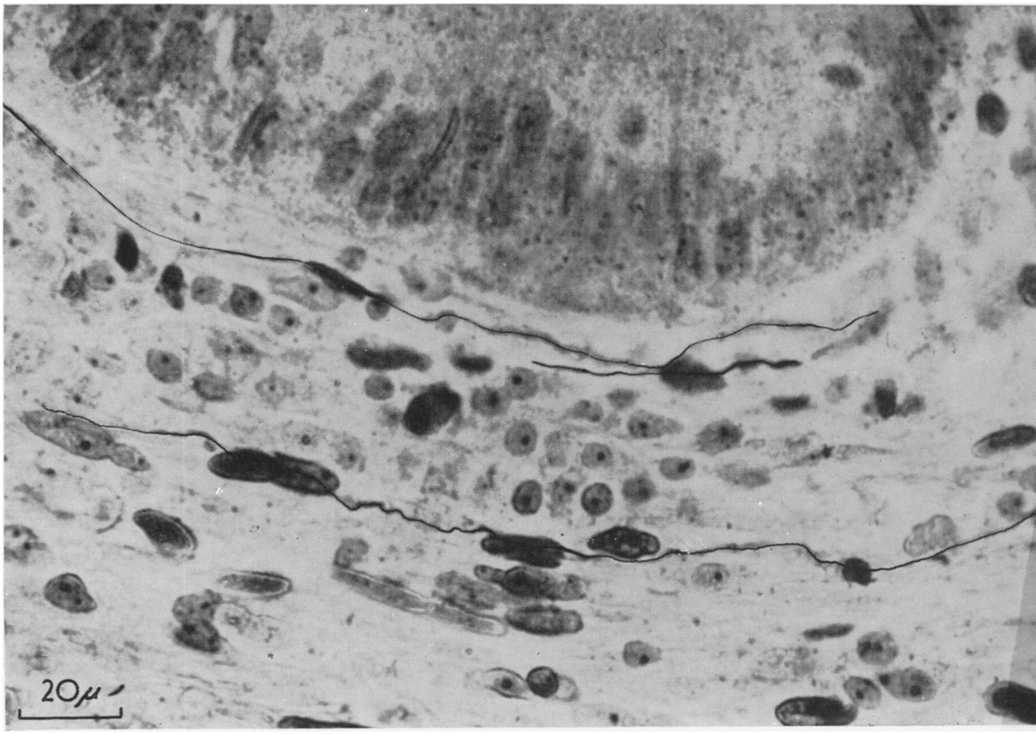

FIG. 9. Cystic duct. Fine nerve fibers forming delicate and loose sub-epithelial plexus. No intra-epithelial nerve endings can be seen. Modified Bielschowsky-Gros, × 750.

association with the nerves of the plexuses (Fig. 8). The cell bodies of these interstitial cells are frequently extremely argentophobe and thus, in many cases, they can be differentiated against the long ovoid nuclei of the Schwann cells only by the more spherical form of their own nuclei. Beneath the lining epithelium, in close proximity to the basement membrane, there is a fairly rich network of nerve fibers, usually of the fine beaded type. We were unable to observe unequivocal nerve endings in an intra-epithelial situation (Fig. 9). This is quite contrary to Hill's findings on the intestinal villi,[36] but probably is in harmony with out own findings in the other layers of the biliary passages, where the exact mode of ending of the nerve fibers and their more detailed relation to the structures supplied by them could not be observed with any accuracy. At present we believe that we should treat any apparent nerve ending as extremely doubtful. These findings are in complete harmony with Richardson's observations on adequately fixed and impregnated intestinal material and with the results of his electron-microscopical studies.[61] They also show agreement with the views expressed by Rash and Thomas.[60]

The distribution of the various nervous components described above is not uniform throughout the whole gallbladder: the nerve fibers and nerve cells are most numerous at the neck of the organ. In general, the wall of the cystic duct has a structure similar to that of the gallbladder, but, especially in its middle third, the musculature is better developed and the innervation is richer.

For the study of the innervation of the hepatic ducts, only fairly fresh postmortem material was at our disposal, consequently the value of our observations is somewhat restricted. Parallel with the relatively scanty, mostly longitudinal, musculature, and the occasional glands, the intramural plexuses show a comparable reduction and their three-tiered arrangement is least well developed. Most of the widely spaced *ganglia* contain only a smaller number of nerve cells. The few larger nerve bundles present in the superficial layers of the subserous coat may well be destined for the liver.

The common bile duct has a thinner and more irregular muscle coat containing bundles of smooth muscle cells running principally, though not exclusively, in a longitudinal direction. The circular muscle bundles become more conspicuous at the lower end of the duct, where they form the more cephalad sphincter of Boyden and the somewhat more caudad sphincter of Oddi. The intrinsic innervation of the common bile duct is basically similar to that of the gallbladder and of the cystic duct, but in association with the scantier amount of muscle tissue, the nerve supply shows a comparable degree of reduction. The three-tiered arrangement of the nervous plexuses is less distinct, *ganglia* are few and contain either single or no more than a small number of nerve cells. In the deeper parts of the mucosa, many of the single nerve cells or small *ganglia* are intimately related to the occasional groups of small mucous glands. The frequent large nerve bundles seen in the most superficial layers of the subserous coat may be destined for innervation of other parts of blood vessels and of the liver. This arrangement represents a second avenue through which, apart from the vascular route, the extrinsic nerve supply of the biliary system and of the liver can reach its destination. At the level of the two sphincters at the duodenal end, the intrinsic nerve supply of the common bile duct shows a noticeable increase in its complexity. The intrinsic nerve plexuses are better developed, are more closely woven, and the *ganglia* are larger and also more numerous. It is probable that in the region of the sphincter of Oddi some of the ganglionic masses actually represent an upward continuation of the myenteric plexus of the duodenum. On the basis of accepted functional theories connected with these regions, one would expect to find some specialized receptor organs in the ampulla of the common bile duct or in the duodenal papilla. However, none of our postmortem material was of good enough quality to enable us to make accurate observations. In this respect, it is of interest that even Grenade,[32] who seemed to have observed intraepithelial nerve endings in the cystic and common bile ducts, was unable to find them in these areas. Murakami and Kawahara,[51] described in the deeper layers of the mucosa of the pars papillaris of the common bile in dogs, specialized groups of epithelial cells, resembling the taste buds, but the single illustration provided by them cannot be taken as reliable evidence, and therefore the conclusion seems to be justified that the existence of any recognized sensory receptor regulating the opening of the common bile duct into the duodenum has not so far been demonstrated.

Discussion

Extrinsic Nerve Supply. It is generally accepted that the gallbladder and the biliary passages, like the gastro-intestinal canal, derive their extrinsic autonomic nerve supply from both sympathetic and parasympathetic sources. An anterior and a posterior hepatic plexus have been described.[1, 33, 34, 42, 59] The anterior hepatic plexus contains postganglionic sympathetic fibers deriving chiefly from the left coeliac ganglion and representing the outflow from the seventh to tenth thoracic segments (the experimental studies of Mallet-Guy, Eicholz and Latreille,[48] emphasize the particular importance of the seventh thoracic level in the sympathetic supply of the gallbladder); it also serves as a pathway for the parasympathetic preganglionic fibers from the left vagus nerve. The posterior hepatic plexus carries sympathetic postganglionic fibers from the right celiac ganglion, and also parasympathetic preganglionic fibers from the right vagus nerve. Nerve fibers from these two plexuses supply the gallbladder and the bile ducts with both autonomic components. These authors also claim that the afferent fibers from the biliary tree pass centrally mainly along the sympathetic nerves, and to a lesser extent, along the right phrenic nerve. Apparently the role of the vagus as a centripetal biliary pathway is negligible. Without doubt there are species variations in some of the details, and therefore it is not surprising that the results of animal experiments are sometimes conflicting, but the existence of sensory fibers in the human right phrenic nerve could provide a sound anatomical basis for the referred pain experienced by patients with gallbladder disease. However, biliary colic can occur in the absence of inflammation of the peritoneum overlying the gallbladder area so that there may well be afferent routes in man other than by the right phrenic nerve. (For example, acute distention of the bile duct with saline in patients without a gallbladder is accompanied with a sickening pain; this is relieved immediately by the inhalation of Amyl Nitrite which brings about relaxation of the sphincter of Oddi and rapid emptying of the bile duct.[11])

Intrinsic Nerve Supply. The first reliable accounts of the presence of nerve cells in the intramural plexuses of the gallbladder and of the extrahepatic biliary passages was given by Dogiel,[19, 21] on the basis of methylene blue stained preparations. However, Oppel[56] points out that Manz, Lee, and Gerlach had already indicated the presence of nerve cells in the gallbladder on the basis of results obtained with somewhat primitive microtechnical methods.[31, 45, 49] Later workers, using mostly silver impregnation methods, have substantiated Dogiel's cautious observations, and added further details to our knowledge of the general structure and distribution of the three ganglionated plexuses in the walls of the biliary passages.[1, 14, 34, 35, 38] The only dissentient voice was raised by Greving[33] whose opinion, though with some reluctance, was accepted as valid by Kuntz.[42] Unfortunately, Greving used for his studies Schultze's silver impregnation, which, as is well known, gives excellent pictures of the finest nerve fibers, but is not so suitable for the demonstration of nerve cells. Consequently his negative findings can be disregarded.

Our views on the three *ganglionated* plexuses are not in complete harmony with those of previous workers. We believe that the inner two, the muscular and mucosal plexuses, are the equivalents of Auerbach's and Meissner's plexuses of the gastro-intestinal tract, and that the subserous plexus is a development intended to cope with certain specialized needs of the extrahepatic biliary system. In this respect, it is of interest, though not generally recognized, that the existence of a definite subserous plexus along most of the length of the gastro-intestinal canal had already been observed by Auerbach.[2] Later workers provided additional details and tried to analyse its function.[36, 65, 67, 70] Various views were expressed, but we believe with Auerbach that the most likely purpose of this subserous plexus, which contains either no nerve cells, or only an occasional one, is to establish connexion between the mesenteric nerves and the myenteric plexus. The ganglionated subserous plexus of the extrahepatic biliary system may be, therefore, a modification of an already existing fundamental pattern, and therefore is not in this respect, a new development. Its purpose may be to provide additional relay stations for the parasympathetic nerve fibers, some of which may even be destined for the liver; or it may be concerned with the innervation of the blood vessels or with some other function.

Like all previous workers, we find two types of nerve cells in the intramural plexuses of the extrahepatic biliary passages. Various opinions have been expressed about the probable function of these nerve cells here and in the alimentary canal. Dogiel [21] and Temesrékási [70] believe that the Type 1 cells are motor while Hill [36] and Jabonero [39] consider them to be associative in function. There is an equal uncertainty with regard to the physiological role of the Type 2 cells. We believe that there is little reliable experimental evidence to support any particular theory. The two cell types show no regularity in their pattern of distribution either in the gastro-intestinal tract, or in the extrahepatic biliary passages, and do not show the same high degree of functional polarization as those of the cerebro-spinal system.

Ever since they were discovered, the *autonomic interstitial cells* have been a subject of fierce controversy. Some workers regarded the interstitial cells as true neurones, which play an important, if not a fundamental, role in the physiology of the autonomic nervous system.[6, 13, 68] Many of the existing theories concerning the nervous control of visceral function are built on this assumption. Nevertheless some dissentient opinions were expressed: Stöhr,[67] Hillarp [37] and Kuntz and Nepolitano [43] believed that they are neurolemmal cells; Hill [36] considered them to be of neuroglial origin; while Dogiel,[21] De Witt,[17] Kuntz,[41] Cole [15] and finally Richardson [61] asserted that they are specially modified connective tissue cells. It is evident from the literature that silver impregnation is not invariably the best method for the identification of these cells and from time to time, frank neurolemmal cells were confused with interstitial cells. The quality of Richardson's illustrations is unquestionable and one hopes that this longstanding controversy will soon be satisfactorily settled. As additional evidence, Richardson quotes the work of Benitez, Murray, and Chargaff,[5] who found that under experimental conditions true fibroblasts growing at certain interfaces can acquire all the accepted morphological characteristics of the interstitial cells of Cajal. In this connexion, it is also of interest that Ottaviani and Cavazzana observed,[58] stored granules in interstitial cells following the injection of trypan blue, a quality which is believed to be the exclusive faculty of cells of mesenchymal origin. If the interstitial cells of Cajal are now to be accepted as modified connective tissue cells, there will have to be reformulation of the ideas on function based on the neuronal nature of these cells.

Experimental Surgery. Histologically, the various components of the intramural autonomic plexuses are nearly undistinguishable from each other but this visual similarity need not necessarily imply a functional similarity. To examine the interrelation between sympathetic and parasympathetic components in the innervation of the extrahepatic biliary system, Sabussow and Ssuslikow [63] and later Alexander [1] studied in animal experiments the histological changes which follow either bilateral sectioning of the vagus nerves or extirpation of the celiac ganglia. These experiments, causing the destruction of the incoming pathways, produced in a broad sense, the expected results and supported strongly the dual nature of the extrinsic nervous control of the gallbladder and the biliary passages. On the other hand, in their finer details the results were by no means uniform, and we are forced to agree with Stöhr [69] that the biliary musculature appears to be innervated simultaneously by both vagal and sympathetic fibers and the terminal effector plexuses represent the final common pathway in which the sympathetic and parasympathetic elements are indivisibly merged with each other. On these grounds the frequently advocated antagonistic influence of the vagal and sympathetic fibers on the terminal effector organs becomes more or less illusory and we are inclined to accept that under normal physiological conditions there is a harmonious collaboration between the two systems.

This further increases the importance of the nerve cells in the intramural ganglia and we support the view, so aptly expressed by Hermann,[35] that they could have the role of transmitting instructions arriving through vagal and sympathetic pathways, and also that they might have the capacity to make a selective choice of the incoming stimuli giving preference to those which suit best the immediate physiological needs of the organism. To many, these views sound almost heretical as they seem to be in contradiction with the orthodox concepts of structure and function of the autonomic nervous system. On the other hand, in recent years the view has been expressed that there were some too facile generalizations in this field. In this respect, it is of interest that Langley had already suggested[44] that the enteric plexuses should be regarded as a separate subdivision of the autonomic nervous system and proposed that they should be designated the *enteric nervous system*. In his fundamental work he wrote: "This classification is, I think, advisable for the central connection of the enteric nerve cells is still uncertain and evidence has been obtained that they have automatic and reflex functions, which other peripheral nerve cells do not possess."

Pathological Changes. This proposed view of nervous control through the intramural plexuses would mean that the various tissue components of the biliary passages are brought into the closest harmony with each other. It would also infer, quite logically, that the intramural nervous system would not only participate in, but be affected by the various pathological processes acting on the gallbladder and the extrahepatic biliary passages, and might itself exhibit detectable morphological changes.

Hermann,[35] after reviewing the small amount of information available on the pathological changes observed in autonomic nerve cells in association with disease, found that up to the time of his own inquiry there was no information whatsoever in the literature concerning the influence of disease of the gallbladder on the morphology of its intramural nervous components. In the 25 gallbladders studied by him, all the seat of cholelithiasis, between half and three quarters of all nerve cells examined showed varying degrees of degeneration. A more surprising result of this investigation was that, despite the high percentage of nerve cells affected, the intramural plexuses showed little detectable deterioration. This may be an additional indication of the syncytial nature of the ganglionated intramural plexuses, and the relative independence of the nerve processes from individual nerve cells, which may have become involved in the disease process.

Our own material, derived principally from cases of cholelithiasis, confirms the validity of Hermann's observations as we also found that many of the intramural ganglionic cells showed signs of incipient, or even advanced neuronal damage. However, this aspect was beyond the scope of the present investigation. We are inclined to think that exploration of this field might give information of considerable value and that this approach, for example, might be particularly fruitful in studies on the confused subject of biliary dyskinesia. It has been already suggested that in this condition, there may be a disorder of the nervous mechanisms relating to reciprocal action between the gallbladder and the sphincter of Oddi.[71] It seems quite possible that the intrinsic nervous reflex apparatus might show some detectable morphological alteration.

Physiological and Clinical Considerations. Physiological and clinical interests in the innervation of the biliary tract are naturally focused on the part played by the nervous elements in the function of the biliary passages, and on the paths followed by the nerve impulses giving rise to pain when the biliary tract is diseased.

It is attractive to imagine that there is a reciprocal relationship between the sphincter of Oddi and the filling and emptying of the gallbladder. This had been mentioned by Oddi in 1887 and further amplified in subsequent communications,[53-55] when he stated that the same stimuli which lead to relaxation of the sphincter cause contractions in the musculature of the gallbladder.

With regard to the role of the vagus nerves in this process, Bainbridge and Dale[4] showed that their role must be motor, as their stimulations usually results in contraction of the gallbladder. Rost observed under experimental conditions that the sphincter of Oddi relaxes with each contraction of the gallbladder.[62] Westphal claimed that he actually observed discharge of bile into the duodenum following vagus stimulation.[72] Despite this, he comes to the final conclusion that the functions of the extrahepatic biliary passages are governed by the intrinsic vegetative elements in a fairly independent fashion. In this respect it is of interest that Lütkens expressed the view[47] that from the point of view of semiautomacy the intrinsic nervous elements of the mid-portion of the cystic duct are of cardinal importance and that removal of this area results in a disturbance of the motility of the bile passages. On the other hand, Eiger found[24] that vagus stimulation in dogs causes contraction of the common bile duct and biliary stasis. If this closure is accompanied by the contraction of the sphincter of Oddi, this would indicate that it receives, similarly to the cardiac sphincter, both motor and inhibitor fibers via the vagi.[42] Although some of the contradictory results may be due to errors in experimental procedure, others may be the result of artificial stimulation of a vagus nerve containing fibers of sympathetic origin. In this respect the experiments of Johnson and Boyden[40] stand out in their well planned elegance. They found that the volume of the gallbladder in cats doubles after vagotomy, undoubtedly due to the loss of vagal tone. If they sectioned only one of the vagus nerves, severance of the right vagus produced a noticeably greater delay in the flow of the bile than did severance of the left vagus. From this they inferred that in the cat, the right vagus sends both motor fibers to the gallbladder and inhibitor fibers to the sphincter of Oddi; on the other hand, the left vagus contains only motor fibers for the gallbladder. In a further experiment, Boyden and Van Buskirk[9] demonstrated that section of all the extrinsic nerves to the sphincter of Oddi, although this destroys all preganglionic fibers to the area, does not cause the ganglionic cells and the postganglionic fibers to degenerate. They put forward the suggestion that it is this intrinsic nervous apparatus which continues to respond to humoral stimuli, and maintains function after both vagi and splanchnics are sectioned.

The part played by the sympathetic innervation in the function of the biliary system also contains contradictions. The general belief is that the sympathetic nerve fibers exert an inhibitory influence on the biliary musculature, and it is in full accord with this view that in Bainbridge and Dale's experiments[4] stimulation of the splanchnic nerves caused relaxation of the gallbladder. They also found that bilateral section of the splanchnics augmented the rhythmic contractions of the gallbladder. Contrary to these findings, Doyon expressed the view that stimulation of the sympathetic fibers causes contractions in the musculature of the gallbladder.[22] Others,[16, 28, 50] found that splanchnic stimulation is followed first by facilitation and only later by hindering of the contraction waves in the gallbladder.

Undoubtedly much of the confusion concerning the role of the vagal and sympathetic fibers in the extrinsic innervation of the extrahepatic biliary passages is due to incomplete anatomical knowledge. Apart from species and individual variations the picture is further complicated by the fact that there is a considerable mixing and interchange between the fibers of the parasympathetic and sympathetic systems. This is shown by the fact that while most of the parasympathetic fibers are cholinergic, in the sympathetic, in addition to the adrenergic fibers, substantial quantities of cholinergic fibers have also been demonstrated.[71] The situation concerning the two vagus nerves is also far from being clear, as there is a tendency to overlook the fact that after the vagi merge in the oesophageal plexus the two nerves can be no longer distinguished from each other. Nevertheless the posterior of the two trunks which emerge at the level of the diaphragm from the oesophageal plexus is still referred to by some anatomists as the right vagus, though it contains fibers from the opposite side as proved by electrical stimulation in the neck.[71]

The results of pharmacological experiments again show no complete unanimity. Flexner, Bruger and Wright,[27] studying the action of autonomic drugs on the biliary system, came to the conclusion that the biliary musculature is controlled by the sympathetic and parasympathetic fibers in identical fashion to that of the gastrointestinal canal. On the other hand, Eppinger and Elek concluded from their pharmacological studies,[26] that the musculature of the gallbladder receives its motor innervation both from sympathetic and parasympathetic sources, and only the inhibitory nerve supply comes entirely from vagal sources.

Apart from these uncertainties and contradictions concerning the role played by the extrinsic nerves in initiating contractions of the gallbladder and thus biliary evacuation, some even questioned whether the gallbladder took active part in this process at all. Auster and Crohn[3] apparently were unable to elicit any contractions in the gallbladder by direct electrical stimulation of the gallbladder muscles of a strength which exceeded that needed to produce contractions in the small intestine. Winkelstein and Aschner were similarly unsuccessful using direct or reflex stimulation.[73] Burget[10] expressed the view that, because of the relatively poor development of its musculature, the gallbladder is incapable of contractions which could play a significant role in the flow of bile.

On the other hand, Elman and McMaster (1926) and McMaster and Elman (1926), in a series of carefully planned experiments,[25, 52] proved that after taking food, part of the bile in the gallbladder is expelled forcibly by active contraction of the viscus. They demonstrated also the reciprocal relationship between gallbladder activity and that of the musculature at the lower end of the common bile duct. They postulated that the escape of bile into the duodenum occurs promptly at the first ingestion of food, then nearly ceases for a short period, and later on continues at intervals strictly parallel with emptying of the stomach. In this respect, it is of interest that one of us has described waves of contraction in the human biliary passages.[12] By the use of image intensification and cineradiography these waves of contraction were clearly seen in the common bile duct, and were apparently concerned with the propulsion of the bile into the duodenum. At that time we assumed that there must be a sufficiently well developed circular and longitudinal muscle coat in the wall of the common bile duct to account for these movements. During the progress of the present work it became evident that, while circular muscle is present in the cystic, and to a much less extent in the hepatic ducts, most of the common bile duct contains only longitudinal muscle and circular muscle appears in appreciable quantities only at its lower end in the region of the sphincters of Boyden and Oddi. As these *peristaltic* waves were observed only in the common bile duct of post-cholecystectomy cases, their origin is obviously unconnected with the gallbladder. They may represent the rhythmic contractions of the scanty longitudinal musculature in synchronous harmony with the relaxing sphincter at its duodenal opening. This assumption, based on the actual anatomical features of the common bile duct, is not only attractive, but also logical. These well-coordinated actions appear to be neurogenic, of reflex type and may well be under the control of the intrinsic ganglionated plexuses which we have described in that area.

Results of some other experimental studies have indicated quite clearly that the discharge of bile from the gallbladder is elicited by the passage of certain kinds of food into the duodenum. Boyden's experiments seemed to suggest that the fatty constituents of ingested food may be responsible for the emptying of the gallbladder and cause "a functional periodicity in relation to meals." [7] Meals of pure protein or carbohydrate did not produce any marked changes in his animals. He also demonstrated the effectiveness of fatty food in the emptying of the gallbladder in man. Later Boyden, Bergh and Layne [8] studied the bile flow and gallbladder evacuation in medical students after instillation of 30 cc. of saturated magnesium sulphate into the duodenum. Changes in the volume of the gallbladder were computed from x-ray shadows, and the results compared with those previously obtained with egg yolk. These two agents were found to act for the same length of time and to have the same qualitative effects on the gallbladder and sphincter of Oddi.

All this seems to show, as we have already stated elsewhere,[12] that there exists a special relationship between the biliary system and the duodenum. Normally duodenal contractions do not begin until a few waves of contraction have occurred in the bile duct and, in the first instance, the waves in the duodenum run craniad, presumably to assist in the mixing of food. Our finding that the duct empties into the duodenum while it is still relaxed is in complete harmony with the results obtained by Lueth in dogs,[46] and we can now put forward a tentative theory of biliary function in man, a theory more or less in keeping with accepted teaching in physiology. In the fasting state, the gallbladder and the biliary passages have their tone maintained through their intrinsic nervous reflex apparatus. During this period, the closed sphincters allow filling of the gallbladder in which there are only occasional spontaneous contractions. The presence of food in the duodenum generates *cholecystokinin*, which causes contraction of the gallbladder. The sequence of events is quite orderly, and the contractions of the gallbladder are not just followed by the passage of the bile along a passively conducting tube. A series of waves of contraction in the extrahepatic biliary channels propel the bile jets through the relaxed sphincters into the duodenum. The duodenum then takes up the running, and waves of contraction mix the contents of the gut with the digestive secretions.

Although we have frequently emphasized the cardinal importance of the intrinsic reflex apparatus in the physiology of the biliary organs and their relative independence from the higher autonomic centers, nevertheless this freedom is by no means an absolute one, and influences emanating from the central nervous system may produce a profound effect on the biliary system.

Finally, why is there an intrinsic nervous reflex apparatus in the extrahepatic biliary tree? A facile explanation would be that, in keeping with their embryological origin, their innervation should be similar to that of the intestines. However, a serious objection to this view is that the duct system of the pancreas, though identical in its origin to the biliary passages, has no intrinsic nervous apparatus. Consequently we must seek an explanation elsewhere. We believe that, contrary to all other glands, where the duct system plays an almost exclusively passive role in the delivery and discharge of secretory products, the extrahepatic biliary passages have a dual role to fulfill. The production of the bile by the liver, is more or less continuous, and the dual process of its storage, as well as concentration by the gallbladder, and its discharge from there into the duodenum has to be carefully attuned to and synchronized with the changing physiological needs of the digestive apparatus. Even if this process is largely controlled by humoral factors, it can be mediated efficiently only through a well developed and highly sensitive intrinsic nervous reflex apparatus.

Summary

Examination of silver impregnated and methylene blue stained material shows that the extrahepatic portion of the human biliary tree has a rich nerve supply.

Associated with the intramural nerve plexuses are numerous groups of nerve cells of a distinctly ganglionated character.

No nerve endings could be found in the terminal effector areas and we believe, in harmony with other workers, that the ultimate extension of the intrinsic plexuses represents a true nervous syncytium. Though the higher autonomic centers exert a remote control over the biliary passages, our results and the data of recent literature indicate the importance of the intrinsic nervous system in responding to humoral and other stimuli.

In contrast with all other glandular passages the biliary system has a dual role to fulfill, storage of bile during fasting and its discharge into the duodenum following meals, and this can be mediated only through an efficient intrinsic nervous apparatus, developed as a result of functional necessity.

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