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Selective destruction of intestinal nervous elements by local application of benzalkonium solution in the rat

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Summary. Intestinal aganglionosis produced by serosal application of 0.1% benzalkonium solution to the colon of the rat was studied electronmicroscopically, and it was concluded that a higher susceptibility to the agent and a lower recovering ability of the nerve elements might be responsible for the phenomenon.

It has previously been reported by us that local serosal application of 0.1% benzalkonium chloride (BC) normal saline solution to the colon of the rat for 30 min produces selective destruction of intestinal nervous elements, and that the aganglionic colonic segment, produced by this method, is histologically and physiologically completely denervated, whereas the smooth muscles per se remain normal, morphologically and functionally[1]. Benzalkonium chloride or Osvan, a product of Takeda Pharmaceutical Co., Osaka, is dimethylalkylbenzylammonium chloride, or $[C_6H_5CH_2N(CH_3)_2R]Cl$, in which R ranges from C_8H_{17} to $C_{18}H_{37}$, about 60% being $C_{12}H_{25}$, about 35% being $C_{14}H_{29}$, less than 1% being $C_{10}H_{21}$ and less than 1% being $C_{16}H_{33}$. In this report, electron microscopic observation was performed to obtain sequential ultrastructural findings following local application of the solution, and to investigate the mechanism of action of the solution on the intestinal structures.

Materials and methods. 27 adult Wistar rats, weighing about 200 g, were used. Under nembutal anesthesia, the rat was laparotomized, and a gauze stick, which was 1.5–2 cm wide and had been soaked in 0.1% BC solution, was rolled around a segment of the descending colon, and was maintained for 30 min, followed thereafter by flushing with copious saline solution and abdominal closure. The animals were sacrificed at intervals ranging from 1 week to 14 months after the procedure and the treated intestinal segment was observed by light and electron microscopy. Specimens for light microscopy were stained by hematoxylin and eosin, Nissl, and Bodian stains. Specimens for electron microscopy were pre-fixed with 1%-glutaraldehyde-4%-paraformaldehyde, post-fixed with 1% osmium tetraoxide, dehydrated through graded acetones, embedded in Epon 812, cut into ultrathin sections with Porter-Blum M-1 ultramicrotome, doubly stained with uranyl acetate and lead acetate, and observed with JEM-100U electron microscope.

Results. Light microscopically the intestinal segment, 1–4 week(s) after the BC treatment, showed more or less manifest findings of inflammation mainly adjacent to the serosa at the early stage. Changes in nervous elements were not remarkable at the early stage, but at 4 weeks after the procedure complete disappearance of intestinal nervous elements, either sparing or not sparing Schwann cells, was observed (figure 1). Smooth muscle cells showed no marked change except for some partial reduction in stainability of outer layer muscles. More than 4 weeks after the procedure, there was no inflammatory change nor smooth muscle abnormality, and the intestinal nervous elements, including nervous networks in the intestinal wall, were found to have disappeared completely.

Electron microscopic findings were as follows. At 1 to 2 week(s) after the BC treatment, inflammatory changes, such as granulocytic infiltration, were observed. At this stage intermuscular nerve plexi showed either such changes as constriction of ganglia as a whole, increase in cytoplasmic electron density of nerve cells and swelling of mitochondria, or, at places, only minor changes. Smooth muscle cells generally tended to show contraction, and diffuse reduction in cytoplasmic electron density, decrease in amount of myofilament and formation of intra- and extracellular vacuoles were observed at places (figure 2, a). At 3 to 6 weeks after the BC treatment, inflammatory changes were found to have subsided, and there was an increase in collagen fibrils in the widened intermuscular spaces and intramuscular intercellular spaces, where nerve cells and nerve fibres had disappeared or degenerated. From 7th week after the BC treatment, degeneration and disappearance of nervous elements continued, while smooth muscle cells became normal, although markedly contracting (figure 2, b). In the widened intermuscular and intercellular spaces, an increase in collagen fibrils was observed. At places Schwann units containing vacuoles, presumably corresponding to degenerative axons, were observed.

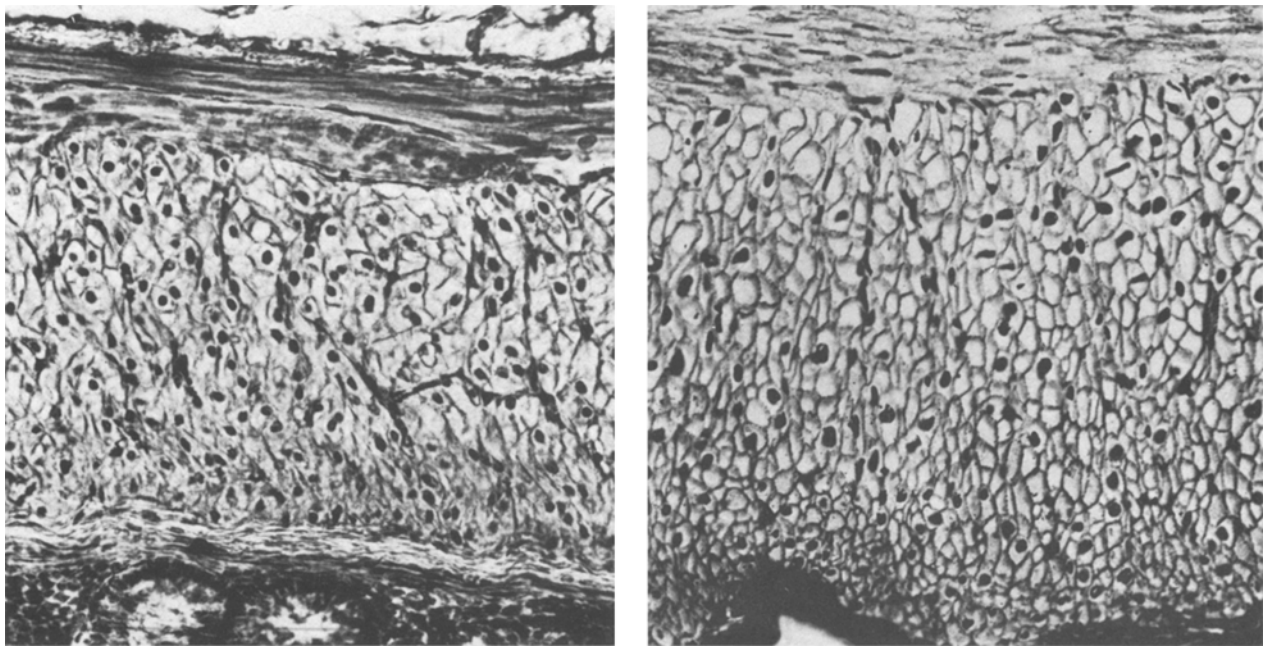

Fig. 1. *a* Photomicrograph of the descending colon of a normal rat. Normal intermuscular ganglion cells and intramuscular nerve fibres are discernible. Bodian stain. × 125. *b* Photomicrograph of the descending colon of a rat, treated with 0.1% BC solution 4 weeks priorly. Complete disappearances of intermuscular ganglion cells and intramuscular nerve fibres are apparent. Bodian stain. × 125.

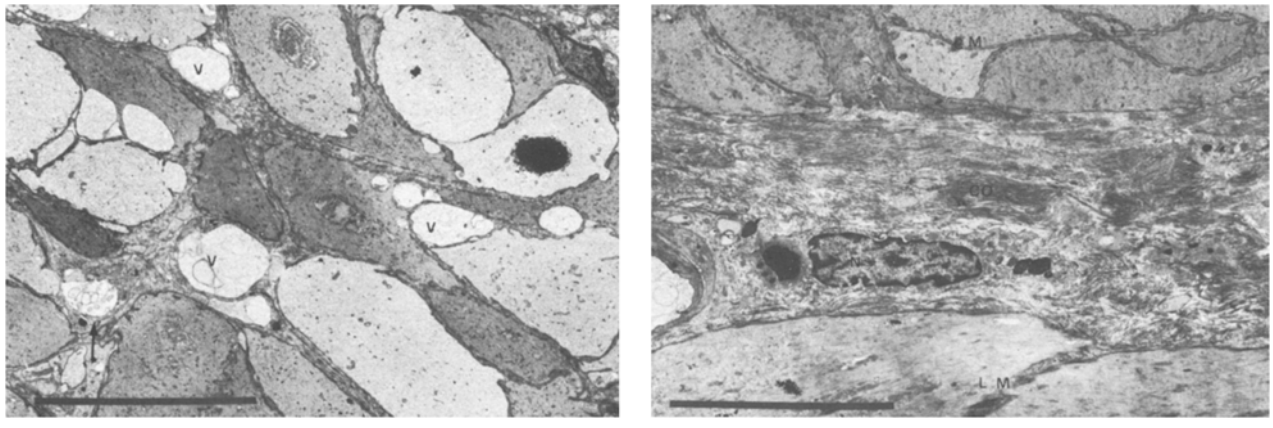

Fig. 2. *a* Electron micrograph of the circular muscle layer of the descending colon of a rat, treated with 0.1% BC solution 2 weeks priorly. Smooth muscle cells show homogenization and partial vacuolation of cytoplasm. Intercellular vacuoles (V) are also discernible at places: A degenerative nerve bundle (arrow) is seen. Bar: 10 µm. *b* Electron micrograph of the muscle layer of the descending colon of a rat, treated with 0.1% BC solution 7 months priorly. The interspace between the longitudinal muscle layer (LM) and the circular muscle layer (CM) is widened, and contains increased amount of collagen fibrils (CO) and scattered residual cells (N), probably originating from degenerative Auerbach's plexi. Cell membranes of circular muscles (CM) show a sawtoothed appearance, suggesting muscular contraction. Bar: 10 µm.

Comment and conclusion. Sato[2] applied BC solution of various concentrations to serosal surface of the descending colon of the rat, and found that application of BC solution at a concentration of 0.5% or more always caused necrosis of the intestinal wall, that application of BC solution at a concentration of 0.01% or less mostly caused no marked change in the intestinal wall, and that application of 0.1% BC solution for 30 min caused selective destruction of intestinal nervous elements. Action of cationic surfactants on living cells has been studied by various authors using various models: Kishimoto et al.[3] studied it using giant axons of the squid, Bonciocat[4] using toe extensor muscles of the frog, Green et al.[5] using cornea of the rabbit, and Futami et al.[6] using skin of the frog respectively. They generally suggest that the agent first exerts an injurious effect on cell membrane, causing its depolarization or its impairment in active sodium transport, and may result in damage of the cell per se. The reason why application of BC solution, under the specific condition used in the present experiment, selectively injured nervous elements of the intestinal wall, may be conjectured as follows. It may be that depolarizing effect of the agent expresses itself more markedly on a cell membrane having higher negative charge than on a cell membrane having lower one, and, since the level of negative charge is known to be higher in nervous tissue than in such tissues as smooth muscles[7], the former is critically injured, sparing the latter, under such specific condition of applying the agent as in this ex-

periment. On the other hand, electron microscopic findings obtained in the present study demonstrated that, at the acute stage after the BC treatment, smooth muscle cells suffered from injurious effect of the agent as did nerve cells, but that smooth muscle cells recovered fully and nerve tissues alone were irreversibly damaged at the chronic stage. Thus, it may be reasonable to suppose that the observed selective destruction of nervous elements of the intestinal wall, undergoing the BC treatment, was partly the result of poorer repairing ability of nervous tissues as compared with other tissues such as smooth muscles.

The fact that BC solution, under certain conditions, may exert considerable injurious effects on the intestinal wall is to be emphasized as a warning, since the drug is commonly used as an antiseptic in the operating room. On the other hand, the agent may be profitably utilized in research fields as a simple means of denervating certain tissues without injuring other tissue constituents.

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Radioimmunoassay of progesterone in uterine flushings of buffalo *(Bubalus bubalis)*

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Summary. The concentration of progesterone as determined by radioimmunoassay varied in accordance with the phase of ovarian activity.

The significant role played by uterine fluid in sperm capacitation, nourishment, development and implantation of embryo is well recognized. As far as qualitative and quantitative aspects are concerned, very little information was available on its ovarian steroid contents. The progesterone content of rabbit uterine fluid has very recently been shown to play some role in implantation of blastocyst[3-5]. However, no report could be found on qualitative and quantitative aspects of this hormone in the buffalo. The present investigation was a step towards screening this hormone in uterine fluid of buffaloes during various phases of ovarian activity.

Materials and methods. Sample collection: 23 genitalia from apparently healthy buffalo cows were collected from the abbatoir. All necessary precautions were made to prevent contamination of uterine fluid with any other fluid, viz. oviducal, cervical and the blood. The organs were classified into follicular, subactive and luteal phase by gross morphological examination of ovary[6]. Each horn was flushed with 20 ml normal saline and the flushings stored at $-20\,^{\circ}C$ pending analysis.

Analysis. Samples were thawed at room temperature. The fluid was centrifuged under refrigeration at 12,000 rpm for 10 min, to remove the cellular debris. The progesterone was assayed in duplicate in 0.5 ml of the supernatant by the method of Aso et al.[7]. The sensitivity of the assay in term of detection limit was 4.70 pg. The coefficient of intra assay variation carried out in the same assay using pooled uterine fluid was 17.3%. The specificity of antiserum has already been reported from this laboratory (Arora et al.[8]).

Results and discussion. The progesterone concentration during follicular, subactive and luteal phases, was observed to be 118.12 ± 6.6, 187.76 ± 23.55 and 782.50 ± 117.89 pg/ml, respectively (table).

The calculated level of progesterone is likely to be a substantial underestimate, since the recovery of the fluid was not 100%. Our values during the luteal phase are quite low compared with rabbits[3] during early pregnancy. The difference is likely to be the species difference. The source of progesterone in the uterine fluid has been shown to be the blood plasma[9]. Patek and Watson[10], however, demonstrated the synthesis of progesterone by sow endometerium. The concentration of progesterone during luteal phase was 5-8 times higher as compared to follicular phase. Batra et al.[11] reported about 5-10 times higher values of progesterone in these 2 phases during oestrous cycle in buffalo. It may therefore be concluded that the source of progesterone in uterine fluid of buffalo is also the blood plasma. At the same time, the active secretion from the endometerium cannot be ruled out.

The progesterone content has been demonstrated in pig blastocysts[12,13]. Rabbit blastocysts have also been shown to undergo steroid conversions[14]. Seamark and Lutwak-Mann[15], however, believed that the progesterone may not necessarily be synthesized by blastocysts but may be conveyed to them by endometerial secretions. The progesterone from the rabbit maternal tissue to the embryo was thought possibly to be carried with a protein, uteroglobin, where it stimulates cell divisions and blastocyt expan-

Progesterone concentration (pg/horn) in uterine fluid during various phases of ovarian activity

Follicular phase	Luteal phase	Subactive phase
96.50	435.0	71.00
108.00	506.50	74.00
120.50	575.00	105.50
130.50	742.00	119.00
111.50	1041.00	122.00
141.75	1060.00	246.00
	1107.50	269.00
		284.00
		400.00
118.12 ± 6.6	782.50 ± 117.89	187.76 ± 23.55

$\pm$ SEM.