It has also been shown that ischemia of the small intestine is accompanied by specific hormonal changes in the blood, which differ from those observed after laparotomy. This fact may evidently reflect the much greater contribution of the intestinal hormonal (enterin) [3] system to the response of the tissue to injury, which interacts very closely through numerous feedback circuits with the general hormonal system.

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NEUROTROPHIC CONTROL OF FROG SKELETAL MUSCLE AFTER PARENTERAL INJECTION OF COLCHICINE

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Disturbance of axoplasmic transport (AT) of colchicine (COL) in motor nerve fibers of mammals and amphibians leads to the appearance of denervation-like changes in the skeletal muscle without any disturbance of neuromuscular transmission or exclusion of the muscle from motor activity [1, 4]. This suggests that substances (trophic factors), carried to the muscle by AT [6], participate in the mechanism of neurotrophic control of skeletal muscle fibers. Meanwhile investigations have shown that denervation-like changes also develop in mammalian muscles in direct contact with COL. In this case AT in motor nerve fibers is not appreciably affected [5, 7]. It can therefore be tentatively suggested that the development of the denervation-like syndrome in muscle fibers in experiments with COL is not necessarily the result of a disturbance of AT but may be the result of the direct action of the alkaloid on the muscle [5, 7]. However, the experiments described above did not solve the problem of how neuromuscular transmission is altered under these circumstances, and at the same time, we know that disturbance of neuromuscular transmission can lead to the appearance of changes of a denervation type in muscle [6].

Consequently, in experiments on frogs in which denervation changes in muscle fibers differ in certain respects from those in warm-blooded animals [8], it is interesting to study whether COL, through direct contact with the muscle, can cause the appearance of denervation-like changes in the muscle fibers and whether the character of myoneural transmission is altered under these circumstances.

The aim of the present investigation was to study the functional state of the pre- and postsynaptic membranes of the neuromuscular synapse after parenteral injection of COL into frogs.

EXPERIMENTAL METHOD

Experiments were carried out on a nerve–muscle preparation of the sciatic nerve and sartorius muscle of frogs (Rana ridibunda and Rana temporaria) in the winter period, using a standard microelectrode technique. Frogs of the experimental group received an injection of 0.1 ml of a 10 mM solution of COL (from Merck, West Germany), made up in Ringer's solution, into the dorsal lymph sac. The dose used is equivalent to the

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TABLE 1. MP, Electrical Properties, Zone and Level of Maximal Sensitivity of Postsynaptic Membrane to ACh, and Frequency of MEPPs of Frog Sartorius Muscle Fibers 13-15 Days after Injection of 0.1 ml of 10 mM COL Solution or Some Volume of Ringer's Solution without COL into Lymph Sac (M ± m)

Experimental conditions	MP, mV	R_{in}, kΩ	ε, msec	Zone of sensitivity to ACh, mm	Maximal sensitivity to ACh, mV/nC	Frequency of MEPPs, Hz
Control (injection of Ringer's solution)	86±0,8 (58)	456±30 (24)	18,2±0,9 (24)	350±50 (5)	23,6±9,3 (5)	0,34±0,115 (20)
Injection of COL	78±1,6* (65)	418±20 (36)	18,1±0,8 (36)	1600±120* (11)	32,7±7,3 (11)	0,24±0,03 (21)

Legend. Number of observations shown in parentheses; *P < 0.05 compared with control.

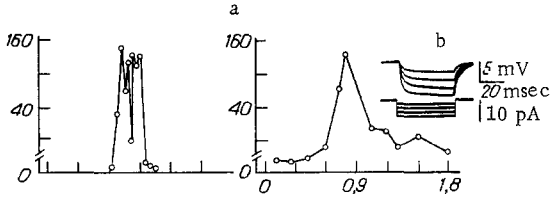

Fig. 1

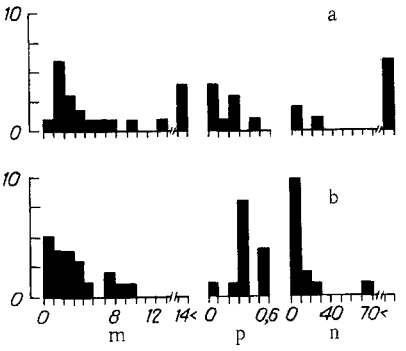

Fig. 2

Fig. 1. Sensitivity to ACh and input resistance of postsynaptic membrane of frog sartorius muscle fibers. a) Control, b) on 13th-15th day after injection of COL into dorsal lymph sac. Abscissa, distance along fiber (in mm); ordinate, sensitivity (in mV/nC). Calibration in millivolts and picoamperes.

Fig. 2. Distribution of myoneural synapses of frog sartorius muscle based on m value of EPPs. a) Control (24 observations); b) on 13th-15th day after injection of COL into dorsal lymph sac (21-23 observations). Abscissa, value of m; ordinate, frequency of sign.

amount of COL applied to the nerve in order to disturb AT [1]. The same volume of Ringer's solution without COL was injected into frogs of the control group. The animals were used in the experiments 2 weeks after the injection, when denervation changes in the muscle after division of the nerve supplying it or after application of COL to the nerve were already pronounced [1]. All animals were kept at room temperature.

The zone and magnitude of maximal sensitivity of single nerve fibers to acetylcholine (ACh) were measured by microelectrophoresis [7, 8]. The input resistance and time constant of the electrogenic membrane [1, 8] of the muscle fibers were determined by means of two microelectrodes by the voltage drop method. The quantum composition (m) of end-plate potentials (EPPs) was determined by the direct method [3]. For this purpose, 200 miniature EPPs (MEPPs) and also 200 EPPs during stimulation of the nerve with a frequency of 0.2 Hz were recorded in succession.

During the experiment the muscle was kept in a transparent plastic bath containing Ringer's solution of the following ionic composition (in mM): NaCl 115, KCl 2.5, $CaCl_2$ 1.8, in phosphate buffer, pH 7.2. The rate of flow of the fluid through the bath was 4 ml/min. To record EPPs uncomplicated by an action potential and contraction of the muscle [3], Mg^{++} ions (4 mM) were added to the Ringer's solution and the Ca^{++} ion concentration was correspondingly reduced (0.75 mM). MEPPs and EPPs were recorded at 19°C and the postsynaptic membrane study was carried out at room temperature.

EXPERIMENTAL RESULTS

The membrane potential (MP) of the sartorius muscle fibers 2 weeks after injection of COL into the dorsal lymph sac of the frog was significantly lower than in the control on average by 8 mV (Table 1). The

input resistance and time constant of the electrogenic membrane of the muscle fibers following injection of COL were the same as in the control (Fig. 1, Table 1). Besides the fall in MP, 2 weeks after injection of COL the zone of postsynaptic sensitivity in the muscle fibers to ACh was appreciably widened, but there was no change in the level of maximal sensitivity (Fig. 1, Table 1).

The frequency of MEPPs in sartorius muscle fibers 2 weeks after injection of COL did not differ statistically significantly from the control values (Table 1).

Meanwhile the number of synapses with lower m values of their EPPs was increased in the muscle (Fig. 2). In the intact muscle, for instance, the number of synapses with low m values (under 5 quanta) was 59% and on the 13th-15th day after injection of COL they already accounted for 77% of the total number of fibers tested.

The experiments showed that injection of COL into the dorsal lymph sac of the frog causes a fall in MP and the appearance of extrasynaptic sensitivity to ACh, but the electrical properties of the plasma membrane are unchanged. Injection of COL into the frog thus gives rise to basically the same effects as surgical denervation of muscle fibers [7, 8]. This may be the result both of the direct action of COL on the muscle and its indirect action through nerve endings, for COL is an agent which affects neurosecretory processes [9].

In fact, in the present experiments the appearance of signs of the denervation syndrome in the muscle fibers was accompanied by a change in functional state of the myoneural synapses. For instance, the number of synapses in the muscle with lower m values of their EPPs was increased. A change in the functional state of myoneural synapses in the experiments with COL may perhaps play an important role in the mechanism of disturbance of neurotrophic control of skeletal muscle fibers, for disturbance of neuromuscular transmission, by botulinus toxin for example, causes the appearance of denervation-like changes in frog muscle [10]. Although the results of the present experiments do not rule out the probability of appearance of denervation-like changes in muscle fibers as a result of the direct action of COL on them, nevertheless the simultaneous change in the functional state of the myoneural apparatus points to the possibility that the phenomena observed may have a different interpretation.

It was shown previously that local isolated application of COL to a motor nerve modifies the properties of the muscle fibers in the same way as division of the motor nerve, but without disturbing transmission of excitation from nerve to muscle [2]. Under those circumstances denervation-like changes do not arise in the contralateral muscles of the experimental animals, evidence against a direct action of the alkaloid on the muscle.

These arguments support the view that in the present experiments with parenteral injection of COL the appearance of denervation-like changes in the muscle takes place as a result of the action of the alkaloid mainly on nerve endings and not directly on the muscle, in good agreement with data in the literature [1, 2, 4].

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