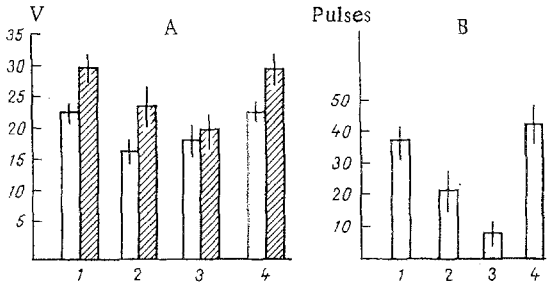

Fig. 2. Effect of L-dopa (150 mg/kg) and apomorphine (5 mg/kg) 30 min after injection on thresholds of emotional responses and orienting motor activity. Ordinate: number of pulses. A) Unshaded columns show threshold of emotional reactivity, shaded columns threshold of aggressiveness (in V); B) ordinate, number of pulses; 1) control; 2) apomorphine (5 mg/kg); 3) L-dopa (50 mg/kg); 4) apomorphine + L-dopa.

when an increased level of functionally active mediator depresses the activity of postsynaptic receptors sensitive to it, as a result of which the effects both of dopamine and of the stimulator of dopamine receptors (apomorphine) are reduced.

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METABOLISM OF NITRAZEPAM IN THE INTESTINE OF ALBINO RATS

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In the rat intestine nitrazepam is transformed to an amine and acetamide. In the duodenum and small intestine the reduction of nitrazepam and its subsequent acetylation are catalyzed by enzymes in the mucosa. In the cecum and large intestine these processes are due to the action of the microflora and tissue enzymes, and in the rectum to the action of the microflora alone.

KEY WORDS: *metabolism of nitrazepam; intestine; antibiotics.*

The pathways of metabolism of nitrazepam have been studied in the liver [6] but not in the intestine, where this substance may have its pharmacological properties modified by the

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action of the intestinal contents. The object of this investigation was to study the role of enzymes in different parts of the intestine and their microflora in the transformation of nitrazepam.

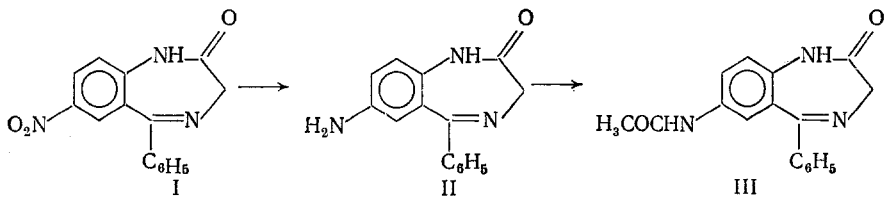
EXPERIMENTAL METHOD

The experiments were carried out on male Wistar albino rats weighing 200-250 g. An intraperitoneal injection of medinal was given to the animals in a dose of 400 mg/kg and, when they had fallen asleep, they were fixed to a frame, laparotomy was performed, and a certain part of the intestine was removed. In all the experiments the part of the intestine isolated was of the same size. At one end of the segment of intestine a ligature was tied, and a cannula was introduced into the other end, through which 1 ml of a Tween emulsion of nitrazepam (10 mg/kg) in physiological saline was introduced. The rats were divided into five groups depending on the portion of intestine studied. After 60 min the segment of intestine was removed, weighed, and homogenized. Nitrazepam and its possible metabolites were synthesized in the writers' laboratory [1]. The compounds were extracted from homogenates of the intestine and separated by the method described earlier [3]. The metabolites were identified by a combination of thin-layer chromatography, UV spectroscopy, and mass spectrometry [2]. The UV spectra were recorded in ethyl alcohol on the Specord UV-vis spectrophotometer. The mass spectra of metabolites eluted from the chromatograms were obtained on the MKh-1303 instrument. For quantitative analysis of the compounds the corresponding spots on the chromatograms were dissolved in 4 ml ethyl alcohol and the optical density was measured on the SF-16 spectrophotometer (nitrazepam at 260 nm, the amine at 240 nm, and acetamide at 248 nm). The quantity of the substances was determined from calibration curves with a correction for the degree of their extractability from the biological samples. The intestinal microflora was inhibited by oral administration of the antibiotics oleandomycin (125 mg/kg), tetracycline (100 mg/kg), and nystatin (100 mg/kg) four times a day for three days. The choice of this combination of antibiotics was made on the basis of counts of the colonies of microorganisms after seeding from the intestinal contents.

EXPERIMENTAL RESULTS

Three compounds were found 60 min after injection of nitrazepam into the duodenum, small intestine, cecum, large intestine, and rectum by thin-layer chromatography from chloroform extracts. The first substance (R_f = 0.75) had absorption maxima in the UV spectrum at 220, 260, and 310 nm. For the second compound (R_f = 0.38) λ_{max} was 220 and 240 nm. In the mass spectrum a peak of a molecular ion with m/e = 251 (100) and peaks of fragmentary ions with m/e values of 252 (11), 250 (21), 223 (49), 222 (50), and 69 (5.0) were observed. The third compound (R_f = 0.28) had λ_{max} 230, 248, and 320 nm. The peaks of a molecular ion with m/e 293 (100) and peaks of fragmentary ions with m/e values of 294 (10), 292 (30), 266 (5.0), 265 (62), 264 (30), 223 (8), 222 (12), and 69 (8) were observed in the mass spectrum.

Comparison of the R_f values for the metabolites discovered with the corresponding properties of the synthesized compounds, their color after irradiation of the chromatograms with UV light with a wavelength of 253.7 nm, and also the results of UV spectroscopy and mass spectrometry enables these substances to be identified as nitrazepam (I), an amine (II), and acetamide (III).



The content of the amine and acetamide formed from nitrazepam in different segments of the intestine is given in Table 1. The intensity of reduction of the original compound in the duodenum and small intestine was about equal. The amine was present in significantly larger amounts in the cecum and large intestine. The highest level of this metabolite was found in the rectum. A similar picture also was found with the acetamide. Whereas the high content of the amine in the cecum and rectum, and also the large intestine, could be attributed to the activity of nitroreductases, the increased level of the acetamide in these seg-

TABLE 1. Content of Amine and Acetamide in Isolated Segments of Albino Rat Intestine under Normal Conditions (N) and after Administration of Antibiotics (A) (M ± m)

Metabolite	Content of nitrazepam metabolites, μg/g intestine									
	duodenum (n=6)		small intestine (n=6)		cecum (n=6)		large intestine (n=6)		rectum (n=6)	
	N	A	N	A	N	A	N	A	N	A
Amine	13,3±2,1	15,7±4,5	8,3±1,8	10,1±3,7	35,0±10,4	13,3±1,6	29,8±12,1	1,8±0,7	53,0±22,3	Trace
Acetamide	6,2±1,5	9,6±2,5	6,1±1,2	5,4±0,2	13,8±4,1	5,0±0,7	8,3±2,4	0,7±0,1	49,0±19,4	"

ments of the intestine was evidently attributable not only to acetylase activity, but also to the high concentration of the amines, acting as the substrate for these enzymes.

In all segments of the intestine studied the amine predominated, whereas in the liver larger quantities of acetamide are found [3]. This can evidently be explained by differences in the activity of the hepatic and intestinal acetylases and to differences in the pattern of competition between acetylases and deacetylases in different tissues.

Administration of the antibiotics to the rats had virtually no effect on nitrazepam metabolism in the duodenum and small intestine (Table 1). Meanwhile, in the cecum and large intestine the content of the amine and acetamide was changed. Depression of the microflora was reflected especially in metabolism of the original compound in the rectum, where only traces of the amine and acetamide were found. In all probability the antibiotics inhibited the action only of microorganisms reducing the nitro compound, for the amine/acetamide ratio under normal conditions and after administration of the antibiotics remained constant in the cecum and small intestine. Absence of the acetylation substrate in the rectum after administration of the antibiotics prevented this rule from being demonstrated in this segment of the intestine. Usually administration of antibiotics to experimental animals leads to an increase in the absorption of natural compounds in the intestine [4]. It may therefore be postulated that the decrease in the content of nitrazepam metabolites was due to this factor. Blood analysis in additional experiments showed no difference in the content of these compounds under normal conditions and after administration of antibiotics.

Considering that the microflora in rats is uniformly distributed along the whole of the digestive tract [5] it can be concluded that the decrease in the contents of the amine after administration of antibiotics to the animals was due not only to quantitative changes in the microorganisms, but also to a disturbance of their relations with the tissue enzymes of the intestine, performing analogous functions.

The reduction of nitrazepam and its subsequent acetylation in the duodenum and small intestine of rats are thus catalyzed mainly by enzymes of the mucosa. In the cecum and large intestine these processes are due to the action of the microflora and tissue enzymes, but in the rectum to the microflora only.

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