Some workers state that types of primary targets for the action of PG can be limited, and as the most probable target they suggest an examination of macrophages [4]. The results given in this paper are evidence that the action of PG, isolated from *L. bulgaricus*, on TAG expression in developing T lymphocytes is mediated through the complement system.

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DISTURBANCE OF CARBOHYDRATE METABOLISM IN EXPERIMENTAL SECRETORY DIARRHEA INDUCED BY CHOLERA TOXIN

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Injection of cholera toxin (CT) into the lumen of the small intestine induces intensive secretion of electrolytes and water, mediated through elevation of the cAMP level in the mucous membrane [7]. It has also been shown that cholerogen and agents increasing the intracellular cAMP concentration potentiate the Na-dependent transport of glucose or its unmetabolized analogs, both in vivo and in vesicles of enterocyte apical membranes [14]. This is facilitated by the fact that the mucous membrane of the small intestine has well marked ability not only to transport and metabolize glucose in the intestinal lumen, but also to recirculate sugars [1]. One pathway of carbohydrate utilization in the mucous membrane of the small intestine is the synthesis of the glycoproteins of mucin [5, 16], whose secretion is sharply intensified under the influence of CT [9].

On the basis of these data it can be postulated that cholera enterotoxin causes increased utilization of glucose in the mucous membrane of the small intestine on account of the use of an intermediate glycolysis product (fructose-6-phosphate) for synthesis of glucosamine — one of the important components of mucin. We previously reported that secretory diarrhea developing in the rabbit small intestine under the influence of CT is accompanied by stimulation of gluconeogenesis in the liver [2]. It is possible that the increased consumption of glucose by the small intestine is compensated by increased glucose production by the liver. The aim of this investigation was to test the above hypothesis.

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TABLE 1. Effect of CT on Secretion of Hexosamines of Mucin, Lactate Concentration in Mucous Membrane, and Accumulation of Fluid

Experimental conditions	Accumulation of fluid, ml/cm	Secretion of hexosamines, μg/cm	Lactate, μmoles/g tissue
Intact animals (mock operation)	—	22,2±1,8 (4)	13,0±1,3 (4)
Experimental loop (CT – 100 μg per loop)	0,97±0,12 (6)	66,2±1,3 (6)	10,5±0,8 (7)
Control loop (1 ml of physiological saline)	—	Not determined	10,1±0,6 (7)

Legend. Number of determinations given in parentheses.

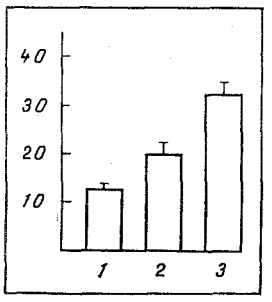

Fig. 1. Effect of CT on glucose utilization (in mg %) in rabbit small intestine. 1) Arteriovenous difference in glucose concentration in isolated loop of small intestine of intact animals; 2) neighboring isolated loops, not treated with toxin, 3) isolated loop of small intestine treated with CT.

EXPERIMENTAL METHOD

CT (Calbiochem, USA) was injected in situ into an isolated loop of the jejunum of rabbits deprived of food for 48 h, in a dose of 100 μg per loop, and 1 ml of physiological saline was injected into a neighboring loop. After 3.5 h, under general anesthesia, blood samples were taken from the mesenteric veins of the animals, draining both the experimental and the control loops, and arterial blood samples were taken from the left ventricle.

The glucose concentration was determined by the orthotoluidine method. The contents of the experiment loop were collected and the loop was washed out with 4 ml of Ca^{++}-free phosphate buffer (pH 7.5). The pooled samples were covered with an equal volume of 20% TCA, containing 2% phosphotungstic acid. The contents of the control loop were collected in the same way. After incubation for 18 h at 4°C the samples were centrifuged and the residue washed 3 times with 10% TCA, containing 1% phosphotungstic acid, after which the residues were hydrolyzed in 2 ml of 6 N HCl for 4 h at 90°C. Isolation and spectrophotometric determination of hexosamines were carried out by the method in [5]. The lactate concentration was measured by the method in [3] in homogenates of mucous membrane made up in Tyrode solution. The cAMP concentration was determined in samples of liver after alcoholic extraction, using kits from Amersham International (England). The rate of gluconeogenesis in the liver homogenates was measured as described previously [2].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that CT induced considerable accumulation of fluid, which contained large quantities of mucin, in the isolated loop of the rabbit jejunum, Secretion of

TABLE 2. Effect of Intraluminal Injection of CT on Rate of Gluconeogenesis and cAMP Concentration in the Liver

Experimental conditions	Glucose, μmoles/g tissue/45 min	cAMP, pmoles/g tissue
Control	4,93±0,68	120±10
CT	8,35±0,39	133±17

TABLE 3. Effect of CT, Injected into Lumen of Small Intestine, on Glucose Concentration in Blood

Experimental conditions	Glucose concentration, mM	
	after 0 min	after 210 min
Control	4,6±0,2	6,2±0,2
CT	4,4±0,3	9,6±0,6

one of the principal components of glycoproteins, namely hexosamines (90% of which is accounted for by glucosamine [7]), was increased by 2.98 times. The lactate concentration in the mucous membrane of the experimental loops fell below that in intact animals and did not differ from the control.

Secretion in the small intestine was accompanied by a considerable increase in the arteriovenous difference in glucose concentration. It was 2.46 and 1.6 times higher, respectively, than in intact animals and in the control (Fig. 1). The difference between the glucose consumption in the control loop and in the intact animals can be explained on the basis of data in Fig. 1. After the action of CT for 3.5 h the glucose level in arterial blood in the mucous membrane of the small intestine rose by 65%.

Measurements of the equilibrium velocity of glucose synthesis, conducted in vitro on liver homogenates, showed that gluconeogenesis in the experimental animals was intensified in these preparations by 60% (Table 2), compared with the control. Under these circumstances the cAMP level in the liver was unchanged.

The hypothesis put forward at the beginning of this paper to the effect that glucose consumption for glucosamine synthesis is intensified in tissue of the small intestine, treated with CT, is confirmed by the data given in Fig. 1. An increase in the arteriovenous difference in glucose concentration may in fact be evidence in support of this view, provided that the blood flow in the tissue is not reduced. It was shown previously [9] that CT potentiates the total blood flow in the wall of the small intestine on account of an increase in blood supply in the mucous membrane and a decrease in the submucosa and muscular coat. The difference in glucose consumption between the experimental and control loops, according to our data, was 12 mg %.

The blood flow in the wall of the rabbit small intestine is known to be about 50 ml/min/100 g tissue [11]. Approximate calculations, based on these data, show that the loop treated with CT extracted from the blood stream 0.18 mg/min more glucose than the neighboring segments of intestine, not treated with toxin. Glycoprotein synthesis in the small intestine under these conditions is evidently the most significant consumer of the utilized glucose, for one of the intermediate products of glycolysis, namely fructose-6-phosphate, is incorporated into the reaction of glucosamine synthesis, limiting further assembly of glycoproteins [16]. Glycolysis itself can hardly play an appreciable role here, for equilibrium between glycolysis and gluconeogenesis must be shifted toward the latter, since fructose-1,6-diphosphatase activity increases under the influence of CT simultaneously with inhibition of pyruvate kinase [15]. Moreover the concentration of lactate, the end product of glycolysis, in the mucous membrane does not rise (Table 1).

The difference in glucose consumption in the control loop and in intact animals undergoing a mock operation can be explained on the grounds of elevation of the blood glucose level under the influence of the toxin (Table 3).

In the present experiments the animals were starved for 48 h before the operation, and this must have led to considerable (up to 90%) emptying of the glycogen depots in the body [4].

Consequently, under these conditions an increase in the glucose concentration in the blood can be linked only with the more rapid synthesis of glucose in the body. The rate of gluconeogenesis in the liver, as follows from Table 2, rose by 60%. Glucose synthesis in the liver can be stimulated by both cAMP-dependent and Ca-dependent groups [4, 13]. Moreover, regulation by substrate accessibility also is possible [12]. In the present case stimulation of glucose synthesis in the liver took place by a non-cAMP-dependent route (Table 2).

On the basis of the above arguments the following scheme can be proposed for changes in carbohydrate metabolism under the influence of CT: intensive secretion of mucin, developing in response to injection of the enterotoxin into the lumen of the small intestine, is largely maintained by substrates on account of glucose utilized from the blood stream, where its concentration is raised, evidently due to acceleration of gluconeogenesis in the liver. The method of regulation of these mutual relations between organs is unknown in this case, and we can only postulate the participation of certain factors released into the blood stream in the small intestine in response to the action of CT, and capable of stimulating gluconeogenesis in the liver by a cAMP-independent pathway.

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