also to Candidate of Chemical Sciences V. K. Mazo, of the Institute of Nutrition, Academy of Medical Sciences of the USSR, for his useful discussion of the experimental results.

LITERATURE CITED

1. Z. M. Gadzhieva and I. Ya. Kon', Byull. Éksp. Biol. Med., No. 1, 108 (1984).
2. I. Ya. Kon', Theoretical and Clinical Aspects of the Science of Nutrition. Vol. 4, Current Problems in Vitaminology [in Russian], Moscow (1983), p. 77.
3. I. Ya. Kon', L. Sh. Gorgoshidze, O. N. Vasil'eva, and S. N. Kulakova, Biokhimiya, No. 1, 70 (1986).
4. V. K. Mazo, V. A. Konyshev, and V. A. Shaternikov, Vopr. Pitan., No. 4, 3 (1982).
5. L. A. Mikhailov, I. V. Gmoshinskii, V. K. Mazo, and V. A. Shaternikov, Byull. Éksp. Biol. Med., No. 6, 656 (1985).
6. I. A. Morozov, S. I. Khvylya, and Yu. A. Lysikov, Fiziol. Zh. SSSR, No. 9, 1261 (1982).
7. J. Pollard, A Handbook of Numerical and Statistical Techniques, Cambridge (1977).
8. V. B. Spirichev and I. Ya. Kon', Zh. Vses. Khim. Ova. im. Mendeleeva, No. 4, 425 (1978).
9. M. Anzano, A. J. Lamb, and J. A. Olson, J. Nutr., 109, 1419 (1979).
10. L. M. de Luca, Vitam. Horm., 35, 1 (1977).
11. R. Meeks, D. Zaharevitz, and R. F. Chen, Arch. Biochem., 207, 141 (1981).
12. M. S. Reinhardt, Ann. Allergy, 53, No. 6, Pt. 2, 597 (1984).
13. O. A. Roels, O. R. Anderson, N. S. T. Lui, et al., Am. J. Clin. Nutr., 22, 1020 (1969).
14. W. A. Walker and K. J. Bloch, Ann. Allergy, 51, No. 2, Pt. 2, 240 (1983).

EXPERIMENTAL DIABETES IN MICE INFECTED WITH COXSACKIE VIRUSES

E. F. Bocharov, Yu. P. Shorin,
I. A. Solodovnikova, L. S. Kazaryan,
V. G. Selyatitskaya, and N. A. Pal'chikova

UDC 616.379-008.64-092.9-06:
616.98:578.835.17

KEY WORDS: Coxsackie A and B viruses; pancreas.

It has been shown that virus infections, together with heredity and autoimmune disturbances, can contribute to the development of insulin-dependent diabetes. The presence of virus-neutralizing antibodies in high titers in the early stages of the disease [1, 2], the discovery of specific antigens in the islets of Langerhans [3], isolation of viruses causing a diabetes-like syndrome in experimental animals from the pancreas of dying patients [5, 6] — all these and other factors confirm the parts played by virus infections in the development of the disease.

Among viruses that are most frequently stated to be a possible cause of human diabetes, Coxsackie viruses of the B group may be mentioned. These viruses can cause a disease resembling diabetes in genetically predisposed strains of mice. In some resistant strains of mice the disease develops after preliminary injection of subdiabetogenic doses of streptozocin into the animals [4]. No information could be found in the literature on the role of infection by Coxsackie A virus in the pathology of the pancreas.

The aim of this investigation was to compare the effect of Coxsackie B4 and A13 viruses on the pancreas of strains of mice sensitive and resistant to diabetes, using subdiabetogenic doses of aloxan in the second case.

EXPERIMENTAL METHOD

DBA/2 and (CBA × C57Bl/6)F_1 mice aged 3-4 months were used in the experiments. Altogether 247 animals were used. Male DBA/2 (sensitive) mice were infected with Coxsackie B4 or A13

Laboratories of Virology and Endocrinology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. I. Borodin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 2, pp. 163-166, February, 1987. Original article submitted July 28, 1986.

 0007-4888/87/0002-0182$12.50

viruses and investigated on the 7th and 21st days after infection. Male and female (CBA × C57Bl/6)F_1 mice resistant to virus-induced diabetes, and also female DBA/2 mice, less susceptible to diabetes than males, were subjected to a twofold procedure: preliminary injection of subdiabetogenic doses of alloxan (5 mg/100 g body weight), and 7 days later, injection of the virus. To infect the animals, prototype strains of Coxsackie A13 (Flores) and Coxsackie B4 (Povers) viruses were used. Coxsackie A13 virus was subcultured on human embryonic fibroblasts (HEF) in a titer of 5.5 log TCD_{50}/0.1 ml, and Coxsackie B4 virus on a culture of HEp-2 cells in a titer of 4.5 log TCD_{50}/0.1 ml. The animals were infected intraperitoneally with 0.2 ml of the virus. To determine the presence of virus antigens in the heart and pancreas, sections were cut on a freezing microtome and examined by the indirect immunofluorescence method (IF assay), using type-specific sera to Coxsackie A13 and B4 viruses and an antispecific luminescent serum.

The biochemical investigation included determination of immunoreactive insulin (IRI) in the blood serum by radioimmunoassay using standard kits from CEA IRE Sorin (France) and determination of the blood sugar in the fasting state and 30, 60, and 120 min after glucose loading (the glucose tolerance test — GTT). A single dose of glucose was given: 2 mgβlg body weight in the form of a 40% solution. The glucose was injected intraperitoneally, and the animals were exsanguinated after the specified time intervals by decapitation, after which the blood sugar was determined by the orthotoluidine method.

EXPERIMENTAL RESULTS

The results of the GTT on male DBA/2 mice showed differences between the experimental groups of mice. In mice infected with viruses a decrease in glucose tolerance was observed both in the acute stage of the infection (7th day) and on the 21st day of infection. Compared with mice infected with Coxsackie A13 virus, higher glucose levels both in the fasting state and throughout the test were found in DBA/2 mice infected with Coxsackie A13 mice (Fig. 1). At the same time it must be noted that on the 21st day of the investigation the GTT levels were somewhat higher in male DBA/2 mice infected with Coxsackie A13 virus than on the 7th day of the investigation, possible evidence of the change to a chronic stage of the pathological process associated with Coxsackie A13 virus, but this requires further study.

Besides a decrease of tolerance to glucose in the male DBA/2 mice infected with Coxsackie A13 and B4 viruses, high plasma levels of IRI were observed, both in the fasting state and during the GTT (Fig. 1).

Lowering of the glucose tolerance and the appearance of large quantities of IRI in the blood are characteristic of the development of the initial stages of diabetes in man and are evidently connected with secretion of proinsulin or insulin with modified biological activity. In the virological investigation (Table 1) Coxsackie A13 and B4 viruses were isolated from the heart and pancreas only in the acute stage (7th day). Higher titers of these viruses (2.75-3.25 log TCD_{50}/0.1 ml) were obtained in the pancreas than in the heart. Coxsackie B4 virus was isolated from these organs somewhat more frequently and in higher titers than Coxsackie A13 virus.

The results of the virological investigation correlated with those of IR assay. Antigens of Coxsackie A13 and B4 viruses were discovered in the organs tested, only on the 7th day (Table 1); Coxsackie B4 virus, moreover, was found in the pancreas rather more frequently than Coxsackie A13 virus. The character of arrangement of the virus antigens in cells of the organs was similar in the case of infection by both viruses. Specific luminescence was found in the pancreas in cells of the vascular endothelium and in the cytoplasm of single cells in both the exocrine and the endocrine part of the gland. Fluorescence of regions of muscle fibers and of small groups of cells located near blood vessels was found in the heart.

Alloxan, like streptozocin, is a specific toxin for β-cells. Partial damage to β-cells by subdiabetogenic doses of this preparation lowers the resistance of mouse strains that are insusceptible to the virus. This may be connected with reduction in the size of and injury to the remaining islets by the virus, which causes overstrain on the insular apparatus and disturbs insulin synthesis.

Administration of subdiabetogenic doses of alloxan to mice of the resistant (CBA × C57Bl/6)F_1 line and of DBA/2 females, which unlike males are insusceptible to virus-induced diabetes, followed by injection of the virus (7 days later), caused the development of overt diabetes in these animals. They became apathetic and disinclined to move, developed thirst, and their

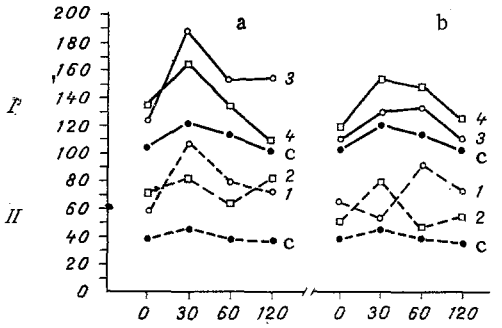

Fig. 1. Glucose concentration (continuous line) and IRI level (broken line) during GTT on male DBA/2 mice infected with Coxsackie B4 (a) and Coxsackie A13 (b) viruses. Abscissa, time (in min); ordinate: I) blood glucose (in mg/100 ml); II) IRI (in μU/ml). C) Control; 1) IRI on 7th day after infection, 2) the same, on 21st day after infection, 3) glucose concentration on 7th day after infection, 4) the same, on 21st day of infection.

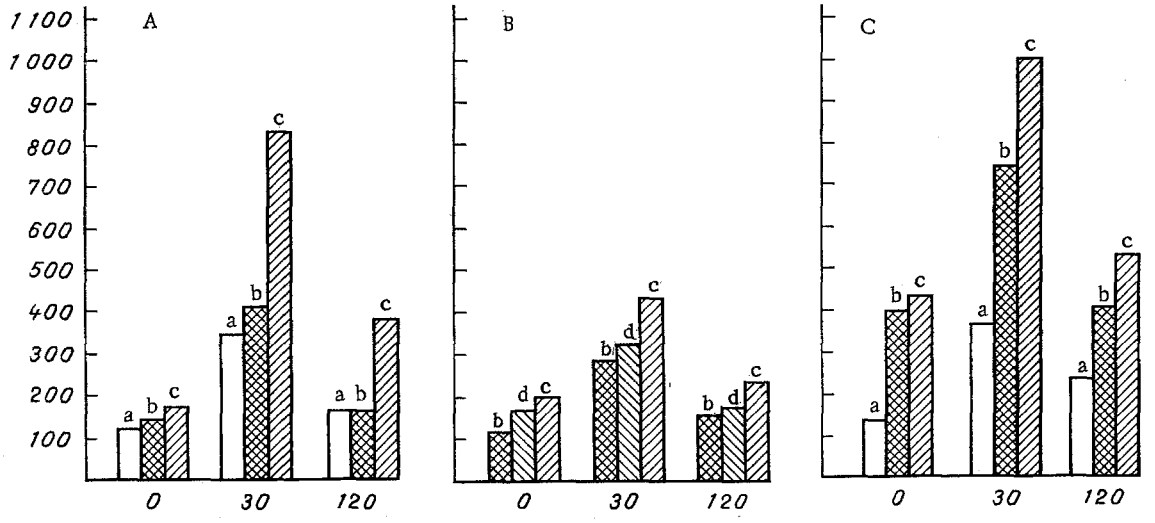

Fig. 2. Blood glucose levels 30 and 120 min after glucose loading in male (CBA × C57Bl/6)F_1 mice infected with Coxsackie A13 virus (A), and Coxsackie B4 virus (B) and in female DBA/2 mice infected with Coxsackie A13 virus (C), 7 days after injection of alloxan. Abscissa, time (in min); ordinate, blood glucose (in mg/100 ml). Columns: a) control; b) alloxan; c) virus + alloxan; d) virus.

body weight was lower than that in the control. The clearest clinical picture was obtained in male (CBA × C57Bl/6)F_1 mice and female DBA/2 mice, infected with Coxsackie A13 virus. In male (CBA × C57Bl/6)F_1 mice infected with this virus after receiving subdiabetogenic doses of alloxan, a considerable rise of the glucose level was observed 30 min after loading (819 ± 102 mg%), which remained quite high (368 ± 97 mg%) by the end of the test (Fig. 2a). In mice receiving alloxan alone, deviations from the results of the GTT obtained in the control were not significant. Values of the GTT test in DBA/2 females in the group of animals infected with Coxsackie A13 virus preceded by injection of alloxan, were significantly higher than in the group of animals receiving alloxan alone, and significantly higher than the control values (Fig. 2c). The maximal glucose concentration in the animals of this group was 992 ± 108 mg% after 30 min and 505 ± 108 mg% after 120 min, compared with an initial level of 298 ± 82 mg% ($P < 0.05$).

A different picture was observed in female and male (CBA × C57Bl/6)F_1 mice infected with Coxsackie B4 virus. In the animals of this group the GTT parameters did not differ significantly from the controls (Fig. 2b). Compared with females, a tendency was observed in the

TABLE 1. Results of Virological and IF Assay during Investigation of Male DBA/2 Mice Infected with Coxsackie A13 and B4 Viruses

Material tested	Time after infection, days	Coxsackie A13		Coxsackie B_4	
		IF assay	titers of virus, log TCD_{50}/ml	IF assay	titers of virus, log TCD_{50}/ml
Pancreas	7	5/10	2,75	7/10	3,25
	21	0/10	—	0/10	—
Heart	7	4/10	1,25	4/10	2,25
	21	0/10	—	0/10	—

Legend. Numerator indicates number of animals with positive findings, denominator — total number of animals tested. Average titers of virus given for these animals; —) virus not isolated.

TABLE 2. Results of Virological and IF Assay during Investigation of (CBA × C57Bl/6)F_1 Mice and Female DBA/2 Mice

Strain of mice	Experimental conditions	Mouse No.	IF assay of pancreas	Titers of virus in pancreas, log TCD_{50}/ml
F_1 (CBA × ×C57Bl/6): males	Coxsackie B4	1	+	2,5
		2	—	+
		3	—	—
		4	+	2,5
		5	—	—
	Coxsackie B4 + alloxan	1	—	—
		2	+	2,25
		3	+	1,25
		4	—	—
		5	—	—
		6	+	—
	Coxsackie A13 + alloxan	1	+	2,25
		2	—	—
		3	—	—
		4	+	2,5
		5	+	2,25
		6	—	—
Females	Coxsackie B4	1	—	—
		2	—	—
		3	+	1,75
		4	—	—
	Coxsackie B4 + alloxan	1	+	—
		2	—	—
		3	—	—
		4	—	—
DBA/2: females	Coxsackie A13 + alloxan	1	—	—
		2	+	2,5
		3	+	1,75
		4	—	+

Legend. —) Neither virus antigen nor cytopathic agent was found.

males for the glucose level to be higher in the fasting stage and 30 min after glucose loading.

The results of the virological and IF assay of mice of the resistant strain given in Table 2 show that virus antigens were found just as often in animals previously treated with

alloxan and in animals infected with the virus only. The isolation rate of Coxsackie A13 virus in animals of this group was rather higher than in the case of Coxsackie B4 virus. The character and localization of fluorescence were the same as in DBA/2 males. The results of IF assay and virological investigation correlated with each other.

The study of the diabetogenic properties of Coxsackie P′ and A13 viruses in mice of sensitive and resistant strains, with the use of subdiabetogenic doses of alloxan in the latter case thus revealed definite biochemical changes, expressed as lowered glucose tolerance and disturbance of IRI synthesis. The most marked biochemical changes were observed in male DBA/2 mice infected with Coxsackie B4 virus, in (CBA × C57B1/6)F_1 mice, and in DBA/2 females infected with Coxsackie A13 virus.

LITERATURE CITED

1. H. Champsaur, E. Dussaix, F. Samolyk, et al., Lancet., 1, 251 (1980).
2. D. R. Gamble, M. L. Kinsley, M. G. Fitzgerald, et al., Br. Med. J., 3, 627 (1969).
3. R. Gladisch, W. Hofmann, and R. Waldherr, Z. Kardiol., 65, 827 (1976).
4. A. Toniolo, T. Onodera, J. W. Yoon, et al., Nature, 288, 383 (1980).
5. J. W. Yoon, M. Auslin, T. Onodera, and A. L. Notkins, New Engl. J. Med., 300, 1173 (1970).
6. J. W. Yoon and A. L. Notkins, J. Metab., 32, 37 (1983).

PROTECTIVE PROPERTIES OF MICROCRYSTALLINE CELLULOSE IN RATS WITH EXPERIMENTAL DIABETES

S. G. Vainshtein, I. V. Zhulkevich, G. A. Petropavlovskii, and N. E. Kotel'nikova

UDC 616.379-008.64-092.9-085.874.263(547.458.81

KEY WORDS: metabolism; diabetes mellitus, microcrystalline cellulose.

Among the many factors which influence the mechanism of function of the digestive organs, one of particular importance, as has recently been shown, is the class of natural regulators, which includes dietary fibers [9]. Microcrystalline cellulose (MCC) a refined preparation of native cellulose [4], has been used as one such fiber. In previous investigations [3] the writers showed that MCC, administered in large doses and for long periods of time, does not disturb homeostasis and does not cause injury to organs of the gastrointestinal tract.

The known protective properties of the various dietary fibers in relation to disorders of carbohydrate and lipid metabolism in diabetic patients, and the use of MCC as a food additive in several countries, provided the motivation for this investigation.

EXPERIMENTAL METHOD

MCC — the purest cellulose preparation, obtained by hydrolysis of native cotton fiber to the "limiting" degree of polymerization — was used as the test object. According to mean viscosity calculations, this is equivalent to about 170 glucose residues to each cellulose macromolecule [4]. Experimental diabetes (ED) was produced in 45 noninbred albino rats weighing 150-170 g by injection of alloxan (Fluka, Switzerland) by the method in [10]. After 2 weeks, when the hyperglycemia stabilized (glucose concentration in blood from the caudal vein 9.03-11.77 mmoles/liter) and on elevation of the glycosylated hemoglobin (Gly-Hb) level, in addition to the standard animal house diet, balanced with respect to the principal parameters, animals of the experimental groups were fed with MCC in a dose of 3 g daily, equivalent to 10%

Department of Internal Medicine, Faculty of Postgraduate Medicine, Ternopol' Medical Institute. Laboratory of Chemistry and Physical Chemistry of Cellulose, Institute of Macromolecular Compounds, Academy of Sciences of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. G. Baranov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 2, pp. 167-168, February, 1987. Original article submitted March 4, 1986.

0007-4888/87/0002-0186$12.50