PG in 22 species of animals, of which 18 were marine invertebrates. In the present investigation the whole organism was used, whereas the Japanese workers studied individual organs (gonads, liver, gills, mantle, and so on). For biological testing they used the fundus of the rat stomach. The quantity of PG in the tissues of the animals they tested was very small, namely 1-45 ng/g wet weight of tissue.

Our results also confirmed data on the discovery of PG in eggs of the sea urchin *Strongylocentrotus intermedius* [2].

To verify the reliability of the data and to exclude any possible effect of other substances present in the PGX, those extracts which exhibited very high activity (+++) were subjected to preparative thin-layer chromatography (TLC). Biological tests of zones corresponding in chromatographic behavior to PGE_2 and $PGE_{2\alpha}$ confirmed the high activity of these extracts, and particularly high activity was exhibited by the trepang *Stichopus japonicus*.

Additional confirmation of the biological activity of total PGX from the trepang was obtained on the obturator muscle of *Cucumaria japonica* [3, 4]. For this purpose the extract also was fractionated by preparative TLC and zones corresponding in their chromatographic behavior to PG were tested. The results obtained on the obturator muscle of *Cucumaria japonica* are shown in Fig. 1.

The screening of this large number of marine invertebrates thus demonstrates the virtually universal presence of PG-like substances. The results justify a more intensive study of these substances from particular organisms.

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HEPATOCYTE PROLIFERATION AFTER BCG STIMULATION OF THE MONONUCLEAR PHAGOCYTE SYSTEM

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UDC 616-003.93:616.36+616.42:576.852.211

KEY WORDS: obesity; phospholipases A; lysosomes; hepatocytes; adipocytes.

Stimulation of the Kupffer cells by the polysaccharide prodigiosan sharply enhances hepatocyte proliferation in rats after partial resection of the liver [1]. It was considered interesting to study how the hepatocytes regenerate during activation of the mononuclear phagocyte system (MPS) and, in particular, the Kupffer cells, by other methods. It must be emphasized that *Mycobacterium tuberculosis* effectively stimulates MPS and also directly stimulates the Kupffer cells [5, 9]. Disinhibition of the functions of the MPS reaches its peak when macrophages initially activate BCG, and later, to potentiate their secretion, old tuberculin is injected [2, 10]. The aim of the present investigation was to study the early stages of regeneration of the liver after repeated injections of preparations of *M. tuberculosis*.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 11, pp. 96-98, November, 1982. Original article submitted April 30, 1981.

0007-4888/82/9411-1585$07.50

TABLE 1. Mitotic Indices of Hepatocytes (in °/oo) in Control and after Injection of BCG in Hepatectomized Rats (M ± m)

Time after operation, h	Control	Experiment
12	Influenced mitoses	Influenced mitoses
16	" "	" "
20	" "	" "
24	1,5±0,17	3,4±0,2**
30	28,13±2,36	41,56±0,65**
48	19,68±1,5	14,8±0,51*
72	2,9±0,42	1,3±0,2**

TABLE 2. ILN of Hepatocytes (in %) in Control and after Injection of BCG in Hepatectomized Rats (M ± m)

Time after operation, h	Control	Experiment
12	0,14±0,024	0,133±0,0338
16	0,32±0,0583	0,3±0,0447
20	2,32±0,446	2,76±0,331
24	16,22±1,212	29,72±2,183**
30	14,42±0,975	21,02±1,151**
48	10,04±0,351	7,14±0,441**
72	2,36±0,242	0,46±0,06**

Legend. Here and in Table 2: *P < 0.05 relative to corresponding group in control; **P < 0.01.

EXPERIMENTAL METHOD

Experiments were carried out on female Wistar rats weighing 160-200 g. BCG was injected subcutaneously into the experimental animals once a week in gradually increasing doses: 0.05, 0.15 mg, then intraperitoneally in a dose of 0.25 mg. Old tuberculin was then injected twice a week in increasing doses in a volume of 0.1 ml and in dilutions of 1:1000, 1:100, 1:10, and whole. In the control 0.85% NaCl was injected. Resection of the liver was performed by the method of Higgins and Anderson [6] under ether anesthesia between 9 and 10 a.m. 2 days after the end of the old tuberculin injections. The animals were killed six at a time 12, 16, 20, 24, 30, 48, and 72 h after the operation. An intraperitoneal injection of 1 μCi ^{3}H-thymidine/g body weight (specific activity 12.8 Ci/mmole) was given to the rats 1 h before sacrifice. Sections of the liver, stained with hematoxylin-eosin, were coated with liquid photographic emulsion (Photographic Chemical Research Institute Project) and exposed in darkness at 4°C for 3 weeks. The relative number of Kupffer cells per 1000 hepatocytes was calculated at the time of the operation after examination of liver sections stained with hematoxylin—eosin. To determine the mitotic indices of the hepatocytes in promille, 5000 hepatocytes were counted under a magnification of 900 times in liver sections 4-5 μ thick, stained with hematoxylin—eosin. To calculate the indices of labeled nuclei of the hepatocytes, in percent, 3000 nuclei were counted on the autoradiographs. Nuclei containing at least 10 grains of reduced silver were considered to be labeled. All the numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Histological examination of the liver at the time of the operation revealed no significant changes in the structure of the organ. The number of Kupffer cells was increased: 39.7 ± 0.8 compared with 35.0 ± 1.7 in the control (P < 0.05). The relative weight of the liver (hepatorenal index) in the experimental group did not differ from that in the control (7.68 ± 0.219 and 7.99 ± 0.31 respectively). The mitotic indices increased in the control, starting with 24 h after resection of the liver (Table 1) and reached a maximum 30 h after the operation. Many mitoses also were observed 48 h after the operation, but thereafter their number fell sharply to 72 h after resection. In the experimental animals an increase in mitotic activity also was observed 24 h after the operation, with a maximum after 30 h, but in this case the peak of mitotic activity was almost 1.5 times higher than in the control; later, 48 h after the operation, there was a sharper decline of mitotic activity. Whereas in the control the reduction amounted to 1.42 times, in the experimental group it was 2.8 times. Mitotic activity after 72 h was lower still, almost 50% lower than in the control. Injection of BCG was thus followed by the more synchronous entry of the hepatocytes into the period of division, and toward the end of 3 days mitotic activity fell to a minimum.

The character of the dynamics of the indices of labeled nuclei (ILN) of the hepatocytes both in the control and in the experimental series largely reflected the dynamics of mitotic activity of the hepatocytes. ILN of the hepatocytes in the control (Table 2) increased gradually from 12 h after resection of the liver, with a peak 24 h after the operation. A very small decrease in this index took place after 30 h. Later the fall was greater, but even after 72 h the index was still sufficiently high. In the experimental group in the period from 12 to 20 h after the operation a slow increase in ILN of the hepatocytes also took place,

followed by a sharp rise in ILN and a more considerable fall at all later times compared with the control. For instance, in the control ILN after 30 h was reduced by 1.12 times, and in the experiment by 1.41 times, whereas after 48 h it was reduced in the control by 1.6 times and in the experiment by 4.16 times. In this case, just as with mitoses, the rise and fall of ILN of the hepatocytes were more synchronized.

After injection of BCG proliferative activity of the hepatocytes was thus enhanced, and this was reflected in the more synchronized DNA synthesis followed by mitotic division of the hepatocytes. If these results are compared with those obtained after stimulation of Kupffer cells with prodigiosan (a polysaccharide from *Serratia marcescens*) the following pattern will be detected. After injection of prodigiosan, besides an increase in the number of mitoses compared with the control, which was also observed in the case of BCG, an earlier rise of DNA synthesis and mitotic activity began, which did not occur after injection of BCG. This difference is attributable to differences in the structure of the stimulators themselves. The stimulating effect of BCG can be explained by functional reorganization of the MPS and directly in the system of liver macrophages. The effect of BCG on macrophages as an activator was demonstrated long ago. A synthetic analog of the active principle of the cell wall of the mycobacteria has now been obtained: N-acetylmuramyl-L-alanyl-D-isoglutamine (abbreviation muramyl dipeptide), and is able to stimulate macrophages, but it is free from the side effects observed when BCG is used. It has been shown [3] that stimulation of macrophages by muramyl dipeptide leads to an increase in their protein content, β-glucosaminidase and lactate dehydrogenase activity, and their intensity of phagocytosis. Activated macrophages potentiate glucocorticoid metabolism [7] and, in addition, they secrete a factor which is an antagonist of these hormones [8], with the result that their blood level falls. It has also been shown that synchronization of hepatocytes after partial resection of the liver depends directly on the glucocorticoid level [4]. All these facts explain the enhanced synchronization of mitotic activity after bacterial stimulation of the MPS in the present experiments.

The author is grateful to S. M. Gordienko for technical help in the course of the experiments.

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