The results are evidence that estrogens can bind with human AFP under certain conditions. Human AFP in biological fluids is evidently bound with estrogens; the binding constant, moreover, is so high that exchange of hormone molecules is virtually impossible and AFP untreated with butanol therefore does not bind the hormone immobilized on sepharose. After treatment of abortion material with butanol, as a result of which dissociation of the estrogen–AFP complex takes place, human AFP becomes capable of binding the immobilized hormone. The binding is very strong in character and the complex does not dissociate during attempts at elution with 1 M and 2 M NaCl solutions and also by the use of a whole range of detergents. A very good eluting agent under these circumstances is a 10% solution of butanol in isotonic sodium chloride or in 0.01 N Veronal–Medinal buffer, pH 8.6. The orientation of the hormone immobilized on sepharose also is important. In the present experiments on sorbents containing estradiol or estrone, immobilized through C-17, the yield of AFP on affinity chromatography was very small (about 0.5-1%) and this was accompanied by a high concentration of ballast proteins, mainly albumin, although these data also are evidence in support of binding of human AFP with estrogenic hormones.

Meanwhile the results of chromatography both of the butanol extract of abortion material and of AFP preparations incubated beforehand with free estrone and estradiol, are evidence that binding of AP with free estrogens does not take place, confirming prevous observations [7]. It can be tentatively suggested that binding of estrogens with human AFP is specific in character, through the participation of unknown protein intermediaries, the discovery of which is an interesting problem, and the microenvironment of the immobilized estrogen thus as it were models the conditions of binding of human AFP in vivo.

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EFFECT OF LOW DOSES OF HYDRA PEPTIDE MORPHOGEN ON PROTEIN SYNTHESIS IN THE INTACT AND REGENERATING RAT LIVER

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Hydra peptide morphogen (HPM), described in 1981 by Schaller and Bodenmüller, accelerates regeneration of injured organs of the Coelenterata and Polychaeta [4, 9]. This oligopeptide has been found by immunochemical methods in the gastrointestinal tract and brain of mammals and also in human blood plasma [4, 8, 9]. Data on the biological effect of this

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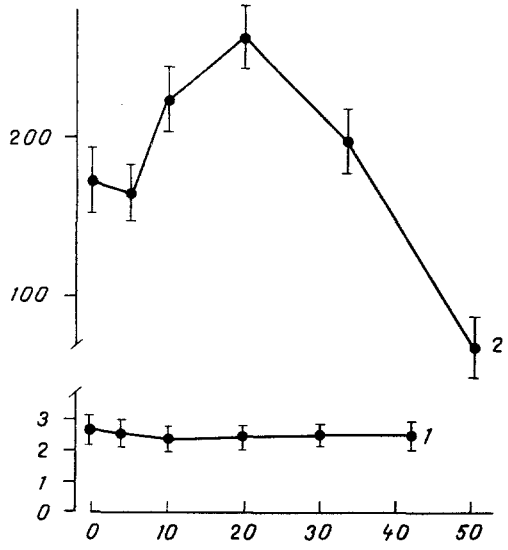

Fig. 1. Dependence of ODC activity on dose of HPM injected. Abscissa, dose of peptide (in μg/kg); ordinate, specific activity of ODC (in pmoles CO_2/mg protein/g). 1) In liver of intact animals; 2) in liver of partially hepatectomized rats.

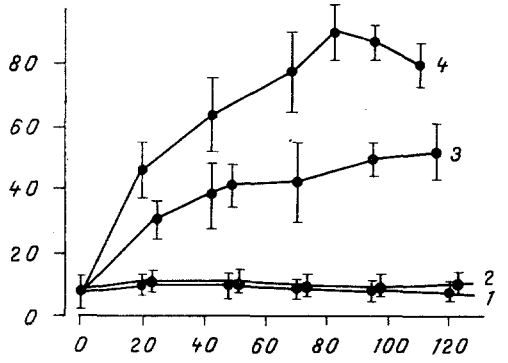

Fig. 2. Incorporation of ^{3}H-leucine into liver protein of intact (1, 2) and partially hepatectomized (3, 4) rats. Abscissa, time after injection (in h); ordinate, quantity of ^{3}H-leucine incorporated (in cpm/mg protein × 10^{-3}). 1, 3) Injection of control solution of equimolar mixture of amino acids; 2,4) injection of HPM.

peptide in experiments on mammals are confined to three communications. Stimulation of pancreatic amylase secretion in rats in vitro under the influence of HPM has been reported [5]. The ability of the peptide to change the parameters of the estrous cycle in rats was described in [2]. Injection of the peptide in a dose of 2 mg/kg activates ornithine decarboxylase (ODC) in the liver of intact rats, and when ODC is activated by partial hepatectomy, injection of the peptide in the same dose stimulates this enzyme additionally [1]. Since ODC is an enzyme which limits the rate of polyamine synthesis, the increase in ODC activty reflects the intensity of tissue anabolic processes [7].

The aim of this investigation was to determine the character of dependence of ODC activity on the dose of HPM and to study the parameters of protein synthesis in the intact and regenerating rat liver under the influence of the peptide in a dose of 20 μg/kg, which activates the enzyme maximally [3].

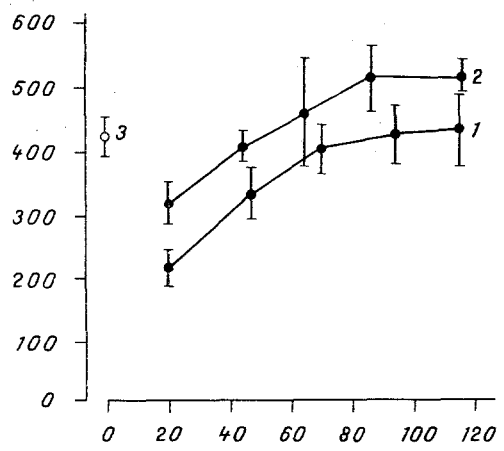

Fig. 3. Protein content in regenerating rat liver. Abscissa, time after injection (in h); ordinate, protein content (in mg). 1) Injection of control solution of equimolar mixture of amino acids; 2) injection of HPM; 3) protein content in liver of intact animals.

EXPERIMENTAL METHODS

Experiments were carried out on 114 male Wistar rats weighing 160-180 g. HPM was synthesized in the Laboratory of Peptide Synthesis, Research Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. The HPM was dissolved in physiological saline and injected intraperitoneally in a volume of 1 ml. The control animals were given an injection of physiological saline containing an equimolar mixture of amino acids. A solution of the peptide or mixture of amino acids was injected into partially hepatectomized animals [6] 10 min after the operation. Activity of the soluble form of ODC was determined by a radioisotope method based on release of $^{14}Co_2$ from L-(1-^{14}C)-ornithine, 6 h after the operation, in supernatant obtained by centrifugation of liver homogenate at 105,000g for 70 min [3].

Protein synthesis in normal and regenerating rat liver was studied by determining incorporation of ^{3}H-leucine with specific activity of 49 μCi/mole, which was injected in a dose of 1 μCi/g body weight intraperitoneally in 1 ml of physioloigcal saline 1 h before sacrifice [10]. To obtain protein containing the labeled amino acid the liver was removed, washed in two changes of cold physiological saline, weighed, and homogenized in two volumes of buffer: 0.9% NaCl, 20 mM Tris-HCl (pH 7.2). Next, 200 μl of the resulting homogenate was treated with 3 ml of a 5% solution of TCA and heated for 20 min at 90°C. The sample was then centrifuged at 20,000g for 20 min and washed successively with a 5% solution of TCA, a mixture of ethanol ether chloroform (2:2:1), and with acetone. Washing the radioactive residue was accompanied each time by sedimentation in the centrifuge. The residue after the last centrifugation was dried and dissolved in 1 ml of a 0.2% solution of sodium dodecylsulfate, made up in 2 N NaOH. The protein solution in a volume of 100 μl was added to 10 ml of dioxan scintillator and radioactivity was determined on a RackBeta-1215 scintillation counter (LKB, Sweden). The results were subjected to statistical analysis by Student's t test for small samples ($p \leq 0.01$). Protein was determined by the method in [11].

EXPERIMENTAL RESULTS

The level of ODC activity in the liver of intact animals was 2.75 ± 0.24 pmole CO_2/mg protein/h. This level was unchanged after administration of HPM to the animals in a dose of between 10 and 50 μg/kg. Injection of the control equimolar mixture of amino acids likewise did not change activity of the enzyme. The operation of partial hepatectomy led to activation of ODC: 6 h after the operation enzyme activity was increased to 167 ± 28 pmoles CO_2/mg protein/h (Fig. 1).

Injections of increasing doses of HPM into partially hepatectomized animals had varied effect on ODC activity. For instance, the lowest dose (5 μg/kg) of HPM did not cause additional stimulation of ODC. Higher doses (10 and 20 μg/kg) activated the enzyme; the effect of additional stimulation of ODC activity was most marked when the peptide was injected in

a dose of 20 μg/kg. A further increase in the dose of HPM (50 μg/kg) inhibited ODC activity. To study protein synthesis in the liver and the effect of HPM on it, a dose of 20 μg/kg was chosen as being most effective for ODC activation in partially hepatectomized rats. Injection of this dose of the peptide into intact animals did not change the level of ^{3}H-leucine incorporation compared with that observed in animals receiving an injection of the control solution of an equimolar mixture of amino acids. After the operation of partial hepatectomy the level of incorporation of the labeled amino acid increased during the period from 20 to 120 h (Fig. 2). Injection of HPM into partially hepatectomized rats caused an additional increase in the intensity of ^{3}H-leucine incorporation into protein of the regenerating liver by 1.4-1.9 times.

The quantity of protein in the liver of the intact animals was 425 ± 28 mg. This value was unchanged after injection of HPM for the control solution of an equimolar mixture of amino acids into the rat at all times studied.

After partial hepatectomy, when about 70% of the weight of the organ was removed, growth of the liver took place and was accompanied by an increase in the protein content in the regenerating organ. The protein content in the liver regenerating under the influence of HPM was higher than the corresponding control values (Fig. 3). However, significant differences were observed in the 20-45 h interval. Under the influence of HPM there was a more rapid increase in protein content in the regenerating organ at these times than in the control (by 1.4 and 1.2 times, respectively).

The results relative to activation of ODC by low doses of HPM, the dome-shaped curves of dependence of ODC activity on the dose of the peptide, and stimulation of ^{3}H-leucine incorporation into protein of the regenerating liver are evidence, in our view, of specific interaction of this protein with regenerating liver tissue and the influence of HPM on anabolic processes through the polyamine system.

The much more intensive incorporation of amino acids into protein of the liver regenerating under the influence of HPM, and unaccompanied by adequate protein accumulation by the regenerating organ, may indicate an increase in secretion of a certain fraction of blood plasma proteins.

The results evidently indicate that HPM can perform functions in mammals similar to its functions in invertebrates, and they are also evidence of the specificity of the effect of this compound.

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