tide, the solubility increased sharply, and at 100 molecules per nucleotide all the DNA went into solution. This relationship may indicate that the conversion to the soluble state requires modification of the critical number of groups in the DNA and protein.

In connection with this hypothesis of the formation of single breaks in the DNA in the complex and the absence of any significant degradation of the DNA, the results of quantitative determination of the accumulation of single breaks in the polynucleotide strands in relation to NMU concentration are of fundamental importance (Fig. 3). Incubation of DNP for 24 h with NMU in the maximal concentration (100 molecules per nucleotide) led to the appearance of about 30 single breaks. As was shown previously [4], during the action of NMU on DNA, one paired break occurs during the formation of 30-40 single breaks. Considering that the results obtained by the viscosimetric method are possibly a little on the high side [7], it can be concluded that under the conditions used there was no significant degradation of DNA.

During the action of NMU on DNP the secondary structure of the DNA remained stable, no ruptures evidently occurred in the double-stranded structure of the DNA, but as a result of the single breaks the DNA molecule became more flexible. This conclusion agrees well with the results of investigation of the chemical properties of DNA after methylation by dimethyl sulfate [6]. The increase in the solubility of DNP in solvents with near-physiological ionic strength was evidently due mainly to labilization or dissociation of the DNA–protein bonds.

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EFFECT OF THE PROTEASE INHIBITOR CONTRYCAL ON GRANULATION TISSUE METABOLISM

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Investigation of the effect of contrycal on granulation tissue metabolism in rats showed that the inhibitor stimulated incorporation of glycine-C^{14} into collagen proteins and modified the lactate dehydrogenase isozyme spectrum; meanwhile maturation of the granulation tissue was delayed despite its well-marked growth.

KEY WORDS: protein synthesis; granulation tissue; protease inhibitor, contrycal; lactate dehydrogenase and its isozymes.

Inhibitors of proteolytic enzymes are extensively used in surgical practice [5]. However, the desirability of using inhibitors to accelerate the healing of clean and infected wounds is still under discussion in the literature. For instance, besides papers giving evidence of the beneficial effect of inhibitors on wound healing [2, 8], there are others describing the absence of any marked therapeutic effect [10]. The contradictory nature of the

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TABLE 1. Effect of Contrycal on Incorporation of Glycine-C^{14} into Proteins of Granulation Tissue and Skin

	Radioactivity, counts/min/5 mg protein, experiment No.				Incorporation, % of control (M ± m)
	1	2	3	4	
	Granulation tissue				
TCA-collagen					
control	4260	4200	1120	1355	123,0± 2,0
experiment	4880	5000	1415	1800	
Noncollagen proteins					
control	1360	1456	915	472	83,0± 3,5
experiment	1104	1280	1018	365	
	Skin				
Citrate-soluble collagen					
control	1180	960	—	318	140,0± 11,0
experiment	1580	1520	—	408	
TCA-collagen					
control	662	631	—	250	131,0± 1,5
experiment	900	789	—	330	

Legend. Each number is mean of values for 5 animals.

TABLE 2. Effect of Contrycal on Activity of LD, MD, and Their Isozyme Spectrum in Granulation Tissue

	Statistical index	LD						MD				
		total activity	isozymes, %					total activity	isozymes, %			
			LD_1	LD_2	LD_3	LD_4	LD_5		MD_1	MD_2	MD_3	MD_4
Control	$M\pm$	496,4	11,50	10,20	8,95	15,90	51,40	22,5	6,1	14,5	35,3	45,1
	$\pm m$	32,2	1,74	1,23	1,42	1,80	3,35	1,59	0,73	1,34	0,96	1,09
Experiment	$M\pm$	530,2	1,96*	2,5*	4,6*	16,0	74,1*	24,8	5,2	11,5	38,3	47,3
	$\pm m$	36,3	0,38	0,22	0,25	0,63	0,83	0,87	0,59	1,46	1,20	0,73

*$P < 0.05$ compared with control.

Legend. Total enzyme activity given in μmoles substrate/mg protein/min.

results obtained by different workers is largely due to the absence of any precise indications for the use of inhibitors, for information on their effect on the body and, in particular, of the metabolism of developing granulation tissue is very limited. It is therefore urgent to study the effect of protease inhibitors on the metabolism of granulation tissue.

In the investigation described below the effect of contrycal was studied on the biosynthesis of collagen and noncollagen proteins, on the content of hydroxyproline, hexosamines, and sialic acids, and also on the activity and isozyme spectrum of malate dehydrogenase (MD), an enzyme of the Krebs cycle, and lactate dehydrogenase (LD), an enzyme of glycolysis, in the granulation tissue. Simultaneous morphological and histochemical investigations were made of the granulations.

EXPERIMENTAL METHOD

Male rats weighing from 150 to 200 g were used. A nichrome wire coil, 35 mm long and 4 mm in diameter, was implanted subcutaneously into the animals under ether anesthesia. The coil was fixed by one suture to the fascia of the spinal muscles. The edges of the wound were sutured without drainage. On the day of the operation and for the next 2 days the control animals received intraperitoneal injections of 1 ml 0.14 M NaCl and the experimental rats received 5000 units (ATU) of contrycal. On the seventh day, 2 h before decapitation, all the animals received an injection of glycine-C^{14} (specific activity 91 or 130 mCi/mg) in a dose of 100,000 counts/min/g body weight. To discover the effect of changes in the reserves of precursor on protein biosynthesis two concentrations of glycine, differing by one order of magnitude, were used.

The rats of both groups were killed simultaneously and granulation tissue with the encapsulated coil and the tissue of the linear scar, freed from hair and subcutaneous fatty

areolar tissue, was removed. The TCA–collagen was removed from the clean granulation tissue [9] and the citrate-soluble [4] and TCA–collagen from the tissue of the linear scar. The radioactivity of the collagen preparations was measured in a gas-flow counter. The content of hydroxyproline [3], hexosamines [1], and sialic acids [11], was determined in the dried and defatted samples of tissue. Activity of LD and MD was determined spectrophotometrically [6, 12]. Fractionation into isozymes was carried out by electrophoresis on agar [7]. Protein was determined by Lowry's method. Material for morphological investigation was fixed in a 10% solution of neutral formalin and embedded in paraffin wax. The tissue was stained with hematoxylin–eosin, by Van Gieson's method, and with alcian and toluidine blue to determine acid mucopolysaccharides.

EXPERIMENTAL RESULTS

As Table 1 shows, administration of contrycal to the animals undergoing the operation stimulated incorporation of glycine-C^{14} into the TCA–collagen of the granulation tissue on the average by 23%, and into the citrate-soluble and TCA–collagen of the skin in the region of the linear scar by 40% and 31%, respectively. Meanwhile the incorporation of labeled precursor into the noncollagen proteins of the granulation tissue was slightly inhibited.

Changes in the specific radioactivity of the isolated proteins were unconnected with changes in the reserves of the precursor, for the specific radioactivity of the collagen and noncollagen proteins of the animals of the control and experimental groups were virtually indistinguishable for two different concentrations of the precursor. Contrycal was found not to affect the content of hydroxyproline, hexosamines, and sialic acids in the granulation tissue or the tissue of the linear scar.

Investigation of the activity of LD, MD, and their isozymes in granulation tissue (Table 2) showed that contrycal caused marked changes in the isozyme spectrum of LD, inhibiting the anodal fractions: The LD_1 level fell by 83% and LD_2 by 75.5%. Activity of LD_3 also was reduced by 48.6%, activity of LD_4 was unchanged, and that of LD_5 was increased by 44.1%. A tendency was observed for the total activity of this enzyme to increase. Meanwhile no marked changes in the activity of MD or its isozymes in the granulation tissue were found under the influence of contrycal.

The results of the morphological investigation showed that on the seventh day a dense connective-tissue capsule consisting of bundles of collagen fibers, with fibroblasts located between them, had formed around the implanted coil in the control and experimental animals. In the control animals collagen fibers in the capsule were more compactly arranged than in the experimental group; the fibroblasts were thin and long, with dark nuclei, and resembled mature fibrocytes; their cytoplasm was weakly basophilic, did not stain metachromatically, and did not contain granules staining with alcian blue. In the central parts of the capsule marked metachromatic staining was observed, evidence of the presence of acid mucopolysaccharides. Immediately beyond the capsule numerous blood vessels, some of them empty, were seen.

In the experimental animals the collagen fibers were more loosely arranged than in the controls, the fibroblasts were more numerous and larger in size, and their cytoplasm, which was strongly basophilic, stained metachromatically and did contain granules stained with alcian blue. Metachromatic staining was discovered in both the central and the peripheral zones of the capsule. In the central zones of the capsule small groups of leukocytes, large macrophages, and mast cells were found. The vessels beyond the capsule were congested, with leukocytes and erythrocytes concentrated in their lumen.

The results point to the less mature character of the connective tissue in the animals receiving contrycal compared with the granulation tissue of the animals of the control groups.

The writers showed previously [2] that contrycal, if administered to burned animals, inhibits inflammatory infiltration and the development of secondary necrosis in the burned area and activates the incorporation of labeled precursor into collagen proteins. Under the conditions of the present experiments, contrycal also led to increased incorporation of glycine-C^{14} into the collagen proteins of granulation tissue, although the total quantity of collagen remained unchanged. Morphologically, the collagen fibers of the granulation tissue of the experimental animals were less mature than in the controls. These changes in collagen biosynthesis were coupled with activation of anaerobic processes, as shown by the modification of the LD isozyme spectrum in the granulation tissue.

It can be postulated that contrycal, by inhibiting protease activity in the early stage of granulation tissue formation, thereby stimulated the incorporation of labeled precursors into certain biological polymers, including into collagen proteins. The simultaneous reduction in the quantity of breakdown products — the natural stimulators of repair processes — evidently was responsible for some delay in the maturation of the granulation tissue formed around the coil implanted under the skin.

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EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON LYSOSOMAL MEMBRANES OF THE EYE TISSUES

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Both in experiments *in vitro* and after intravenous injection of prostaglandin (PG) $F_{2\alpha}$ labilization of the lysosomal membranes takes place in the sclera and ciliary body but not in the cornea of the rabbit eye. Under the influence of PG, glycosidase activity appears in the vitreous body, where it cannot be found normally.

KEY WORDS: prostaglandin $F_{2\alpha}$; lysosomal membranes; rabbit eye tissues.

Prostaglandins (PG) are present in nearly all mammalian and human tissues and have a broad spectrum of action. Recently two types of PG ($F_{2\alpha}$ and E_1) have been discovered in the eye tissues [9, 11], and their localization [4, 5, 11] and their effect on certain tissues of the eye [6, 7, 14] have been demonstrated. Prostaglandins can act on membranes and on cell metabolism both through a change in the ionic permeability of the membranes and through their action on the cyclic AMP system and on certain hormones [8, 15]. However, the effect of PG on the membranes of the eye tissues has not been studied, with the exception of the ciliary body, for which an increase in the permeability of the cell structures under the influence of PG has been demonstrated [6, 13].

In this investigation the effect of PG $F_{2\alpha}$ on the lysosomal membranes of the eye tissues was studied. Changes in the activity of enzymes hydrolyzing glycoside bonds in mucopolysaccharides (β-galactosidase, β-glucosidase, hyaluronidase) were investigated in the sclera, ciliary body, cornea, aqueous humor, and vitreous body.

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