origin and very nearly the same in amount in both aerobic and anaerobic experiments. Thus the proposition is appealing that the influence of DNC on some aerobic reaction other than aerobic phosphorylation, possibly reduced pyridine nucleotide oxidation, underlies the difference between aerobic and anaerobic values for maximal stimulation at 37° and 25°. In fact, interference with reduced triphosphopyridine nucleotide (TPNH) oxidation as a mechanism of DNC action for both aerobic and anaerobic stimulation seems a reasonable speculation on which to base further work. Some evidence pointing in this direction has been obtained with the eggs of *Arbacia punctulata* in which it has been found(9) that DNC alters the pathway of glucose utilization from one requiring a continuing supply of oxidized triphosphopyridine nucleotide (phosphogluconate pathway) to one requiring a supply of oxidized diphosphopyridine nucleotide (glycolytic pathway). In addition, substituted phenols can inhibit flavin nucleotide oxidations in general(10) and TPNH-cytochrome-c reductase in particular(11).

Summary. Conversion of glucose to lactate by ascites tumor cells derived from the Ehrlich carcinoma, Crocker sarcoma 180, and Freund sarcoma is stimulated by 4,6-dinitro-o-cresol under both aerobic and anaerobic conditions. Degree of stimulation increases as temperature is lowered from 37° to 15°, although the absolute rate of lactate production diminishes. Aerobic lactate production is stimulated to a greater degree than anaerobic and the former exceeds the latter under certain conditions. The implications and possible mechanisms of these phenomena are discussed.

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Mucopolysaccharide, Protein and Desoxyribosenucleic Acid Concentration of Granulation Tissue Induced by Polyvinyl Sponges.* (24369)

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Granulation tissue free of inflammatory infiltrate has been observed to enter polyvinyl sponges inserted under skin of various animals (1,2). This tissue provides proliferating, metabolically active connective tissue suitable for experimental study. Protein and collagen content of tissue which enters these sponges and some of the enzymatic activities of this tissue have been studied(2,3). We explored the metabolism of mucopolysaccharides in this tissue. The present report concerns mu-

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TABLE I. Analyses of Tissue in Polyvinyl Sponges.

Determination	Units	1 wk	1½ wk	2 wk	3 wk	4 wk	6 wk	12 wk
Wt of granuloma	mg	176 ± 35.1 * (7)	211 ± 17.0 (12)	172 ± 15.8 (10)	159 ± 11.4 (8)	133 ± 13.1 (6)	76.2 ± 10.5 (4)	92.5 ± 5.4 (5)
Protein	g/100 g	14.1 ± .58 (4)	14.5 ± 1.59 (10)	11.7 ± .33 (10)	14.4 ± .46 (7)	12.8 ± 1.09 (6)	12.7 ± .76 (4)	9.39 ± .45 (6)
Desoxyribosenucleic acid	mg/100 g	708 ±120 (12)	845 ± 55.0 (10)	857 ± 59.3 (8)	830 ± 95.5 (6)	820 ± 82.5 (4)	986 ±120 (6)	
Uronic acid of acid mucopolysaccharide (carbazole method)	"	96.5 ± 21.4 (7)	139 ± 15.2 (12)	192 ± 22.9 (10)	168 ± 19.4 (8)	139 ± 35.3 (6)	103 ± 14.8 (4)	125 ± 8.0 (6)
Ratio of carbazole/orcinol†		.9 ± .22 (5)	1.1 ± .07 (12)	.8 ± .10 (9)	7 ± .08 (7)	.5 ± .04 (6)	.6 ± .05 (4)	.5 ± .16 (5)

No. of observations in parentheses.
* Mean ± stand. error of mean.
† This ratio compares values obtained for uronic acid content of acid mucopolysaccharide fraction by carbazole and orcinol methods of determination.

copolysaccharide, protein and desoxyribosenucleic acid concentration of tissue which forms in sponges inserted under dorsal skin of guinea pigs. Subsequent reports will deal with enzymes involved in synthesis of mucopolysaccharides in this tissue(4).

Materials and methods. Polyvinyl sponges (Ivalon, Clay-Adams) weighing 130 ± 10 mg were inserted under dorsal skin of male guinea pigs weighing 500 ± 50 g anesthetized with pentobarbital. Two sponges were inserted in lumbar regions of each animal. After one to 12 weeks, sponges were removed and dissected free of adjacent connective tissue. They were cut into 1-2 mm particles, defatted and dried. The details of this procedure and subsequent determination of acid mucopolysaccharide content were reported previously (5). The dry, defatted tissue was weighed; weight of granulation tissue was obtained by subtracting original weight of sponge used, since weight of sponge was not altered by prior steps. After grinding in a Wiley mill, weighed aliquots of sponge tissue were taken for chemical analyses; weights were corrected for content of inert sponge material and results were calculated/100 mg of dried, fat-free granulation tissue. Acid mucopolysaccharide was determined by carbazole and orcinol determinations of uronic acid in the mucopolysaccharide fraction(5). Desoxyribosenucleic acid (DNA) was determined by extraction with hot trichloracetic acid, color development with indole, and removal of nonspecific color with isoamyl alcohol, according to the method of Keck(6). Protein was determined by extraction with alkaline copper solution ("reagent C") of Lowry, *et al.*(7) for 2 hours at room temperature. An aliquot was then used for protein determination using the Folin-Ciocalteau reagent(7). Samples of sponges not used for quantitative studies were fixed in 40% formaldehyde and microscopic sections were made and stained with hematoxylin and eosin. Chromatography of mucopolysaccharides in extracts of sponge tissue was done by methods described previously (8). Chromatography of hexosamines was done by the method of Stoffyn and Jeanloz (9) after hydrolysis and isolation on a Dowex-50 resin column.

Results. The results are given in Table I. Maximum weight of granulomas occurred at 10 days, then decreased; the sponges became progressively more shrunken, firm and less vascular. Cellularity of sponges, as reflected by desoxyribosenucleic acid concentration, reached a maximum at 2-3 weeks while protein content was near maximum at one week. The acid mucopolysaccharide concentration climbed rapidly between one and 2 weeks, then fell gradually thereafter. As the content of mucopolysaccharide in sponges fell, a decrease in carbazole/orcinol ratio occurred. Since chondroitin sulfate B gives a low carbazole/orcinol ratio(10), this presumably indicated an increase in relative concentration of this mucopolysaccharide.

Chromatography of the mucopolysaccharide fraction of extracts of sponge tissue revealed metachromatically staining components near the solvent front and near the origin in both buffered ethanol and buffered propanol. Markers of chondroitin sulfate A migrate near the solvent front in both systems; hyaluronate remains near the origin in both systems. Heparin and chondroitin sulfate B stay near the origin in ethanol, but migrate near the solvent front in propanol. Therefore, the spot near the solvent front in ethanol probably contains chondroitin sulfate A or C (or both), and that near the origin in propanol probably contains hyaluronate. In view of the carbazole/orcinol ratios, chondroitin sulfate B is probably also present but its mobility overlaps that of one of the other components in each system.

Chromatography of hexosamines isolated from mucopolysaccharide fraction revealed the presence of both glucosamine and galactosamine.

Incubation with testicular hyaluronidase, following the procedure described(5), resulted in disappearance of up to 90% of the mucopolysaccharide in extracts of sponges which had been *in situ* 1-2 weeks.

Histological appearance of tissue in sponges is illustrated in Fig. 1. No infiltration by inflammatory cells was seen. Multinucleated giant cells were present, particularly in the older granulomas.

Discussion. Concentration of acid mucopolysaccharide in connective tissue which formed in polyvinyl sponges reached a level higher than that found in either normal guinea pig skin or subcutaneous tissue, but not as high as that found in aorta or cartilage. Protein concentrations were higher than the total protein of 9 g/100 g of wet tissue reported by Kao *et al.*(2) for 120 day-old sponge specimens from rats.

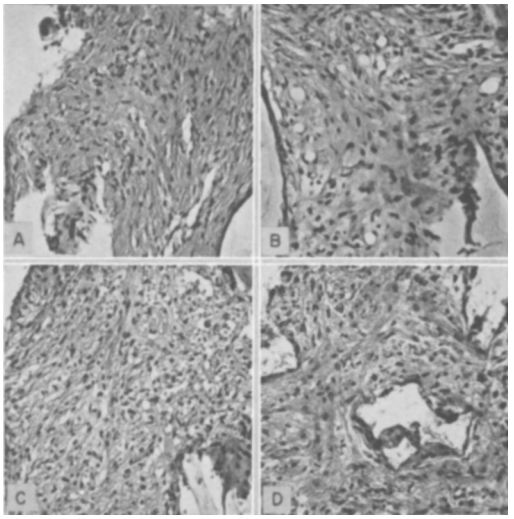

FIG. 1. Microscopic appearance of sections of sponges which had been inserted under the dorsal skin of guinea pigs. A, 10 days; B, 3 weeks; C, 4 weeks; D, 6 weeks. Magnification 113 ×, hematoxylin and eosin stain.

Jackson(11) and Slack(12) reported collagen and sulfated polysaccharide content of granulomas induced by subcutaneous injection of carrageenin. Their results resemble the findings in the polyvinyl sponges, except that in granulomas induced by carrageenin the peak growth is reached earlier and resorption is more rapid and more complete. We found that granulomas induced by polyvinyl sponges are more suitable for polysaccharide analyses than those induced by carrageenin,‡ since the latter is a sulfated polygalactose which interfered with some of the carbohydrate analyses.

The tissue found in polyvinyl sponges synthesizes significant quantities of mucopolysaccharide, and is suitable for study of intermediary steps in the metabolism of these sub-

‡ Carrageenin was kindly supplied by Seaplant Chem. Corp., New Bedford, Mass.

stances(4). Further information concerning degradation of mucopolysaccharide might also be gained by study of the sponge tissue in view of the decreasing mucopolysaccharide content of this tissue after the second week.

Summary. Polyvinyl sponges inserted under the dorsal skin of guinea pigs fill with cellular connective tissue. Dry weight of the granulation tissue, concentration of protein, desoxyribosenucleic acid and acid mucopolysaccharide at intervals between 1 and 12 weeks are reported. Both glucosamine and galactosamine were present in the mucopolysaccharide fraction. Evidence for the presence of 3 types of mucopolysaccharide components was obtained by chromatography and carbazole/orcinol ratios. A decrease in carbazole/orcinol ratios suggested an increased proportion of chondroitin sulfate B in the older granulomas.

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Chemotherapeutic Studies with 2,4-Dimethoxy-6-sulfanilamido-1,3-diazine (Madribon*) (24370)

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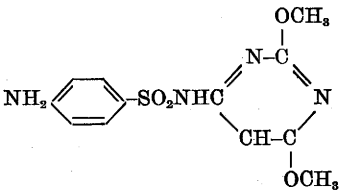
Comparatively high activity of 2,4-dimethoxy-6-sulfanilamido-1,3-diazine in experimental streptococcal infections has been described by Semenitz(1). The results appeared promising although the experiments were carried out with organisms of low virulence and evaluation of the effect of subcutaneous administration was based on very short observation time. More extensive studies on chemotherapeutic activity carried out recently in these laboratories confirmed the remarkable activity of this compound in a series of experimental infections with gram-positive and gram-negative bacteria. The experiments are here described.

Chemical and physical properties. 2,4-Dimethoxy-6-sulfanilamido-1,3-diazine (I; Madribon), first synthesized by Bretschneider and Kloetzer(2), is a white, odorless and almost tasteless crystalline powder with a melting range of 200-202°C. Two Kpa values at 2 and 6 were observed.† The substance is slightly soluble in water, dependent on H-ion concentration. Solubility values determined at 37°C of 4.6 to 7.5 mg/100 ml at pH 4.10 and 5.7 rose to 29.5 mg/100 ml at pH 6.7, 58.0 mg/100 ml at 7.06, 920 mg/100 ml at 8.19 and reached 5170 mg/100 ml at pH 8.71. Under the same conditions, the N^4-acetyl de-



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† We are greatly obliged to Dr. A. Motchane of the Physico-chemical Lab. for permission to include these values as well as data on solubility, in this paper.