Microautoradiographic Study of the Intracellular and Extracellular Distribution of Glycerol-1,3-C^{14} Labeled Lipids in Experimental Connective Tissue

GILES G. BOLE, JR.

A WIDE VARIETY of morphologic and chemical studies have contributed to an underestanding of the biochemistry and physiologic functions of connective tissue.[1] All available evidence would suggest that mucopolysaccharides and collagen, as extracellular constituents of connective tissue, are synthesized locally by the connective tissue cells. It is now clear that the fibroblast and macrophages,[2,3] like other cell types, also synthesize lipids. To date, this synthetic capacity has been thought to relate to the renewal of subcellular constituents,[4] to serve as an immediate or potential source of metabolic energy,[5] or to be involved in modifications of the cell membrane during the physiologic processes of transport or phagocytosis.[6] Previous studies reported from this laboratory have shown that the cells in the polyvinyl sponge granuloma are capable of net synthesis of triglycerides and phospholipids when glycerol-1,3-C^{14} is used as precursor.[7,8] During cellular organization of the polyvinyl sponge implant, varying proportions of fibroblasts, macrophages, foreign body giant cells, and endothelial cells are present in the granuloma. The concentration of these cells and of other vascular elements change with age of the tissue.[9] Due to this cellular heterogeneity, the contribution of each major cell type to lipid synthesis has not been established. To answer this question the present histological studies were carried out on tissue aliquots removed from the same incubation flasks which contained the tissues used for the chemical analysis of glycerol-1,3-C^{14} incorporation by granuloma slices 7–450 days of age.[7,8] Using the technic of quantitative microautoradiography, it has been possible to identify incorporation of this isotope and study its localization within the several cell types present in polyvinyl sponge and open wound granulomas. In addition, the presence of glycerol-1,3-C^{14} in extracellular location has been demonstrated. Using standard histologic methods designed to preserve or extract lipids from the tissue, it has been found that 75 per cent of the incorporated isotope is labile to such procedures. These findings suggest that lipids (lipoproteins) occur as constituents of connective tissue "ground substance."

METHODS

Polyvinyl sponge implants were removed from adult mongrel guinea pigs 7 to 450 days postimplantation and open wound granulomas were excised after 12 to 14 days.[8] The tissues were immediately sliced free hand (thickness ± 1.5 mm.) and incubated aerobically at 37 C. in vitro in 3 ml. Krebs-Ringer bicarbonate buffer pH 7.4 supplemented with glucose, 2 mg./ml. Five microcuries of glycerol-1,3-C^{14}, with a specific activity of 3 μc per μmole, was added to each flask. Zero time and 3 hour incubations were carried out with granuloma slices at each tissue age. Incubations 1½, 3, and 5 hours were carried out with granulomas 7–42 days of age. In this group of exper-

From the Department of Internal Medicine, the University of Michigan, and the Rackham Arthritis Research Unit. *(The Rackham Arthritis Research Unit is supported by a grant from the Horace H. Rackham School of Graduate Studies.)*

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GILES G. BOLE, JR., M.D.: *Senior Investigator, The Arthritis Foundation, Rackham Arthritis Research Unit; Associate Professor of Internal Medicine, Department of Internal Medicine, The University of Michigan Medical School, Ann Arbor, Mich.*

iments tissue slices subjected to repeated freezing and thawing or predried in vacuo were also incubated for 1 to 5 hours.

Tissues before and after incubation with glycerol-C^{14} were fixed in Carnoy, Bouin, McManus fluid, or Baker formalin and sections, 4 microns (μ) thick, prepared.[9] Formalin and McManus fixed tissues were also prepared for sectioning using the carbowax 1000 embedding technic.[9] Additional aliquots were quick-frozen with isopentane-liquid nitrogen and sectioned at 4 μ with a cryostat. These preparations were post-fixed with formalin, ethanol, or dried in air. All tissue sections were washed for 30 minutes in distilled water just prior to application of the autoradiographic stripping film. Autoradiography was performed using the Kodak AR-10 stripping film technic.[10] It was established that an exposure time of 30 days at 4 C. yielded satisfactory autoradiograms with low background. The microautoradiograms were developed in Kodak D-19 developer at 20 C., washed, and fixed in Kodak acid fixer or 24 per cent sodium thiosulfate. Some sections were stained prior to application of the stripping film with Mayer hematoxylin and eosin, Sudan black-B in propylene glycol, carmalum, or basic fuchsin. Most of the preparations were post-stained with Ehrlich hematoxylin.[10] At least 6 slides were prepared from each tissue.

All fresh-frozen, formalin, and McManus fixed tissue specimens after cryostat, carbowax, or paraffin sectioning were directly prepared for microautoradiography while duplicate slides were subjected to one of the lipid extraction procedures. Extraction with 95 per cent ethanol was followed by pyridine for 30 minutes at 22 C., for 24 hours at 60 C., and then washed in water for 2 hours.[10] The method of Keilig was also employed using acetone at 22 C. and 60 C. for 3 hours followed by ether: chloroform: methanol (1:1:1) for 12 hours at 60 C.[10] Bouin, Carnoy, and cryostat sections post-fixed in 80 per cent ethanol were in some instances also subjected to one of these lipid extraction technics. More vigorous methods employing organic solvent combinations at higher temperatures were employed, but they resulted in unsatisfactory histologic and autoradiographic preparations.

Quantitative silver grain counts were carried out under oil immersion using calibrated linear and grid micrometers fixed in the microscope eyepieces. The grid contained 49 equal squares, each encompassing a 10.2 x 10.2 μ area, with a total area of 4998 μ^2. On the linear micrometer the smallest division equaled 0.51 μ. Counting was done at random in at least 4 areas of the slide to determine background grain counts. Slides were rejected from the study if background scatter exceeded 15 grains per 1000 μ^2. Four areas of each slide preparation were selected at random under low magnification. Under oil immersion, grain counts were enumerated three ways in each 5000 μ^2 area: a total grain count was determined; all grains over cells or within 5 μ of any identifiable cell margin were counted, and total grain count over each cell was individually recorded. Total cells per 5000 μ^2 were counted and classified as fibroblast, macrophage, polymorphonuclear leukocyte, lymphocyte, plasma cell, mast cell, endothelial cell, foreign body giant cell, or undetermined. In order to establish a basis for comparing grain counts over foreign body giant cells with the other cell types, each giant cell was measured using the linear micrometer. Total grains per giant cell has been calculated per 30 μ^2 area. The total grain count per tissue area has been corrected for background grain count and reported as net grains per 1000 μ^2. The total grain count over cells, as defined above, has been expressed as a decimal fraction of the total grain count per 5000 μ^2 area of tissue. Since no uniform method was available for correction of background grain count over individual cells such counting was done only on preparations with background counts less than 10 per 1000 μ^2.

Radioactivity was also measured in tissue sections and aliquots from the fixatives and histologic solvents using the Packard Tricarb Spectrometer and a liquid scintillation system.[7] These measurements were used to follow changes in radioactivity that occurred during the process of preparation of the microautoradiograms.

Results

Quantitative grain counts were carried out on preincubation tissue sections, sections incubated for zero time, and specimens prepared from material subjected to cryogenic shock or desiccation. Irrespective of tissue age, method of fixation or preparation of these sections, net mean grain counts and S.E.M.* was less than 2.1 $\pm$ 1.5 per 1000 μ^2 above background. Individual preparations with total background grain counts greater than 15 grains per 1000 μ^2 were rejected from the study. In these control preparations the proportion of silver grains found over cells per

*Standard error of the mean.

Table 1.—*Comparison of Mean Grain Counts on Duplicate Preparations After Various Periods of in Vitro Incubation*

Tissue Age, Days	Fixative	Incubation Hours	Net Grains 1000 μ^2	Cell Grain Fraction
14	Formalin	0	0	
		1.5	31	0.72
		3	34	0.88
		5	36	0.65
26	Formalin	0	2	
		1	99	0.38
		2	36	0.37
		3	95	0.54
	McManus	0	0	
		2	14	0.47
		3	24	0.47
		5	47	0.33
	Bouin	0	0	
		1	31	0.24
		2	61	0.38
		3	67	0.57
	Carnoy	0	0	
		1	15	0.51
		2	8	0.46
		3	42	0.42
42	Formalin	0	0	
		1.5	35	0.43
		2	38	0.50
		3	140	0.36
		5	103	0.43

unit area was 0.41 ± .04, (mean ± S.E.M.). In the preincubation and zero time sections the mean and S.D.† for maximum total background grain count over fibroblasts was 1.0 ± 0.6, macrophage 0.9 ± 0.5, and all other cell types 1.2 ± 0.8. Localization of silver grains over individual cells was undetected in a majority of the control tissue sections. Based upon these data, grain counts over individual cells in the in vitro incubated specimens have been reported as total values with no attempt made to correct individual values for background. To further reduce any contribution from background scatter, only individual cell counts made on preparations with less than 10 grains per 1000 μ^2 background activity have been included in this study.

Since no difference in quantitative grain counts was observed between the various control specimens exposed to glycerol-C^{14}, the procedures used to prepare the autoradiographs appear to have successfully removed unincorporated isotope from the tissue sections. In each incubation preparation grain counts over the sponge prosthesis were no greater than the background grain count, while the tissue itself contained measurable activity. Similarly "wound fluid" in the centrum of 7–14 day preparations, which had not been completely penetrated by tissue ingrowth, yielded grain counts no greater than background. These observations added additional evidence that passive diffusion or entrapment of unincorporated glycerol-C^{14} did not contribute to recorded radioactivity in the tissue sections.

Microautoradiograms of in vitro incubated open wound granuloma slices were included in this study so that any unusual effect of the polyvinyl sponge prosthesis on glycerol-C^{14} incorporation by new formed connective tissue elements could be excluded. Results of this comparative study are to be found in each group of data included in this report.

No significant difference in net grain counts or individual cell counts was found for microautoradiograms of formalin-fixed tissue sections prepared by the cryostat or carbowax embedding technics. The results obtained by these two methods will be presented jointly, and represent the histologic procedures presumed to have best preserved tissue lipids. In Table 1 the duration of in vitro incubation, method of tissue fixation, and effect of tissue age on net grain counts and cell-localized radioactivity are summarized. An increase in mean net grain count was noted as the time of in vitro exposure was lengthened. Retained radioactivity in formalin-fixed tissue increased with tissue age between 14 and 42 days. The method of fixation and preparation influenced the net grain count as illustrated for

†Standard deviation.

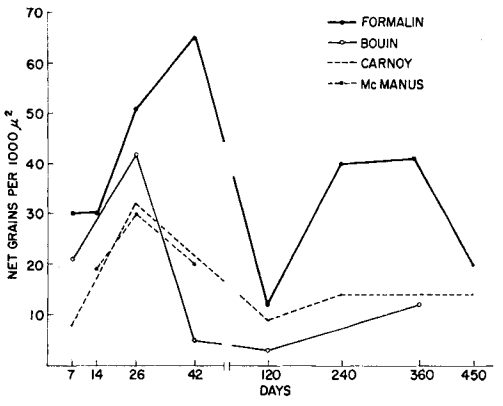

Fig. 1.—The effect of tissue age and method of tissue fixation on net grain counts following in vitro exposure of polyvinyl sponge granuloma slices to glycerol-C^{14}.

the 26-day specimens. In the 14 day specimens cell-localized grain counts were higher in formalin-fixed material than recorded at 26 and 42 days ($p < .01$). No significant difference between the individual values for cell-localized radioactivity was found at 26 days when the methods of fixation were compared. These results indicated that 3 hour in vitro incubations yield essentially maximal grain counts, and have been used to evaluate microautoradiographic differences between tissue slices 7–450 days of age. As indicated in Figure 1, formalin-fixed preparations contained the greatest amount of radioactivity per unit area. The changes in mean grain counts for the several methods of preparation followed a similar pattern with increase in tissue age. A significant difference between the mean value of formalin and all other fixative procedures was found at 42, 360 and 450 days ($p < .05$). The lowest values recorded occurred in 120 day granulomas. At this tissue age a series of spontaneously reversible retrogressive changes, as well as suppression of lipid synthesis,[8] have been consistently observed (56–180 days) in the polyvinyl sponge granuloma.[9]

A complete summary of the results of quantitative microautoradiography is presented in Table 2. Data on 14 day open wound granulomas is also included. The formalin-fixed material prepared to preserve tissue lipids gave the highest grain counts per unit area, and per individual cell type. Fixation with Bouin, Carnoy, or McManus solution followed by routine preparation of paraffin sections, which are known to extract tissue lipids, reduced radioactivity in an independent manner both over cells and in extracellular areas. Cryostat sections of fresh frozen 26 day tissue when post-fixed in 80 per cent ethanol retained the least amount of radioactivity. Total grain mean counts for fibroblasts and tissue macrophages in formalin preparations are graphically presented in Figure 2, and in Table 2 are compared with the other methods of tissue preparation. Essentially equal radioactivity was found at each tissue age for both cell types in the formalin preparations, although the mean value differed by more than one standard deviation at 42 and 450 days. Cells in 14 day open wound granulomas were labeled as heavily as those in 42 day sponge granulomas. Individual cells, irrespective of the method of tissue preparation, demonstrated wide variation in total grain counts. This variation in grain count followed a consistent pattern where cells were either highly labeled, contained minimal activity, or were unlabeled. To compare grain counts over the large foreign body giant cells with other cell types, the area occupied by these cells was measured with a linear micrometer. When total grain counts per 30 μ^2 were compared with other cells, the foreign body giant cell generally contained less activity per unit area. An exception is noted for 14 day formalin-fixed preparations. These cells are most numerous and randomly distributed in granulomas 14–42 days of age, and tend to be absent in the older sponge implants.[9]

Cells derived from the blood, mast cells, plasma cells, and endothelial cells could be identified in the granulomas. Grain

Table 2.—*Variation in Net Tissue and Cell Grain Counts with Tissue Age and Method of Tissue Preparation (Three Hour In Vitro Incubations)*

Tissue Age, Days	Fixative	Net Grains 1000 μ^2*	Cell Grain Fraction*	Fibroblast†	Macrophage†	FBGC‡ per 30 μ^2†	Total Cells 5000 μ^2§	% Cells Identified§
7	Formalin	30§	.46	5 ± 1.5	6 ± 2.5		20	63
14		30 ± 2	.61 ± .05	6 ± 3	4 ± 1	16 ± 2	27	47
26		51 ± 18	.45 ± .04	5 ± 2	5 ± 1	5 ± 1	29	35
42		65 ± 17	.44 ± .05	10 ± 1.2	15 ± 1.3	4 ± 3	15	47
120		11 ± 2	.38 ± .06	2 ± 1	2 ± 1		17	42
240		40 ± 21	.29 ± .05	3 ± 1	2 ± 1		17	41
360		41 ± 15	.42 ± .05	5 ± 2	7 ± 2		12	75
450		20 ± 4	.37 ± .01	3.7 ± 0.5	5.3 ± 0.5	4	17	53
7	Bouin	21 ± 1	.64 ± .12					
26		42 ± 9	.41 ± .05	9 ± 1	8 ± 1	2	11	68
42		5 ± 2	.72 ± .09	1 ± 0	1 ± 0.5	0.3	30	46
120		3 ± 1	.40 ± .06	2 ± 1	3 ± 1		11	52
360		12 ± 4	.32	3 ± 1	2 ± 0.5	1.1	9	44
7	Carnoy	8 ± 4	.42 ± .16	2 ± 1	2 ± 1		22	54
14		18 ± 11	.42 ± .12					
26		32 ± 9	.45 ± .04	5 ± 2	7 ± 3	2	21	51
120		9 ± 4	.44 ± .08	2 ± 2	6 ± 3		24	58
240		14 ± 7	.34 ± .02	3 ± 1	4 ± 1		11	36
360		0	.24	2 ± 2	2 ± 0.5		9	66
450		14 ± 1	.46 ± .04	7 ± 1	8 ± 4	2 ± 1	19	25
14	McManus	19 ± 7	.31 ± .06	7 ± 1	8 ± 1		19	37
26		30 ± 7	.34 ± .03	9 ± 3	9 ± 2	3 ± 2	17	43
42		20 ± 9	.49 ± .13	9 ± 3	7 ± 3		17	58
26	Ethanol	24 ± 8	.34 ± .04	3 ± 1	3 ± 0.3		16	37
14	Formalin	150 ± 27	.46 ± .02	12 ± 3	13 ± 2		22	43
(Open Wd.)	McManus	41 ± 7	.33 ± .05	6 ± 1			11	54

*Mean value and standard error.
†Mean value and standard deviation.
‡Foreign body giant cell.
§Mean value.

counts for these cells were similar to those recorded for labeled fibroblasts and tissue macrophages. Red blood cells present within small blood vessels contained no net radioactivity in any preparation examined. The total number of cells per 5000 μ^2 was highest in the younger granulomas and in general decreased with tissue age. Since the four standard fields on each slide preparation were selected at random, individual counts varied considerably from these mean values, reflecting the low cell density per unit area which is typical of these granulomas. Positive identification of the morphological cell type was possible in approximately one-half of the cells per area examined. There was no essential difference between the several methods used to prepare the tissue for histologic study. Grain counts were recorded for all cells per unit, and there was no evidence that unidentified cells as a group contained more radioactivity than those classified by morphological cell type. Except for preparations stained with hematoxylin and eosin, monochromatic dyes which preferentially stained nuclei were most successfully used in processing the microautoradiograms. The results obtained wtih 26 day tissue incubated zero time (Fig. 3), and for 3 hours in vitro (Fig. 4) are illustrated. In these Carnoy fixed specimens the silver grains reflecting

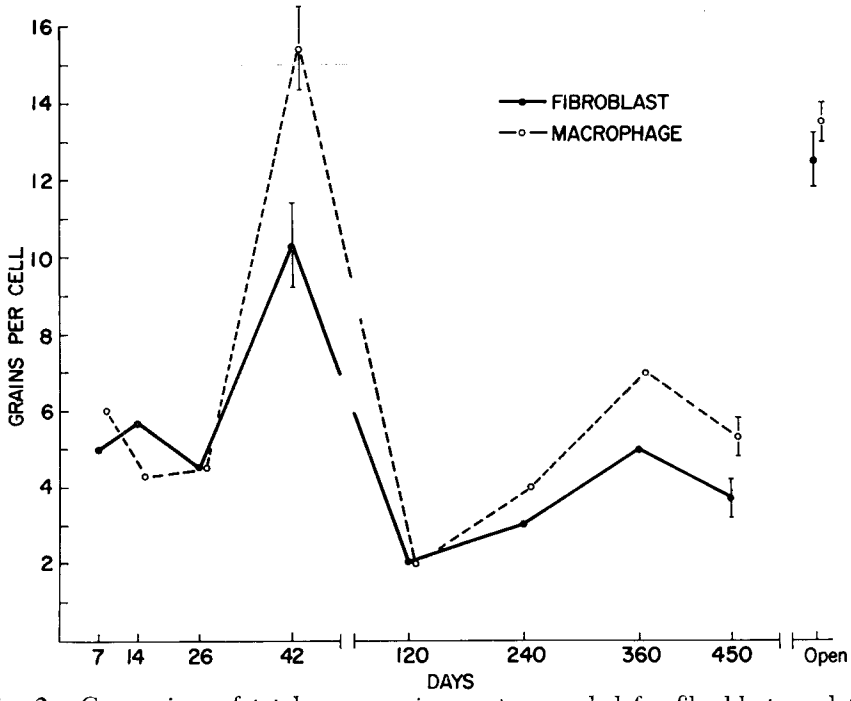

Fig. 2.—Comparison of total mean grain counts recorded for fibroblasts and tissue macrophages present in 7–450 day polyvinyl sponge and 14 day open wound granulomas after 3 hour in vitro incubation with glycerol-C^{14}. Tissue specimens were formalin fixed and tissue sections prepared on a cryostat or by the carbowax embedding technic. One standard deviation of the mean is indicated by the vertical lines.

background activity in the zero time preparation were easily distinguished from hemosiderin deposits present in the tissue and within macrophages. In the 3 hour specimen, radioactivity was present over fibroblasts, a foreign body giant cell, and in extracellular areas. In Figure 5 the grain distribution found with 42 day formalin-fixed material was essentially equal over cells and in the interstitium. Individual cells demonstrated the usual variation in cell-localized total grain counts. Due to restriction of the depth of field of the photographic image at high magnification, the visible grain counts are lower than those obtained on the same preparations when they are viewed under the microscope where the entire thickness of the autoradiographic emulsion could be examined.

In this study all silver grains identified over cells or within 5 μ of a cell margin have been enumerated as cell-localized radioactivity. This grain count has been referred to as the cell grain fraction (Table 2) and represents that portion of the total grain count per unit area assumed to be derived from intracellular localization of isotope. Irrespective of the method of tissue preparation approximately one-half of the total grain count was found in extracellular locations. By employing special photographic masking procedures the actual silver grain density as observed in the microscopic preparations is shown in Figure 6. In this 42 day specimen stained with Sudan black-B, cytoplasmic margins of individual cells are more sharply defined so that the tissue distribution of total grain count is more clearly demonstrated. Results obtained by quantitative microautoradiography on sections stained with Sudan black-B agreed with those performed on sections

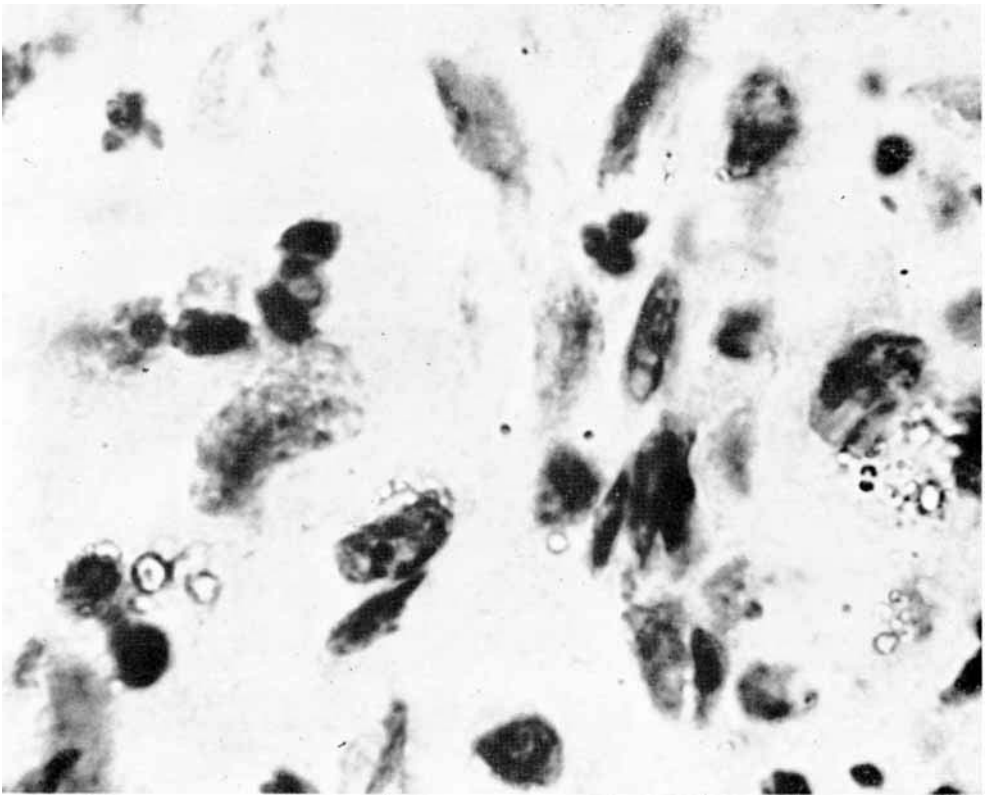

Fig. 3.—An autoradiograph of a 26 day sponge granuloma incubated for zero time with glycerol-C^{14}. This tissue specimen was fixed in Carnoy fluid and 4 μ sections prepared after routine paraffin embedding. Only background silver grains are present in this preparation stained with Ehrlich hematoxylin (1134×).

where the more nucleophilic dyes were employed.

At each tissue age duplicate preparations were subjected to classic histologic methods designed to remove lipids from tissue sections. Use of these organic solvents just prior to application of the autoradiographic stripping film resulted in high background silver grain counts in some of the microautoradiograms. In all specimens that had been incubated in vitro with glycerol-C^{14} a net reduction in grain count was demonstrated with the tissue sections exposed to one of the lipid extraction technics. This was observed whether or not the method of histologic preparation had been designed to preserve the lipid constituents in the tissue specimen. Representative results obtained with duplicate preparations of sponge granulomas 26–120 days of age and 14 day open wound granulomas are presented in Table 3. In all instances in which the net mean grain count was greater than 40 per 1000 μ^2, over 75 per cent of the radioactivity was removed following exposure to one of the methods of lipid extraction. No consistent pattern of preferential removal of isotope from cells or extracellular structures was established by changes in the cell grain fraction.

At the time of histologic preparation of specimens for microautoradiography other sections were placed in a liquid scintillation system containing toluene and radioactivity determined in a spectrometer. Using this method to measure tissue radio-

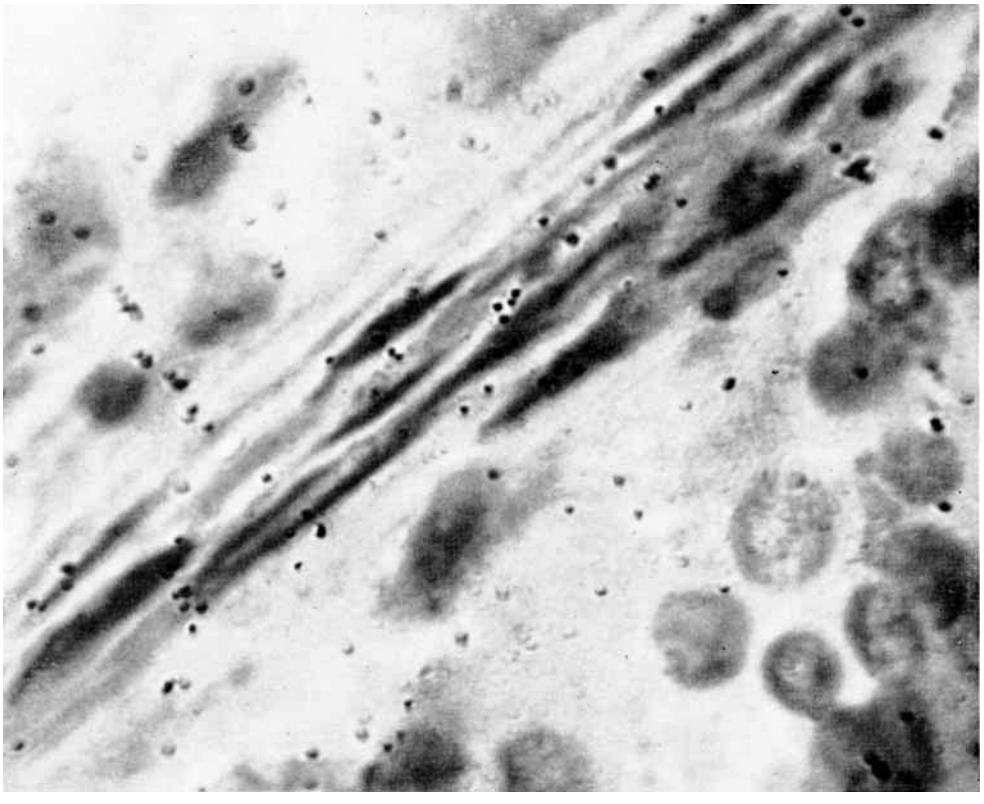

Fig. 4.—This autoradiograph was prepared by the same histologic technic used with the control section shown in Figure 3. This 26 day specimen was incubated for 3 hours with glycerol-C^{14}. A group of fibroblasts are present, and a portion of a foreign body giant cell is included in the lower right section of the photomicrograph (1134×).

activity the results were completely comparable to those recorded by microautoradiography.

Discussion

Interpretation of results obtained with the technic of quantitative microautoradiography requires an appreciation of the factors which influence the development and localization of precipitated silver grains over biological structures. Detailed reviews of the technical variables inherent in the use of this procedure have been presented.[11-14] Of particular importance in the present study is information bearing on the resolving power of the photographic emulsion. Pelc defines this as the smallest distance between two labeled structures which gives two distinct images. For C^{14}-labeled compounds in specimens less than 5 μ thick, using fine-grain stripping film, he reports[12] that resolution of 2 μ can be achieved. Both Pelc and Levi[13] suggest that approximately 2 silver grains are produced per incident C^{14} beta particle. Although the maximum range of C^{14} particles in certain photographic emulsions has been reported as 20 μ, the effective range is considerably less than the theoretical value.[11] In the present study silver grains found within 5 μ of a cell have been assumed to originate from beta particles released from isotope contained within cells. This value appears to be a reasonable approximation based

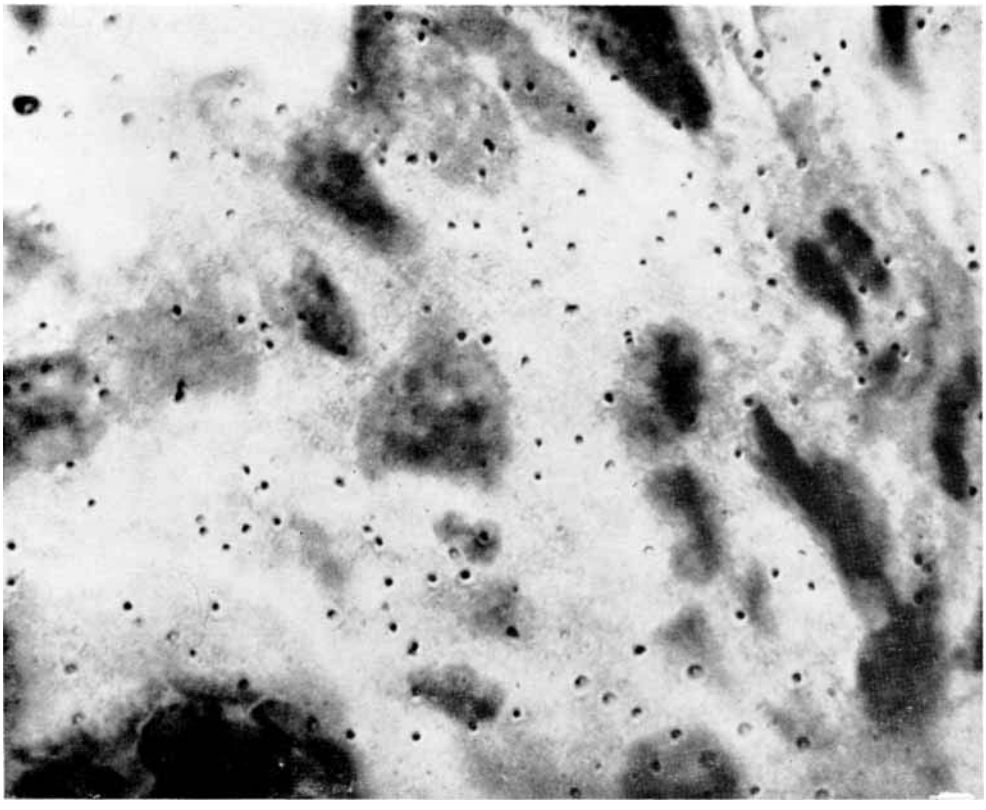

Fig. 5.—This 42 day sponge granuloma was incubated with glycerol-C^{14} for 3 hours, cryostat sections were post-fixed in formalin, and the microautoradiogram was prepared. A foreign body giant cell (lower left), tissue macrophages (center), and fibroblasts (upper right) are present in this tissue section. Stained with Ehrlich hematoxylin (1134×).

upon current theoretical and experimental evidence bearing on this point.[11,12,14]

The present microautoradiographic findings complement previous chemical and radioisotope tracer studies which indicated that the cells in polyvinyl sponge and open wound granulomas were able to synthesize both phospholipids and triglycerides in situ.[7,8] Since the relative proportions of morphological cell types present in these granulomas change with tissue age, the degree of participation of the fibroblast, macrophage, foreign body giant cell or other cellular elements in net lipid synthesis could not be established. The present data indicates that each cell type will incorporate glycerol-C^{14}, and based upon the histologic methods employed the variation in retained radioactivity suggests that at least three-fourths of the isotope has been incorporated into tissue lipids. It was previously demonstrated that no detectable randomization of C^{14}-labeled glycerol to sterols or sphingomyelin occurred in the in vitro system used in this study.[7] Some degree of incorporation of C^{14}-labeled precursor into nonlipid constituents cannot be excluded. The residual net grain counts recorded for tissue sections subjected to classic methods of lipid extraction could be interpreted as indicating that some of the detected radioactivity was in nonlipid structures. At the same time presently available histochemical technics are not absolute

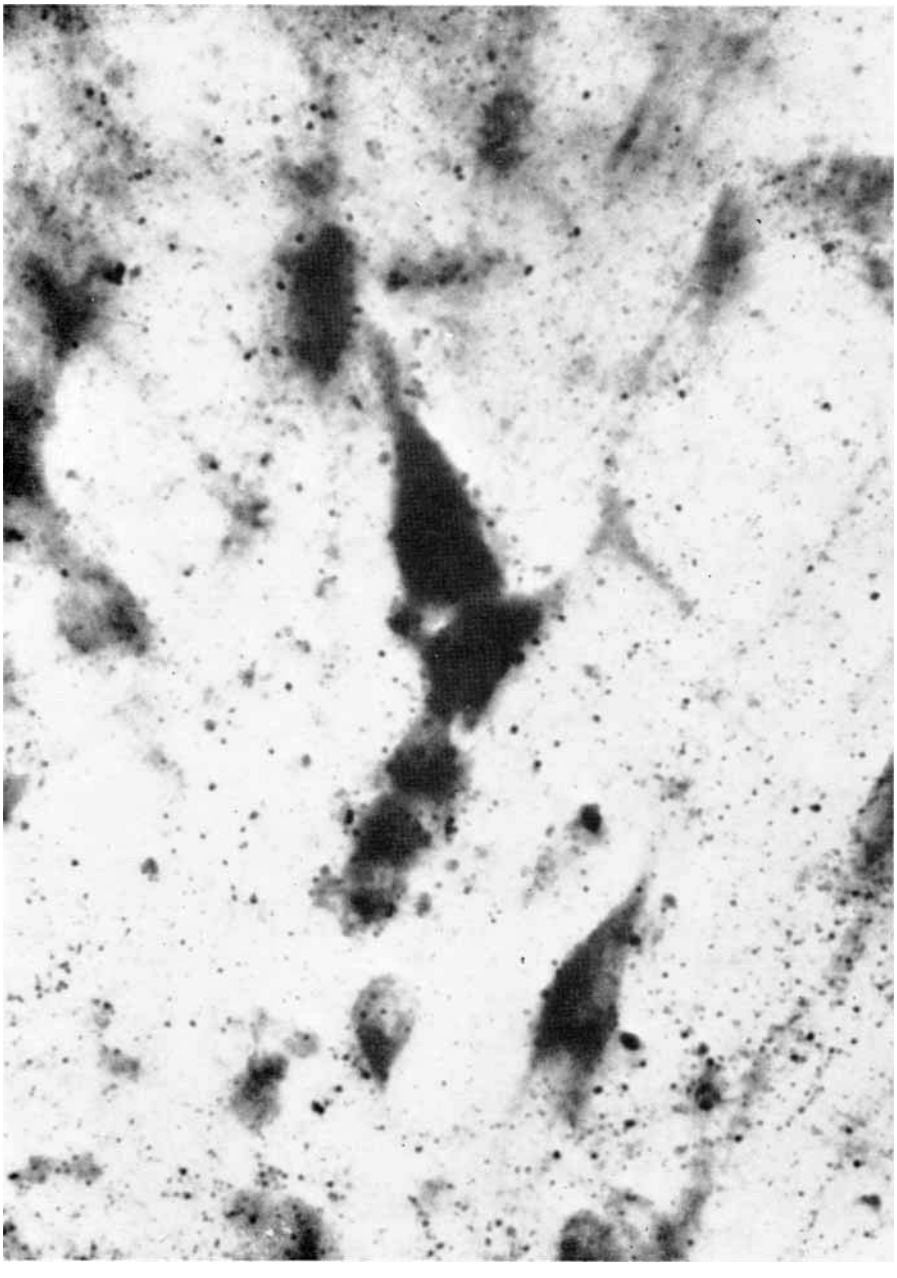

Fig. 6.—This autoradiogram of a 42 day sponge granuloma incubated for 3 hours with glycerol-C^{14} was photographed using a special masking technic to fully visualize the total silver grain content and the cell margins. The formalin-fixed specimen was sectioned using the carbowax embedding technic. Staining was carried out with Sudan black-B, and the coarse nonspherical extracellular particulates are sudanophilic droplets which should be distinguished from the intracellular and extracellular silver grains (512×).

Table 3.—*Mean Reduction of Grain Counts in Paired Preparations Following Extraction of Sections with Organic Solvents*

Tissue Age, Days	Animal	Fixative	Incubation Hours	Net Grains 1000 μ^2	Cell Grain Fraction	Extraction	% Reduction
26	167	McManus	3	24	0.47	None	79
				5	0.30	Pyridine	
	168		5	47	0.33	None	
				10	0.16	Pyridine	79
	154		5	67	0.32	None	
				13	0.67	Pyridine	81
26	251	Formalin	2	95	0.61	None	
				8	0.46	Keilig	91
	167		3	18	0.47	None	
				9	0.42	Keilig	50
26	251	Ethanol	2	34	0.28	None	
				12	0.25	Pyridine	65
42	161	Formalin	3	140	0.36	None	
				20	0.48	Pyridine	86
			5	108	0.43	None	
				23	0.41	Pyridine	79
	166		5	41	0.63	None	
				2	0.57	Pyridine	95
120	254	Bouin	3	4	0.51	None	
				0	0.29	Keilig	100
		Carnoy	3	16	0.44	None	
				12	0.60	Keilig	25
		Formalin	3	14	0.31	None	
				6	0.34	Keilig	57
14	173	Formalin	3	141	0.53	None	
(Open Wd.)			3	0	0.42	Pyridine	100

methods for the identification of all tissue lipids. Sidman[15] and other investigators[16,17] have critically explored these technical limitations.

One long-term objective of the study of lipids and lipid synthesis by the cells of connective tissue has been to accumulate evidence for the extracellular distribution of lipids (lipoproteins). Comparison of the results of chemical studies on experimental granulomas[7,8] and characterization of the lipid constituents of in vitro cultivated fibroblasts[18] suggested that some of the lipid content of inflammatory granulomas must occur in the interstitium. The microautoradiographic findings support this impression and are interpreted as indicating that over a wide span of granuloma age (7–450 days) approximately one-half of the incorporated glycerol-C^{14} was identified in extracellular areas. Similar results were obtained with open wound granulomas.

It has been demonstrated that a rapid change in the specific activity of phospholipids occur during the process of phagocytosis.[6] Evidence has been presented suggesting that mucopolysaccharides will alter lipid composition in in vitro cultivated cells,[19] and physiochemical studies have been reported which demonstrate that chemical interactions occur between lipoproteins and polysaccharides.[20] Most recently a potential biochemical interrelation between glycolipid and glycoprotein synthesis has been proposed.[21] Studies of this type suggest that a series of important physiologic relationships exist between lipid biosynthesis and the other well-defined

functions of cells present in connective tissue. The current study indicates that lipid synthesis occurs in each cell type present in experimental connective tissue, and that lipids (lipoproteins) are present as "ground substance" constituents of connective tissue.

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SUMMARY

The in vitro incorporation of glycerol-1,3-C^{14} by polyvinyl sponge and open wound granuloma slices was studied by the technic of quantitative microautoradiography. Silver grain counts over cells changed with the age of the tissue. Each morphologic cell type present in the granuloma was found to incorporate glycerol-C^{14}. The method of histologic preparation of the tissue sections directly influenced the net grain count per unit area. By comparison of procedures designed to preserve or to extract lipids selectively from the tissue it was shown that over 75 per cent of the radioactivity was present in the lipid constituents of the tissue. At least 50 per cent of the total grain count was distributed in extracellular areas, which suggests that lipids occur as "ground substance" constituents in experimental granulation tissue.

SUMMARIO IN INTERLINGUA

Le incorporation in vitro de glycerol-1,3-C^{14} per sectiones de granuloma a spongia polyvinylic e de vulnere aperte esseva studiate per le technica de quantitative microautoradiographia. Numerationes del granos de argento super le cellulas variava con le etate del tissu. Esseva trovate que omne typos morphologic de cellula intra le granuloma incorporava glycerol-C^{14}. Le methodo del preparation histologic applicate al sectiones tissular exerceva un directe influentia super le nette numeration de granos per unitate de area. Per medio de un comparation del technicas visante a preservar o a extraher lipidos selectivemente ab le tissu il esseva monstrate que plus que 75 pro cento del radioactivitate esseva presente in le constitutuentes lipidic del tissu. Al minus 50 pro cento del numeration total de granos esseva distribuite in areas extracellular lo que suggere que lipidos occurre como constituentes de "substantia de base" in experimental tissu de granulation.

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