Wound Healing: Investigation of Proteins, Glycoproteins, and Lipids of Experimental Wound Fluid in the Dog.*† (24937)

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Wound fluid provides an external environment for proliferating fibroblasts and remains in contact with repair tissue of experimentally induced wounds for prolonged periods of time. Due to technical difficulties in obtaining this fluid, little is known of its biochemical composition and alteration during time-course of healing. A method of obtaining wound fluid was developed by Schilling *et al.*(2,3). Stainless steel mesh cylinders were implanted subcutaneously in animals and used to collect bacteria-free wound fluid which accumulates within the cylinders at interface of fibroblastic response. Previous work with guinea pigs (3) indicated that such fluid differed in chemical composition from corresponding blood plasma. It appears likely that time-related changes may occur in content of this fluid during healing. This study was designed to investigate wound fluid throughout 1 to 4 week post-wound period in the dog where larger amounts of fluid are available from single animals.

Materials and methods. Cylinders. All cylinders were made from rectangular pieces of #46 stainless steel surgical foundation wire mesh (60 mm x 30 mm). Each was carefully shaped and secured around 2 lucite rings for support. Male dogs of mongrel, short-haired type weighing about 20 kg were bathed, clipped, and provided with adequate diet. Sterile surgical procedures were employed for implantation and removal of cylinders. Details of surgical method used are reported elsewhere(4). Groups of 16 to 20 cylinders were implanted into subcutaneous tissue along each dog's back. At designated times during healing period (7, 14, 21 or 28 days) cylinders were removed and fluid contents aspirated and pooled. Blood samples were drawn from femoral artery before implantation and before removal of cylinders. In this way changes in wound fluid components could be observed and compared with serum of the wounded animal during all periods. All equipment for collecting fluid was sterilized by autoclaving. Bacteriological checks were made to detect contamination in any samples. The few samples that were bacterially contaminated were discarded. During the first week fibrous tissue encapsulated the cylinders and clear yellow fluid could be aspirated from the interior; about 0.5 ml of fluid/cylinder was present at 5th day after implantation. This increased to a maximum of 2 ml about the 14th day. The volume then decreased as the lining of dense fibro-collagenous connective tissue proliferated inside the cylinder until the 6th week when 1.25 ml of fluid remained. One cylinder which remained in a dog for 8 months still contained nearly 0.5 ml of fluid. Paper strip electrophoretic studies were carried out using Spinco Model R electrophoresis cells by the method of Block, Durrum, and Zweig(5) using .075 M barbital buffer, pH 8.6. Whatman 3 mm paper strips and a current of 5 MA/8 strips were employed. After drying at 110° for 30 minutes, strips were stained with bromphenol blue for protein estimation. For glycoprotein, duplicate strips were stained using periodic acid-Schiff method of Koiw and Gronwall(6) as currently used by Shetlar *et al.*(7). Strips for lipoprotein studies were stained with a saturated solution of Oil Red O in 60% ethanol by the method of Jenck, Durrum, and Jetton(8). A Spinco Analytrol was used for quantitation of dyed strips using #550 interference filter for glycoprotein strips and #500 filter for both protein and lipid strips. Total area under the protein curve was equated to total protein as determined by the biuret method(9), and the glycoprotein curve was equated to total glycoprotein (protein-bound hexose) as estimated by tryptophan method of Shetlar, Foster, and Everett (10). The lipoprotein curve was equated to

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TABLE I. Comparison of Some Constituents of Wound Fluid with Blood Serum during 28-Day Post-Wound Period in the Dog.

Days post-wound	Group	Total protein, g %	Total glycoprotein	Seromucoid	Total lipid	Total phosphatide	Total cholesterol	Free cholesterol
			mg %					
0	Control serum	6.34 ± .12* (18)	121 ± 4 (18)	14.9 ± .3 (3)	607 ± 15 (7)	353 ± 13 (7)	128.3 ± 6.6 (7)	32.2 ± 2.2 (7)
1–14	Serum	6.28 ± .13 (13)	122 ± 5 (13)		615 ± 23 (4)	355 ± 21 (4)	127.0 ± 9.9 (4)	33.1 ± 3.1 (4)
	Fluid	4.02 ± .11† (13)	79 ± 4‡ (13)		327 ± 45 (4)	158 ± 31 (4)	74.8 ± 5.0 (4)	21.4 ± 2.7 (4)
15–28	Serum	6.21 ± .15 (10)	108 ± 6 (10)	14.4 ± .8 (3)	585 ± 29 (4)	337 ± 17 (4)	110.8 ±10.3 (4)	27.5 ± 1.4 (4)
	Fluid	3.28 ± .15† (10)	66 ± 3‡ (10)	12.3 ± .8 (3)	224 ± 25 (4)	94 ± 8 (4)	60.5 ±11.2 (4)	14.5 ± 3.6 (4)

Mean values ± stand. error; No. of animals studied in parentheses. † Protein significantly decreased between these 2 time periods ($P < .01$). ‡ Glycoprotein significantly decreased between these 2 time periods ($P < .021$).

total lipid content as determined by method of Sperry and Brand(11). Total and free cholesterol were determined using revised method of Sperry and Webb(12), and total lipid phosphorus was done with modification of Youngburg method as described by Hawk, Oser, and Summerson(13).

Results. Wound fluid had significantly lowered amounts of protein, glycoprotein, and lipid when compared to blood serum during the entire period (Table I). In the first 2 weeks after wounding, this fluid contained 64% as much protein, 65% as much glycoprotein, and 53% as much lipid as did serum. Significant decreases were demonstrated between protein and glycoprotein contents of fluid at 1 to 2 weeks and those at 3 to 4 weeks. By 3rd to 4th week post-wound, wound fluid had decreased by 19% in protein, 17% in glycoprotein, and 32% in lipid. Lipid values of fluid when considered relative to protein showed the greatest changes in phosphatide:protein ratio (1:25 at 1-14 days and 1:35 at 15-28 days). However cholesterol:protein ratio (1:54) was relatively unchanged.

All electrophoretically classified groups of proteins found in serum were also present in wound fluid. In some of the electrophoretic fractions, however, quantitative differences were found between fluid and serum. At all times during healing, a greater proportion of albumin was found in wound fluid as compared with serum (Table II). The per cent of alpha-2 and beta-1 globulins in wound fluid was lower than in serum. Total glycoprotein content of fluid showed a smaller decrease during healing than either protein or lipid. Since glycoprotein content did not decrease as much in fluid as did protein, then total glycoprotein to protein ratio increased (Table III). However, the seromucoid fraction of Winzler and Weimer was at the same level (in terms of mg/100 ml of fluid) in both

TABLE II. Electrophoretic Distribution of Proteins in Wound Fluid as Compared to Serum in Dog.

Days post-wound	Group	No. dogs	% albumin	% globulins				
				$Alpha_1$	$Alpha_2$	$Beta_1$	$Beta_2$	Gamma
0	Control serum	18	41.5 ± 1.8*	5.4 ± .3	10.8 ± .6	14.3 ± .7	16.0 ± .9	12.2 ± .9
1–14	Serum	13	41.4 ± 2.2	5.3 ± .3	10.7 ± .7	14.5 ± .7	15.4 ± .7	12.9 ±1.1
	Fluid	13	45.2 ± 1.8	5.8 ± .4	9.8 ± .6	12.7 ± .7	15.8 ± .7	10.8 ±1.0
15–28	Serum	10	43.6 ± 2.6	5.8 ± .1	9.6 ± .4	13.6 ± .7	15.6 ±1.2	12.0 ±1.1
	Fluid	10	48.4 ± 2.3†	6.0 ± .4	8.6 ± .4‡	11.8 ± .7‡	15.2 ±1.2	10.2 ± .7

* Mean ± stand. error. † Significantly higher than in serum at 0-14 days ($P < .02$).
‡ Significantly lower than in serum at 0-14 days ($P < .02$ and $P < .01$).

TABLE III. Electrophoretic Distribution of Glycoproteins in Wound Fluid as Compared to Serum in the Dog.

Days post-wound	Group	No. dogs	Albumin	Bound hexose* associated with Globulins: Alpha$_1$	Alpha$_2$	Beta$_1$	Beta$_2$	Gamma	Total
0	Control serum	18	.46 ±.03†	4.90 ±.34	5.75 ±.25	2.65 ±.14	1.93 ±.09	1.54 ±.11	1.92 ±.06
1–14	Serum	13	.51 ±.04	5.58 ±.36	5.83 ±.29	2.62 ±.14	1.88 ±.17	1.51 ±.12	1.95 ±.09
	Fluid	13	.49 ±.04	5.14 ±.38	6.07 ±.28	3.08 ±.20	1.97 ±.14	1.64 ±.12	1.97 ±.08
15–28	Serum	10	.46 ±.04	4.24 ±.43	5.20 ±.25	2.51 ±.15	1.82 ±.12	1.45 ±.12	1.75 ±.09
	Fluid	10	.59 ±.06	4.92 ±.28	5.88 ±.33	3.47 ±.24‡	2.45 ±.16‡	1.99 ±.16‡	2.03 ±.07

* Expressed as % of protein moiety. † Mean ± stand. error. ‡ Glycoprotein higher in fluid than in serum at this time. ($P < .01$, .01, and .05 respectively.)

fluid and serum (Table I). When corrected for seromucoid content, the wound fluid glycoprotein:protein ratio was similar to that of corresponding serum. Electrophoretic studies also indicated that beta and gamma globulin fractions of wound fluid had comparatively more protein-bound hexose. Table IV shows results from lipid stained strips, which were evaluated in terms of 2 well-defined zones visible called alpha and beta lipoprotein. Wound fluid showed lowered amounts of both fractions compared with the corresponding serum in the 3rd to 4th week post-wound group.

Discussion. Changes in amounts of various components in wound fluid suggest that alterations in permeability for certain molecules in the wounded area have occurred at cell-wall or capillary interfaces. Albumin is significantly elevated in wound fluid, whereas larger protein components, particularly beta and gamma globulins, tend to be lower. The same level of seromucoid in this fluid and serum seems to indicate that this component passes freely from blood stream into the wound fluid in cylinders. Similar findings in comparative studies of lymph and serum of dogs following thermal injury have been reported(14).

TABLE IV. Protein-Bound Lipid Stained with Oil Red O after Paper Electrophoresis.

Days post-wound	Group	No. dogs	Lipid: Expressed as % of total protein in each: Alpha	Beta
0	Control serum	7	6.95 ± .17*	2.29 ± .21
1–14	Serum	4	7.20 ± .37	2.26 ± .39
	Fluid	4	6.99 ± .54	1.25 ± .22
15–28	Serum	4	7.39 ± .34	1.74 ± .18
	Fluid	4	5.66 ± .42†	1.03 ± .16†

* Mean ± stand. error.

† Significantly lower in wound fluid than in serum at this time ($p < .01$ and $p < .04$).

In previous work with guinea pigs(3) a comparison was made of wound fluid with plasma using a moving boundary electrophoresis method. Elevations were found in alpha-2, beta, and gamma globulins of 7th day wound fluid samples. Species differences, or degree of injury to animal, may explain differences noted between the 2 studies.

Elevated glycoprotein values of wound fluid as compared with corresponding serum are of interest. The increase of bound carbohydrate (when expressed as percentage of protein) in the alpha globulin fraction may be explained by comparatively high seromucoid. However, the elevated bound carbohydrate of beta and gamma fractions requires other explanations. One possibility might be that the reparative process utilizes, preferentially, proteins from these fractions with low carbohydrate content leaving those fractions with higher bound carbohydrate. On the other hand, these glycoproteins may be elaborated by cells of the reparative tissue. Additional studies will be necessary to clarify these points.

Summary. Surgical implants of stainless steel mesh cylinders were made into subcutaneous tissue of dog's back. These were removed at intervals of 1 to 4 weeks. Wound fluid was aspirated from inside these cylinders encapsulated with fibrous tissue. When compared with animal's serum, this fluid was lower in protein, glycoprotein, and lipid; and these components decreased with time during post-wound period. As studied by paper elec-

trophoresis, wound fluid had a higher per cent of albumin and lower alpha-2 and beta-1 globulin. An elevation of protein-bound hexose was found in beta and gamma globulin fractions at 3 to 4 weeks. Seromucoid was present at same level in wound fluid and serum. The lipid partition revealed especially low phosphatides but similar cholesterol:protein ratios. It appears that time-related changes occur in large molecular constituents of fluid found in wound area which may be significant in wound healing phenomenon.

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Alterations of Gamma Globulin with Plasma Cell Neoplasm in Mice.* (24938)

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Potter, Fahey and Pilgram described a transmissible plasma cell neoplasm in C3H mice, which was distinguished by production of gamma globulin(1). Nathans, Fahey and Potter measured uptake of *l*-lysine-C^{14} by tumor and demonstrated that gamma globulin formed within tumor was released into general circulation(2). The following investigation was carried out with the intent of further characterizing alterations of plasma protein after partial ablation of tumor and during the course of homologous transplantation of tumor.

Materials and methods. The plasma cell neoplasm, X5563, transfer generation 15, was obtained in its subcutaneous form in 2 living C3H mice.‡ Continuous subcutaneous transmission for 5 generations has been carried out in C3H mice,§ ranging from 6 weeks to 3 months in age at time of transplantation. Subcutaneous transplantation of minced tumor tissue was completed within 1 hour following removal from donor animal. Swiss mice have been used in homologous transplantation experiments. Blood was collected from tail vein in heparinized capillary tubes. Follow-

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