Biochemical Changes Occuring during the Development of a Turpentine Granuloma

A series of models has been suggested for the study of the non-specific inflammatory reactivity of the connective tissue. Biochemically and morphologically best known is the so-called carrageenin granuloma induced by subcutaneous injection of the extract of the alga, *Chondrus crispus*, which proved best for these purposes. Its biochemical profile passes *via* accumulation of desoxyribonucleic acid and of mucopolysaccharides of the ground substance to maximal formation of insoluble scleroproteins from soluble precursors and to slow absorption of the granuloma[1,2]. We intended to ascertain if similar non-specific changes will be observed in the connective tissue components during the development of an experimental granuloma induced by subcutaneous injection of turpentine oil.

Methods. The trial was performed on 75 male Wistar rats, weighing 100 g each. The animals were kept on Larsen diet and water *ad libitum*. Experimental granuloma was induced by subcutaneous injection of 0.2 ml of turpentine oil (*Oleum terebinthinae rect.*). One injection was given into the subcutaneous tissue of the thorax, the second into the abdominal area so as to obtain sufficient amount of tissue. The animals were divided into 5 groups of 15 rats. Different groups were killed by decapitation on the third, fifth, seventh, tenth, and fourteenth day of the trial. The granulomas were extirpated and the granuloma tissue from the thoracic and abdominal area was collected and homogenized with 0.5 ml of 0.1 *N* NaOH in a glass high-speed homogenizer. The homogenate was then dried to a constant weight at 105°C. In the dry material we determined:

(1) The total hydroxyproline after hydrolyzing the whole unmodified tissue with 6 *N* HCl at 105°C during 20 h. For the determination of hydroxyproline, we employed the method according to STEGEMANN in the modification of CHVAPIL[3,4]. (2) Alkali soluble substances containing hydroxyproline (hereinafter ALOH) were obtained with the use of repeated extraction of a part of the homogenate with 0.1 *N* NaOH at room temperature during 26 h. (3) Insoluble connective tissue proteins (scleroproteins) were determined in the tissue remained after extraction of ALOH, by estimation of hydroxyproline content in its hydrolyzate. (4) The desoxyribonucleic acid content was determined, subsequent to extraction with trichloracetic acid, using the method according to SCHNEIDER[5]. (5) The hexosamine content was determined in the tissue using the method according to BLIX[6]. (6) Total nitrogen was determined using the microdifusion method of CONWAY[7].

Results. The results are summarized in the Table. The total hydroxyproline content curve culminates between the fifth and seventh day with subsequent decline toward the tenth and fourteenth day where the granuloma is absorbed (Figure 1). The ALOH levels culminate on the fifth day with subsequent rise in the content of insoluble connective tissue proteins up to the maximum on the seventh day. Then the ALOH levels fall. Later a parallel fall of the levels of ALOH and of insoluble connective tissue proteins occur. On the fourteenth day, the levels of ALOH are higher than the levels of insoluble connective tissue proteins. The desoxyribonucleic acid content reaches its highest peak on the fifth day when also hexosamine content shows its maximum (Figure 2). There is then a continual fall of both substances. It is possible to distinguish three stages of the inflammatory reaction which can be observed also in the development of a turpentine granuloma. The development of this reaction lasts, in some cases, several months (experimental silicosis), in other instances from days to weeks (carrageenin

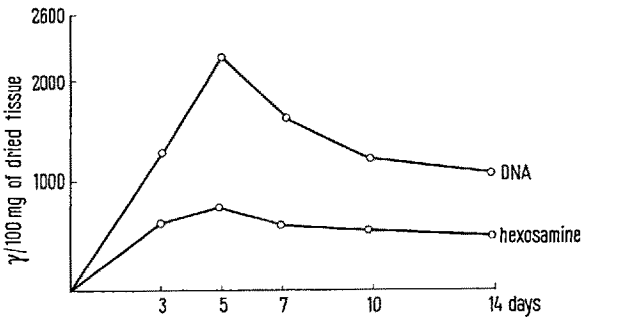

Fig. 1. Biochemical changes occuring during the development of a turpentine granuloma.

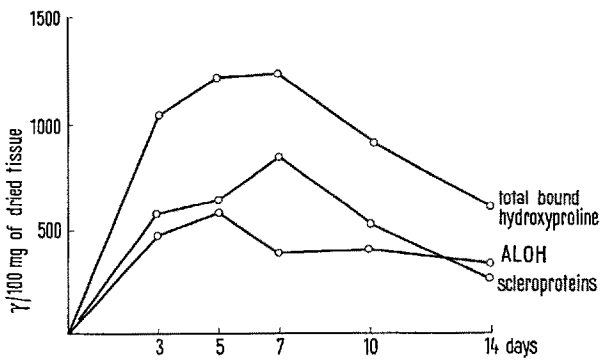

Fig. 2. Biochemical changes occuring during the development of a turpentine granuloma.

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Days	Total hydroxyproline /100 mg of dried tissue	ALOH /100 mg of dried tissue	Insoluble scleroprotein /100 mg of dried tissue	DNA /100 mg of dried tissue	Hexosamine /100 mg of dried tissue	Nitrogen /100 mg of dried tissue
3	1054 ± 97,5	475	579,5 ± 28,6	1262 ± 125,0	646 ± 41,3	4,93 ± 0,33
5	1225 ± 53,0	586	638 ± 39,1	2239 ± 122,8	805 ± 12,5	7,96 ± 0,44
7	1233 ± 47,2	388	845 ± 56,6	1663 ± 72,1	612 ± 78,2	4,88 ± 0,25
10	925 ± 52,0	409	516 ± 38,7	1280 ± 49,0	593 ± 34,6	4,50 ± 0,40
14	600 ± 45,0	330	269 ± 46,6	1143 ± 37,6	562 ± 40,0	5,50 ± 0,65

or turpentine granuloma). In the first stage, activation of cellular elements and mucopolysaccharides of the ground substance occurs—the apex of the desoxyribonucleic acid and hexosamine content in turpentine granuloma attained on the fifth day of the trial. The cellular component and the mucopolysaccharides form the basis for the development of connective tissue proteins. The second stage sets in—as shown by biochemical results—on the seventh day of the development of a turpentine granuloma when the levels of insoluble connective tissue proteins culminate. Finally in the third and last stage, the involved tissue is restored. The results show the turpentine granuloma to be a suitable model for the study of biological functions of the connective tissue in organism.

Zusammenfassung. Die Bildung eines Versuchsgranuloms, bei Ratten mittels subkutaner Injektion von Terpentinöl hervorgerufen, wurde verfolgt. Im Verlauf der Bildung des Granuloms erreichten die Spiegel der Desoxyribonukleinsäure und der Hexosamine am fünften und die Spiegel der unlöslichen Bindegewebeproteine am siebenten Tag des Versuches ihren Höhepunkt. Das Terpentinölgranulom zeigt sich als ein geeignetes Modell für das Studium der Bindegewebereaktivität.

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Effect of X-Irradiation on Triglyceride Metabolism of the Rabbit

The exposure of various animals species to lethal X-irradiation results in significant alterations in plasma lipid and lipoprotein metabolism[1-3]. This is characterized in the rabbit by a pronounced hyperlipemia[4-5].

This study was undertaken to determine if radiation lipemia was due to an impairment in the removal rate of plasma triglycerides. In view of the intimate relationship between various plasma lipids, whereby an elevation in one results in concomitant increases in other lipids[6], the initial accumulation of plasma triglyceride could explain the rise in various other lipid fractions which characterizes radiation lipemia[2,7].

The intravascular removal rate of an injected I^{131} labeled triolein emulsion was measured in normal and X-irradiated rabbits. The concentrations of plasma triglycerides and free fatty acids (FFA) and the extent of hepatic deposition of I^{131} triolein were also determined.

Methods. Blood samples were obtained from the central ear artery of New Zealand Albino rabbits of either sex and weighing approximately 2.5 kg, prior to, and 16 h following sham-irradiation or exposure to 1000 r whole body X-irradiation. The X-ray unit was operated at 250 KVP, 15 ma with HVL of 2.0 mm Cu. The dose rate was 32 r/min at a mid-point distance of 70 cm, control and experimental rabbits were then fasted for 16 h. Plasma triglyceride[8] and free fatty acids[9] were determined. The I^{131} labeled triolein emulsion was prepared as an anhydrous base[10] employing corn oil. The emulsion was administered intravenously 16 h after radiation exposure, as a 15% solution in the amount of 0.5 g of triglyceride/kg. The disappearance of the phospholipid and triglyceride components of this emulsion has been shown to be similar to that of homologous chylomicrons[10] and has been employed as a quantitative index of the intravascular removal rate of emulsified triglyceride[11].

Serial intracardiac blood samples were obtained from 2 to 30 min following the injection of the emulsion in unanesthetized rabbits and total radioactivity determined. The I^{131} labeled lipid in whole blood was precipitated in the presence of carrier iodide by trichloroacetic acid[12] and counted in a scintillation counter. The data was analyzed according to the t-test for paired observations or employing a pooled variance.

Discussion and Results. In both control and irradiated groups the plasma FFA concentrations after 16 h of fasting were significantly higher than the initial values. Since no significant differences were noted between groups, the increased levels reflect the fasting state.

Plasma triglyceride concentrations in all control rabbits were significantly decreased below their initial values. In contrast, the plasma triglyceride concentrations in all the X-irradiated rabbits were significantly increased by a mean of 337% with increases ranging from 18 to 2140% above the pre-irradiation value.

A study of the plasma lipid alterations prior to and during the development of radiation lipemia indicated that plasma triglyceride, phospholipid and cholesterol

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Effect of X-irradiation on lipid metabolism of rabbits

Group and No.		Plasma FFA mEq/L 0 h	Plasma FFA mEq/L 16 h	Plasma Triglyceride mg% 0 h	Plasma Triglyceride mg% 16 h	I^{131} Triolein t/2 min
Control	(6)	0.430 ± 0.041	0.692 ± 0.078	70.7 ± 13.3	40.5 ± 6.1	3.8 ± 0.7
1000 r	(6)	0.434 ± 0.063	0.754 ± 0.112	66.8 ± 17.8	291.9 ± 73.8	4.0 ± 0.6

Values are expressed as means ± standard error. The half-time (t/2) is equal to the time at which the amount of radioactivity present in blood is reduced to one-half its zero-time concentration. This is determined by plotting, semilogarithmically, the blood radioactivity against time.