Diphtheria Antitoxin Formation in the Horse at site of Injection of Toxoid and Adjuvants. (19976)

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Numerous attempts have been made to demonstrate the formation of antibodies at the site of injection of antigen in the skin or subcutaneous tissue. The literature on this subject has been reviewed by Burnet and Fenner(1) and also by Oakley and his associates(2). Employing alum precipitated diphtheria or tetanus toxoids the latter authors found that after a single *secondary* injection of these antigens into the skin, fat or voluntary muscle of rabbits or guinea pigs, specific antitoxin may be formed at the site of the deposition of the toxoids. However, the local formation of antitoxin was not demonstrable in a group of seven horses given a single secondary intracutaneous injection of alum precipitated diphtheria or tetanus toxoids. Evidence for the formation of antibodies in the rabbit at the site of intracutaneous injection of killed tubercle bacilli in paraffin oil was obtained by Westwater(3). He found antibodies fixing complement in the presence of tubercle bacilli in the extracts of the cutaneous nodules before they appeared in the blood.

It may be justified to report the following observation in part because it does not involve the extraction of tissue.

Horse No. 848 was injected repeatedly with concentrated diphtheria toxoid, combined with Falba, paraffin oil and heat killed and dried tubercle bacilli into the deep subcutaneous tissue(4). Some of the injections were made omitting the mycobacteria and in one instance concentrated broth was substituted for toxoid. The 7th, 8th and 10th injections produced areas of inflammation from which fluid could be withdrawn by hypodermic syringe. The fluid was slightly turbid and contained mononuclear cells, polymorphonuclear leucocytes, lymphocytes and a few red blood cells. The eosinophiles were numerous. There were also fragmented, pyknotic cells. The antitoxin titer of the samples of centrifugalized fluid and that of the blood were compared. The titrations were carried out at intervals of from 20 to 50 units by the intracutaneous technic in the rabbit(5). The material injected and the results of titrations are given in the tables.

The results show the concentration of antitoxin was from two to seven times higher in the fluid from the sites of injections of toxoid plus adjuvants than in the blood, while in the fluid from an inflammatory area injected with concentrated broth and adjuvants the anti-

TABLE I. Material and Schedule of Injections.

Inj. No.	Day after 1st inj.	Toxoid Lf*	Falba, ml	Paraffin oil, ml	Killed tubercle bacilli, mg†
1	0	100	.35	1	1
2	1	100	.35	1	1
3	2	200	.7	2	2
4	5	200	.7	2	0
5	6	400	1.4	4	0
6	9	400	1.4	4	0
7	12	800	2.8	8	8
8	14	1000	5.6	16	0
9	72	2000	5.6	16	3
10C	72	Conc. broth	5.6	16	3

* Toxoid was prepared from cultures of diphtheria bacillus grown on broth. The formalized preparation was concentrated by precipitation with acetone. The precipitate was dissolved in saline solution. The vol inj. was twice the vol of Falba.

† Dry wt.

TABLE II. Diphtheria Antitoxin Units per ml of Fluid from Sites of the Injection and of Serum.

Days after inj. of sites			Units/ml Fluid from sites			
No. 7	No. 8	No. 10c	No. 7	No. 8	No. 10c	Serum
12	10		225	375		60
	16			525		75
	18			425		75
	21			350		65
43			375			75
46			170			75
						425*
		8			175	425
		13			175	450

* 68 days after 1st inj.

toxin concentration was lower than in the blood. This observation supports the conclusion that antibodies are formed at the site of injection of antigen combined with adjuvants and is in harmony with the assumption that the role of these adjuvants is to evoke an accumulation of cells about the antigen which produce antibodies(6).

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Occurrence of Tapered Collagen Fibrils from Human Sources with Observations on Mesenchymal Neoplasms.* (19977)

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Collagen fibrils were first recognized in the electron microscope by Hall, Jakus, and Schmitt(1). Since then attention has been focused on their width and striations but there has been little mention of the occurrence of tapered ends. The ends of collagen fibrils from most sources have been described as square or frayed. Gross(2) has noted the presence of an occasional tapered end in preparations of skin collagen, and Porter(3) has mentioned collagen fibrils which he described as having tapered ends in tissue cultures of fibroblasts. It is the purpose of this note to indicate and discuss the relative frequency of occurrence of tapered collagen fibrils from a number of human sources.

Methods. Pieces of tissue, fresh or fixed in 10% formalin were homogenized in a standard Waring blendor or a micro Waring blendor. A drop of the resulting suspension was placed on a collodion-covered specimen grid, allowed to dry and shadowed with palladium. Preparations were examined in the RCA EMU 50 kV electron microscope.

Results. Adult human tissues. Collagen fibrils with tapered ends were encountered only rarely in preparations of Achilles tendon, dura and skin of adult human beings. Tapered fibrils were found in most samples of human endometrium taken at various intervals during the menstrual cycle.

Embryonic human tissue. Almost every collagen fibril from the skin of human embryos (6 and 10 cm crown-rump measurement) was tapered at one end and a significant number were tapered at both ends (Fig. 1). Tapering was present but less frequent in the specimens of embryonic Achilles tendon.

Benign and malignant neoplasms. Seven human fibrosarcomas were examined and tapered collagen fibrils were present in all. The number of tapered fibrils varied considerably among the fibrosarcomas. They were com-

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