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Effect of Chlorophyll Derivative upon Experimentally Produced Lymphedema in Rats. (23674)

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It has previously been shown(1) that edema of the foot and lower leg in the rat can be produced by injecting a suspension of colloidal kaolin about the ankle. If, at the height of the edema, thorotrast be injected subcutaneously into the dorsum of the foot, dilated lymphatic channels can be demonstrated by roentgenogram. These channels extend upward toward the regional lymph nodes, and it is believed, therefore, that the edema is due to lymphatic block near the lymph nodes. This edema will subside 14-17 days after the initial injection and the foot will appear grossly normal, unless infection involves it, in which case the edema may continue indefinitely, and ulceration may result.

Beef hydrolysates might be expected to exert an effect upon the lymphatic channels comparable with the products of infection. In preliminary studies it was found that no sustained edema appeared following injection of beef hydrolysates into the foot of the rat. However, if the foot had been previously conditioned by an injection of colloidal kaolin, an injection of beef hydrolysate either caused the appearance of edema (if none was originally present) or augmented an already

present edema. Edema due to beef hydrolysate also tended to disappear after 14-17 days, but if repeated injections were given, some chronic swelling might be grossly apparent, or, if not grossly apparent, might be shown by surface area measurements.

Materials and methods. Colloidal kaolin was suspended in 3 times its volume of distilled water. At the beginning of the experiment, 1 cc was injected in the form of a ring completely encircling each ankle. At no time was chlorophyll given along with the kaolin. Beef hydrolysate was prepared from Bacto Beef Powder (Difco). The powder was hydrolyzed in aqueous solution for 3 hours at 37°C, using papain as the digestant. A portion of this was mixed with a derivative of chlorophyll (potassium sodium copper chlorophyllin) so that the resultant solution contained 0.2% chlorophyll by weight. Injections were given into the dorsum of the foot, always paired, so that one foot received the beef hydrolysate without chlorophyll, and the other beef hydrolysate with chlorophyll. When repeated injections were given, the same order was maintained in the feet. The volume of each injection was 0.2 cc.

Healthy male rats were used, 18 in number, 8 being included in series #1, and 10 in series #2. The strain, originally Wistar, has perpetuated itself in our colony for 10 years. All rats weighed at least 200 g. Comparisons were always made between the right and left hind feet of the same animal. The rats in series #1 received one kaolin injection at the beginning of the study, and a second injection 54 days later. Series #2 received but one kaolin injection. Injections of beef hydrolysate (with or without chlorophyll) were given on the 7th, 73rd and 100th day in series #1, and on the 19th and 46th day in series #2. The series was terminated on the 119th day for series #1, and the 63rd day for series #2, at which time surface area measurements were made on the paired legs as follows: Under deep nembutal anesthesia, leg circumference was measured to the nearest mm, beginning at the base of the toes and repeatedly up the leg at 3 mm intervals for a distance of 2.4 cm. These circumferences were then plotted on graph paper against the distances up the leg, and the area thus delimited cut out and weighed on an analytic balance. These weights (of graph paper) were then reconverted to sq mm of leg surface area in the usual way.

Results. Visual observations on the paired legs were made 75 times on the 18 rats within the 14 day period following injections of beef hydrolysate. During this acute phase it was noted that the foot receiving the chlorophyll was less swollen than its mate 31 times, more swollen 3 times, and no difference was noted between the two feet on 41 occasions. From observations made at the time surface areas were measured, it appears that the surface area of one foot-leg must be 9-11% greater than its fellow before a difference can be detected grossly.

Table I shows that there is usually a difference between the chlorophyll treated and non-treated leg, the non-treated leg being the

TABLE I. Effect of Chlorophyll Derivative upon Lymphedema.

Series #	Surface area of leg segments in mm ²		Difference in mm ² between 2 legs*
	No chlorophyll	Given chlorophyll	
1	636	590	+ 46
	650	590	+ 60
	646	636	+ 10
	626	604	+ 22
	664	628	+ 36
	640	598	+ 42
	650	556	+ 94
	656	620	+ 36
2	610	604	+ 6
	632	560	+ 72
	646	570	+ 76
	648	560	+ 88
	604	608	— 4
	640	624	+ 16
	552	470	+ 82
	652	652	0
	710	646	+ 64
	654	590	+ 64
Totals	11,516	10,706	+ 810

* + = non-chlorophyll leg is larger; — = chlorophyll leg is larger.

larger. That this is statistically significant* is shown by analysis of paired observations, from the last column of the Table. The computed value of "t" is 6.05, which exceeds the tabular "t" of 3.97 (the value required for significance at the .001 level of probability).

Summary. Under conditions of the experiment (lymphatic block + injection of protein irritants) locally injected chlorophyll diminished the extent of lymphedema, both acutely and chronically. While the mechanism is not established, a heparin-like effect in preventing protein agglutination may be suspected.

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