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Effect of Hyaluronic Acid on Skin Lesions Produced by Staphylococci.* (21133)

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The increasing interest aroused by recent biochemical and enzymological researches on the mucopolysaccharides of the connective tissue ground substance has led us to carry out a series of investigations on the significance of such substances in inflammation processes and experimental carcinogenesis. In this first paper some observations are reported on the action of hyaluronic acid and its enzymatic split products on an inflammation experimentally produced in the rabbit skin by inoculation with pathogenic staphylococci. These investigations follow the observations of Scott *et al.*(1) who reported a slight shortening of incubation period in experimental syphilis, if spirochetes are inoculated in the rabbit testis together with hyaluronidase. According to these authors, this is due either to direct action of hyaluronidase on spirochetes or on the ground substance. On the other hand, evidence has been repeatedly given (Meyer and Chaffee(2), Campani(3), Caputo(4), Cutinelli(5), McClean *et al.*(6)) of the participation of mucopolysaccharides of the connective tissue ground substance in inflammation processes and in the formation of granulation tissue (Caselli(7), Baggi (8-12)).

Methods. The following materials have been used: A) 24-hr agar cultures of *Staphylococcus aureus*.† Immediately before the experiment the bacterial growth of each culture was suspended in 10 ml of saline. B) Potassium hyaluronate solution (7.5 mg/ml) in saline, solution pH 7.3.‡ C) Split products of potassium hyaluronate obtained by incubating for 2 hours at 37°C the solution listed as B with hyaluronidase§ (10 Schering units/1 ml of hyaluronidase solution), then inactivating the enzyme by keeping mixture in water bath at 80°C, 30 minutes. D) 24-hr agar cultures of the same strain of *S. aureus* A, seeding from a broth culture after 5 successive cultures in broth (4 ml) to which one ml potassium hyaluronate had been added at concentration of 2 mg/ml. The fifth broth culture was then seeded in agar and the growth suspended in 10 ml saline. Experiments were made on 9 albino rabbits from the same breed, weighing approximately 2.5 kg. The back was carefully shaved, not to produce abrasions. Each animal was given 2 intradermal injections on both sides at 8 cm distance from each other. The animals were divided into 3 groups: *1st group.* 4 rabbits injected in left side skin with 0.4 ml (2 animals) and 0.2 ml (2 animals) of a mixture made with equal volumes of bacterial suspension A and potassium hyaluronate solution B. Controls were injected in the right side with the same amount of a mixture containing the bacterial suspension and saline in equal volumes. *2nd group.* 3 rabbits were injected in the left

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† A strain of staphylococcus has been used recently isolated from an abscess.

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§ Throughout our experiments hyaluronidase (Kinaden) has been used prepared by the Schering Laboratory, Berlin.

TABLE I. Inflammation Areas in mm² Produced by Intradermal Inoculation of a Mixture of Staphylococci and K Hyaluronate, and by a Control Mixture of Staphylococci and Saline.

*	1†			5†			9†			11†		
ml	A	B	△	A	B	△	A	B	△	A	B	△
0.4	567	273	294	223	50	173	196	0	196	190	0	190
	693	532	161	400	81	319	111	0	111	111	0	111
0.4	858	180	678	1189	101	1088	310	40	270	250	40	210
	1008	396	612	989	50	939	756	44	712	528	40	488
Mean	781	338	436	700	70	629	343	21	322	269	20	249
0.2	255	96	159	129	27	102	89	0	89			
	610	48	562	200	16	184	69	0	69			
0.2	49	34	15	133	29	104	51	22	29	31	7	24
	139	31	108	286	30	256	71	29	42	81	11	70
Mean	263	52	211	187	25	161	70	12	57			

* Amount of injurious agent used.

† Days elapsed between inoculation of injurious agent and evaluation of inflammation area in mm².

A—Inflammation area produced by inoculation of staphylococci and K hyaluronate.

B— " " " " " " staphylococci and saline.

△—Difference in mm² between the 2 inflammation areas.

side with 0.2 ml of mixture containing equal volumes of bacterial suspension and the solution of potassium hyaluronate split products C. Controls were injected in the right side with the same amount of a mixture containing bacterial suspension and saline in equal volumes. *3rd group.* 2 rabbits were injected in the left side with 0.2 ml of staphylococci suspension grown in potassium hyaluronate, D. Controls were injected in right side with the same amount of a staphylococci suspension previously cultivated in plain broth.

In order to make sure that no independent inflammatory action was induced by either potassium hyaluronate or its split products, these materials were injected in quantities from 0.1 ml to 0.5 ml into the skin of 2 more rabbits. The extension of the inflammation areas was daily kept by drawing their outline on cellophane and calculating the result in square millimeters. The results are shown in the Tables and Figures. The skin lesion produced in animals of first, second and third groups was represented by a well-defined papular zone. On the third day following injection of the injurious agent, rabbit No. 2 showed the formation of large crusts on the center of the papular lesions.

The results obtained from the first group (see Table I and Fig. 1) give a clear demonstration that addition of potassium hyaluronate to the staphylococci brought on a constant remarkable intensification of the inflammatory reaction. This is particularly evident in animal 2 of Table I (see Fig. 2). For on the 5th day following injection of the larger dose of the injurious agent, the inflammation

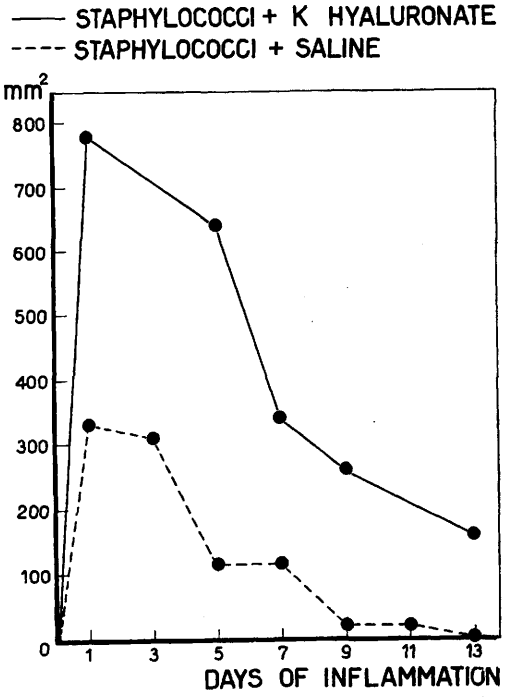

FIG. 1. Average measure of the inflammation areas in rabbits. 1-2 of first group.

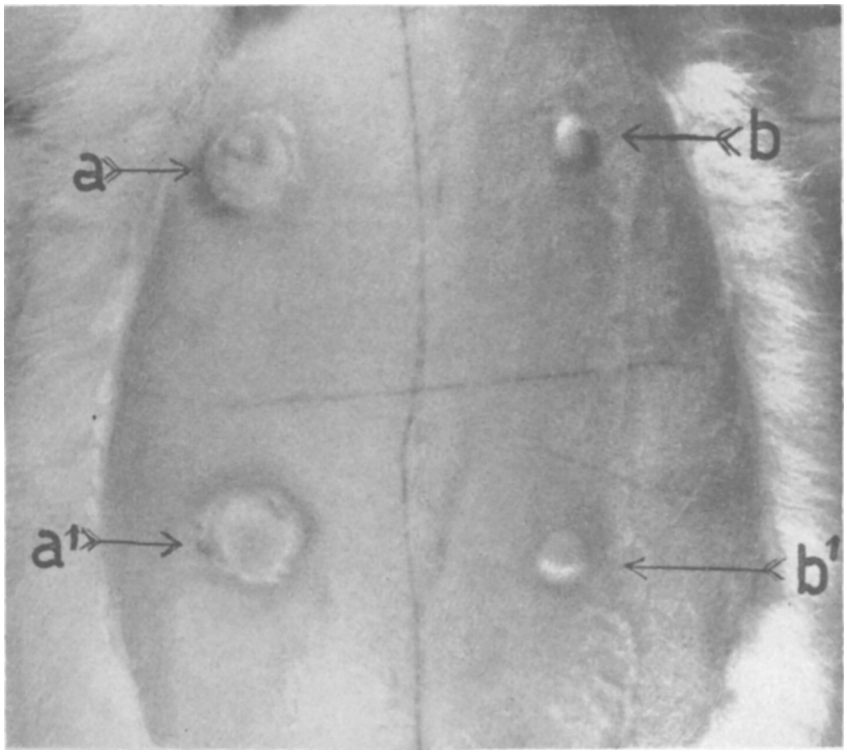

FIG. 2. Lesions produced by staphylococci + K hyaluronate, and control lesions produced by staphylococci + saline. A, 0.4 ml staphylococci + K hyaluronate; B, 0.4 ml staphylococci + saline; A^1, 0.2 ml staphylococci + K hyaluronate; B^1, 0.2 ml staphylococci + saline.

area measured 1189 mm^2 in comparison to 101 mm^2 of the control area, while the lesion induced by the smaller dose of staphylococci had an area of 989 mm^2, the control only 50 mm^2.

The addition of hyaluronic acid split products to the staphylococci likewise produces an increased cutaneous inflammatory reaction, though in much slighter degree than by addition of hyaluronate. The greatest differences between the inflammation areas are represented by rabbit 1 on the third day after injection of causative agent and by rabbit 2 on the fifth day: the former showing 117 mm^2

TABLE II. Inflammation Areas in mm^2 Produced by Intradermal Inoculation of a Mixture of Staphylococci and K Hyaluronate Split Products and by a Control Mixture of Staphylococci and Saline.

	*	1†			3†			5†			7†			9†			11†			13†		
	ml	A	B	△	A	B	△	A	B	△	A	B	△	A	B	△	A	B	△	A	B	△
	0.2	76	72	4	117	85	32	47	20	27	58	32	26	37	13	24	18	6	12	15	0	15
		78	65	13	128	58	70	79	23	56	50	22	28	43	11	32	30	13	17	10	0	10
	0.2	247	120	127	86	72	14	109	36	73	88	39	49	49	24	25	35	14	21	22	0	22
		105	81	24	71	41	30	75	35	40	96	35	61	41	26	15	51	13	38	18	5	13
	0.2	280	132	148	187	102	85	146	100	46	95	40	55	32	19	20	11	0	11			
		90	115	25	173	70	3	65	62	3	55	30	25	21	9	12	7	0	7			
Mean		146	97	48	110	88	39	86	46	40	73	39	40	37	17	21	25	7	17			

* Amount of injurious agent used.
† Days elapsed between inoculation of injurious agent and inflammation area in mm^2.
A—Inflammation area produced by inoculation of staphylococci and K hyaluronate split products.
B— " " " " " " staphylococci and saline.
△—Difference in mm^2 between the two inflammation areas.

vs. 85 mm^2 and 128 mm^2 vs. 58 mm^2, while the latter showed 109 mm^2 vs. 36 mm^2 and 75 mm^2 vs. 35 mm^2 (Table II).

The inflammatory lesions produced by the staphylococci cultivated in presence of potassium hyaluronate showed no significant differences in comparison with the control lesions produced by staphylococci cultivated in normal media. Neither potassium hyaluronate nor its split products induced any appreciable lesion in the 2 rabbits inoculated as controls.

No definite statement can be made about the mechanism with which hyaluronic acid, and in lesser degree its split products, caused the remarkable spreading of inflammation processes. We assume that the mucopolysaccharide, by causing alterations of the tissue where it was inoculated together with the staphylococci, possibly originated interactions between these microorganisms and the tissue, thus favoring the development of the lesions.

Summary. Intradermal inoculation of a mixture of K hyaluronate and staphylococci constantly produces larger lesions than by staphylococci alone. A similar but slighter increase in lesions is also observed after inoculation of a solution containing staphylococci and K hyaluronate split products.

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A New Method for Direct Recording of Cardiac Output.* (21134)

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No simple method is available to measure under physiological conditions directly and accurately total blood flow in the main pulmonary artery. It is the purpose of this communication to report a method for the determination of cardiac output which makes it possible to measure directly in experimental animals main pulmonary artery flow and its fluctuations from one heartbeat to the next. This has been accomplished with the aid of a bristle flowmeter(1-3) which is suitably modified for use in the pulmonary artery trunk.

Method. Fig. 1 shows a diagram of the flowmeter cannula. The device consists of 3 brass parts (A,B,C) which are assembled by threaded joints. The tip of part A is inserted into the vessel wall by slipping the lip D through a "button hole" opening in the artery wall using a technic similar to that employed with the non-suture aortic shunt developed in this laboratory(4,5). The lip D is secured firmly to the intima by the pressure of plate E on the outside of the wall. Plate E is held in place by the screw F. In the shaft of part A lies a snugly fitting stainless steel cylinder G, which can be pushed forward or retracted by means of a cable release H, suitably attached to the outside of the cylinder. When the cylinder G is in the retracted position as shown in Fig. 1, the bristle I, protruding into the lumen of the pulmonary artery, can be

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