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QUANTITATIVE MEASUREMENTS ON SPREADING PHENOMENA IN SKIN

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Intradermal injections are often used in experimental and clinical studies of the skin, but little is known of the pressure changes which they cause. Hechter (1947) believes that raised interstitial pressure, whether caused by intradermal injection or accumulation of inflammatory fluid, is responsible for the spreading of substances in skin, and that the action of hyaluronidase cannot be demonstrated if there is no pressure gradient. He concluded that the action of hyaluronidase was increased when it was injected in large volumes of fluid. The interstitial pressure was also presumed to increase as the volume injected was increased, but no measurements of pressure were made.

The present work was undertaken to determine the pressures produced in normal skin when substances are injected into it, and an attempt was made to test Hechter's idea quantitatively. Rapid serial radiography has been used to determine the time relations of hyaluronidase action in skin, and spreading phenomena in skin have been studied after the administration of cortisone and salicylate.

METHODS

Rabbits were dry-shaved 24 hr. before use, and were anaesthetized with pentobarbitone intraperitoneally (40 mg./kg.). The skin of the back, flank, abdomen, and ears was used, control and test injections being given at comparable sites on either side of the midline. The occasional very thick patches found in rabbit skin were avoided. To measure the intradermal pressures during an injection of fluid, a condenser manometer was connected between a micrometer syringe and needle by means of a brass T-piece; the T-piece was joined by short lengths of plastic tubing which were bound with wire to prevent expansion. The output of the condenser manometer was displayed on a cathode ray oscilloscope and recorded photographically. The rate of injection was controlled by a velodyne motor (Williams and Uttley, 1946). The expansibility of the system was tested and found to be very low; leaks were negligible. The needles used were of such a bore that there was no pressure loss due to the passage of fluid through them at normal rates of flow. In a few experiments exceptionally rapid injections caused a slight change in pressure in the system when detached from the skin; this pressure change was allowed for in subsequent calculations.

Spreading was studied by a method previously described (Barer, 1952), in which thorotrast with and without hyaluronidase (Benger's Hyalase) is injected into the thin part of rabbits' ears. Radiographs are taken at intervals and the area of the injection mass is measured. Injections were either made slowly by hand as steadily as possible (0.1 ml. in 1 min.) or by the mechanical method described above. Cineradiograms at 2/sec. were taken to determine the time relations of the action of hyaluronidase. The injection mass fills the whole depth of skin between epidermis and cartilage and no significant spread occurs in a downward direction; spreading therefore takes place radially from a disc-shape mass of thorotrast and the measurement of area rather than volume is justified. Lymphatics are also filled when rabbits' ears are injected in this way, so that a certain quantity of thorotrast is carried away. This error is, however, present in all methods using intradermal injections.

Cortisone acetate was obtained through the generous gift of the Merck Foundation to the Medical Research Council.

RESULTS

Interstitial Pressures Produced by Injection.—The pressures reached in the skin when saline was injected intradermally at various speeds were measured in 28 experiments, and Fig. 1 and Table I illustrate the

TABLE I

INTERSTITIAL PRESSURE PRODUCED IN SKIN BY THE MECHANICAL INJECTION OF SALINE AT VARIOUS RATES

(Typical experiment)

Rate of Injection (ml./min.)	Plateau Pressure (mm. Hg) (Each value represents one injection)							Mean
0·0016			42	28				35
0·0025		50	32	45				42
0·005	75	48	40	54	60	42		53
0·012		120	88	38	63			77
0·06	138	190	180	170	123	150	94	149
0·13			127	190				159
0·19	345	406	312	324	382	138		318
0·35		326	294	246				289

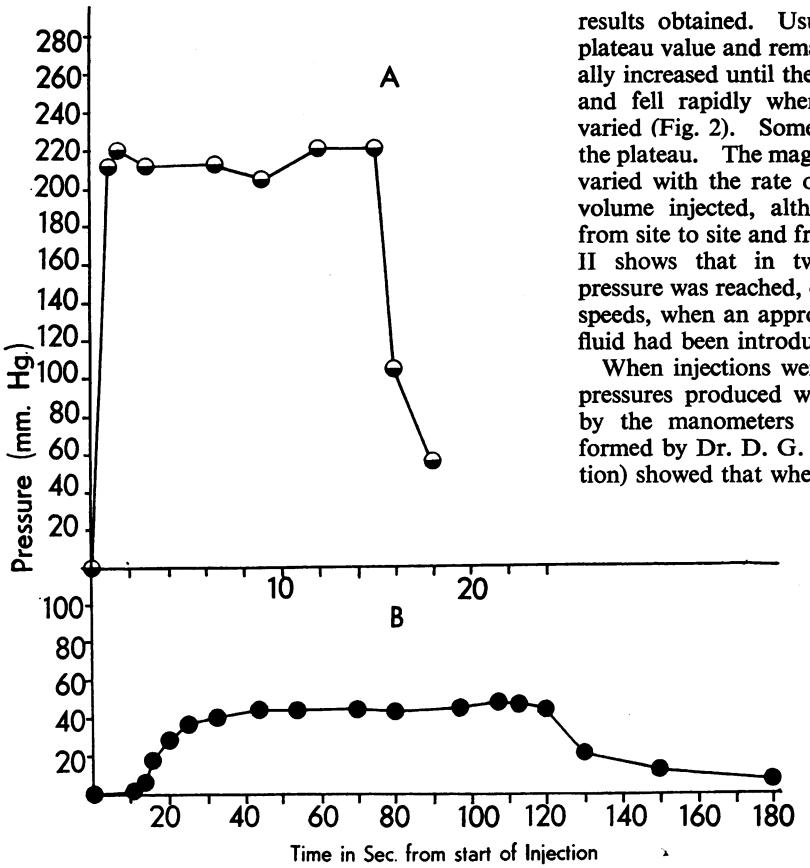

FIG. 1.—Rise in intradermal pressure caused by intradermal injections of saline. (A) Fast mechanical injection (0.088 ml. in 15 sec.). (B) Slow mechanical injection (0.0048 ml. in 2 min.).

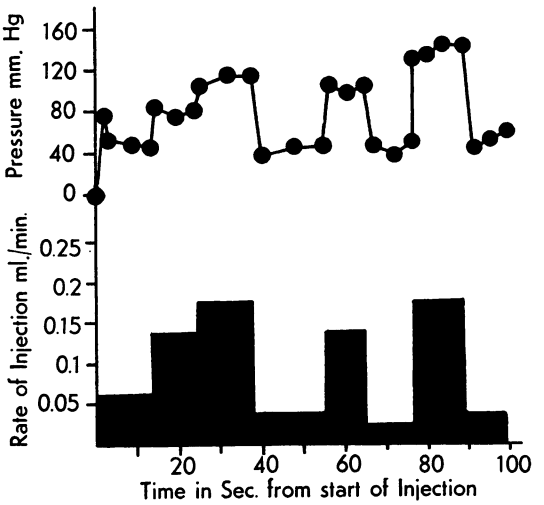

FIG. 2.—Rise in intradermal pressure caused by an intradermal injection of saline. The speed of injection was varied as shown in the lower part of the graph.

results obtained. Usually the pressure rose to a plateau value and remained constant or very gradually increased until the end of the injection; it rose and fell rapidly when the rate of injection was varied (Fig. 2). Sometimes a slight peak preceded the plateau. The magnitude of the plateau pressure varied with the rate of injection and not with the volume injected, although it varied considerably from site to site and from animal to animal. Table II shows that in two experiments the plateau pressure was reached, over a wide range of injection speeds, when an approximately constant volume of fluid had been introduced.

When injections were made rapidly by hand the pressures produced were too high to be recorded by the manometers available. Experiments performed by Dr. D. G. Wyatt (personal communication) showed that when a syringe whose piston was 53 mm. in diameter was compressed against a mercury manometer by hand, a mean pressure of 300 mm. Hg was produced. With syringes of smaller bore the pressure would be greater ($P \propto 1/d^2$).

No clear relationship was found between the rate of fall of pressure after an injection had ceased and the volume injected, but for technical reasons the pressure was not followed to very low levels.

The pressures recorded during and after the mechanical injection of hyaluronidase in saline

TABLE II

TO SHOW THAT THE VOLUME OF SALINE INJECTED WHEN THE INTERSTITIAL PRESSURE REACHES A PLATEAU IS INDEPENDENT OF THE RATE OF INJECTION

	Rate of Injection (ml./min.)	Volume Injected (ml.) (When interstitial pressure reaches a plateau)
Experiment I	0·06	0·0025 0·0025 0·0035
	0·036	0·0027 0·0036 0·0033
	0·024	0·0024 0·0036 0·0034
	0·011	0·0035 0·0037
Experiment II	0·09	0·0021
	0·06	0·0025
	0·01	0·0024
	0·005	0·0025
	0·002	0·0016

(1 mg./100 ml.) into the skin fell within the same range as those observed after administration of saline alone. The plateau pressure varied with the rate of injection, and the rate of fall of pressure after injection was as variable as it was after saline only.

The Action of Hyaluronidase when Injected at Different Pressures.—A constant volume (0.1 ml.) of hyaluronidase (1 mg./ml.) dissolved in thorotrast was injected mechanically into rabbits' ears at different speeds and the rate of spreading was studied from serial radiographs. Interstitial pressures were measured simultaneously in only a few experiments, for it has been shown already that the pressure during injection varies with the rate of injection. Thorotrast and hyaluronidase were injected at rates varying from 0.1 ml. in 18 sec. to 0.1 ml. in 2.75 min. As Table III shows, the mean radius of the injected mass 30 sec. after the end of the injection was greater for the slow injections than the fast. However, the radius at 5 and 20 min. after injection was similar for all rates of administration.

Time Relations of the Action of Hyaluronidase in Skin.—The action of hyaluronidase in skin is extremely rapid. In 11 experiments manual injections were made as quickly as possible (taking about 1 sec.) and radiographs were taken at the rate of 2/sec. from the moment of completion; it was found that in the first picture (immediately after the end of the injection) the areas of hyaluronidase injections were greater than those of saline controls. When injections were made mechanically (0.1 ml. in 1 min.) and radiographs were taken during the injection, the hyaluronidase areas were all greater than those of controls in the first picture, taken at 15 sec., when only 0.025 ml. had been injected (2 experiments, Table IV).

Reasonably accurate estimations of the area of the injection mass may be made at frequent intervals (Fig. 3). Spreading is very rapid at first; it continues more slowly for several hours after hyaluronidase + thorotrast injections, whereas injections of saline + thorotrast reach their maximum area in about 90 min. Hechter (1947)

TABLE III

RATE OF SPREAD OF HYALURONIDASE IN THOROTRAST* WHEN INJECTED INTO THE SKIN OF RABBITS' EARS AT VARYING RATES

Expt. No.	Time taken to Inject 0·1 ml. (sec.)	Radius of Injection Mass (mm.) at 30 sec.	5 min.	20 min.
1	18	5·6	7·2	9·3
2	18	7·7	9·6	11·5
3	18	8·5	*ca.*9·8	*ca.*11·2
4	20	7·1	9·1	10·1
5	20	6·2	7·5	9·0
6	23	8·0	9·8	11·6
7	24	6·2	7·7	10·3
Mean for injection times less than 30 sec. ..		6·8	8·5	10·3
8	35	7·9	9·1	
9	60	5·8	6·9	
10	62	8·1	9·7	10·8
11	110	6·7	7·8	8·9
12	110	7·7	8·9	
13	150	9·1	10·2	11·2
Mean for injection times greater than 30 sec. ..		7·5	8·8	10·3

* 1,000 Benger units = 1 mg. in 1 ml. thorotrast.

TABLE IV

AREAS (MM.^{2}) OF INJECTION MASS DURING AND AFTER MECHANICAL INJECTIONS INTO RABBITS' EARS

Substance Injected	Interval from Start of Injection (sec.) 15	30	45	60	90	150
Thorotrast 0·1 ml. in 60 sec.	29	55	73	87	125	131
	25	47	62	83	97	109
	33	45	68	84	112	123
Thorotrast + hyaluronidase* 0·1 ml. in 60 sec.	52	91	124	151	188	220
	62	97	117	145	171	199
	44	68	93	132	165	185
	54	87	128	160	193	215

* 1 mg. = 1,000 Benger units/ml.

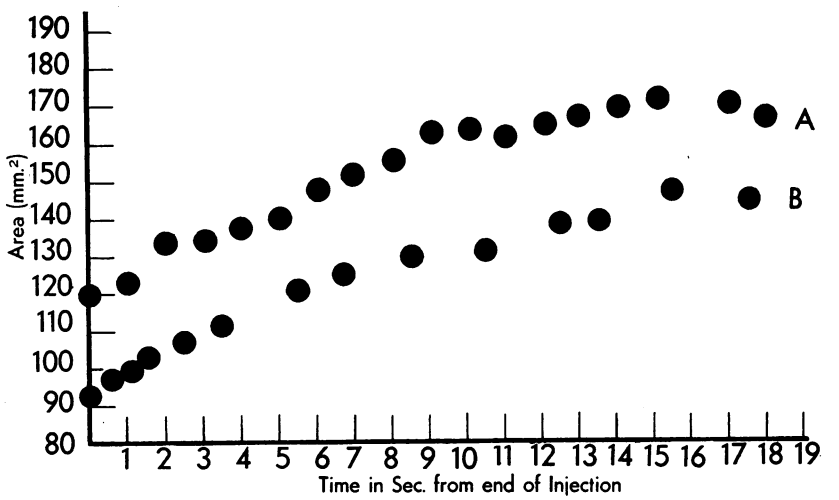

FIG. 3.—Rapid action of hyaluronidase in skin. (A) Spreading of thorotrast + hyaluronidase (0.1 ml. of 1 mg. enzyme/ml.) in a rabbit's ear. (B) Spreading of thorotrast alone in a rabbit's ear. The area occupied by the thorotrast was measured from rapid serial radiographs.

TABLE V

THE EFFECT OF CORTISONE AND SALICYLATE ON SPREADING PHENOMENA IN THE SKIN OF RABBITS' EARS

Area (mm.²) after injection of thorotrast.*

Treatment	Thorotrast Only (0·1 ml.)				Thorotrast + Hyaluronidase† (0·1 ml.)			
	After 0	5	10	60	0	5	10	60 min.
(1) Saline controls	95±24 (9)	122±33	144±41	242±81	207±26 (10)	274±48	321±72	529±138
(2) Cortisone 10 mg./kg. 1½–3 hr. previously	97±14 (5)	125±27	144±37	205±50	215±25 (5)	260±41	296±32	522±109
(3) Cortisone 10 mg./kg. daily for 5 days	125 106 (2)	180 125	212 140	323 235	175±49 (7)	233±72	268±79	374±114
(4) Na Salicylate 0·4 g./kg. i.p. or 0·1 g./kg. i.v. ½–2 hr. previously	100±20 (11)	140±24	167±30	249±71	202±12 (5)	288±40	358±50	—

Number of ears used are indicated in brackets. * Measured from radiographs. The mean area and standard deviation are given. In the first 4 columns of line 3 actual values are given instead of the mean, owing to the small number of animals used. † 1 mg. enzyme dissolved in 1 ml. thorotrast.

states that the action of hyaluronidase is complete in 10–20 min. unless the preparation contains substances increasing the permeability of capillaries so that spreading is prolonged by the advent of oedema fluid. Thorotrast causes no visible inflammation in the first few hours after injection into the skin.

Cortisone and Salicylate.—Table V compares the rate of spread of thorotrast with and without hyaluronidase in five rabbits treated with a single dose of cortisone (10 mg./kg. intramuscularly 1½–3 hr. before injection), seven rabbits treated with five similar daily doses of cortisone (the last 1½–3 hr. before test), and 10 untreated rabbits. The values for the treated animals fall within the range of the controls. Large numbers could not be used, because of a limited supply of cortisone.

The low values sometimes observed in the later stages of spread after hyaluronidase injections might be due to suppression of an inflammatory reaction, since cortisone is known to have this effect. However, a dose of cortisone in one rabbit did not alter the changes in spreading phenomena in skin produced by inflammation (early turpentine lesion) which have been described previously (Barer, 1952). Cortisone (0.2 ml.) was also injected locally into one ear and the proprietary medium in which it was suspended was injected into the other ear (0.2 ml.) of each of 11 animals. Subsequent injections of thorotrast at intervals of 15 min. to 4 hr. showed that spreading on both the control and cortisone sides resembled that seen in early inflammation (Barer, 1952). Instead of being a clear-cut disc the injection mass became blurred at its margins, which extended outwards as finger-like processes. Lymphatics filled less well than usual, and small quantities of thorotrast which entered lymphatics failed to drain away normally to lymph glands. These changes suggest that the suspending medium causes a fairly severe inflammation. In confirmation of this, pontamine sky blue injected intravenously leaked out into both cortisone and suspending medium areas.

The results obtained when spreading was studied in animals treated with one dose of sodium salicylate (0.4 g./kg. i.p. or 0.1 g./kg. i.v.) are also shown in Table V. The rate of spread of both control and hyaluronidase areas falls within normal limits.

The Action of Hyaluronidase on Lymphatic Filling.—Lymphatics filled less when rabbits' ears were injected with thorotrast + hyaluronidase as compared with thorotrast alone. It is not possible to put this observation on a quantitative basis, since lymphatics may be directly entered by chance. However, in 10 rabbits all the ears injected with thorotrast alone showed good lymphatic filling; the corresponding ears injected with hyaluronidase and thorotrast showed moderate filling in one, much less filling than that of the controls in two, and negligible or no filling in seven.

DISCUSSION

The high pressures which were recorded during intradermal injections are in keeping with those found by previous workers (Miles and Miles, 1952; Winter and Flataker, 1952), and are consistent with the belief that the matrix of the dermis is comparatively rigid and without "free" fluid in the physiological state. The present method has enabled the pressure changes which follow intradermal injections to be analysed more completely than hitherto, and has shown that the interstitial pressure bears little relation to the volume introduced in the range of volumes tested, but varies with the rate of injection. This suggests that with these volumes the tissue elements which contain the injection mass

are stretched beyond their elastic limit, and that skin structure is grossly disorganized by an intradermal injection (Bensley, 1949–50). When skin properties are studied by injection methods, microscopic volumes of fluid should therefore be used whenever possible. From Fig. 1B, it can be deduced that volumes of the order 0.0004 ml. and less would be desirable if large increases in pressure are to be avoided.

No significant difference was found between the pressures attained when saline and saline + hyaluronidase were injected. This conflicts with the findings of Winter and Flataker (1952), who used a single rate of injection in rats. No explanation can be offered except that gross species differences occur in skin properties (Bangham, 1951). It is possible that such differences may only be present at very low pressures before the tissue elements are disorganized.

The very rapid quickly diminishing action of hyaluronidase in skin which has been demonstrated is consistent with the conception of Day (1950, 1952) in which connective tissue is pictured as being " waterproofed " by large molecules which are rapidly removed by hyaluronidase.

The fact that injection pressures were within normal limits during hyaluronidase injections suggests that hyaluronidase does not reduce the resistance to flow throughout the entire connective tissue matrix. It may simply open up fibres of molecular dimensions, possibly alongside the fibres of the protein network recently demonstrated by Day (1952b). The resistance of such channels would be high, so that even the large pressures caused by injections would not produce much flow.

Hechter's prediction that interstitial pressure would rise with the volume of fluid injected into the skin was not borne out by measurements, at least in the range of volumes tested. It remains possible that the pressure stays raised for longer periods after large than after small injections, since the fall of pressure was not followed to very low levels.

Hechter compared the difference between the areas attained by spreading from a large hyaluronidase injection and a large control injection, with the difference between the areas attained by spreading from a small hyaluronidase injection and a small control injection, and concluded that hyaluronidase was more effective when the volume injected was larger. But the rate of increase of area is a function of the initial area while the rate of increase of radius is not. Geometrically, the difference in the final areas will be greater for the large than for the small injections, even if the difference between the rate of advance of the margins (rate of increase in radius) of hyaluronidase and control areas is the same. The rate of increase in radius (calculated from Hechter's data) after intradermal injection increased with the volume of the injection after both hyaluronidase and control injections. Taking the data as a whole there was no significant difference between them.

Reports have appeared in which cortisone (or less pure adrenal products) decrease (Winter and Flataker, 1950; Mahaux and Stienlet, 1951; Opsahl, 1949a, b, and c; Ducommun, Timiras, and Dordoni, 1951), increase (Hayes and Bridgman, 1951; Coste and Bourel, 1951), or have no effect (Holborow and Keech, 1951) on intradermal spreading. Hayes and Baker (1951) and Coste and Bourel (1951) found the direction of the effect to depend on the dosage, and the latter workers found that it depended on the test animal. Some have reported a specific inhibiting action on hyaluronidase (Opsahl, 1949), while others consider this to be no greater than the effect on controls. Adrenalectomy was found to increase spreading (Opsahl, 1949; Winter and Flataker, 1950). In the present work cortisone was found to have no significant effect either on hyaluronidase or controls. Many workers have not measured the areas of injected substances until several hours, sometimes 24 hours after injection. Earlier readings are more reliable, since with the passage of time the onset of mild inflammation or the ingestion of foreign material by macrophages will influence the area occupied by the injected material. The considerable variation in control observations might obscure small differences due to cortisone, and the necessity of using different animals for the control and treated series is also a drawback. The answer to the question as to whether cortisone has any action on the matrix of the connective tissue may have to await the development of more delicate methods.

The local " spreading " action of cortisone is due to the suspending medium, a conclusion reached by Menkin (1951), who also found that the suspending medium caused an increase in capillary permeability.

Conflicting reports have been published on the effect of sodium salicylate on spreading in skin with and without hyaluronidase. Guerra (1946) claimed that hyaluronidase is inhibited, but Swyer (1948a and b) in both *in vivo* and *in vitro* studies found that salicylate inhibited the increase of capillary permeability caused by histamine and snake venoms but not the action of pure hyaluronidase. Jacot, Ducommun, Timiras, and Selye (1951) claim that large doses of salicylate decreased the permeability of skin and that this effect cannot be obtained after

adrenalectomy. In the experiments which have been described salicylate did not alter spreading in rabbits' skin.

Summary

1. The pressure changes accompanying and following intradermal injections have been measured. When fluid is injected continuously at a constant rate the pressure reaches a plateau which varies with the rate of injection and not with the volume injected.

2. The effect of pressure on the action of hyaluronidase and the time relations of hyaluronidase action in rabbit skin have been investigated.

3. Spreading in skin with and without hyaluronidase was not significantly altered by preadministration of cortisone or salicylate.

4. Changes in spreading properties of skin near local injections of cortisone were found to be due to the suspending medium.

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