ON THE INHIBITORY EFFECT OF ANTISTIN ON HYALURONIDASE

by

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In 1947 MAYER AND KULL[1] reported their findings which showed the antihyaluronidase effect as exerted by antistin and pyribenzamine within the living body. Their experiments were carried out as follows: India ink containing hyaluronidase was intradermally injected into albino rabbits. The area stained by the India ink in a certain time was measured. There upon a substantial amount of antistin was given to the animal and the procedure outlined above was repeated. The diminished size of the discoloured area proved the inhibition of the spreading effect of hyaluronidase.

In 1946 GUERRA[2] carried out similar studies using sodium salicylate which was found to diminish the activity of hyaluronidase *in vivo*. SWYER[3], however, pointed out that sodium salicylate could only exert an influence upon the mucolytic property of hyaluronidase in such an excessive concentration when the reduction of p_H, caused by the substance, was in itself sufficient to bring about the same effect. Therefore SWYER concluded that in GUERRA's experiment sodium salicylate did not affect the hyaluronidase itself but that it exerted a kind of antihistamine effect on the histamine present as impurity in GUERRA's hyaluronidase extract.

Morover SWYER succeeded in demonstrating in his own experiments that certain hyaluronidase compounds such as that obtained from snake venom increased the permeability of the capillaries in the same manner as histamin which could be shown with the aid of pontamin blue, that the spreading effect of a hyaluronidase preparation completely free from histamine was proportionally enhanced by the admixture of histamine, and that sodium salicylate only affected hyaluronidase solutions containing histamine and it lowered the activity of such preparations in so far as the excess in the spreading effect was due to histamine.

The same objection can be raised against the observations of MAYER AND KULL. It remains to be made clear whether the antihistamines can be accepted as real hyaluronidase inhibitors or whether the whole phenomenon is merely based on the antagonistic effect of antihistamines on the histamine detected as impurity in some hyaluronidase extracts.

Our main target was the solution of this problem. In the course of the present studies, however, a new question invited attention. *In vitro* antistin was found to precipitate India ink in such a manner that the latter could not pass filter paper as it was completely retained. Even after separate subcutaneous administration of antistin and India ink, when both were subsequently injected at the very same spot, histological examination revealed precipitated India ink in the tissues. Therefore we were tempted to assume that in the experiments of MAYER AND KULL the large doses of antistin injected into the subcutaneous tissues simultaneously with, though at a considerable

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TABLE I

CHANGES IN THE SPREADING EFFECT DUE TO ANTISTIN

Without antistin		With antistin		Staining material
controls	with hyaluronidase	controls	with hyaluronidase	
1.03 cm^2	2.02 cm^2	0.85 cm^2	1.50 cm^2	India ink
1.20 cm^2	1.42 cm^2	1.20 cm^2	1.17 cm^2	India ink
1.38 cm^2	4.67 cm^2	1.38 cm^2	2.25 cm^2	India ink
1.50 cm^2	3.44 cm^2	1.29 cm^2	2.22 cm^2	India ink
1.68 cm^2	3.68 cm^2	1.48 cm^2	2.11 cm^2	haemoglobin
1.25 cm^2	2.46 cm^2	0.94 cm^2	1.96 cm^2	haemoglobin
average 1.34 cm^2	3.06 cm^2	1.19 cm^2	1.97 cm^2	
gain in per cent	+ 128%		+ 65%	

distance from India ink also led to the precipitation of the latter and thus resulted in its fixation in the tissues.

First we repeated the experiments of MAYER AND KULL. Their results could be confirmed. In fact the size of the discoloured area was much smaller after the administration of antistin. Histological examination made after autopsy failed to detect any deposited precipitate of India ink in the skin.

In the next step a haemoglobin solution was used instead of India ink. It had the advantage over India ink of being unaffected by antistin *in vitro* whereas the inhibitory effect of antistin on the spreading effect could be demonstrated as easily using haemoglobin as with India ink. The results obtained are shown in Table I.

In the experiments with hyaluronidase 0.2 ml of solution containing 0.25 g in 100 ml was given intradermally, in the control experiments it was substituted by 0.2 ml of saline. The dose of antistin was 75 mg per kg body weight. All figures except those in the bottom row indicate skin areas in square centimeters stained by India ink or by haemoglobin.

Besides the *intradermal* technique the following method was devised to record the effect of antistin. Two identical drip containers were mounted at the same height, fitted with rubber tubes of the same length and diameter, with hypodermic needles of identical gauge attached to both. Thus the apparatus was designed to deliver identical amounts of fluid through each tube during a definite time. Both containers were filled with equal amounts of saline containing hyaluronidase but to one antistin was also added. The needles were inserted into the hind legs of the animal (dog and rabbit) and the volume of the fluid infused into each leg during a certain time was recorded. Table II indicates

TABLE II

THE EFFECT OF ANTISTIN ON THE INFUSION OF HYALURONIDASE SOLUTION

Time of infusion min.	Volume in ml of the fluid infused		Hyaluronidase content of containers No. 1 and No. 2	Antistin content of container No. 2
	from container No. 1 containing hyaluronidase	from container No. 2 containing hyaluronidase and antistin		
5′	75	15	0.5% hyaluronidase	0.1% ant.
3′	120	80	0.5% hyaluronidase	0.066% ant.
3′	74	61	0.5% hyaluronidase	0.1% ant.
3′30″	96	41	0.5% hyaluronidase	0.2% ant.

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how clearly the inhibitory effect of antistin was also demonstrated by this technique.

In order to detect the occurrence of histamine, if any, in our hyaluronidase extract, which in all cases was prepared from bovine testicles, proper pharmacological experiments were performed instead of the unreliable staining process. An excessive dose of 10 ml of a saline solution containing 2% hyaluronidase injected into guinea pigs, known to be highly sensitive to histamine, produced no ill effects whatsoever. Similarly it was shown that our enzyme preparation rather increased the blood pressure of the cat instead of diminishing it.

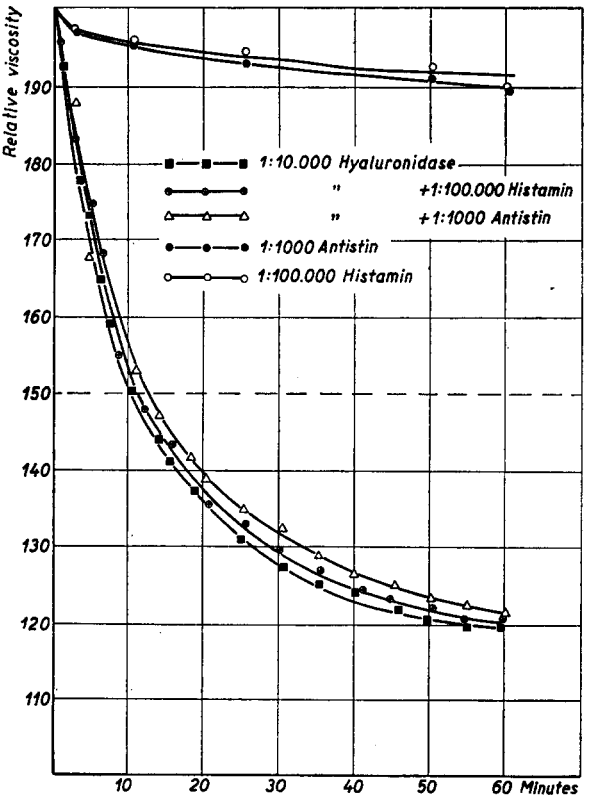

Fig. 1

In vitro tests — performed by the viscosimetric technique described by McLEAN AND HALE[4] as modified by McLEAN[5] — failed to detect any influence of antistin on the mucolytic effect of hyaluronidase, see Fig. 1.

Nevertheless, in spite of the purity of the hyaluronidase preparation the observation that antistin impedes the activity of hyaluronidase *in vivo* only and not *in vitro* certainly remains questionable. For the possibility can not be precluded that histamine set free by the insertion of the needle was neutralized by antistin. Furthermore there is another aspect. As antistin is apt to precipitate colloidal solutions, it may make the tissues more firm and impermeable, thus creating a false antihyaluronidase effect.

Before discussing how to overcome these new difficulties it appears important to remark that in SWYER's experiments[1] histamine was added to hyaluronidase in large

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doses (10 γ) as compared to the negligible amounts of traumatic origin[2]; there was no marked correlation between hyaluronidase effect and histamine content. Their influence on the spreading effect was additional because of histamine also increases the permeability of the capillaries[3]. Though *in vitro* histamine was found incapable of affecting the activity of hyaluronidase as shown in Fig. 1, further investigation was necessary to exclude the effect of histamine and or antistin on the infusion time of saline.

Therefore, in the next experiments pure saline was hypodermically infused into one hind limb and the same solution, containing histamine, was brought into the other limb. The results obtained are presented in Table III. So histamine apparently accelerates the inflow of saline.

TABLE III

THE EFFECT OF HISTAMINE ON THE INFUSION OF PHYSIOLOGICAL SALINE

Time of infusion	Volume of the infused fluid in ml		Place of infusion
	150 ml saline and Histamine 20 microg	150 ml saline	
4′ 05″	150	115	thigh
4′	150	135	
4′ 15″	150	100	
13′	150	95	paw
10′	64	46	

Antistin was tested by the same experimental technique. There was not, however, any significant difference between the pure saline and the antistin solution, as can be seen from Table IV.

TABLE IV

THE EFFECT OF ANTISTIN ON THE INFUSION OF SALINE

Time of infusion	Volume of the infused fluid in ml		Place of infusion
	150 ml saline and 0.1 g antistin	150 ml saline	
5′ 15″	150	143	thigh
7′ 07″	125	150	thigh
6′ 00″	125	120	thigh
5′ 30″	125	120	thigh
3′ 30″	150	150	thigh

DISCUSSION

In spite of our initial unfavourable prejudice the present experiments furnished sufficient evidence that *in vivo* the inhibitory affect of antistin on the spreading effect of hyaluronidase, as first shown by MAYER AND KULL, cannot be attributed to methodical errors. The more so as similar results were obtained by an entirely different technique, the hypodermic infusion.

A negative answer must be given to the suggestion brought forward that perhaps antistin would merely neutralize the histamine impurity of the hyaluronidase extract. The hyaluronidase solution used was biologically tested and found to be completely free from histamine.

Furthermore another objection, that antistin would merely act on the histamine

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set free from damaged tissue following the insertion of the needle or other mechanical injury, was overruled by the observation that substantial amounts of histamine, such as 20 micrograms, which cannot possibly be produced by trauma, increased the velocity of the hypodermic infusion by a negligible 25%.

Antistin alone was incapable of lowering the infusion rate of saline. On the other hand, if significant quantities of histamine would have been produced by mechanical trauma, the inflow of fluid would necessarily be slowed down due to the neutralization of histamine by antistin.

Therefore we must conclude that antistin indeed possesses an antihyaluronidase property which explains the inhibitory effect exerted on both the infusion rate and the spreading effect of hyaluronidase solution.

SUMMARY

The observation of MAYER AND KULL concerning the antihyaluronidase effect of antistin has been confirmed by the present experiments.

The possibility that antistin affects histamine occurring as impurity in the hyaluronidase solution or the other histamine like by-products of cellular decomposition can be discarded.

By hypodermic infusion it was also proved that *in vivo* antistin exhibits a definite antihyaluronidase effect which, however, is not observed *in vitro*.

Histamine alone was found to accelerate the inflow of the hypodermic infusion; pure antistin does not reduce the infusion rate of saline.

RÉSUMÉ

Nous avous confirmé par nos expériences les observations de MAYER ET KULL concernant l'effet antihyaluronidasique de l'antistine.

Nous pouvons exclure la possibilité que l'antistine affecte l'histamine (qu'on rencontre comme impureté dans la solution d'hyaluronidase) ou d'autres produits secondaires de la décomposition cellulaire semblables à l'histamine.

Il a été démontré par infusion hypodermique que l'antistine a un effet antihyaluronidasique bien défini *in vivo*, effet qui, cependant, n'est par observé *in vitro*.

Nous avons trouvé que l'histamine seule accélère la pénétration de l'infusion hypodermique; antistine pure ne réduit pas la vitesse de pénétration de solution physiologique.

ZUSAMMENFASSUNG

Die Beobachtungen von MAYER UND KULL über den Antihyaluronidase-Effekt des Antistins werden durch unsere Versuche bestätigt.

Die Möglichkeit einer durch Antistin bewirkten Hemmung des Histamins (das als Verunreinigung in der Hyaluronidaselösung vorkommt) oder histaminähnlicher Nebenprodukte der Zersetzung der Zelle kann ausgeschlossen werden.

Auch durch hypodermale Infusion wurde bewiesen, dass Antistin *in vivo* eine deutliche Antihyaluronidase-Wirkung besitzt, die aber *in vitro* nicht beobachtet worden ist.

Wir haben gefunden dass Histamin allein das Eindringen der hypodermalen Infusion beschleunigt; reines Antistin verlangsamt das Eindringen von physiologischer Lösung nicht.

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