

Department of Pharmacology, University College, London.
Wright-Fleming Institute, St. Mary's Hospital, London.

THE HISTAMINE BINDING POWER OF HUMAN SERUM FROM ALLERGIC AND NON-ALLERGIC SUBJECTS

By

P. KIRTCHEV & A. W. FRANKLAND

The effect of human sera on the biological activity of histamine has been studied both *in vivo* and *in vitro* and found to be related to certain clinical conditions. The *in vivo* experiments of R. Benda & D. A. Urquia (1948) demonstrated that 2 ml of certain "non-allergic" human sera administered subcutaneously to guinea pigs protected the animals from the broncho-constriction produced by a histamine aerosol. The same dose administered to humans appeared to produce a marked improvement in 70 per cent of 2,400 patients suffering from allergic asthma (Benda 1958).

Parrot and his colleagues on the other hand measured the "histamine binding" of human sera using as an *in vitro* test the contraction of the guinea pig ileum. They compared allergic and non-allergic subjects and found that 174 out of 185 allergic patients had sera which did not alter the activity of a histamine solution. Non-allergic individuals, on the contrary, were found to possess sera which had a histamine binding action. In the presence of 5 per cent serum the activity of a 10^{-6} solution of histamine was reduced by about 30 per cent in every one of 70 subjects tested. This power of serum to bind histamine they called "histaminopexy". (C. Laborde, J. L. Parrot & D. A. Urquia (1953)). J. L. Parrot & C. Laborde (1953).

Our experiments followed closely those of *Parrot et al.* and were designed to investigate further the mechanism of histaminopexy using quantitative methods. We were not able to proceed very far because of our failure to reproduce the basic observations.

METHODS

Venous blood (6 ml) was taken with a sterile glass syringe and left for several hours at room temperature for the clot to contract. After centrifugation the serum was dialysed in the cold for 24 hours against a large volume of 0.9 per cent sodium chloride with a change of solution every 8 hours. Some sera were stored in a deep-freeze and retested after 2 months.

The histamine assays were done on guinea pig ileum using a 2×2 design (*Schild 1942*) and an automatic assay apparatus (*Boura, Mongar & Schild 1954*).

The "standard" histamine solutions were made up in Tyrode (NaCl 8, KCl 0.2, CaCl_2 0.2, MgCl 0.1, NaHPO_4 0.05, glucose 1.0, NaHCO_3 1.0, atropine 0.002 $\mu\text{g/ml}$) at a concentration of 0.02 and 0.01 $\mu\text{g/ml}$. The test solutions of histamine were identical except that they were made up in cold Tyrode diluted with 5 per cent of the serum under test. Normally the histamine was allowed to equilibrate with the serum for at least 10 min in the cold before being warmed up just before use. In some experiments, however, it was incubated at 37° C for $\frac{1}{2}$ hr to test for the presence of histaminase in the serum. One ml of undiluted equilibrated histamine solution (test or standard) was added to the bath after complete emptying of the Tyrode washing solution. When a maximum response was obtained the test solution was removed and the gut was given a succession of Tyrode washes.

Five groups of the four solutions added in random order constituted an assay. The potency of the test solution expressed in terms of the standard as unity was calculated assuming a logarithmic relation between dose and response. The limits of error were calculated from the analysis of variance (*Schild 1942*).

RESULTS

The histamine binding power of serum from 10 non-allergic subjects is summarized in Table 1. One had a test to standard ratio significantly greater than unity (1.16) and two significantly less than unity (0.90 and 0.91). The average ratio is 0.97: *i.e.* the gut-contracting activity of histamine is not appreciably affected by the presence of 5 per cent serum taken from non-allergic individuals.

TABLE 1

Histamine Binding by Sera from 10 non-allergic Individuals. Ratio of Histamine plus Serum (test) to Histamine alone (std).

<i>Ref.</i>	<i>Age</i>	<i>Sex</i>	<i>Test/std.</i>	<i>1 % limits of error</i>
51548	24	M	0.98	1.12-1.21
DN	26	M	1.16	1.12-1.21
PSM	24	M	0.99	0.98-1.03
AS	25	F	0.98	0.94-1.04
HE	29	M	0.98	0.93-1.09
LO	30	M	0.96	0.90-1.03
51548	35	M	0.94	0.82-1.08
51545	25	M	0.93	0.87-1.02
TH	30	M	0.91	0.75-1.11
LE	28	F	0.91	0.83-0.99
GA	26	F	0.90	0.81-0.98
Mean			0.97	

TABLE 2

Histamine Binding by Sera from 22 Allergic Individuals

<i>Ref.</i>	<i>Age</i>	<i>Sex</i>	<i>Test/std.</i>	<i>1 % limits of error</i>
WD	25	M	1.09	107-111
CD	38	F	1.08	97-125
PK	48	M	1.01	95-108
WS	37	F	0.97	96-98
SD	34	F	0.97	85-112
TN	48	F	0.97	81-118
DS	21	F	0.89	71-114
PA	38	F	0.89	81-100
CM	28	F	0.89	82-98
TG	50	F	0.89	81-99
CM	28	F	0.89	82-98
BT	35	F	0.88	76-98
TU	60	F	0.88	87-89
RM	53	F	0.87	86-90
GG	31	F	0.85	80-90
SK	54	F	0.84	83-85
R ₂	40	F	0.83	75-92
ME	24	F	0.80	74-88
NM	35	F	0.77	76-80
BG	65	F	0.69	60-79
BE	55	F	0.69	64-76
TJ	50	F	0.69	55-87
BM	70	F	0.63	55-73
Mean			0.87	

Table 2 presents results of similar measurements from 21 patients from an allergy clinic. Only one is significantly greater than unity (1.09) and 13 are significantly less (0.89–0.63). The average ratio is 0.87. It would appear from these results that the serum of some allergic patients has a small histamine bindings capacity that is lacking in sera of normal individuals.

Two model experiments were carried out to measure the binding of histamine to human γ -globulin again using the guinea pig ileum as a test object for the activity of the histamine. A 5 per cent γ -globulin solution gave a test to standard ratio of 0.93 and 10 per cent solution gave a ratio of 0.89. Thus the γ -globulin normally present in serum (about 3 per cent) would not be expected to give more than a 5 per cent reduction in histaminic activity.

All tests on allergic and non-allergic sera were done with fresh samples after dialysis for 24 hours in the cold. Four samples which had been stored in a deep-freeze at -15°C were retested two months later. All had a decreased ratio (average initial ratio 1.19, average final ratio 0.85). The decrease was greatest in those that initially had the highest ratio (Table 3).

TABLE 3
*Effect of Storing in the Cold (-15°C for 2 Months)
or Incubation (37°C for 2 Hours) on Histamine Binding of sera.
Ratio Histamine + Serum: Histamine alone.*

<i>Treatment</i>	<i>Diagnosis</i>	<i>Initial ratio</i>	<i>Final ratio</i>	<i>Change</i>
Cold	non-allergic	1.42	0.95	—0.47
Cold	non-allergic	1.20	0.81	—0.39
Cold	non-allergic	1.16	0.82	—0.34
Cold	non-allergic	0.99	0.84	—0.15
Incubation	non-allergic	0.93	0.90	—0.03
Incubation	non-allergic	0.94	0.99	+0.05
Incubation	hay-fever	1.09	1.18	+0.09
Incubation	hay-fever	1.15	1.38	+0.23
Incubation	hay-fever	1.15	1.26	+0.11
Incubation	hay-fever	1.18	1.27	+0.09
Incubation	hay-fever	1.10	1.10	+nil

Tests were also done to measure the stability of histamine in the presence of serum. Sera from 2 non-allergic individuals and 5 patients with hay fever were tested before and after incubating with histamine for 2 hours at 37° C. The results are shown in the second part of Table 3. There was no appreciable destruction; on average the results after incubation were slightly higher than before.

DISCUSSION

Our results, based on careful quantitative biological assays of histamine activity, provide no evidence that sera from allergic individuals are less able to bind histamine than sera from non-allergic individuals. On the contrary, 22 "allergic" sera gave, on the average, a small binding of histamine (ratio 0.87) but 10 "non-allergic" sera showed on the average no binding (ratio 0.97). This difference between the two series is only just significant ($0.02 > P > 0.05$). *Parrot et al.*, it will be recalled, obtained ratios of 0.7 for non-allergic and 1.0 for allergic individuals.

The finding that a few sera enhanced the gut contracting activity of histamine solutions was surprising; it cannot be explained in terms of protein binding. This anomalous behaviour had disappeared when the sera were retested after 2 months storage at -15° C. The test samples showed no activity when 2×10^{-9} mepyramine was added to the Tyrode solution in the assay bath. The enhancement, though not due to histamine, could be attributed to an unstable non-dialysable histamine-potentiating substance in the sera. While this possibility invalidates any conclusions concerning the *absolute* values of the test to standard ratios the *relative* values between the allergic and non-allergic groups are still valid.

The question arises whether the difference between our results and those of Parrot and his colleagues can be attributed to a difference in method. It is difficult to conceive of any variable in experimental techniques which would affect allergic and non-allergic sera differently, but this cannot be ex-

cluded for there are small differences between the two methods. In the present investigation the 5 per cent solution of serum in Tyrode was equilibrated with 0.01 and 0.02 $\mu\text{g/ml}$ of histamine and the solution was then warmed up and added to the ileum without further dilution; in Parrot's method the 5 per cent diluted serum was equilibrated with 1 $\mu\text{g/ml}$ histamine and a further 5–10 fold dilution was obtained by adding a measured volume of this mixture to the Tyrode in the isolated organ bath containing the ileum. Our technique provides the maximum opportunity for histamine binding as the ratio of serum proteins to histamine bath during the equilibration before assays and during the assay was 50–100 times higher. The only other difference appears to be in the composition of the Tyrode used in the bioassays. Three constituents were absent from the Tyrode solution used by Parrot (glucose 1 g/l, NaH_2PO_4 0.05 g/l and atropine 1 $\mu\text{g/l}$).

Throughout this report we have assumed that any decrease in histamine activity was due to protein binding. The decrease could also have been due to enzymic destruction. However, no decrease in activity was found when sera from allergic and non-allergic individuals were incubated with histamine (0.02 and 0.01 $\mu\text{g/ml}$) for 2 hr. at 37° C before assay (Table 3). Destruction of histamine does occur with pregnancy serum as we found in a series of experiments to be elsewhere. Added histamine was almost completely destroyed in 15 min at 37° and it is possible that in this case appreciable destruction would result during the few seconds that elapsed between warming up the solution and the completion of its action on the ileum. None of the results reported here was obtained with pregnancy serum.

We found that normal human γ -globulin needed to be present in a high concentration (5 per cent or 10 per cent) to give even small reduction in the biological activity of a very dilute (0.01 $\mu\text{g/ml}$) histamine solution.

Histamine produced in allergic conditions may come from stores in the tissue (*Schild, Hawkins, Mongar & Herxheimer* 1951) or it may be continuously formed from histidine in the

walls of capillaries (Schayer 1963). In either case the protection afforded by sera could not be achieved until the histamine had diffused into the blood. But in this case it would be expected that the histamine binding action of the sera would only be exerted against generalised toxic effects such as would be produced by an intravenous infusion of histamine and not against localised allergic reactions. The ineffectiveness of highly active synthetic antihistamines in the treatment of allergies also argues against the possibility of any histamine antagonist being effective.

SUMMARY

It has been reported that non-allergic human serum differs from allergic in that it has a histamine binding power which has been called "histaminopexy". This finding could not be confirmed.

It was found that histamine is relatively stable in the presence of serum except that histamine destruction does occur with pregnancy serum. In 10 non-allergic subjects, the gut contracting activity of histamine was not affected by the presence of 5 per cent serum. In 21 allergic patients the sera had a small histamine binding activity which was not present in normal sera.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Dr. J. L. Mongar for valuable discussions and for providing facilities.

REFERENCES

- Benda, R. & Urquía, D. A.: C. R. Soc. Biol. Paris 142, 167 & 1507, 1948.
 Benda, R.: Presse Méd. 66, 455, 1958.
 Boura, A., Mongar, J. L. & Schild, H. O.: Brit. J. Pharm. 9, 24, 1954.
 Laborde, C., Parrot, J. L. & Urquía, D. A.: Presse Méd. 61, 1151, 1953.
 Parrot, J. L. & Laborde, C.: Presse Méd. 61, 1288, 1953.
 Schayer, R. W.: Progress Allergy 7, 187, 1963.
 Schild, H. O.: J. Physiol. 101, 115, 1942.
 Schild, H. O., Hawkins, D. F., Mongar, J. L. & Herxheimer, H.: Lancet ii, 376, 1951.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.