BIOASSAY OF HISTAMINE

By

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The usually accepted mothods for bioassay of histamine according to Schild *et al.* (1951), employ (i) cat's blood pressure, (ii) guinea pig's uterus or (iii) the guinea pig's ileum. Code (1952) has reported that the most convenient tissue for the bioassay of histamine is guinea pig's terminal portion of the ileum and on this account it is the most widely employed pharmacological preparation for the work. In this laboratory also for the above reason, guinea pig's ileum was employed for the bioassay of histamine but the tissue was found not reliable as its sensitivity changed with repeated small doses of histamine. This obvious fallacy was more pronounced with locally obtained spotted guinea pig's ileum. This led us to think for some other tissue for the work. Guinea pig's uterus was also not preferred as the spontaneous rhythmic contractions rendered correct interpretation of the results difficult and cats B. P. method was not employed due to non-availability of the animal. Guinea pig's tracheal chain was then employed for the purpose and the present communication is the result of these experiments. These preparations were further employed for testing the blood histamine in 25 normal human subjects.

MATERIAL AND METHOD

Tracheal chain was prepared by following Ackasu's (1952) modification of Castillo and de Beer's (1947) method from guinea pigs of either sex and weighing more than 350 gm. The chain was then mounted in a five ml. bath of modified Burn and Dale apparatus. A weight of about 200 to 300 mg. was hung on the long arm of a previously counterpoised light pith wood lever for 3 hours to obtain maximum relaxation of the tracheal muscle. The magnification produced by the lever was ten times. The best results were obtained in the temperature ranging between 30 to 34 degree centigrade.

Blood histamine was extracted by Code's modified method (1937) from 25 normal healthy individuals belonging to different strata of life and vocations. Systemic infections were ruled out in these patients by examination of blood, urine, stool and sputum.

RESULTS

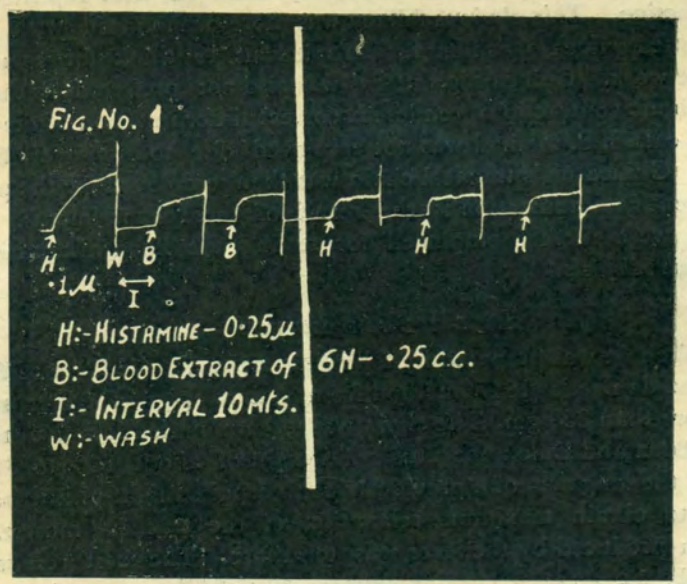
(a) *Guinea pig's ileum.*

The same dose of histamine acid phosphate solution caused a gradual increase in the sensitivity of tissue for about six or seven doses each at an interval of ten minutes and thereafter the sensitivity of the tissue was either found to be lost all of a sudden or gradually decreased to nil.

The same type of results were obtained with the ileum of 20 guinea pigs even after following different experimental conditions with regard to (i) temperature variations between 28 to 42 degree Centigrade (ii) variations in relaxation time from 0 to 4 hours (iii) using atropinised tyrode's solution, (iv) increasing or decreasing the calcium contents of the tyrode solution, and (v) with different doses of histamine ranging from 1 mcg. to 30 mcg. in a 65 ml. bath.

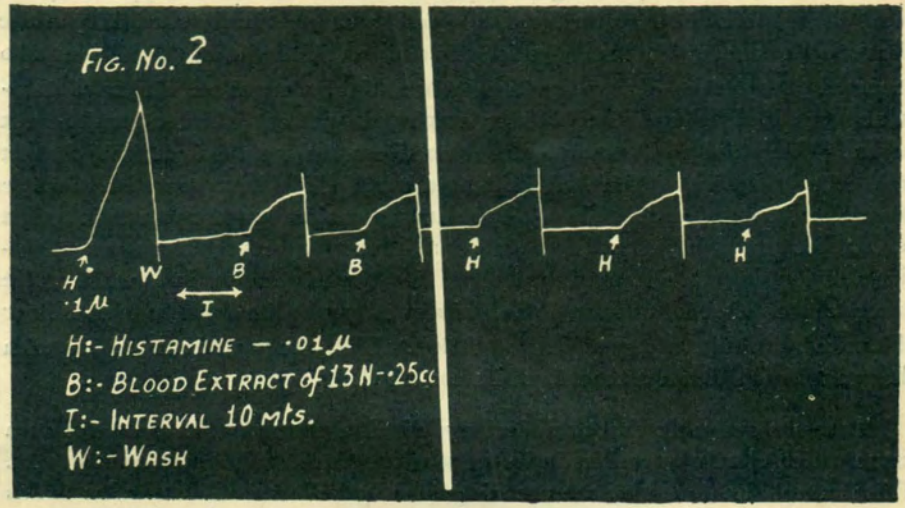
(b) *Guinea pig's tracheal chain.*

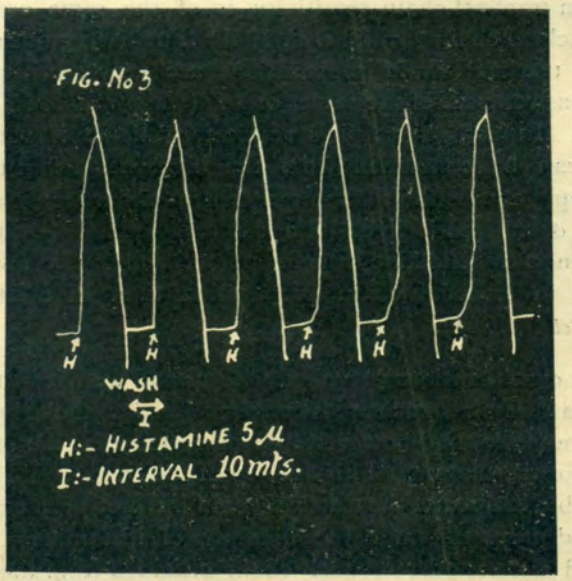
The results obtained with this tissue are shown in figures 1, 2 and 3. The point worth noting in these cases was that the tracheal chain obtained from those guinea pig's whose ileum showed the change in the sensitivity with repeated doses of histamine, responded very well by showing no change in the sensitivity (Fig. 3)



In the present series of 25 normal individuals, the blood histamine (as base) concentration was found to range from 0.003 mcgl ml. to 0.0512 mcgl ml. with an average value of 0.02632 having a standard deviation of 0.01584 and

a standard error of 0.003168. Histamine base has been calculated as $\frac{1}{3}$rd by weight of its salt, histamine acid phosphate (Schild *et al*, 1951).





DISCUSSION

The most widely used pharmacological preparation for the bioassay of histamine as has already been mentioned above, is the terminal portion of guinea-pig's ileum. The recent method of bioassay of histamine by super-

fusion technique is also done on guineapig's ileum (Gaddum 1953; Mongar and Schild 1950; Adam *et al*, 1954). In our laboratory, the tissue obtained from white as well as spotted guinea pigs of either sex and supplied by the local dealers was not found reliable due to the change in the sensitivity of the tissue with the repeated doses of histamine. Valle *et al*, (1954) had also reported that the sensitivity decreased when higher repeated doses of histamine were used, when these workers employed guineapig's ileum as the test organ. They had also observed that sometimes the responsiveness of the gut may even be blocked. Further Valentine *et al*, (1950) have reported that the bioassay of histamine on guinea-pigs ileum "is subject to the whims inherent in biologic assays in general, but with careful technique provides a very sensitive and reproducible method of determining the histamine in blood". The exact cause of this abnormal behaviour is yet to be found out as all our efforts to get uniformly good results failed. Thus the obvious fallacy with the guinea pig's ileum discouraged its use for the bioassay of histamine.

It seems desirable that the bioassay of histamine should be done on a tissue which closely resembles in its responsiveness to its human counterpart. This is amply achieved by the use of guinea pig's tracheal chain as it has already been observed that the responsiveness of bronchial tissue to histamine is same in human beings and guinea pigs (Ackasu, 1952; Sinha, 1956); and also that human tracheal chain sensitivity is of the same order as that of guinea pig's tracheal chain (Schild *et al*, 1951). Further, guinea pig's isolated bronchial and tracheal chain preparations are very sensitive to histamine and their responsiveness is of the same order (Isagowa, 1941; Castillo and de Beer, 1947). In fact, guinea pig's respiratory tissue is very sensitive to histamine as can be concluded from the observation that the respiratory organ of guineapig acts as the shock organ when either exogenous histamine is administered or antigen is given to a sentised animal (Dragstedt, 1941). This responsiveness to histamine can be potentiated by a few histaminase inhibitors more in tracheal chain preparation than in uterus and ileum (Arunlakshana *et al*, 1954).

The above considerations prevailed with us and we tried the guinea pig's tracheal chain as the pharmacological preparation for the bioassay of histamine. Gaddum (1948) had however, also suggested that the bronchial tissue could be used for the bioassay of histamine but so far, as could be ascertained from the available literature, the tracheal chain has not been employed as the pharmacological preparation for the bioassay of histamine. In the experiments reported above the tracheal chain obtained from those guinea pigs whose ileum showed changes in sensitivity with repeated doses of histamine, gave uniformly good results. The range of blood histamine obtained by employing the tracheal chain preparation can be grossly compared with that obtained by the earlier workers. (Table 1). The lower values of the blood

histamine obtained in the present series appears to be due to the fact that the tracheal chain preparation is more sensitive than the terminal portion of the gut.

TABLE 1

Showing Values of blood histamine obtained by different workers.

Author.	Blood histamine mcg/ml.	No. of cases	Method of assay.
Randolph & Rackemann (1941).	0.019 to 0.071	20	Guinea pig's ileum.
Haworth & MacDonald (1937).	0.01 to 0.08	103	Guinea pig's ileum.
Valentine et al. (1950).	0.03 to 0.11	17(fasting)	Guinea pig's ileum.
,,	0.057 to 0.140	16	,,
Jha (1957)	0.02 to 0.13	12	,,
Present work	0.003 to 0.0512	25	Guinea pig's tracheal chain.

It is thus felt that guineapig tracheal chain might prove, a more reliable tissue than the widely used ileum. Further work in this direction is indicated.

SUMMARY

A fallacy with guinea pig's ileum for the bioassay of histamine has been pointed out and the usefulness of guinea pig's tracheal chain as a reliable pharmacological preparation is reported and discussed.

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