EFFECT OF ULTRADILUTIONS ON NEUROTRANSMITTER/ENZYME

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Most if not all the biological phenomena can now be explained at the molecular level in terms of enzyme catalyzed reactions. There is no need to postulate some mysterious vital force to account for these phenomena. The enzymes are highly specific in their action and are subject to regulation by certain specific activators and inhibitors. Enzyme synthesis can also be regulated at the genetic level as far as the synthesis of each constituent polypeptide chain of the enzyme molecule, a specific gene is involved. The concentration of a particular enzyme in different systems, say, nervous system, liver or blood etc., is more or less fixed. Detection of any marked change in their normal value may aid in diagnosis and prognosis of the particular disease related to that particular organ. Thus as the acetylcholinesterase, synthesized in liver is distributed in brain cells, and nerve fibres, the assay of acetylcholinesterase is of diagnostic value in various liver diseases, malignant tumours, bronchial asthma, pulmonary tuberculosis, malnutritoin, etc. The value of acetylcholinesterase is raised in case of obesity, nephrosis whereas the decreased value is obtained in case of malignancy, after obstructive jaundice or advanced cirrhosis, etc.

Homoeopathic drugs are believed to act by correcting impulses. An impulse is produced by the sequential stimulation of synaptic junction in the nerve fibres and travels in the form of electrochemical waves. In the process of transport across the synaptic junction, certain chemicals, the neurotransmitters, are involved. Out of these neurotransmitters, acetylcholine is the most widely studied. Acetylcholine is released from neuron. Then it travels through the synapse, carrying the information to the next neuron which has acetylcholine receptors in its membrane facing the synapse, i.e. the post-synaptic membrane. Two enzymes are involved in this process. One is acetylcholinesterase, which hydrolyzes acetylcholine into acetate and choline immediately after its function is over. The other is choline acetyl transferase which resynthesizes acetylcholine. We expected the study of acetylcholinesterase could provide us some insight in homoeopathic drug function. The source of this enzyme was goat brain and human blood serum; and it was purified in this laboratory.

ASSAY OF ACETYLCHOLINESTERASE ACTIVITY

The removal hydrolysis of acetylcholine per unit time is measured by comparison of the initial concentration in a reference tube (minus the non-enzymatic hydrolysis) with the final concentration in the experimental tube.

The acetylcholine is converted with hydroxylamine to the corresponding acylhydroxamic acid, which forms a strongly coloured ferric hydroxamate with ferric salts. The colour is read at 490 nm.

Conditions: pH optimum 8.6; incubation time 30 to 60 minutes; temperature 37°C.

Equipment: (1) Constant temperature incubator mixer. (2) Pyc Unican SP500.

Reagents: (1) Veronal buffer (0.1 M; pH 8.6): 49.2g Sodium veronal and 32.4g sodium acetate dissolved in about 3000 ml distilled water, 30 ml of 1N HCL and diluted to 5000 ml with distilled water.

(2) Acetylcholine stock solution (200 mM): 1.8167 g acetylcholine chloride added to a 50ml volumetric flask, dissolved in distilled water and made up to the volume.

(3) Substrate acetylcholine (1-33 nm) : 150ml veronal buffer (1) and required volume of acetylcholine stock (2) is mixed by shaking.

(4) NaOH (2.4 N): 100 g NaOH in 1000ml of distilled water.

(5) Hydroxylamine (1 N): Dissolve 70g hydroxylamine hydrochloride dissolved in distilled water and made up to 1000ml; stored in polythene flask in refrigerator.

(6) Alkaline hydroxylamine: Equal volume of (4) and (5) mixed before use.

(7) Iron solution (0.7 M): 337.5g or FeSO $12H_2O$ dissolved in about 700ml distilled water with gentle warming. 25g of potassium nitrate dissolved in a little distilled water were transferred to a 1000ml volumetric flask and diluted to mark.

(8) Citrate fubber (1N, pH 1.4): 10.5g of citric acid and 4.0g of NaOH were dissolved in the minimum distilled water in a 500ml volumetric flask; 445 1N HCL were added and diluted to mark. The pH of the solution adjusted to 1.4 to 1.2.

PROCEDURE

In a reference tube the initial concentration of the substrate is measured and in the test the final concentration; temperature 37°C; incubation volume 27ml; read against a blank cuvette.

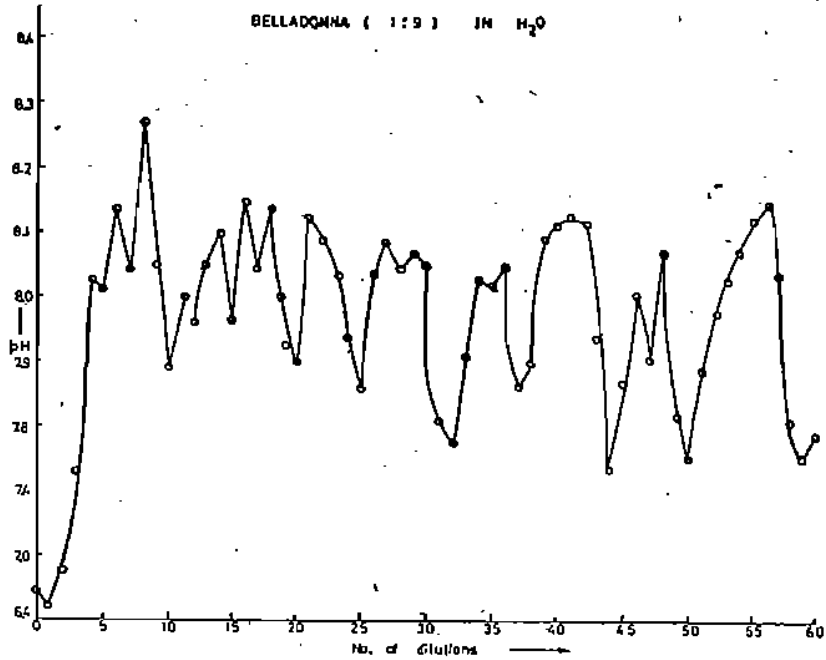
Calculation: The extinction of the dye at 490 nm is 0.961cm per mole. Hence the amount of dye formed from the non-hydrolyzed acetylcholine is 50ml.

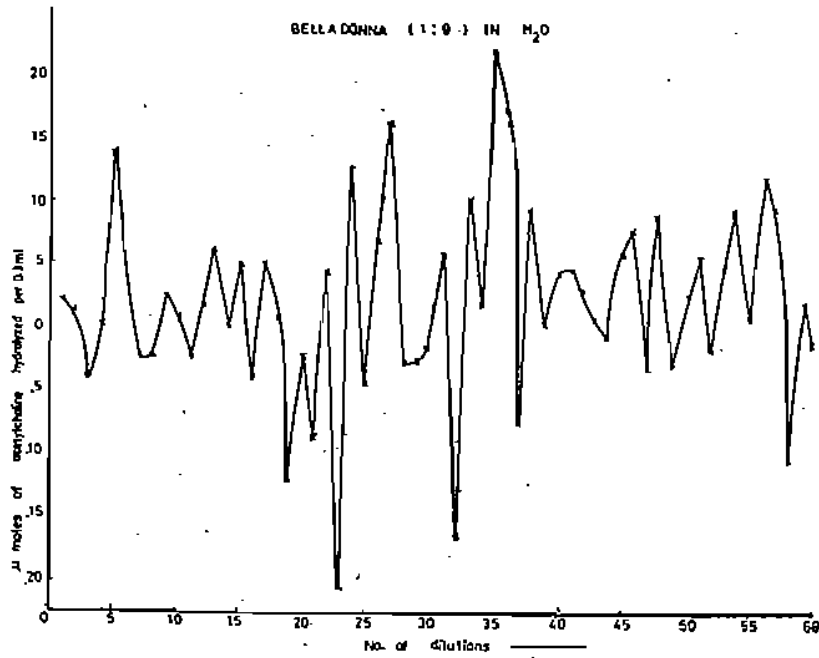
$$C = \frac{\triangle E \times 50}{0.961 \times 1.0} \text{ mole/50ml}$$

The acetylcholinesterase activity in whole blood is given by volume activity.

First method: Acetylcholinesterase was purified from goat brain and the

rate of acetylcholine hydrolyzed under the influence of ultradilutions of Belladonna is shown in the graph No. 1.





Second method: Estimation of activity of acetylcholinesterase in serum as given in the *Standard Methods of Clinical Chemistry* volume 344, Bergmeyer was used.

Collection of sample: Blood collected from cubital vein was allowed (by staying) to coagulate at room temperature and centrifuged 3000g. Serum was collected before haemolysis started.

Quantity used: 0.2ml serum added to 5.0 ml of distilled water.

Stability of sample: Undiluted serum is stable for several days at 4°C. Diluted serum does not stay longer than 4 to 6 hours.

OBSERVATIONS

Experiment I: Assay of serum acetylcholinesterase activity.

The procedure as described in the assay system was followed and following observations were made:

Exp. No.	ER O.D. of Ref.	ET O.D. of Test	$E=E_r-E_t$	Enzyme activity units/litre=E×10833
1.	0.21	0.08	0.13	1408.29
2.	0.248	0.850	0.163	1765.779
3.	0.29	0.12	0.17	1841.61

Hence average activity of the enzyme = 1672+265 units/litre
Normal value=2500 ± 265 U/L serum

Experiment II: Effect of ultradilutions of Arsenic album on enzyme activity.

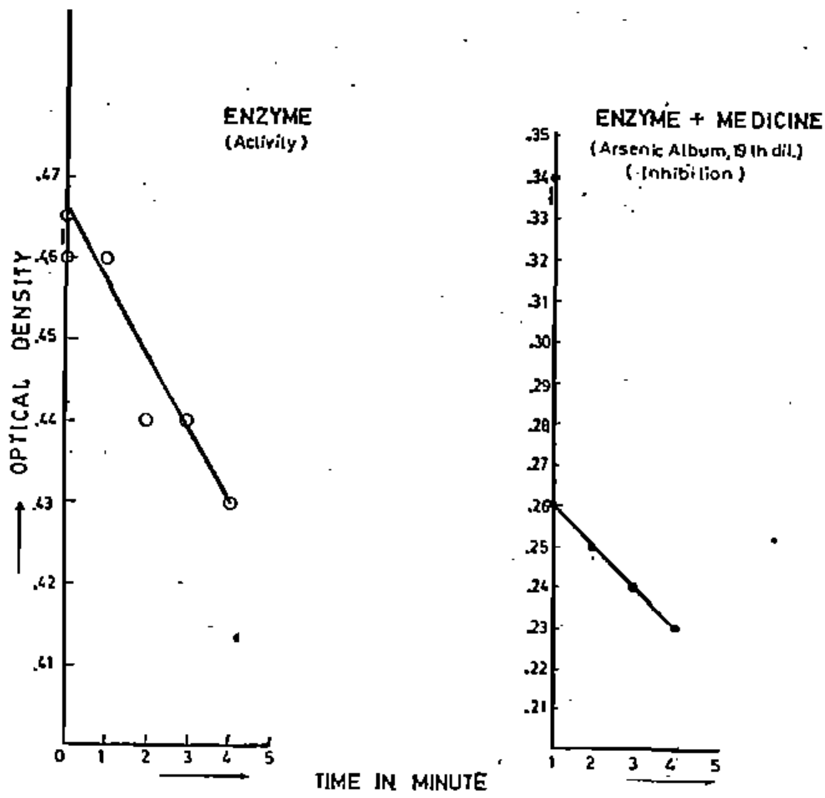
Representations used in the following table:

T=Test: 2. 5ml substrate+2.5ml distilled water+1.0ml DW+0.4ml enzyme, these incubated for the given time; then were added 1.0ml NaOH.HCL+ 1ml citrate+2.0ml ferric ammonium sulphate. (Keep for 20 min. centrifuge, Read O.D.).

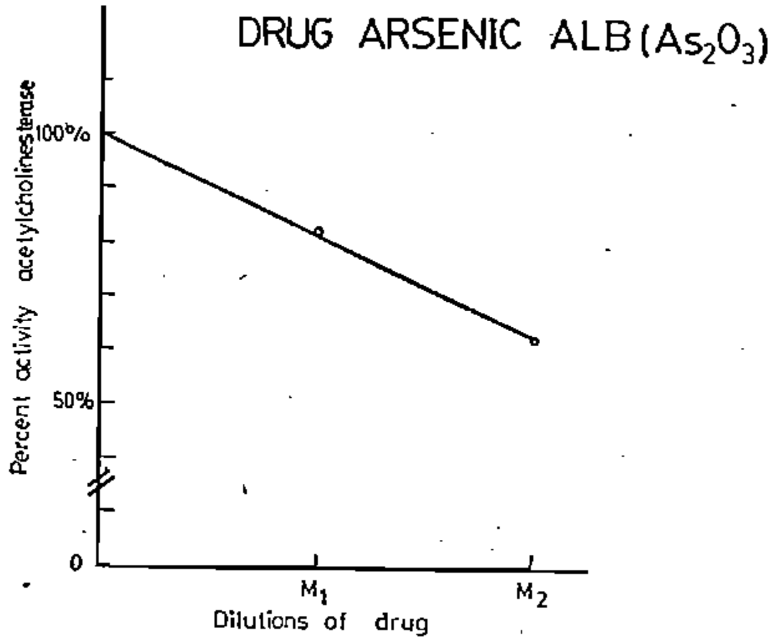
A : 10% alcohol (instead of distilled water)
M_1 : 10% solution of Arsenic alb. 27 } These dilutions were prepared
M_2 : 10% solution of Arsenic alb. 28 } in our laboratory.

Time in minutes for incubation	Enzyme activity in terms of acetylcholine hydrolysed					
	T	A	M_1	M_2	Percentage of M_1	Percentage of M_2
15 minutes	1.85	2.00	1.65	1.25	82.5	62.5

Value of "A" has been taken as 100%.
(Please see graphs)



DRUG ARSENIC ALB (As_2O_3)



1 Control is taken as 100 % alcohol
2 Each value is mean of four or more experiments
3 M_1 is 27th dilution of medicine and M_2 28th dilution

CONCLUSIONS

The above results indicate a definite and strong inhibition of acetylcholinesterase activity by Arsenie used in virtually infinite dilution and suggest further investigations or a dilution at which Arsenic would have no effect.

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REFERENCES

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