ENHANCEMENT OF THE ANTIGENIC ACTIVITY OF TOXOIDS BY SYMPATHOMIMETIC DRUGS

BY

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The immunization of children with fluid toxoids involves repeated injections of the antigen to achieve a satisfactory degree of protection. Research has been proceeding for some time into ways of improving antigenic efficiency, and a number of successful means have been found. Added water-insoluble mineral carriers—such as alum, aluminium or calcium salts, tapioca, oils and fats, and, lately, bacterial suspensions—have all had an enhancing effect on the antigenic efficiency of fluid toxoids. Their presence in prophylactic toxoids causes local irritation at the site of inoculation, the degree of irritation varying with the type of adjuvant used. It is very marked, for instance, with alum or insoluble calcium salts, but rather mild with bacterial suspensions.

For some years we have been interested in the problem of enhancing the antigenic action of toxoids, and to this end have so far been using *H. pertussis* suspensions with success. We realize, however, that there are occasions when the addition of pertussis vaccines is superfluous, as, for example, to toxoids required for booster doses to older children previously immunized against diphtheria, or to children known to be allergic, into whom the less foreign protein is injected the better.

Our attention has therefore been directed to drugs known to have a local tissue effect, either decreasing the rate of absorption or involving local inflammation. Of substances with the former pharmacological action we have studied the sympathomimetic drugs; with the latter, histamine. We have found that histamine had only a slightly stimulating action on the antigenic response to diphtheria toxoid, whereas the vasoconstrictor effect of the sympathomimetic group had a considerable enhancing effect.

As sympathomimetic drugs we used (1) adrenaline hydrochloride, at a dilution of 1/1,000 or 1/2,000; (2) benzedrine hydrochloride, at a dilution of 1/250; and (3) ephedrine hydrochloride, at a dilution of 1/250. In addition, two amines with no vasoconstrictor action, β -phenylethylamine (at a dilution of 1/250) and phenylethylamine (at the same dilution), were tested and found to be without stimulating effect on the antigenicity of diphtheria toxoid. The method of testing the different antigens was the same as used in earlier experiments (Ungar, 1954). Our new results are given in the Table.

Mean Antitoxin Titres in Groups of Ten Guinea-pigs After Giving Purified Diphtheria Toxoid with Various Substances

Group No.	Substance Added to Diphtheria Toxoid	Antitoxin Response (u./ml.) (Geometric mean of 10 animals)
1	Control. Plain toxoid	0.25
2	β -phenylethylamine 1/250	0.17
3	Phenylethylamine acetate 1/250	0.23
4	Benzedrine hydrochloride 1/250	1.96
5	Ephedrine hydrochloride 1/250	2.21
6	Adrenaline 1/1,000	2.87
7	Adrenaline 1/2,000	3.36
8	Histamine 0.5 μ g./ml.	0.70

Doses: 1 ml. diphtheria toxoid (2.5 Lf./ml.) injected by subcutaneous route. Two doses with four-weeks interval. Animals bled two weeks after second dose.

It should be noted that the amount of the vasoconstrictor added to the toxoid must be carefully chosen, for we have observed that higher concentrations of vasoconstrictors than those quoted above may cause local damage to the injected tissue.

From our results it may be concluded that the addition of vasoconstrictors had a considerable enhancing effect on the antigenic action of diphtheria toxoid. As adrenaline hydrochloride is stable only in an acid medium, it cannot be kept in contact with the toxoid for any length of time. On the other hand, ephedrine and benzedrine, being stable at a neutral pH, can be mixed with the toxoid and stored at a suitable temperature.

It seems that the addition of a vasoconstrictor to a toxoid might be useful for immunizing children against diphtheria; it has the additional advantage that its anti-allergic action would be of value for children subject to allergic manifestations after injections of antigens.

REFERENCE

Ungar, J. (1954). *Proc. roy. Soc. Med.*, 47, 355

ACTIVITY OF PIPERAZINE, IN VITRO, AGAINST ASCARIS LUMBRICOIDES

BY

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Since the introduction and acceptance of piperazine as the drug of choice for the treatment of threadworms much interest has been taken in the possible value of this drug against other intestinal helminths. Piperazine hydrate and certain salts of piperazine have been shown to possess a high efficiency against *Ascaris lumbricoides* in man (Fayard, 1949; White, 1954; Brown, 1954; Brown and Serman, 1954; O'Brien, 1954; Rearden, 1954; Goodwin and Standen, 1954). Little work has been reported on the *in vitro* activity of piperazine against ascarides. Poynter (1955), using piperazine adipate *in vitro* against *Parascaris equorum*, showed that drug-affected worms recovered when they were placed in drug-free medium. The present series of experiments, described below, was carried out preparatory to trials with piperazine citrate against *A. lumbricoides* in man (Goodwin and Standen, 1954), and the experience so gained led to the observations made by these workers on the recovery of drug-affected worms passed by treated patients.

Materials and Method

The worms used in these experiments were *A. lumbricoides* from the pig. The culture medium was a modified Baldwin's solution, as described by Cavier and Savel (1952), in which these workers found it possible to keep ascarides alive for as long as 22 days. The composition of the medium was: NaCl, 0.8%; KCl, 0.02%; CaCl₂, 0.02%; NaHCO₃, 0.15%; MgCl₂, 0.01%; glucose, 0.5%; phosphate buffer to bring the solution to pH 8.4-8.6.

The worms were obtained from the abattoir, where they were removed from the gut immediately after the pig was slaughtered. They were placed at once in culture medium contained in a vacuum flask, and upon arrival at the laboratory were transferred to 8 by 5-in. (20 by 12.5-cm.) circular glass troughs containing fresh medium at 37° C. The worms were maintained overnight in this way, after which the most vigorous specimens of each sex were selected for experiment. For each experiment, two male and two female worms were placed in each trough and fresh medium was added to provide 125 ml. of fluid per worm. The drugs were added, in solution, to provide equivalent quantities of piperazine base as contained by 1:200, 1:280, 1:400, 1:560, 1:800, 1:1,120, and 1:1,600 dilutions of piperazine

hydrate. The object of this procedure was to provide a means of observing any difference in efficiency of the drugs that could be related to the varying character of the acid radical. The temperature was maintained at 37° C. throughout the experiments. The medium was changed every 24 hours, either with or without addition of the drug, according to experimental requirement. pH readings of the medium were taken before and after addition of the drug and again just before the change of medium, at the end of each 24-hour period. Since the worms were found to be sensitive to touch and to bright light, these stimuli were applied to drug-affected worms to determine their degree of vitality. The worms were examined frequently after addition of the drug in order to observe any drug effects, and also after removal to drug-free medium for signs of recovery.

Three salts of piperazine—the citrate, the adipate, and the phosphate—were tested to compare their activity, *in vitro*, against *A. lumbricoides*. The experiments were divided into two groups: (1) to determine the minimum drug concentration required to produce an effect on the worms, and (2) to observe the relative rates of recovery from the drug effect.

Results

Effects of Piperazine upon the Worms.—The introduction of piperazine salts in solution had no immediately apparent effect upon the worms. There was no evidence of increased activity due to irritation. With all three salts the first effects were apparent five to six hours after introduction of the drugs. At this time the worms began to show signs of reduced activity. Gradually their movements became slower until, by 24 hours, they had ceased altogether. Both sexes were affected equally. At 24 hours the colour of the worms was unchanged. They did not respond to bright light—a stimulus to which normal worms proved highly sensitive—but most worms gave a just discernible response to mechanical stimulation of the anterior ends. These effects were observed at 24 hours in drug dilutions of 1:200–1:560. After 72 hours' immersion in drug medium some of the worms in drug dilution of 1:800 showed signs of reduced activity, but still responded to light stimulus. No one salt of piperazine was more effective than any other.

Recovery of Drug-affected Worms.—In this series of experiments the worms were exposed to the drug for 48 hours, with one change of drug medium, and were then placed in drug-free medium. Completely torpid worms recovered their full activity in a half to two hours. There was little difference in the rate of recovery from the effects of the different salts of piperazine, although the worms affected by piperazine phosphate appeared somewhat less active than those affected by the citrate or the adipate.

pH of the Medium.—Cavier and Savel (1952) observed that the pH of a medium containing living worms decreased over 24 hours and that the fall from pH 8.0 to pH 5.8–7.1 was more rapid when the medium contained glucose. Control experiments confirmed these observations, but the addition of the drug to the media lowered the initial pH. It was found that, after addition of the drug, the pH fell from an initial 8.4 to 6.0–6.9. In the greater dilutions, piperazine adipate gave less reduction of pH than did the citrate or the phosphate. During the course of the experiments the pH fell progressively to 5.4–6.3 during each 24-hour period. At the beginning of the experiments citric acid was added to the medium in one of the two control groups in sufficient quantity to produce an initial pH equivalent to that of the test medium containing most drug. In this instance the pH continued to fall, and it was found that such a medium, with pH even as low as 4.8, did not materially affect the worms by the end of the 24-hour period.

In test media in which the drug addition had produced complete torpidity in the worms the pH continued to fall. This provided the first evidence that the worms may not have been affected in any vital manner by the action of the salts of piperazine, but that although they were reduced to immobility their metabolic processes continued.

Discussion

The appearance of worms in the stools of patients treated less than 24 hours previously and the recovery of such worms when placed in warm Ringer solution (Goodwin and Standen, 1954) suggest that the action of piperazine against *A. lumbricoides* in man is very similar to that against *A. lumbricoides* from the pig *in vitro*. If these similarities may be interpreted to imply a mode of drug action common to both conditions of environment then it is perhaps possible to accept the time relationship of drug action to movement *in vitro* as a basis for speculation upon the most advantageous time to give treatment to infected patients. Obviously, to achieve successful treatment drug-affected and narcotized worms must be voided in the faeces before the effects of the drug have worn off. Also, as is seen from the *in vitro* experiments, the narcotic effect *in vivo* is likely to persist for only a half to two hours after the worm is freed from the influence of the drug. Similarly, it seems almost certain that the worms in the small bowel are likely to be affected five to six hours after the drug has been given and will remain fully narcotized for as long as a piperazine concentration of 1:560 or more persists. On the other hand, it may well be that the drug effects, apparent under conditions of *in vitro* experiments, would be real much earlier *in vivo*, where peristaltic movements would assist in dislodging worms whose normal ability to maintain position in the bowel was even slightly impaired. It would appear, therefore, that successful treatment depends largely on the size of the dose, the rate of excretion of drug from the bowel once the worms have been affected, and the rate of bowel movement.

It may be argued that the most certain way of achieving worm clearance would be a series of doses each large enough to induce and maintain narcosis in the worms over the period of a normal bowel clearance. However, for ascariis-infected populations of most tropical and subtropical countries the administration of a single dose is obviously preferable to continuous and controlled treatment, providing the dose is large enough to affect the worms and is given at a time of day when the duration of worm narcosis is most likely to extend to the time of the usual morning bowel movement. In persons with normal daily bowel movements this can be achieved by the single large dose in the morning, but it is likely that worm clearance would be more certain if the drug was given before the evening meal (Goodwin and Standen, 1954). Obviously, in costive patients the opportunity for a return to normality of affected worms would be much enhanced, and it would appear desirable that the normal rate of bowel movement should be assisted by a purge timed to take effect within the limits of duration of worm narcosis.

Conclusions

From the above experiments it is concluded that piperazine citrate, adipate, and phosphate are equally active, *in vitro*, against *A. lumbricoides* from the pig. The drugs induce a state of narcosis in the worms, which recover in drug-free medium. The rate of recovery of the worms in drug-free medium is much the same—namely, a half to two hours—for all three salts of piperazine tested. The effect of the drug is gradual and does not stimulate violent movements of the worms. It is considered that this is a most desirable feature of the use of piperazine in ascariasis, since it may preclude the possibility of rupture of the bowel wall or occlusion of the common bile duct as a result of the efforts of large and muscular worms to escape from an antagonistic environment.

Summary

Piperazine citrate, adipate, and phosphate were tested *in vitro* against *A. lumbricoides* from the pig. All three salts of piperazine were equally efficient in inducing a state of narcosis in the worms.

The drug effect was gradual and did not irritate the worms.

The drugs did not kill the worms. When placed in drug-free medium all affected worms recovered in half to two hours.

In view of the similarities observed between the drug effects of piperazine against *A. lumbricoides* from the pig, *in vitro*, and clinical use of piperazine citrate against ascariasis in man, the relationship between the beginning of drug action, duration of narcosis, rate of drug excretion, and the intestinal emptying time of the host is discussed.

REFERENCES

- Brown, H. W. (1954). *J. Pediat.*, 45, 419.
— and Sierman, M. M. (1954). *Amer. J. trop. Med.*, 3, 750.
Cavier, R., and Savel, J. (1952). *C.R. Acad. Sci. (Paris)*, 234, 1216.
Fayard, C. (1949). *Thèse*, No. 889. Paris. Abstract in *Sem. Hôp. Paris*, 35, 1778.
Goodwin, L. G., and Standen, O. D. (1954). *British Medical Journal*, 2, 1332.
O'Brien, D. P. (1954). *Ibid.*, 2, 246.
Poynter, D. (1955). *Vet. Rec.*, 67, 159.
Rearden, J. (1954). *British Medical Journal*, 2, 872.
White, R. H. R. (1954). *Lancet*, 2, 315.

CINERADIOGRAPHY OF THE URINARY TRACT

BY

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The great advantages which would accrue from being able to visualize the actual movements of the renal pelvis, calices, and ureter have been obvious to urologists since the advent of contrast media and the fluorescent x-ray screen. Many urologists perform their ascending pyelograms under screen control, but, although much valuable information can be obtained by this means, the small volume of opaque medium which can be used provides poor contrast as compared with a barium suspension in the bowel, while the light intensity is insufficient to provide really clear definition of the calices.

The fluorescent screen can, of course, be made brilliant enough for clear definition, and even for photography, by greatly increasing the screening current, and this is how the ordinary excretion urogram film is exposed. A high screening current is used for a very short exposure. Unfortunately the x-ray dosage absorbed by the patient during such an exposure is high and can be repeated only a few times with safety. Up to the present time fluoroscopy, and certainly photography, has been limited by this problem of having to choose between a low x-ray dosage with poor illumination for a reasonable length of time or a high x-ray dosage with brilliant illumination for a few seconds only.

An X-Ray Image Amplifier

However, this problem has now been solved by the use of an x-ray image amplifier, which, as its name implies, amplifies a poorly illuminated screen image by electronic means (Coltman, 1948; *British Medical Journal*, 1955).

The instrument is attached to an ordinary x-ray apparatus in place of the usual viewing screen, so that the x rays strike a metal screen inside an evacuated glass tube. Instead of producing a fluorescent image, this screen emits electrons which are greatly accelerated through the tube by a potential difference of 25 kV, and are kept in focus by several "electron lenses" until they strike a small, 1-in. (2.5-cm.) fluorescent screen at the other end. This is similar to an ordinary fluorescent viewing-screen except that the image is reduced in size to 1 in., but is extremely brilliantly illuminated. An optical system can now project this image either

through an eye-piece, on to a mirror system or, of greater importance still, into the lens of a cinecamera (Fig. 1).

A bright visual image of the renal shadow can be seen with a screening current as low as a half to one milliamperere, while cineradiography can be carried out with a current as low as 6 to 7 milliamperes in some cases.

Fig. 1.—Diagrammatic representation of Marconi image amplifier, showing path of electrons through tube, together with optical system of mirrors for visual screening. The retractable mirror, which is of a semitransparent nature, is used to reflect some 90% of the light into the 16-mm. cinecamera while still allowing the operator to see what is being photographed.

At the present time, using the Marconi or the Philips instruments, the area to be screened is limited to 5 in. (13 cm.) in diameter by the size of the image amplifier tube, but it is hoped that this will be increased in the not too distant future. For urological work this small field is only just large enough to cover the whole kidney in the average patient, but the potentialities of this new method of investigation are very great indeed.

Potentialities of the Method

Using one of the new rapidly excreted opaque media such as "hypaque," with its radiologically denser molecule, the slow movements of the calices can be clearly seen during excretion urography. The very marked influence which gravity exerts on the emptying of the pelvis has proved a great surprise, at least to me. For example, if the pelvis is filled from below with the patient lying flat, the calices may remain filled for an appreciable time after the catheter is withdrawn. If the table is now tilted towards the vertical position, the calices immediately increase their movements and the pelvis may empty in a matter of seconds. The study of delayed emptying in pelvi-ureteric junction lesions, of decubitus calculi, and many other problems will surely be stimulated by these new possibilities (Fig. 2).

As one would expect, a calix damaged by a calculus or by inflammation loses its contractile power, and the possibility of detecting early tuberculous lesions is being studied with some success.

The argument whether peristalsis is present in the ureter or not can now be answered. One can see and film the column of medium moving down the ureter from one segment to the next. One can also see the very active but