together with 3 tablets of diodoquin t.d.s. for 20 days, in a controlled series of primary acute amoebiasis over-seas, to be compared with a similar series treated with emetine, E.B.I., and yatren. In chronic established infections a cure will eventually be effected by a blunderbuss therapy of courses of E.B.I. orally and yatren enemata, followed by the 20-days standard course of diodoquin and ending with a 10-days course of stovarsol or carbarsone. The diet, a non-residue one, should be as nourishing as possible; it should contain a relatively high proportion of animal protein supplemented by vitamin preparations containing the B₂ complex. A course of sulphasuxidine, to eliminate secondary bacterial invaders, should be given, and recently penicillin has been successfully used in severe cases for the same purpose, although neither penicillin nor sulphasuxidine has any direct amoebicidal effect.

Summary

Diodoquin appears to be the best of the oxyquinoline group of drugs in the treatment of amoebiasis, and is a valuable addition to the therapeutic remedies available for the treatment of this insidious and intractable disease. It is non-toxic in therapeutic doses, and is well tolerated in all but a small minority of cases.

The practice of treating amoebiasis with emetine alone cannot be too strongly condemned; only a small percentage of cases are cured, and the vast majority are rendered more resistant to further treatment with emetine and less amenable to treatment with any other available drugs.

I wish to thank the Director-General of the R.A.F. Medical Services for his interest in these cases and for obtaining the supplies of diodoquin. I am indebted to Fl. Lieut. Daly, R.A.F.V.R., who was the medical officer in charge of the wards, for his careful recording and helpful criticism; to Miss Sage, senior laboratory technician, for her painstaking examination of countless specimens; and to Sgt. Greenfield, A.G., laboratory technician, for his technical assistance.

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RESTORATION OF DIPHTHERIA IMMUNITY WITHOUT INJECTIONS

TOXOID PASTILLES BY MOUTH

BY

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I have long sought a means of immunization whereby injections could be avoided entirely, but up to the present time I have not met with this degree of success. If, however, one could devise a method of *re-immunization* which could be applied to older children and adults without the production of functional disturbances of any kind a very considerable advance would have been made.

So far as can be seen at present, the future of diphtheria immunization will be concerned mainly with the treatment of infants aged 1 year, or slightly less. At this time of life the method of administration is likely to continue to be the injection. Considerable dosage of such antigens as A.P.T. can as a rule be given without causing constitutional disturbance of any kind, at the same time bestowing an effective basal immunity. It is usually considered desirable, at the beginning of school life, to boost this immunity by means of a further injection of A.P.T., and this can generally be administered to young subjects without fear of producing sore arms or other ill effects. Nevertheless, there are fairly numerous persons of more mature age—e.g., older school-children, hospital nurses, troops, etc.—who, owing to various circumstances, may require re-immunization. Then one is confronted with the possibility of sharp local or general reactions in some subjects, even when such a mild antigen as T.A.F. is employed, but more especially when injections of A.P.T. are given. Such results may occur when only Schick-positive reactors are inoculated, but when re-immunizing injections are given to all and sundry, without previous Schick-testing to establish the need, the incidence and severity of local reactions and constitutional disturbance rise sharply in adult age groups.

Jensen's (1937) method of instilling drops of formol-toxoid into the nostrils might have solved the problem if the procedure had not been rather impracticable, and if the danger of producing rhinitis could have been overcome (*Lancet*, 1941). It has not been thought worth while to pursue that form of treatment to any extent in this country.

In a limited number of subjects I demonstrated (Bousfield and King-Brown, 1938) that deep inhalation of the air of a room which was impregnated with finely atomized formol-toxoid was capable of producing an excellent rise in the antitoxin titre of the blood: this method I could not follow up, as experience showed that most of the volunteers became highly sensitized and developed allergic symptoms. I then attempted to pass formol-toxoid through the skin, in a neutral glycerin medium, with inconclusive results (Bousfield, 1944).

The present series of experiments is concerned with attempts to apply stimuli to the upper portion of the alimentary tract. The idea was suggested by the fact that, during an attack of diphtheria, absorption of some toxin from the mouth, fauces, and oesophagus might be a factor in the production of any subsequently acquired active immunity. If toxin is thus absorbed during the disease it seemed logical to investigate whether formol-toxoid, given in sufficiently large dosage, would be absorbed by *healthy* membranes in amounts which would provide an adequate stimulus.

I conceived the idea of a fairly firm gelatin sweet or pastille, containing about 100 Lf units of formol-toxoid, suitably sweetened and flavoured with some fairly neutral agent such as peppermint. I am variously indebted to Sir Alexander Fleming, Mr. L. B. Holt, of the Inoculation Dept., St. Mary's Hospital, and to Dr. Stanley White and the chief chemist of Messrs. Parke, Davis and Co., for the final production of the pastille, which I required, in the form of a gelatin disk. In preparing the disks the toxoid was added to the melted gelatin at a low temperature—about 40° C.—and the pH was adjusted to 6.5. The formol-toxoid which Mr. Holt supplied for the purpose was highly concentrated (2,000 Lf per c.cm.) and was of great purity: it was no less than 75% pure, in terms of protein, having been prepared by a method developed by Mr. Holt at St. Mary's Hospital.

Serum-antitoxin Tests on Adult Volunteers

As I personally am highly allergic to diphtheria antigens, I tried the effect on myself of sucking the toxoid disks—four each day, for seven days—and experienced no inconvenience of any kind. I then obtained five volunteers, bled them for preliminary antitoxin titration, and gave each of them 28 toxoid-gelatin disks to suck slowly, at intervals, four a day, for seven days. Fourteen days after the conclusion of treatment they were bled again, to see whether any rise in circulating antitoxin could be demonstrated as a result of the treatment.

Sir Percival Hartley kindly undertook all serum-antitoxin titrations for me; at his request neither the main purpose of the inquiry nor the details of the individual experiments were communicated to him, in order that the antitoxin determinations should be done "blind." The importance of this fact in considering results will not escape notice. The changes in the blood serum titres in my first five cases will be found in Table I.

TABLE I.—*Dosage, Four 100-Lf Disks Daily for 7 Days*

Case	Sex and Age (Years)	Previous Immunization or History of Diphtheria	Original Serum Titre (Units)	Titre 14 Days After Treatment (Units)
1	F 19	Diphtheria at 5 years; Schick-neg. at age 17	> 0.1 < 0.2	> 0.2 < 0.4
2	F 40	3 inj. diph. prophylactic 15 years ago. No post-Schick test	> 1.5 < 2.0	> 4.0, nearly 8
3	M 17	2 inj. 2 years ago, probably A.P.T. No post-Schick test	> 0.05 < 0.1	> 8.0 < 16.0
4	M 33	Had diphtheria at age of 6 years	> 0.02 < 0.05	> 1.5 < 2.0
5	F 45	Naturally Schick-negative 10 years ago	> 1.5 < 2.0	> 4.0, nearly 8

At this point it may be stated that none of the subjects who took the disks by mouth, in this or in subsequent experiments, experienced any inconvenience whatever, with the exception of one adult female who complained of persistent repetition of the

flavouring element in the mouth. Constitutional symptoms were entirely lacking.

It will be noticed that all the subjects in Table I had previous experience of diphtheria antigens or of the disease, except Case 5, which was naturally immune and in which such experience can be assumed. All the cases showed a definite increase in antitoxin titre at the second examination, the change being very marked indeed in subjects 3 and 4, in which rises are of the order of 160 and 50 times respectively. These five subjects seem to demonstrate fairly conclusively that an effective boosting stimulus of toxoid had been passed across the mucous membranes, as I was unaware of any other form of extraneous stimulus to account for the increase of antitoxin. It seemed unlikely that five subjects, living in different homes, would all have chanced to come in contact with a stimulating dose of diphtheria infection.

The next step was to see whether a reduction of the number of toxoid disks would yield similar results. In this series only three of the disks were sucked daily by a further five volunteers. The results are shown in Table II.

TABLE II.—*Dosage, Three 100-Lf Disks Daily for 7 Days*

Case	Sex and Age (Years)	Previous Immunization or History of Diphtheria	Original Serum Titre (Units)	Titre 14 Days After Treatment (Units)
6	M 24	Blood antitoxin 1 year ago, 0.1 to 0.2 unit	> 0.1 < 0.2	> 8.0
7	M 18	3 inj. T.A.M., 1933; blood antitoxin, 1943, 0.01 to 0.02	> 0.01 < 0.02	> 0.05 < 0.1
8	F 19	3 inj. T.A.M. and Schick-neg., 1931	> 0.05 < 0.1	> 0.4 < 0.8
9	F 16	Blood antitoxin, 1943, 0.001; not immunized	< 0.001	< 0.001
10	F 18	Never Schick-tested or immunized	> 0.02 < 0.05	> 1.0 < 2.0

Four out of the five subjects gave a definite response to this reduced dosage, Nos. 6, 8, and 10 showing most marked improvement in antitoxin titre. Case 9 is of interest. This subject had apparently never had an effective primary stimulus in her life, and proved, furthermore, to be relatively difficult to immunize. When she failed to respond in the above experiment, I attempted to immunize her by means of three well-spaced injections of 1 c.cm. of T.A.F. A Schick test performed three months later was still definitely positive, and she required a fourth injection of T.A.F. to render her Schick-negative.

I then inquired whether the dosage of toxoid could be reduced still further. The original highly concentrated and purified toxoid, which Mr. Holt had supplied, had become exhausted. It was clearly not practicable to utilize such a refined product on a large scale on account of expense, and a relatively crude formol-toxoid, of about 50 Lf per c.cm. potency, was used for the preparation of the next supply of disks. Messrs. Parke Davis overcame the difficulty of including the larger volume of antigen in a disk of similar size to those supplied formerly. On account of the use of this relatively impure formol-toxoid, I again controlled the possibility of allergic trouble by sucking a number of the disks personally before handing them to others, without ill effect. Table III shows the effect of administering only two of these new disks (each containing approximately 100 Lf units) daily, for seven days, to a further group of five subjects.

TABLE III.—*Dosage, Two 100-Lf Disks Daily for 7 Days*

Case	Sex and Age (Years)	Previous Immunization or History of Diphtheria	Original Serum Titre (Units)	Titre 14 Days After Treatment (Units)
11	F 15	Immunized 2 years ago	> 0.1 < 0.2	> 0.2 < 0.4
12	F 14	Immunized early in life	> 0.05 < 0.1	> 0.05 < 0.1
13	F 16	Immunized at age of 4; re-immunized at 11 years	> 0.005 < 0.01	> 0.01 < 0.05
14	F 18	Immunized 5 years ago	> 0.01 < 0.02	> 0.05 < 0.1
15	F 19	Immunized at age of 11	> 0.04 < 0.02	> 0.05 < 0.1

It will be noted that the reduced dosage produced definite rises in the antitoxin titre in four cases out of five, though the improvement is not as great as was obtained by the use of three or four disks daily. No change was found in the titre of Case 12. This may indicate that the stimulus was insufficient or that the subject was unusually resistant to a stimulus the size of which would ordinarily be found to be moderately satisfactory. On the whole I am of the opinion that three 100-Lf disks daily for one week is the smallest dosage which should be applied to adults if a marked increase in the antitoxin concentration of the blood is sought.

It was now desirable to determine if the stimulation took place only between the lips and the lower end of the oesophagus, or whether, in any circumstances, absorption of any undestroyed toxoid took place through the gastro-intestinal membranes. I expected that rapid destruction would occur in the stomach. My first attempt was a failure. I dipped a large number of the disks into melted gelatin to coat them, the dipping being so rapid that I did not expect to harm the toxoid. One group of volunteers swallowed some of these coated disks rapidly with water, thrice daily, while others sucked them slowly in the same dosage, the total taken being 21 disks in each case. Clearly I had damaged the toxoid in the dipping process, as neither those who swallowed nor those who sucked showed any change at all in their antitoxin titrations, and I wasted a number of very valuable volunteers.

The problem was then approached from a different angle. Instead of dipping the disks in gelatin after manufacture some toxoid was put up in capsules in a gelatin mass. These could be swallowed at once with a draught of water. Four subjects undertook to do this, and the experiment was controlled by giving the same toxoid dosage in plain disk form, to be sucked slowly by another group. The results are given in Table IV.

TABLE IV.—*The Effect of (a) Swallowing 100 Lf. of Toxoid in Gelatin Mass, Thrice Daily for 7 Days; and (b) the Effect of Sucking Three 100-Lf Disks Daily for the Same Period*

(a) Effect of Swallowing Toxoid				
Case	Sex and Age (Years)	Previous Immunization or History of Diphtheria	Original Serum Titre (Units)	Titre 14 Days After Treatment (Units)
26	F 19	Immunized 4 years ago	> 0.4 < 0.8	> 0.4 < 0.8
27	F 20	Nat. Schick-neg. 18 months ago	> 1.0 < 2.0	> 1.0 < 2.0
28	F 17	No history of either	> 0.1 < 0.2	> 0.1 < 0.2
29	F 32	Nat. Schick-neg. 6 months ago; reported to have had diphtheria twice	> 0.4 < 0.8	> 0.4 < 0.8
(b) Effect of Sucking Toxoid				
30	F 37	Diphtheria as a baby	> 0.1 < 0.2	> 1.0 < 2.0
31	F 30	No history of either	> 0.1 < 0.2	> 0.4 < 0.8
32	F 19	Immunized 4 years ago	> 0.005 < 0.01	> 0.1 < 0.2
33	F 55	Diphtheria at 10 years	> 0.2 < 0.4	> 2.0 < 4.0
34	F 39	Nat. Schick-neg. 2 years ago	> 0.2 < 0.4	> 0.4 < 0.8
35	F 33	Positive diph. swab during attack of scarlet fever 10 years ago	> 0.05 < 0.1	> 1.0 < 2.0
36	M 24	Nat. Schick-neg. 2 years ago	> 0.4 < 0.8	> 0.4 < 0.8

From the above it will be seen that none of the subjects who swallowed the toxoid showed any antitoxin rises, whereas all those who sucked the disks showed an increase, with the exception of Case 36. It is interesting that he, sucked the residue of an early supply of disks, all the other control cases having been given a new batch. Case 36 may thus have been one of those individuals who respond to stimuli only with difficulty, or the disks he sucked may have lost Lf value.

Consideration of the results in the above table suggests most strongly that the stimuli responsible for increases of titre take place only between the lips and, at the lowest, the lower end of the oesophagus. The method will probably be limited in use to such subjects as are old enough or intelligent enough to suck the disks slowly.

I also desired to find out whether, in the case of persons who had only negligible quantities of antitoxin in their bloods, the titre could be raised by repetition of the toxoid-sucking procedure at intervals. In my abortive gelatin-coated disk-swallowing and sucking experiment I had come across three volunteers who had less than 0.001 of a unit of antitoxin per c.cm. in their bloods. This suggested that they might never have possessed any basal immunity, in spite of the fact that they were of mature adult age. Some weeks after the first experiment I gave them a further 21 disks—one to be sucked thrice daily—waited a fortnight after completion of the treatment, and then repeated the 21-disks course. After a further 14-day interval a third course of disks was given, and a fortnight later the subjects were again bled. Sir Percival Hartley once more reported less than 0.001 unit of antitoxin in all three cases. It thus seems probable that the method, at any rate in its present form, does not provide a sufficiently powerful

stimulus for the purpose of primary immunization. A higher concentration of toxoid in the disks, or a method of prolonging the stimulus, such as some form of chewing-gum, might still be made to operate as a primary stimulus. Further work on such lines is indicated.

Investigations on Children, using a Modified Schick Test

The next stage was to see if the treatment could be applied to children who, having been satisfactorily rendered Schick-negative by means of prophylactic injections some years before, had been found to have relapsed to the Schick-positive state. Circumstances did not permit of bleeding these children, so I had to work by the Schick standard. It was first necessary to determine the effect of the stimulus provided by the amount of toxin present in the test dose of 0.2 c.cm. of Schick toxin. I Schick-tested a large number of children immunized some years before and found that 21 cases had relapsed to Schick-positive. Without giving any further injections I retested these 21 children three weeks from the date of finding them Schick-positive. To my surprise no fewer than 16 of the 21 had become Schick-negative as a result of the stimulus provided by the former test. It was thus clear that a routine Schick test for the purpose of finding positive cases on which to test the toxoid disk was a complicating factor, making it difficult to select groups suitable for comparative tests. Accordingly I resolved to find out whether testing with a half-dose of Schick toxin produced a lower conversion rate. Twenty-six children who had relapsed to Schick-positive were thus identified from another large group of subjects who had been previously immunized by myself. When I tested these twenty-six children again three weeks later I found that 13 had become Schick-negative as a result of the stimulus provided by the previous test in the reduced dosage of 0.1 c.cm. This result was disappointing; but I could not take bloods, so I was forced to work on the half-Schick dose for sorting-out purposes if I wished to try the effect of the disks on children.

Using the half-dose Schick test, I identified a further group of children who had relapsed to Schick-positive; I gave 17 of them three toxoid disks daily for a week, and retested them 14 days after completion of the treatment. All proved to be Schick-negative then. Three other relapsed children were given only two disks per day for a week, and 14 days after conclusion of treatment two had become Schick-negative, while one remained positive.

Thus, so far as it is possible to infer anything from Schick-test-controlled work, the observations on the volunteers who were bled are supported by these experiments in the children. This was only to be expected, but it was necessary to find out whether children of from 4 to 14 years of age could, on the whole, be relied upon to suck the disks slowly enough. There seems to be good reason to believe that they can be trusted to do so. Experience might enable one to produce the disks as a really attractive sweet, and this would ensure that the parent would not be allowed to forget to administer the treatment.

I attempted to amplify the evidence by investigating children resident in an institution who had a history of some previous immunization or of having been Schick-negative in the past. In my sorting-out process to find relapsed cases the few Schick-positive readings which I obtained were mostly so pronounced that I felt doubtful about their alleged original natural Schick-negative state. In many years of practical experience I have not found that natural Schick-negative reactors relapse to the intense degree of positive reaction observed in these cases, though most of the subjects were supposed to have been naturally immune formerly. Administration of disks to the children was largely a failure, and, on the evidence which I have offered above in the blood-antitoxin tables, I am of the opinion that these children had probably never reached the Schick-negative level in the past. This may serve to confirm the opinion which I have stated above—that the disk treatment is insufficient as a primary stimulus, and will prove adequate only when the subject has, at some time previously, reached the degree of training in antitoxin production which is revealed by a negative Schick test. All relapsed cases which were previously immunized by myself, and which had three disks daily for seven days, proved to be Schick-negative 14 days later, as compared with the

control group, in which one-half of 26 cases became Schick-negative as a result of the 0.1 c.cm. Schick-toxin stimulus when tested again at the same interval as the children who sucked the disks.

Effect of Disk Treatment on Subjects having only Small Quantities of Circulating Antitoxin

There will be those who will be especially interested in this aspect of the question, and, to save a further summarizing table, I would draw attention to the following cases: Cases 7 and 9 (Table II), Cases 13, 14, and 15 (Table III), and Case 32 (Table IV). I repeat that I was unable to obtain a response from any of my four cases which had less than 0.001 of serum-antitoxin.

The above work demonstrates, I believe for the first time, that antitoxin-producing stimuli can be passed effectively across the mucous membranes of the upper portion of a healthy alimentary tract. Much work remains to be done to verify my results and to determine whether the method is applicable in the case of other antigens, such as tetanus toxoid, haemolytic streptococcus toxin, and the like. I feel that the findings should be made known at this stage so that others, who may have greater opportunities, may pursue the matter.

Summary

A method of providing a stimulus to the upper part of the alimentary tract by means of toxoid-impregnated gelatin disks is described.

The method seems to be generally effective in subjects who have any demonstrable amount of antitoxin present in the blood, or who are definitely known to have reached the Schick-negative level at some time in the past.

Rises in serum-antitoxin content are very striking in some of the cases.

While I find no evidence that the method will suffice to provide an adequate stimulus for primary immunization, it appears to be generally applicable to children and adults who have ever been immune in the past, provided that the age and intelligence of the subject are sufficient to ensure that the disks are sucked slowly. Modification of technique might still render primary immunization feasible.

The treatment can be administered to immune and non-immune subjects impartially without fear of producing local or general reactions, and thus, in cases which are known to have been immune in the past, a Schick test is not essential before using this method of re-immunization.

In view of the findings in the case of diphtheria formal-toxoid there seems to be an indication for pursuing the investigations in the case of other antigens, such as tetanus toxoid, where a rise in antitoxin titre is sought in order to boost a previously bestow immunity.

While attractive to needle-shy children, the greatest value of such a method might be in dealing with adults—e.g., nurses or men in the Services—when it is so important that constitutional disturbance shall be avoided.

More research will be necessary in the matter of stabilizing the disks, so that they could safely be given a long period of legitimate use before expiry date. It would also be necessary to eliminate more of the formalin in order to produce the disks in the form of a sweet which would be really attractive to children.

In addition to the help I have already gratefully acknowledged, I wish to thank Sir Alan Daley, Dr. Anderson (M.O.H., Heston and Isleworth), Dr. O. W. Roberts (medical superintendent, Dulwich Hospital), and Drs. Smith and Taylor, of the London County Council medical staff, for the kind provision of facilities. I am also greatly indebted to the nurses, health visitors, public health department staff, and sanitary inspectors who volunteered for the blood investigation experiments.

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According to reports in the Swedish press, Prof. Ragnar Granit, of the Neurophysiological Institution at the Carolinian Institute in Stockholm, has received a donation of 200,000 kronor (£11,765) from the Rockefeller Foundation. The grant is to be distributed over a period of five years. Recently it was decided to incorporate Prof. Granit's institution in the Nobel Institute of Medicine and to erect a special building for the institution in the neighbourhood of the Carolinian Hospital on the northern outskirts of Stockholm.