SUMMARY

Possible examples of spread of postinoculation jaundice to contacts who had not been inoculated with icterogenic yellow fever vaccine are reported.

The incidence of infective hepatitis in childhood and adolescence in a sample of British troops has been ascertained. From this, evidence has been deduced that a previous attack of infective hepatitis gives a certain measure of protection against an attack of postinoculation jaundice, though the protection is not absolute.

A complement-fixation test has been developed, the results of which tend to show that there is an antigenic relationship between the agents responsible for infective hepatitis and postinoculation jaundice.

Efforts to transmit postinoculation jaundice to man and various animals are described.

A comparison is made of infective hepatitis and postinoculation jaundice; the conclusion is reached that they are due to the same or to very closely allied agents.

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LABORATORY AND CLINICAL TRIALS OF PATULIN

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A SUPPLY of patulin was made available to the Army in March, 1943, by the courtesy of Prof. H. Raistrick, for laboratory and clinical trials. These trials, which were sponsored by the Director of Pathology and the Consulting Physician, War Office, were begun in March, 1943, and continued until October, 1943. The results were briefly referred to in a letter to THE LANCET (1943, ii, 684). Since it is understood that the good results recorded by Hopkins [1] have not been confirmed by other workers, it has been thought desirable to record the results of the Army investigations. This article is a condensed version of the report submitted to the Director of Pathology.

BACTERIOSTATIC ACTIVITY

Patulin was found to have a bacteriostatic action on a wide range of bacteria, both gram-positive and gram-negative. Eighteen strains, members of the genera *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Neisseria*, *Bacterium* and *Hæmophilus* were tested and all were inhibited for 24 hours at 37° C. in fluid media by concentrations of patulin ranging from 1/40,000 to 1/160,000 for different strains. With one exception, *Bact. typhosum* Rawlings "rejuvenated," the addition of 10% horse serum reduced the activity of patulin by about three-quarters, while the overnight incubation of dilutions prepared in tryptic digest broth at pH 7·4 almost completely destroyed its activity.

TOXICITY TO ANIMALS

(*a*) *Mice.*—The toxicity for mice was ascertained by inoculating groups of mice intravenously, intraperitoneally and subcutaneously with graded doses. The mice were all of the Swiss strain, and weighed 16–19 grammes. All doses were given in 0·25 c.cm. saline.

Intravenous route.—Mice which received 1·0 mg. showed immediate excitement and jerky movements. Thirty seconds later they lay on their sides, became unconscious and stopped breathing. After a few minutes respiration restarted and within 5 minutes all the mice got up, but looked ill and remained so. They all died within 5 hours. Those mice given 0·5 mg. looked a little rough after one hour; one died after 5 hours and the remainder within 3 days. Mice given 0·25, 0·125 and 0·0625 mg. appeared well after one hour, but rough after 5 hours, though all survived. Mice which died showed plum-coloured lungs with a hæmorrhagic exudate and one had an axillary hæmorrhage in addition. Their livers appeared to be unusually pale.

Intraperitoneal route.—Mice given 1·0 mg. looked sick after 1 hour, and those given 0·5 mg. looked somewhat rough, while the others looked well. After 5 hours those given the smaller doses all looked rough, while those given 1·0 and 0·5 mg. were all dead. All the mice which died showed considerable ascites; in one the fluid was bloodstained. Mice given 0·25 mg. all died in 3 or 4 days.

Subcutaneous route.—All mice given subcutaneous injection appeared rough after 1 hour, and those which received 0·0625 mg. and 0·125 mg. were scratching the site of inoculation. After 5 hours all had developed œdema at the site of injection, which was very massive, forming tumours as large as the head of the mouse in those given 0·5 and 1·0 mg. All the latter died within 18 hours, and those given 0·25 mg. within 2 days.

It thus appeared that patulin was more toxic when given subcutaneously or intraperitoneally than by the intravenous route.

(*b*) *Rabbits.*—The effect of patulin on the rabbit's eye and skin was ascertained as a guide to the possible use of the substance in infections of the conjunctiva or skin in man. 1% patulin in pH 6·0 phosphate buffer dropped into the eye produced intense, apparently painless, œdema of the conjunctiva in each of two rabbits. Purulent conjunctivitis and opacity of the cornea developed, but there was ultimate complete recovery. The opposite eye of each rabbit received the phosphate buffer without effect. 0·1% patulin produced slight reddening of the conjunctiva without œdema, while weaker solutions had no effect.

Intradermal injections of 0·1 c.cm. of 1/1000, 1/10,000 and 1/20,000 patulin solutions produced a small swelling of the skin without reddening, and this swelling persisted during the next 24 hours. Corresponding solutions of buffer without the patulin were absorbed without giving rise to any swelling.

(*c*) *Chick embryos.*—A small number of 12-day developing chick embryos were inoculated with patulin solution via the allantoic sac. 0·25 c.cm. of 1/400, 1/800 and 1/1600 solutions of patulin all killed the embryos in 1–4 days, whereas embryos receiving weaker solutions survived. 0·5 c.cm. of 1/400 and 1/800 solutions of patulin dropped on the chorio-allantoic membrane of 12-day embryos produced no immediate vascular response, but resulted in the death of the embryos within 24 hours.

CHEMOTHERAPEUTIC EXPERIMENTS

Two chemotherapeutic experiments were made on mice, employing influenza virus A and *Bact. typhosum* respectively. Influenza virus was then thought to be a suitable test for patulin in view of the use of the drug in the treatment of the common cold, and *Bact. typhosum* was chosen for the bacterial infection because of the high in-vitro bacteriostasis of patulin for that organism.

(*a*) *Influenza virus A.*—Groups of six mice of 12–14 g. were treated by inoculation intraperitoneally of either phosphate buffer solution alone, or similar solution containing patulin. Treatment was given 1 day before, immediately after, 1 day after, and 2 days after the intranasal inoculation of a mouse-lung emulsion from mice infected with influenza A (PR 8 strain). The treated mice received 0·1875 mg. patulin in all. The virus inoculum was given in three dilutions of the original lung emulsion and the several dilutions were each administered to a group of patulin-treated and a group of control buffer-solution-treated mice. The mice were killed on the 10th day.

The treated and the control groups of mice showed a considerable similarity in number and extent of lesions, and the patulin treatment did not appear to have exerted any influence on the virus infection.

(*b*) *Bact. typhosum* ("rejuvenated" Rawlings strain).—This strain was chosen as it was inhibited by a dilution of 1/160,000 of patulin in broth cultures, even when 10% horse serum was added. Groups of mice were treated with buffer or with patulin solution intraperitoneally 24 hours before, 2 and 10½ hours after infection with measured quantities of an 18-hour culture of *Bact. typhosum* also given intraperitoneally. The treated mice each received 0·156 mg. patulin in all.

The treated mice had a higher death-rate during the 48 hours after injection than did the controls.

1. Hopkins, W. A. *Lancet*, 1943, ii, 631.

Treatment of the Common Cold

ORGANISATION OF CLINICAL TRIALS

During March, 1943, preliminary experiments were carried out at a primary training wing for infantry to ascertain whether patulin had any demonstrable effect on the severity or duration of the ordinary afebrile coryza or common cold. The investigator was supplied with two solutions, A and B, but he was not told which solution contained the active compound. Nearly 50 patients with coryza were treated with the solutions by nasal instillation of drops of the solutions, alternate cases being treated with the two solutions. It was found difficult to assess the effect of treatment, in view of the lack of real objective signs which could serve as a check to the patient's subjective feelings. Among both treated and controls some colds showed some improvement but this varied a good deal. Improvement coming on in 2 days and lasting for a week after the start of treatment might be classed as "cure." On this basis, "cures" were recorded among 5 of the 25 patients treated with patulin and in none of the 25 controls. Therefore although only one-fifth could be regarded as "cures" we felt that further trials were indicated.

The later trials were undertaken at a different primary training wing in August and September, 1943. The investigator was given solutions labelled C and D, F and G. One of each pair of solutions contained patulin. The procedure was that the technician allotted alternate patients for treatment with each solution and handed the appropriate solution to the clinician. Neither patient nor clinician knew at the time of treatment, or when the results were being recorded, whether patulin or control solution had been used. The technique of administration was changed to nasal spraying in order to conform to that used by Surgeon-Commander Hopkins. The solutions used were supplied from a military laboratory, and were prepared from a single sample of patulin, sent by Professor Raistrick, who had stated that the stability of the compound in solution was largely controlled by pH and that the substance should be dissolved in phosphate buffer at pH 6·0. He also stated that in his view the exact composition of the buffer was not of importance, and a mixed disodium phosphate and monosodium phosphate was therefore used throughout the work.

PREPARATION OF SOLUTIONS

During the preliminary trials with solutions A and B a stock solution of 1/1000 patulin in M/50 phosphate buffered sterile distilled water of pH 6·0 was supplied to the clinician who diluted it 1/10 with sterile normal saline on the day of use. The control solution consisted of the same buffered water without patulin, diluted freshly in saline.

On August 5, 1943, four bottles were sent to the clinician. Two consisted of 250 c.cm. volumes and were labelled "solution C" and "solution D," and also "ready for use." Solution C was a 1/5000 solution of patulin in M/500 sodium phosphate buffer, pH 6·0 in normal saline made from dry powder on August 5. D was buffered saline only. Two other bottles consisted of 25 c.cm. amounts labelled "concentrated 10 times." C contained 1/500 patulin in M/50 sodium phosphate buffer pH 6·0 in normal saline prepared from dry powder on August 5, while D was buffered saline only.

The clinician used the "ready for use" or dilute patulin and buffer solutions for 2 weeks after receipt, there having been a period of 2 days while the bottles were in the post. Thus the dilute solutions were used between August 7 and 21. The clinician then diluted the concentrated solutions to 250 c.cm. with sterile normal saline and used these until they were exhausted about August 29.

On August 27 six bottles of concentrated solutions were sent to the clinician, labelled F and G, three of patulin and three of buffered saline. The clinician then diluted each solution to 250 c.cm. as required for use. The concentrated patulin solution had been made up freshly from powder on August 27.

On Sept. 20 six further similar bottles of 25 c.cm. freshly prepared concentrated solutions were sent, three of patulin and three of control solution. These were diluted as required, and used up to Oct. 6 when the experiment terminated. That this technique did not impair the activity of the patulin was shown by the following test, carried out on Nov. 24, 1943. The first solution diluted by the clinician from the batch prepared on Sept. 20 had not been exhausted, and two bottles of the concentrated solutions remained. These were compared with a stock 1/1000 patulin solution in M/400 phosphate buffer solution prepared on March 11, 1943, and with one freshly prepared on Nov. 24, the day of the test. All four solutions inhibited the growth of *Staph. aureus* and of *Bact. typhosum* (Watson) to within the limits of error, taken as half a tube-reading, of a final concentration of 1/80,000 patulin.

This has been set out at some length, for early criticism of the Army investigations was mainly directed at the minutiæ of the preparation of solutions.

CLINICAL PROCEDURE

First day.—Cases were first seen at 09.00 hours. A detailed history was then taken and recorded on a special form. Temperature and pulse-rate were noted, and an examination of the conjunctivæ, nose (with speculum), nasal sinuses for tenderness on pressure, fauces and chest was then made. The amount of nasal discharge and obstruction were next assessed. Two smears of the nasal discharge were made, and a swab taken for culture. The patient was then told to lie on a couch, where he was sprayed (using a de Vilbiss spray) up both nostrils and into the throat, and a few drops of the solution used were also instilled into each nostril with a pipette. About 3–5 c.cm. of either patulin or control solution were thus given. The patient was instructed not to blow his nose for half an hour, but simply to dab his nostrils with a handkerchief if required. He was instructed to report again at 12.00 hours, 14.00 hours and 16.00 hours, and again the next day. On each occasion the amount of nasal obstruction and discharge were assessed and recorded, and then the treatment was repeated.

Third day.—No treatment was given, but further smears of the nasal discharge were made, and in some cases a further swab for culture. He was also given a form on which to record the daily progress of his cold until his final attendance on the 7th day. On the 7th day his answer to the question "Do you think the treatment has done your cold any good?" was recorded, and if the cold was still present, he was handed a slip on which to record the day on which he considered his cold ended.

SPECIAL INVESTIGATIONS

Two smears of the nasal discharge were taken on the 1st day of attendance and again on the 3rd day. One of each was stained by Gram's method and one with Leishman's stain. The bacteria and types of cells present were thus observed before and after treatment. A culture was made from a swab, on the 1st day only in most cases, though in a few cases it was also made on the 3rd day. If the history or the physical signs indicated that a chest lesion was possibly present, a skiagram of the chest was taken.

An attempt was made to correlate the findings in smears and cultures with the clinical progress of each case. It would be expected that those cases which were clinical successes would be found amongst those whose nasal discharge showed a definite decrease in pus or bacteria or both. This was not obviously the case. There was no correlation between the type of organism present in cultures and the clinical response or the appearances in films after treatment, and it could not be said that either solution acted on any special organism or failed because any special organism was present.

ANALYSIS OF CASES AND RESULTS

During the trial the investigator did not gain any impression that one solution was better than the other; so personal bias in assessing results was eliminated as far as possible. All the data were obtained before an analysis of the results was begun.

In all, 130 cases of coryza were investigated, alternate cases being controls. The first 19 cases were not adequately followed up and occurred only spasmodically during the summer. They were useful in getting the procedure running smoothly. They have been excluded from the analysis. Some of the remaining cases were unable to attend for the requisite number of times, owing to leave, postings and so forth. There remained for analysis 100 cases, 50 treated with patulin and 50 with the control buffer solution.

Type of cases.—The subjects were all men, with the exception of one ATS private. No officers were treated. The majority were young recruits for the armoured corps in their twenties, but the ages ranged from 17 to 43 years. The colds were of the acute type commonly seen in recruit establishments and conformed to the ordinary type of cold frequent in this country in the autumn. Only 3 were associated with pyrexia, which in no case exceeded 99·6° F. The symptoms, previous history, nasal signs and complications of the treated and the control groups were analysed, and the two groups were found to be closely comparable in these respects.

Effect of treatment.—The cases were classified into groups according to the clinical response. Group 1 contains all the obvious successes whose colds improved on all days, or which improved on the first 3 days and had gone by the 7th. Group 2 consists of those cases who, although they did not show improvement at the end of the 1st day—i.e., within 7 hours—had improved on the next two mornings and showed either continued improvement or absence of the cold on the 7th day. Group 3 consists of cases showing no improvement on the first two days, but improved continuously thereafter. Group 4 contains cases which showed some temporary improvement which was not maintained. Group 5 failed to show any improvement during the whole week. Of group 1, only 1 case, treated with the control solution, showed clinical cure within 48 hours (table I).

TABLE I

Group	Patulin		Control	
1. Improved on all days	5	9	7	14
2. Improved after the 1st day	1		4	
3. Improved after the 2nd day	3		3	
4. Temporary improvement, but relapsed	13		7	
Total cases considered improved	22		21	
Group 5. Cases not affected	28		29	
Totals	50		50	
Number of cases whose colds had gone on or before 7th day	7		10	

Duration of the colds.—When last seen on the 7th day, patients were given slips to fill in and return when their colds eventually went. Only those whose colds had gone by the end of the week after starting treatment were examined for the absence of signs of coryza. The patients' estimates of the duration of their colds were probably variable. The results of 42 treated cases and the 42 controls from whom the necessary figures were obtained are given in table II.

TABLE II

	Patulin	Control
Duration of the cold before treatment—		
Colds over a week old before treatment	0	4
Average duration of remainder before treatment	2·6 days	2·74 days
Duration of the cold after treatment—		
0 – 7 days	7	10
8 –14 ,,	12	11
15–21 ,,	11	11
Over 21 days	12	10
Totals	42	42
Total duration of the cold—		
1– 7 days	4	5
8–14 ,,	9	8
15–21 ,,	13	14
Over 21 days	16	15
Totals	42	42

Opinion of the patients treated.—Answers given on the 7th day to the question "Do you think the treatment has done your cold any good?" were probably to some extent unreliable, for the honesty of the replies was perhaps tempered by a kindly desire not to give offence to the investigator whom they regarded as the sponsor of a new treatment. Further, they were for the most part impressed by the tests done and the interest taken in them, in contrast to the usual attitude to colds in the medical inspection room. The figures are :—

	Patulin	Control
Undecided, or believed that the treatment had no effect	13	13
Believed that the treatment had had a good effect, temporary or lasting	36	37

(The answer of one patient treated with patulin was not recorded).

Treatment of Conjunctivitis

Nine cases of bilateral conjunctivitis were treated by Brigadier Sir Stewart Duke-Elder with patulin which was sent to him in the form of a 1/1000 solution in M/400 phosphate buffer. He was advised to use it in final concentrations of 1/10,000 or 1/20,000 diluted with saline. The bottle of 1/1000 stock solution was returned after the trials and found to be fully active against *Staph. aureus* and *Bact. typhosum*. The report, which is quoted with his permission, is as follows :—

(*a*) In each case one eye was treated with patulin, the other with saline; in case 4 only one eye was affected. In cases 1, 2, 3, 5, 6, 7, 8 no clinical difference was seen between the two eyes. In case 9 the eye on saline improved considerably.

(*b*) The only organism which disappeared was the streptococcus, case 4. (It was noted, however, that *Proteus vulgaris* appeared in the second culture.)

(*c*) Cases 1 and 2 had 1/20,000 patulin; cases 3 to 9 had 1/10,000. The latter produced some irritation.

Summary and Conclusions

Patulin is bacteriostatic against a wide range of gram-positive and gram-negative bacteria. Its bacteriostatic activity is materially reduced by preliminary incubation at 37° C. overnight of dilutions in broth at pH 7·4, or by the addition of horse serum. It is stable in solution at pH 6·0 for several months at room temperature.

Toxicity experiments in mice have shown a relatively small margin between concentrations which kill the animals and those which produce bacteriostasis in vitro. Lethal and toxic effects are more readily produced by subcutaneous or intraperitoneal inoculation than by intravenous injection. Two experiments in mice resulted in failure to cure infections with influenza virus A or with *Bact. typhosum*.

Controlled clinical trials in the treatment of the common cold with patulin have shown no advantage from the use of this substance as compared with the use of a control buffer solution without patulin, nor did patulin appear to be of value in the treatment of human cases of conjunctivitis.

These therapeutic trials emphasised the great difficulty of assessing the effect of treatment in view of the lack of real objective signs which, in the common cold, can serve as a check of the patient's subjective feelings. A serious attempt was made in the main trial of 100 cases to eliminate personal bias, both during treatment and in recording results. Neither investigator nor patient knew whether patulin or control solution had been used for treatment, and all results were obtained before any analysis was begun.

The main trial was carried out on 100 men at a recruit establishment during the season of autumn colds. Patulin was given to 50 and the control solution to 50. The treated and the control groups were comparable as regards age, symptomatology, duration of the cold before and after treatment, and the bacteriological findings. Neither the control nor the patulin solution appeared to produce any effects which could be described as either immediate or dramatic.

The actual solutions used in the final series of trials were tested for bacteriostatic power 9 weeks after preparation and after the conclusion of the trials. When compared with a freshly prepared solution it was found that they retained full activity.

It had to be concluded that patulin had no demonstrable effect on the course of this series of colds as compared with the natural evolution of the disease.

Coffee-beans, whether roasted or not, contain about 1·3% of caffeine. If 2 oz. of the ground beans are used to make a pint of coffee, a teacupful of the beverage will contain some gr. 1¾ of caffeine—about the same as an equal quantity of tea. Café au lait usually contains about one part of coffee to one of milk, so that a breakfast-cup of this will not contain more caffeine than a teacupful of tea. The Ministry of Food is now drawing up standards for liquid essences, under which coffee essences must contain not less than 0·5% and coffee-chicory essences not less than 0·25% of caffeine derived from coffee. To contain this amount, coffee essences will have to be prepared from not less than 4 lb. and coffee-chicory essences from not less than 2 lb. of roasted coffee per gallon.