CHEMOTHERAPY OF CHOLERA WITH A NEW SULPHONAMIDE COMPOUND ("6257")

LABORATORY INVESTIGATIONS AND FIELD TRIALS

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For a number of years Rogers's hypertonic saline and palliative drug therapy has formed the sheet anchor for the treatment of cholera. Recently sulphaguanidine has been aligned to it, particularly through the efforts of workers in Bengal, and has given encouraging results (Chopra *et al.*, 1941; Carruthers, 1942; Misra, 1944; Huang, 1944; Lahiri, 1945; Seal, 1946, 1947; and others).

An observation by one of us (S.S.B.) since 1939, that hexamethylenetetramine in a 10% solution in normal saline killed cholera vibrio in less than half an hour up to a concentration of 1 in 10^{12} , resulted in the production of a few compounds of hexamine linkage to sulphanilamide experiments with which on animal and human cholera infection, though interrupted by conditions of field service, gave hope of successful chemotherapy against this disease. From a chance discussion with the scientific department of Ciba (Basle) it transpired that their investigators (Meier, 1946; Druey, 1948), after studying a large series of sulpha derivatives of low solubility, had condensed a product—"6257"—of two molecules of sulphathiazole ("cibazol") and three molecules of formaldehyde with the formula $C_{21}H_{22}O_6N_6S_4$, the molecular configuration not having yet been worked out. The present study relates to *in vitro* and *in vivo* experiments with this compound against the cholera vibrio and to field trials on human cholera infection.

Laboratory Investigations

Culture.—The Inaba and Ogawa strains of *Vibrio cholerae*, grown on meat-infusion alkaline "bacto" agar (pH 8) at 37° C. for 18 to 24 hours, provided the test inocula for all the experiments. After suitable dilution with normal saline the initial bacterial concentration was estimated by opacity and controlled by pour-plate counts.

Drug Concentration.—The compound "6257" being only slightly soluble in water, it was suspended by means of 2% gum arabic in distilled water. A 20% drug suspension—the "standard drug suspension" of the text—was initially prepared and sterilized at 100° C. for 10 minutes only, this exposure being sufficient for the purpose. This suspension was suitably diluted as required.

Test Mixtures.—All *in vitro* experiments were carried out in 10 ml. of "difco bacto" peptone water (pH 8) to which was added the requisite amount of the drug and the required

number of bacterial cells, each suspended in 0.1 ml. volume. Peptone water not only is an excellent medium for the growth of the cholera vibrio, but its use also eliminates the possibility of erroneous results due to acid fermentation of the carbohydrate likely to be found in a meat-infusion substrate. The test mixtures and the control tubes were incubated at 37° C. ($\pm 0.5^\circ$ C.). It was not found necessary to raise the temperature of incubation any higher to witness bactericidal and growth-inhibiting activity, as has been the experience with other sulpha derivatives (White, 1939). At various periods after incubation (6, 12, and 24 hours) a measured amount of the fluid was withdrawn and serially diluted to seed alkaline agar plates for the purpose of counting the number of viable organisms.

Animal Experiments.—Adult mice of uniform stock, 20–25 g. in weight, were employed in groups of 20 as an experimental unit, the controls being one-half this number. The Inaba strain was mouse-passaged till the cholera vibrio attained a virulence resulting in 100% deaths of untreated mice with a dose of 2,000 million organisms administered intraperitoneally. Twice this number (4,000 millions) was the test dose in the experiments reported here. Higher multiples of M.L.D. were given up after a few preliminary experiments on account of the danger of mortality from toxic action alone. The test dose consisted of organisms freshly isolated from the intraperitoneal fluid of dead mice and grown only once on alkaline agar, since the cholera vibrio loses its virulence quickly when subcultured frequently.

In relation to the time of infection, the drug therapy was divided into (i) a pre-infective period of 48 hours, and (ii) a post-infective period of 96 hours. The dosage is indicated in Table II. Oral feeding was carried out directly into the stomach of the animal with a special curved bulb-ended needle and syringe. This procedure was found to be simpler and more accurate than the drug-diet intake method. No local irritation was observed after subcutaneous therapy.

Determinations of the blood concentration of the drug were carried out on non-infected animals given identical dosage, since frequent handling and bleeding of infected mice produced inconsistent results. The methods employed were those advocated by Bratton and Marshall (1939) and Churg and Lehr (1941).

In vitro Activity of "6257"

Serial dilutions of the sterile "standard drug suspension" were titrated against inocula of different size. The results of one of these experiments are reproduced in Table I.

TABLE I.—*In vitro Activity of "6257" on the Inaba Strain of Vibrio cholerae: Bactericidal and Bacteriostatic Action*

Dilution of 20% Standard Drug Suspension	Total Concentration of Drug (mg.)	Inoculum: Total No. of Bacterial Cells	Growth at 37° C. Colony Counts Hours after Incubation		
			6	12	24
Control	0	1 × 10 ⁶	C	C	C
1: 50	200.00	"	—	—	—
1: 100	100.00	"	—	—	—
1: 200	50.00	"	4 × 10 ⁴	4 × 10 ⁴	—
1: 300	33.33	"	9 × 10 ⁵	1.5 × 10 ⁶	—
1: 400	25.00	"	6.5 × 10 ⁶	18 × 10 ⁶	9 × 10 ³
1: 500	20.00	"	8 × 10 ⁶	—	26 × 10 ⁶
1: 600	16.66	"	C	C	34 × 10 ⁶
1: 700	14.28	"	C	C	44 × 10 ⁶
1: 800	12.50	"	C	C	60 × 10 ⁶
1: 1,000	10.00	"	C	C	—
1: 2,000	5.00	"	C	C	C

C Indicates confluent growth. — Indicates no growth.
Columns 4, 5, and 6 show the total number of viable organisms in the test mixture.

The Table shows that a marked bactericidal and inhibitory activity is exercised by "6257" on the Inaba strain of *Vibrio cholerae*. Similar results were obtained with the Ogawa strain.

When an inoculum of one million cholera vibrios was incubated with 200, 100, 50, and 33.3 mg. of the compound for 24 hours, samples plated on alkaline agar remained sterile. Further confirmation of this finding was obtained by subjecting the total volume of 10.2 ml. of the test mixtures to two other viability tests—namely, fractional division over 10 plates by the pour-plate method, when there was complete absence of colonies after 48 hours' incubation, and fractional transfer into 100 tubes of glucose-peptone water, with Andrade's indicator. In the latter test the absence of turbidity and acid fermentation, after similar incubation, was taken as an index of non-viability.

Two other possible pitfalls in these tests were also excluded—namely, (a) there was no adverse effect of increase in hydrogen-ion concentration consequent on bacterial multiplication, since the pH values of the test mixtures were found not to exceed 8.6, and (b) there was no bactericidal activity of gum arabic *per se*.

It would appear that the drug has both bactericidal and bacteriostatic activity.

In vivo Activity of "6257"

The experiment recorded in Table II confirms the result of the *in vitro* tests. When the drug was administered by the

within a few hours, the infecting organism being isolated from heart-blood, liver, and spleen. Biochemical examination of the blood showed that the absorption of "6257" by the parenteral routes was slow, and still more so when given by mouth. The excretion of the drug being equally slow, a much flatter concentration curve in comparison with other sulpha derivatives was the result. Prior to infection, therefore, an effective blood level was initiated with 80–90 mg. of the drug, and was maintained with a daily dosage of 40 mg. in the post-infective period for four days, in the belief that, *vis-à-vis* rapid mouse septicaemia, the therapeutic results could be conditioned only by the concentration of the drug in the blood and the tissues. That this assumption was correct was borne out by the results obtained—i.e., 100% protection by the intraperitoneal and subcutaneous routes and only 20% survival through oral therapy.

Field Trials

In all, 85 cases of cholera were treated in 27 villages in the "talukas" of Kumbhakonam, Tiruvavur, and Nega-patam of the Tanjore district, in the south-east of Madras Presidency, during November and December. Records for the past seven years show that the disease could be considered to be endemic in that area, and its highest incidence was from September to February, with a case mortality in the region of 60%. The patients were treated in their homes without the benefit of nursing and general medical care, and no complementary treatment in the form of saline and palliative drug therapy was resorted to.

Bacteriological Diagnosis.—In all the cases the diagnosis was established by the following procedure, minor details being omitted.

A capillary-pipette drop of a saline suspension of the stool, standardized by opacity, was transferred to Dunham's peptone water (pH 8) and incubated at 37° C. The pellicle from the growth developing in 8 to 24 hours was plated on to Mac-Conkey's agar. This medium offered the advantage that the non-lactose-fermenting cholera vibrio could be easily differentiated from lactose fermenters for the purpose of quantitative studies, referred to later. After morphological examination five to six suspected colonies were transferred on to an alkaline agar slope and the growth tested against a mixed Inaba–Ogawa agglutinating rabbit serum (titre 1 in 5,000).

Classification of Cases.—The typical symptoms of cholera—purgation, rice-water stools, vomiting, dehydration, anuria, algidity, cramps, imperceptible pulse, and an apathetic-to-comatose mental state—were encountered in different combinations. The clinical picture could be differentiated into three stages of infection according to severity and, what was important from our point of view, to the period at which the treatment was started: (1) The primary

TABLE II.—*In vivo Activity of "6257"*

Treatment												Test Dose Inoculated Intraperitoneally 4,000 million Organisms	No. of Mice Infected	Survivors	
Route of Administration	Daily Dosage in mg.													No.	%
	Before Infection No. of Days		After Infection No. of Days												
	1	2	1	2	3	4									
Intraperitoneal	50	40	40	40	40	40	40	2 M.L.D.	20	20	100				
Subcutaneous	50	40	40	40	40	40	40	2 "	20	20	100				
Oral	50	40	40	40	40	40	40	2 "	20	4	20				
Controls	..	..	..	..	..	..	..	}	2 "	10	0				
									2 " live boiled culture	10	10	100			

intraperitoneal and subcutaneous routes it exercised a powerful protective action on cholera-infected mice. With the oral method, however, it was not possible to save more than 20% of the animals.

Post-mortem examination revealed that intraperitoneal cholera infection was followed by invasion of the tissues

stage, in which purgation was the main feature with little or no vomiting and no prostration—the "moderately severe" cases. It was difficult to contact these patients because the difficulties of transport caused delay in notification (2) The secondary stage, characterized by passage of rice-water stools, frequent vomiting, and

prostration but no anuria—referred to as “severe” cases. (3) The critical stage, of which anuria and dehydration were the marked features, with a semi-comatose condition, algidity, imperceptible pulse, and body cramps. The term “serious” is here applied to these cases.

Table III summarizes some of the salient features of the cases treated during the course of a cholera outbreak in an endemic area. The great majority of the patients belonged to the “untouchable” labouring class, many of

in the course of two to three hours. In six cases where this practice did not succeed, on account of hyperemesis and a comatose condition, the drug was given per rectum in a 6-g. dose suspended in gum arabic in 50 ml. volume, high up the colon, by the employment of a long piece of rubber tubing. The beneficial effect of this measure made it possible to proceed with the second dose orally.

The earlier cases were given the drug more frequently, in smaller doses. Experience, however, showed that to get

TABLE III.—*Analysis of Treated Cholera Cases*

Clinical Course	No. Treated	No. Surviving	Sex		Age Groups (Years)								Inoculated		Uninoculated
			Male	Female	5-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	No. Attacked	No. Attacked in Negative Phase	
Moderately severe cases (primary stage)	12	12	4	8	2	7	2	1	—	—	—	—	2	—	10
Severe cases (secondary stage)	16	16	7	9	3	2	5	4	1	1	—	—	3	—	13
Serious cases (critical stage)	57	54	24	33	10	8	15	9	11	2	1	1	11	11	46
Total	85	82	35	50	15	17	22	14	12	3	1	1	16	11	69

them women and children, with one feature common to all—malnutrition. The outbreak was characterized as severe by the local health authorities. This was borne out by the galloping march of symptoms, the patient being dangerously ill six to eight hours after the initial clinical manifestations, with dehydration and all its sequelae.

The scarcity of the drug precluded us from treating more than 85 cases: the total quantity available for the trial was 1,800 g. in the form of powder and in tablets of 0.5 g.

Treatment and Results

The average amount given was 16 g. for a child, 23 g. for an adult female, and 25–30 g. for an adult male, according to the scheme shown in Table IV.

TABLE IV.—*Dosage of “6257” for Oral Treatment of Cholera Patients*

Day of Treatment	Daily Dosage (Grammes)		
	Children	Women	Men
1st	6	10	10
2nd	4	4	6
3rd	2	4	4
4th	1	2	2
5th	1	1	1
6th	1	1	1
7th	1	1	1

The subcutaneous route was first tried in view of the results of the experiments in mice. It soon became clear, however, that parenteral therapy did not bring about any clinical amelioration. Obviously the treatment of cholera septicaemia in the mouse could not serve as a guide to the treatment of human disease, which in the bacteriological sense is confined to the intestinal tract alone.

Oral therapy was therefore practised and it immediately gave gratifying results. The pleasant observation also was made that “6257,” in sharp contrast to other sulpha derivatives, including sulphaguanidine, had no appreciable toxic effects. As much as 50 g. was administered to some of the cases without causing or accentuating nausea or vomiting. On a few occasions when blood was examined for agranulocytosis the findings were negative. Four women in advanced stages of pregnancy were treated successfully without any ill effects.

The retention of the drug in the presence of frequent vomiting was achieved by substituting 0.5-g. tablets in place of the powder, two tablets being given fractionally every 10 to 15 minutes until the full dose of 6 g. was ingested

the best and quickest results it was essential to start with a large dose of 6 g. and to follow it with another large dose of 4 g. four hours later, and then to watch carefully the progress of the case. Six to eight hours after starting treatment purgation diminished and vomiting stopped. By the ninth hour, as a rule, the patient had passed water, although in three seriously ill cases it took as long as 20 to 24 hours. Nourishment in the form of barley or rice “kunji” (decoction) greatly helped to restore the kidney function.

There was distinct improvement by the end of 24 hours. Purgation was much reduced and nausea, vomiting, and cramps were absent. Although the patient was weak and dehydrated, the pulse was perceptible and interest was taken in the surroundings. By the 48th hour the body was warm and dehydration reduced in proportion to fluid intake, which was insisted upon. By the morning of the fourth day the patient was convalescent and well on the way to recovery. As an illustration, the case histories of two patients—one treated orally and the other per rectum, with subsequent oral therapy—are here given.

Illustrative Cases

Case 27.—A man aged 40, uninoculated. Symptoms started at 3 a.m.; visited 12.45 p.m.; patient in critical stage; 5 motions; 7 vomits; dehydrated; comatose; anuria, cramps, pulse imperceptible, body cold. Treatment was as follows:—Dec. 3: 1 p.m., 6 g. powder, all vomited; 1.40 p.m., 6 g. tablets, retained; 8 p.m., 4 g. tablets, retained. Dec. 4: 8 a.m., 2 liquid motions during night; anuria persisted; 4 g. tablets, retained; 1 p.m., urine passed; no vomit; one liquid motion; dehydrated; apathetic. Dec. 5: 9.30 a.m., sitting up; pulse perceptible; one motion during night; still dehydrated and weak; 2 g. powder, retained; 5 p.m., cheerful; answers questions; fluids taken; dehydration less; 2 g. powder, retained. Dec. 6: 8 a.m., solid motion; free urine; body warm; rapid low-volume pulse; 1 g. powder; 6 p.m., 1 g. tablets. Dec. 7, 8, 9, 10: 0.5 g. tablet morning and evening; patient recovered.

Case 11.—A man aged 29, inoculated Nov. 23; symptoms. Nov. 25, 10 a.m.; visited, 6 p.m.; 10 motions; vomiting started 2 p.m.; no urine since 4 p.m.; body cold; pulse imperceptible; semi-comatose. Treatment was as follows:—Nov. 25: 6 p.m., powder by mouth not retained; tablets also brought up as vomit every 10 minutes. 9 p.m., 6 g. drug per rectum, most of it retained. 12 p.m., 4 g. tablets fractionally; about 2 g. retained. Nov. 26: 8 a.m., 4 rice-water motions during the night; nausea but no vomiting; body cold; pulse imperceptible; 6 g. tablets fractionally retained; 4 p.m., 2 motions; free flow of urine; dehydrated; semi-comatose; 4 g. powder, retained. Nov. 27: 9 a.m., 2 motions; free urine; dehydrated; faint

pulse; cold; 4 g. powder; 5 p.m., no motion; no vomiting; cheerful but weak; 2 g. powder. Nov. 28: 8 a.m., much better; semi-solid motion; body warm; pulse perceptible; dehydration much less; 2 g. powder. Nov. 28, 29, 30: 0.5 g. tablet morning and evening; patient recovered.

Mortality

Table III shows that all the cases in the primary and secondary stages of infection recovered, but three out of 57 cases in the critical stage could not be saved. In two of them the symptoms developed during the negative phase, within 12 hours after the inoculation of cholera vaccine, and were serious in character.

The first fatal case was that of a female, 25 years of age, who was visited 8 hours after the onset and responded well to 14 g. of the drug. Next morning, however, she was found by her neighbours in a state of collapse on the river bank, where she had gone to fetch water. She survived only two hours thereafter.

The second case was that of a man aged 63 with a long history of chronic diarrhoea who passed blood-stained stools in spite of treatment. He died within 10 hours as a result of anuria.

The third patient, a man aged 35, was visited within six hours of onset and given 11 g. of the drug in the next eight hours, the evening report being no motion and no vomit, but anuria persisting. The sanitary inspector, however, reported next morning that the patient had died in the middle of the night. A necropsy not being possible, it was presumed that he had succumbed to uraemia.

Epidemiological Significance of 7-day Therapy

As a rule, patients could be said to have recovered by the end of 72 hours; nevertheless, a schedule of treatment extending over seven days is advised. This recommendation is based on observations regarding the persistence of cholera vibrios in the stools of cases under treatment (for details see Fernandes, 1948). In the present series of cases the excreta could be said to be free from vibrios on the sixth day.

Another observation of epidemiological significance was the freedom of contacts from subsequent attacks of the disease though living in the restricted areas of cholera-infected hamlets. The seven-day drug therapy outlined in Table IV cannot therefore be too strongly recommended in the interest of the patient as well as that of the community.

Prophylactic Treatment in the Negative Phase following Cholera Vaccination

A matter that has not received sufficient consideration is the danger of possible precipitation of a serious attack of cholera from the common practice of protective inoculation of contacts, amongst whom may be persons who are harbouring the cholera vibrio or who may actually be in the incubation period of the disease. Similar experiences are known in the case of typhoid fever (Bhatnagar, Freeman, and Dhillon, 1937). Table III shows that of the 16 cases of cholera amongst the inoculated population as many as 11 were attacked in the negative phase, the infection being severe in all of them and responsible for two out of three deaths in this series.

In co-operation with the local health authorities, 4 g. of the drug, in two equal doses, was given morning and evening for two days immediately after inoculation to the contacts of treated cases in one of the villages. Although the figures are not sufficient for any conclusions to be drawn, the health authorities were struck by the freedom from infection of persons subjected to this simple therapy in comparison with those not so treated.

Discussion

The evidence presented here, in spite of its limitation in scope, clearly indicates the role of the compound "6257" in the treatment of cholera as well as in its prophylaxis. To deal with an epidemic effectively, the form of treatment at present practised necessitates the mobilization of a considerable amount of hospital accommodation, medical equipment, and personnel, all of which are limited in countries where the disease is endemic. In the absence of these amenities the results of our field trials emphasized the economic advantage of the method advocated. Consider-

ing the survival rate alone, our experiences compare very favourably with those of others obtained from the application of combined sulphaguanidine, saline, and palliative drug therapy.

Without minimizing the curative aspect of this treatment we attach very great importance to the *complete* freedom from cholera vibrios of the stools of treated cases—accomplished, as we have shown, by seven-day drug therapy. It was our experience that this simple measure brought about a very material reduction in the number of carriers, who, along with the prevailing insanitary conditions, are the backbone of any cholera epidemic, be it in an endemic area or following in the wake of big religious gatherings. If in addition the contacts of a case of cholera were protected by prophylactic treatment with the drug as indicated, particularly in the short negative phase after concomitant cholera inoculation, there is a possibility that this disease would cease to be a major problem of preventive medicine. Future trials on a more extensive scale will prove if the thesis advanced here is correct.

The preparation of "6257" by the molecular combination of two different chemical components is a departure from the common practice of obtaining sulpha derivatives by additions to, and the substitution of, the radicals of sulphanilamide. There are two references in the literature to attempts in the same direction—namely, the preparation of "septurit," a compound manufactured in the early days of sulphanilamide chemistry, and "strepturin" (Astrakhansev and Vasil'ev, 1940), found by Russian scientists to be effective against streptococcal infection.

The two outstanding properties of "6257" that are in sharp contrast to those of other sulpha derivatives are: (a) the absence of toxicity within the zone of therapeutic action, tested both in laboratory animals and in human beings; and (b) its slow absorption from a subcutaneous "depot" and equally slow excretion from the system, so that blood concentration can be maintained at a higher level for a longer period of time. The first of these properties would presumably be attributable to the formaldehyde radical, a well-known detoxicant of bacterial toxins, whereas the second would appear to be related to the high molecular weight of the compound.

The absence of both these attributes, particularly the former, was responsible for relegating the sulpha compounds to a position in clinical therapy secondary to that of the antibiotics. If future experience showed that the therapeutic value of "6257," and that of other similarly prepared compounds possessing these two qualities, extends over a wider field of pathogenetically important bacterial species, it might be that sulpha derivatives would through these new compounds regain their place of priority in clinical therapy—a welcome reversion from the point of view of production as well as of economy.

Summary

The new compound "6257"—a condensation product of sulphathiazole ("cibazol") and formaldehyde—shows *in vitro* a marked bactericidal and growth-inhibiting action against *Vibrio cholerae*.

When administered parenterally to mice, it offers 100% protection against septicaemia resulting from intraperitoneal cholera infection. When given by mouth it is, however, not so successful. The discrepancy is due to low blood-concentration value of the drug.

When patients suffering from cholera in an endemic area during the course of an epidemic were treated in their homes in villages with this drug alone, in the absence of nursing, general medical care, and any form of adjuvant treatment, the survival rate compared very favourably with that obtained by present-day recognized therapy.

In the control of an epidemic of cholera the importance of complete freedom from cholera vibrio of the stools of cases treated by seven-day drug therapy, and the prophylactic treatment of contacts with the drug, is emphasized.

The role of two properties of this drug—namely, the absence of toxic action and slow absorption and slow excretion—is discussed in its bearing on future sulpha therapy as opposed to the antibiotics.

Our grateful thanks are due to Major-General S. L. Bhatia, Surgeon General to the Government of Madras, Drs. Mathews and Pillai, Director and Assistant Director of Public Health, for their co-operation, and to Dr. R. Ramaswamy, District Health Officer, Tanjore, and his staff for their excellent help, which made this investigation possible.

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TREATMENT OF RINGWORM OF THE SCALP

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Until recent years there was no reliable treatment of ringworm of the scalp which did not involve removal of infected hairs from their follicles by the use of x rays or thallium acetate or by producing a purulent folliculitis. The removal of infected hairs with masses of spores was in itself inadequate to effect a cure, but it so enhanced the effect of local treatment with fungicides that x-ray epilation became the accepted practice of dermatologists and reduced the duration of treatment from an indefinite number of years to one or two months. For example, a series of 100 cases of ringworm of the scalp were treated from Feb. 13 to Sept. 29, 1947, by Dr. McKenny, at St. John's Hospital for Diseases of the Skin, by x-ray epilation followed by the application of Whitfield's ointment and bi-weekly paintings with tincture of iodine. The x-ray technique was the irradiation of the usual five areas (Kienböck-Adamson) with a dose of approximately 400 r to each area at 90 kV, the half-value layer equivalent to 1.4 mm. of aluminium, and focal skin distance 9½ in. (24 cm.), the diameter of the active field being 5 in. (12.5 cm.). All the patients were examined by Wood's filtered ultra-violet light or microscopically, or both, to confirm the diagnosis and again about one month later, and then at intervals of two weeks until three examinations had been negative.

The average number of days for a cure was 38.4, but four cases required treatment for 85, 85, 151, and 160 days; thus the average period for cure in the other 96 was 35 days. These results are considered to be highly satisfactory, but they were obtained in a very well equipped and specialized clinic. Moreover, the x-ray technique is tedious and exacting and occupies at least an hour. Under such conditions the risk of any permanent alopecia is negligible and certainly much less than that of toxic reactions to thallium acetate, but the risks have to be borne in mind, and they have always brought anxiety to the doctor and to the parents of children under treatment.

It was therefore disappointing to find in our own experience and in that of Carrick (1946) and others that new and potent fungicides were no more effective in the treatment of scalp ringworm than the older remedies, and it was obvious that this must be due to their failure to penetrate the infected follicles. At first it was hoped to overcome this difficulty by using fat solvents such as acetone, benzene, carbon tetrachloride, chloroform, and alcohol containing iodine or benzoic and salicylic acids, but these met with little success.

The interesting fact that while microsporon infections of the scalp are common in children they are very rare in adults led to the discovery by Stephen Rothman and his co-workers (1946, 1947) that a certain fraction of the free fatty acids extracted from adult human hair was capable of inhibiting the growth of *Microsporon audouini* in concentrations of 0.0002–0.0005%. Pelargonic acid was the main constituent of this fraction, but no success followed its therapeutic use, probably because of its lack of penetrating power.

Penetrating Vehicles

Attention was therefore directed to the use of penetrating vehicles which might carry an effective fungicide to the base of the hair follicle and make possible the cure of scalp ringworm by simple inunction or even by painting the scalp without preliminary epilation. In recent years this problem has received considerable attention in America. Herrmann, Sulzberger and Baer (1944) and MacKee, Sulzberger, Herrmann, and Baer (1945) have reported on the use of vehicles containing wetting agents to promote skin penetration. MacKee, Herrmann, and Karp (1946) found that the most promising results in the treatment of tinea capitis were obtained with a preparation containing cetyltrimethylammonium pentachlorophenate 3.5% in "intraderm." The latter consists of a combination of sodium alkylbenzene sulphonate 5%, phenazone 12.5%, propylene glycol 10%, sulphonated castor oil 10%, and distilled water. The pH was adjusted to 4 by the addition of citric and propionic acids. In addition to the application of the solution the treatment included the use of an acid shampoo containing sodium alkylbenzene sulphonate and sulphonated castor oil. In a series of 59 cases of infection with *M. audouini*, 54% of the children were cured, the average duration of treatment being approximately 14 weeks. More recently Foley and Lee (1947) have reported on the fungicidal action of combinations of cetyltrimethylammonium pentachlorophenate with fatty acids. The combination found to be most active against pathogenic fungi was cetyltrimethylammonium pentachlorophenate and undecylenic acid.

Fungicidal Agents

In the preliminary experiments of our series phenylmercuric nitrate and salicylanilide have been used as the fungicidal agents. Phenylmercuric nitrate was investigated clinically in 1933 by Levine, who reported favourably on its use in aqueous ointment bases for the treatment of epidermophytosis and tinea. It possesses low toxicity, and