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CONTENTS.

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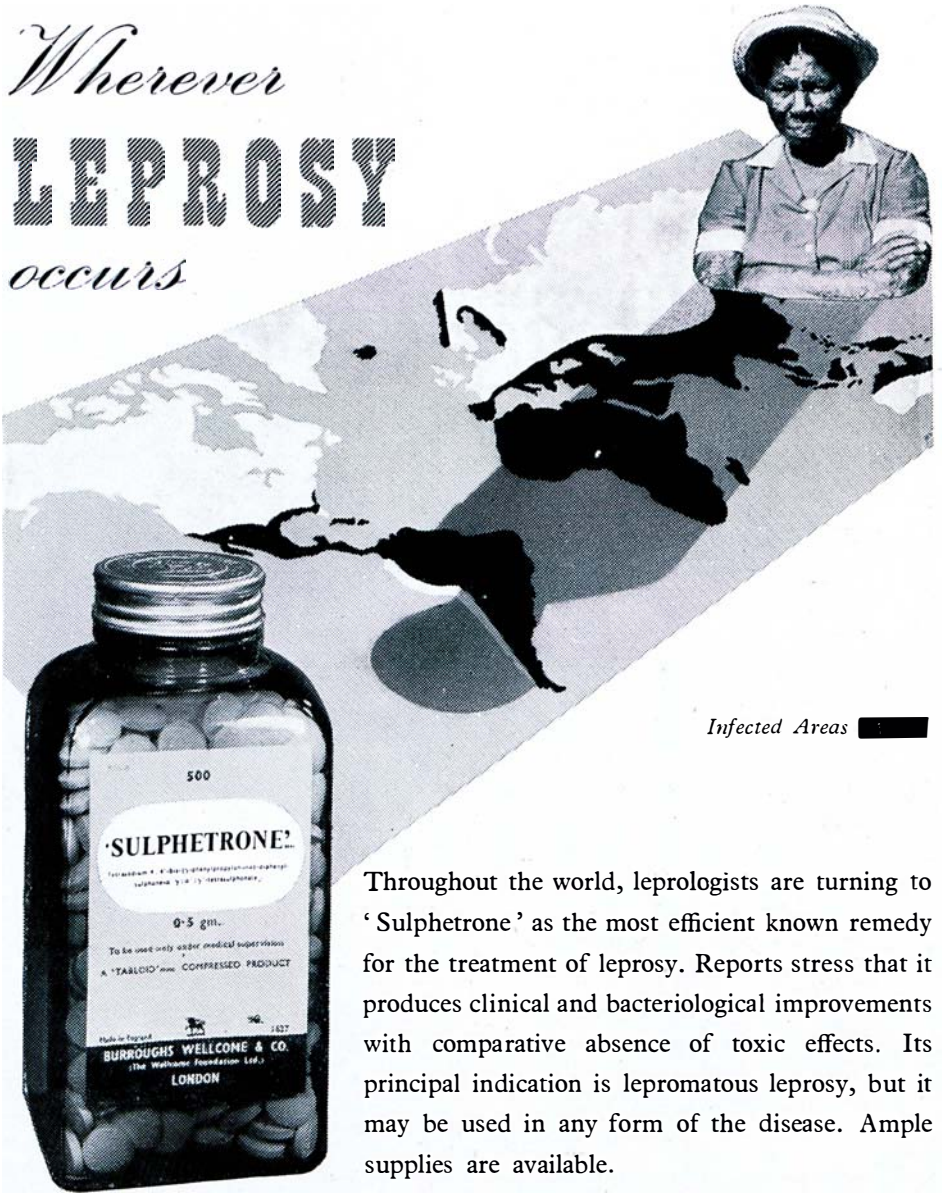
Wherever

LEPROSY

occurs



Infected Areas

Throughout the world, leprologists are turning to 'Sulphetrone' as the most efficient known remedy for the treatment of leprosy. Reports stress that it produces clinical and bacteriological improvements with comparative absence of toxic effects. Its principal indication is lepromatous leprosy, but it may be used in any form of the disease. Ample supplies are available.

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EDITORIAL

In this issue we republish an article from Vol. 16, No. 4 (Oct.-Dec. 1948) of the *International Journal of Leprosy* giving an account of two American Marines who apparently developed tuberculoid lesions on the site of tattooing on the forearm which was done in Sydney, Australia. These two cases are cited as evidence that leprosy can be spread by direct inoculation, and it is inevitable that they will be quoted in future textbooks as clinical evidence of one method of leprosy transmission.

How far does this claim and other accounts of leprosy due to direct inoculation stand up to intelligent scrutiny? The answer is that in no recorded case is there direct and infallible evidence of infection by inoculation.

The evidence is admirably summed up in Rogers and Muir, *Leprosy* (1946). The cases divide themselves into three categories; firstly, those which invite open disbelief; secondly, those whose lesions manifest themselves on a site of previous injury and, thirdly, where the evidence cannot be regarded as scientifically valid.

1. *Cases which invite open disbelief.* Rogers & Muir report the following cases: "a man who abraded his shoulder when carrying the coffin of a recently deceased ulcerated leper woman, liquid matter from which contaminated the wound and was not washed off until his return home. Some months after he felt unwell and developed leprosy of which he subsequently died." . . . . "a European child in Borneo became infected after thrusting a thorn into himself immediately after a leper boy had thrust it into himself" . . . . "a boy of six years had a negro leper playmate aged eight years. The latter introduced needles into nodules on his arms and legs, and his master, the younger boy, took the same needles directly out of the negro boy and forced them into his own flesh, with the result that soon after he began to get febrile attacks and pains in his limbs and one year later he was covered with a typical nodular leprotic eruption." . . . . "a medical student who cut the tip of his left index finger and abraded his right hand at a post mortem on an advanced leper. This was followed *in a few days* by nodular thickening." (The italics are mine—Ed.)

The first and fourth stories are so fantastic that comment is unnecessary. In the cases of the two children we must ask what possible reliance can be placed on the evidence of minors who are obviously abnormal, or of their parents who are so criminally negligent about their children's playmates.

2. *Cases where the scientific evidence is not sufficiently valid.* Rogers & Muir quote the following case:—" strong evidence of the infection of the human subject by inoculation of leprosy-bacillus-containing material from another case has been brought forward by Lagoudaky (1936 and 1937) who has reported typical development of the disease in himself after inoculation into his own veins on three occasions of the blood of two Greek and one Egyptian leper respectively; the first small lepromata appeared after forty days."

There are two important points in connection with this case. One is that Dr. Lagoudaky worked for many years as a leprosy officer prior to this experiment. He may therefore have been infected at any point before the alleged inoculation. The second is that the writer examined Dr. Lagoudaky closely in the Spring of 1938 and came to the definite conclusion that the duration of his disease was very much longer than that suggested by the dates of his self inoculation. It must be laid down as an essential fact that no worker in contact with cases of leprosy is a suitable subject for experimental purposes.

Rogers & Muir, on page 86 have very properly stressed the fact that inoculations, particularly for vaccination, may cause an acute flare up, or lepra reaction, in cases where the previous signs of the disease have been cryptic. This and similar phenomena have undoubtedly accounted for a large number of recorded cases where leprosy has apparently broken out as a result of inoculation. Let us see then to what extent the evidence of these two marines is, as is stated, " strong evidence for the spread of infection by inoculation."

1. Both men were resident of the same town in civil life. No evidence is quoted as to whether this town was in an endemic area of leprosy. If it were the evidence is thereby qualified.

2. No evidence is given as to whether friends or relatives of these two patients had ever suffered from leprosy. Again the evidence cannot be judged without this information.

3. As the article points out, tattooing causes considerable

trauma. In leprosy the area of previous trauma is frequently the first manifestation of systemic leprosy. We require evidence that these men were not previously infected with leprosy. In any case, whatever the source of infection, the first visible source of manifestation would normally be in the traumatic area.

4. Leprosy in connection with tattoo marks is not an unknown phenomenon. The writer has seen a number of cases where tattooing has been done over a previously depigmented area in order to hide the disfigurement. If this applied to either of these cases, the marked coincidence of their both developing leprosy is thereby considerably reduced.

It will be noted that other cases quoted in the literature are of extremely doubtful significance. There is the often quoted case of the barber who developed leprosy of the forearm through stropping his razor on it after shaving a lepromatous case. It is obvious (a) that the evidence of such a man cannot be accepted and (b) that our whole knowledge of leprosy indicates that the first systemic lesion in this case appeared on an area of chronic trauma.

In 1935 the writer was enabled to examine a case of leprosy in Sydney, Australia. The story was as follows:—the victim had been bitten by a crocodile on the left arm and left leg in an effort to save a child. Two years afterwards inflamed areas appeared round the tooth bites, and these were positive for lepra bacilli. Now, in harmony with the ideas of inoculation, it could be claimed that the crocodle had suffered from leprotic pyorrhoea, and it must be left to the reader to judge whether this would be likely or not. Whatever the final judgment the case of these two Marines is a remarkable coincidence. They are cases which deserve much more thorough, and much more careful investigation.

TWO SIMULTANEOUS CASES OF LEPROSY DEVELOPING IN TATTOOS [1]

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(Reprinted from *Intl. Jl. of Leprosy*, 16, No. 4.)

There is still uncertainty concerning the transmission of leprosy, though it is one of the oldest known diseases. Close and prolonged association with leprous persons has always been recognized as dangerous, yet minimal precautions seem adequate to prevent infection. Various theories of transmission have enjoyed popularity, including those of fish as intermediate hosts and insects as vectors, infection by sexual intercourse and by wearing contaminated clothing, and inoculation by person to person vaccination; also that leprosy is acquired through the nasal mucous membrane.

During the past century it has been a debated question whether or not infection can be induced by inoculation into the skin. Klingmüller (1) reviewed well the evidence for and against experimental inoculation. Jeanselme (2) cited the negative experments of Danielssen and Boeck (3), Profeta (4) and Mouritz (5), and concluded that there was no adequate proof of transmission by inoculation. Rogers and Muir (6) accepted transmission by inoculation. They questioned the case of Keanu who was inoculated by Arning (7) and subsequently developed leprosy, but accepted as valid among others that of Marchoux (8) who, while operating on a leprosy patient, pricked the finger of his assistant who developed leprosy after several years. De Langen (9) reported an accidental inoculation by a physician using for a hypodermic injection a syringe whch had been used on a person with leprosy. Lagoudaky (10) was repeatedly injected with blood and developed cutaneous lesions.

Negative results are difficult to evaluate because of the long

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[1] The original article was published in the *American Journal of Pathology 23* (1947) 805-817, which periodical by established policy does not permit identical reprinting. Republication in modified form was not agreed to by the authors on the ground that it might cause confusion in the literature. The report being regarded as of too much importance to be dismissed with an ordinary abstract this extended one, made with more liberties in arrangement than in phraseology, is run in this way. The plates are from the original cuts, loaned for the purpose.—EDITOR.

incubation period and the possibility of subclinical or dormant infections. Different persons, age groups, races and the sexes vary widely in susceptibility. Since leprosy tends to localize in scars and tattoos and to be activated by trauma, it is difficult to prove that an infection following inoculation actually resulted from it.

REPORT OF CASES

The remarkable coincidences which occurred in the two cases here presented offer very strong evidence favoring transmission by inoculation. The men, friends and residents of the same town in civilian life, were in the same unit of the U. S. Marine Corps; they were tattooed together in Melbourne, Australia by the same man on the same day in June 1943, they and the tattooer being inebriated; and they developed maculo-anesthetic leprosy in the tattoos about $2\frac{1}{2}$ years later. A third man tattooed at the same place, but at a different time, shows no evidence of leprosy.

Case 1.—A white male, 24 years old, with nothing significant in the family or personal history except for malaria in 1942 and some weakness of the left arm ascribed to accidental chipping of the left scapula in 1945, complained chiefly of increased pigmentation and numbness of a tattooed area on the extensor surface of the lower left forearm. Nothing untoward had taken place after the tattooing in 1943 until March or April 1946, when the area and a zone about 1.5 cm. wide around it were noticed to have become pale red and to be insensitive to light touch and pain. A deliberate burn with a lighted cigarette caused no sensation.

On physical examination, the distal one of the two tattoos on the extensor surface on the left forearm (i.e. the one made in Melbourne) presented a uniform, pale, fawn-coloured appearance over its entire tattooed area and a narrow zone around it. In this discoloured area, the total diameter of which was 9 cm., there was loss of sensation to pain and light touch. The other tattoo showed no evidence of disease. A slight congestion of the usual mucous membrane was noted, and a nontender swelling, about 0.5 x 4 cm., on the lateral surface of the juncture of the middle and lower thirds of the left upper arm. The axillary and inguinal lymph nodes were small, and there was no palpable evidence of lesion of the nerve trunks.

Routine laboratory examination (hemoglobin, red and white cell counts, and urinalysis) gave normal results. The Kahn reaction was negative.

A biopsy specimen was removed (November 2, 1946) from the pigmented area outside the tattoo, without anesthetic and with no discomfort to the patient. Section showed tuberculoid tissue reaction, and occasional acid-fast bacilli were found averaging about four per section. Smears from the nasal mucosa were negative. Intradermal injection of 0.1 cc. of O.T. (1:10,000) caused a positive reaction. In view of the history of microscopic findings, a provisional diagnosis of cutaneous tuberculoid leprosy was made. This first case was informally described to Dr. Claude Behn, of Detroit, who without seeing the patient made the original suggestion of leprosy as a probable diagnosis.

Case 2.—A white male, 25 years old, with nothing pertinent in his history except for malaria in 1942 and occasional malarial chills since then, complained chiefly of numbness and pigmentation of the tattooed area on the flexor surface of the left forearm, made in 1943. About January 1946 he noticed that that area and a zone about 1.5 cm. wide about it was becoming dusky red and numb, since when the color had gradually darkened. Two new areas had appeared on the extensor surface of the upper left arm 7.5 cm. above the elbow, and had become confluent; they were dark violaceous in color and numb, with no elevation of the surface.

On physical examination the skin of the entire tattooed area and a zone 1.5 cm. wide about it showed a violaceous discolouration with loss of sensation of pain and light touch. The two coalescent lesions above the elbow made an hour-glass shaped area about 2.5 by 4 cm. with the same color and loss of sensation as the tattooed area. There was no palpable abnormality of the nerve

there was found a violaceous, flat lesion, 1 cm. in diameter, which had normal sensation and was not definitely related to the present illness. The axillary and inguinal lymph nodes were normal in size.

Routine laboratory examinations gave normal findings, including a negative Kahn reaction.

A biopsy specimen, removed (November 11, 1946) from the pigmented area outside the tattoo, without an anesthetic, was divided into two parts. One part was sent in saline solution to the Michigan Department of Health, which reported no acid-fast bacilli found in direct smears or in culture, and no evidence of tuberculosis after seven weeks in two guinea-pigs inoculated with the tissue. Sections of the other part presented the same microscopic appearance as in Case 1. Acid-fast bacilli were also demonstrated, but they were fewer, averaging only about one per section. Nasal smears were negative. The result of an intradermal inoculation with 0.1 cc. of O.T. (1:10,000) was negative. The tentative diagnosis was cutaneous tuberculoid leprosy.

DISCUSSION

Clinically, the lesions in these two cases could well be tuberculous. Microscopically, the presence of epithelioid tubercles with Langhans' giant cells, lymphocytic and plasma cell infiltration and occasional acid-fast bacilli is as characteristic of tuberculosis as of leprosy. The history, however, is more suggestive of the latter. The loss of sensation to pain and light touch, the negative tuberculin test, cultures and guinea-pig inoculation in Case 2, the presence of vacuolated cells, and the positive diagnosis by the U. S. Marine Hospital at Carville,[3] established these cases as maculo-anesthetic or tuberculoid leprosy.

The long incubation period suggests resistance to the disease. The extensive traumatization of the skin incident to tattooing might favor its development. As noted by other observers, cinnabar (red mercuric sulphide) in the tattoos, which discourages spirochetal activity in syphilis of the skin, has no apparent effect on the bacillus of leprosy.

A single description suffices for both biopsy specimens. Each was taken from near the edge of the pigmented lesion, outside the tattoo. The microscopic picture so closely resembled tuberculosis of the skin as to be almost, if not quite, indistinguishable. The characteristic lesion consisted of a center of epithelioid cells with a rim of lymphocytes, a few plasma cells, and even fewer polymorphonuclear leucocytes, with occasional eosinophiles. These nodules were largely avascular. Many of them suggested Boeck's sarcoid, but occasional large ones showed an appreciable degree of caseous necrosis. The Langhans' giant cells were of all sizes, and in appearance and distribution they did not differ from those of tuberculosis; they occurred both in the epithelioid foci and scattered through the areas of lymphocytic infiltration.

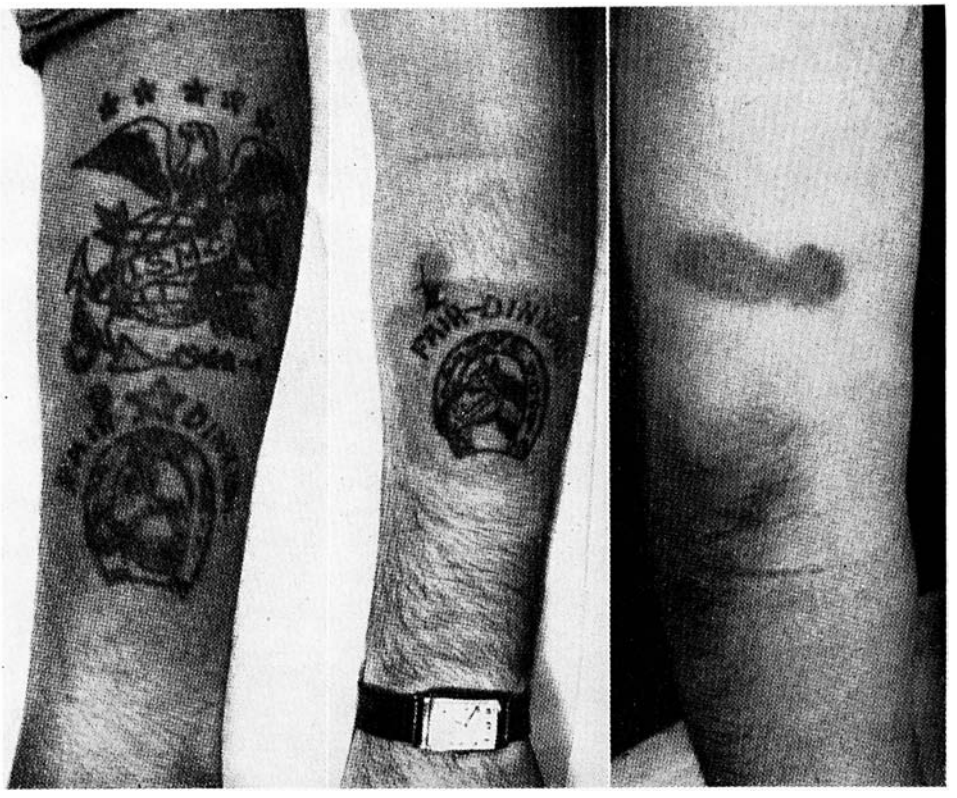

FIG. 1. CASE 1. The distal tattoo, made in Melbourne, is the only one with pigmentation and anesthesia. The pigmentation is so light that it does not appear in the photograph. The dark spot by the star indicates the site where tissue was excised for biopsy.

FIG. 2. CASE 2. Tattoo on the left forearm showing the extent of the pigmentation. The skin suture is still present where tissue was taken for biopsy.

FIG. 3. CASE 3. Secondary lesions on the extensor surface of the left upper arm.

These tuberculoid foci were present throughout the specimen, in both corium and subcutaneous fat, the process apparently extending beyond the depth of the excised tissue. The largest were in the deep layer of the corium, with smaller ones and extensive lymphocytic infiltration in the superficial layer. The foci in the subcutis were more discrete than those nearer the epithelial surface, and were smaller than those in the deep corium. There were no characteristic leprous foam cells, but there were occasional suggestive vacuolated cells.

The epidermis was irregularly atrophic, and there was flattening and partial loss of the dermal papillae, with lymphocytic invasion of the basal and prickle cell layers in some places. The hair follicles showed lymphocyte infiltration, the picture entirely compatible with the loss of hair characteristic of lepromas. There was a granulomatous involvement of the sweat glands, some of which had almost completely disappeared. The cutaneous nerves were involved, but not more so than other structures; there was no particular evidence of extension by way of the nerves. There was considerable involvement of the small vessels, but they did not show the swelling and proliferation of the endothelium found in syphilis.

After Ziehl-Neelsen staining of the sections, occasional acid-fast bacilli were found in the first case and rare ones in the second. They were found most often in or about the largest foci in the deep corium. Usually they occurred in pairs, or two single organisms in one oil-immersion field. They showed no significant variation from tubercle bacilli in either morphologic or staining characteristics.

([3] Unstained section from both cases were submitted to the U.S. Public Health Service, and the Carville, Louisiana, leprosarium reported unequivocally "typical tuberculoid leprosy," though acid-fast bacilli were not demonstrated.)

SUMMARY

Two men from the same community, while serving in the U. S. Marine Corps, were tattooed by the same man on the same day in June 1943, in Melbourne, Australia. They both developed maculo-anesthetic or tuberculoid leprosy in their tattoos during the first half of 1946. One man had multiple tattoos but developed leprosy only in the one made in Melbourne the day when his friend was tattooed. A third Marine, tattooed at the same place but not on the same day, has shown no evidence of leprosy. These two cases provide strong evidence for the spread of leprosy by inoculation.

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EFFECT OF SUNLIGHT ON THE STAINING PROPERTIES OF THE LEPROSY BACILLUS

By DHARMENDRA and MUKERJEE

INTRODUCTION.

At the suggestion of Dr. Ryrie, Medical Secretary of the British Empire Leprosy Relief Association, we investigated the effect of sunlight on the staining properties of the leprosy bacillus. In view of his investigations on the subject he suggested that unstained smears containing the bacilli be exposed to sunlight for varying periods up to 30 minutes, and then stained by Ziehl-Neelsen's method to see if the exposure made any difference to the property of acid-fastness of the bacilli. No difference was however noted after exposure for 30 minutes, but on extending

the period of exposure some interesting observations were made, which form the subject of this preliminary note.

EFFECT OF SUNLIGHT.

A number of smears were made from a suspension prepared from leprous tissue and rich in the leprosy bacilli. Some of the smears were stored in the dark to serve as controls, while the others were exposed to bright sunlight for periods varying from 1 to 12 hours. The unexposed and the exposed smears were then stained with the Ziehl-Neelsen method and examined. The unexposed smears showed large number of bacilli stained red in every field. No appreciable difference was observed in the acid-fastness of the bacilli in the smears exposed for less than six hours. However, in the smears exposed for six hours the number of bacilli stained red, was considerably lower, and with further exposure this number gradually became less and less, till at the end of eighteen hours there were hardly any acid-fasts left.

The experiment was repeated with fresh batches of smears made from suspensions prepared from several other patients. Similar results were seen, but it was noted that if the suspension contained excess of tissue matter, the bacilli lying within this tissue matter were protected from the effect of sunlight, and still stained red even at the end of 18 hours.

EFFECT OF COVERING SMEARS BEFORE EXPOSURE.

The question was then investigated as to the particular rays in the sunlight which were responsible for the above noted effect. Smears prepared from a suspension of leprous material were divided into two lots; those in one lot were wrapped in pieces of black paper, and the other lot was left uncovered. Both the covered and the uncovered smears were exposed to sunlight for varying periods from 6 to 18 hours. They were then stained with Ziehl-Neelsen and examined for the acid-fastness of the bacilli. In the covered smears the bacilli remained acid-fast even after 18 hours exposure; in the other series, however, there was a gradual decrease in the number of bacilli stained red, and in the smears exposed for 18 hours there were hardly any acid-fast bacilli to be found. Similar findings were made on repeating the experiment.

It was therefore concluded that it was not the heat rays but the actinic rays at the ultra-violet end of the spectrum that were responsible for the effect noted above.

EFFECT OF ULTRA-VIOLET AND INFRA-RED RAYS.

Smears made from suspensions of leprous materials were then exposed to ultra-violet and infra-red rays, for varying periods and

then stained with the Ziehl-Neelsen's method. The smears were placed at a distance of 12" from the ultra-violet and infra-red lamps, and the rise in temperature at the end of exposure was to 33°C and 68°C respectively. It was found that in the smears exposed to ultra-violet there was a gradual reduction in the number of bacilli stained red, till there were hardly any left after 12 hours' exposure. The exposure to infra-red, however, did not make any difference to the acid-fastness of the bacilli.

The Rat Leprosy Bacilli and the Effect of Light.

The above experiments were repeated with smears made from suspensions of rat leprosy material obtained from white rats in an advanced stage of experimental rat leprosy. It was found that the property of acid-fastness of these bacilli was not affected by either sunlight or ultra-violet and infra-red rays.

Discussion.

The above observations have a bearing on certain important matters such as the staining of smears taken from leprosy patients during field investigations, the possible therapeutic value of ultra-violet light in leprosy, and the difference between the organisms of human leprosy and rat leprosy.

In the course of field work in leprosy, it is often necessary to send the skin smears for staining and examination to a laboratory at some distance. If the unstained smears are left lying about exposed to sunlight, either in the field or in the laboratory, it may result in positive smears being reported on as negative, especially in the case of smears with small number of bacilli. However, if the smears are carefully stored, preferably after being wrapped in paper, they can be later stained and examined in the laboratory without vitiating the results.

Regarding the possibility of the use of ultra-violet rays in the treatment of leprosy, it can no doubt be said that previous trials in this connection have not given encouraging results. But in view of the action of the ultra-violet rays on the leprosy bacillus in smears, the matter appears to deserve further investigation. It may be that because of the bacilli being deeper in the skin, or because of some other reason the ultra-violet rays have no effect on the leprosy bacillus in the body of the patient.

The difference in their susceptibility to sunlight is yet another evidence in support of the view that the organisms of leprosy and rat leprosy are two separate organisms. As a matter of fact there is no point in naming the disease in rats as rat leprosy and its causative organism as rat leprosy bacillus.

SUMMARY.

1. Exposure to sunlight of unstained smears containing leprosy bacilli interferes with the property of acid-fastness of these bacilli.
2. If the smears are covered with black paper before being exposed to sunlight, the staining property of the bacilli remains unchanged.
3. Exposure to ultra-violet rays has the same effect as exposure of uncovered smears to sunlight, while exposure to infra-red does not affect the staining properties of the bacillus.
4. Exposure of the rat leprosy bacillus to sunlight, ultra-violet, or infra-red rays, does not affect its staining properties.
5. The above observations have a bearing on certain important matters, such as the staining of smears taken from leprosy patients during field investigations, the possible therapeutic value of ultra-violet light in leprosy, and the difference between the organisms of human leprosy and rat leprosy.

ELICITATION AND INTERPRETATION OF TACTILE ANAESTHESIA.

G. A. RYRIE.

It is a common belief that the elicitation and interpretation of tactile anaesthesia in leprosy requires no special skill and experience. This is a delusion which has caused frequent errors in diagnosis. During the last twelve months I have seen the following 3 cases:—

1. Male, Englishman, aged 39, who returned from the tropics after residence for some years as a welfare worker in a leprosy institution. He had marked anaesthesia of both arms and legs, and acid-fast rods were found in the nasal mucous membrane. He was diagnosed as suffering from leprosy by (a) a doctor in this country with fair experience of the disease; (b) a professor of tropical medicine; and (c) a well known neurologist. On examination I found that the acid-fast rods in the nasal mucous

membrane were not lepra bacilli, and that the anaesthesia was due to hysteria. In this case the deep nature of the anaesthesia, compared with the marked lack of any muscle wastage, and the absence of any skin lesion or nerve thickening, made the diagnosis of leprosy improbable. Further examination elicited the classical symptoms of hysteria.

2. White colonial subject, aged 30. Had been diagnosed as a case of leprosy for the last six years and had lived a life of incarceration and misery during that time. Examination showed areas of anaesthesia over the outer sides of both lower legs. The patient gave a history of being brought up on a cattle ranch, where he had been subject to constant trauma from the hooves of cattle or falls from horseback. There was no wasting of muscle, no nerve thickening and no skin lesion. Diagnosis traumatic anaesthesia.

3. European, male, aged 27, with a history of a short residence in the tropics. On examination I found he had widespread macular lesions over the trunk and arms, showing typical anaesthesia to light touch. I confirmed the diagnosis of leprosy. He had been seen by more than six doctors, all of whom, after testing with pins and needles, had stated that there was no anaesthesia. Had the disease not been discovered in time the patient would almost inevitably have drifted into a dangerous lepromatous state. The mistake here lay in the patient's medical advisers being unaware of the correct method of eliciting anaesthesia.

In different leprosy institutions one sees occasionally the most extraordinary methods of testing for anaesthesia. These include the use of a folded piece of stiff paper, pins, needles, and, in some cases, the use of a large feather attached to the end of a walking stick, presumably in order to prevent any possibility of contact. The elicitation of anaesthesia in a well marked tuberculoid case of some duration is relatively easy. The difficult cases are (1) the early anaesthetic lesion where pathological findings are indeterminate, and (b) the early tuberculoid lesion where gross anaesthesia has not yet become stabilised.

The only instrument that is of any real value in the elicitation of tactile anaesthesia is a very fine point of cotton wool. There are, however, several points which must be remembered. The first is a need for inviting the patient to co-operate, combined with a complete explanation to the patient of what is happening. In some languages the word for " feeling " is not clearly differentiated from the word for " pain," and it is therefore necessary to explain exactly to the patient what is expected of him. A large number

of patients will almost inevitably give the " wrong " answers to begin with; this for various reasons including ignorance, stupidity, and the desire to say what will apparently please the doctor.

The effort of concentration required by the patient may be quite rapidly fatiguing, and there should be careful intervals allowing for a short mental rest between each test. Apart from exceptional cases the patient should not be blindfolded, and in no case is there any excuse for the attendant putting his thumbs over the patient's eyes. In most cases there is no reason why the patient should not watch the procedure, at any rate during the first part of the examination. He is just as interested in it as you are. Blindfolding and eye blocking can only add to the patient's sense of apprehension and therefore to the margin of error in the elicitation of anaesthesia.

Any emotion, such as fear, can effectively inhibit the normal sense of touch, and the accuracy of the patient's response. It should be remembered that the actual definite diagnosis of leprosy often brings a sense of relief to a patient, who for days and weeks may have been harrassed by tormenting doubts or fears. During this stage a patient may be quite incapable of giving a rational, coherent response to any test for anaesthesia unless he is treated with the utmost consideration and understanding. Physical tiredness may affect the response to light touch. A patient who has come to the clinic from a distance, carrying a fairly heavy load, may be incapable of recognising light touch until he has had a rest.

Variations in touch response in normal skin. To elicit this the writer suggests making the following simple tests:—

Take a fine wisp of cotton wool and, after eliciting normal touch response on the back of the hand, then test the back of the knuckles of the same hand. It will be found that there is no response to light touch, and that this area is normally anaesthetic. Examination of the whole body in normal subjects shows that the skin on the outer aspects of the joints in general has a varying, but dulled delayed response to light touch. This is a most important, but little known observation, ignorance of which has led in a number of cases to a wrong diagnosis.

The hair on the skin may be sensitive to light touch where the underlying skin is anaesthetic. In every case, therefore, it is necessary to shave both the suspected area and the control area before examination for anaesthesia. The act of shaving itself may cause a temporary numbness, so that the patient should be left for at least ten minutes after the shaving has been completed.

If a patient has been sitting with one knee over the other for

some time, the lower part of the leg which has been superimposed on the other will be found completely anaesthetic to light touch. Similarly, in patients who have been squatting cross-legged for some time, there may be partial anaesthesia in both lower limbs.

In testing for anaesthesia of the forearm the limb should not be rested on a desk, or kept in any position where there may be pressure on the ulnar nerve, or compression of the limb. The patient should not be allowed to sit in a draught during examination, as this may inhibit a normal response.

Perhaps the most difficult place for the elicitation of anaesthesia is on the face. Here it must be remembered that the skin is extremely sensitive indeed, and anaesthesia may only be discovered by the mere touch, not stroke, of a single hair of cotton wool.

Trauma, particularly round the ankles and feet, is a common cause of anaesthesia, and is not infrequently mistaken for leprosy. It should be kept in mind that almost everyone has, from childhood, suffered repeated trauma in this area, and that no diagnosis of leprosy should be made without confirmatory signs and symptoms.

I am indebted to Dr. J. Barnes, of the Ogoja Leprosy Settlement, Nigeria, for a detailed study of relative insensitivity over joints in ten Africans. He reports as follows:—

Insensitive skin areas in the normal African.

(+ = Stimulus appreciated. O = Stimulus not appreciated.)

Patient	Stimulus	Elbow tip	Middle knuckle of fingers	Knees	Remarks
1. Adult female	Light touch	O	O	+	
	Pin prick	+	+	+	
2. Adult male	Light touch	O	O	+	Tendo-achilles O
	Pin prick	O	O	+	,, O
3. Adult male	Light touch	O	O	O	Tendo-achilles ,, O
	Pin prick	+	+	+	+
4. Adult male	Light touch	O	O	+	
	Pin prick	+	+	+	
5. Boy aged 15	Light touch	O	O	+	
	Pin prick	+	+	+	
6. Adult male	Light touch	O	O	O	
	Pin prick	O	O	O	
7. Girl aged 12	Light touch	O	O	O	
	Pin prick	O	+	+	
8. Adolescent boy	Light touch	+	+	+	Dorsum of feet O
	Pin prick	+	+	+	,, +
9. Boy aged 13	Light touch	O	O	+	
	Pin prick	+	+	+	
10. Adolescent boy	Light touch	O	O	+	Full sensation in tinea patches.
	Pin prick	+	+	+	

The whole skin area of ten normal Africans was studied, and the appreciation of pin prick and light touch noted. All parts of the body appeared to be sensitive except where the skin was stretched or thickened on elbows, knees, middle knuckle of fingers and soles. Most, but not necessarily all, of these sites were insensitive to light touch in the cases examined.

SUMMARY

1. The most effective test for anaesthesia in leprosy is by the use of a fine point of cotton wool.

2. The areas of the outer aspects of the joints (ankles, etc.) normally show a relative anaesthesia in a healthy person.

3. Hair is not insensitive to light touch where underlying skin is anaesthetic in a case of leprosy.

4. The skin of the human face is extremely sensitive to light touch and anaesthesia should be elicited with a single hair of cotton wool.

5. In cases of doubt diagnosis of leprosy should not be made where there is (a) no skin lesion; (b) no thickened nerve, and (c) no muscle wasting.

SULPHETRONE: THERAPEUTICS AND TOXICOLOGY *

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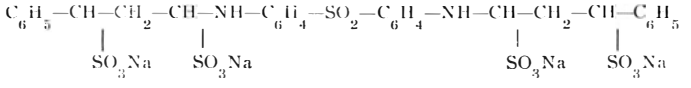
The discovery in these laboratories of the chemotherapeutic activity of diaminodiphenysulphone (Buttle et al. 1937) stimulated a search for derivatives having the efficiency of the parent drug

* The development of sulphetrone is part of a programme of work on Antituberculous Compounds carried out by the Therapeutic Research Corporation of Great Britain.

but not its undesirable toxicity. One derivative, tetrosodium 4 : 4-bis (γ-phenylpropylamino)-diphenylsulphone-α : γ : α : γ'-tetrasulphonate, given the registered name 'Sulphetrone,' was first prepared in 1936 and attracted attention on re-examination in 1941 by reason of its freedom from toxicity and its potent antituberculotic activity.

Brownlee et al. (1948) have described its structure, chemical and physical properties, pharmacology, experimental therapy, and possible clinical uses; and Brownlee and Kennedy (1948a) have described its suppressive effect on progressive experimental tuberculosis in guinea pigs The drug was found to be bacteriostatic and, in common with other sulphones, incapable of eliminating the infective organism from animal tissue. In a second study Brownlee and Kennedy (1948b) have shown that sulphetrone is more efficient than 'Promin' in protecting the laboratory animal against experimental tuberculosis, and that it is synergic with streptomycin. A clinical evaluation, unfortunately restricted to a limited series, was made by Madigan et al. (1947). The chemical preparation of sulphetrone has been described by Gray and Henry (1936) and by Buttle et al. (1938).

Sulphetrone is a cream-coloured amorphous material containing 5—7% water. Its molecular weight is 892.5 and its probable constitution is as follows:



It is insoluble in alcohol and other organic solvents but exceedingly soluble in cold water; 40% (w/v) solutions are stable when neutral or slightly alkaline, and a 60% solution may be autoclaved; 10% solutions are isosmotic, but 20% and even 40% solutions are well tolerated intramuscularly.

ESTIMATION

Sulphetrone may be estimated in blood, urine, cerebro-spinal fluid, and tissue by diazotisation and coupling to N-(1-napthyl)-ethylenediamine hydrochloride (Bratton and Marshall 1939), the resultant dye being measured colorimetrically or, better, absorptiometrically. Since sulphetrone mixes intimately with precipitated proteins, the conditions governing its optimal recovery were determined experimentally. They are critical and must be rigidly respected to obtain 90% recoveries of sulphetrone.

To 5 ml. of N/1 hydrochloric acid 0.5 ml. of blood or other body fluid is added and mixed well; 2.0 ml. of 12% (w/v) trichloracetic acid is added, and the well mixed solution is filtered immediately through a no. 5 Whatman paper and repassed until brilliant; 3 ml. of filtrate is mixed with 0.05 ml. of 0.3% fresh (weekly) sodium nitrate and left for 3 min.; 0.05 ml. of 1.5% ammonium sulphamate is now added, and the solution left for 2 min. Finally 0.05 ml. of 0.1% N-(1-napthyl)-ethylenediamine hydrochloride is added and mixed well.

The colour is allowed to develop for 30 min. before being read colorimetrically, or absorptiometrically with a Wratten 61 filter. All

estimates of sulphetrone are given in terms of the anhydrous compound. By collaboration with the Tintometer Ltd., a standard Lovibond colour disc is available for the reading of blood and body-fluid estimates falling within the range of 0—9 mg. per 100 ml.

It has been shown (Brownlee et al. 1948) that the visible absorption spectra of the naphthyl-ethylenediamine derivatives of diaminodiphenylsulphone and of sulphetrone are substantially different in preparations containing equivalent amounts of diaminodiphenylsulphone.

TOXICITY

The *acute toxicity* of sulphetrone when given by mouth experimentally to animals is so slight that it cannot be determined with any certainty (Brownlee et al. 1948). Comparison of blood levels at death between ordinary sulphonamides and sulphetrone show the latter to be many times less acutely toxic than sulphanilamide. In the dog acute toxic symptoms are absent with blood-sulphetrone levels as high as 160 mg. per 100 ml. This fact, together with the observation that conjugated derivatives cannot be detected in blood or urine, is a strong link in the chain of evidence that sulphetrone is not easily hydrolysed. Acute toxic effects are not observed in man.

Chronic Toxicity

Very large doses of sulphetrone can be given by mouth for a long time to mice and dogs without producing ill effects. In rabbits, however, a diet containing 4% sulphetrone causes blood changes: (1) a slight but continuous hæmolytic anæmia marked by a concurrent reticulocytosis: (2) a progressive anæmia due to lack of iron owing to competition for alimentary iron by sulphetrone, with which iron forms a non-absorbable complex, and which may be treated, or prevented, by the administration of iron parenterally or by mouth; and (3) an anæmia of slower development but precipitate onset which seems to be of nutritional origin, since it may be both prevented and cured by the simultaneous exhibition of brewers' yeast, and is probably caused by the limitation and alteration of the bacterial flora of the gut by sulphetrone (Brownlee and Tonkin 1941).

That an essentially similar condition develops in man is implicit in the case reported by Madigan (1948), Anderson and Strachan (1948), and Clay and Clay (1948). The problem was studied intensively in 2 female patients presenting bilateral fibrocaseous disease, and both at first gravely ill. Sulphetrone-balance experiments were made for twelve months, involving daily estimates of blood-sulphetrone concentrations and of the amounts excreted in urine and fæces. Daily estimates were made of

reticulocytes, red cells, white cells, and hæmoglobin concentrations; differential cell counts were also made. In all, estimates were made on 298 occasions.

The urine was examined for urobilin (Watson 1936) and abnormal pigment; total porphyrin estimates were made weekly on 24-hour specimens, subsequently to be abandoned because the estimates fell within normal limits. Reference is made below to a chemical study of metabolic products of sulphetrone. The information collected has allowed the study of several related problems also to be discussed. The figure summarises the typical response of a patient to sulphetrone by mouth; the dose was at first 3 g. daily (0·5 g. every four hours) and thereafter raised at seven-day intervals by increments of 1 g. until a therapeutic blood level of 7·5-10 mg. per 100 ml. was reached. The weekly estimates are means of seven daily observations. As with the rabbit, the residual effect is a hæmolytic anæmia, which stimulates reticulocytosis. The red cells were crenated, and hæmolysis in fragility tests were observed at 0·45 and increased at 0·48% NaCl.

In all the 94 cases examined no distinguishable effects on the numbers of distribution of white cells were observed. Consistent with the reticulocytosis observed in man there was an increase in the primitive red cells of the bone-marrow,

Typical response in man treated with sulphetrone 7.5 g. daily for 52 weeks, with increments of iron and fresh yeast to diet. Urobilin was estimated spectroscopically (Watson 1936).

The range within which the differential red bone-marrow counts fell in 11 tuberculosis patients treated with sulphetrone was as follows:

	%		%
Polymorphs ...	3·5—34·4	Lymphocytes ...	3·8—22·8
Metamyelocytes ...	9·0—33·4	Monocytes ...	0·4—10·8
Myelocytes ...	1·8—11·5	Plasma cells ...	0·2— 2·6
Premyelocytes ...	1·2— 4·0	Normoblasts ...	24·0—37·5
Myeloblasts ...	1·2— 4·0	Erythroblasts ...	0·4— 1·0

The proportion of cells of the myeloid series (A) to nucleated red cells is 1·24-2·5 to 1. This falls within the limits for normal marrow counts (Young and Osgood 1935). Estimates made on 2 of the 11 patients before treatment started gave ratios of 6·4 to 1 and 9·25 to 1.

Abnormal Blood Pigments

The blood of animals and man containing therapeutic concentrations of sulphetrone is usually dark brown, and the pigment is restricted to the red cells, even though sulphetrone is distributed within plasma and cells. Examined spectrometrically (Beckman) the blood may show no specific absorption characteristic of known abnormal blood pigments. In a proportion of cases, which may be

as high as 20%, the specific absorptions of methæmoglobin (630 mμ) may be detected. Sulphæmoglobin (618 mμ) is absent. Methæmoglobin may be identified visibly by spectroscope but only in the strongest solutions of laked blood which will just transmit light. When it is present, calculations from the extraction coefficients of the wave-length corresponding to oxyhæmoglobin (540 mμ) and methæmoglobin (630 mμ) suggest that the amount-present is about 4-12% of the total hæmoglobin.

Blood containing concentrations of sulphetrone above 10 mg. per 100 ml. may contain additional abnormal blood pigments. For example, of 904 spectrometric estimates in this category 23 were abnormal: 18 contained suphæmoglobin, and 5 appreciable amounts of methæmoglobin. In all other cases in which sulphæmoglobinæmia was observed in patients with blood-sulphetrone levels below 10 mg. per 100 ml. other drugs were implicated, phenacetin five times and methylacetanilide once. An additional abnormal pigment may be identified by a generalised absorption in the area 600—620mμ. This may be a hæmoglobin-sulphetrone complex or a hæmoglobin-sulphetrone degradation product.

Oxygen-capacity of the Blood.—Whatever the precise nature of the abnormal pigment, its ultimate importance depends on whether or not there is variation in the oxygen-carrying capacity of the blood. A series of 8 patients, with blood-sulphetrone levels of 7·4-9·2 mg. per 100 ml., were observed for ten weeks in which five repeated estimates were made of the oxygen-capacity of blood, togther with spectrometric estimates. The analyses were made in the constant-volume Van Slyke apparatus. In this small series the estimates of oxygen-capacity were lower than normal in 5 cases, though associated with the presence of methæmoglobin in only 2 (table 1). The rate of oxygen (air) uptake was always slower than in normal blood.

Blue Coloration of Patients.—Associated with these facts is the clinical observation that many patients treated with sulphetrone show a leaden-blue coloration of skin and mucosæ. The tint and distribution of the colour are reminiscent of sulphæmoglobinæmia, which, as noted above, is absent. The intensity of pigmentation is not related to the blood-sulphetrone level but is greatest during the early period of adjustment. It is less obvious in the young, and most pronounced in those with low vital capacity. There is at the same time a quanity of violet-blue dye in the urine. Clinically the condition does not contra-indicate the exhibition of sulphetrone.

Specific Toxic Effects

Sulphetrone shares with the sulphonamides the property of causing hyperæmia and hyperplasia of the thyroid gland in laboratory animals. The hyperplasia is thought to result from diminished synthesis of thyroid hormone. The tests showed sulphetrone to be the least and sulphaguanidine the most toxic, with

sulphadiazine intermediate. The effect has not been observed in man.

Effect on Alkali Reserve

The lack of acute toxicity and the low chronic toxicity, with freedom from the characteristic nervous sequelæ seen in experiments on animals after the administration of diaminodiphenylsulphone, make it clear that sulphetrone is not degraded to any extent to diaminodiphenylsulphone in the body.

A point which must not be overlooked is the possible hydrolysis of one or more of the four sulphonated side-chains, and animal experiments were made to study this point (Brownlee et al. 1948). Simple hydrolysis with liberation of sodium acid sulphate appeared likely, but experiments on rabbits given sulphetrone by mouth and parenterally, and on dogs given sulphetrone parenterally, showed increased alkali in the blood. Comparison with the effects of administering alkali parenterally showed the increase in alkali reserve to be consistent with the liberation of one molecule of sodium hydroxide from each molecule of sulphetrone. This would involve the production of a condensation product of sulphetrone, and there is experimental evidence in man pointing to the excretion of a coloured complex of this kind in the urine.

It is significant that tests made on rabbits given sulphetrone for long periods showed the animals to be capable of establishing an equilibrium in plasma-alkali balance.

The alkali reserves of untreated and treated tuberculous patients are compared in table II. In the first column are the estimates of alkali reserve after seven days' treatment; in the second, two months later. Included within the second series are 3 patients who had received sulphetrone for seven days only. The estimates were made within 30 min. from freshly drawn blood centrifuged under paraffin; the CO_2-capacity was determined by the volumentric method of Van Slyke. It will be seen that there is no significant change in the CO_2-combining capacity of the plasma after protracted treatment with sulphetrone.

Sulphetrone is well tolerated if the exhibition of the drug is gradual. Nevertheless, during this period of adjustment mild symptoms of toxicity are seen (Madigan 1948)—slight nausea, mild headache, and difficulty in reading. These may be associated with the physiological accommodation the patient is called on to make in alkali reserve. The symptoms are relieved best by sodium bicarbonate and not so well by lactate or citrate. The bicarbonate ion (HCO_3)— may therefore be involved.

ABSORPTION AND EXCRETION

Experiments on mice and dogs in which single small or large doses were given showed that, after a certain point is reached, large increases in dose do not lead to either a higher blood-sulphetrone level or increased absorption. Similar observations

have been made with the more complex sulphonamides at somewhat higher doses. The rabbit is anomalous, for increase in dosage increases the absorption of sulphetrone and its concentration in the blood. In dogs the same total amount of sulphetrone given in divided doses at intervals of a few hours led to a higher blood-sulphetrone level than when given as a single dose. A similar effect was seen in man in ten experiments restricted to 96 hours. The results of an experiment on dogs in which sulphetrone was injected first into the large and then into the small intestine, the ileoæcal junction having been tied, support the view that absorption is largely confined to the small intestine. It follows that optimal conditions of absorption are likely to follow uniformly divided doses.

Sulphetrone and Metabolism Products in Urine and Fæces

Two female tuberculous patients given sulphetrone were the subject of drug-balance experiments during a continuous period of twelve months. The intake of sulphetrone averaged 6·5 g. daily; the amounts of sulphetrone in urine and fæces were estimated. As already indicated, the recovery of sulphetrone from urine was good, 90-95%, but only 75-82% could be obtained from fæces. Sulphetrone in the fæces does not indicate only the amounts which remain unabsorbed, since some secretion takes place into the bile and from the ileum. It is impossible to relate the amount of sulphetrone in the urine to the amount absorbed, for still other channels of excretion are open to it. After the initial period of adjustment, as little as two-fifths, and later as much as three-fifths, passed through the kidneys, as much as two-fifths and as little as a fifth was recovered from the fæces.

TABLE I.—OXYGEN-CAPACITY OF BLOOD OF 8 TUBERCULOUS PATIENTS TREATED WITH SULPHETRONE. TWO NORMAL ESTIMATES ARE INCLUDED. THE PRESENCE OR ABSENCE OF METHÆMOGLOBINÆMIA IS INDICATED.

Case no. and sex	Oxygen found (vols. %)	Hb % (Haldane)	Oxygen calculated from Haldane value (vols. %)	Mephæmoglobinæmia (spectrometric)
Normal:				
1 (F)	15·3	82	18·6	o
2 (F)	16·4	90	18·3	o
Tuberculous:				
3 (F)	12·0	86	14·0	+
4 (M)	10·6	76	13·5	+
5 (F)	16·3	90	18·1	o
6 (M)	16·3	86	18·8	o
7 (M)	14·5	94	15·4	o
8 (M)	16·7	88	18·8	o
9 (M)	14·8	88	16·6	o
10 (M)	12·8	92	13·8	o

TABLE II—EFFECT OF SULPHETRONE IN PLASMA CO_2-COMBINING CAPACITY, IN VOLUMES % IN MAN

Case no.	Oct. 8, 1947 Blood-sulphetrone (mg. per 100 ml.)	Oct. 8, 1947 Carbon-dioxide capacity of plasma (vols. %)	Dec. 8, 1947 Blood-sulphetrone (mg. per 100 ml.)	Dec. 8, 1947 Carbon-dioxide capacity of plasma (vols. %)	Dec. 8, 1947 Normal patient	Dec. 8, 1947 Carbon-dioxide capacity of plasma (vols. %)
1	3	61·7	7·5	64·3	A	64·8
2	5	61·1	8·1	63·0	B	65·5
3	4	63·6	5·3	64·9	C	58·0
4	4	63·0	7·2	58·6	D	65·5
5	4	53·0	*2·0	58·6	E	57·2
6	...	...	4·0	55·4	F	63·9
7	...	...	4·1	57·3	...	...
8	...	...	3·2	56·7	...	...

* Drug being withdrawn.

The urine of animals and man receiving sulphetrone contains a diazotisable substance which, when coupled with naphthylethylenediamine, forms a dye with an absorption similar to that of sulphetrone. Heating with $N/1$ hydrochloric acid for 30 min. does not increase the amount of diazotisable material; thus there is probably no conjugation. The concentration of sulphetrone in urine is high—usually 10 to 20 times that in the blood.

The urine of patients treated with sulphetrone is a dark smoky-brown. The pigment may be separated chromatographically on a column of activated alumina (Brockmann: Messrs. Savory and Moore).

Most of this pigment separates as a dark brown $\frac{1}{2}$ in. band at the top. After washing with a similar volume of distilled water about 95-97% of the total diazotisable material passes through the column. The pigment may now be developed with 0.5% hydrochloric acid to give a blue-violet narrow band, followed closely by a mauve-pink broader band, with brown (bile) pigments left behind. The violet-blue pigment is soluble in 0.5% hydrochloric acid and, diazotised and coupled, appears to account for about 2% of the total sulphetrone in the urine. The mauve-pink solution in hydrochloric acid couples with difficulty; calculated as sulphetrone it appears to account for about 0.5% of the total.

It is interesting to speculate on the part played by these dye-stuffs in the leaden-blue coloration of sulphetrone-treated patients excreting these dyes in their urine. The dyes appear to be condensation products of two or more molecules of sulphetrone, which, it will be recalled, loses alkali in the blood-stream, probably by just such a condensation procedure.

Renal Clearance

Sulphetrone is excreted by the kidney very fast. In rabbits the clearance was 58% of that of creatinine, or two or three times as fast as that of sulphanilamide, while in the dog the clearance was five times as fast as that of sulphanilamide (Brownlee et al. 1948).

The opportunity arose in a patient, repeatedly chatheterised for the diagnosis of renal tuberculosis, to determine the clearance-rate of sulphetrone by direct comparison with sulphanilamide. Both drugs were given intravenously in a dose of 0.05 g. per kg. and five estimates were made at intervals of 30 min. The mean clearance for sulphanilamide ± S.D. was 12·2±2·4, whereas for sulphetrone, tested after an interval of eight days, it was 62·1±11·3.

It seems that, in man, tubular resorption of sulphetrone is small, and that the rate of clearance is about four times as fast as with sulphanilamide.

TABLE III — PENETRATION OF SULPHETRONE INTO VARIOUS TISSUES IN MAN, EXPRESED AS MG. PER 100 ML. OF BLOOD OR OTHER FLUID AND AS MG. PER 100 G. OF TISSUE.

Tissues	Case 1	Case 2	Case 3	Case 4	Case 5
Blood	12·4	4·1	...	22·4	17·7
Plasma	8·1	2·2	...	13·3	10·3
Corpuscles	4·3	1·9	...	9·1	7·4
Cisternal fluid	1·8	1·3	1·6	3·7	2·9
Bile	140·0	19·9	130·0	254·0	171·4
Liver	14·0	11·5	3·7	30·0	29·4
Kidney	17·6	20·2	23·7	97·5	73·1
Spleen	30·5	9·8	2·4	40·0	46·0
Normal lungs	10·4	7·4	4·9	37·0	27·2
Caseous lungs	...	...	7·3	...	44·2
Bone-marrow	...	0·0	0·2	...	0·4
Heart muscle	14·0	6·0	...	...	19·2
Striated muscle of thigh	...	17·8	...	42·7	28·0
Ileum	21·0	...	22·7	...	22·1
Cerebal hemispheres	...	5·9	2·0	6·1	3·7
Peritoneal fluid	66·0	...	...	...	27·4
Pericardial fluid	6·3	...	...	...	8·2
Medistinal glands	...	5·6	...	...	...
Pus from mediastinal gland	...	16·0	...	...	...

DISTRIBUTION OF SULPHETRONE IN TISSUE

Sulphetrone rapidly penetrates all tissues except brain, but it appears in cerebrospinal fluid more slowly than do sulphonamides. The tissues of animals show similar concentrations whether the sulphetrone is given in a sufficient dose intravenously or by mouth over a period of ten weeks (Brownlee et al. 1948). In experiments on animals concentrations of sulphetrone in liver, kidney, and spleen are always greater than in plasma; the same is true for man (table III). The relation between absorbed sulphetrone, sulphetrone in transport, and sulphetrone in kidney clearance does not seem to be a simple one, and conditions other than the concentration of sulphetrone in the plasma appear to coöperate in determining the amount in the tissues. Plasma-sulphetrone levels obtained in nephrectomised rabbits are many times those seen in normal rabbits, and many times the level attained in other tissues. The concentrations in the bile of both normal and nephrectomised

animals are very high; in the normal animal the concentration in bile is about twelve times the plasma value and must be considered to be sulphetrone in transport, so that biliary excretion makes a significant contribution to the clearance of the drug.

The study of the distribution of sulphetrone in the tissues in man has necessarily been confined to necropsy specimens. Cases 1, 2, and 3 of table III show the distribution of sulphetrone in different tissues when the blood-sulphetrone was at a therapeutic level, at death after tuberculous meningitis (2 cases) and hæmoptysis (1 case). Cases 4 and 5 show the distribution of sulphetrone in different tissues after excessively high and long-maintained blood-sulphetrone levels caused by constipation allowing sulphetrone to accumulate in the small intestine. In both cases the blood-levels were maintained at 17·5 mg. per 100 ml. for more than five days. Death from anoxia followed from the inability of the red cells to transport oxygen.

SUMMARY

The chemical and physical properties of sulphetrone and its estimation in body fluids are described.

The acute toxicity of sulphetrone in experiments on animals is very slight. No acute toxic effects have been observed in man.

The chronic hæmatotoxic effects in rabbits and in man include a hæmolytic anæmia, an anæmia of iron lack, and an anæmia of nutritional origin.

Sulphetrone has a goitrogenic effect similar to that of sulphonamides, but this is only slight in experiments on animals and has not been observed in patients receiving the drug.

Sulphetrone given by mouth or parenterally raises the alkali reserve of the plasma of rabbit and dog. The probable mechanism is discussed. Given over a long period it brings about some tolerance. It is thought that the period of adjustment seen during the first three weeks of sulphetrone therapy is associated with adjustments of alkali reserve. The minor discomforts encountered during this period are relieved by bicarbonate, but not so well by other alkalis.

Though very soluble in water, sulphetrone is slowly absorbed from the intestinal tract—most from the small intestine, little from the large.

Sulphetrone is cleared by the kidney very quickly, and fluid must be limited to maintain blood-sulphetrone concentrations; there is also a substantial excretion into the bile and to a lesser extent into the ileum. The drug is not conjugated; so there is no danger of crystaluria.

Sulphetrone penetrates all tissues, except brain, very rapidly. It passes into the cerebrospinal fluid more slowly than do sulphonamides.

Thanks are due to Dr. A. I. Ross, of Farnborough County Hospital, who was responsible for the bone-marrow biopsis; Dr. T. Anderson and Dr. D. G. Madigan for permission to draw on their clinical necropsy findings; Dr. E. I. Short for some of the oxygen-capacity and spectrometric observations; Dr. C. L. Oakley for the histological reports.

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A PHARMACOLOGICAL STUDY OF THREE SULPHONES.

MICHAEL SMITH

PART II.

HYDROLYSIS AND THE SPECIFIC TOXIC PHENOMENA.

BLOOD LEVELS AND THERAPY.

No information has been presented which indicates that high blood levels of any of the sulphones promotes greater rapidity of clinical response. The present dosage regimens are based upon empirical assumptions and no leprologist can say with certainty that it is necessary to maintain a definite prestated level of a sulphone in the blood (Ref. 1). It has yet to be shewn that a blood level of 6mg% of sulphetrone is any more effective than a blood

level of 2mg%; since the former blood level requires 8-10g. daily of sulphetrone and the latter 2g. daily, the matter is obviously of some importance both from a medical and economic point of view.

Recent work (Ref. 2) has shown that clinical response is obtained with extremely small doses of diaminodiphenylsulphone. Doses of 0.05. every other day are reported to give good results (Ref. 3); blood levels on this dosage are $<$ 0.1mg% therefore it would seem that the attempt to achieve and maintain bloodlevels of the order of 1-2mg% with diaminodiphenylsulphone is unnecessary.

This attempt was made because of the assumption that if a blood level of 5mg% of sulphetrone and 2mg% of diasone produced clinical response then the molecularly equivalent blood level of diaminodiphenylsulphone would do so too. (It was assumed, ad hoc, that 100% hydrolysis of the proprietary sulphone occurred though in fact it is improbable that this degree of breakdown does occur.) Fig 1 presents the constructed table shewing the chemical method of computing the molecular ratio of the sulphones.

FIG. 1.

Col. 1. Sulphone.	Col. 2. Molecular Wt.	Col. 3. Ratio of Col. 2 to DADPS*	Col. 4. Molecular Ratio.
DADPS*	248	248/248	1
Diasone	448	248/448	0.56
Sulphetrone ...	892	248/892	0.28

* Diaminodiphenylsulphone.

Therefore the "therapeutic" blood level of diaminodiphenylsulphone may be calculated empirically, as follows, using the blood levels of the proprietary sulphones that are maintained on the usual dosage regimens.

Diasone — 0.56 x 1.8mg% = 1.0mg%
Sulphetrone — 0.28 x 5.0mg% = 1.4mg%

It was found that a blood level of 1mg% of diaminodiphenylsulphone could be established and maintained by a daily oral dose of 0.2-0.3g. (v. Part I).

The ad hoc assumption that 100% *in vivo* hydrolysis occurs is obviously not correct since concentrations of sulphetrone are found in the urine of the order of 1000mg *in solution* (v. Part I). The solubility of diaminodiphenylsulphone being only 30mg% in urine, the sulphetrone must be present largely as the complete molecule. The degree of breakdown that does, if at all, occur with the proprietary sulphones is obviously a point of fundamental importance, and the following work was undertaken to establish

the evidence of, and degree of, *in vivo* hydrolysis of the proprietary sulphones. Brownlee states (Ref. 3) that breakdown of sulphetrone to diaminodiphenylsulphone does not occur. Similar statements are not made concerning other proprietary sulphones.

IN VIVO HYDROLYSIS.

a. *Therapeutic evidence.*

In Part I theoretical evidence was presented to shew that the proprietary sulphones probably owe their activity to their content of diaminodiphenylsulphone. For the purposes of this discussion if 100% hydrolysis of the proprietary sulphones occurs we may construct, as in Fig 2, a table to demonstrate how much diaminodiphenylsulphone would be available to the body from the usual dosage regimens of the proprietary sulphones.

FIG. 2.

Col. 1. Sulphone.	Col. 2 Daily oral dose.	Col. 3. %age Absorbed from gut.	Col. 4 Amount metabolised. (Col. 2 x Col.3)	Col. 5. Am'nt DADPS* liberated (Col. 4 x Molecular ratio)
DADPS*	... 0.3g.	85	0.26g.	0.26g.
Diasone	... 1.5g.	46	0.69g.	0.38g.
Sulphetrone	... 5.0g.	15	0.75g.	0.21g.

* Diaminodiphenylsulphone.

From this table it is interesting to see that the amount of diaminodiphenylsulphone available to the body when the proprietary sulphones are used is of the same order as the dose of diaminodiphenylsulphone which we have established to be the maximum therapeutic dose capable of continuous administration without toxic sequelae. If only 20% hydrolysis of the proprietary sulphones occurred it is obvious that the amount of diaminodiphenylsulphone available would be c.0.05g. That this figure is capable of giving clinical response is shewn by the work referred to above (Ref. 2).

b. *Experimental evidence.*

When any of the proprietary sulphones are ingested, blood and urine levels may be ascertained by diazotisation and coupling of the circulating sulphone as in the Bratton & Marshall procedure. However, this procedure does not give any information as to whether the sulphone present is present as the proprietary substance, the monoamino compound, the doubly hydrolysed compound (i.e., 4,4′ diaminodiphenylsulphone) or a various mixture of all of these. A blood level of 5mg% obtained from a patient

receiving sulphetrone therapy may represent not 5mg of sulphetrone but only 2mg% of sulphetrone and the rest diaminodiphenylsulphone. In order to determine the degree of *in vivo* hydrolysis of the proprietary sulphones the following method was developed.

Method for Urine. A sample of urine from a patient undergoing therapy with a proprietary sulphone is analysed for its concentration of sulphone by the usual technique (see previous paper). In order to facilitate the procedure a urine of >100mg% sulphetrone is obtained and >50mg% diasone is obtained, and after analysis the urines are adjusted exactly to some convenient figure (e.g., 100mg% and 50mg%) by the addition of water. Control solutions of sulphetrone and diasone are then prepared by weighing the pure powders and dissolving in a fresh urine obtained from a person not on sulphone or sulphonamide therapy. 100ml. portions of both the control and the urine to be tested are then extracted with three aliquots representing a total volume of 100ml. of ethyl acetate. The extracts are then dried over anhydrous sodium sulphate and 1ml. of each is estimated for diaminodiphenylsulphone content as described previously (Part I), except that the trichloracetic acid is replaced with a 50% solution of ethyl alcohol. The concentration of diaminodiphenylsulphone found in the "control" tube (usually negligible with sulphetrone) is subtracted from that obtained in the urine. This figure represents the amount of diaminodiphenylsulphone in mgs%. This over the figure obtained from the first analysis, will give the degree of hydrolysis that has occurred *in vivo*. The following figures show the amount of *in vivo* hydrolysis occurring in patients undergoing sulphone therapy with this Unit.

Drug.	No Observations.	mg% total sulphone.	mg% as DADPS*	Partition.
Diasone ...	5	50	2-10	4-20%
Sulphetrone ...	8	100	3-6	3-6%

* Diaminodiphenylsulphone.

Method for Blood. c.20ml. of blood from a patient are withdrawn. 1ml. of this sample is estimated for the sulphone in question as described in Part I. 10ml. of the remaining blood are mixed with 50ml. satd. $MgSO_4$ and then centrifuged until separation is obtained. 10ml. of the supernatent fluid is then shaken with 10mls. of ethyl acetate. 1ml. of the ethyl acetate extract is then estimated as for the extract obtained from urine.

A "blank" estimation is performed upon blood extracted from a person not undergoing sulphone therapy and to which blood has been added a known equivalent quantity of the sulphone to be estimated.

Result. Using this technique no diaminodiphenylsulphone could be discovered in the blood of patients undergoing sulphetrone therapy. With diasone, equivocal results were obtained, due to some grossness of the technical procedure. Further studies are contemplated: the above results and methods are reported in order that the work may be repeated in other research laboratories.

DISCUSSION.

That diaminodiphenylsulphone may be extracted from the urine of patients undergoing therapy with proprietary sulphones is evidence from the partial hydrolysis of these compounds in the body. The grossness of the methods used do not allow of any conclusions regarding the partition of the sulphones in the blood.

The presence of diaminodiphenylsulphone in the urine however is strong evidence for the presence of diaminodiphenylsulphone in the blood. The author holds the view that in the light of the evidence presented, both experimental and theoretical, the *in vivo* partial hydrolysis of the proprietary sulphones must be accepted.

TOXIC EFFECTS OF THE PROPRIETARY SULPHONES.

Since the sulphones are in some measure degraded to diaminodiphenylsulphone the toxic effects of any proprietary drug may be due to a combination of two factors:

a. The toxic action of diaminodiphenylsulphone.

b. The toxic effect of the whole molecule of the sulphone or portion of the molecule remaining after liberation of diaminodiphenylsulphone.

In this article the toxic phenomena of three sulphones will be studied in an attempt to discover whether diaminodiphenylsulphone possesses intrinsic toxic qualities which renders its use in leprosy therapy inadvisable.

BLOOD DYSCRASIAS.

Anaemia.

That the administration of the sulphones whether orally or parentally results in an anaemia (Ref. 4), is well known. That the anaemia produced may differ quantitavely with (a) the type of sulphone and (b) the mode of administration is not generally realised.

Sulphetrone. The anaemia produced is in the majority of cases not severe on a dosage of not more than 5.0g. daily *when the exhibition of the drug is gradual.* A fall of $>$ 3g. Hb% in the first six weeks is usual, and if iron is given concurrently with the sulphetrone the body stabilises itself within a matter of two or three months. The anaemia is hypochromic in type, no evidence for a marked haemolytic process has been found from studies of plasma bilirubin, red cell fragility, urobilinogen excretion or the presence of methaemalbuminaemia.

If the exhibition of the drug is rapid there occurs in Southern Nigeria a syndrome of glandular enlargement, splenomegaly, pyrexia and disturbance of liver function exhibition the blood picture similar to that of infectious mononucleosis. This effect is seen with all the sulphones when the exhibition of the drug is rapid. So far as is known it occurs only in this area—no such similar syndrome has been reported elsewhere. This particular action of the sulphones will be made the subject of a separate

report. No patient exhibiting this phenomenon is included in the group for purposes of the present report.

Brownlee (Ref. 3) reports that sulphetrone combines with alimentary iron and also depresses the bacterial flora of the gut. Both these factors may be responsible for the anaemia producing effect of sulphetrone administered orally.

In an effort to study the degree of responsibility of both the diaminodiphenylsulphone part of the sulphetrone molecule and the remaining cinnamyl-like portion for the anaemia produced by sulphetrone the following experiment was performed. In Fig. 2, Col. 4 it is shewn that the amount of sulphetrone available to the body on a daily oral dose of 5g. is 0.75g. Sulphetrone was given intramuscularly in a dosage of 0.75g. daily to a group of five patients. The resultant anaemia was not of significance. In the five cases under test no loss of Hb greater than 0.5g. was recorded.

It is realised that this study is by no means as satisfactory as desired.

Diasone. When diasone is given orally in doses of 1.8g. daily an anaemia rather greater than that produced by 5g. of sulphetrone daily is produced. When the dose is raised above 2.0g. daily then a definite haemolytic anaemia is detectable by means of raised urobilinogen, excretion, raised icterus index, and the occasional presence of methaemalbumen in the blood plasma.

The fall in the red cell count with oral diasone is of a much greater degree than with sulphetrone. Red cell count decreases of the order of 1.5 to 2.0 million are not uncommon when the daily dose is 1.8g.

Fig. 2, Col. 4 shews that the amount of diaminodiphenylsulphone available to the body is c.0.7g. when the daily oral dose is 1.5g. When diasone is given parenterally in a dosage approximating to 0.7g. daily (5ml. of a 40% solution thrice weekly) the anaemia produced is (as with parenteral sulphetrone) of nothing like the same degree. In 14 patients treated for two months with parenteral diasone as described above, the anaemia was not severe enough to warrant the administration of iron or the cessation of treatment.

Diaminodiphenylsulphone. In a dosage of 0.2-0.3g. daily the anaemia produced by this drug is of a mild self-correcting type occurring mainly in the first month of treatment. The anaemia is of a haemolytic nature, though perhaps other factors also enter into its production.

General Comment. The study of anaemia in Southern Nigeria

is made difficult by the many intercurrent haemolytic infections to which the patients are exposed. There is also the unknown effect of the various degrees of liver dysfunction present in a large percentage of primitive African people in this area. Thus the effect of any drug upon the body may not be a pure cause and effect relationship. The anaemia produced by the sulphones is always more severe and treatment more difficult in patients with liver dysfunction. Diaminodiphenylsulphone cannot be shewn however to be more anaemia-producing in these cases than diasone or sulphetrone.

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CORRESPONDENCE

The Editor, 6th November, 1948.
"Leprosy Review."
Sir,

There have been suggestions in the literature of the sulphone treatment of leprosy that sulphones may be concentrated in the skin (1, 2). The importance of the study of such an observation is obvious and has been emphasised at the recent International Conference of Leprosy (3).

Some months ago in this laboratory a biopsy specimen of skin was submitted for analysis of sulphone concentration. The result was 35 mg. of sulphone per 100 g. of skin. This result was not in agreement with other values obtained previously, and investigation revealed that the biopsy had been performed under Planocaine local anaesthesia, whereas previous biopsies had been made from nodular material not necessitating the use of a local anaesthetic. A repeat biopsy of this same patient shewing a reported concentration of 35 mg. per 100 g. of skin was made under cocaine local anaesthesia. This second biopsy was made within a few hours of the first and at a site as near to the first as was possible. Analysis of this specimen shewed a concentration of < 2 mg. sulphone per 100 g. of skin, a figure in accordance with the values obtained from previous skin biopsies.

DISCUSSION.

The routine method in general use for quantitative estimations of the proprietary group of substituted diaminodyphenylsulphones is substantially that of Bratton and Marshall (4). The principle is that of diazotisation of the amino grouping and subsequent reaction with a coupling component to produce a compound capable of colourimetric estimation. The method was devised originally for the sulphonamides, but a colour reaction is obtained with any compound having a diazotisable amino grouping. The aromatic anaesthetics, e.g., Procaine and Planocaine, possess such a grouping and will therefore give a colour under the conditions of the test. Infiltration local anaesthesia with this group of anaesthetics is contra-indicated when biopsies of skin for analysis of sulphone concentration are required.

Cocaine is an anaesthetic not possessing a diazotisable amino group and is therefore satisfactory for biopsy work.

We have learnt (5) that in one other leprosy centre high skin concentrations of sulphones have been found to be due to the use of the type of anaesthetic indicated above. In view of this it has been thought desirable to publish our experience together with a method for the estimation of skin concentrations that we have found to work satisfactorily. The whole question of skin concentrations of sulphone will be dealt with more fully in a further article.

METHOD.

Anaesthesia. 1% aqueous solution of cocaine hydrochloride. 1 ml. of this will produce a satisfactory degree of anaesthesia in from 5-10 minutes.

Biopsy. With dark skins it is advantageous to outline the biopsy area with a skin pencil. An elliptical area 2 cm x 0·5 cm extending down to the subcutaneous fat will afford about 0.3 g. of skin, and this amount is the minimum that it is convenient to work with.

Estimation. The excised skin should be rapidly washed in physiological saline to remove any adhering blood, and then pressed dry between several thicknesses of filter paper. Weigh on an analytical balance and transfer to a glass mortar of about 5 cm. diameter. Add 2 or 3 grammes of acid-washed sand and one-third of the calculated volume of N/1 HCl. The calculated volume of N/1 HCl is ten times the weight of the skin excised. Grind until the skin is entirely macerated. With a Pasteur pipette remove the supernatant HCl from the sand and tissue debris, and transfer the HCl to a 10 ml. graduated centrifuge tube. Add to the mortar

the second one third volume portion of N/1 HCl and grind, transferring the supernatant acid to the centrifuge tube as before. The remaining one third volume of N/1 HCl is added to the mortar and transferred, after grinding, as before. In effect the skin is extracted with three portions of HCl. To the centrifuge tube is then added a 12% trichloracetic acid solution to bring the final volume to 15 times the weight of the skin taken.

Example: Weight of skin ... ... 0·5 g.
Calculated volume of
N/1 HCl = 5·0 m. (10 x 0·5 g.)
1/3 calculated volume = 1·6 ml
(skin extracted 3 times with 1·6 ml. of N/1 HCl each time)
Trichloracetic acid added to make final volume up to
7·5 ml. (15 x 0·5 g.)

The tube is then centrifuged, the supernatant fluid poured off into a 10 ml. comparator tube, diazotised and coupled by the method described by Brownlee et al. (6). The above method for skin estimations is applicable to any of the proprietary sulphones, but for each proprietary compound a different standard is necessary. For Sulphetrone, the Lovibond comparator disc is available, and the colour produced by the skin extract may be read directly on the disc as milligrammes per 100 grammes of skin. Promin and Diasone may be estimated approximately by reading in the Lovibond Comparator calibrated for Sulphetrone and applying the following correction factor.

$$\text{mg\% Promin} = \frac{\text{Sulphetrone disc reading}}{1{\cdot}1}$$

$$\text{mg\% Diasone} = \frac{\text{Sulphetrone disc reading}}{1{\cdot}7}$$

Using the above technique we have found no evidence that the sulphones are concentrated in the skin.

Yours faithfully,

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REVIEWS

Leprosy in India. Vol. XX, No. 4, October, 1948.

The editorial in this volume is a study by S. N. Chatterji on the vexed question of leprosy and divorce. It is a careful study that should be read by everyone interested in the subject.

Frequency of the presence of leprosy bacilli in nasal smears of leprosy patients by Dharmendra and N. Sen. After careful and tabulated study, the authors conclude as follows:

"From the above study the following conclusions are made:

1. In case of leprosy of all types the bacilli are found more frequently in the skin lesions than in the nasal smears. This difference is specially marked in cases of the neural type.

2. In cases of the 'neural' type with bacteriologically negative skin lesions, it is rare to find leprosy bacilli in the nose. Out of the 2,834 cases this finding has been made in only 5, two of them being 'tuberculoid' cases in the stage of reaction.

3. Although the finding of leprosy bacilli in the nose, in the absence of bacilli in the skin, is not a common feature, because this is possible, though rarely, it is important to make a bacteriological examination of the nose in addition to the skin lesions before declaring a patient a 'closed' or non-infectious case."

Leprosy in India. Vol. XXI No. 1, January, 1949.

This is the Havana Congress Number giving the official findings of the Congress on classification, therapy and epidemiology control. This material has also been published in the **International Journal of Leprosy** April June 1948 and the **Leprosy Review** of July 1948. Besides these, however, there are a number of short abstracts of the more important papers presented to the Congress.

Leprosy in India, Vol. XXI, No. 2, April 1949.

This has an account of the All-India Leprosy Workers' Conference held in Calcutta from the 29th-31st December 1948. After the official opening, speeches and messages of goodwill, the scientific session began.

Session 1. The Treatment of Leprosy. Papers were given by Doctors R. G. Cochrane, Dharmendra and S. N. Chatterjee on sulphone drugs. Papers by Dr. Teichmann and Dr. Roy were also given on hydnocarpus oil. These papers were followed by a stimulating discussion on the part of various members.

In Session 2 the *Control of Leprosy* was discussed and in the third session the histopathology and classification of leprosy. It is obvious, from this last session, that a great deal more work and clarification is necessary before we can come to any established basis for classification which will be both international and permanent. It is obvious from the findings of this Congress that

India is facing the problem of leprosy with considerable anxiety, zeal and aptitude, and this whole number could be studied with profit by leprosy administrations in other territories.

Injection of sulphetrone and diasone in leprosy by Dr. S. N. Chatterjee. The author used a 3·3 per cent solution of both sulphetrone and diasone, working up from 1 to 5 c.c. intramuscularly twice a week. This method is considered definitely more economical and less toxic than when these drugs are given ordinarily, and the author suggests that more extensive trials of this method should be used.

General principles in the treatment of leprosy with particular reference to the sulphones by Dr. R. G. Cochrane. This is a vigorous study of Dr. Cochrane's views on the use of sulphones, and the author's intentions and feelings make it worthy of the closest study. It is not everyone, however, who will agree that " no case showing neural macules, tuberculoid or neural anaesthetic lesions should be given sulphone therapy." The author ends on a timely note of warning against undue optimism and lack of perspective.

Some brief comments on the classification of leprosy by Dr. R. G. Cochrane. In this study Dr. Cochrane proposes one or two changes. He introduces the term " Dimorphous Leprosy " to describe the border-line case. He would then alter the South American uncharacteristic classification and use this latter phrase for the pre-lepromatous macule. He also advocates the avoidance of the terms ' neural ' and ' tuberculoid.'

Leprosy and its relief in the industrial town of Jamshedpur by Dr. B. Dalal. This is an important steel centre in Bahar with a total incidence of leprosy of 4·9 per cent per thousand. The work of a leprosy clinic is briefly described, and the need is stressed for an agricultural colony.

A note on the histopathology of leprosy by Dharmendra. The author summarises as follows:

1. A plea is made for creating interest in histological studies in leprosy on a wider scale in India, though of course priority should be given to the studies on treatment and control of the disease.
2. A description is given of the typical findings generally met with in the ' simple ' or ' uncharacteristic ' tuberculoid, and lepromatous histological pictures, and in the borderline, intermediate, or doubtful group it is pointed out that a particular histological picture, with perhaps the exception of the lepromatous histology, is not confined to one particular clinical variety of the disease.
3. In the tuberculoid and lepromatous histology, certain findings may sometimes be seen which are not typical of the respective type, but the subsequent course of the disease in a long-term study of selected cases has shown that these variations are not of any special significance.
4. It is suggested that classification of leprosy should not be based

primarily on histology. In the opinion of the writer the use of histological terms to indicate clinical types results in confusion in many instances.

Some comments on the classification of leprosy recommended by the Havana Congress by Dharmendra. This studies the classification which was recommended by the Classification Committee of the Havana Congress, and which was subsequently rejected at a general meeting. The author summarises his views in the following conclusions:

"It can therefore be concluded that the recommendations of the Classification Committee of the Havana Congress mark an improvement in certain respects over the Cairo Classification and the Pan-American Classification, but that in certain other respects they introduce features which create more difficulties. The recommendations merit serious consideration of all workers, and if it is possible to remedy the defects, they can form the basis of a universal classification.

"Regarding the main classification, the conception of two main types and a 'group' appears to be acceptable to all, the only difference of opinion is about the term 'tuberculoid' used to designate one of the main types. A search has to be made to find a more suitable and less objectionable term, though it appears to be a difficult task to find a word likely to meet general approval. To the writer the term maculo-anaesthetic appears to be less objectionable, the main objction against it being that the term macule in general dermatology refers only to a flat patch. However, leprosy workers are familiar with its use in a wider sense as it is being at present used to designate both the flat and thick patches of the neural type (vide the term neuro-macular). If the term maculo-anaesthetic or some equivalent term is acceptable, then the cases with purely polyneuritic changes without any skin lesions will have to be taken out from this type and placed in the 'indeterminate' group. This arrangement has much to be said for it because on the one hand there is considerable amount of justification, since the prognosis in purely polyneuritic cases is uncertain, and secondly, it will do away with the difficulty of splitting up such cases into tuberculoid, lepromatous, and indeterminate (or alternately these purely polyneuritic cases may be placed in a separate class but that would mean 4 main classes in place of 3).

Regarding the clinical subdivision of the main types the main changes which appear to be necessary would be to find a place in the indeterminate group for the thick borderline, intermediate, or unclassified patches of leprosy and, as suggested above, to do away with the terms like tuberculoid-polyneuritic, lepromatous-polyneuritic, and intermediate-polyneuritic.

According to the above suggestions the three main classes (2 types and 1 group) would be maculo-anaesthetic, lepromatous, and indeterminate. In the maculo-anaesthetic, there will be included flat and thick patches of the present neuro-macular variety; in the lepromatous, the various clinical varieties in this type; and in the indeterminate the flat, and thick patches (not covered by the maculo-anaesthetic or the lepromatous types), and the purely polyneuritic cases (in case the purely polyneuritic cases are placed in a separate class, the number of classes will be 4 and of course these cases will be excluded from the indeterminate group)"

Some observations on the treatment of leprosy with the sulphone drugs by Dharmendra. The author points out that the use of sulphones in leprosy is now established beyond the experimental stage. Dr. Dharmendra's main plea, however, is for a scientific trial of combined treatment with sulphones and hydnocarpus oil. (Such a trial of the two main drugs known to be efficacious in leprosy is by now overdue. The experiment should be made under

the aegis of an efficient scientific body such as the Indian Council of the British Empire Leprosy Relief Association. It might even be preferable if combined experiments were carried out on the same scientific basis by authorities in such different territories as India, Malaya, East Africa and West Africa. Only by a correlated study of such experiments can we arrive at authoritative conclusions on the most effective methods of leprosy treatment.—Ed.)

If tomorrow we were sure of a specific for leprosy? by T. N. Jagadisan. This article is a deeply moving account of the human problem in leprosy, pointing out that the mere introduction of a remedy cannot by itself remove the human misery and stigma of the disease. It is a plea for a wide scale campaign of education, prevention and rehabilitation. In the author's own concluding words: " I like to think that a specific (cure for leprosy) would throw out a wider challenge and show leprosy to be what it is, a test of social sincerity, and that we shall answer the challenge and obtain the victory.

Control of Leprosy by Dr. E. Muir. In this article Dr. Muir summarises his well known views on the social steps which should be taken to prevent the spread of leprosy. He suggests that there are seven factors in India which facilitate the spread of the disease —concentration and increased contact due to migration and movement of population, congested housing, defective cleanliness, defective diet, the weakening effects of other diseases, climate, and lastly, ideas and attitude. He advocates a widespread campaign of increased knowledge to replace the superstition and dread of the disease which at present exists. This could be done by village societies in whom he believes the hope of the future of the new India rests.

Welfare work for leprosy patients by Shri N. Ramakrishnan. This article deals with the very pressing problem of the psychological and physical rehabilitation of a patient. The task of the actual leprosy settlement is dealt with under the headings of vocation, effective treatment, health, education and social life. The task of dealing with out-patients is dealt with under care of family, follow-up, rehabilitation, out-patients and publicity and propaganda. This is a carefully written paper and deserves attention.

Leprosy in Calcutta by Dr. P. Sen. The leprosy problem in India has, in the past, been considered mainly from the point of view of its rural aspect. Urban leprosy has not so far received the attention that it merits. This is possibly due to the fact that surveys are very much more easily conducted in villages than in cities. Dr. Sen's contribution in this article therefore is extremely

welcome. The number of cases of leprosy in Calcutta, with a population of roughly four million, is variously estimated as between 20,000 and 40,000. The author pleads the very great seriousness of the problem of leprosy in Calcutta, and the richness of the field for the study of epidemiology and control. He summarises as follows:—

1. Even though the incidence of leprosy is low in Calcutta, diffusion of leprosy and population pressure per square mile is highest in the province. Hence the risk of spread of the disease is considered greatest in the city, particularly under the prevailing conditions of acute housing shortage, extreme congestion in transport services, unawareness of communicability of leprosy and malnutrition.

2. Calcutta is considered eminently suitable for the proposed All-India Leprosy Institute and the grounds thereof are indicated.

3. The urgency of the problem calls for a comprehensive undertaking of measures for control and treatment of the disease by the authorities.

4. Leprosy being a long-term sickness entailing a large expenditure of money, advisability of exploration of sharing the burden between the Provincial and the Central Government is suggested.

5. In a city like Calcutta private generosity, if properly canalized, should be a great help in grappling with the problem.

International Journal of Leprosy, Volume 16, No. 3, July-September, 1948.

Haematologic Study of Leprosy Patients Treated with Diasone, by Drs. J. M. M. Fernandez, E. A. Carboni, Pedro Tommasino and M. M. Gimenez. This is an important study of the red cell alterations occurring under diasone therapy, with a comparison of patients treated with hydnocarpus oil. This is the authors' summary:—

"The clinical symptoms, frequency and relation to intensity of treatment, and the course of the anaemia produced by diasone are described. It is shown that the changes in the blood picture are limited to the red blood count, without disturbance of other components. The anaemia begins early in the first weeks of treatment and as a general rule abates with complete recovery during the periods of rest from the drug.

Investigations made in the treated patients have included the reticulocytosis, urobilinuria, bilirubinemia, and red blood cell resistance; the results of biopsy of the bone marrow in certain cases are also given. The findings show that the anaemia is not due to a toxic action of the drug in the bone marrow, since there is good reticulocyte response in all cases and no leucopenia; furthermore, bone marrow biopsy shows a normal or increased erythroblastic activity, and granulopoiesis in also normal or increased. It is therefore deduced that the drug acts as a haemolytic toxin, as shown by the decrease in the red-blood-cell resistance, the presence of urobilin in the urine of almost all the cases, the absence of bile pigments in the urine, of direct bilirubin in the blood, and the absence of indirect bilirubinemia in the great majority of cases.

Taking these facts into account adequate therapeutic measures, based on clinical experience, are suggested.

CONCLUSIONS.

As a result of this study we have come to the following conclusions.

1. Diasone produces an early anaemia, which begins in the second week of treatment.

2. The anaemia is probably due to a toxic haemolytic effect on the drug, because: (a) there is a definite decrease in the red-cell resistance; (b) urobilin is consistently found in the urine; (c) bile pigments are not found in the urine; and (d) the indirect bilirubin determination has proved negative in almost all cases.

3. The anaemia is not due to a toxic effect on the bone marrow, because: (a) there is a satisfactory reticulocyte response in all cases; (b) bone-marrow biopsy shows normal or increased erythroblastic activity; and (c) granulopoiesis also is normal or increased.

4. The toxic effect is transitory, as shown by the fact that the blood changes disappear when the drug is withdrawn.

5. Clinical experience has shown that the anaemia is usually benign and seldom requires interruption of the treatment.

6. Use of the common antianaemic remedies—iron liver and vitamin B complex—produces a favourable reaction, increasing the tolerance of the organism for the drug."

Effects of Sulphone Treatment on the Larynx in Leprosy, by Dr. N. R. Sloan. The author points out in this brief article that laryngeal improvement is one of the most dramatic results of sulphone therapy, whatever form of sulphone is used. He concludes:—

"Improvement of laryngeal lesions is one of the most striking results of sulphone treatment, perhaps the most striking one. The reason for this is undetermined; possibly it is the generous vascular supply. It appears to make no difference whether the drug is promin or diasone, if it is used in adequate dosage over a sufficient period of time; and improvement may be seen in only a few weeks or months. We feel that patients who are beginning to show laryngeal dyspnea should be carefully watched for the first months of treatment, so that emergency tracheotomy may be performed if necessary; but often it may be avoided. It is our firm conviction that the response of laryngeal lesions to treatment would justify use of the sulphones even if no other benefit were derived from them.

SUMMARY.

The sulphones—promin and diasone—are of great value in the care of patients suffering from lepromatous laryngeal involvement, both in those who have not required tracheotomy and in those who are wearing tracheal tubes. In an experience of less than two years with these drugs at the Kalaupapa Settlement, 9 patients have been able to remove tubes as a result of that treatment, and at least two (perhaps 5) have been saved from tracheotomy. The last death from the chronic bronchitis which follows tracheotomy was in February, 1947; the last tracheotomy was in May, 1947."

The Intradermal Metholyl Test for Anidrosis; A Diagnostic Aid in Leprosy, by Dr. Harry J. Arnold, Jr. The author describes the technique of the metacholine test, and the following is an extract:—

"The method of performing this test (1) does not differ in any essential respect from that employed by earlier workers, except that it involves (a) the use of metacholine, which is a relatively stable and readily available substance, and (b) the demonstration of the sweat response in control areas outside the lesion by Minor's method.

The materials required for the test are as follows:—(1) a bottle of Minor's solution (crystalline iodine 2 grammes, castor oil 10 cc. absolute alcohol to make 100 cc.); (2) ordinary cotton applicators for applying it to the skin; (3) metacholine chloride in 1 per cent. aqueus solution (conveniently prepared from a 25 mg. ampoule of macholyl chloride by dissolving it in 2.5 cc. of sterile saline in a rubber-capped vaccine bottle); (4) a hypodermic syringe graduated in 0.01 cc.; (5) a 26 gauge, one-half inch hypodermic needle; (6) dry gauze for blotting off the drop of solution which back-leaks from the injection site; (7) powdered starch (ordinary cornstarch seems to be quite as satisfactory as the rice starch powder recommended by Minor; and (8) a powder-blower type of atomizer for application of the starch.

The lesion or area to be tested, plus a roughly equal area of adjacent normal skin, is first painted with Miner's solution; this will dry rapidly, but it is not necessary to wait for it to do so. Approximately 0.05 to 0.1 cc. of metacholine chloride solution is then injected intradermally at the border of the lesion, so that the elevated wheal will be partly inside and partly outside the involved area. In larger lesions two additional injections may be made, one entirely within, the other entirely without, the lesion. These merely make the demonstration more dramatic and in general add little to the ease of interpreting the test. The droplet of solution that leaks back from the injection site should be gently blotted (not wiped) off, and the whole area quickly and lightly dusted with powdered starch, blown from the atomizer.

Within a few seconds, also, sweat droplets will begin to appear at the mouths of those sweat glands which are still functionally intact. They moisten the dry white-over-tan iodine-starch combination, which immediately turns deep blue-black and remains so. This clearly visible sweat secretion spreads rapidly over an area concentric with the intradermal wheal, to a radial distance of about 1 to 3 cm. The response reaches its maximum within two or three minutes.

If more than three injections of 0.1 cc. are made, the patient may experience transient systemic discomfort from the absorption of metacholine. If the dose does not exceed, say, double that amount, the result is almost always limited to generalized sweating, flushing of the face, some salivation and slight malaise. Urination and defection may be stimulated by larger doses, or in the rarely-encountered hypersensitive individual. Atropine, in the usual therapeutic doses given by hypodermic injection, is the precise pharmacologic antidote."

Report of Leprosy Lesions of the Fundus, by Dr. David C. Elliott. This is a most interesting clinical account of a case with leprous lesions in the posteror segment of the eye. This appears to us to be an original advance in our knowledge of the evolution of leprotic ocular lesions. The author's summary is as follows:—

"1. In a case of leprosy classified as of advanced lepromatous type, an acute ocular disturbance resulted in an examination which disclosed lesions on the fundus, which are believed to be lepromatous in character.

2. These lesions are identical ophthalmoscopically with those seen on the iris in this patient and in others; iris lesions of this kind have been histologically proven to be true lepromas.

3. The relatively transient character of these "pearl" formations, both on the iris and on the fundus, is illustrated by this case, in which these lesions have developed and receded within a period of eight months of observation.

4. It is believed that unless such an eye could be obtained accidentally at the time such a manifestation is observed clinically, histological confirmation of the nature of these lesions will be difficult to obtain. There may be no distinct pathological condition remaining to be observed in the eyes which come to examination many years after the acute process has passed."

A Comparative Study by Electron Microscopy of the Morphology of Mycobacterium Leprae and Cultivable Species of Mycobacteria. by Drs. F. W. Bishop, L. G. Suhrland and C. M. Carpenter.

The Effect of Fixatives on Staining Procedures for Lepra Bacilli in Tissues, by F. Leon Blanco and G. L. Fite. In this brief article it is recommended that for a demonstration of leprosy bacilli in paraffin sections the following are recommended:— (a) Fixation in Zenker's fluid; (b) the use of thin blocks, with minimal time in dehydrating and clearing fluids; (c) staining by the oil fuchsin method, and (d) mounting in one of the modern synthetic mediums, 'clarite' or 'permount,' never in balsam.