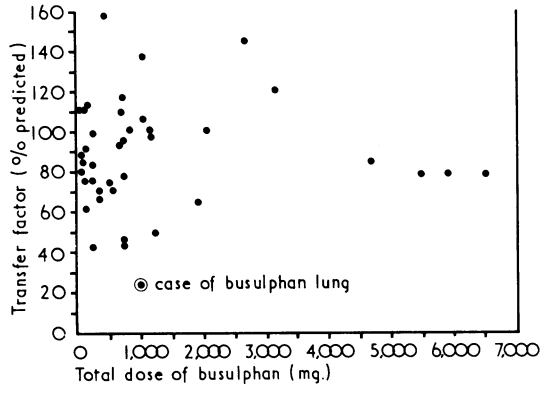
TABLE VII.—*Transfer Factor and Necropsy Findings Related to Busulphan Therapy in the Five Patients who Died*

Case No.	Total Duration of Busulphan (Months)	Total Dosage of Busulphan (mg.)	Transfer Factor (% Normal)	Necropsy Findings
3 ..	8	612	85	No fibrosing alveolitis
5 ..	60	4,704	86	No fibrosing alveolitis
4 ..	19	1,000	25	"Busulphan lung"
9 ..	25	2,361	101	No necropsy. Antemortem chest radiograph—clear
1 ..	13	748	96	No necropsy. Antemortem chest radiograph—clear

to 3% of normal people have an unusually high transfer factor, and A. Seaton (personal communication, 1970) found high transfer factors in women taking the contraceptive pill.

Five patients (Cases 1, 3, 4, 5, and 9) have died since the study was begun. One death (Case 4) resulted from fibrosing



Relationship between transfer factor and total busulphan dosage.

alveolitis due to "busulphan lung." The other four patients died after a myeloblastic crisis, and in two of these no histological abnormality of the lung was found at necropsy. In the other two cases permission for necropsy was refused, but antemortem chest radiographs and physiological studies were normal. Two of these patients had received a much larger dose of busulphan than the patient who developed fibrosing alveolitis (Table VII), and a further six patients who are alive and well also received a dose in excess of 1,000 mg. (see Table II). The lack of any relationship between busulphan dosage and transfer factor is illustrated in the Chart. Hence the development of fibrosing alveolitis may be related to the genetic or immunological constitution of the patient rather than to the dose or duration of busulphan therapy.

The preliminary results of this study suggest that lung damage, detectable during life, is a relatively uncommon complication of busulphan treatment, occurring in only 1 out of 23 patients closely studied over an average period of nearly two years.

We are indebted to Dr. David Weatherall for his constant encouragement and for allowing us to study so many patients under his care. We would also like to thank Dr. J. Forshaw, Dr. P. Robb, Dr. J. Meecham, Dr. J. Campbell, Dr. J. Carmichael, and Professor Donald Heath and his department for their help with various aspects of this work. We are grateful to Miss S. Williams and Mrs. M. Deakin for technical assistance.

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Interference of Iron with the Absorption of Tetracyclines in Man

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British Medical Journal, 1970, **4**, 532-534

Summary: Ferrous sulphate administered together with tetracycline and three of its derivatives—oxytetracycline, methacycline, and doxycycline—was found seriously to impair the absorption of these antibiotics. Thus even small doses of iron taken simultaneously should be avoided during tetracycline treatment.

Introduction

Most tetracycline derivatives are rapidly but incompletely absorbed from the gastrointestinal tract. As these substances are generally considered to be bacteriostatic, their plasma levels during therapy should never be allowed to fall below the minimum inhibitory concentration. Thus it is of great importance to avoid situations that may impair absorption and lead to therapeutically inadequate plasma concentrations. Milk, antacids, and bivalent and trivalent cations are known to reduce the absorption of tetracyclines (Waisbren and Hueckel, 1950; Dearborn *et al.*, 1957; Rosenblatt *et al.*, 1966), possibly by formation of chelates (Albert and Rees, 1956).

Since it is not uncommon for patients on tetracyclines also to receive preparations containing iron, we considered it valuable to study the effect of iron given simultaneously on the absorption of various tetracyclines.

Methods

The subjects were healthy medical students aged 20–24, weighing 60–80 kg., who volunteered for the experiment. Each

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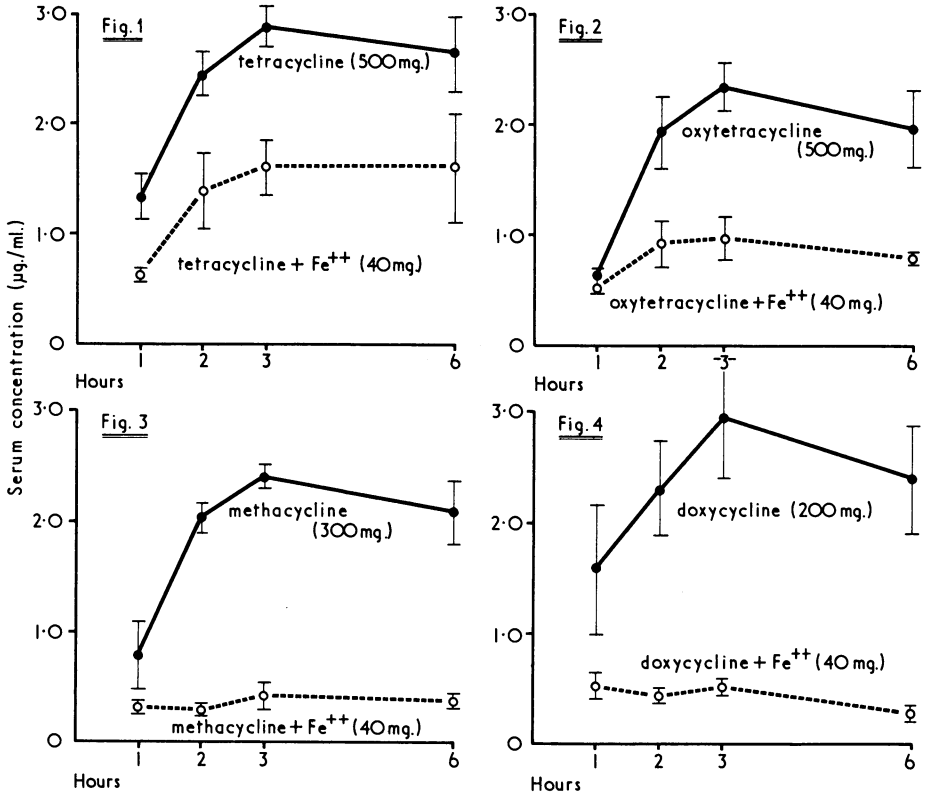

FIGS. 1-4.—Mean serum levels (±S. E.) after single doses of tetracycline, oxytetracycline, methacycline, and doxycycline taken either alone or simultaneously with 200 mg. of ferrous sulphate. Number of subjects in each group = 5.

tetracycline derivative was tested on 10 students. Five took the drug alone and five with 200 mg. of ferrous sulphate. After overnight fasting the tetracyclines were given as single oral doses with about 200 ml. of water. Blood samples were collected from the cubital vein one, two, three, and six hours after drug ingestion. After the third venepuncture the subjects had a light lunch without milk or dairy products. Serum antibiotic levels were assayed microbiologically by the agar-plate method according to the *Pharmacopoea Nordica* (1964), which corresponds in general to the *British Pharmacopoeia* (1968) on Bacto Antibiotic Medium 1 (Difco) with *Bacillus subtilis* A.T.C.C. 6633 as test organism.

The following tetracycline derivatives (in capsules) were studied: tetracycline chloride, oxytetracycline chloride, methacycline chloride, and doxycycline chloride. The iron was given as ferrous sulphate, each 200-mg. tablet containing 40 mg. elemental iron. Means with standard errors (S.E.) were calculated, and Student's *t* test was applied in the statistical evaluation of the results.

Results

After 500 mg. of tetracycline a maximal serum concentration of 2·9 μg./ml. was reached in three hours (Fig. 1). When this drug was taken with 200 mg. of ferrous sulphate the serum levels attained at one, two, and three hours were significantly reduced ($P<0.05$). The average decrease was 40–50%.

Ingestion of 500 mg. of oxytetracycline and 200 mg. of ferrous sulphate resulted in distinctly lower ($P<0.02$) serum levels compared with the value of 2·3 μg./ml. obtained with oxytetracycline alone. The serum concentrations fell by 50–60% (Fig. 2).

The peak level for serum methacycline reached about 2·4 μg./ml. in three hours after 300 mg. of this drug. When 200 mg. of ferrous sulphate was taken with the antibiotic the average serum concentration of methacycline did not exceed 0·5 μg./ml. The difference between the values attained without and with iron was highly significant ($P<0.001$) at two, three, and six hours, the average reduction being 80-85% (Fig. 3).

When 200 mg. of doxycycline was given the serum concentration rose to 3·0 μg./ml. The same dose of doxycycline taken with 200 mg. of ferrous sulphate never caused the average serum concentration to exceed 0·6 μg./ml. This inhibitory effect of iron was highly significant ($P<0.005$) at two, three, and six hours, being 80–90% on average (Fig. 4).

Discussion

We chose the doses of tetracyclines according to the recommendations of each manufacturer. Thus methacycline and doxycycline were used in smaller doses because of their reportedly more complete absorption and/or longer half-lives. Indeed the mean peak serum values of the different derivatives in the control subjects reached the same level of about 2·3 to 3·0 μg./ml. The binding of Fe^{++} to tetracyclines apparently takes place in equimolar ratios (Mitscher *et al.*, 1969), which may explain why methacycline and doxycycline, owing to their smaller doses, suffered the greatest reduction in serum concentrations when given with iron.

The amount of Fe^{++} taken by the subjects was quite modest; in the treatment of anaemia doses containing up to 100 mg. of Fe^{++} are often used. On the other hand, the definite effects obtained with small doses emphasizes the importance of the tetracycline-iron interaction. Moreover, single doses of some tonics contain an equivalent amount of iron to that shown by us to influence tetracycline absorption and lead to serum levels below the usually accepted minimum therapeutic concentration of 0·6 μg./ml. Not only does iron inhibit absorption of tetracyclines but the reverse also has been shown to hold true (Greenberger *et al.*, 1967).

In some preliminary tests with other iron preparations containing ferrous or ferric salts we found the same inhibitory action on absorption of tetracyclines. Hence to avoid inadequate plasma levels and therapeutic failures tetracyclines should not be taken simultaneously with any preparation liberating ferrous or ferric ions in the upper part of the gastrointestinal tract.

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Recovery of Adrenocortical Function During Long-term Treatment with Corticosteroids

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British Medical Journal, 1970, 4, 534-537

Summary: The recovery of adrenocortical function during very slow withdrawal of corticosteroids was studied in a homogeneous group of patients suffering from sarcoidosis. All patients had been treated with gradually decreasing doses of prednisone for at least two years. The initial dose had been 40 mg. daily in all cases. Determination of the cortisol production rate and of plasma fluorogenic corticosteroids was done under basal conditions and after tetracosactrin stimulation. There was good correlation between cortisol production rate and plasma fluorogenic corticosteroids throughout all the tests. Cortisol production rate and plasma fluorogenic corticosteroids started to rise when the dosage of prednisone was lowered to 7·5 mg. daily and reached normal values when the dosage was reduced to 2·5 mg. The response to tetracosactrin began to increase at the same dosage level, but was not normal at 2·5 mg., or when prednisone treatment was stopped. At a dosage level of 7·5 mg. of prednisone plasma fluorogenic corticosteroids already showed a nyctohemeral rhythm.

It may be calculated that even very low dosages of prednisone given during the last stage of a treatment schedule enhance total corticosteroid activity beyond the normal level, which would account for their therapeutic value.

Introduction

The present study was undertaken to investigate the recovery of adrenocortical function during very slow withdrawal of corticosteroids in a homogeneous population of patients who had been on this therapy for a number of years. Furthermore, we aimed to ascertain whether the very low dosages of prednisone given during the last stages of such a treatment were therapeutically active.

The 43 subjects who participated were part of a large group of patients suffering from pulmonary sarcoidosis who had been treated with prednisone for at least two years according to a uniform schedule (shown in Table I). Besides determining the plasma fluorogenic corticosteroids, as usually undertaken in similar investigations, we measured the cortisol production rate by isotope dilution, in order to obtain the most reliable quantitative assessment of adrenocortical function.

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In a pilot study the cortisol production rate was determined at a time when the dosage of prednisone had been decreased to 12·5 mg. or less. Further investigations, including measurement of plasma fluorogenic corticosteroids in the morning and afternoon and the cortisol production rate and plasma fluorogenic corticosteroids during and after tetracosactrin stimulation, were made on 26 patients when they had reached a dosage level of 7·5 mg., of prednisone or less. All investigations were made after the dosage level indicated in the charts had been maintained for at least four months. In addition, eight of these patients were studied within six months after cessation of treatment. In 18 patients the cortisol production rate could be determined more than once.

Patients and Methods

The diagnosis of sarcoidosis was confirmed in all patients by extensive laboratory examinations, *x*-ray studies, and biopsies (lymph nodes, liver, lung, and skin, or bronchial mucosa, or several). Details concerning diagnostic procedures and indications for corticosteroid therapy have been described elsewhere (Deenstra and van Ditmars, 1968).

Of the patients studied 22 were men aged 25-63 and 21 were women aged 27-63. Twenty-seven were in hospital for six days. On the second day the cortisol production rate was determined; plasma fluorogenic corticosteroids were measured at 8.30 a.m. and 4.30 p.m. on days 3 and 4. On day 5 the patients received an intravenous infusion of 0·25 mg. of tetracosactrin (Synacthen) in 500 ml. of saline during eight hours. On the same day the cortisol production rate was again determined. Plasma fluorogenic corticosteroids were measured at the beginning and at the end of the infusion. Prednisone treatment was continued during all tests. Furthermore, 29 patients aged 20-61 with pulmonary sarcoidosis were investigated before prednisone treatment was started—in six the whole series of tests were done and in 23 the cortisol production rate only. For comparison the cortisol production rate was determined in 23 normal volunteer subjects (staff

TABLE I.—*Schedule for Treatment with Prednisone of Patients with Sarcoidosis. Treatment Starts with 40 mg. of Prednisone Daily during the Time Indicated followed by a Gradually Decreasing Dose, as shown from Top to Bottom. Total Time of Prednisone Medication is about 6 Years*

	Dose per day
6 weeks	40 mg. of prednisone
32 days	Dosage reduced by 2·5 mg. every fourth day to a final level of 20 mg.
8 months	20 mg.
8 months	17·5 mg.
8 months	15 mg.
8 months	12·5 mg. (5–2·5–5 mg.)
8 months	10 mg. (5–0–5 mg.)
8 months	7·5 mg. (5–0–2·5 mg.)
8 months	5 mg. (5–0–0 mg.)
8 months	2·5 mg. (2·5–0–0 mg.)
8 months	2·5 mg./2 days