

REPORTS

ASSAY OF THERAPEUTIC DOSES OF METHOTREXATE IN BODY FLUIDS OF PATIENTS WITH PSORIASIS

W. C. NOBLE, PH.D., M.R.C.PATH., PAMELA M. WHITE, A.I.M.L.T., AND H. BAKER, M.D., F.R.C.P.

Department of Bacteriology, St. John's Hospital for Diseases of the Skin, London, and the Department of Dermatology, The London Hospital, Whitechapel, England

A microbiologic technique for the assay of methotrexate (MTX) in urine, serum, erythrocytes, feces, and skin is described. The accuracy of the method equals that of routine microbiologic assays of folic acid. Important differences in serum MTX levels in psoriatic patients during the 24 hr after standardized intravenous and intramuscular administration were demonstrated. Repeated intravenous doses tended to be cleared from the blood uniformly.

After oral doses many patients achieved peak serum levels within 2 hr, with fall of level by 4 hr. Others achieved lower levels and responded less well clinically. Persistence of high serum levels at 24 and 48 hr did not confer obvious clinical benefit or necessarily give rise to toxicity. The pattern of clearance from serum was not influenced by serum albumin levels or by renal function when the creatinine clearance was greater than 50 ml/min. However, impaired renal function was clearly correlated with slow clearance.

Routine measurement of MTX blood levels is of value in patients with suspected malabsorption or partial renal failure.

Methotrexate (MTX) remains unchallenged as the most effective systemic drug available for the management of certain categories of severe psoriasis unresponsive to topical therapy [1,2]. However, dosage schedules remain empirical, based on the clinical response and clinical or laboratory evidence of toxicity.

Patients might be expected to metabolize a drug in different ways, leading to varying blood, urine, and tissue levels. The safety margin between effective and toxic doses is often small and there is no routinely available method for detecting accumulation of the drug in the tissues. A study of the absorption and excretion of MTX might enable us to understand some of these variations. With this in mind, we have investigated the fate of MTX prescribed to psoriatic patients.

There are several methods available for estimating MTX levels, including fluorometric analysis [3], inhibition of folic acid reductase [4], and use of tritium-labeled MTX [5]. However, for routine estimation (on potentially large numbers of patients) the microbiologic method of Burchenal and

his colleagues [6] seemed to be the most satisfactory, and the assay method was adapted from their work.

MATERIALS AND METHODS

Assay Procedure

Difco folic acid assay (FAA) medium was made up as follows:

FAA	37.5 gm/liter
Oxoid agar no. 3	12 gm/liter
Folic acid (Analar)	10 ng/liter

The medium was autoclaved at 15 lb (121°C) for 15 min and allowed to cool at 42°C.

Cultures of a folic acid-dependent organism were added to the cooled medium and "pour" plates were made on the assay day in 9-cm Petri dishes at 20 ml/plate. Cultures of *Streptococcus faecalis* NCIB 6459, NCIB 8123, NCIB 8966, and *Lactobacillus casei* var. *rhamnosus* NCIB 6395 and NCIB 8010 were maintained lyophilized or, for short periods, in maintenance medium. Subcultures were made on blood agar to check purity, and nutrient broth cultures were made from the blood agar. Overnight broth cultures were added to the molten FAA agar at a concentration of 1 ml/liter. After the plates had set they were dried for 1 hr at 37°C. A hole 2 mm in diameter was punched in the center of the plate and filled with the fluid under assay.

A diffusion period of about 30 min was allowed (although serum diffused very little in this time). Plates were then incubated aerobically—streptococcus plates for 18 hr and lactobacillus plates for 36 hr, at 37°C. The diameter of the zone of inhibition surrounding the holes

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Reprint requests to: Dr. W. C. Noble, Department of Bacteriology, St. John's Hospital for Diseases of the Skin, Homerton Grove, London, E9 6BX, England.

was measured to the nearest millimeter. All cultures were found to give good assay, and zone diameters were in proportion to known amounts of MTX. However, lactobacillus cultures gave larger zones for given quantity of drug, making measurement easier, and all assays reported here were made with NCIB 8010.

Preparation of Specimens

Blood. The time of venepuncture was noted. The cells were removed by centrifugation to leave serum which was normally assayed unautoclaved. (Autoclaving had no significant effect; 13 specimens were assayed in parallel, mean zone diameter unautoclaved 39.6 mm, autoclaved 38.6 mm, SD = 1.8 mm.) Red cells were washed three times with saline and lysed in distilled water made to the same volume as the original blood specimen. This material is referred to as "red cell lysate."

Urine. The time of voiding and the total volume were noted. A 25-ml sample of urine was autoclaved and stored at -20°C prior to assay. Autoclaving proved necessary to prevent urinary-tract contaminants from overgrowing the assay plates. (There was no apparent effect on the level of MTX assayed; 74 specimens were assayed in parallel, mean zone diameter 43.3 mm unautoclaved, 43.7 autoclaved, SD = 1.9 mm.)

Tissue. Pieces of tissue, squamous epithelial debris, or liver biopsies, were examined. (Liver biopsies were taken for histologic examination for evidence of hepatotoxicity. Material examined for MTX was that in excess of histologic requirement and was received prior to fixation.) In skin it was found that autoclaving at 121°C in phosphate buffer, pH 8.4, gave the greatest recovery. Liver biopsies were too small to allow a range of conditions to be tried; they were therefore extracted under the optimum conditions found for skin. All specimens were stored at -20°C prior to assay.

Homogenized fecal samples of approximately 100 gm were dialyzed against water for 24 hr and the dialysate concentrated to approximately 5 ml.

Clinical Material

Many of the 82 patients contributing to this study were inpatients at The London Hospital or at St. John's Hospital for Diseases of the Skin. Others were outpatients attending Psoriasis Clinics run by one of us (H.B.) at the two hospitals. Before MTX was given, the following tests were completed in addition to an ordinary clinical assessment: full blood count; urinalysis, including blood urea and creatinine clearance tests; liver function tests, including bromsulphthalein (BSP) retention and liver biopsy in many patients; and chest radiograph.

Standard MTX doses of 0.22 mg/kilo were generally used when blood sampling was intended. Small test doses were given to patients with impaired renal function.

RESULTS

Standardization of Assay

To construct the initial correlation curve, weighed amounts of MTX were added to distilled water, suitable dilutions prepared, and the inhibition-zone diameter measured. Separate curves obtained using pure MTX (from Lederle Ltd.) and commercial vials containing 5 mg or 50 mg of MTX and varying amounts of parabens and sodium chloride did not differ significantly. In the final analysis a composite curve, derived from the pure

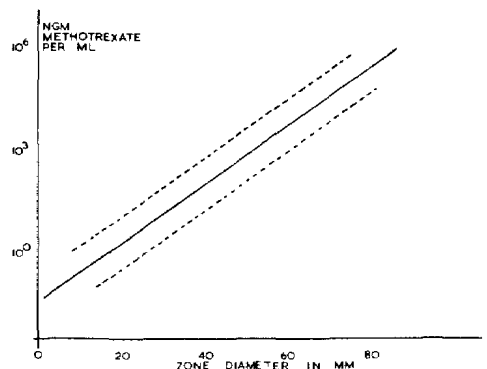


Fig. 1. Correlation curve with 95% confidence limits between inhibition-zone diameter and quantity of methotrexate.

MTX and 50-mg commercial vials, was used and checked for validity against data from 5-mg vials. The final correlation curve is shown in Figure 1; it is based on 328 observations (correlation coefficient, 0.99; residual SD, 2.5 mm; mean-zone diameter, 50.9 mm). The standard deviation is 5% of the mean reading, which is comparable with the results for assay of folic acid [7].

Validity of Assay

Attempts to validate the assay were important in view of the results obtained with specimens from patients. Since assay of 5-mg commercial vials dissolved in distilled water, human serum, or normal urine gave results exceptionally close to those of the standard curve, the assay was judged to be valid for laboratory specimens. Clinical specimens assayed in duplicate on the same day gave results which were very close with a small standard deviation (68 pairs, mean (a) = 35.5 mm, (b) = 35.8 mm, SD = 1.9 mm). Assays repeated 1 week later were also close, although, as expected, the standard deviation was greater (24 pairs, mean week 1 = 40.8 mm, mean week 2 = 40.5 mm, SD = 3.7 mm).

Stability of Drug in Solution

Solutions in distilled water showed no loss of potency over a period of 1 month when stored in the dark at 4° , 25° , and 37°C . Specimens stored in the light at 25° and 37°C lost 50% of potency in 1 month.

Influence of Natural Folate Levels

Neither normal human urine (12 individuals) nor normal serum (37 individuals) contained any substance which inhibited the growth of *L. casei*. There remained the possibility, however, that the normal folate levels of the specimen might interfere in the test. Burchenal et al [6] used higher

loses of folic acid in their medium without invalidating the assay. This was confirmed in the present series by raising the quantity of folic acid added to the FAA medium by 10-fold. Two alternative methods were used to further clarify this point. Samples of distilled water to which both MTX and folic acid had been added in a checkerboard pattern were assayed; a ratio of folic acid to MTX of 500:1 was required to eliminate the zone of inhibition in the assay. In addition, high levels of serum folate were induced in humans by the introduction of excess folic acid or folinic acid by the oral or intramuscular route. (Serum levels achieved by the oral route were 6.3 to 35.9 ng/ml [mean 24.2 ng/ml] and 162 to 560 ng/ml [mean 432 ng/ml] by the intramuscular route in 15 non-psoriatic patients.) None of these levels interfered with the assay of MTX added *in vitro*.

Protein Binding

Although the degree of protein binding was not directly investigated, it appears likely that bound material was released during the assay. Autoclaving of serum from patients with therapeutic MTX levels failed to reveal any increase in level (see above). About 50% of serum folic acid is bound and can be released by autoclaving [7].

Red cell lysates (RCL) were found to contain MTX in the absence of detectable serum levels. This MTX was presumably in a bound or intracellular form prior to assay. No binding could be demonstrated when MTX was added to human serum, horse serum, human urine, or distilled water.

Assay of Clinical Material

Over 9000 assays on clinical material were performed. It was possible to detect MTX in concentrations as low as 0.5 ng/ml, but below 4 ng/ml the assay was quantitatively less reliable. The peak level measurable was 23,000 ng/ml, corresponding to a 70-mm inhibition zone. Above this level nonstoichiometric results were obtained and specimens were diluted 1:10 for assay.

Urine levels. Recovery of MTX from the urine showed that about 90% of the drug excreted by this route appeared in the first 24 hr, although most patients still had detectable levels in the urine 2 to 3 weeks after a dose. Assuming a normal urinary concentration factor, the blood levels required to produce these low urine levels would be below the limit of detection.

Using the product of total urine volume voided and the level of drug assayed, it was possible to compute the total urinary excretion. This was considerably less than the dose administered. Individual patients were fairly consistent in the proportion of drug excreted by the urinary route after repeated doses.

Feces. Excretion via the bile was investigated by sampling feces from patients given intramuscular MTX. MTX was detected in 6 of 7 samples from 3

individuals in amounts varying from 25 ng/gm to 1000 ng/gm. This method was not practicable for routine assay but suggests a route via which some or much drug may have been excreted.

Tissue. Samples of skin obtained 3 or 4 days after a routine weekly dose of MTX were assayed from 5 individuals. Epidermis from psoriatic plaques of 1 patient and normal epidermis from another failed to yield an inhibitory zone. Both normal and psoriatic epidermis from the others gave levels between 200 and 400 ng/mg.

Liver biopsies were obtained shortly before a standard weekly dose of MTX from 12 patients; all were negative for MTX. One further specimen which proved positive was taken within 3 hr after administration of the drug and probably reflects the blood level.

Assay of Therapeutic Material

During the course of the investigation it became desirable to determine whether there was any gross variation between different batches of tablets supplied for therapeutic use. Accordingly, 8 tablets with different batch numbers were dissolved in distilled water and assayed. At about 250 ng/ml the tablets assayed gave zone diameters between 43 and 51 mm, mean 47.6 mm. The standard curve read 47.5 mm at this point with 95% confidence limits of 42.5 and 52.5 mm.

Clearance of Single Intravenous Doses

The initial level was usually measured by sampling venous blood from the arm opposite to that used for the intravenous injection, 90 sec after administration of the drug. Further samples were collected at intervals. With 0.22 mg/kilo doses, serum levels up to 10,000 ng/ml were present after 90 sec. Falloff was apparent within 10 min and progressive over 24 hr; a typical pattern is shown in Figure 2. Up to 15-fold differences were observed between levels found in different patients at 1 and 6 hr and up to 30-fold differences were seen at 24 hr (Fig. 3). These variations could not be correlated with serum albumin levels. There was no correlation between red-cell uptake of MTX and rapidity of clearance of the drug from the serum.

In 3 patients given 0.44 mg/kilo of MTX, serum levels were no higher than with the smaller dose at 6 hr and thereafter. This suggests that the initial high levels seen for up to 1 hr were cleared very rapidly, presumably into the tissues and excreted by the kidneys and liver. Such single doses only rarely seriously disturbed the patient. One woman (twice given 0.22 mg/kilo) developed hallucinations which ceased promptly when MTX was withdrawn. There was nothing atypical about her serum levels after each dose. Several patients complained of variable nausea within 12 hr of an intravenous dose but there was no correlation between presence of nausea and blood levels of the drug. One 73-year-old woman developed inflammation, "burning" skin, pain, and erosion of psoriasis.

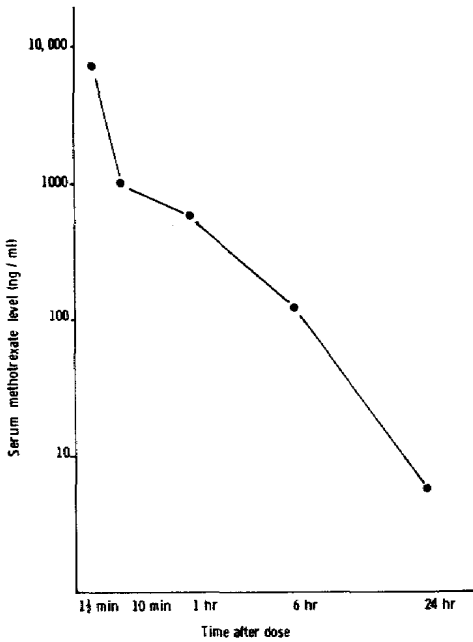


FIG. 2. Serum methotrexate levels after an intravenous dose.

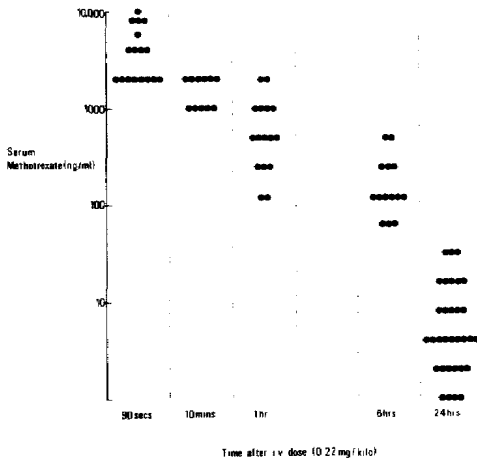


FIG. 3. Serum methotrexate levels after single intravenous doses (32 patients).

riatic plaques 36 to 48 hr after a single intravenous dose of 0.5 mg/kilo. These findings were associated with high persisting MTX levels. Serum MTX was 500 ng/ml after 24 hr, and 16 ng/ml were still detectable in the serum after 6 days. Single doses higher than 0.44 mg/kilo have not subsequently been used. Repeated measurements were made after identical doses in some of these patients at weekly or greater intervals. Figures 4 and 5 show

that such repeated doses were cleared rather uniformly with only minor variations in the measured serum levels. This suggests that the interpatient variation is real.

Clearance of Single Intramuscular Doses

Single intramuscular doses of MTX were cleared from the serum more slowly than intravenous doses; the findings are summarized in Figure 6 which shows the considerable variations in levels for up to 24 hr after standard doses. These patients include 3 in whom use of maintenance MTX was unavoidable, although renal function was known not be normal.

Initial levels are lower but high levels are often maintained for several hours longer, perhaps because of delayed release from the infection site.

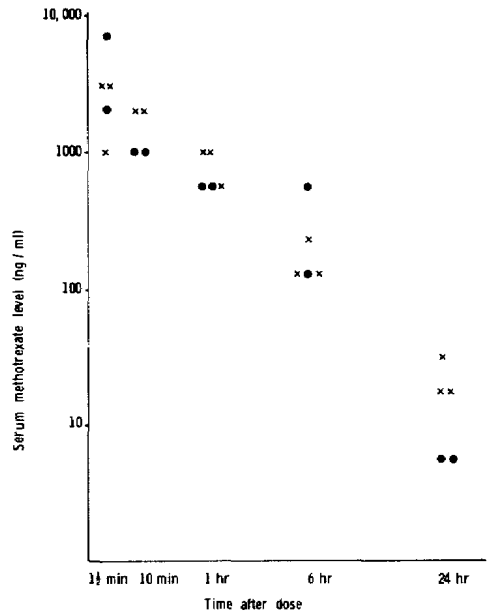


FIG. 4. Consistent handling of intravenous doses of methotrexate. x and ● represent 2 patients.

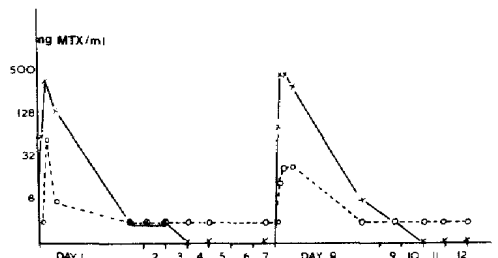


FIG. 5. Consistent handling of two intramuscular doses of methotrexate by 1 patient. The solid line represents the serum level and the broken line the red-cell lysate level.

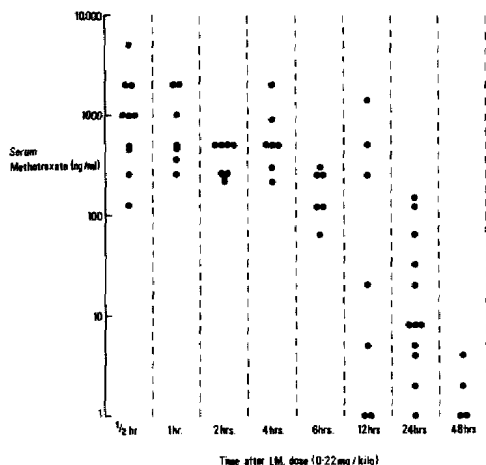


FIG. 6. Serum methotrexate levels after single intramuscular doses (12 patients).

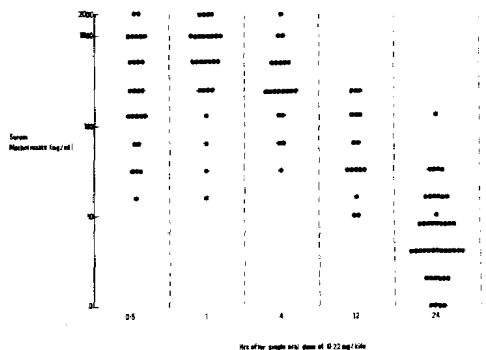


FIG. 7. Serum methotrexate levels after single oral doses (44 patients).

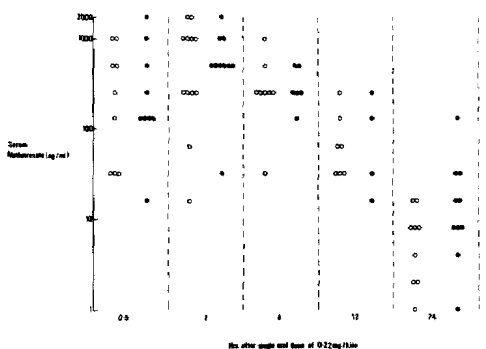


FIG. 8. Comparison of serum methotrexate levels in patients with comparable psoriasis who had a "good" (O) or a "poor" (●) response to the drug.

but persistent, 4-hr levels being comparable with those in patients with high initial levels. Three of these latter patients had persistent high levels at 12 and 24 hr; they showed an excellent clinical response but mild clinical toxicity. All 3 had some impairment of renal function with mild cardiac failure, which might explain the persistent high levels but not the initial low ones. The 2 patients with the highest 12-hr levels both suffered from troublesome headaches for a day after the injection, a symptom found in a minority of patients on intramuscular therapy.

Absorption of Oral Doses

Blood level data were obtained for 44 patients after oral administration of MTX, based on 102 doses of MTX. Most of these patients were exposed to MTX for the first time and none had received the drug in the previous 3 months. Oral absorption as indicated by serum levels was often rapid but, as with clearance of intravenous and intramuscular doses, much variation was seen. Figure 7 shows the great variations in serum levels in the first 24 hr after single oral doses of 0.22 mg/kilo.

An attempt was made to correlate pooled serum level data as shown in Figure 7 with overall clinical response to treatment. Figure 8 shows the serum MTX levels for a number of patients with comparable patterns of psoriasis who were given 0.22 mg/kilo doses. The data are divided according to whether clinical response was "good" or "poor." It can be seen that there are no significant differences between the levels at various intervals after the dose in the two "response groups."

Interpatient Variation (Oral Doses)

Three fairly distinct patterns of blood levels and a mixed group were seen after single oral doses of MTX, and these have been arbitrarily delineated as follows. Types I and II are naturally exclusive but Type III overlapped with both.

Type I—Rapid Absorption Leading to High Levels and Rapid Fall

- Criteria: 125 ng/ml or more at 30 min
- 500 ng/ml or more at 1 hr
- 1000 ng/ml or more at 2 hr
- 500 ng/ml or less at 4 hr

Figure 9 shows three examples of this pattern of response. The details of clinical response and toxicity are shown in Table I. Throughout the period of treatment not one of these patients exhibited serious clinical or laboratory evidence of toxicity. Four patients complained of mild anorexia and nausea, lasting 12 to 36 hr and usually beginning about 18 hr after each dose. One (the patient who failed to respond) suffered abdominal pain. After 0.44 mg/kg doses, 7 patients showed this blood level pattern, including 3 who had responded similarly to 0.22 mg/kilo doses. Of these 7, 3 responded very well and 3 moderately well to treatment. Only 1 patient failed to improve.

Two distinct patterns were seen in these patients. In some, high initial levels fell rapidly to 300 ng/ml or less within 6 hr and to 20 ng/ml or less within 12 hr. In others, initial levels were uniformly lower

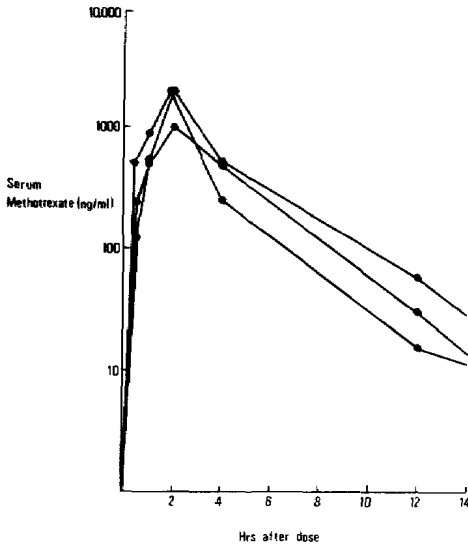


FIG. 9. Type I response to oral methotrexate, showing high peak levels and rapid fall.

TABLE I. Patterns of methotrexate blood levels, clinical response, and toxicity after oral doses of 0.22 mg/kilo

	No. of patients	Response			Toxicity	
		Very good	Moderate	Poor	Serious	Mild
Type I Rapid rise and fall	12	5	6	1	0	4
Type II Low rise and low peak	11	3	3	5	0	6
Type III Delayed fall	9	4	3	2	0	0

Type II—"Slow Rise and Low Peak" Curves

Criteria: 64 ng/ml or less at 30 min
125 ng/ml or less at 1 hr
250 ng/ml or less at 2 hr

Figure 10 shows three examples of this pattern of response. Two of the patients who failed to respond (both with erythrodermic psoriasis) had exceptionally low blood levels and it seems possible that their lack of response was due to low absorption. One patient had previously responded well to parenteral therapy, supporting this contention. At first it was suspected that he had not in fact taken the MTX tablets, but repeated administration (when the tablets were swallowed under supervision) gave the same results.

Type III—Delayed Fall in Blood Levels

Criteria: 100 ng/ml or more at 12 hr
16 ng/ml or more at 24 hr
8 ng/ml or more at 48 hr

After 0.22 mg/kilo doses, 9 patients showed delayed fall, including 4 who had high initial peaks and 4 with low peak curves (Table I). After 0.44 mg/kilo doses, 12- to 48-hr levels were higher and 5 of 12 subjects given doses of this order showed such levels; 3 of these latter patients had a good response and the other 2 a moderate response.

Mixed patterns. Nineteen patients did not fit any of the above categories and showed various mixed patterns of response.

Comparison of Oral Absorption Tests Before and After Prolonged Therapy

Five patients with a Type I absorption pattern, whose psoriasis was well controlled by MTX, had oral absorption studies repeated after 6 to 18 months of continuous treatment with the drug. No alteration in their absorption pattern was seen. One other patient, originally studied in a phase of widespread discoid psoriasis, showed a flatter absorption curve during an acute phase of erythrodermic psoriasis when her psoriasis was out of control at a later date (after a period without MTX). This may have been a reflection of an acute "dermatogenic enteropathy."

Relationship of Serum MTX and Albumin Levels

Sixteen patients had serum albumin levels greater than 4.2 gm% at the time of MTX serum level studies. Ten responded very well, and 4 moderately well to treatment. Only 2 of these patients were receiving salicylate or sulfonamides at the same time, which might have competed for albumin-binding sites. Four patients, all with

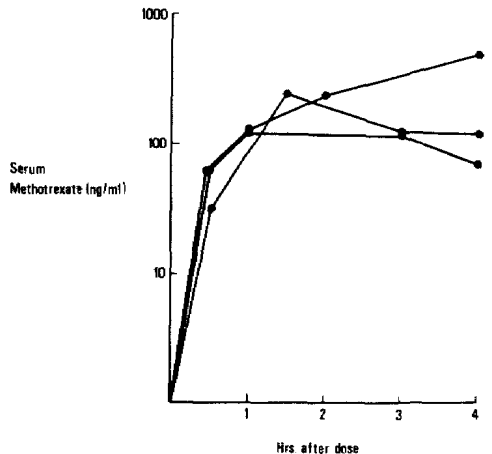


FIG. 10. Type II response to oral methotrexate, showing slow rise and low peak.

erythrodermic or generalized pustular disease, had serum albumin levels of 3.0 gm% or less at the time of study. All 3 responded excellently to repeated doses of 0.22 mg/kilo, orally or parenterally, and had Type I serum level patterns.

Relationship of Serum MTX Levels to Renal Function

In patients with a renal creatinine clearance greater than 50 ml/min no correlation was observed between serum MTX levels (peak or duration) and the creatinine clearance. In 4 patients with poor renal function, slow excretion of the drug was clearly related to low creatinine clearance and dosage was adjusted accordingly. One 83-year-old man with intractable and disabling, widespread discoid psoriasis, whose creatinine clearance was 23 ml/min, was kept well controlled for over a year by 2.5 mg MTX by mouth weekly, after a test dose had suggested delayed excretion. Six- and twelve-hour serum MTX measurements after a small intravenous test dose provide a useful guide to subsequent therapy in patients with kidney disease whose psoriasis cannot be managed by topical measures. We have detected no evidence that such treatment itself causes further renal damage.

Relationship of Red-Cell Levels of MTX and Clinical Response and Toxicity

The assay system demonstrated that MTX is taken up by red cells, peak levels reflecting peak serum levels. After 0.22 mg/kilo doses, many patients achieved peak red-cell levels of 32 ng/ml RCL or greater. In most of these subjects the level had fallen to zero after 24 hr. However, a number of patients failed to show peak levels greater than 4 ng/ml RCL. Contrasting groups of patients with peak erythrocyte levels greater than 32 and less than 4 have been compared in respect to clinical response of psoriasis and toxicity (Tab. II). Of 15 patients with the high peak levels, 10 responded well and only 1 failed to improve significantly. Of 10 patients with peak levels of 4 or less, 4 patients were completely resistant to therapy which was later abandoned (see Tab. II). Five patients had persistent erythrocyte levels of 4 ng/ml RCL or more 24 hr after single doses of 0.22 mg/kilo; all responded very well clinically. Four of the 5 are on successful long-term maintenance treatment. No

relationship was apparent between erythrocyte MTX levels and incidence or type of toxicity.

DISCUSSION

Laboratory Studies

A variety of results was obtained from the urinary excretion studies, some patients apparently excreting much and others little of the drug by this route. These results may be compared with those of Johns et al [8] who, in 4 subjects, found the excretion of tritium-labeled MTX to range from 38 to 57% of the intravenously administered dose in the first 48 hr. After 48 hr the substances excreted by this route were metabolites rather than unchanged MTX. Johns and his colleagues found that further labeled material could be flushed from the tissues by compounds which competed for the enzyme dihydrofolate reductase; clearly some tissue binding occurs. The extent of this binding is difficult to assess for Johns and his co-workers took no account of excretion via the bile, although acknowledging that such excretion may occur. We have demonstrated biliary excretion in patients receiving parenteral doses. Henderson et al [5] found that, in 10 cancer patients, from 54 to 88% of a small intravenous or oral dose of MTX was excreted via the urine within the first 24 hr, and that from 4 to 24% appeared in subsequent urines or in the stool. Some of the patients in the present series of experiments excreted significantly less even than the 38% reported by Johns et al, but it is important to note that the microbiologic method measures an active folic acid antagonist. It may be speculated that some MTX is inactivated in the body and thus not detected in this assay. Very high recovery rates from some patients, however, suggest that this phenomenon may be expressed only in some individuals. No other studies on patients without cancer appear to be available.

Henderson et al [5] reported that about 50% of MTX is bound to serum proteins. It was not possible to demonstrate such binding in the present study. In vitro addition of MTX to distilled water, normal urine, and horse serum revealed no differences in fluid levels of MTX on assay. Autoclaving had no significant effect on the levels assayed in serum from patients treated with the drug (although this releases folic acid [7]).

Clinical Studies

Our studies established that variations in activity of the different commercial batches of injectable and tablet forms of MTX available to us were small and could not contribute significantly to the variations in clinical response and toxicity observed.

Considerable variation has been found in the measured blood levels after standard oral and parenteral MTX administration. These presumably reflect variations in the balance between rate and extent of absorption from the gut, rates of excretion by the kidneys and the liver, and uptake

TABLE II. Relationships of peak red-cell lysate methotrexate levels to clinical response and toxicity

Red-cell lysate (ng/ml)	No. of patients	Very good response	Moderate response	No response	Toxicity
High peak levels (≥ 32)	15	10	4	1	5
Low peak levels (≤ 4)	10	5	1	4	7
High persistent levels (≥ 4 at 24 hr)	5	5	0	0	1

and release of the drug by the tissues. Hepatic excretion is itself complicated by the existence of an enterohepatic cycling of MTX. The fact that we have observed just as much variation in blood levels after parenteral administration suggests that variation in absorption is the least important of these factors. This is consistent with the observation of Halprin et al [9] in a small number of patients that there was a direct relationship between the amount of MTX administered orally and the blood levels achieved 2 hr later. Nevertheless, there may be occasional patients, especially those with psoriatic erythroderma, who do not absorb MTX normally, although we cannot prove that their low blood levels were not due to avid and exceptional uptake by the dihydrofolate reductase of the erythrodermic skin.

Our assay is thought to measure total free and protein-bound MTX in the blood, but it is a matter of conjecture how these levels are related to the intracellular levels in skin and other organs, on which therapeutic benefit and toxicity must depend. Our inability to correlate our assay data with clinical response or toxicity (see Fig. 8) confirms that serum levels are a crude indicator of drug metabolism and of little value except in gross situations such as renal disease. However, the data in Table II suggest that red-cell level measurement may be a better parameter.

Much work in the last two decades has led to the realization that the distribution of MTX in the body depends on multiple and complex factors. Renal function must be of prime importance in view of the evidence that much of an administered dose is eventually excreted by this organ [5]. Hepatic function is concerned at the levels of the extent of biliary secretion, enterohepatic cycling, and cellular function. At the terminal level, the cellular reproductive activity of the skin, the extent and type of the psoriasis, the skin's blood supply, and the cellular levels of dihydrofolate reductase must be important—similar considerations applying to other organs where toxicity may prejudice continued therapy. Tissue levels of folates, particularly in the liver, may be important; this aspect is complicated by the recent observation that MTX increased the renal excretion of folate [10].

Routine control of MTX therapy in psoriasis by measurement of blood levels of the drug may not

be worthwhile. The microbiologic assay is too tedious and time consuming although accurate and reproducible. Although results can be available within 36 hr, clinical manifestations of toxicity may appear earlier. Blood levels can be valuable, however, in three clinical situations:

1. Where severe psoriasis is associated with severe renal disease. Blood levels following a very small intravenous test dose provide a guide to the appropriate dosage. Our limited experience provides no evidence that small maintenance doses further aggravated renal function.
2. Where psoriasis is apparently refractory to oral dosage and parenteral therapy is difficult or impossible. Blood studies distinguish between failure of absorption and refractoriness for other (unknown) reasons.
3. To confirm that the tablets are being taken as prescribed.

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