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The Metabolic Fate of CIBA 32644-Ba

J.W. FAIGLE * and H. KEBERLE *

Introduction

Investigation of the metabolic fate of drugs is today a necessary complement to conventional biological and clinical tests. This is particularly true in the case of preparations with a new type of activity because in such instances no conclusions can be drawn from the behaviour of known, analogous compounds.

Preparation CIBA 32644-Ba undoubtedly represents a new type of compound as regards its schistosomicidal effect (1, 2). It was therefore essential to submit the fate of this compound in the organism to detailed study in the hope of finding answers to the following two questions in particular:

1. In what way does the compound exert its selective attack on the schistosome?
2. What dosage schedule is likely to produce an optimal schistosomicidal effect when the compound is used therapeutically in man?

The investigation of these questions calls for the accurate determination of many different metabolic parameters of the compound, such as absorption, blood levels, distribution, biological degradation, and excretion. The most reliable way of obtaining this information is to use a radioactively labelled preparation.

Chemically, CIBA 32644-Ba is 1-(5-nitro-2-thiazolyl)-2-imidazolidinone. It was labelled by introducing C^{14} into the 4 position on the imidazolidinone ring, a position which in all probability cannot be split off from the molecule during biological degradation.

Fig. 1 shows the method of synthesis employed. This labelled CIBA 32644-Ba obtained as the end product was radiochemically pure and displayed a specific radioactivity of 3.5 μc/mg. For certain experiments, the preparation was diluted to yield lower levels of radioactivity and due attention was paid to safety regulations in the case of tests in man.

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The fate of 32644-Ba in the organism

Initial information about the fate of CIBA 32644-Ba in the organism was obtained with the aid of animal experiments. Since the compound is invariably administered by mouth for therapeutic

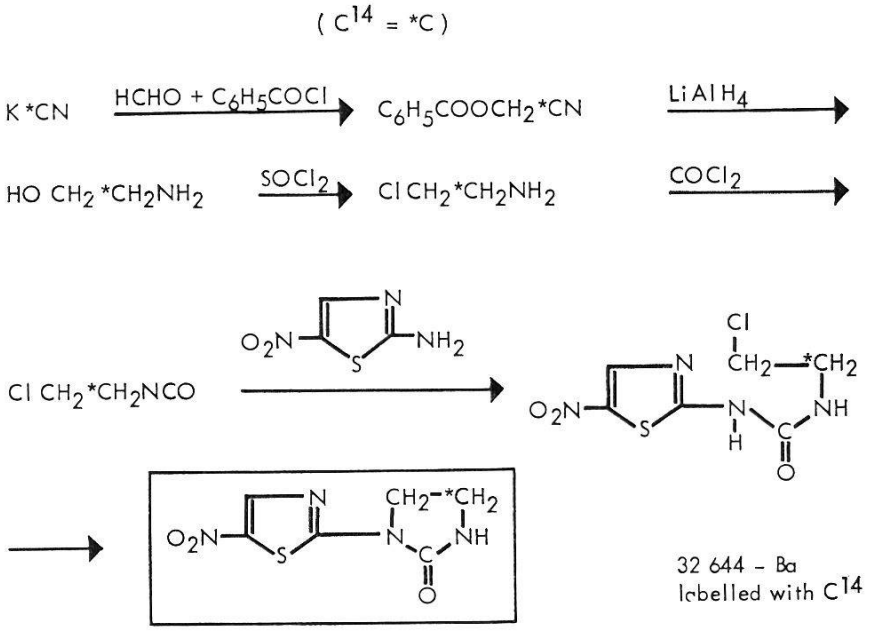

Fig. 1. Labelling of 32644-Ba with radioactive carbon.

purposes, attention was focussed first of all on the extent of its absorption from the gastro-intestinal tract.

In the rat, it was found that following a single oral dose of 200 mg/kg of the labelled compound the radioactivity was excreted quantitatively and in approximately equal proportions in the urine and faeces over a period of several days. Since the proportion contained in the urine can only have consisted of absorbed material, it must be assumed that at least 50% of the dose was absorbed from the intestinal tract.

Since the radioactivity in the urine and faeces is present in the form of transformation products, CIBA 32644-Ba must be virtually completely metabolised in the organism. The fact that the compound is excreted in the faeces in the form of metabolites leaves open the possibility that this material, too, has been absorbed and eliminated via the bile. Consequently, the amount of the dose actually absorbed may possibly far exceed the 50% determined in the urine.

The radioactivity is remarkably evenly distributed in the rat organism and no particularly marked accumulations are found in anyone site.

TABLE 1

*Concentrations * of 32644-Ba and/or its metabolites in various organs of rats following a single oral dose (200 mg/kg)*

Time after administration	Peripheral blood	Liver	Lungs	Heart	Kidney	Testicle	Spleen	Brain
3 hrs	9.7	12.4	7.0	5.6	28.5	5.8	8.2	3.4
6 hrs	28	44	20	23	105	28	26	26

* Expressed in μg 32644-Ba per ml of blood or per g of wet organ tissue.

This is shown by the organ concentrations listed in Table 1, which were determined 3 and 6 hours after administration of the dose already mentioned. The relatively high value for the kidneys is due to the renal excretion of metabolites which was at its height at the times the assays were performed.

The absorption of CIBA 32644-Ba in man follows a pattern similar to that observed in the rat, as can be deduced from the curve in Fig. 2 which is representative of the excretion of radioactive substances in the urine following administration of a dose of 25 mg/kg of the labelled compound.

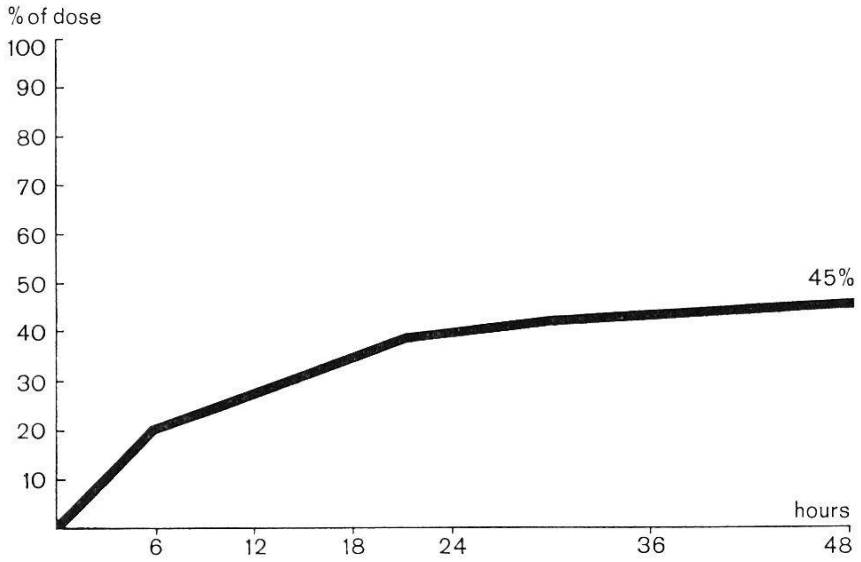

Fig. 2. Excretion of radioactivity in the urine following a single oral dose of C^{14}-labelled 32644-Ba (25 mg/kg) in man.

The excretion takes place chiefly on the first day; it then declines, but is still not completed after 48 hours. The pattern of the curve shows that altogether about half the dose is eliminated by the kidneys—that is to say, here once again the minimum absorption rate is approximately 50%.

In man, too, the radioactivity in the urine and faeces is present almost exclusively in the form of metabolites of CIBA 32644-Ba.

The results of the experiments described above suggest therefore that the compound is well absorbed, that it is quantitatively metabolised, and, finally, that its metabolites are excreted completely, though relatively slowly.

In the human test in which the processes of absorption and excretion were studied, the chronological pattern of the blood concentration was also ascertained. The total radioactivity in blood samples was measured first, thus giving the sum of the concentrations of both CIBA 32644-Ba and any of its metabolites that might be present. Then, with the aid of the isotope dilution technique, the concentration of unchanged CIBA 32644-Ba was determined in each blood sample. The results are plotted in the form of blood-level curves in Fig. 3.

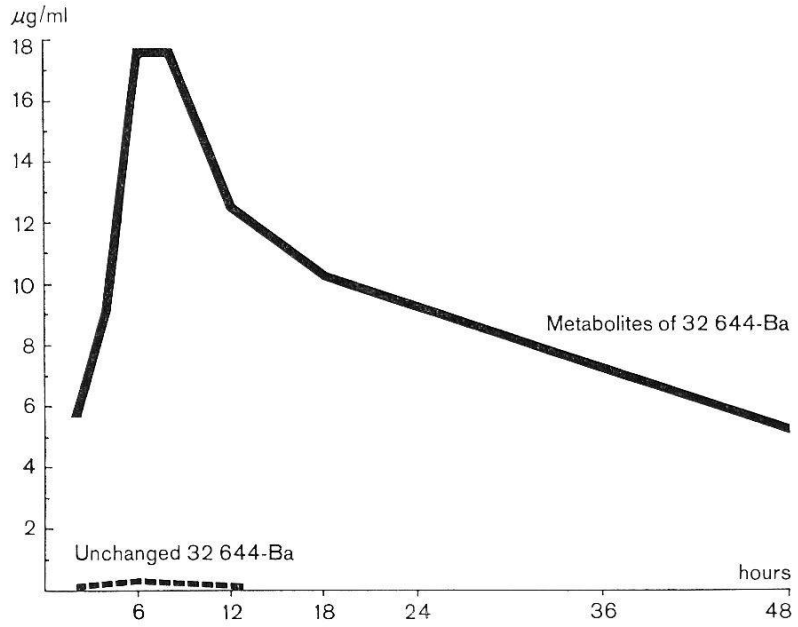

Fig. 3. Blood levels of 32644-Ba and its metabolites in man following a single dose (25 mg/kg).

It is surprising to see that in the blood, too, the radioactivity is due predominantly to metabolites and only to a small extent to the unchanged compound. The blood concentrations of the metabolites are relatively high, attaining a maximum of 18 µg/ml. The half-life of the metabolites is about 40 hours. The levels of unchanged CIBA 32644-Ba, on the other hand, are low from the very outset, attain a maximum of only 0.3 µg/ml (i.e. only about 2% of the corresponding metabolite concentration), and are no longer demonstrable after about 15 hours.

These blood-level patterns do not fit in with the usual picture of the degradation of a drug. As a rule, the body endeavours to convert a foreign substance into metabolites which can be easily

excreted. Although CIBA 32644-Ba, too, must be rapidly broken down, the resulting metabolites are nevertheless eliminated only slowly from the blood.

Paper electrophoresis of blood plasma from a patient treated with labelled CIBA 32644-Ba provided an explanation for the slow elimination of the metabolites. Fig. 4 shows the electrophoretic separation of the plasma proteins, together with the distribution of the radioactivity on the electropherogram.

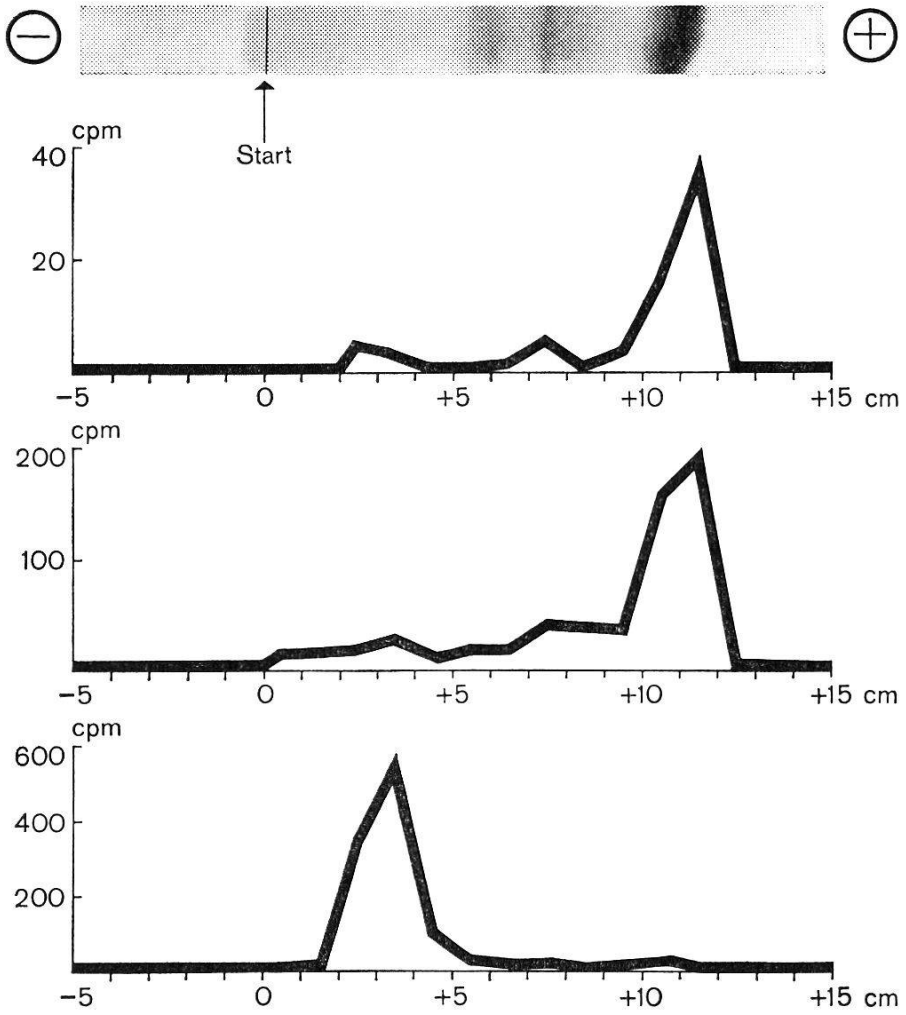

Fig. 4. Radiometric analysis of paper electropherograms of plasma containing metabolites of C^{14}-labelled 32644-Ba (15 hrs pH 8.6, 200 V).

This figure indicates that the maximum radioactivity coincides with the albumin fraction, from which it may be concluded that the metabolites of CIBA 32644-Ba are bound to albumin. Separate extraction tests performed on lyophilised metabolite-containing plasma revealed in addition that the albumin bond is stronger than is normally the case with drug metabolites. This would account for the delayed excretion of the degradation products of CIBA 32644-Ba.

It can also be seen from Fig. 4 that the metabolites behave similarly in rabbit plasma, which suggests that the fate of CIBA 32644-Ba is basically the same in both humans and rabbits. Finally, the results of a control experiment, which are included in this same figure, show that unchanged CIBA 32644-Ba dissolved in the plasma of an untreated rabbit displays an entirely different distribution of radioactivity. This means that only the metabolites and not the unchanged compound become firmly bound to albumin.

The low blood level of unchanged CIBA 32644-Ba and the high concentration of metabolites in the blood only a few hours after the administration of the drug suggest that CIBA 32644-Ba is rapidly metabolised. As to the question of where the drug is transformed in the organism, there are two possibilities:

1. Either the drug is absorbed slowly, but in an unchanged form, from the intestine and is then carried in the portal blood to the liver where it is almost completely broken down, except for a small portion, during its first passage.
2. Or else it is broken down in the gastro-intestinal tract itself, and the material absorbed consists principally of metabolites —in addition to very small amounts of the initial compound.

It should be possible to decide which of these two possible explanations is correct by comparing the concentration of CIBA 32644-Ba in the portal and peripheral blood of an experimental animal, since only in the event of the compound being broken down in the liver would its concentration be likely to show a sharp decrease after it had passed through this organ. An experiment of this type, performed in the rabbit, is described below:

Two rabbits were given a dose of 25 mg/kg CIBA 32644-Ba and two others a dose of 100 mg/kg. The first animal of each pair was

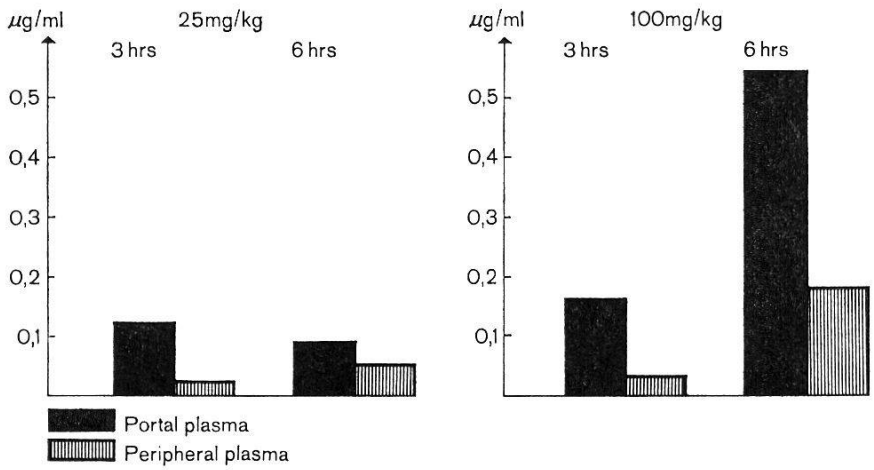

Fig. 5. Concentrations of 32644-Ba in the plasma of portal and peripheral blood following a single oral dose in rabbits.

bled after 3 hours, the other after 6 hours, samples being taken simultaneously of the portal and peripheral blood in every case.

The results illustrated in Fig. 5 clearly show that the concentration of CIBA 32644-Ba was invariably much higher in portal blood plasma than in peripheral blood plasma. The largest difference observed, expressed as the quotient

$$\frac{\text{portal plasma concentration}}{\text{peripheral plasma concentration}}$$

was 6 and the smallest about 2.

For reasons of clarity, the metabolite levels have not been included in this figure. As was to be expected, these levels were considerably higher than those of the unchanged compound, but were approximately equal in both portal and peripheral plasma. This is readily understandable since the metabolites are excreted slowly and thus pass into the portal system again from the greater circulation.

The difference between the concentration of CIBA 32644-Ba in portal blood and in peripheral blood clearly indicates that the first of the two possible answers to the question about the site of the compound's biological degradation is the correct one. In other words, CIBA 32644-Ba is absorbed slowly, but in an unchanged form, and during its very first passage through the liver it is largely broken down into metabolites which are then only slowly eliminated from the blood.

The various plasma samples in this rabbit experiment were submitted not only to radiometric analysis but also to a biological assay for active substances. In this assay *Trichomonas foetus* was used as a test organism. The results showed that the biological activities in the portal and peripheral plasma run roughly parallel to the concentrations of unchanged CIBA 32644-Ba at these two sites and not to the concentrations of metabolites, indicating that the active agent is the unchanged drug.

Interaction between CIBA 32644-Ba or its metabolites and the schistosome

Although the findings described above furnish a comprehensive picture of the fate of CIBA 32644-Ba in the organism of the host, they do not supply any information about the metabolic interaction between the drug and the schistosome.

In the blood of the host organism the parasite is invariably exposed not only to the initial compound but also to the latter's metabolites, and it is impossible to decide *a priori* which substance is responsible for the effect. It is essential, however, that this question should be clarified if a proper assessment of the clinical dosage schedule is to be made. True, the trichomonad test already mentioned does suggest that CIBA 32644-Ba itself is the biologically active compound, but this finding cannot be considered as sufficient proof.

A further contribution to the solution of this problem has been supplied by an autoradiographic observation: following the administration of labelled CIBA 32644-Ba to mice infected with *S. mansoni,* micro-autoradiographs revealed that radioactive substances had accumulated and become fixed in the worms (3). This accumulation is probably correlated with the schistosomicidal effect because the highest radioactivity was found in those areas where the first morphological changes appear (4).

The autoradiographs, however, provide no information about the nature of the fixed substances and we therefore still do not know whether the biological activity lies in the initial compound or in its metabolites. In order to decide between these two possibilities, attempts were then made, with the aid of an incubation technique (2), to study the processes occurring during and after penetration.

In this experiment schistosomes were kept in physiological solutions containing either CIBA 32644-Ba or its metabolites. In the case of CIBA 32644-Ba, the medium was prepared by dissolving

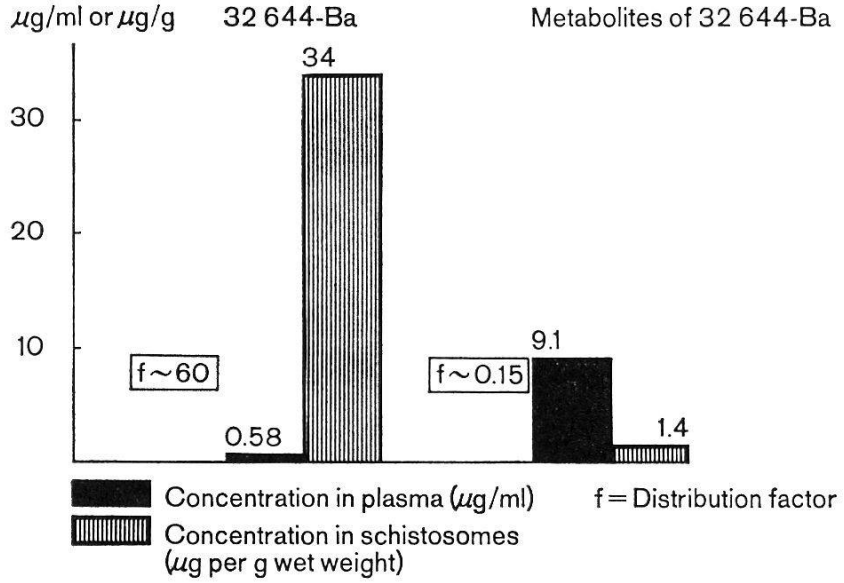

Fig. 6. Comparative incubation of schistosomes *(S. mansoni)* in rabbit plasma containing either C^{14}-labelled 32644-Ba or metabolites thereof (24 hrs, 37°C).

the compound in plasma taken from an untreated rabbit. For the comparative incubation, a so-called metabolite plasma was used, which had been obtained from a rabbit 48 hours after treatment with labelled CIBA 32644-Ba (100 mg/kg orally). Living schistosomes *(S. mansoni)* from untreated mice were added to the two media and left there for 24 hours at 37°C. On completion of the period of incubation the schistosomes, which were in both cases still alive, were washed and their radioactivity determined.

The results are illustrated in Fig. 6, in which the concentrations of radioactive substances in the incubation media (in μg/ml) are compared with those found in the schistosomes [in μg per g worm (moist weight)]. This figure clearly shows that only unchanged CIBA 32644-Ba causes an accumulation of radioactive substances in the worm and that this accumulation is very marked, corresponding to a distribution factor

$$= \frac{\text{concentration in schistosome}}{\text{concentration in plasma}}$$

of about 60. In the case of the metabolite plasma, on the other hand, no accumulation of metabolites occurs in the worm. Even though the plasma level is far higher, the level of radioactivity attained in the worm is much lower than in the case of the initial compound. The distribution factor (0.15) is very small. It appears that the metabolites cannot penetrate into the schistosome at all.

The accumulation observed was confirmed by running a further series of incubations in the presence of various concentrations of CIBA 32644-Ba. The results are illustrated in Fig. 7.

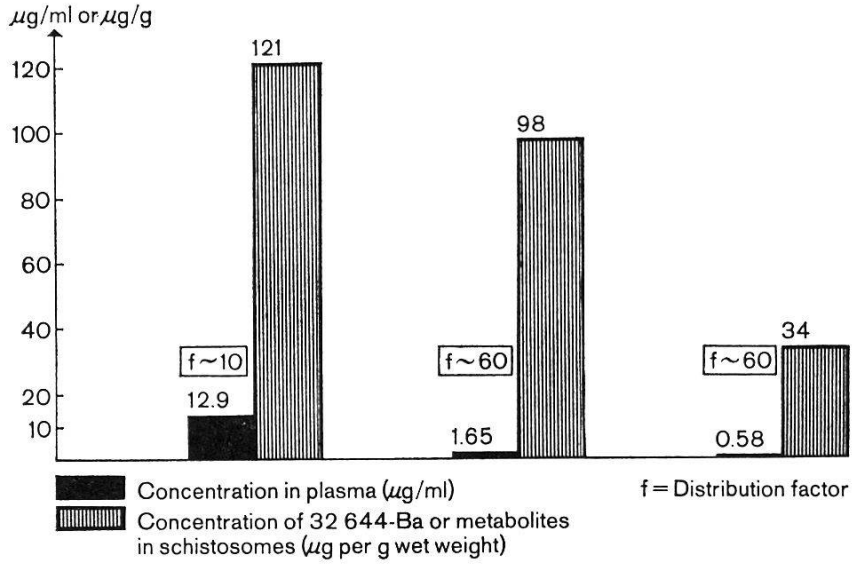

Fig. 7. Accumulation of radioactive material in schistosomes *(S. mansoni)* during incubation in rabbit plasma containing various concentrations of C^{14}-labelled 32644-Ba (24 hrs, 37°C).

The distribution factors rise as the concentrations fall, thus suggesting that the compound is relatively better utilised at low concentrations. The blood levels observed in man lie within the region of optimal utilisation. Consequently, the incubation tests show that only the initial compound is responsible for the accumulation in the worm and thus probably for the schistosomicidal effect as well. It would appear that despite their high concentration in the blood, the metabolites play no part in the action of the drug.

An explanation for the accumulation described above was found by analysing the radioactive substances in the worm. It was observed that the radioactivity in the worm stemmed almost exclusively from metabolites—a finding which can only be accounted for by assuming that the initial compound is rapidly transformed following penetration.

The processes taking place during and after penetration of the schistosome are summarised diagrammatically in Fig. 8.

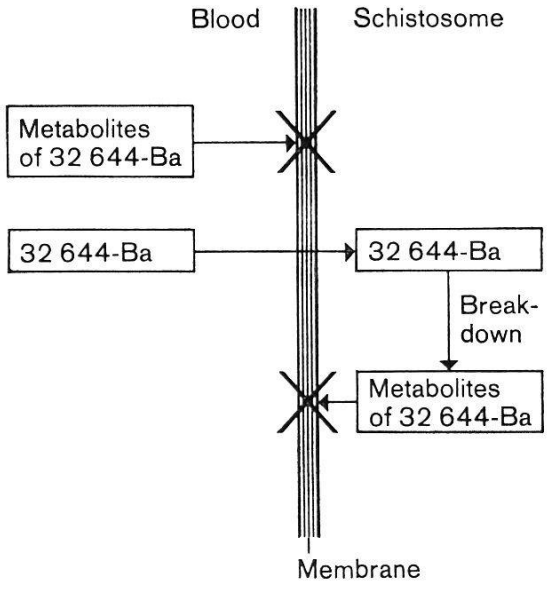

Fig. 8. Diagram showing how radioactive material accumulates in the schistosomes.

Of the two different radioactive substances present in the blood of the host, only the initial compound can penetrate into the parasite; it may be that CIBA 32644-Ba has a high degree of affinity for the worm and that this facilitates penetration. Inside the parasite the initial compound is broken down into metabolites which, for their part, can get out of the parasite only slowly, if at

all. The initial compound continues to enter the parasite from the blood and is gradually metabolised in the worm. The cumulative effect thus becomes readily understandable. The immediate cause of the actual damage to the schistosome may possibly result from an overloading of the enzyme system responsible for degradation of the compound. It is of course also possible that the schistosomicidal effect is in fact exerted by a metabolite formed after CIBA 32644-Ba has penetrated the membrane.

The chemical structure of the metabolites formed in the host and in the schistosome has not been definitely elucidated. It is, however, known that the degradation of CIBA 32644-Ba is initiated by a reduction of the nitro group in the molecule. This principle of breakdown by reduction also applies to chemically related therapeutic agents of the nitrofurane series (5).

These experimental findings on the fate of CIBA 32644-Ba in the organism of the host and of the schistosome make it possible to answer the first of the questions posed at the beginning of this paper—namely, in what way does the compound exert its selective attack on the schistosome?

For the sake of clarity, we would like once again briefly to summarise the processes occurring during the absorption and degradation of CIBA 32644-Ba.

Following oral administration the compound is absorbed from the intestinal tract over a period of several hours. It passes with the portal blood into the liver, where it is largely transformed into metabolites during its very first passage. Owing to slow absorption on the one hand and to rapid breakdown on the other, a uniformly low, but therapeutically active level of CIBA 32644-Ba is maintained in the peripheral blood. This automatic regulation of the peripheral blood levels may explain why the compound is well tolerated in man.

On the other hand the metabolites resulting from the breakdown of CIBA 32644-Ba attain high blood concentrations because they are excreted slowly, but they apparently exert no effect on the host and cannot penetrate into the parasite.

The biological activity of CIBA 32644-Ba is due principally to the continuous influx of the compound into the worm over a prolonged period of time. The fact that the compound is more highly concentrated in portal blood than in peripheral blood is fortunate because the various species of schistosome live mainly or exclusively in the vascular bed supplied by portal blood. The compound is therefore selective in two ways—firstly, with regard to the schistosome itself, and secondly, with regard to the regions of the host organism in which the schistosomes are chiefly localised.

The dosage schedule for the clinical use of CIBA 32644-Ba

The data obtained on the fate of CIBA 32644-Ba in the human and schistosomal organism also make it possible to answer the second of the questions posed at the beginning of this paper—that is to say, the question of the dosage schedule.

The purpose of a dosage schedule is to keep the blood concentration of active substance at a therapeutic and, if possible, uniform level throughout the period of treatment. The dosage schedule for CIBA 32644-Ba which has been tested with success in clinical trials provides for a treatment period of 5-10 days with daily doses of 15-25 mg/kg, the daily amount being given in two fractional doses with an interval of 12 hours. The question to be examined is whether this schedule meets the demand for uniform blood levels of active substance.

The assessment of this question is based on the finding that, of the substances present in the blood, only unchanged CIBA 32644-Ba itself attacks the schistosome and that its blood concentration is maintained at a therapeutic level as long as the substance is being absorbed. Hence, only the duration of absorption has to be ascertained and compared with the interval between doses.

The duration of absorption of CIBA 32644-Ba can, surprisingly enough, be established from the blood-level curve of the metabolites (cf. Fig. 3). This is possible because the rate at which the metabolite levels rise is determined by the slow absorption of the initial compound and not by its rapid transformation into metabolites. For the purposes of evaluation, the metabolite curve in Fig. 3 is converted to a semilogarithmic scale (log. conc. against time) (Fig. 9).

The time at which new radioactive substance ceases to flow into the blood is reached at the point where the curve changes into the straight line indicating elimination only. In the present case this point was reached about 13 hours after medication. In other subjects values of between 10 and 15 hours were found. An absorption time of 10-15 hours tallies well with the blood-level pattern of CIBA 32644-Ba actually determined by direct radiometric analysis (cf. Fig. 3).

In man, therefore, the duration of absorption following a single dose of CIBA 32644-Ba amounts to 10-15 hours; during this period a therapeutic blood level of the compound is maintained. In the event of repeated administration of CIBA 32644-Ba, the intervals between doses amount to 12 hours—that is to say, each new dose is given approximately at a time when the blood level attained by

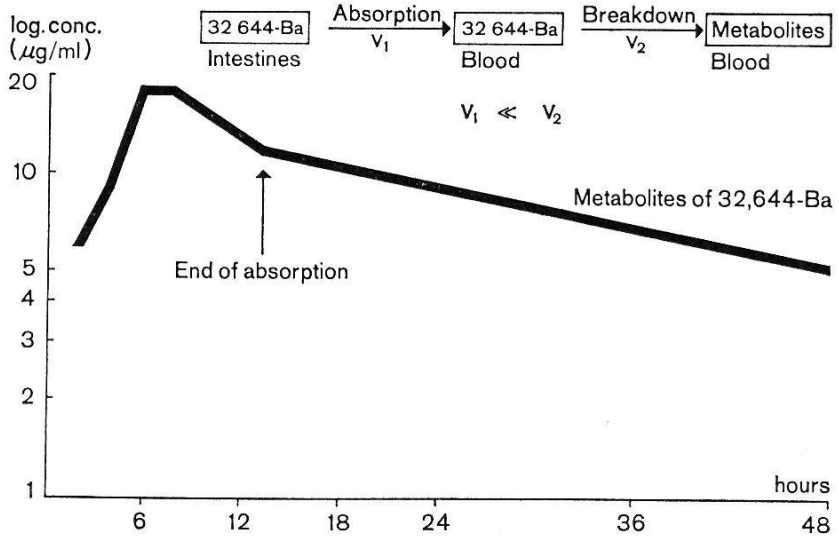

Fig. 9. Duration of absorption of 32644-Ba in man following a single oral dose (25 mg/kg).

the previous dose is just beginning to disappear. This shows that the clinical dosage schedule fully satisfies the requirement for a uniform blood concentration of active substance.

The slow excretion of the metabolites of CIBA 32644-Ba which has already been frequently referred to, suggests that the blood level of the metabolites will rise following repeated administration of the compound. This is in fact the case, as shown in Fig. 10. In the light of clinical experience to date, however, this increase in the metabolite concentration seems to be of no significance in man.

In conclusion, these studies on the metabolic fate of CIBA 32644-Ba yielded one finding which is particularly striking, i.e.

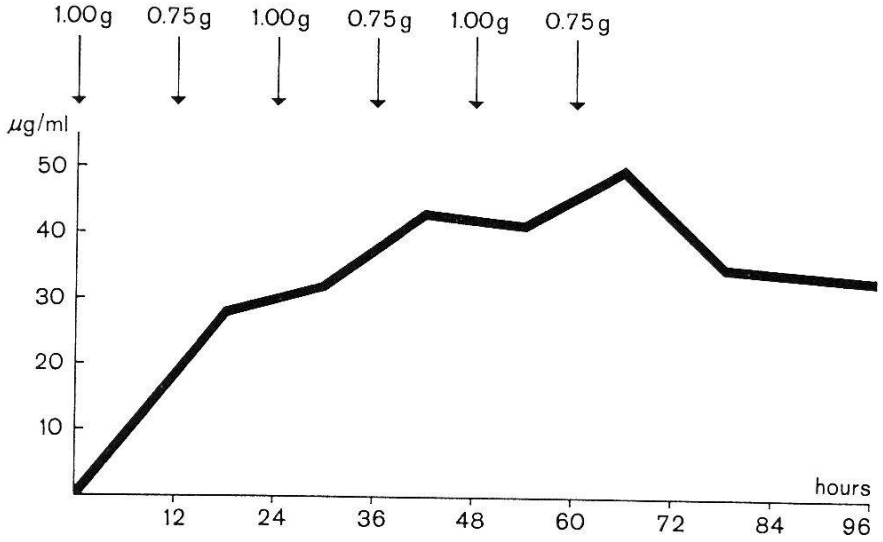

Fig. 10. Plasma levels of metabolites of 32644-Ba in man following repeated doses of the drug (25 mg/kg daily. The daily dosage was given in two fractional doses).

the adjustment of the blood concentration to a level which exerts a therapeutic effect on the parasite, but is quite safe for the patient. This, in conjunction with the well-chosen dosage schedule, ensures an optimal schistosomicidal effect.

Summary

The metabolism of CIBA 32644-Ba has been studied in various animal species and in man by administering the product labelled with C^{14}.

Following oral administration, the product is absorbed from the gastro-intestinal tract over a period of several hours; a large proportion of the absorbed substance is broken down rapidly. Consequently, during the process of absorption there is a constant, low, but therapeutically effective, concentration of unchanged CIBA 32644-Ba in the blood. The metabolites are biologically inactive and are excreted in the urine and faeces. None or virtually none, of the non-metabolised substance is excreted.

CIBA 32644-Ba is rapidly absorbed and broken down by the schistosomes *(S. mansoni),* and the metabolites accumulate in the parasites. There is probably a connection between this process and the appearance of lesions in the schistosomes.

The dosage schedule for clinical treatment (15-25 mg/kg daily for 5-10 days) ensures that there is a constant concentration of unchanged CIBA 32644-Ba in the blood throughout the entire period of treatment.

Résumé

Le métabolisme du CIBA 32644-Ba a été étudié dans différentes espèces animales et chez l'homme à l'aide du produit marqué C^{14}.

Après administration orale, le produit est résorbé au niveau du tractus digestif pendant plusieurs heures et est ensuite rapidement métabolisé dans une proportion importante. Pendant ce processus, la concentration sanguine de la substance non dégradée reste à un taux égal et bas, mais actif du point de vue thérapeutique. Les métabolites sont biologiquement inactifs ; ils sont excrétés par l'urine et les selles ; l'excrétion du produit sous forme non dégradée est nulle ou extrêmement faible.

Le CIBA 32644-Ba est rapidement résorbé et métabolisé par les schistosomes *(S. mansoni)* ; les métabolites sont alors accumulés par le parasite. Vraisemblablement ce processus correspond à l'apparition des lésions chez le schistosome.

Le schéma thérapeutique chez l'homme (15-25 mg/kg/jour, pendant 5-10 jours) donne un taux sanguin égal de CIBA 32644-Ba non dégradé pendant toute la durée du traitement.

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