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Discussion

a) Chimie

YOGORE: Is there any possibility of finding an easy test to determine blood levels of the drug?

FAIGLE: Up to now we have only one method for measuring blood levels of the non-metabolised drug, that is the use of radioactive tracers. At present we are trying to find another method which does not require labelled material, but so far we have not succeeded. This is difficult because the levels of the non-metabolised compound are always relatively low and those of the metabolised rather high, and this interferes with the method of determination. But I hope that in a few months we shall be able to determine the blood levels of CIBA 32644-Ba using the non-labelled compound.

YOGORE: We think that the blood levels are very important, since we have had several cases of vomiting, and we should like to find out whether the drug really remains in the body. It would therefore be helpful to have some idea of the concentration of the drug in our patients.

HUGONOT : Je voudrais poser au chimiste une question : au Maroc, dans le traitement de masse de la tuberculose par la thiosemicarbazone, des accidents cutanés graves furent observés au niveau des tatouages des femmes, sous forme de réactions cutanées ressemblant à des brûlures profondes. Or, ces tatouages sont à base de colorants d'aniline et certains pensent qu'une réaction chimique pourrait se produire entre l'aniline des tatouages et la thiosemicarbazone. Est-ce que ce risque pourrait exister avec le nitro-thiazolyl-imidazolidinone ?

WILHELM : Une interaction entre les colorants d'aniline et le nitro-thiazolyl-imidazolidinone est hypothétiquement possible. Les colorants d'aniline sont normalement basiques et le produit pourrait réagir, si le réactif basique est donné en même temps. Les colorants pourrait alors décomposer le CIBA 32644-Ba en sous-produits de structure et d'action inconnues. Mais il ne s'agit là que d'une spéculation purement hypothétique.

BLAIR: I should like to raise this point: if you remember the history of the Miracil group, only the sulphur-containing derivative Miracil-D was active in man (although, I think, Miracil-B, without sulphur, was active in monkeys and mice). Now, we see this sulphur atom again in this compound and Dr. LAMBERT will remember that I asked him in Salisbury whether this was very significant. I am interested to know whether in fact the compound would be active or not if it were to be made without sulphur.

WILHELM: As regards Dr. BLAIR's question on the role of the sulphur atom and the molecule, it is not yet known how the sulphur acts, but it seems to us

that is necessary, because compounds in which sulphur is replaced by carbon or nitrogen are less active; this is probably due to the fact that sulphur is easily oxidised whereas nitrogen is reduced to the amino-group. However, compounds containing only sulphur and no nitrogen are also inactive. This matter of structure appears to be very specific, but we do not know exactly why.

BLOCH: I should like to ask you whether you think it is worth while to spend time and effort on developing an injectable form which perhaps would permit a one-shot treatment? I do not know whether this is possible, or what it would involve, but I do not believe it is worth trying if the majority of the investigators think that the present oral medication is satisfactory.

WILHELM: To Professor BLOCH's question I should like to add one thing: The highest concentration in aqueous solution that we have been able to make up to now was 0.5%. On the other hand, one could prepare a suspension, but, as far as I know, experiments with intramuscular injections of suspensions have shown that the substance is not well absorbed. If the concentration has to be higher, a suspension is the only answer.

PETERS: May I just make a small point here? I think this is a question that we should keep a very open mind on, because we are beginning to realise that with a number of chemotherapeutic agents which are being given these days as depots, the therapeutically effective concentration is far less than we should have anticipated from earlier animal studies on blood levels. I do not think we should be prejudiced by poor solubility or by what we think ought to be the blood concentration. We should try experimentally to find out whether such compounds can be used if people think that it is worth doing from a clinical point of view.

POWELL: From the point of view of amoebiasis, I should say that severe cases of amoebic dysentery require parenteral therapy. Oral therapy is impossible, and therefore an injectable preparation would be useful. But before it could be given, one would have to know how effective this drug is in the oral form. In other words, we need to conduct more trials to find out whether it would be worth while to produce an injectable form.

SADUN: One question that occurs to me is this: if an injectable form were available, don't you think that some of the toxic effects previously mentioned might be alarmingly severe, and we would have no antidote? Or do you think, on the basis of your experience with the oral treatment where the side-effects were so mild and infrequent, that this is not an important question?

DAVIS: I agree with Dr. POWELL, I think this is rather the top of the cake. But we have got quite a good cake.

b) Métabolisme

BLAIR: Another point is raised by the remarks of Dr. FAIGLE who said in his paper that it was very important to have the two daily doses spaced 12 hours apart. I should point out that if this drug comes into practical use the difficulty of dosing people every 12 hours will be immense. Human society is not organised on a 12-hour basis; it is on 8-hour shifts; so, although you recommend that doses be taken at 12-hour intervals, people will not follow this schedule. So I think you ought to consider whether a smaller dose should be given in the morning and a bigger dose at night, to carry patients through the 16 hours until they are ready for their morning dose again. This is im-

portant because one hopes that, as CIBA 32644-Ba is obviously going to be used on a large scale, much of the treatment will be outside institutions.

LAMBERT: Concerning this question, Dr. BLAIR, I quite agree with the adoption of an 8- or 9-hour schedule in practice, but our dosage schedule was fixed at 12 hours because absorption takes between 10 and 15 hours. I think therefore that the drug can be given twice a day, and whether it is given early in the morning or late in the evening, does not matter. I do not think that it is important to keep exactly to the 12-hour schedule.

BLAIR: Another point is that we have observed that the metabolite in the urine may be toxic to the miracidium itself. The metabolite as it appears in the urine may not be toxic to the adult worms, but it is extremely toxic to the miracidia on hatching. It is very interesting to observe that the moment treatment starts, miracidia cease to be active. In other words one does not see them swim about in the hatching water. But on examining the deposit, one finds many eggs that have hatched with the miracidia in the larval state with a typical "beaked" form. One does not, at this stage, see the typical "greyhound" form that one normally expects to find in healthy miracidia swimming about in the water. So that if it is not toxic to the adult worms, it seems to be extremely toxic to the miracidia. Miracidia do not recover and become active again, if they are going to be active, until about 2 days after the cessation of treatment, presumably until the metabolite is cleared out of the urine.

LAMBERT: As regards the urinary metabolite and its action on miricidia, our findings are not quite the same as yours; our method was to concentrate the urine by centrifugation in order to obtain a very small amount of residue and then resuspend this in 70 to 100 ml of water. If you do this, you can see the miracidia swimming in the water even during treatment.

BLAIR: Another point has arisen from the clinical follow-up of cases. Several of my European patients have commented on the fact that their urine clears. The intense colour appears very quickly, but then the urine becomes lighter before the end of the 5-day course of treatment, which is rather astonishing. The colour disappears quickly after treatment, so much so that 24 hours after the end of the treatment the urine is clear of the drug colour.

BLOCH: I am very interested in Dr. BLAIR's remarks about urine clearance and I wonder if other investigators found the same. If I understood correctly, the colouring of the urine is more intense after the first or second day than it is later and it clears faster. Does this mean that the drug has some sort of threshold activity and that elimination is more rapid towards the end of the treatment, or do you think it is owing to a change in metabolism? I wonder if anybody else has comments or other explanations?

BLAIR: I should like to point out that these observations about the clearance of the urine were not solicited by me, but made to me by a European patient receiving treatment. No African patients—and I have treated over 100—have ever commented about the colour of the urine. But I think the African is very uninterested in the colour of his urine.

FAIGLE: I should like to say something about Professor BLOCH's and Dr. BLAIR's questions. We have observed that there are apparently 2 different types of metabolite excreted in the urine after administration of CIBA 32644-Ba. One type is coloured and is excreted very quickly—I think during the first day after treatment. The second type is non-coloured, and these non-coloured metabolites are probably those which remain for a long time in the blood because they are bound to proteins. Hence, it is possible that during prolonged treatment with the drug some change in the pattern of metabolism takes place,

so that more of the coloured product is obtained after the first dose whereas following the third, fourth or fifth dose more of the non-coloured product is excreted. This might be possible.

DAVIS: We found in African patients that the colouration is maintained until the end of the treatment or until about 2 or 3 days after treatment. Hatching continued throughout the period of treatment, but we are going to talk about this in detail at a later stage.

DA SILVA: I think the table presented here by Professor COUTINHO showed that dark urine was observed in only 15 out of the 20 patients. I think one very important point here was that described by Professor COUTINHO relating to dark urine. We observed dark urine in all our cases, but our cases were of the intestinal form with perhaps only mild damage to the liver. Perhaps one of the reasons why he did not find dark urine in 5 cases may be that there was some difficulty in metabolising the drug. Perhaps one of the pharmacologists present could give us some information on this point which might shed some light on important factors.

FAIGLE: As to the question of the brown-coloured urine, we have observed this in all treated animals and humans; it is independent of the degree of infection and of the success of the treatment. The colour is due solely to the metabolism of the product, since there are metabolites which have this dark-brown colour. I don't know why Professor COUTINHO did not find similar discoloration in 5 of his patients.

COUTINHO: We thought there were too few patients to draw any conclusions. We had 8 patients with one form of infection and 12 patients with the other form to provide enough statistical data for comparison. So I am not sure whether the non-appearance of dark urine is explained by alteration of liver function or perhaps by unreliable information on the part of the patients, because a few of them were out-patients.

BLAIR: Mr. Chairman, I hesitate to suggest this, but my explanation of the 5 cases that did not show dark urine is that they did not take the drug. Now I should like to know whether the doctor concerned actually administered the drug himself and gave the patient a drink of water to wash the tablets down or whether it was left to a nurse or somebody else. I think in trials of this nature, unless the person responsible actually does the drug-giving, anything may happen.

COUTINHO: I am not sure about the 8 out-patients; they were given the drug and instructed how to take it, which I assume they did. I don't know whether the absence of a dark urine is more common in the group of out-patients or in the group of in-patients, in which the drug was given by the nurse.

BLAIR: Dr. JORDAN is suggesting that I should raise another point, I did not think it came under the heading of biochemistry, but perhaps it could best be raised here. I refer to the smell of the patients. I do not know whether any other people have observed this; but it can be quite unpleasant. I had two men who said that their wives objected sleeping with them during the treatment, because of this. It is a rather heavy musty smell. One might think that the man has not been bathing or washing, but it is not a dirty smell. It is so bad in some cases that even members of the family complain about their father.

CRUZ FERREIRA: It seems to me that you are right, but that is the price of the cure.

BLAIR: Yes, but this is a point which should be kept in mind, otherwise clinicians treating patients in the future may be worried by such observations from their patients.

c) Transaminases

DODIN : Quelle technique, Dr. LAMBERT, avez-vous employée pour les transaminases ? Quels sont les taux normaux de vos transaminases chez les sujets normaux ?

LAMBERT : Nous avons utilisé les réactifs mis au point par « Dade Reagents » et, chez les sujets normaux, les taux de 20 à 50 sont considérés comme normaux.

DODIN : Et vous ne les avez pas dosés dans le schistosome lui-même ?

LAMBERT : Non, je n'ai rapporté que les travaux traitant des enzymes trouvées chez les schistosomes. Nous n'avons nous-mêmes rien fait à ce sujet.

DA SILVA: I have a comment on the transaminases in 27 patients treated with CIBA 32644-Ba; no difference was found between the results obtained before treatment and those obtained soon after the end of the treatment.

POWELL: I should like to add my comment to Professor DA SILVA's. We have also done transaminase estimations in 10 patients with acute amoebic dysentery and have found no change.

d) Immunologie

SADUN: I should like to congratulate Dr. DODIN on a highly imaginative and thought-provoking presentation. I only wish that we had fewer barriers and limitations with time and language, so that we might be able to dwell on this at some length. I am not so much intrigued by the results as by their interpretation and I wonder whether I might ask a few questions. One of them is: I see no bands were observed when adult schistosome extracts were reacted with the serum of patients treated for less than 14 days. In other similar studies that I know, conducted with extracts of adults against sera from infected animals or humans, a number of bands appeared against them. What about reactions against eggs or cercaria extracts? At one time I understand that I heard the term "anti-cercaria serum" and I was wondering how was that serum prepared against cercaria?

One point which needs clarification is this: I understand that the heaviness of lines was related to titres. It seems to me that the zone of equilibration between antigen and antibody is what determines the heaviness of the line rather than the titre. In fact the opposite may be true; i.e. a serum with a very high antibody titre may give very thin or no precipitation lines at all. In this connection I wonder whether the quantitative Ouchterlony technique was used throughout so as to determine the optimal equilibration zone.

One more point: I heard the term "protective antibodies" (and here maybe I am mistaken because of the language barrier) in connection with the antibodies detected by agar gel diffusion two or more weeks following treatment. Why "protective"? What evidence was there these antibodies in any way protected the host against super-infection? I don't want to monopolise the conversation, but I should like to emphasise that we are dealing with very complex antigenetic mixtures and that final interpretation of the results must await the acquisition of further evidence about the relative appearance, proportion, function and stability of the various antigens and antibodies involved.

DODIN : Nous avons préparé le sérum anticercaire, avec des cercaires traitées par un système générateur de radicaux libres, lyophilisées et injectées aux

lapins ; chaque injection contient un nombre de cercaires de l'ordre de 500 millions. Les cercaires sont tuées par la poudre de radicaux libres, c'est-à-dire acide ascorbique + sulfate de cuivre dans une très faible proportion ; il y a alors émission d'ions qui stérilisent les cercaires, mais qui n'empêchent pas l'activité enzymatique pendant 12 heures.

SADUN: Since you observed no bands when serum from infected untreated patients was reacted with the adults, my question was: were there bands observed against cercaria?

DODIN : J'ai une seule bande de précipitation anticercarienne dans le sérum des malades, et 5 à 6 bandes chez les lapins injectés par les cercaires traités aux radicaux libres.

LAMBERT : Quant à l'évidence de la protection par les anticorps sur l'hôte, je pense que la réponse est donnée par l'école traitée par DODIN.

DODIN : Oui, c'est l'école qui par le retard à l'infestation permet de parler de protection. Les élèves sont restés dans leur milieu naturel. Le premier groupe qui était non-infecté, s'est infecté dans la proportion de 15 à 17 % au bout de 120 jours, alors que les enfants traités se sont infectés dans la proportion de 1 à 7 % du 100e au 120e jour. Je ne pense pas que ce soit le médicament qui ait un effet rémanent. Je pense qu'il s'agit plutôt d'une protection immunologique, puisqu'on trouve des bandes anti-cercariennes. A Rome (1964) Hsü a vacciné ses animaux avec des cercaires traitées aux rayons X ; j'ai pu également vacciner des souris avec des cercaires traitées aux radicaux libres. On peut aussi protéger des souris en injectant des extraits de mollusques + cercaires ou même sans cercaire et l'on arrive à un taux de protection convenable de la souris, avec bandes communes de précipitation. Cela nous entraînerait très loin de discuter ce problème maintenant, mais je vais reprendre cette école et faire des injections cercariennes sous couvert d'antihistaminiques.

HUGONOT : Je voudrais simplement demander une précision : quelle est la dose employée pour les enfants de l'école ?

DODIN : La dose fut de 25 mg/kg/jour. On n'a eu aucun accident.

SADUN: In calling attention to the use of the term "protective antibodies", my question was not, of course, to cast doubt on whether or not an acquired immunity can be produced in schistosomiasis. I am aware of the fact that one can induce an acquired immunity to schistosomiasis, in experimental animals at least. My question was: what evidence do we have that those antibodies which had been found in the serum of treated individuals and which produced the bands observed in the Ouchterlony plates are indeed protective antibodies?

DODIN : Je pense comme vous : on ne peut pas appeler ces anticorps «anticorps protecteurs» ; la seule chose qu'on a pu mettre en évidence dans le sérum des enfants de cette école, ce sont des anticorps et les enfants ont été protégés. Partout ailleurs, dans toutes les autres maladies infectieuses, on considère que parmi les anticorps présents dans le sérum, certains sont des anticorps protecteurs. Dans la peste on connaît très bien les anticorps protecteurs. A la suite même du traitement de la peste, on connaît les anticorps protecteurs qui apparaissent.

BLOCH : Ce que vous venez de dire est certainement correct. D'autre part vous connaissez aussi bien que moi des quantités de maladies infectieuses où les taux d'anticorps circulant ne sont en aucun rapport avec le degré de protection ou de susceptibilité. Alors je crois que pour l'instant on pourrait limiter la définition de l'éventuel effet protecteur de la façon suivante : Les anticorps

apparaissent sous traitement, disparaissent après un certain temps et semblent correspondre à une période d'immunité relative à la réinfection.

DODIN : Il y a une chose curieuse : dans ces anticorps on trouve tout de même une bande anticercarienne. Si l'on place sur la plaque d'Ouchterlony un sérum de lapin anticercaire et un extrait cercarien, on va trouver un certain nombre de bandes. Si l'on ajoute le sérum d'un malade traité depuis 1 mois par exemple, on obtient une bande de précipitation commune avec l'extrait cercarien. On peut admettre quand même que cette bande pourrait peut-être avoir une action protectrice contre les cercaires. Il est évident qu'il pourrait exister une immunité tégumentaire, une immunité cutanée. Il est possible qu'une immunité cutanée, que personne n'a pu mettre en évidence jusqu'à présent, serait justement celle qu'il faudrait obtenir dans le problème de la protection bilharzienne ; elle empêcherait la cercaire de franchir la peau et, s'il doit y avoir des vaccinations contre la bilharziose, il est bien évident que ce ne sera pas en essayant de provoquer des anticorps dans le sérum, mais en essayant de provoquer un anticorps cercarien, correspondant peut-être à une protection cutanée. Je pense que là est le problème de la vaccination contre la bilharziose. Ce serait cet anticorps qu'il faudrait essayer de relever après traitement, car il est peut-être protecteur contre la cercaire. Il faudrait mettre au conditionnel les propositions que vous avez faites.

DA SILVA: One question concerning Dr. DODIN's remark relates to eosinophilia. I should like to know the response in non-schistosomal patients treated with the drug. We know that in schistosomiasis about 40% of the patients treated with antimonials show an increase in the eosinophil count. Some later publications have shown that simply giving antimonials to any normal patient may cause this increase in the number of eosinophils. I wonder whether the same thing would happen with this drug.

LAMBERT : Si j'ai bien compris, vous aimeriez savoir si la substance elle-même est capable de donner une éosinophilie. Je peux répondre de façon absolument catégorique ; grâce à ce qui fut observé chez les amibiens traités et chez les malades qu'on peut considérer comme des contrôles, car n'ayant pas réagi à la thérapeutique, il n'y a aucune réaction éosinophilique due au traitement lui-même.

PRATA: Our observations in endemic areas of *S. mansoni* infection show that immunity is lost 2 months after cure with TWSb. Reinfection can be acquired quickly, 2 months after the cure. We have carried out a comparative study with TWSb in Salvador: in a non-endemic area, the patients were cured in about 80-90% of cases; in the hyperendemic area, 2 months after treatment, the patients again acquired a reinfection and there was almost 100% reinfection in patients treated, 2-3 months after the end of the treatment.

SADUN: Since it takes 2-3 months to produce eggs, it means that then they are becoming reinfected almost immediately. Is that correct?

PRATA: I suppose that 1-2 months after the treatment they are reinfected.

SADUN: Then again, are you sure that the worms were not already there, but in the migratory phase so that they were not being eliminated by the drug? Then they started to lay eggs a couple of months later, at the time they reached the mesenteries and became full adults.

PRATA: One month after the end of treatment the stool examination was negative. In the second and the third months the stool examination became positive.

e) G6PD

LAMBERT : J'aimerais encore demander au Docteur DODIN les observations qu'il a faites chez les malades où la G6PD était déficiente. Est-ce qu'on a déjà observé chez l'un ou l'autre malade traité, en particulier chez ceux où la G6PD avait été testée et trouvée déficiente, une hémolyse ou des phénomènes indiquant une action toxique de la substance elle-même ?

DODIN: Sur 5 bilharziens mansoni traités et ayant une déficience en G6PD, je n'ai pas observé un seul cas d'hémolyse et sur les enfants de l'école, où je crois il y avait 12 % de déficients en G6PD parmi les enfants traités, on n'a eu aucun accident.

LAMBERT : Je me demande également s'il est possible de faire une relation avec ce qui a été observé sur la G6PD dans la cuticule des mâles et le vitellogène des femelles, et ce que STRIEBEL a trouvé sur le point d'attaque du CIBA 32644-Ba ; en d'autres termes, pourrait-on par là expliquer la phagoctyose précoce des vers femelles après traitement, alors que le mâle est d'abord pénétré et entouré de tissu conjonctif avant d'être tardivement attaqué par les leucocytes.

DODIN : Je ne sais pas. Est-ce que la substance a une action sur le cycle des hydrates de carbone ou sur un autre métabolisme absolument inconnu encore ? Il y a certainement une action carbohydrate, puisque BUEDING a parlé de phosphofructokinase qui est inhibée par les dérivés de l'antimoine ; il est probable que s'il y a une action schistosomicide, elle devrait porter sur un cycle des hydrates de carbone.

LAMBERT : Tous les tests que BUEDING a jusqu'à présent tentés, pour déterminer l'action biochimique du CIBA 32644-Ba, n'ont encore donné aucune réponse explicative du mode d'action biochimique.