DETERMINATION METHOD OF PANS-NO. 610 (4-ACETYLAMINO-NAPHTALENE-LAUROYLSULFONAMIDE)

(Studies on the Chemotherapeutica against Viruses, Part 7)

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We found the determination method of PANS-No. 610 (4-acetylaminonaphthalene-lauroylsulfonamide), which is effective against Japanese encephalitis virus, Nakayama strain.

The principle of this method is as follows: deacetylated PANS-No. 610 is coupled with diazotized-*p*-nitroaniline, and the resulting compound shows red color. There is a parallel relationship between the concentration of PANS-No. 610 and the color-intensity.

Consequently, we can determine PANS-No. 610 colorimetrically by the use of this principle.

This method was applied to the determination of PANS-No. 610 in blood, in urine and in brain.

In this paper, the fundamental data for the determination of PANS-No. 610 in these tissues were described.

It may be said that this determination method is applied to the various studies on the effect of PANS-No. 610 against viral-diseases.

ON THE RELATIONSHIP BETWEEN THE CONCENTRATION OF PANS-NO. 610 IN VARIOUS TISSUES AND VIRUS MULTIPLICATION

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In this report, we described the results of the experiments on absorption, distribution and excretion of PANS-No. 610 in animal body with our determination method descrbied in previous report.

PANS-No. 610 injected into rabbit intravenously remained at the maximal level of the blood concentration during half an hour, and disappeared after 3-4 hours from the blood, and then excreted into the urine from the tissues within 48 hours completely.

When PANS-No. 610 was injected intravenously into the mice inoculated Japanese encephalitis virus, its amount in the brain increased in proportion to the progress of the virus multiplication, and then decreased on the period of cell decomposition.

From the above described facts, we consider that, there is the definite balance between PANS-No. 610 amount and virus content in brain.

STUDIES ON CHLORAMPHENICOL BENZOATE. I.

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(1) Chloramphenicol benzoate (CMBA) is easily hydrolyzed by pastes of viscera of rats and guinea pigs to yield free chloramphenicol. However, the drug is decomposed only slightly when commercially available digestive enzymes such as lipase B, pancreatin, pepsin or Taka-diastase are used. Chloramphenicol palmitate (CMPA) is readily hydrolyzed by pancreatin or lipase B.

(2) Blood levels following oral administration of CMBA tend to vary according to the animals tested. In human being CMBA gives somewhat lower blood levels and is excreted less in the urine than CMPA. When administered to the rabbit CMBA produces higher blood levels than CMPA.

(3) Chloramphenicol, CMBA and CMPA were tested for their anti-infective effects on mice, with results as analyzed by T. AKIBA's method as well as BÜLBRING-BURN's showing that any one of the three was not significantly different from others in the effect.

(4) It would appear from the foregoing facts that CMBA undergoes hydrolysis *in vivo* in a different way from that of CMPA, though the both gave chloramphenicol when hydrolyzed. The hydrolysis and the absorption of CMBA takes place rapidly enough to warrant the same therapeutic use as for CMPA.

STUDIES ON CHLORAMPHENICOL BENZOATE. II.

CMBA-Hydrolyzing Enzyme.

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The catalytic action of CMBA-hydrolyzing enzyme contained in various organs of animals was greatly inhibited by the cholinesterase inhibitors. The fraction with potent hydrolyzing activity obtained from rabbit-liver by fractional precipitation with ammonium sulfate corresponds to the fraction obtained by TAMAI* as cholinesterase from dog-liver using the same fractionation process. The properties of the former are as follows :

(1) Its hydrolyzing activity is inhibited by the cholinesterase inhibitors such as DFP, TEPP, Eserine or Neostigmine, and by Atoxyl and Quinine as well.

(2) When CMBA and acetylcholine are used together as substrates, its activity is far smaller than expected additional activity of two.

(3) Although presence of an SH-group was suspected in the enzyme, Na-nitroprusside test proved negative.

(4) The optimum pH of the action lied between 7.0 and 7.5, which was activated in the presence of Ca^{++} or Cd^{++}

(5) The hydrolyzing activity was sharply decreased by treatment with acetone. In view of the fact that the above mentioned properties are largely in line with those of non-specific cholinesterase known todate, the CMBA-hydrolyzing enzyme may belong in the non-specific cholinesterase.

* Tamai A. The Jour. Japanese Biochem. Soc. 22, 29 (1950).

UPON THE BACTERIOSTATIC EFFECT OF TETRACYCLINE METAL CHELATES BY DIFFUSION PROCEDURE

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The authors studied the bacteriostatic effect of tetracycline metal chelates by a sort of diffusion procedure, and found that it depends upon the stability of their chelate, ——the more stable the chelate, the lower the effect is.

And the diminished effect could be found to be revived when EDTA (Ethylenediamine tetraacetic acid) was added to the tetracycline metal chelates.

This experiment shows that the metal chelate group of tetracycline is in close connection with the bacteriostatic functional group in it.

STUDIES ON THE ACTION OF SULFONAMIDES AND HOMOSULFAMINE ON THE RESPIRATION OF MYCOBACTERIA. I

Mechanism of Inhibition of Respiration by Sulfonamides and Homosulfamine

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A cell-free enzyme preparation that oxidizes reduced diphosphopyridine-nucleotide (reduced DPN) has been extracted from washed cells of *Mycobacterium avium*, strain Jucho. The method used for extraction was a modified one which was used by A. F. BRODIE (J. Biol. Chem., 199:835-844, 1952) for extracting a bacterial DPN-linked cytochrome c reductase. The effect of sulfathiazole and homosulfamine on the enzymatic oxidation of reduced DPN was observed by using spectrophotometer at wave length 340 mμ.

The oxidation of reduced DPN by the extracted enzyme preparation was considerably inhibited by the presence of sulfathiazole. The inhibitory effect of sulfathiazole on the enzymatic oxidation of reduced DPN could be regarded as a mechanism of inhibition of aerobic respiration, that of picric acid-reduction (the activity of respiration observed by using picric acid as hydrogen acceptor) and that of neotetrazolium-reduction of the living whole cells of *M. avium*.

(The reduction of picric acid by cell-free crude extracts obtained from *M. avium* was also inhibited by the presence of sulfathiazole. The reduction of picric acid was previously reported by M. TSUKAMURA (Medicine and Biology (Japan), 33 : 59-62, 270-273, 1954) as being produced by reduced DPN and flavoprotein (or plus x) and as occurring under aerobic conditions. The activity of reducing picric acid is readily measurable by adding acetone to a given system and determing optical density of the acetone extract at 530 or 550 mμ.)

The enzymatic reduction of reduced DPN was not influenced by the presence of homosulfamine. The reduction of methyleneblue by cell-free crude extracts under anaerobic conditions was increased by the presence of homosulfamine. However, since homosulfamine inhibits oxygen uptake, reduction of picric acid and reduction of neotetrazolium by living cells of *M. avium*, it would be

considered that homosulfamine inhibits an enzyme wich is not responsible for the oxidation of reduced DPN and which is possibly present in the latter part of the redox system (probably between flavoprotein and cytochrome oxidase).

STUDIES ON THE ACTION OF SULFONAMIDES AND HOMOSULFAMINE ON THE RESPIRATION OF MYCOBACTERIA. II.

Modes of action

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The action of sulfathiazole and homosulfamine (Marfanil) on the respiration of whole cells of *Mycobacterium avium* strain Jucho was studied on the present paper.

(1) The action of sulfathiazole and homosulfamine on the respiration was at first observed by the method using picric acid as hydrogen acceptor according to M. TSUKAMURA (Medicine and Biology, 33 : 59–62, 270–273, 1954 ; 34 : 111–115, 1955). The oxidation of glycerine, glucose, fructose, xylose, lactate, etc. was most significantly inhibited and the oxidation of succinate, pyruvate and malate was most poorly inhibited by sulfathiazole. On the other hand, the oxidation of succinate and acetate was most significantly inhibited and the oxidation of the sugars and lactate was only poorly inhibited by homosulfamine.

(2) The action was secondly observed by the conventional Warburg method. The increase of oxygen uptake which occurred with addition of glycerine, that is, the oxidation of glycerine was more significantly inhibited than that of pyruvate by sulfathiazole. On the other hand, the oxidation of succinate was very significantly inhibited by homosulfamine, although that of glycerine was scarecely inhibited by the drug. The endogeneous respiration of the organism that represented always a large amount of the endogeneous respiration was significantly increased by the addition of sulfathiazole, and, on the other hand, decreased by homosulfamine.

(3) Loci of action of the drugs have been suggested as being different from each other.

(4) The results obtained by the method using picric acid as hydrogen acceptor were entirely similar to those obtained by the conventional Warburg method. The method, in which picric acid is used as hydrogen acceptor and the redution of picric acid to picramic acid is quantitatively estimated by using electric photometer (filter 550 mμ), has been considered as very useful for studies of the action of drugs on the respiration of bacteria (for example, *mycobacteria* and *E. coli*), because the activity of respiration in many samples are readily measurable at the same time and the method does not require any anaerobic conditions.

STUDIES ON THE ACTION OF SULFONAMIDES AND HOMOSULFAMINE ON THE RESPIRATION OF MYCOBACTERIA. III.

Significance of the Action

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The relationship between the inhibition of growth and the inhibition of respiration by sulfathiazole was studied. The respiration of *Mycobacterium avium* was measured by the method using picric acid as hydrogen acceptor as shown previously. The respiration of a small amont of the organism was measurable by the method, and 72% inhibition of the reduction of picric acid by *M. avium* of 0.2 mg per ml in the presence of 1-glutaminate was shown by the presence of sulfathiazole at a concentration of 100 mcg per ml. On the other hand, the growth of the organism of 0.15 mg per ml was inhibited by the presence of 50 mcg of sulfathiazole per ml. The less the amount of the organism, the less the amount of the drug necessary for the inhibition of respiration and growth.

In experiments, in which the large amount of the organism was used and the inhibition of respiration and growth was simultaneously observed, it was demonstrated that the visible growth followed the occurrence of the reduction of picric acid.

In view of the results, the inhibition of respiration by sulfathiazole has been considered to be related to the inhibition of growth, although it may be of accessory significance.

CLINICAL RESULTS OF SARKOMVCIN

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Twenty-seven cases were treated with sarkomycin. As their number was a few and most were hard of complete surgery, it was difficult to decide its effect promptly. However, the antitumor activity of sarkomycin can be somewhat expected. Although it is said that sarkomycin has no reaction or verv slight, reduction of leucocyte was observed in one-third cases with malignant tumors.