Résumé

STUDY ON DEVELOPMENT OF RESISTANCE OF BACTERIA TO ANTIBIOTICS IN ORGANISM, I

Acquisition of Resistance due to Separate Administration of Various Antibiotics

HARUAKI HOSHIZAKI

Internal Medicine 2nd Clinic
(Director Prof. T. KIKUCHI)
Faculty of Medicine, Kyoto University

The author examined on the acquisition *in vivo* of resistance of *staphylococci* to various antibiotics separetely administered to rats by forming abscesses on their abdominal wall and comparing it with the test *in vitro*.

Resistance acquired *in vivo* by *staphylococci* to penicillin, streptomycin, chloramphenicol (chloromycetin) and chlortetracycline (aureomycin) was found to be at almost the same level as that *in vitro*. But this is not the case for erythromycin.

To penicillin, the bacteria acquired rather slowly a resistance in terms of about 2～3 times higher concentration of the antibiotic; to streptomycin, very rapidly in terms of 10～200 times higher concentration ; to chloromycetin, very slowly in terms of 1.5～2 times higher concentration. To erythromycin, whereas resistance is aquired rather rapidly *in vitro* the bacteria acquired it rather slowly *in vivo* in terms of 3～4 times higher concentration.

Comparison between the groups receiving daily administrations and those receiving intermittent administrations disclosed us a tendency to delay the acquisition of resistance in the latter groups. However, it was found on the other hand that no less strong resistance was acquired in the groups intermittently administered with 100 mcg and 1,000 mcg of streptomycin, 100 mcg of aureomycin and 100 mcg of erythromycin than in the groups of daily administrations.

As to difference in acquistion of resistance according to the doses administered, it was learned that a marked acquisition was observed with the administrations in a dose of 10 mcg of penicillin, 1,000 mcg of streptomycin, 1,000 mcg of chloromycetin, 100 mcg of aureomycin or 10 mcg of erythromycin, particularly in the groups of daily administrations. In other words, acquisition of resistance was found to be the maximum when the antibiotics were administered in a dose not capable of sufficiently inhibiting the bacteria. It seems that *staphlococci* acquire less resistance with a dose larger or smaller than the above-mentioned.

STUDY ON DEVELOPMENT OF RESISTANCE OF BACTERIA TO ANTIBIOTICS IN ORGANISM, II

Acquisition of Resistance due to Combined Administration of Various Antibiotics

HARUAKI HOSHIZAKI

Internal Medicine 2nd Clinic
(Director : Prof. T. KIKUCHI)
Faculty of Medicine, Kyoto University

The author conducted an experiment on resistance acquired *in vivo* by *staphylococci* to antibiotics administered in combination. Acquisition of resistance of *staphylococci* to penicillin was not inhibited by its combined use with streptomycin, while inhibited by its combined use with streptomycin, while inhibited by its combined use with each of chloramphenicol (chloromycetin,) 'chlortetracycline (aureomycin) and erythromycin. Acquisition of resistance of *staphylococci* to streptomycin was inhibited by its combined use with penicillin, but little inhibited by its combined use with the others. *Staphylococci* acquire resistance to chloromycetin very slowly even when administered alone, but their resistance acquisition by its combined administration with each of penicillin, streptomycin and aureomycin remained almost at the same level as the case of chloromycetin administered alone, or somewhat inhibited.

Acquisition of resistance of *staphylococci* to aureomycin by its combined use with each of penicillin, streptomycin and chloromycetin was found to be inhibited in each case. To erythromycin in combined administration with penicillin or streptomycin, their resistance acquisition was also found to be inhibited in each case. Intermittent administrations of antibiotics in combination provided resistance to the bacteria more slowly than in case of daily administrations.

The results obtained *in vivo* were often different from those obtained *in vitro*.

STUDY ON DEVELOPMENT OF RESISTANCE OF BACTERIA TO ANTIBOTICS IN ORGANISM, III

On Return to Sensitivity

HARUAKI HOSHIZAKI

Internal Medicine 2nd Clinic
(Director : Prof. T. KIKUCHI)
Faculty of Medicine, Kyoto University

The author examined the manner of return both *in vivo* and *in vitro* to sensitivity to antibiotics of so-called "artificial resistant bacteria" which have been made resistant *in vitro* and of so-called "spontaneous resistant bacteria" isolated from human subjects.

In vitro, the "artificial resistant bacteria" regained sensitivity of the primitive strain by the successive culture on bouillon for 15 generations, but "spontaneous resistant bacteria" showed no such indication.

In vivo, "artificial resistant bacteria", transplanted on the abdominal wall of rats, regained sensitivity of the primitive strain, or nearly just like in case of the experiment *in vitro*. "Spontaneous resistant bacteria", which showed no indication of return to senisitivity *in vitro*, again acquired sensitivity to the fair extent.

STUDIES ON THE ASSAY METHODS OF NITROGEN MUSTARD N-OXIDE (NITROMIN) IN BODY FLUIDS

YAEMON SHIRAHA, MATAICHIRO MINAMI, JUN TAKASU, KAZUO ODA & MICHIKO ISHIDA

From the Department of Surgery, Osaka City University Medical School
(Director : Prof. YAEMON SHIRAHA)

Studies are made on various methods for assaying a small amount of nitrogen mustard N-oxide (nitromin) in body fluids both biologically and chemically with the following results :

1) A small amount of nitromin in body fluids can not be detected by such method as to be used for the inhibitory action of the drug upon the growth of malts of either *Phaseolum hirtus* Retz or *Brussica chinensis* as an indicator.

2) When the one dimensional superposition method is applied for the bioassay of nitromin in body fluids, with reference to diffusion of the drug in agar media and its inhibitory action against pathogens, *Staphylococcus aureus* F. D. A. 209 P is the most sensitive as test organism. Nevertheless, if the test materials should contain 1 . 2 or 1 : 4 diluted tissue extracts, one can not detect 500 mcg/ml or less nitromin in body fluids.

3) One can not detect also a small amount of nitromin, utilizing the inhibitory action of the drug as an indicator, which is calculated through the influences upon the growth of the connective tissue cells originated from the chick embryonal heart. But the biological assay using the growth of large mononuclear leucocytes of chick blood as an indicator is suitable for the purpose, if the concentration of nitromin is over 10 mcg/ml in body fluids.

4) Among the chemical assay methods of nitromin, bromcresol purple method is the most suitable, enabling to detect 5~10 mcg/ml nitromin in body fluids. One can extract tissue nitromin most effectively with benzene (single) as a solvent, and either urine or tissue nitromin with hexane (double).

5) The blood level of nitromin reaches the peak (30~50 mcg/ml) within 5 or 10 minutes after intravenous administration of the drug at a dosage of 20 mg per kg of body weight, then it decreases gradually till it becomes zero 2 hours later.

6) Immediately after intravenous injection of nitromin, the drug is excreted into the urine. The excretion continues about 6 hours after its injection, occupiing 15~16 percent of the administered drug.

INHIBITION MODE OF PARA-AMINOSALICYLIC ACID TO INAH RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*

KOUJI MIURA, TAKASHI ABO & MICHIO TSUKAMURA

Obuso National Sanatorium
(Director : Dr. R. KATSUNUMA)

Mycobacterium tuberculosis var. *hominis* and 1% OGAWA's egg medium were used. Prior to sterlization, isoniazid and PAS were added to the media to give an appropriate final concentration of drug. Each tube was poured with 10 ml of the medium and slanted by sterilizing at 85 or 88°C for 50 minutes.

Isoniazid concentrated in 0.00045 mcg per ml of medium and PAS in 0.0 mcg, 0.05 mcg, 0.01 mcg and 0.1 mcg per ml were used. Besides, isoniazid of 0.0035 mcg per ml and PAS of 0.0 mcg, 0.05 mcg, 0.01 mcg and 0.1 mcg per ml were employed.

After inoculated tubes were cultured for 4 weeks, the number of isoniazid-resistant mutants to 1.0 mcg per ml of the number of viable cells were compared each other. The following result

was obtained :

The former showed 3.44×10^{-7}, 27.5×10^{-7}, 1.35×10^{-7}, 6.05×10^{-7} and 0.978×10^{-7}, and the latter 16.2×10^{-7}, 35.6×10^{-7}, 2.59×10^{-7} and 2.41×10^{-7}.

As mentioned above, it was observed that the appearance rate of isoniazid-resistant was decreased with PAS.

STUDY ON REVERSION OF DRUG-RESISTANT VARIANT OF TUBERCLE BACILLI

TAKETOSHI KURIMURA

The Third Clinic of Internal Medicine,
Osaka University, School of Medicine.
(Director : Prof. IMASATO DONOMAE)

The patients expectorating positive sputa of tebercle bacilli resistant to drugs after conclusion of the treatment were divided into two groups according to successive use of the chemotherapeutic agents.

On each groups, changes of resistance to drugs were experimentally investigated.

On the other hand, by *in vitro* experiments, the proportion of the partial resistant variants to the total population each showing the different grades of resistance are affected by subculture, were also studied.

The results of experiments are as follows.

1) Reversion of the resistance to drugs is easily obtained from the partial resistant variants than the total resistant variants. Similar phenomena are easily observed in susceptibility condition by use of other antituberculous drugs.
2) The partial drug resistant variants cultivated on media comprising, which is under minimal drug concentration for inhibiting bacterial growth, are promptly able to turn to sensitive than those on media without drugs by making decrease the proportion of higher resistant variants in bacterial population.