Abstracts of "Tuberculosis Research"

Vol. 34, 1974

Reduction of Tetrazolium Salts containing Heterocyclic Compounds by Tubercle Bacilli

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In the method using tetrazolium salts containing a heterocyclic compound to diffefentiate between Mycobacterium bovis and Mycobacterium tuberculosis, the affinitive group of tetrazolium salt and its position must be the same as in the compound showing antituberculous activity only against M. bovis.

Clubbed finger in the patients of pulmonary tuberculosis

— Some physiologic considerations —

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Eighty patients of pulmonary tuberculosis were divided into two groups, of which one had complicated with clubbing and other without clubbing. These were comparatively examined.

4 physiologic findings were observed:

1) no significant differences on the PaO_2, Sat O_2 and $PaCO_2$ of the peripheral blood in two grougs,

2) a small peripheral arteriovenous oxygen differences indicative of the presence of arterio-venous shuntings across the digits with clubbing,

3) a small reduction of peripheral vasomotor reflex believed to be present some arterio-venous shunting in the clubbed finger, and

4) no significant differences of the value of A-aDo_2 during 100 per cent oxygen breathing indicative of little relationship between intra-pulmonary arteriovenous shunting and appearence of clubbing.

Complete amino acid analysis of crystalline creatine kinase from horse skeletal muscle

Toshihide TAKASAWA and Hiroyuki SHIOKAWA

Complete amino acid composition of crystalline creatine kinase from horse skeletal muscle was determined, after the hydrolyses by three different methods of acid hydrolysis. There were no big difference of amino acids between horse enzyme and rabbit one, except glutamic acid.

Fractionation of proteins in culture filtrate of *Mycobacterium tuberculosis*

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and Hiroyuki SHIOKAWA

The culture filtrate of *Mycobacterium tuberculosis*, $H_{37}Rv$ strain, which was grown on modified Sauton's medium for 6~10 weeks, was passed through a DEAE-cellulose column, which had been equilibrated with phosphate buffer (30 mM, pH 6.5). Most of the proteins in the filtrate were adsorbed on the column and eluted with the phosphate buffer which contained NaCl 0.5 M. Nucleic acids and polysaccharides, which were present in the filtrate, were not adsorbed on this column and removed as break-through peaks before the fractions of proteins.

The eluted fraction of proteins mixture was concentrated by ammonium sulfate precipitation at 90% saturation and dialyzed against phosphate buffer (30 mM, pH 6.5). The dialyzed solution was chromatographed on Sephadex G-200 column. Of 4 peaks obtained, only the first peak contained polysaccharides. These peaks were sub-fractionated by ion-exchanger chromatogrphy and/or solubility chromatography with decreasing gradient of ammonium sulfate concentration. 6 proteins were homogeneous on disc electrophoresis among 14 proteins obtained from the culture filtrate, of which the final pH was alkaline, while 5 proteins were homogeneous among 12 proteins obtained from the culture filtrate, of which the final pH was acidic.

Studies on the Reactivity of Rabbit IgM and IgG Antibodies against HSA, with Special Reference to the Variations of their Antigen-Binding Capacities or Avidities

Takuro KIIMURA and Masahide SHIMIZU

1) The antibodies against HSA were titrated at various time after immunization in rabbits immunized with varying dose (0.04 mg, 4 mg and 20 mg) of the antigen. The titers were found nearly in the same level over a wide range of immunizing dose that differed as much as 500-fold in quantity, excepting that the appearance of antibody was delayed when small amounts of HSA were injected.

2) The immune response of rabbits immunized with HSA coupled to S. typhimurium or with the mixture of bacterial LPS and antigen were greater than that of animals immunized with HSA alone. Antibodies in the former case showed relatively high avidities at early stage of immune response.

3) IgM antibody seemed to be more avid than IgG counterpart.

Serological Reactions by Purified Phospholipids of Mycobacterium Tuberculosis

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Saburo KUREMATSU and Yoshitame NAGAYAMA

Passive hemagglutination reaction and its inhibition test using several purified phospholipids of

tubercle bacilli were carried out with sera from sensitized rabbits and tuberculous patients.

Of four phospholipids purified by Pangborn et al., only two dimannoside fractions (A and M) gave similar hemosensitizing capacities. However, their phospholipids were not pure on thin-layer chromatograms.

All of six phospholipids purified in this laboratory up to chromatographically pure states had similar hemosensitizing capacities. All of them were phosphatidylinositol olygomannosides with extra acyl residues (PIM_XA_Y): PIM_6A_2, PIM_6A_1, PIM_4A_1, PIM_3A_1, PIM_2A_2 and PIM_2A_1. Although some serological differences did exist within the phospholipids, the most significant difference was found between PIM_6 group and PIM_3 group.

In summary, it is given that there are at least two different antigenic determinants in the total phospholipids of tubercle bacilli. Serologtcal difference observed within the six phospholipids was discussed.

Polykaryocyte Formation of Rabbit Alveolar Macrophages in vitro

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Alveolar macrophages obtained from normal or $H_{37}Rv$ sensitized rabbits showed development of polykaryocytes after 12 hours of incubation in Sykes-Moore tissue culture chamber containing either MIF rich culture supernatants of lymphocytes from $H_{37}Rv$ sensitized rabbits or heat-killed $H_{37}Rv$ itself.

Polykaryocytes induced by MIF rich supernatants contained over 100 nuclei and showed large irregular pseudopods. In the electron microscopic observations network products consisted of numerous villi of cell membrane were found in the cytoplasma of polykaryocytes. It is suggested that mechanism of polykaryocpte formation by MIF rich supernatants in vitro is cell fusion.

On the other hand, polykaryocytes induced by $H_{37}Rv$ contained less nuclei than those by MIF rich supernatants and showed smooth cytoplasmic membrane. In the electron microscopic observations many clear and dense phagosomes were found in the center of these cells. No findings of network products of cell membrane were found.

Polykaryocytes were formed more easily from sensitized alveolar macrophages than from normal macrophages. The addition of immune sera (anti-$H_{37}Rv$) potentiated the phenomenon of polykaryocyte formatron in vitro by $H_{37}Rv$.

These results suggest that polykaryocyte formation in vitro of alveolar macrophages from $H_{37}Rv$ sensitized rabbits is mediated by an immunological mechanism and MIF rich supernatants may act as a fusion factor for alveolar macrophages.