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INDUCTION OF CYTOCHROME P-450 AND AN IMMUNE RESPONSE BY PHENOBARBITAL, FREE AND COVALENTLY BOUND WITH ALBUMIN

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Definite interaction exists between the induction of microsomal enzymes and induction of the immune response to foreign substances. The loss by phenobarbital of its ability to induce the cytochrome P-450-hydroxylase system when bound covalently with albumin, coupled with the acquisition by phenobarbital of ability to induce a lymphocytic immune system reflects the ability of the two defensive systems of the body — hydroxylase and immune — to interact with foreign compounds of low and high molecular weight. The cytochrome P-450-hydroxylase system of the liver and other tissues is designed to protect the body against the action of low-molecular-weight hydrophobic compounds. The immune system is responsible for the protective effect against high-molecular-weight foreign substances.

KEY WORDS: induction; phenobarbital; albumin; cytochrome P-450; immune response.

Many investigations have shown that administration of phenobarbital (PB) to animals leads to the induction of cytochrome P-450 in the liver, thereby stimulating reactions of hydroxylation both of PB itself and of many other foreign compounds [1]. Induction of the hydroxylase system is accompanied by synthesis of mRNA and enzyme proteins of membranes of the endoplasmic reticulum [7]. Meanwhile injection of PB bound covalently to a macromolecular carrier (a protein, for example), into animals leads to induction of synthesis of immunoglobulins specifically binding with PB and certain structurally related compounds, and inactivating them [3-6], in the lymphocytes. It has been suggested that definite interaction exists between the induction of microsomal enzymes and induction of the immune response to foreign substances. Low-molecular-weight foreign substances induce the hydroxylase systems of the liver, high-molecular-weight substances induce the immune system of the lymphocytes. According to this view, the hydroxylase and immune systems can be regarded as a single system of protection of the organism against the action of foreign substances of low and high molecular weight.

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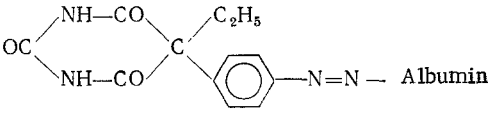
TABLE 1. Effect of PB Bound Covalently with Albumin on Induction of Cytochrome P-450-Hydroxylase System

Compound injected into rats	Cytochrome P-450, mmoles/mg protein (mean values of 5 experiments)	Aminopyrine N-demethylase, nmoles formaldehyde/mg protein/min (mean data from 3 experiments)
0.9% NaCl solution	1,4	4,1
PB	2,1	6,1
PB bound covalently with albumin	1,4	3,9
Mixture of PB and albumin	2,0	6,0
Albumin	1,3	3,8

To test this hypothesis it was decided to study the ability of PB, free and conjugated with albumin, to induce cytochrome P-450 and a receptor system of immunocompetent lymphocytes. It was postulated that binding of PB with the albumin macromolecule leads to loss of its inducing activity relative to cytochrome P-450 and to the appearance of ability to induce the immune system of animals.

EXPERIMENTAL METHOD

PB from Merck (West Germany) and a PB-bovine serum albumin (PB-BSA) conjugate, synthesized as described previously [5], the formula for which is given below, were used.



The PB content of the conjugate was 17 molecules to 1 molecule of albumin (PB_{17} BSA). The preparation was stored in lyophilized form and dissolved before use in 0.9% sodium chloride solution. All experiments were performed on male Wistar rats weighing 180-200 g. Cytochrome P-450 was induced by intraperitoneal injection of a solution of the sodium salt of PB or the conjugate in a dose of 20 mg/kg body weight daily for 3 days. The rats were killed 24 h after the 3rd injection. Control animals received NaCl, PB with albumin, and albumin alone, by the same scheme. The microsomal fraction was isolated and N-demethylase activity measured as described previously [2]. The cytochrome P-450 concentration was measured on the Hitachi-356 spectrophotometer by a differential scheme [8]. Induction of the immune response to injection of PB into the rats (twice a week, 20 mg/kg intraperitoneally at each dose, for 2 months) was determined by a method enabling separate antigen-binding (rosette-forming) cells (RFC) in the spleen to be detected. PB was bound covalently to sheep's red blood cells (SRBC) [6]. The SRBC, sensitized in this way, were used to detect cells carrying receptor molecules binding PB on their surface. During interaction between these receptors and PB the SRBC were fixed with the formation of rosettes visible under the microscope [9].

EXPERIMENTAL RESULTS AND DISCUSSION

Data on the effect of free PB and PB conjugated with BSA on the cytochrome P-450 content and N-demethylase activity are given in Table 1. They show that injection of free PB increased the specific content of cytochrome P-450 and aminopyrine N-demethylase activity in the liver microsomes by 1.5 times. PB, bound covalently with albumin, lost its ability to induce enzymes of microsomal hydroxylation. Loss of ability of the PB_{17} BSA conjugate to induce was not the result of the inhibitory effect of albumin on this process. Injection of BSA alone had no effect on the cytochrome P-450 content or on microsomal hydroxylase activity. Combined injection of PB and albumin had the same inhibitory action as injection of PB alone. The loss by PB of its ability to induce microsomal hydrolases when bound covalently with albumin was accompanied by the acquisition, by the conjugate, of ability to induce the appearance of PB-binding receptors on the surface of lymphocytes. In rats immunized with PB_{17} BSA conjugate, for instance, among 10^3 nucleated spleen cells 23 cells binding SRBC sensitized with PB were found. In control animals receiving free PB there were only three such cells, corresponding to "background" nonspecific binding of unmodified SRBC.

The fact that PB loses its ability to induce microsomal enzymes on conjugation with albumin, but at the same time acquires ability to induce an immune response specific for PB, in the writers' view, reflects the ability of the two defensive systems of the body — hydroxylase and immune — to interact with foreign compounds of low and high molecular weight. The cytochrome P-450-hydroxylase system of the liver and other tissues is designed to protect the body against the action of hydrophobic low-molecular-weight compounds. The immune system is responsible for the protective effect against high-molecular-weight foreign substances.

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ADSORPTION OF PHAGE λ DNA ON *Escherichia coli* CELLS TREATED WITH Ca^{++} IONS AND ON FROZEN AND THAWED BACTERIA

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Investigation of adsorption of biologically active tritiated phage λ DNA on *Escherichia coli* cells treated with Ca^{++} ions in the cold and on frozen-thawed bacteria revealed no correlation between the increase in adsorption and the efficiency of transfection. The level of adsorption of infectious DNA, for instance, was unchanged by freezing and thawing the *E. coli* cells, whereas after treatment with Ca^{++} ions in the cold it was increased tenfold; the level of transfection of phage λ DNA on both types of recipients was the same.

KEY WORDS: phage; adsorption; DNA; *Escherichia coli*.

Treatment of *Escherichia coli* cells with calcium cations in the cold, and also freezing and thawing the cells are known to form a state of competence in the recipient with respect to isolated phage, plasmid, and chromosomal DNA [1, 3-5]. However, the mechanism of induction of such competence is not yet clear. It has been suggested that as a result of the treatment mentioned above the permeability of the cell membranes is increased on account of structural changes arising therein. Such changes have been found by the fluorescent probe method in cells treated with Ca^{++} cations. Nevertheless it is not yet clear how these changes affect interaction between molecules of infectious DNA and recipient bacteria.

The object of this investigation was to study adsorption of isolated, biologically active phage λ DNA on *E. coli* cells treated with Ca^{++} cations in the cold and on frozen and thawed cells.

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