Effects of Hepatic Enzyme Inducers and Mitogens on Experimental Tyzzer's Disease in Rats

Aisuke NII*, Kôsaku FUJIWARA[1)], and Naoaki GOTO[2)]

Safety Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd., 1-1-8 Azusawa, Itabashi-ku, Tokyo 174, [1)]Department of Veterinary Pathology II, Nihon University Veterinary School, 1866 Kameino, Fujisawa 252, and [2)]Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

(Received 8 January 1993/Accepted 6 April 1993)

ABSTRACT. We studied the effects of chemicals which induce liver enlargement and can induce hepatic protein synthesis on experimental Tyzzer's disease in rats. Plasma transaminase values were evaluated as an indicator of the severity of the disease. Chemicals used were phenobarbital sodium and 3-methylcholanthrene as hepatic enzyme inducers, and lead nitrate and ethylene dibromide as mitogens of hepatocytes. In rats non infected with Tyzzer's organism, liver weight in those treated with these chemicals was higher than that in rats without chemical treatment. Tyzzer's disease-infected rats treated with these chemicals showed higher plasma transaminase values and more severe histopathologic liver lesions than infected rats without chemical treatment. The results indicated that the growth of Tyzzer's organisms in hepatocytes was accelerated in the course of hepatocytic metabolic changes during liver enlargement, and that protein synthesized in the hepatocyte may play an important role in the nutritive requirements of the organism.—KEY WORDS: enzyme inducer, liver enlargement, mitogen, protein synthesis, Tyzzer's disease.

J. Vet. Med. Sci. 55(4): 601–606, 1993

Tyzzer's organism, *Bacillus piliformis*, is an obligate intracellular parasite which produces hepatic multifocal necrosis in various species of animals [8, 9, 12, 25, 27, 29]. The nutritive requirements and metabolic characteristics of the organism remain unknown because cultivation in artificial media has not been achieved.

In the livers of mice under conditions of compensatory regeneration after treatment with CCl_4 [26] or partial hepatectomy [19], hepatic lesions of Tyzzer's disease become more severe. The formation of hepatic focal necrosis in mice infected with Tyzzer's organisms, however, is inhibited by fasting or feeding with a low-protein diet after inoculation [16, 19], and the disease develops in parallel with casein and amino acid content in the diet [16]. It has therefore been suggested that the organism propagates in close relation to the metabolic activity of the host hepatocyte, especially to protein metabolism [8, 16].

Many chemicals induce morphological and biochemical changes in hepatocytes [4, 14, 15, 20, 21]; however, little attention has been paid to the relationship between these changes and the growth of Tyzzer's organism. An exception is one study using phenobarbital sodium, which enhanced the disease in mice [28].

In the present study, we examined the effects of hepatic enzyme inducers and mitogens which induce liver enlargement, and have the ability to induce hepatic protein synthesis, in experimental Tyzzer's disease in rats. Phenobarbital sodium and 3-methylcholanthrene, as different types of enzyme inducers [4–7, 13, 23], and ethylene dibromide [17] and lead nitrate [3] as mitogens were used.

* PRESENT ADDRESS: NII, A., Veterinary and Tumor Pathology Section, Office of Laboratory Animal Science, National Cancer Institute, NCI-FCRDC, Fairview 201, Frederick, MD 21702–1201, U.S.A.

MATERIALS AND METHODS

Animals: Four-week-old female F344 rats were purchased from Charles River Japan Inc., Atsugi, Kanagawa and used for experiments after a one-week acclimatization period. They were housed in polycarbonate cages and given CRF-1 chow (Oriental Yeast Co., Tokyo) and tap water *ad libitum*.

Inoculation: Before experimental inoculations to rats, the infectivity of Tyzzer's organism was amplified in mice. Blocks of liver from female ICR mouse (Charles River Japan Inc.) infected with the RT strain [11] of Tyzzer's organism, after storage at −80°C, were homogenized in phosphate-buffered saline, pH 7.4 (PBS). The homogenate was intravenously (i.v.) inoculated into mice with a subcutaneous injection of 5 mg hydrocortisone acetate (Scherosone F®, Nihon Schering, Osaka). A few days later, moribund mice were killed and livers with multifocal necrosis were sampled. In inoculations to rats, mouse liver homogenate in PBS (× 20 to 40) was inoculated (1 ml/100 g body weight) i.v. into rats without cortisone treatment. Inoculation to each rat was made in the same order between groups. The number of organisms in the inoculum was counted as previously described [24].

Chemicals: Phenobarbital sodium, 3-methylcholanthrene, and lead nitrate were purchased from Wako Pure Chemical Industries Ltd., Osaka, and ethylene dibromide from Tokyo Kasei Kogyo Co., Ltd., Tokyo. Olive oil as vehicle was obtained from Wako Pure Chemical Industries.

Experimental design: In all experiments 20 rats were divided into 4 groups of 5 each. Two of the 4 groups were treated with test chemical, while the other 2 were treated with corresponding control vehicle or solvent. One group each from the test and control chemical-treated groups

were infected with Tyzzer's organisms and the remaining groups were non-infected. The infected and non infected rats were killed on the same day by exanguination via the caudal vena cava under ether anesthesia. Body and liver weight was measured at this time and liver tissues and blood samples were collected. Body weight was also measured at the commencement of experiments and before inoculation with Tyzzer's organism.

Experiment 1: The effect of phenobarbital sodium (PB) on Tyzzer's disease was studied. Two groups of rats were given daily intraperitoneal (i.p.) administration of PB at a dose of 100 mg/kg dissolved in physiological saline (50 mg/m*l*) for 4 days, one group of which was infected with Tyzzer's organisms at 6.0×10^7/kg shortly after the second treatment. These rats were killed three days after inoculation (the day after the final PB dose). The remaining two groups of rats were treated with saline and subjected to the same procedure as the PB-treated groups.

Experiment 2: To examine the effect of 3-methylcholanthrene (3-MC) on the disease, rats were given daily oral (p.o.) administration of 3-MC at a dose of 30 mg/kg suspended in olive oil (6 mg/m*l*) for 4 days. Tyzzer's organisms at 4.4×10^8/kg were inoculated shortly after the third treatment. All rats were killed two days after inoculation (the day after the final 3-MC dose).

Experiment 3: The mitogenic effect of lead nitrate on the disease was examined. Rats were given a single i.v. administration of lead nitrate at a dose of 100 μM/kg in saline (20 μM/m*l*). These rats were inoculated with 2.1×10^8/kg of Tyzzer's organisms on the following day and killed 3 days later.

Experiment 4: Rats were given daily p.o. administration of ethylene dibromide (EDB) at 100 mg/kg in olive oil (20 mg/m*l*) for 2 days. Shortly after the second treatment, rats were inoculated with 3.3×10^8/kg of Tyzzer's organisms and killed 2 days later.

Measurement of transaminases: Blood samples were collected from the caudal vena cava. Plasma glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) values were measured by the NADH-UV method using a Type 736 Hitachi autoanalyzer system (Hitachi, Tokyo).

Histopathology: Tissue samples from the liver were fixed in 10% neutral buffered formalin and embedded in paraffin. Two-μm sections were stained with hematoxylin and eosin (HE) and periodic acid Schiff (PAS). Immunohistochemical staining for the RT strain of Tyzzer's organism was made by the avidin-biotin-peroxidase complex (ABC) method using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) as previously described [18, 29].

Statistical analysis: The vegetative phase of Tyzzer's organism is highly unstable and the infectivity of inocula declines rapidly with time [8, 12]. Since intravenous administration to rats takes some time, plasma transaminase values generally diminish in accordance with the order of inoculation. Therefore, parametric distribution of the data was not hypothesized, and data for transaminase values were subjected to calculation of group mean values only including the data for body weight gain during the experiment, liver weight, and relative liver to body weight. Statistical significance of differences at $p<0.05$ was analyzed for transaminase data, with intergroup differences determined using Wilcoxson's two-sample rank test. The intergroup differences were analyzed for four categories; infected groups, non infected groups, test chemical treated groups, and control substance treated groups.

RESULTS

Experiment 1: As shown in Table 1, rats treated with PB and infected with Tyzzer's organism showed approximately two- to three-fold higher GOT and GPT values than infected rats without PB treatment. In the liver of the PB-treated rats, many necrotic foci larger than those observed in rats without PB treatment were seen. In non infected groups, rats treated with PB showed higher absolute (124%) and relative (124%) liver weight than rats without PB treatment. Rats in the PB group showed

Table 1. Effect of PB treatment

Treatment[a)]	Body weight gain (g)	Liver weight (g)	Relative liver weight to body weight (%)	GOT [range] (IU/*l*)	GPT [range] (IU/*l*)
PB[b)]+infection	7.2	5.67	6.66	700.0[c),d)] [336–1252]	643.4[g),h)] [294–1152]
PB[b)]	10.8	5.01	5.52	83.0[c),e)] [78–89]	49.2[g),i)] [47–53]
Saline +infection	8.8	4.72	5.37	289.8[d),f)] [164–545]	214.0[h),j)] [110–376]
Saline	12.0	4.05	4.44	74.0[e),f)] [71–77]	40.4[i),j)] [39–43]

a) Five rats in each group.
b) Administered i.p. at a dose of 100 mg/kg for 4 days.
c), e), f), g), i), j) Significant difference of $p<0.01$ between the two groups.
d), h) Significant difference of $p<0.05$ between the two groups.

Table 2. Effect of 3-MC treatment

Treatment[a)]	Body weight gain (g)	Liver weight (g)	Relative liver weight to body weight (%)	GOT [range] (IU/l)	GPT [range] (IU/l)
3-MC[b)] +infection	1.2	6.20	7.18	1285.4[c),d)] [1011–1690]	642.6[f),g)] [495–833]
3-MC[b)]	8.0	5.18	5.55	74.0[c)] [70–80]	28.8[f),h)] [26–31]
Olive oil +infection	8.0	4.74	5.08	335.4[d),e)] [260–435]	281.0[g),i)] [215–359]
Olive oil	13.2	3.96	4.06	75.4[e)] [67–88]	37.8[h),i)] [35–41]

a) Five rats in each group.
b) Administered i.p. at a dose of 30 mg/kg for 4 days.
c)-i) Significant difference of $p<0.01$ between the two groups.

Table 3. Effect of lead nitrate treatment

Treatment[a)]	Body weight gain (g)	Liver weight (g)	Relative liver weight to body weight (%)	GOT [range] (IU/l)	GPT [range] (IU/l)
Lead nitrate[b)] + infection	−15.5	6.01	8.20	7747.5[c),d)] [3600–18200]	3347.8[g),h)] [1589–7980]
Lead nitrate[b)]	4.4	5.88	6.36	175.8[c),e)] [132–224]	147.8[g),i)] [109–180]
Saline + infection	0.4	5.69	6.42	1103.2[d),f)] [814–1543]	829.4[h),j)] [666–1197]
Saline	12.8	4.21	4.19	92.6[e),f)] [79–124]	52.0[i),j)] [41–68]

a) Five rats in each group, except the lead nitrate + infection group in which one rat died before sacrifice.
b) Single i.v. administration at a dose of 100 μM/kg.
c), d), g), h) Significant difference of $p<0.05$ between the two groups.
e), f), i), j) Significant difference of $p<0.01$ between the two groups.

slight elevation of plasma GOT and GPT values and a greater number of mitotic figures in hepatocytes when compared with rats in the PB-nontreated group.

Experiment 2: Rats receiving 3-MC and infected with Tyzzer's organism showed two- to four-fold higher transaminase values than infected rats without 3-MC treatment (Table 2). Many necrotic foci were seen in the livers of both infected groups. Rats treated with 3-MC, however, showed a greater number of organisms in hepatocytes near the necrotic foci than the nontreated rats. In non infected groups, rats treated with 3-MC showed lower body weight gain and higher liver weight (absolute; 131%, relative; 137%) than rats administered olive oil alone. There was no difference in GOT value between the two groups, whereas GPT value was significantly lower in the 3-MC group than the olive oil group. Hepatocytes of the 3-MC-treated rats were hypertrophic.

Experiment 3: Rats receiving lead nitrate and infected with Tyzzer's organism lost body weight and showed remarkably higher plasma GOT and GPT values (approximately four- to seven-fold elevations) than infected rats without lead nitrate treatment (Table 3). Histopathologic findings supported the plasma transaminase results; large necrotic lesions with many organisms were detected in the livers of rats treated with lead nitrate, while in the control rats, small necrotic foci with few organisms were observed. In non infected groups, rats receiving lead nitrate showed lower body weight gain and higher liver weight (absolute; 140%, relative; 152%) than rats administered saline. Rats treated with lead nitrate showed significantly higher GOT and GPT values than those with saline, but the degree of difference was smaller than in the infected cases. Rats receiving lead nitrate showed cloudy swelling of hepatocytes, with occasional single-cell necrosis.

Experiment 4: As shown in Table 4, rats treated with EDB and infected with Tyzzer's organism showed higher GOT and GPT values than infected rats without EDB treatment, but differences in this experiment were smaller than those using other chemicals. Rats in both infected groups showed many necrotic foci in the liver, but those treated with EDB had a slightly greater number of

Table 4. Effect of EDB treatment

Treatment[a)]	Body weight gain (g)	Liver weight (g)	Relative liver weight to body weight (%)	GOT [range] (IU/l)	GPT [range] (IU/l)
EDB[b)] + infection	2.8	5.23	5.90	321.0[c),d)] [280–369]	259.8[f)] [229–289]
EDB[b)]	5.2	4.28	4.78	72.0[c)] [68–74]	44.8[f)] [37–54]
Olive oil + infection	4.4	4.04	4.54	238.4[d),e)] [177–297]	211.6[g)] [147–276]
Olive oil	6.0	3.49	3.86	71.4[e)] [66–76]	43.4[g)] [38–49]

a) Five rats in each group.
b) Administered twice p.o. at a dose of 100 mg/kg.
c)-g) Significant difference of $p<0.01$ between the two groups.

organism than those treated with olive oil alone. In the non infected groups, rats receiving EDB showed higher liver weight (absolute; 123%, relative; 124%), as well as a greater number of mitotic figures and slight swelling of hepatocytes, than nontreated rats. No difference was seen in GOT and GPT values between the two groups.

DISCUSSION

Plasma GOT and GPT values in Tyzzer's disease directly indicate the severity of lesions and reflect the number of organisms in the liver [10]. When GOT and GPT values were compared in groups of rats inoculated with Tyzzer's organism, values in those rats treated with the hepatic enzyme inducers PB and 3-MC or the mitogenic agent lead nitrate were elevated severalfold compared with values in rats receiving control substances. GOT value in infected rats treated with EDB was also higher ($p<0.01$) than that in infected rats not treated with EDB, though the difference was small. In addition, non infected rats treated with the test chemicals showed little or no elevation in transaminase values when compared with non infected rats treated with the vehicle or solvent, except in the case of lead nitrate. These results indicated that all chemicals used showed enhancing effects on Tyzzer's disease. Histopathological evaluation of the infected livers supported these blood chemical results.

Many drugs and chemical compounds induce liver enlargement associated with various biochemical changes, including those involving proteins, lipids, glycogen, DNA and RNA, though the pattern of changes varies among chemicals [4, 14, 15, 20, 21]. All chemicals used in the present study stimulated liver enlargement, indicating that the growth of Tyzzer's organism in hepatocytes was enhanced by some hepatocytic biochemical change during liver enlargement. In experimental Tyzzer's disease in mice, glycogen, fat, vitamin, and salt content in the diet have much less influence on the severity of the disease than that of protein [16]. Changes in protein metabolism may therefore be the most important determinant of the propagation of Tyzzer's organism among several biochemical changes in hepatocytes during liver enlargement induced by the chemicals in this study.

PB and 3-MC stimulate the activity of NADPH-dependent enzymes in liver microsomes [4, 7]. In addition, PB not only induces synthesis of these enzymes but also exerts a marked anabolic effect on the liver, resulting in a net increase in measurable microsomal protein per g of liver [1, 4–6, 23]. When administered to rats for several days, 3-MC also stimulates the synthesis of total microsomal or liver protein, though it shows little or no increase in microsomal protein per g of liver [4, 6, 23]. Thus, PB and 3-MC induce slightly different patterns of increased protein synthesis activity. However, the common stimulatory effect of these chemicals on the synthesis of microsomal enzymes and protein *in vivo* is evidenced by a parallel increase in the incorporation of amino acids into microsomal protein *in vitro* [13]. The enhancing effect of PB and 3-MC on proliferation of Tyzzer's organism in the liver might be closely related to the course of microsomal protein synthesis.

The enhancement of DNA synthesis in hepatocytes by the mitogenic agents lead nitrate and EDB is an adaptive rather than a toxic response [3, 17]. In rats treated with lead nitrate, total protein in the liver is increased, although protein content per g of liver is unchanged [3], suggesting that protein content in the liver is related to the proliferative process of Tyzzer's organism. Most of the compounds known to induce liver enlargement enhance the NADPH-dependent P-450 oxidative chain as PB [3, 4, 21]. On the contrary, lead is known to inhibit the mixed-function oxidase system [3, 22]. This indicates that the induction of microsomal NADPH-dependent enzymes is not an essential factor for proliferation of the organism, and that more general anabolic activity in hepatocytes may be of importance. Since there is no conclusive evidence that EDB induces hepatic protein synthesis, we are unable to discuss here the relationship between hepatic protein metabolism and the growth of Tyzzer's organism. However, EDB induces hepatocyte prolifera-

tion which is characteristically similar to that seen in the regenerating liver after partial hepatectomy. Liver weight, DNA content of the liver, as well as the number of mitotic figures in hepatocytes increase in the early stages after EDB administration [17]. Washed microsome particles from regenerating liver incorporate ^{14}C-leucine into protein more actively than similar preparations from normal liver [2], as previously described with PB or 3-MC. As PB also acts as a mitogen of hepatocytes [1, 6], some metabolic factors including protein synthesis, activated by the accelerated cell kinetics associated with increased DNA synthesis, may play a role in the enhancing effect of these chemicals on Tyzzer's disease. This is consistent with the results of our previous report describing the enhancing effect of partial hepatectomy on the disease in mice [19].

As a heavy metal, lead exserts a variety of toxic effects in many organs [3]. Non infected rats treated with lead nitrate showed a few instances of single hepatocellular necrosis, as well as a two- to three-fold elevation of GOT and GPT values, indicating that the dose used in this study was at a toxic level. The toxic effects of lead nitrate, such as decreased resistance to infection, should therefore be considered when evaluating the effects of the compound on Tyzzer's disease.

ACKNOWLEDGEMENTS. The authors wish to thank a number of members of the Safety Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd., for their expert technical assistance, especially Messrs. Masashi Sakazume and Hajime Tabata for assistance in blood chemistry, and Messrs. Hisayoshi Kasai, Takao Shishido, and Koichi Kobayashi for their excellent immunostaining preparations.

REFERENCES

1. Argyris, T. S. 1968. Liver growth associated with the induction of aminopyrine demethylase activity after phenobarbital treatment in adult male rats. *J. Pharmacol. Exp. Ther.* 164: 405–411.
2. Campbell, P. N., Lowe, E., and Serck-Hanssen, G. 1967. Protein synthesis by microsomal particles from regenerating rat liver. *Biochem. J.* 103: 280–288.
3. Columbano, A., Ledda, G. M., Sirigu, P., Perra, T., and Pani, P. 1983. Liver cell proliferation induced by a single dose of lead nitrate. *Am. J. Pathol.* 110: 83–88.
4. Conney, A. H. 1967. Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* 19: 317–366.
5. Conney, A. H., Davison, C., Gastel, R., and Burns, J. J. 1960. Adaptive increases in drug-metabolizing enzymes induced by phenobarbital and other drugs. *J. Pharmacol. Exp. Ther.* 130: 1–8.
6. Conney, A. H. and Gilman, A. G. 1963. Puromycin inhibition of enzyme induction by 3-methylcholanthrene and phenobarbital. *J. Biol. Chem.* 238: 3682–3685.
7. Fouts, J. R. and Rogers, L. A. 1965. Morphological changes in the liver accompanying stimulation of microsomal drug metabolizing enzyme activity by phenobarbital, chlordane, benzpyrene or methylcholanthrene in rats. *J. Pharmacol. Exp. Ther.* 147: 112–119.
8. Fujiwara, K. 1978. Tyzzer's disease. *Jpn. J. Exp. Med.* 48: 467–480.
9. Fujiwara, K., Takagaki, Y., Maejima, K., Kato, K., Naiki, M., and Tajima, Y. 1963. Tyzzer's disease in mice: Pathologic studies on experimentally infected animals. *Jpn. J. Exp. Med.* 33: 183–202.
10. Fujiwara, K., Takagaki, Y., Naiki, M., Maejima, K., and Tajima, Y. 1964. Tyzzer's disease in mice. Effect of corticosteroids on the formation of liver lesions and the level of blood transaminases in experimentally infected animals. *Jpn. J. Exp. Med.* 34: 59–75.
11. Fujiwara, K., Yamada, A., Ogawa, H., and Oshima, Y. 1971. Comparative studies on the Tyzzer's organisms from rats and mice. *Jpn. J. Exp. Med.* 41: 125–133.
12. Ganaway, J. R., Allen, A. M., and Moore, T. 1971. Tyzzer's disease. *Am. J. Pathol.* 64: 717–732.
13. Gelboin, H. V. and Sokoloff, L. 1961. Effects of 3-methylcholanthrene and phenobarbital on amino acid incorporation into protein. *Science* 134: 611–612.
14. Iglesia, F. A., Sturgess, J. M., and Feuer, G. 1982. New approachs for the assessment of hepatotoxicity by means of quantitative functional-morphological interrelationships. pp. 47–102. *In*: Target Organ Toxicology Series, Toxicology of the Liver (Plaa, G. L. and Hewitt, W. R. eds.), Raven Press, New York.
15. Kunz, W., Schaude, G., Schimassek, H., Schmid, W., and Siess, M. 1966. Stimulation of liver growth by drugs. II. Biochemical analysis. *Proc. Eur. Soc. Stud. Drug Toxicity* 7: 138–153.
16. Maejima, K., Fujiwara, K., Takagaki, Y., Naiki, M., Kurashina, H., and Tajima, Y. 1965. Dietetic effects on experimental Tyzzer's disease of mice. *Jpn. J. Exp. Med.* 35: 1–10.
17. Nachtomi, E. and Farber, E. 1978. Ethylene dibromide as a mitogen for liver. *Lab. Invest.* 38: 279–283.
18. Nii, A., Fujiwara, K., and Goto, N. 1991. Growth of Tyzzer's organisms in preneoplastic hepatocytes of rats. *J. Vet. Med. Sci.* 53: 847–854.
19. Nii, A., Nakayama, H., and Fujiwara, K. 1986. Effect of partial hepatectomy on Tyzzer's disease of mice. *Jpn. J. Vet. Sci.* 48: 227–235.
20. Plaa, G. L. 1980. Toxic responses of the liver. pp. 286–309. *In*: Toxicology, 3rd ed. (Klaassen, C. D., Amdur, M. O., and Doull, J. eds.), Macmillan Publishing Company, New York.
21. Schulte-Hermann, R. 1974. Induction of liver growth by xenobiotic compounds and other stimuli. *Crit. Rev. Toxicol.* 3: 97–158.
22. Scoppa, P., Roumengous, M., and Penning, W. 1973. Hepatic drug metabolizing activity in lead-poisoned rats. *Experientia* 29: 970–972.
23. Seidegård, J., DePierre, J. W., Morgenstein, R., Pilotti, Å., and Ernster, L. 1981. Induction of drug-metabolites and structural analogues of stilbene. *Biochim. Biophys. Acta* 672: 65–78.
24. Takagaki, Y. and Fujiwara, K. 1968. Bacteremia in experimental Tyzzer's disease of mice. *Jpn. J. Microbiol.* 12: 129–143.
25. Takagaki, Y., Ito, M., Naiki, M., Fujiwara, K., Okugi, M., Maejima, K., and Tajima, Y. 1966. Experimental Tyzzer's disease in different species of laboratory animals. *Jpn. J. Exp. Med.* 36: 519–534.
26. Takenaka, S. and Fujiwara, K. 1975. Effect of carbon tetrachloride on experimental Tyzzer's disease of mice. *Jpn. J. Exp. Med.* 45: 393–402.

27. Tyzzer, E. E. 1917. A fatal disease of the Japanese waltzing mouse caused by a spore-bearing bacillus (*Bacillus piliformis* N. sp.). *J. Med. Res.* 37: 307–338.
28. Xian, M., Inoue, S., Matsunuma, N., Goto, N., and Fujiwara, K. 1988. Enhancing effect of phenobarbital sodium on Tyzzer's disease in mice. *Exp. Anim. (Tokyo)* 37: 311–316.
29. Yokomori, K., Okada, N., Murai, Y., Goto, N., and Fujiwara, K. 1989. Enterohepatitis in Mongolian gerbils (*Meriones unguiculatus*) inoculated perorally with Tyzzer's organism (*Bacillus piliformis*). *Lab. Anim. Sci.* 39: 16–20.