THE EFFECTS OF ORALLY ADMINISTRATED ENTEROCOCCI ON CERTAIN PLASMA ENZYME LEVELS AND ANTIBODY TITERS OF AXENIC, GNOTOBIOTIC, AND CONVENTIONAL CHICKENS

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ABSTRACT

THE EFFECTS OF CERTAIN ENTEROBACTERIACEAE ON CERTAIN PLASMA ENZYME LEVELS AND ANTIBODY TITERS OF AXENIC, GNOTOBIOTIC AND CONVENTIONAL CHICKENS

by Lois Brenda Allen

Axenic and conventional chickens ranging from 4 to 8 weeks of age were orally inoculated against *E. faecalis* or *E. faecalis* var. *zymogens*. The purpose of the study was to detect changes in the level of plasma enzymes which might accompany or precede antibody production. The number of organisms per gram of feces were observed at various time intervals after inoculation.

Most gnotobiotic chickens produced plasma antibodies by 4 days after inoculation. In gnotobiotic and inoculated conventional chickens there was a rapid increase in plasma antibodies until 3 weeks post inoculation. During the period between 3 to 5 weeks after inoculation there was a slight decrease in antibody titers. Fecal antibodies were detected in the plasma of gnotobiotic chickens at 3 to 4 weeks post inoculation.

The number of organisms per gram of feces increased to 1×10^{13} at 3 weeks after inoculation and then decreased to 1×10^{9} between 3 to 5 weeks post inoculation.

There were changes in the levels of each of the 4 plasma enzymes studied and these tended to be concurrent with detectable antibody production.

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The decrease in the number of fecal organisms between 3 to 5 weeks after inoculation is thought to result from the action of fecal antibody and/or other host defence mechanisms. It is concluded that feeding *f. fragilis* affects not only antibody production but produces concurrent changes in the levels of plasma peptidase, phosphatase, and transaminases.

THE EFFECTS OF ORALLY ADMINISTERED ERYTHROCOCCI ON CERTAIN PLASMA ENZYME LEVELS AND ANTIBODY TITERS OF AXENIC, GNOTOBIOTIC, AND CONVENTIONAL CHICKENS

by

Lois Brenda Allen

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The author

M. C. K. Smith

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INTRODUCTION

The axenic animal is a unique research tool because it has had no experience with viable microorganisms. Reyniers et al. (1949, 1960) characterized the axenic chicken using morphological, physiological, serological, nutritional, and biochemical studies. In this paper the term axenic is used to denote animals which are reared free of any other form of life, and the term gnotobiotic signifies animals reared with a known microflora. Germfree life or animal is used interchangeably with axenic life or animal.

The environmental conditions of the axenic animal can be controlled to such an extent that it is possible to change some variable in the conditions and be confident that the responses in the animal are a result of that variable.

In this study the variable which was changed in the conditions of the axenic chicken was that of adding a bacterial organism which is a normal inhabitant of conventional chickens' intestinal tract. One purpose of the study was to observe antibody responses of chickens after inoculation with enterococci and to determine the number of these organisms which could be isolated from fecal samples of gnotobiotic chickens at various time intervals after inoculation.

Westmann and Gordon (1960) found that the total serum protein of germfree and conventional animals were of the same magnitude.

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The greatest difference in the serum proteins of 90 day old germfree and conventional rats was that in the serum of germfree rats the albumin fraction was 17 % greater than their conventional counterparts while the total globulin content was only one-half that of the conventional rat. Wostmann (1959) shows that 75 day old germfree chickens have 17 % more serum albumin than conventional chickens and that the total serum globulin content is 36 % less than the conventional chickens.

Wostmann and Gordon (1960) exposed germfree rats to a conventional flora and found at the end of 1 week there was an increase in the α-2-globulin content of these rats. During the second week there was an increase in β-globulins. The γ-globulins start to increase only after 2-4 weeks of exposure to the conventional flora. As these fractions of serum proteins increased there was a steady decrease in albumin concentration. They stated that the decrease in albumin compensates for the increase in globulins. Their work also shows that antibacterial antibodies appear concurrently with the increase in globulins.

One of the greatest advances in diagnostic medicine has been the development of biochemical methods for detecting serum enzymes and changes in these enzymes associated with certain disease states.

If these changes of serum protein are associated with antibody production, a great number of enzymes will be required to bring about these alterations of protein. In this work 4 plasma enzymes were studied to detect mechanisms of serum protein anabolism and catabolism.

Rearing Germfree Animals

In a review of the history of germfree studies Reyniers (1959) stated that the first experimentation on germfree life was done in 1885 by Nuclaux, who attempted to raise guinea pigs and horses as pure cultures in a sterile holder. Following this, Pasteur became aware of Nuclaux's work and commented that he believed microbes were necessary to the life of an animal, and that life in the absence of microbes would be impossible. Nencki challenged this on the grounds that microorganisms produced toxic substances. Suttal and Thierfelder attempted to prove Nencki's theories by trying to raise germfree guinea pigs and chickens. Schottelius, then set out to disprove the ideas of Nencki, Suttall, and Thierfelder. Another who supported the thesis that microorganisms are not necessary and actually are harmful was Metchnikoff.

Reyniers (1959) stated that Schottelius, working between 1899 and 1906, was the first to successfully rear bacteria-free chickens, up to 31 days of age.

Colombo, working about 1912, raised healthy chickens for 40 days. Gordon (1946) gave the procedure of Colombo. He devised a glass cylinder with metal end-plates which were held in place by long bolts. One end-plate was built together with a hatching compartment, which was separated from the rearing compartment by a curtain. The air in the rearing compartment was dried and

and cooled by condensation of the moisture on a water cooled coil. Equipment was placed in the glass cylinder and the entire apparatus was sterilized by autoclaving at 121 C. Eggs were decontaminated by the method of Lilly, which consisted of brushing the surface with a 0.5 % $HgCl_2$ solution at 40 C, rinsing with sterile and drying.

The chicken diet of Lehendy contained Spratt's flour, chopped eggs, and dead flies which had been autoclaved. Using the above procedure he reared 15 germfree chickens for 45 days.

Reynolds (1946) initiated the work on germfree chickens while at Vanderbilt between 1941 and 1945. At that time Reynolds and associates undertook the study of germfree chickens as biological and medical research tools.

The axenic chicken has been found by Reynolds et al. (1954) to be relatively unstimulated by heat stable bacterial antigens in the diet. Antibodies were detected in the serum of axenic chickens only after 21 or more days of age. These were antibodies that had been stimulated by antigens of micrococci and lactobacilli, which were the organisms in highest concentration in the diet before sterilization.

Antibody Production of Germfree and Conventional Animals

General Primary Response. In order to evaluate the response of axenic chickens to an antigenic stimulus, it is valuable to consider general characteristics of the primary response. Carpenter (1956) stated that three phases usually follow the injection of a single antigen into a previously unstimulated animal. The first phase is a latent period during which antibody can not be detected

in the circulating blood. This period may last for a few hours to several days. During the second phase antibodies appear in the circulating blood and increase to a peak or plateau of varying duration. The amount of antibody in the circulating blood decreases during the third phase. The time period that they may persist varies with the animal, the antigen and other factors.

Carpenter indicated that antibodies against particulate antigens such as bacteria and erythrocytes frequently appear sooner than antibodies to soluble antigens.

The opinion of Wilson and Miles (1957) on the effect of dose was that in each animal there is a threshold value of antigen necessary to stimulate antibody production. If the dose of antigen given to an animal is lower than the threshold value, antibody response will not occur. For increasing doses above this threshold value the antibody titer will vary with the dose administered, but in a manner such that the increase in titer is relatively much smaller than the increase in dose required to produce it. Eventually a point is reached after which increase in dose will result in little or no increase in antibody production.

The response of an animal which has received many closely spaced injections of a particular antigen is discussed by Carpenter (1956). He stated that the titer of circulating antibody will increase toward a maximum beyond which additional injections have no effect and that the titers which are attained may vary widely depending on the animal and the antigen used.

Apparently the very young of any animal species is not capable of antibody production. Holle and Hirtz (1960) studied variations in precipitin response to bovine serum correlated with the age of chickens. They studied precipitin production in newly hatched chickens and in chickens of various ages up to 12 weeks of age. Less than one-half of the newly hatched birds which were studied did not produce antibodies and the others showed only low titers. The number of chickens producing antibodies increased until 4 weeks of age. The average titers of birds which were 5 weeks old were considerably higher than those of the 4-week-old birds. This increase was attributed to the fact that most birds at 5 weeks of age were producing antibodies while those of the 4-week-old birds were still unable to synthesize antibody in detectable amounts. The authors indicated that in their opinion, chickens reach complete "maturity" at about 5 weeks of age, since the titers of the groups between 6 and 12 weeks of age were similar to the 5-week-old birds.

Hoffman, Luth, and Holle (1962) studied cellular changes in the spleen during precipitin production. Circulating antibody appeared on the 6th day and attained a maximum on either the 7th or 8th day following intravenous injection of 50 mg of bovine serum albumin (BSA) into chickens.

Reynders et al. (1961) observed that cystic chickens developed antibacterial antibodies after 20 days of age. This indicated that small numbers of dead bacteria present in the diet might, when fed to cystic chickens, stimulate an antibody response. To determine if large numbers of dead bacteria in the diet would lessen this response, three investigators provided a diet containing 1.5 by

weight of [illegible] material. This was autoclaved and fed to chickens. Axenic and conventional chickens, one month of age, received the diet for a period of 3 weeks, while one axenic chicken and a group of conventional chickens received a normal diet lacking the bacteria. E. [illegible] is a normal intestinal inhabitant but is not normally found in the diet. The cecum of the axenic chicken which had not received the bacteria containing diet did not contain detectable antibodies. The cecum of axenic and conventional chickens that had received the bacteria containing diet were found to contain agglutinin to E. coli. The authors concluded that since the only contact the axenic chickens had with E. coli was through the killed bacterial cell, the agglutinins were produced in a manner similar to antibodies produced in response to oral immunization. Intestinal levels of antibody were demonstrated in cecum of conventional chickens which had not received the bacteria containing diet. It was the opinion of these investigators that the production of agglutinins had been stimulated by these organisms as a part of the normal intestinal flora.

Watkins et al. (1956, 1960) found that by using Streptococcus faecalis as a nonpathogenic in gnotobiotic chickens, serum globulin and antistreptococcus agglutinin values comparable to those in conventional chickens could be obtained.

Antibody Production of Orally Introduced Antigen. Farr et al. (1957) found that rabbits could be stimulated to produce antibodies against BSA by incorporating it in drinking water in various concentrations. Antibodies were detected as early as 7 days after the

first feeding of a 0.1 % solution. Their studies on the concentration of BSA or of bacterial BSA fragments revealed that at no time during oral immunization with 0.1 % BSA did the sera contain as much BSA as was present in the sera of rabbits 7 days after an intravenous injection of a non-antigenic dose of BSA. The animals receiving this non-antigenic dose did not produce antibodies despite the fact that the spleen, bone marrow, and peripheral lymphoid tissues were exposed to higher concentrations of BSA than were similar tissues of the orally immunized animals. The authors suggested that in oral immunization the lymphoid elements of the intestinal tract may initiate antibody response since they have available high concentrations of antigen.

Farr and Nicholson (1961) identified antibody producing cells using fluorescent microscopy. Following oral immunization of rabbits with BSA he was able to detect low numbers of antibody-containing cells in the intestinal wall. When orally immunized rabbits were challenged with 50 mg BSA by intravenous injection, a typical anamnestic response was observed and antibody producing cells were found in sections of spleen, lymph nodes, and intestinal lymphatic tissue.

Local Immunity. Kochland (1959) also Harrow et al. (1947) and Burrows and Bunnell (1948) as finding that local antibodies appeared sooner, disappeared more rapidly, and that the maximum titer was reached earlier than serum antibodies. They concluded from these findings that local antibody was not derived from serum antibody.

Kochland (1959) found that if she used Freund adjuvant with a killed cholera vaccine to immunize guinea pigs, only serum antibody could be found. When she used the vaccine without the adjuvant

both serum and local antibodies could be detected. To determine if adjuvant alters the distribution of the antibody, guinea pigs were positively immunized by intraperitoneal injections of antibody formed in guinea pigs by using the cholera vaccine and adjuvant. Antibody was detected in the lower and it was concluded that its ability to diffuse into the lumen of the local had not been altered by use of the adjuvant in immunization. Koslowski felt that the adjuvant localized the antigen so that sufficient amounts reached the sites of local antibody production to stimulate an antibody response. He concluded that local antibody is synthesized independently of serum antibody and probably in cells of the intestinal tract.

Intestinal and Intestinal Lesions of Guinea Pig

Normal Anatomy and Physiology. Hester and Schwarte (1952) stated that in the guinea, lymph nodules are present throughout the entire intestine, often replacing parts of the mucous membrane, and that the wall of the intestinal tract comprises the following layers; serous tunic, muscular tunic, a very thin submucosa, and mucosa.

The intestine is the area of the digestive tract where most digestion and absorption takes place. Gastric juice is secreted by the proventriculus, and the contents of the duodenum are acidic. The first stage of proteolytic digestion takes place there. The jejunum begins at the point where the pancreatic and bile ducts enter the intestine. The ileum is the lower part of the small intestine. The pancreatic juice contains enzymes for further digestion of proteins and for hydrolysis of carbohydrates and fats.

The absorptive structures are the villi which possess rich supplies of lymphatics (lacteals) and various epithelia. The core of the villus is formed of tissue proper.

Intestinal Histology of Germfree, Germ-contaminated, and Conventional Animals. Gordon and Pleasants-Gordon (1956-1957) investigated the distribution of reticulo-endothelial elements in the intestinal mucosa and submucosa of germfree, monoinfected, and conventional chickens before and treatment with penicillin. They found that the portion of lymphocytes in the reticulo-endothelial system in the intestinal tract of germfree and conventional chickens were the numbers of polyblast lymphocytes, lymphocytes, and plasma cells of the mucosa and submucosa. If germfree chickens were monoinfected with L. fecalis or Bacterium coprophilum, the reticulo-endothelial cells would increase in numbers and approach the levels seen in the conventional animal. Conventional chickens that had been orally treated with penicillin which were found to have reticulo-endothelial cell counts which approximated those found in germfree chickens. Germfree chickens treated with penicillin showed no change in the cellular constituent of the intestine. This would indicate that bacteria in the intestinal tract have a stimulatory effect on the reticulo-endothelial cells of the intestine, and that in penicillin fed chickens this stimulation is reduced. They found that the lymphocyte numbers, weight of the small intestine and intestinal reticulo-endothelial cell count could be positively correlated, and that the high values were associated with the conventional chickens and the lower values with the germfree chickens.

Gordon and Mackaness-Wilson (1953-1955) stated that despite the fact that the nuclei of reticulo-endothelial elements in the later life of the guinea pig are reduced, there is still a relative plentiful supply of these cells which can readily be found. They pointed out that viral and rickettsial reproduction might occur and that the dividing cells might contribute (and probably did) which could account for the stimulation and proliferation of these cells.

Mayer and Lehrman (1954-1955) stated that the increase of reticulo-endothelial cells, the increase in the amount of gamma globulin, and the detection of homologous lymphoid proliferation in primary lipid granulomas with L. donovani occur as concomitant phenomena. When these granulomas-bearing mice were treated with penicillin the parasites all count in the interstitial cells, the serum gamma globulin level, and probably also of the mononuclear cells to normal-bearing mice not receiving the treatment. The authors stated that no explanation could be offered for this inconsistency, except that antibiotics given for prolonged periods to older mice may slightly reduce serum gamma globulin levels.

Summary

Lymphomas. Wilde (1954) has given three possible explanations which might account for a true neoplastic process: (1) dividing concentrations or activity of the various antigens in different tissues; (2) different rates of release of an antigen from different tissues, or different rates for the release of the various antigens for a single tissue; or (3) different rates of the liberated antigens in serum, primarily in relation to the rate

also produced an acid phosphatase staining reaction in normal and tumor cells. Holmes et al. (1952) studied cells from the cervix in order to differentiate in normal from that in malignant tissue by means of histochemical techniques and found a higher acid phosphatase activity.

The histochemical study (1951) of normal human pituitary tissue and pituitary adenomas by Purves-Stewart (1953) revealed a positive reaction.

Prostate. Gutman et al. (1941) found high concentrations of acid phosphatase in normal, hyperplastic, and cancerous tissue, and erythrocytes and stated that these enzymes are sources of the acid phosphatase activity found in lymph and semen. They also suggested that it is possible that the level of acid phosphatase activity in lymph and semen may be an index of the rate of destruction of these tissues and cells in the body. Gutman et al. (1947) found that a single, subcutaneous injection, in rats, of normal cortical adrenotropic or of pituitary adrenocorticotropic hormone caused an appreciable rise in the serum acid phosphatase level of these animals. They suggested that the increase in serum acid phosphatase activity is a result of the stimulation of lymphoid tissue throughout the body by the adrenal cortex.

Gutman et al. (1951) stated that human prostatic tissue contains appreciable amounts of acid phosphatase, as do the seminal vesicles.

Immunology. White (1950) stated that acid phosphatase is very widely distributed throughout the body in relatively high concentrations. Pollard and Kraus (1950) found total histamine-destroying

[illegible] (1.1) and [illegible] (1.2) in heart, liver, [illegible], kidney, pancreas, brain, lung, testis and [illegible]. Proteinase K (1.3) and [illegible] (1.4) are [illegible] in [illegible] and [illegible].

The [illegible] (2.1) [illegible] (2.2) [illegible] with [illegible] and [illegible]. It [illegible] [illegible] [illegible].

MATERIALS AND METHODS

Isolators. Flexible film isolators similar to those described by Trexler and Reynolds (1957) and Trexler (1959) were used in this study. Modifications were a reduction of the size of the chamber to 24"x24"x6" and substitution of a fiberglass sleeve type door in place of the door described.

Perforated stainless steel cages 1'x16"x12" were used to house the chickens within the isolator chamber.

Chickens. The chickens used in this study were White Leghorns, hatched from eggs that had been purchased from a local distributor. Four groups were observed. The group I axenic chickens were 4 weeks of age when fed a 24 hour 1.0 ml broth culture of S. faecalis var. zymogenes. The inoculated chickens termed gnotobiotic were observed for serum antibody production and the number of organisms per gram of fecal sample during a period of 5 weeks following inoculation. Observations on uninoculated conventional control chickens were included.

The group II axenic chickens were 8 weeks of age when S. faecalis was introduced into their drinking water. These gnotobiotic chickens were observed for serum and fecal antibody production and the number of S. faecalis per gram of feces for a period of 4 weeks after inoculation.

The group III chickens were 5 weeks of age when they were orally inoculated with _E. tenella_. The resulting pathologic lesions were observed for plasma enzyme activity, plasma and local antibody production, and the number of organisms per gram of feces. Unimmunized non-infected birds of the same age were observed for plasma enzyme activity and plasma and local antibodies. These birds were observed for a period of 12 days following the inoculation of the oocyst chickens.

The group IV chickens were 5 weeks of age when fed _E. tenella_. Pathologic changes were observed for plasma enzyme activity, plasma antibody production, and the number of organisms per gram of feces. Conventional chickens of the same age were inoculated with _E. tenella_ and observed for plasma enzyme activity, and plasma antibodies for a period of 11 days after inoculation.

Diet. The diet used was L-2[1] of Lofgren (as published). The formula given in Table 1 was employed for preparation of 100 gram batches.

Sterilization Procedures

Sterilization of glassware. Sterilization of 1/2" diameter and 1/4" length were used to sterilize capillaries. The 3" diameter were covered with air filter media[1] provided for air and steam exchange during the sterilizing period. Double-layered tissue paper (3) and 47[2] were used with 1. 2" square filter[3] to close

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1. Owens-Corning Fiberglas Corporation, Toledo, Ohio
2. Whatman Filter and Chromatography Co., Fair Oaks, Conn.
3. E. I. Du Pont De Nemours and Co. Inc., Wilmington, Del.

TABLE 1. The formula for preparation of [illegible] quantities of the chicken diet L-[illegible] F.

Corn Meal	40.62 G
Soya Meal	20.35 G
Alfalfa Leaf Meal	2.79 G
[illegible]	[illegible] G
Fish Meal	2.33 G
Wheat Germ G	2.33 G
Salt (Mineral)	.47 G
Vitamin (1, [illegible] I. U. A., 1, [illegible] I. U. D.)	.23 G
[illegible]3	1.87 G
$MnSO_4 \cdot H_2O$	2.6[illegible] MG
[illegible]	7.[illegible] G
Cornstarch	130.[illegible] MG
Arsanilic Acid	13.[illegible] MG
Riboflavin	.01 MG
[illegible]	.[illegible] MG
Nicotinamide	.5[illegible] MG
[illegible]	21.[illegible] MG
Pyridoxine HCl	.01 MG
[illegible]	13.[illegible] MG
Biotin	.[illegible]7 MG
Folic Acid	.[illegible] MG
Thiamin HCl	.69 MG
.1 % Vitamin B_{12} in mannitol	.45 MG

the open end of the cylinder. Diet was placed in aluminum pans 11 1/4"x7 1/2"x1 1/2" which were placed in the stainless steel cylinder for sterilization.

Water. Water was sterilized in 2 liter Square-Pak[4] flasks.

Peracetic Acid Solution. A 2 % solution of Peracetic acid[5] was used as the germicide throughout this study. An anionic synthetic detergent, an alkyl aryl sulfonate commercially known as Nacconol[6], was incorporated into the peracetic acid solution.

Procedure for Sterilization. Air filters were sterilized by dry heat at 150 C for a period of 4 hours. Chambers and cages were sterilized by spraying with 2 % aqueous peracetic acid solution containing 0.1 % Nacconol which were then allowed to stand for 30 minutes. At that time the air in the isolator space was displaced using air sterilized by filtration. The diet was sterilized by placing 700 g quantities into aluminum pans. These pans were placed in the cylinder, and the open end of the cylinder sealed with mylar film using number 853 polyester tape and number 471 vinyl tape. The cylinders were placed in an autoclave and subjected to the following sterilization procedure: (a) evacuation of the autoclave chamber to 28 inches of vacuum, (b) admission of steam to chamber until a pressure of 10 pounds was reached, (c) evacuation of the

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4. American Sterilizer Company, Erie, Pa.
5. Becco Chemical Company, New York, New York
6. National Aniline Division of Allied Chemical Corporation, New York, New York

autoclave chamber to 25 inches of vacuum, and (d) admission of steam to the chamber. The materials were autoclaved for 15 minutes at a temperature of 250° F. Two liters of tap water were added to the Pyro-Pak flasks and autoclaved separately for 30 minutes at 250° F.

Transfer of Sterile Material into The Isolator Chamber. The water and diet were transferred into the isolator using the following procedure. A warm sealed Pyro-Pak flask containing sterile water was sprayed externally with the peracetic acid solution and placed into a flexible plastic film sleeve that had been attached to the isolator door. This sleeve was then attached to the supply cylinder and the 2 % solution of peracetic acid was sprayed into the sleeve through openings on the side of the sleeve. A 30 minute waiting period was allowed before transfer was begun. The inner door of the isolator was then removed and the flask of water was brought into the isolator chamber. The Pyrex shield was punctured and the diet was removed from the cylinder and transferred into the isolator chamber. The inner door of the isolator was then closed and the sleeve and cylinder removed. The doors were sprayed with the peracetic acid solution and the outer door of the isolator was sealed in place with vinyl tape.

Transfer of Eggs into The Isolator Chamber. Chick, unincubated eggs were incubated for 19 1/2 days in a Jamesway Incubator, candled to insure that they contained living embryos, and then prepared for decontamination. A peracetic acid solution was prepared by adjusting the solution to pH 5.0 and then diluting to a final peracetic acid concentration of 2 %. Hexanol was added to give a final concentration

of 0.2 %. The eggs were scrubbed with an alkaline detergent[7] and with the peracetic acid solution. The scrubbed eggs were placed on a stainless steel grid within the sleeve door of the incubator and the door sleeve was sprayed with the 2 % peracetic acid solution. The eggs were transferred into the incubator chamber after a 30 minute drying period and were incubated at 35 C until after hatching.

Sterility Testing. Sterility testing was carried out at each lock entry by collecting fecal samples which were used to inoculate fluid thioglycollate medium. The inoculated thioglycollate medium was incubated at 24 C, 37 C, and 55 C for a period of 3 weeks. Smears were made from the same samples and stained by the method of Gram. After the test organism had been introduced into the incubator, each sample was streaked on brain heart infusion agar. After incubation some of the colonies were chosen at random for making Gram stains, inoculating into ethyl violet azide broth, mannitol, and gelatin.

The fecal samples were also used to determine the number of organisms per gram of feces. One gram samples were weighed into dilution bottles which contained 99 ml of 0.85 % NaCl. The number of organisms was determined by using the standard plate count technique employing brain heart infusion agar.

Test Organisms and Inoculation Procedures

Test Organisms. The chickens were inoculated with Clostridium feseudis or Clostridium fusiformis var. strains as classified by

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7. Tide, Procter and Gamble, Cincinnati, Ohio

Breed et al. (1957). These organisms were isolated from the feces of conventional chickens. At the beginning of a testing period 10 ml of a 24 hour culture of the test organism which had been grown in brain heart infusion broth, was inoculated directly into the drinking water of the test chickens.

Collection of Blood. Blood was obtained by cardiac puncture allowed to clot at 37 C for 1 hour to harvest serum or 10 ml quantities were placed in 50 ml Erylenmyer flasks which contained 20 mg of potassium oxalate according to the method of Stern et al. (1950). The plasma was separated from the cells by centrifugation and was removed by means of a capillary pipette. Enzyme analyses were carried out immediately, but plasma for antibody analysis was stored at -17 C until tested.

Antigen Preparation. Antigen was prepared by growing the test organism in brain heart infusion broth for 24 hours at 35 C. Formalin was added to the broth to a concentration of 0.5 % as suggested by Pelczar (1957) and glass beads were added to the culture which was shaken for 1 hour at 35 C to break up clumps of cells. The cultures were then incubated 19 hours at 35 C and centrifuged at a relative centrifugal force of 9750 x Gravity. The cells were then resuspended in a 0.85 % NaCl solution at pH 7.0 as suggested by Liao (1949). The cells were washed three times in this saline solution before they were standardized to a density corresponding to Tube 2 of McFarland (1909). The standard suspension of bacterial cells was stored at 4 C.

Collodion particles. The method of preparing collodion particles to be examined on the resolving power test of Bovarnick and Lloyd (1939), with the exception that triple distilled water was used in place of double distilled water. Collodion was precipitated out of an ether solution by adding to the ether-collodion mixture to distilled water. The precipitate so obtained was a dried fine white powder suspended in acetone. Collodion particles were precipitated from the acetone by adding a solution of 3 parts of triple distilled water and 1 part acetone. The stock suspension of collodion particles was made up so that a full division corresponded to the density of a Bacterial Filter A (1/2).

A suspension of rabbit red cells was prepared by adding 1 part cellular particles with 4 parts of saline. This suspension was then used for comparing the resolving titers of the filters. A new suspension of rabbit red cells was prepared for each group of filtrates compared.

Antibacterial Serum. Normal bacterial filtrates of plasma were made in buffered saline (p. 7) as used by Kuhn (1938). Equal volumes of the antigen-antibody suspensions were added to the filtrate and kept under dilute acid phenol in an 37° C water bath for 2 hour before shaking at 4° C over night. The titers of the plasma were recorded as the reciprocal of the highest dilution in which clearing of the antigen-antibody suspension had occurred.

Due to the difficulties of found a pH zone were made in 0.05 phosphate buffered saline and then buffered to pH 7.0. The diluted antisera were centrifuged, and the supernate was stored at -17° C for antibody assay. The

enzyme to wash thrice, centrifuged, and antibody analysis performed on four plasma.

Plasma Enzyme Assay

Total Plasma Acid Phosphatase. Total plasma acid phosphatase was assayed by the method of Tietz and Fiereb (1958). This enzyme catalyzes the reaction:

p-nitrophenyl phosphate + H_2O ⟶ p-nitrophenol + H_3PO_4.

The substrate p-nitrophenyl phosphate is colorless, but the hydrolysis product is yellow in alkali. Plasma was incubated with the buffered substrate for 30 minutes before the reaction was stopped by the addition of alkali. The amount of p-nitrophenol was measured by comparing the optical density of the test solution to that of a standard at 410 mμ. One Bodansky unit of phosphatase will liberate 1 μM of p-nitrophenol per hour.

Glutamic-Oxaloacetic Transaminase. The method used for assaying plasma glutamic-oxaloacetic transaminase was that of Tietz and Fiereb (1958). This enzyme catalyzes the reaction

α-ketoglutarate + L-aspartate ⟶ glutamate + oxaloacetate.

The amount of oxaloacetate formed in one hour is determined by the formation of a hydrazone from oxaloacetate which was measured at 515 mμ. One Reitman-Frankel unit of glutamic-oxaloacetic transaminase will form 4.0×10^{-4} μM of glutamate/min. at pH 7.5 and 38°C.

Glutamic-Pyruvic Transaminase. The activity of plasma glutamic-pyruvic transaminase was measured in the same manner as the glutamic-oxaloacetic transaminase and was recorded in Reitman-Frankel units.

Inhibitors. Peptidase activity was measured by the microtitration technique of Garman and Hogle (1968). The reaction mixture was prepared as described by Stern et al. (1952). The substrate was glycylglycylglycine which was hydrolyzed to glycylglycine and glycine.

RESULTS

Antibody Titers and Fecal Sample Bacterial Counts

In Table 2 and Figure 1 the number of organisms per gram of feces and the serum antibody titers of the group I gnotobiotic chickens are given. The antibody titers and the number of organisms in the feces increased until 3 weeks after inoculation, after which there was a decrease in the antibody titers and a marked decrease in the number of S. faecalis var. zymogens in the feces.

TABLE 2. Serum antibody titers and the number of Streptococcus var. zymogens in the feces of the gnotobiotic chickens in group I after inoculation.

Weeks after Inoculation	Antibody Titers	Mean Titer	Organisms per g feces
0	0 4 8	4	
1	64 64	64	1.9×10^{9}
2	256 128	192	2.7×10^{11}
3	256 512	384	1.0×10^{13}
4	320 320	320	1.0×10^{10}
5	160 160	160	1.0×10^{9}

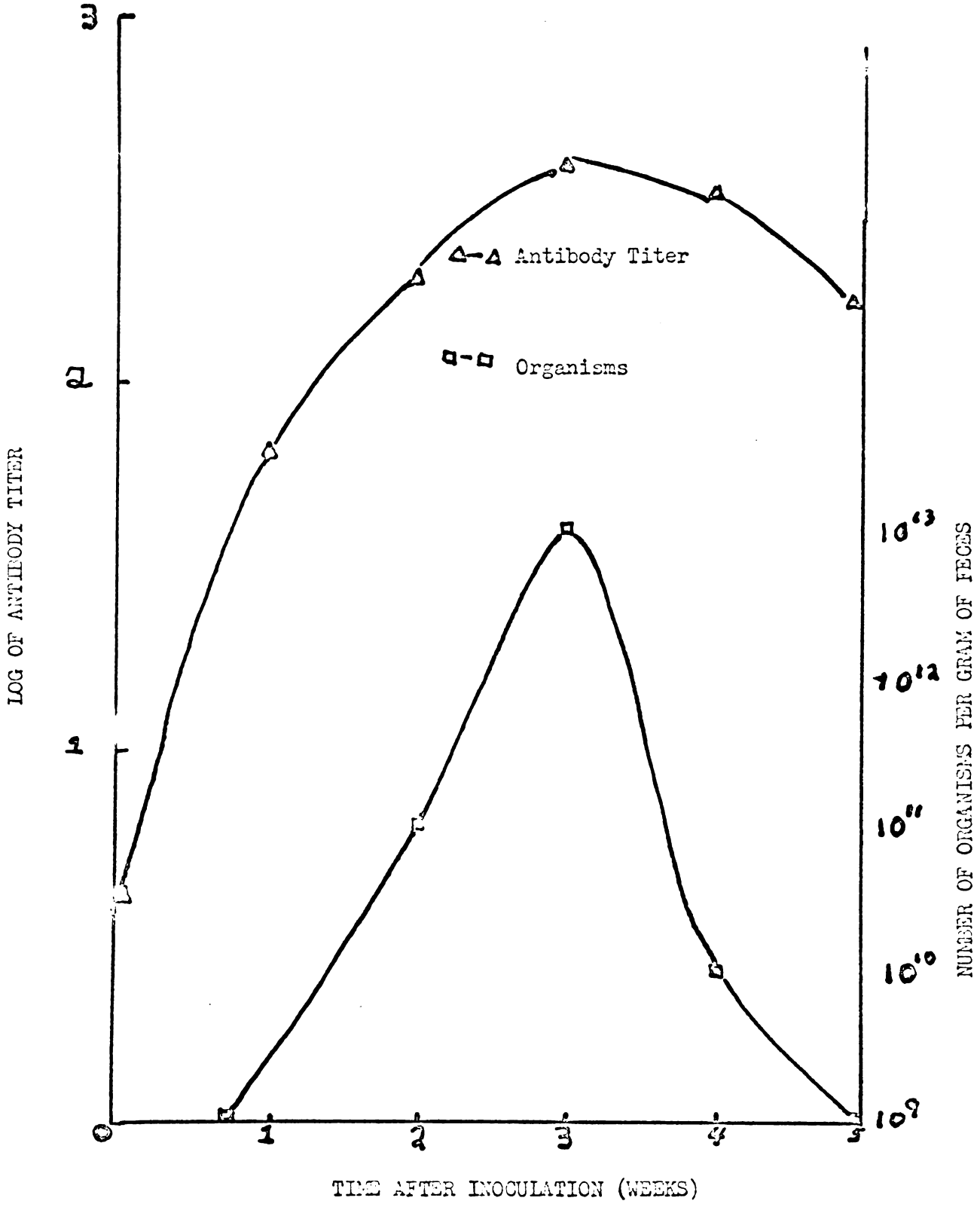

Figure 1. Mean serum antibody titer and the number of S. faecalis var. zymogens in the feces of the chickens from group I.

Group II chickens were used to observe fecal antibodies as well as plasma antibodies, and the number of organisms in the feces. The antibody titers and bacterial populations of the feces obtained in this group were of a magnitude similar to those of group I chickens. Fecal antibodies were detected twice during the period after inoculation. When plasma antibody reached a peak at 3 weeks following inoculation fecal antibody was detected at a titer of 16. Antibodies were detected again in the feces at 4 weeks after inoculation at a titer of 32. These data are given in Table 3.

TABLE 3. Fecal antibody production by gnotobiotic chickens orally immunized with *Streptococcus faecalis*.

Days	4	7	9	14	21	28
Group II Titers	0	0	-*	0	16	32
Group III Titers	0	0	0	-	-	-

* No samples collected

Antibody titers of group III and IV inoculated and uninoculated chickens are given in Table 4. Group III conventional chickens were not inoculated with *S. faecalis* while those of group IV were fed *S. faecalis*. These experiments were of short duration and no fecal antibodies were detected in the group III or IV chickens. In Figure 2 mean plasma antibody titers of the group III and IV chickens are shown. Atypical antibody reactions were detected in the plasma of the group IV gnotobiotic and conventional chickens 4 days after inoculation with *S. faecalis*. Figure 2 shows that a 4 days the

TABLE 4. [illegible] titers of protozoitic and conventional chickens fed [illegible] mix of untreated conventional chickens.

Group	Protozoitic* III	Protozoitic* IV	Protozoitic* Mean	Conventional III	Conventional IV	Conventional Mean
Days						
0	0	0	0	60	60	60
4	0	0 [illegible]	100	90	400 243	320
[illegible]	400 100	700	433	60	400 420	410
[illegible]	400	[illegible] [illegible]	427	60	60 60	60
11		400 [illegible]	[illegible]		400 [illegible]	[illegible]

* E. [illegible] inoculated.

inoculated conventional chickens showed similar titers than the protozoitic and uninoculated conventional chickens. During the remainder of the experimentation period the titers of the protozoitic and inoculated conventional chickens were comparable. The peak of the plasma antibody titers occurred 6 to 8 days after inoculation. Plasma antibody titers of uninoculated conventional chickens showed only slight variations during the period they were observed.

Plasma Lysozyme Activities

Protozoa. [illegible] lysozyme activities of protozoitic chickens, inoculated uninoculated conventional chickens [illegible].

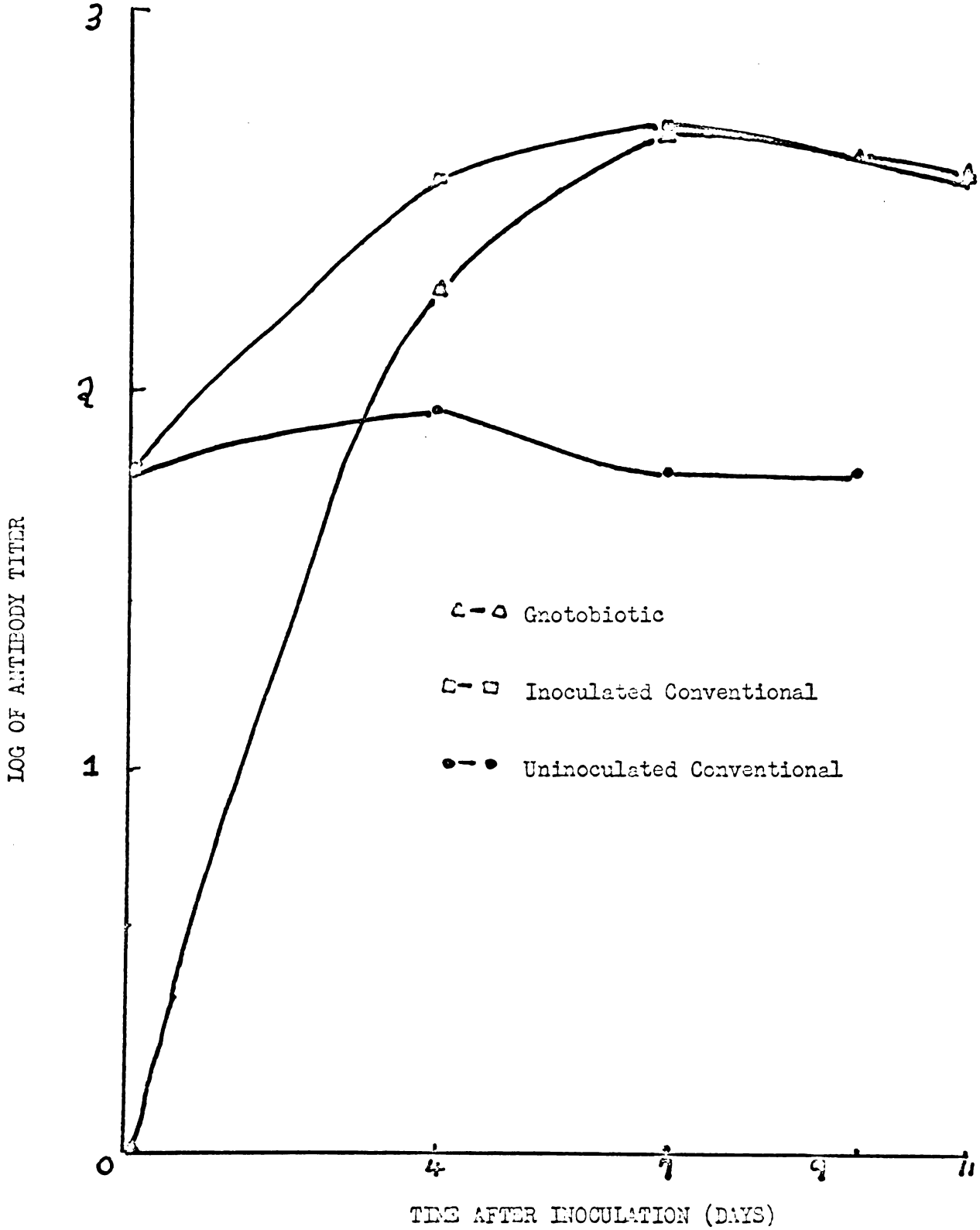

Figure 2. Mean plasma antibody titers of gnotobiotic, inoculated and uninoculated conventional chickens.

TABLE 5. Plasma peptidase activities of gnotobiotic and conventional chickens administered S. faecalis orally, and of uninoculated conventional chickens.

Group	Gnotobiotic* III	Gnotobiotic* IV	Gnotobiotic* Mean	Conventional III	Conventional IV*	Conventional Mean
Days						
0	2.32	3.82 2.64	2.72	2.31	2.72	2.72
4	2.36	5.39 4.83	4.71	3.54	2.92 6.36	4.64
6-8	2.89 7.47	4.14 5.47	4.84	3.53	3.62 4.14	3.83
9-10	4.62	4.03 4.83	4.44	3.23	5.11 5.24	5.17
11		1.37 1.94	1.60		2.41 3.60	3.21

* S. faecalis inoculated.

The mean plasma peptidase values of gnotobiotic and conventional chickens are shown in Figure 3. Peptidase activity of gnotobiotic and inoculated conventional chickens increased over a period of 6 to 8 days. In the gnotobiotic chickens the plasma peptidase activity peaked at 6 to 8 days, and then decreased. In the conventional chickens which had received S. faecalis the plasma peptidase activity continued to increase until 9 to 10 days, after which time it decreased. Some increase was noted in the plasma peptidase activity of uninoculated conventional chickens, but the values were much lower than the gnotobiotic or inoculated conventional chickens. The peak activity of the gnotobiotic birds preceded that of the inoculated conventional by approximately 2 days.

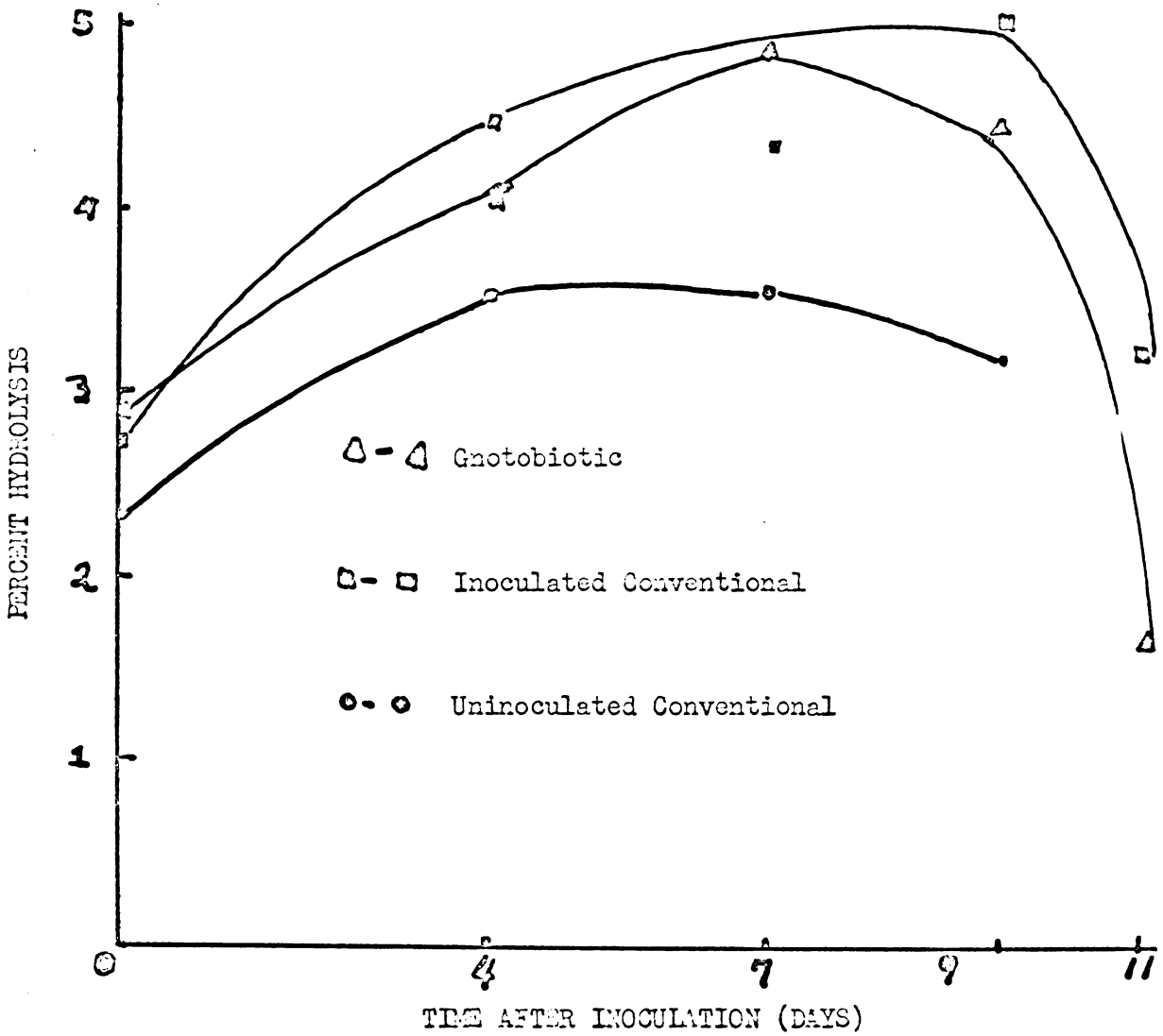

Figure 3. Mean plasma peptidase values of gnotobiotic and conventional chickens administered S. faecalis orally, and of uninoculated chickens.

Acid Phosphatase. The plasma acid phosphatase activities of gnotobiotic, inoculated and uninoculated conventional chickens are shown in Table 6. Graphic representation of the mean values is shown in Figure 4. Four days following with *L. fecalis* gnotobiotic

Table 6. Plasma acid phosphatase values of gnotobiotic and conventional chicks after inoculation with *L. fecalis*, and of uninoculated conventional chicks.

Group	Gnotobiotic* III	Gnotobiotic* IV	Gnotobiotic* Mean	Conventional III	Conventional IV	Conventional Mean
Days						
0	2.[illegible]	[illegible]				
		[illegible]	[illegible]	2.27	1.55	1.55
4	[illegible]	[illegible]			1.[illegible]	
		[illegible]	[illegible]	1.47	1.[illegible]	1.[illegible]
7	1.[illegible]	[illegible]			1.[illegible]	
	[illegible]	[illegible]	[illegible]	1.75	1.[illegible]	2.[illegible]
9-11	1.[illegible]	[illegible]			[illegible]	
		[illegible]	3.17	1.25	[illegible]	[illegible]
11		[illegible]			[illegible]	
		[illegible]	[illegible]		[illegible]	3.87

* *L. fecalis* inoculated.

and conventional chicks showed a slight drop in plasma phosphatase activity. After this the activity of these groups gradually increased until 11 days following inoculation. The plasma acid phosphatase activity of uninoculated conventional chicks showed only minor changes.

Plasma Glutamic-oxaloacetic Transaminase. Plasma glutamic-oxaloacetic transaminase activities of gnotobiotic, inoculated and

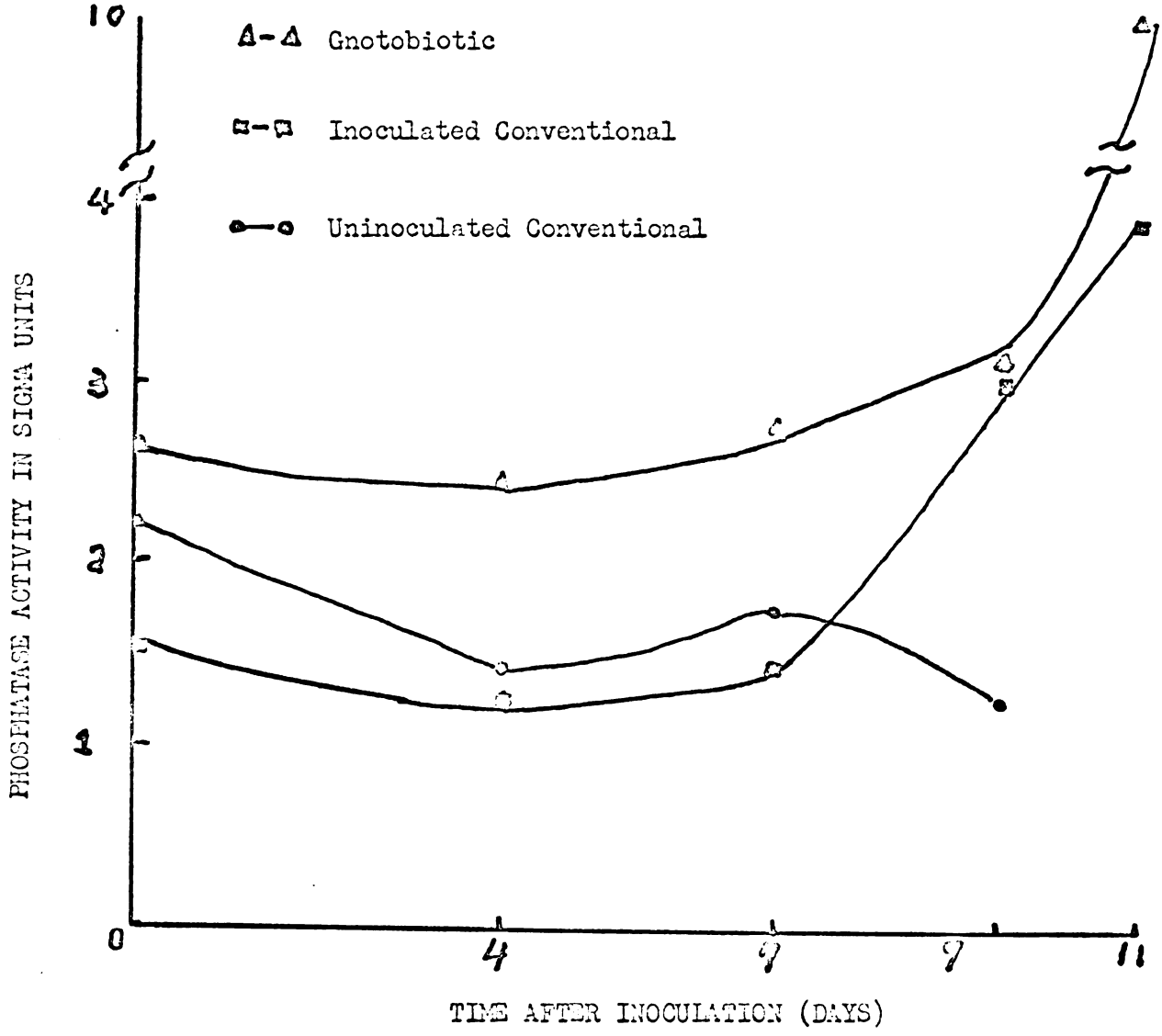

Figure 4. Mean values of plasma acid phosphatase of gnotobiotic and conventional chickens after inoculation with S. faecalis, and of uninoculated conventional chickens.

uninoculated conventional chickens are shown in Table 7.

TABLE 7. Plasma glutamic-oxaloacetic transaminase activities of uninoculated conventional chickens, and of gnotobiotic and conventional chickens administered S. faecalis orally.

Group	Gnotobiotic* III	Gnotobiotic* IV	Gnotobiotic* Mean	Conventional III	Conventional IV*	Conventional Mean
Days						
0	200	200 280	293	280	170	170
4	400	40	260	200	80	80
6-8	200 700	120 160	300	210	80 60	80
9-10	600	400 960	500	280	280 240	260
11		80 300	190		300 420	360

* S. faecalis inoculated.

Mean values of the enzyme activities are graphed in Figure 5. The activity of the gnotobiotic and inoculated conventional chickens decreased for a period of 4 days following inoculation with S. faecalis. This was followed by a gradual increase in plasma glutamic-oxaloacetic transaminase activity in gnotobiotic chickens until 9 to 10 days, after which the activity decreased. During the period of 4 to 8 days after inoculation there was no change in the plasma GOT activity of the conventional chickens, but a four-fold increase had occurred by 10 to 11 days. The peak activity of plasma GOT in the gnotobiotic chickens was found approximately 2 days before that of the conventional chickens.

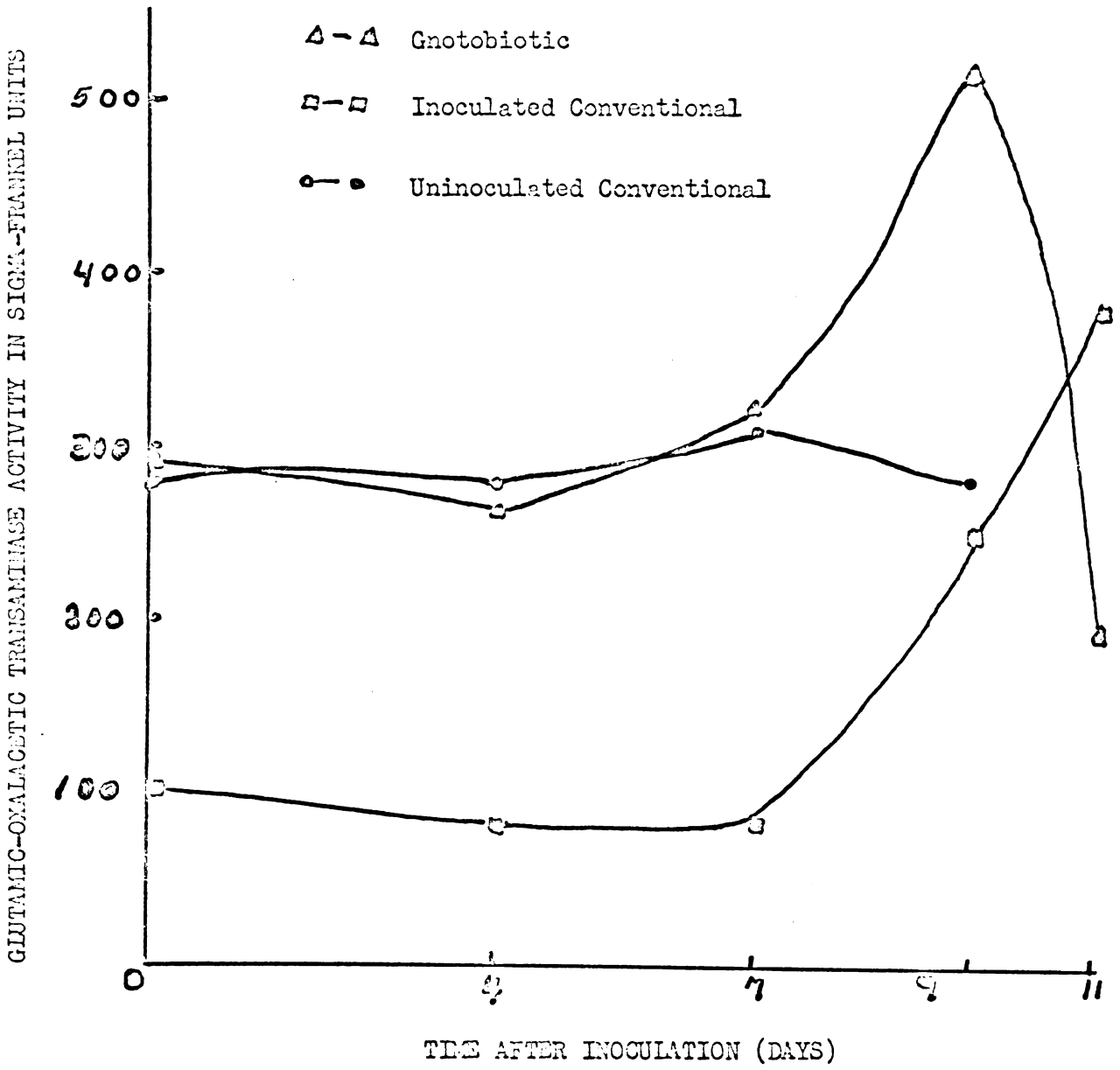

Figure 5. Mean plasma glutamic-oxalacetic transaminase values of uninoculated conventional chickens, and of gnotobiotic and conventional chickens administered S. faecalis orally.

The plant [illegible] the [illegible] activity of the [illegible]

Plant-bacterial interaction. The [illegible] growth-promoting activities of protobiotic, [illegible] and [illegible]

Table [illegible]. Plant growth-promoting [illegible] activities of [illegible] and [illegible], and of protobiotic and conventional [illegible] cultivated i.e. [illegible] only.

Group	Protobiotic[a] III	IV	Mean	Conventional III	IV	Mean
Days						
0	16	3 0	1.6	16	16	16
4	4	26	2.0	4	18	20
(-3)	8 7	21 1	7.0	9	8 21	21
(-8)	0	24 24	8.7	0	22 22	22
11		1 0	9.0		3 0	6

[a] i.e. [illegible] inoculated.

[illegible] from [illegible] with i.e. [illegible] [illegible] activity [illegible] and [illegible] to 2 day period. The plant-growth promoting activity of the [illegible] and protobiotic [illegible] [illegible] [illegible].

The plasma phosphatase activity in various stages of the natural and experimental infections in mice only minor changes.

Blood phosphatase activity. The plasma phosphatase activity in normal mice, infected with *T. gambiense* and with *T. rhodesiense* is recorded in Table 6. All

Table 6. Plasma phosphatase activity in normal mice, mice of group III and IV of Experiment 1 and mice of Experiment 2 infected with *T. rhodesiense* only.

Group	Experiment 1 III	Experiment 1 IV	Experiment 1 Mean	Experiment 2 III	Experiment 2 IV	Experiment 2 Mean
Normal						
0	16	2 0	1.6	16	24	24
4	4	24	2.0	4	24	24
6	0 0	2 24	7.0	9	6 24	24
8	0	2 24	6.7	0	2 24	24
11		2 0	0.0		8 0	8

* *T. rhodesiense* infected.

There is a high degree of variation in the *T. rhodesiense* infected mice as to the activity but on the whole, a rise is seen during the 9 to 12 day period. The pathophysiologic significance of the rise of the enzyme activity is not clear at present.

The plants [illegible] to determine the viability of the [illegible] and [illegible] only four days.

[illegible]. The plants [illegible] to determine the viability of [illegible], [illegible] and [illegible] are given in Table 6. [illegible]

Table 6. Plants [illegible] to determine viability of [illegible] of [illegible], and of pollen [illegible] and [illegible] only.

Group	Controlled* III	Controlled* IV	Controlled* Mean	Convoluta I III	Convoluta I IV	Convoluta I Mean
Days						
0	31	2 8	1.6	16	36	31
4	4	24	2.0	4	16	24
(-3	8 7	21 1	7.0	9	8 21	1
(-2)	1	32 22	6.7	0	21 21	22
22		2 8	9.0		8 8	8

* i.e. female [illegible].

Plants from [illegible] with i.e. female [illegible] [illegible] activity [illegible], and those [illegible] only 0 to 2 day [illegible]. The plot [illegible] the viability of the [illegible] of the convoluta and [illegible] [illegible] during [illegible].

DISCUSSION

The results show that in the inoculated children there was sufficient production of antibodies in 4 days to give high antibody titers. This would indicate that adequate concentrations of antigen reached the site of antibody production.

The area of the alimentary tract in which most absorption takes place is the small intestine. If the intact bacterial organisms did not pass into the intestinal wall, the antigen may have been any of a variety of products of the S. flexneri cells.

The studies of antibodies and fecal organisms in groups I and II showed that plasma antibody titers and the number of organisms increased for a period of 3 weeks after inoculation. In the two week period following these peaks the number of S. flexneri in the feces declined sharply. This indicates that some mechanism has controlled the increase in the number of the organisms and brought about the decrease in concentration of these organisms in the feces. Fecal antibodies were first detected at 3 weeks after inoculation when both plasma antibody and fecal organisms were at their highest level. Fecal antibodies persisted until the fourth week after inoculation during which time there was a 3 log decrease in the number of organisms in the feces. It would appear as if fecal antibodies and/or other host defense mechanisms such as phagocytosis of the bacterial cells by cells of

the lymphatic system of the intestine, were responsible for this decrease in the number of *E. faecalis* in the feces. Koudland (1955) indicated that fecal antibodies appear earlier and disappear sooner than serum antibodies. The conditions under which the current studies were carried out varied considerably from those of Koudland. She determined antibodies by the complement fixation test, and was not dealing with an orally administered living antigen which might react with a large variety of fecal antibodies.

Even though the defense mechanisms of the conventional chickens had been stimulated there was only a difference of 10% in the titer at 4 days after inoculation, between the gnotobiotic and conventional chickens. This indicates that the axenic chicken has normal antibody producing potential.

Some variation in individual antibody titers was found. Factors which may have influenced these variations are differing concentrations of antigen in the plasma that might react with antibody, and possible loss of antibody into the intestinal tract.

The graphic expression of the plasma peptidase activity of the inoculated chickens is a curve (Figure 3) which is similar in appearance to that of the antibody curve. Changes in peptidase activity appear to reflect changes in antibody titer to a certain extent. Holman *et al.* (1947) indicated that cells of the lymphoid tissue might be sources of serum peptidase. The work of Gordon and Bencheir-Kendous (1956-1958) showed that monoassociation with *E. faecalis* of axenic chickens increased the reticulo-endothelial cell contingent of the intestine to the level seen in conventional

chicken. Since the prohibition of antibodies and the stimulation of reticulo-endothelial cell components in the fetus, were shown by these writers to be the result of injection of cancer chicken with B. fusiformis, these changes might be reflected in changes of plasma peptidase. It would be interesting to investigate the origin of this peptidase in order to study any possible connection with antibody producing cells.

Changes in the activity of plasma acid phosphatase in both prostatectomized and normal and conventional chicken followed a considerably different pattern than that of the peptidase activity curves. This may indicate that the source, rate of release, or fates of these enzymes are different.

There is a similarity between the curves of plasma acid phosphatase and those of plasma glutamic-oxaloacetic transaminase. The decrease in level of both enzymes could result from the concentration of the enzymes by the cells which synthesize them in order that they could be used within the cells. The later increase may indicate that the enzymes are no longer needed within the cells of their origin and/or that during the time of their greatest activity within the cells and lowest plasma activity, there was active synthesis of these enzymes followed by a release when they were no longer needed.

Fishman et al. (1950) found that testosterone will inhibit the activity of acid phosphatase which are known in the prostate, and that copper will inhibit the activity of bone acid phosphatase. Studies such as these and the work of Becker and Hom (1950) who

have used electron beams to find and characterize different forms of glutamic dehydrogenase in tissues, may permit the identification of the source of the enzyme. If the source of these enzymes can be determined it would then be possible to explore further the question of this (1.4) concerning the reasons for changes in plasma enzyme activity.

It is difficult to explain the observed increase of the activity of glutamic-pyruvic transaminase, as a result of viral hepatitis in the plasma activity value. The activity of this enzyme in clinical plasma samples is generally considered to be quite low. Plasma activity levels may be lowered by the use of anticoagulants which have no pronounced effects on the data. Some of the viral stress in the release of this enzyme may have been significantly involved. It was there was a decrease in activity between 4 and 8 days following injection this might indicate that the cells from which this enzyme originated were releasing enzymes at a slower rate. Apparently G P has lowered in these cells and/or was released at a greater rate in order the plasma showed increased activity at the 0 to 4 day period, after the initial decrease at 4 to 8 days.

These studies did not show a significant difference in activities of any of the enzymes studied between control and experimental animals.

At this time it is possible to say that the feeding of either of these carbonated effects not only the production of gallstones, but produces concurrent changes in the plasma enzyme levels of glutamic-pyruvic transaminase, and transaminase. It would be also further study

[illegible] to find to add characteristic different forms of [illegible] possible transmittance in tissue, may permit the identification of the source of the enzyme. If the source of these enzymes be known it will then be possible to explore further the theories of Hitz (1947) concerning the reasons for enzyme [illegible] in an organism.

It is difficult to evaluate the significance of the activity of glycolytic [illegible], as a result of variations in the plasma activity value. The activity of this enzyme in diluted plasma is considered to [illegible] and is quite low. When activity levels are low any type of experimental error which might be applied would have pronounced effects on the data. None of the variations in the values of this enzyme may have been experimentally introduced. Since there was a decrease in activity between 4 and 8 days following irradiation this might indicate that the cells from which this enzyme originates were killed during or at a slower rate. Any activity that was introduced in these cells and/or was released at a greater rate since the plasma above known activity at the 0 to 2 day period, after the initial decrease at 4 to 8 days.

These studies did not show a significant difference in activities of any of the enzymes studied between exotic and conventional animals.

At this time it is possible to say that the feeding of either of these experimental diets not only the production of antibodies, but produces concurrent changes in the plasma enzyme levels of peptidase, acid phosphatase, and transaminase. It would be also [illegible] study

[illegible] to isolate and characterize different forms of [illegible] transaminase in tissues, may permit the identification of the source of the enzyme. If the source of these enzymes is established it may be possible to correlate the elevation of this (SGOT) according to the source for changes in plasma enzyme activity.

It is difficult to explain the significance of the activity of glutamic-pyruvic transaminase, as a result of wild type in the plasma activity value. The activity of this enzyme in clinical plasma is considered to contribute to nutrition in acute hepatic activity [illegible] any type of environment [illegible] which might be explained [illegible] permanent effects on the body. Some of the wild types in the values of this enzyme may have been experimentally introduced. Since there was a decrease in activity between 4 and 8 days following inoculation this might indicate that the cells from which the enzyme originated were replacing enzyme at a slower rate. Enzyme activity of the liver cells and/or was replaced at a greater rate since the plasma showed an activity at the 8 to 10 day period, after the initial decrease at 4 to 8 days.

These studies did not show a significant difference in activities of any of the enzyme studied between normal and cancerous animals.

At this time it is possible to say that the feeding of either of these carcinogenic diets not only the production of neoplasms, but produce concurrent changes in the plasma enzyme levels of peptidase, acid phosphatase, and transaminase. It would be [illegible] study

In order to clarify the mechanism or mechanisms which are responsible for the alluvium of placer origin available.

Axenic and conventional chickens were orally inoculated with *L. fermentii* or *L. fermentii* var. *alvei*. Plasma antibodies were detected 4 days after inoculation of the gnotobiotic chickens. The plasma antibody titers of gnotobiotic and conventional chickens increased until 3 weeks after inoculation. The highest plasma titer was observed at 3 weeks after which both gnotobiotic and inoculated conventional chickens showed slight decreases in antibody titers. Fecal antibodies were detected in stool specimens from gnotobiotic chickens at 3 and 4 weeks after inoculation. During this time plasma antibody titers were at their highest level.

The number of organisms per gram of feces were enumerated from 1 to 5 weeks after inoculation. These organisms increased until 3 weeks post inoculation. During the period between 3 and 5 weeks post inoculation there was a marked decrease in numbers of organisms in the feces.

Plasma peptidase activity of gnotobiotic and inoculated conventional chickens increased over a period of 6 to 8 days following inoculation. Plasma peptidase activity of the gnotobiotic chickens peaked at 6 to 8 days, and then decreased. In the inoculated conventional chickens the plasma peptidase continued to increase until 9 to 10 days post inoculation after

with time it decreased. Some increase was noted in the plasma peptidase activity of unfasted but conventional chickens, but the values were much lower than the gnotobiotic or inoculated conventional chickens.

Four days following the administration of L. faecalis gnotobiotic and conventional chickens showed a decrease in plasma acid phosphatase activity. After this time the level of this plasma enzyme increased until 11 days after inoculation. The plasma acid phosphatase level of unfasted conventional chickens showed only minor changes.

The plasma glutamic-oxaloacetic transaminase activity of gnotobiotic and inoculated conventional chickens decreased for a period of 4 days following inoculation with L. faecalis. This was followed by a gradual increase in activity by the gnotobiotic chickens until 9 to 10 days after which it declined. During the period of 4 to 8 days after inoculation there was no change in the plasma GOT activity of the inoculated conventional chickens, but a four-fold increase had occurred by 10 to 11 days post inoculation.

All plasma glutamic-pyruvic transaminase activities from birds inoculated with L. faecalis decreased between 4 and 6 days, and then increased until the 9 to 11 day period. The GPT activity of the plasma of the conventional and gnotobiotic chickens showed considerable variation.

These studies indicate that the gnotobiotic chicken has normal antibody producing potential.

The great decrease in the number of organisms in the feces during the period of 3 to 5 weeks after inoculation may be a result of the action of fecal antibodies and/or other host defense mechanisms

such as phagocytosis of *L. fusiformis* by cells of the lymphatic system of the intestine.

It can be assumed that the feeding of either of these stereoisomers not only the production of antibodies, but produces concurrent changes in the plasma enzyme levels of peptidase, acid phosphatase, and transaminase.

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