THE RELATIONSHIP OF PLASMA GLUTAMIC OXALACETIC TRANSAMINASE TO LIVER LESIONS FROM HISTOMONIASIS IN TURKEYS

by 1264

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INTRODUCTION

Blackhead (Enterohepatitis) disease has been considered one of several economically important diseases which infect both turkeys and chickens. United States Department of Agriculture in 1965, estimated that the losses from this disease in 10 years approximated $10,000,000 (Peardon, 1967). Blackhead disease is caused by infection with Histomonas meleagridis (Smith, 1895, Tyzzer, 1920). This protozoan primarily affects the caeca and is carried by the hepatic portal vein to the liver (Clarkson, 1961) where it causes degeneration of the hepatic tissue.

A considerable number of studies have demonstrated the efficacy of different drugs against histomoniasis in turkey either prophylactically or therapeutically. In association with several diseases, studies have been conducted on the changes in the serum glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and lactic dehydrogenase (LDH) levels in mammals such as cattle, dogs, mice, pigs, rodents, horses and man. Changes in the levels of these blood enzymes have been considered a good means for evaluating damage done by these diseases, especially those which infect the heart or the liver. GOT elevation in the blood of turkeys infected with H. meleagridis has been reported by McDougald (1969).

Because increased levels of GOT are related to histopathological damage in the liver, there is a possibility that GOT levels could be an indicator of arrested disease following therapeutical treatment or an indicator of prophylactic value of new drugs.

The objectives of this study were 1) to relate GOT levels with liver damage (number of lesions) and 2) to relate the findings in (1) to those

obtained when using an effective histomonastat.

REVIEW OF LITERATURE

Blackhead, a disease of chickens, turkeys and some other gallinaceous birds has been studied by many workers. Smith (1895) reported that Amoeba meleagridis, a protozoan, was the etiologic agent causing this disease. Tyzzer (1920b) showed that A. meleagridis was a flagellate and he renamed it Histomonas meleagridis. Many investigators have reported various ways by which fowl may be infected with the protozoan. Turkeys have been infected with histomoniasis by feeding embryonated eggs of Heterakis gallinarium (Tyzzer and Fabyan, 1920; Graybill and Smith, 1920; Malewitz, Runnells and Calhoun, 1958). Tyzzer and Collier (1925) succeeded in infecting turkeys per os and per rectum using infections of liver material. Delaplane (1932) succeeded in producing blackhead disease in turkeys with organisms cultured in vitro. Farmer and Stephenson (1949) obtained a cecal emulsion from infected turkeys and injected this material rectally into turkeys which later showed a high rate of infection.

Few workers have reported the effect of age of the host on its susceptibility to histomoniasis. Desowitz (1951) reported that chickens of various ages rectally infected with liver and cecal material from infected turkeys showed different mortality rates. The highest mortality was 71% for 21-day-old birds, whereas, less than 30% died among 34-day-old birds. Ohara and Reid (1960) reported that one-month-old chickens infected orally with embryonated heterakid eggs had a higher number of liver lesions than did birds of one and two days old or three months old. Among a group of chickens infected intrarectally, the two-month-

old birds were more susceptible to infection than were those one-day-old or three months old. Ohara and Reid (1961) reported 32-day-old chickens fed embryonated heterakid eggs were more susceptible to histomoniasis than were chickens 1, 46 or 64 days of age. They hypothesized that very young birds and those three months old might develop some type of resistance to histomoniasis.

Most studies have concentrated on the pathogenicity of H. meleagridis. Clarkson (1961) found that H. meleagridis was transferred from the caeca via blood stream to the liver which later developed lesions. The number of lesions varies according to the severity of the infection. Smith (1895) recorded several changes in both caeca and liver in the advanced stages of the disease. Also he showed that the distal end of the caeca was the first place to be invaded by histomonads. Hadley (1916a) reported that infected livers of turkeys showed circular, yellowish or grayish, slightly depressed narcotic foci. These foci in an early infection were characterized by separated boundaries which later became aggregated. Allen (1941) differentiated between lesions of histomoniasis and trichomoniasis in turkeys. The lesions produced by trichomoniasis are characterized by a granular, cream-colored necrosiswithout regular outlines and appear as a group of very small rice-shaped granules extending star-like from the center. The lesions resulting from histomoniasis are almost circular, depressed and cream-colored. Sometimes, in severe infections, the liver lesions coalesce to form large necrotic areas. Bayon (1937) described the liver in turkeys infected with histomoniasis as enlarged, swollen, dark and congested with the surface showing several irregularly dirty-green to muddy-yellow round lesions. Harrison, Hansen, DeVolt, Holst and Tromba (1954) reported that in poults

a small portion of infected liver was representative of the distribution of lesions over the entire liver.

Farmer, Hughes and Whiting (1951) reported the development of histomoniasis and changes in the caeca and liver in a group of experimentally infected poults. Microscopic studies showed a slight changes in the cellularity of the caeca but not in the liver at one day post-infection. On the fourth day post-infection, microscopic studies showed several changes in the liver tissue. On the fifth day post-infection, the liver surface showed small lesions which were large and clear by the sixth day. Lund (1955) found that liver lesions in infected turkeys appeared by the sixth day post-infection and became common by the seventh day. Malewitz (1956) found in a group of experimentally infected turkeys that their livers showed a number of lesions which were round and circumscribed while the caeca were highly necrotic with cecal cores. Microscopic studies showed hyperemia, hemorrhage, lymphocytic infiltration, presence of giant cells, and necrosis. Malewitz, Runnells, and Calhoun (1958) reported that the livers of infected turkeys showed an increase in size with several greenish-yellow, necrotic areas. These necrotic areas were infiltrated with lymphocytes, macrophages and a large number of giant cells.

Treatment and prevention of histomoniasis has been studied by many workers. Blount (1938) reported that spircoid oral tablets and mapharside intramuscular injections showed promise for controlling blackhead disease. Farmer (1950) demonstrated that Acetarsol, Vioform, Arsphenamine and Digluocide had therapeutic value. Harton, Smith and Long (1951) found that Enpheptin-T in a concentration of 0.1% in food was effective against histomoniasis in turkeys.

Grumbles, Boney and Turk (1952) reported that 2-amino-5-nitrothiazole in concentrations of 0.05%, 0.04% and 0.03% as a feed additive prevented deaths, but it was not effective therapeutically. Lucas (1961) found that 1,2-dimethyl-5-nitroimidazole was effective in turkeys prophylactically when fed 48 hours prior to exposing them to infection. Sullivan, Kingan, Grace and Kelley (1964) examined the prophylactic potential of four compounds in turkeys. The compounds were 0.025% 4-nitrophenylarsonic acid, 0.0115% furazolidone, 0.0375% P-uriedobenzenearsonic acid and 0.035% nithiazole. They found that 0.025% 4-nitrophenylarsonic acid was more effective against histomoniasis in turkeys than the other drugs. Other workers reported that P-uriedobenzenearsonic acid as a feed additive was effective therapeutically against histominiasis in chickens at a concentration of 0.025% or 0.050% (Peardon and Eoff, 1967; Peardon and Ransay, 1967). Morehouse, Rude and Vatne (1968) reported that the administration of 125 mg of 1,2-dimethyl-5-nitroimidazole given orally was therapeutically effective against H. meleagridis in turkeys. They found its effects appeared within two days when a remarkable remission of lesions occurred in the liver and caeca. Furthermore, it was difficult to find traces of liver lesions in turkeys necropsied three and four weeks post-medication.

Studies on the activities of several enzymes (GOT, GPT, LDH) in the blood of diseased animals have demonstrated the relationship between liver and heart and the activity of certain blood enzymes. Serum glutamic oxalacetic transaminase changes in the blood of mammals are considered as a very sensitive index of damage to the heart and liver. Amador, Massod and Franey (1967) showed that the upper limit of serum glutamic oxalacetic transaminase of normal humans was 25 units/ml at

25 C. Ranke, Tauber, Horonick, Ranke, Goodhart, and Chow (1960) reported that glutamic oxalacetic transaminase level in healthy young people (20-30 years old) was 16.5 units/ml, while 13.0 units/ml is usual at 70-80 years old.

Conconi, Menenti and Benatti (1963) showed that when glutamic oxalacetic transaminase activities were determined in 100 people according to their ages, there was a high level of GOT in 65-74 year olds. Christiansson and Josephson (1960) found that the activities of GOT in normal one-week-old babies was about 35 units/ml, dropping to 23 units/ml after the first week, while in adults the level was about 18 units/ml. Stejskal and Teyschl (1964) reported that GOT activities in children was high at 1-6 months but decreased at 6-12 months. Molander, Sheppard and Payne (1957) considered GOT activities as a good index of liver cell functions, and any disturbance to the liver would elevate the GOT level in the blood. Musser, Ortigoza, Vazquez and Piddick (1966) stated that any morphological changes in the liver could lead to an elevation of the GOT in the blood stream.

Toxic damages in human liver and high level of GOT in the blood have been reported following CCl_4 poisoning (Toeppich, Straubband Minden, 1961; Wröblewski, John and LaDue, 1955; Ratnam, 1958). Rietschmann (1960) reported the GOT level increased to 400 units/ml in patients with hepatitis, cirrhosis, neoplasm and other liver disorders. Talavadeker (1966) reported the elevation of GOT in several patients with hepatitis and obstructive jaundice. Brante, Jonsson, Bromo and Ericsson (1956) recorded that the elevation of GOT in the blood stream could be considered as a means of evaluating the degree of damages in hepatitis, mononucleosis, and ligation of the hepatic artery. Molander, Wröblewski and LaDue

(1955) found that the GOT activities are a good index of liver cell damage in rat. They also reported a relationship between the liver necrosis and GOT activities. Dessi and Gianni (1959) reported a lowering of GOT level during liver regeneration in rats following a partial hepatectomy.

Braun, Papp and Horvath (1958) reported elevation of GOT level in dogs showing liver damage. Trincao, Trincoa, Breda, Gaspar and Gomes (1961) reported elevation of GOT in dogs and rabbits as a result of toxication with CCl_4 and concomitant liver damages. Cornelius (1959) reported the elevation of GOT in the blood stream after administering CCl_4 to horses, cows, pigs and dogs. Fahmy, Talaat and Zachary (1960) reported the elevation of GOT in dogs as a result of ligation of the hepatic artery.

Ford and Boyd (1962) reported cattle liver lesions and an increased GOT level after experimentally administering dimidium bromide to cattle.

Studies on enzyme activities of the blood in avian diseases are few. McDaniel and Chute (1959) reported levels of several enzymes in chickens between 5 weeks to 6 months. They also reported that the GOT level was elevated in coccidiosis, leukosis and sulfonamide toxicity. Cornelius, Law, Julian and Asmundson (1959) observed an elevated GOT level in the blood of chickens having an inherited muscular dystrophy disease. Brown and Abrams (1965) reported an increase in GOT level in the chicken's blood because of toxicosis. Banerjee and Rao (1966) conducted studies on the effects of several diseases on GOT activities in the chicken's blood. They found the GOT level increased markedly with ortherosclerosis and hypercholesteremic infections. McDouglad (1969) noted a marked elevation of several enzymes including GOT in the blood of both chickens

and turkeys having histomoniasis.

MATERIALS AND METHODS

Experimental birds

One-day-old Hy-white turkey poults were obtained from commercial hatcheries.[1,2] Upon arrival, all birds were intranasally inoculated with Newcastle disease vaccine.[3] The birds were kept in electrically heated cages and fed an antibiotic-free ration designed for poults.

Experimental technique

Infective Histomonas meleagridis used in all experiments were cultured in vitro using a modified De Volt's medium (De Volt, 1943; Ostlind, 1966; Ruff, 1968).

At the beginning of these studies, a dose of 30,000 H. meleagridis was inoculated intrarectally into 2-month-old male turkeys in order to verify the pathogenicity of the strain. Eleven days post-infection, the infected turkeys were sacrificed and H. meleagridis plus associated bacteria were isolated from the caeca. The livers of these birds were necrotic, thus verifying the pathogenicity of the strain. Two to four drops of caecal contents were inoculated into several culture tubes of medium. Subcultures were made at 5-day intervals. Enumerations of the population of histomonads were done using a hemacytometer.

In experiment 1, eighteen 15-day-old poults were divided into an infected group of 12 birds and a non-infected control group of 6 birds. Each bird in the group to be infected with histomonads and controls

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[1] Sunshine State Hatchery, Watertown, South Dakota.

[2] Stormking Hatchery, Holstein, Iowa

[3] Live virus, B, Type, Salsbury Laboratories, Charles City, Iowa.

was anesthetized with ether prior to performing a laparotomy. When the left caecum was exposed, 10,000 Histomonas were injected into the cecal lumen, the wound was sutured and the bird placed in a cage to recover. The birds in the control group were treated the same as the infected group except that a 1% saline solution instead of histomonads was injected into the caecum. Eleven days post-infection all birds were sacrificed by decapitation. Blood was collected in beakers previously washed with 1% heparin solution to prevent clotting. The blood was centrifuged, and the plasma was collected with a disposable pipette and then transferred into small vials for storage at -20 C. The liver of each bird was removed from the abdominal cavity, photographed and then placed in a jar containing 5% formalin for later enumerating the number of lesions.

In experiment 2, 21 birds, 30-day-old, were divided into three groups; 1) non-infected control group of 3 birds injected with 1% saline solution via laparotomy, 2) non-infected control group of 6 birds and 3) infected group of 12 birds. The plasma and livers were collected from these birds as previously described.

In experiment 3, thirty 15-day-old birds were divided into three groups, an infected control group of 12 birds, an infected treated group of 12 birds and a non-infected control group of 6 birds.

The drug Salfuride,[1/] (histomonastat) was used in this experiment as a feed additive. In order to get an even distribution of the drug in the turkey feed, Salsbury Laboratories suggested that the feed granules be ground to pass a 4/64" mesh. Salfuride was used in a

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[1/] New histomonastat in turkey, Salsbury's Laboratories, Charles City, Iowa.

concentration of 10 parts per million which would give partial protection against H. meleagridis. This ground feed was divided into two parts, one part was used to feed all birds until the test was initiated when the second part was mixed with the drug to be fed to the treated group only.

The formulation of medicated feed was 150 mg of Salfuride, 100 mg of starch as a carrier to 14.90 kg of feed. Because of the mechanical V- mixer would not hold more than 5 kg, only one third of the formulation was processed at a time. When the three portions had been mixed, they were combined and stored. All birds of the three groups were started on unmedicated feed one week prior to infection. Beginning on the 8th day post-infection, the treated group was given the medicated feed for 4 days, and then on the 11th day post-infection all birds of the three groups were necropsied, the plasma collected and tissue damage assessed.

Plasma Glutamic oxalacetic Transaminase (GOT)

Plasma glutamic oxalacetic transaminase activities were determined using a commercial assay kit[1/] and a Spectrophotometer 20 adjusted for a wavelength of 366 mu. In order to prepare the plasma samples for photometric analysis, 3 ml of double distilled water were first added to the substrate and then 0.5 ml of plasma. This mixture was transferred to a one cubic millimeter cuvette used in the spectrophotometer. After 1 minute, the absorption was recorded followed by three more readings at 1-minute intervals. The mean optical density difference per minute ($\Delta E/min$) was determined by substracting the last reading

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1/ Boehringer Corporation, New York, New York.

from the first reading and dividing by 3. The (ΔE/min) was used for calculation either by using the table of values provided with the test kit or by converting these values (ΔE/min) to Boehringer-Mannheim milli-units (m /ml) of plasma. These units were calculated by multiplying the (ΔE/min) x 2120. According to Wrōblewski, each Boehringer-Mannheim milli-unit corresponds to two units of Wrōblewski.

RESULTS

Data in experiment 1 show an elevation of the average GOT level (138-215 mU/ml) in turkeys 11 days post-infection (Table I). The infected turkeys showed severe liver damage characterized by variable numbers of circular, small, cream-colored or yellow-green lesions with depressed centers. These lesions covered all surfaces of the liver lobes and ranged in average numbers from 169 to 632 (Table I, Fig. 1). The livers from the control group had no lesions and the plasma of these birds had a normal amount of GOT (65-85 mU/ml), (Table II, Figs. 1, 6).

The statistical analysis using correlation coefficient in experiment 1 showed a significant positive relationship ($P<0.01$) between GOT levels in the plasma and the number of lesions in the infected birds (Fig. 10).

Data from experiment 2 show an elevation of GOT levels in 30-day-old infected turkeys 11 days post-infection when compared with the controls (69-79 mU/ml), (Tables III, IV, Fig. 11). Liver damage occurred in all the infected turkeys as shown by typical small and large lesions (Figs. 2, 3, Table III). The control group had normal livers without lesions and normal amounts of GOT (Table IV, Figs. 2, 7). As in experiment 1 there was a significant positive correlation between the GOT level and the number of small lesions on the livers. Likewise, there was a significant positive correlation ($P<0.05$) between the GOT levels and the number of large lesions (Table III, Figs. 2, 3, 11).

Data in Table V show the results of experiment 3. The infected non-treated (no histomonastat) turkeys had higher levels of GOT than did the infected treated (Table V, VI, Figs. 12, 13) and control group

(Table VII, Fig. 8). Liver lesions were observed in all birds in the infected non-treated group (Table V). The controls were negative. There were significantly ($P<0.01$) fewer lesions and lower levels of GOT in the infected treated group than in the infected non-treated group (Tables V, VI, VII).

Experiment 4 was a replication of experiment 2 except that the turkeys were obtained from another source and that a different sub-culture of H. meleagridis was used to infect the birds. Again, the GOT levels of infected birds was significantly ($P<0.01$) higher than the control group (Tables VIII, IX). Likewise, there was a significant correlation between the number of lesions (Figs. 4, 5) and the level of plasma GOT (Fig. 14).

Table I. EXPERIMENT 1. Plasma glutamic oxalacetic transaminase activities and number of lesions in 15-day-old infected Hy-white turkeys, 11 days post-infection.

Bird No.	First Reading (mU/ml)	Second Reading (mU/ml)	Average (mU/ml)	No. of Lesions (small)
6451	218.36	212.00	215.18	632
6467	205.64	205.64	205.64	498
6431	195.04	197.10	196.07	471
6473	197.16	190.80	193.98	459
6463	184.44	175.96	180.20	421
6498	175.96	169.60	172.78	402
6471	163.24	169.60	166.42	380
6495	154.76	163.24	159.00	361
6477	154.76	148.40	151.58	330
6465	148.40	148.40	148.40	316
6468	142.04	148.40	145.22	305
6479	142.04	133.56	137.80	169

Table II. EXPERIMENT 1. Plasma glutamic oxalacetic transaminase activities in 15-day-old control Hy-white turkeys.

Bird No.	First Reading (mU/ml)	Second Reading (mU/ml)	Average (mU/ml)
6458	85.00	85.00	85.00
6454	85.00	85.00	85.00
6492	80.50	80.50	80.50
6497	80.50	80.50	80.50
6488	67.50	67.50	67.50
6489	67.50	63.50	65.50

Table III. EXPERIMENT 2. Plasma glutamic oxalacetic transaminase activities and number of lesions in 30-day-old infected Hy-white turkeys, 11 days post-infection.

Bird No.	First Reading (mU/ml)	Second Reading (mU/ml)	Average (mU/ml)	No. of Lesions (small)	No. of Foci (large)
6448	195.04	184.44	189.74	470	301
6439	169.60	175.96	172.78	421	350
6429	169.60	169.60	169.60	371	332
6440	163.24	173.84	168.54	382	321
6494	175.96	159.00	167.48	365	333
6485	163.24	163.24	163.24	315	218
6453	165.36	159.00	162.18	325	265
6457	152.64	163.24	157.94	300	275
6490	163.24	142.04	152.64	290	273
6493	152.64	142.04	147.34	282	230
6449	137.80	142.04	139.92	263	221
6472	142.04	133.56	137.80	252	195

Table IV. EXPERIMENT 2. Plasma glutamic oxalacetic transaminase activities in 30-day-old control Hy-white turkeys.[1/]

Bird No.	First Reading (mU/ml)	Second Reading (mU/ml)	Average (mU/ml)
6452	85.00	80.50	82.75
6478	80.50	80.50	80.50
6486	80.50	78.44	79.47
6462	78.44	69.96	74.20
6483	69.96	67.50	68.73
6466	67.50	63.50	65.50

[1/] Three controls injected with saline only had a range of GOT 68.73-79.47 mU/ml.

Table V. EXPERIMENT 3. Plasma glutamic oxalacetic transaminase activities and number of lesions in 15-day-old infected non-treated Hy-white turkeys, 11 days post-infection.

Bird No.	First Reading (mU/ml)	Second Reading (mU/ml)	Average (mU/ml)	No. of Lesions (small)	No. of Foci (large)
6593	212.00	201.40	206.70	585	441
6596	205.64	205.64	205.64	578	511
6582	197.16	190.80	193.98	519	470
6597	190.80	184.44	187.62	498	450
6581	169.60	163.24	166.42	400	303
6598	142.04	133.56	137.80	315	282
6595	131.44	133.56	132.50	314	260
6576	133.56	131.44	132.50	299	218
6575	131.44	127.20	129.32	280	230
6589	116.60	122.96	119.78	210	191

Table VI. EXPERIMENT 3. Plasma glutamic oxalacetic transaminase activities and number of lesions in 15-day-old infected treated (histomonastat) Hy-white turkeys, 11 days post-infection.

Bird No.	First Reading (mU/ml)	Second Reading (mU/ml)	Average (mU/ml)	No. of Lesions (small)	No. of Foci (large)
6586	163.24	154.76	159.00	431	313
6577	144.16	142.04	143.20	376	325
6591	133.56	142.04	137.80	315	215
6584	133.56	127.20	130.38	281	260
6590	112.36	106.00	109.18	205	171
6592	112.36	110.24	111.30	181	101
6587	99.64	99.64	99.64	168	119
6594	95.40	99.64	97.52	135	79
6588	99.64	91.16	95.40	111	89
6599	101.76	99.64	100.70	75	62

Table VII. EXPERIMENT 3. Plasma glutamic oxalacetic transaminase activities in 15-day-old control Hy-white turkeys.

Bird No.	First Reading (mU/ml)	Second Reading (mU/ml)	Average (mU/ml)
6515	85.00	85.00	85.00
6580	85.00	78.44	81.72
6514	80.50	78.44	79.47
6511	80.00	78.44	79.22
6579	78.44	74.26	76.35
6513	74.26	69.96	72.11

Table VIII. EXPERIMENT 4. Plasma glutamic oxalacetic transaminase activities and number of lesions in 30-day-old infected Hy-white turkeys, 11 days post-infection.

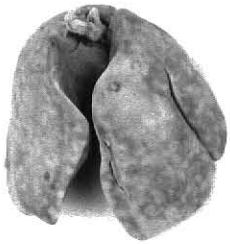
Bird No.	First Reading (mU/ml)	Second Reading (mU/ml)	Average (mU/ml)	No. of Lesions (small)	No. of Foci (large)
6500	424.00	453.68	438.34	997	605
6516	424.00	436.72	430.36	910	780
6524	373.12	390.00	381.56	805	635
65xx	296.80	309.52	303.16	735	513
6578	250.16	269.00	259.58	631	401
6522	226.84	233.20	230.02	570	365
6510	212.00	216.24	214.14	515	430
6512	212.00	207.76	209.88	509	400
6521	205.64	212.00	208.82	500	315
6517	195.04	197.16	196.10	462	352
6519	184.44	197.16	190.80	403	301
6520	190.80	184.44	187.62	312	281

Table IX. EXPERIMENT 4. Plasma glutamic oxalacetic transaminase activities in 30-day-old control Hy-white turkeys.

Bird No.	First Reading (mU/ml)	Second Reading (mU/ml)	Average (mU/ml)
7985	85.00	80.50	82.75
6548	78.44	80.50	79.47
6531	78.44	78.44	78.44
4443	74.20	67.84	70.02
7982	67.84	69.96	68.90
7990	63.50	69.96	66.73

EXPLANATION OF FIG. 1

Figure 1. Lesions on livers of infected 15-day-old turkeys (Nos. 6451,-67,-79, -63); controls (Nos. 6488,-58). Experiment 1.



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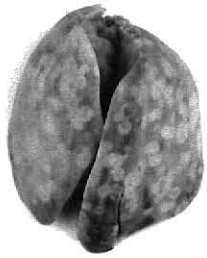

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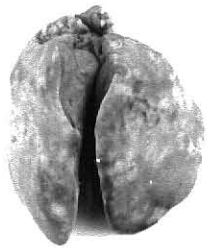


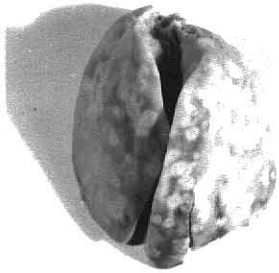
6463

6488

6458

EXPLANATION OF FIG. 2

Figure 2. Lesions on livers of infected 30-day-old turkeys (Nos. 6440,-48,-90,49); controls (Nos. 6462,-86). See Fig. 3 for enlarged photograph of Nos. 6440 and 6448. Experiment 2.



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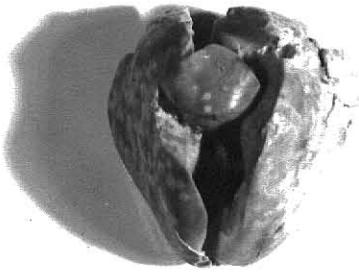

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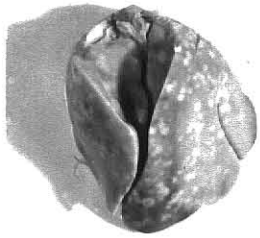

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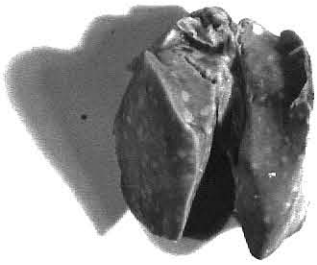

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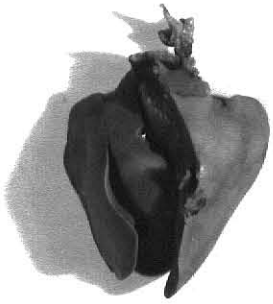


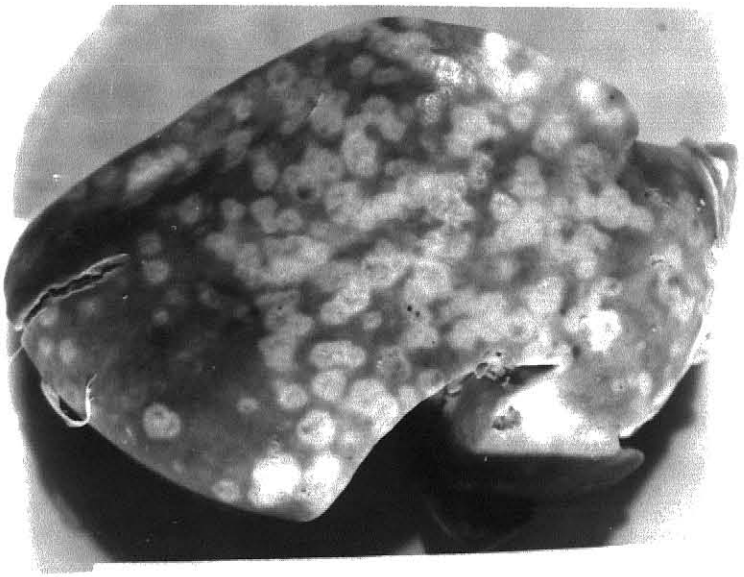
6462

6486

EXPLANATION OF FIG. 3

Figure 3. Lesions on livers of infected 30-day-old turkeys. Experiment 2.

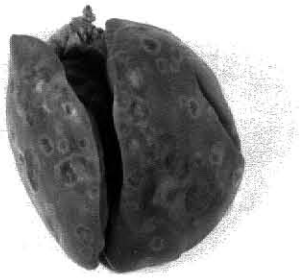
6440



6448

EXPLANATION OF FIG. 4

Figure 4. Lesions on livers of 30-day-old infected turkeys (Nos. 6516,-00, -10,-19); controls (Nos. 6509,-23). Experiment 4.



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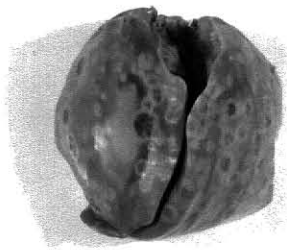
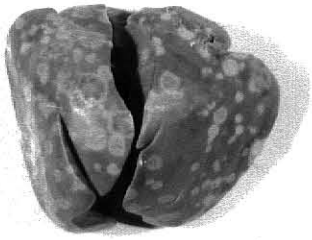
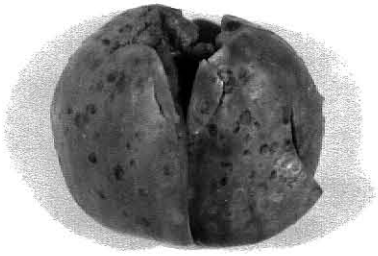


6500



6510



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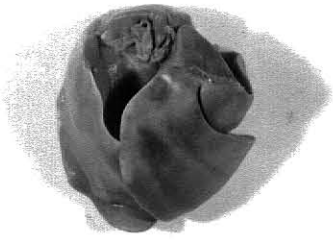
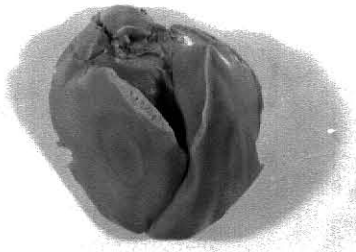


6509



6523

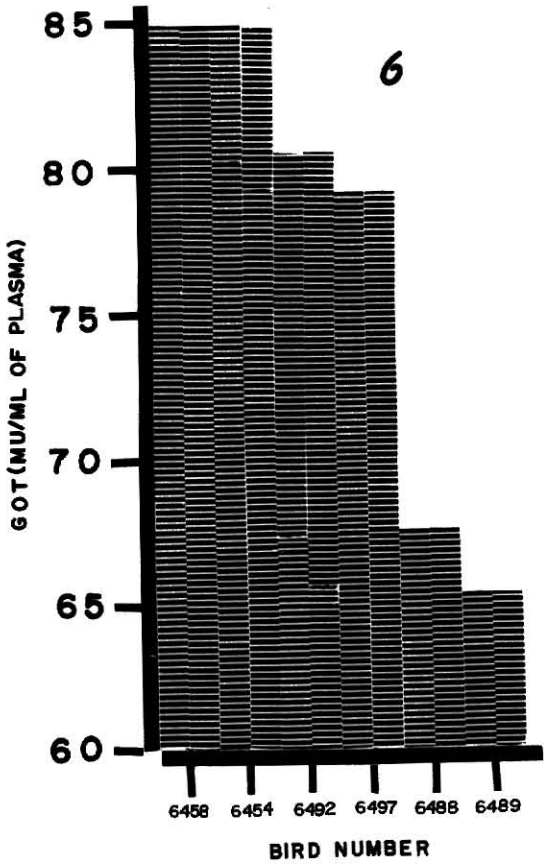
EXPLANATION OF FIG. 5

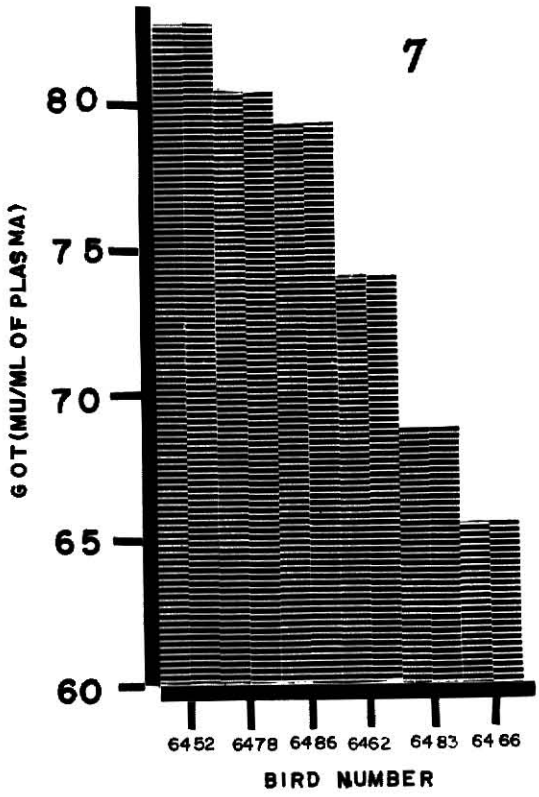
Figure 5. Lesions on infected liver of 30-day-old turkey. Note small and large lesions. Experiment 4.

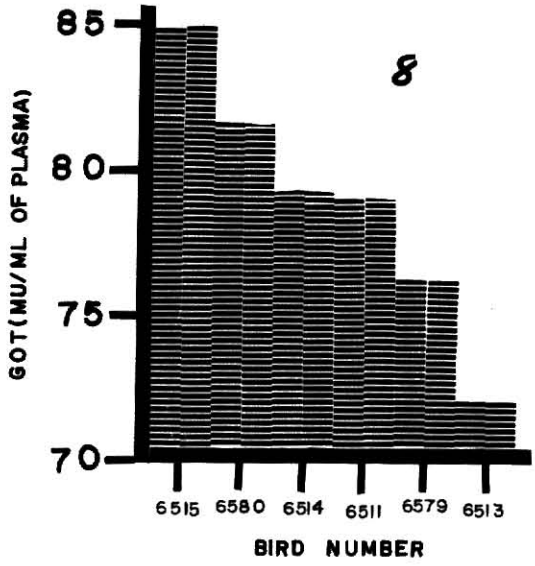

6510

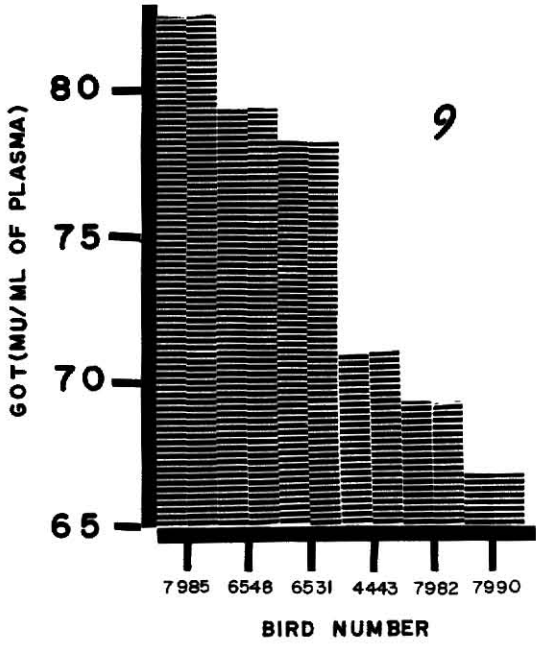
EXPLANATION OF FIGS. 6, 7, 8 and 9

Figures 6, 7, 8 and 9. Glutamic oxalacetic transaminase levels in uninfected control turkeys. Experiments 1, 2, 3 and 4, respectively.


6
85
80
75
70
65
60
GOT(MU/ML OF PLASMA)
6458 6454 6492 6497 6488 6489
BIRD NUMBER

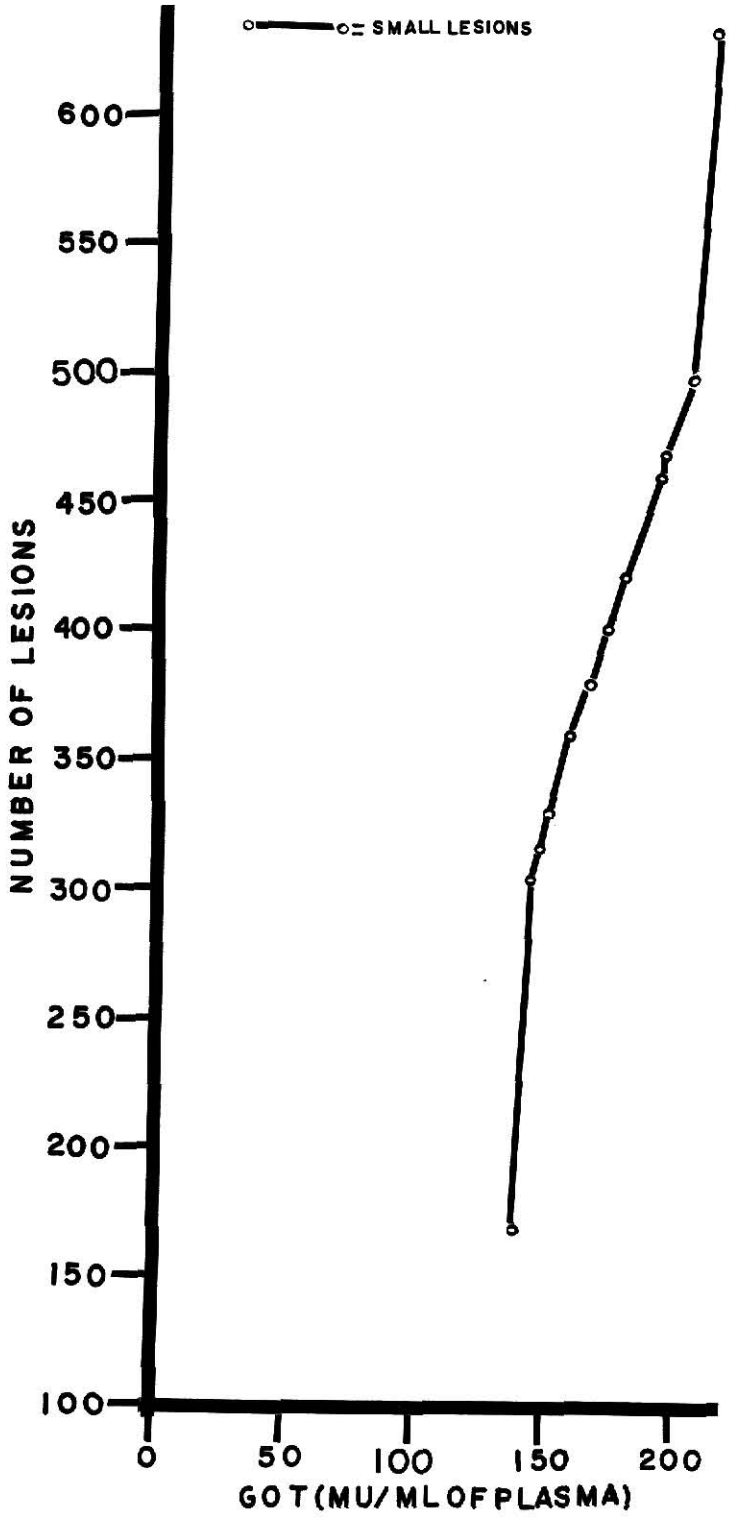

7
80
75
70
65
60
GOT(MU/ML OF PLASMA)
6452 6478 6486 6462 6483 6466
BIRD NUMBER


8
85
80
75
70
GOT(MU/ML OF PLASMA)
6515 6580 6514 6511 6579 6513
BIRD NUMBER


9
80
75
70
65
GOT(MU/ML OF PLASMA)
7985 6548 6531 4443 7982 7990
BIRD NUMBER

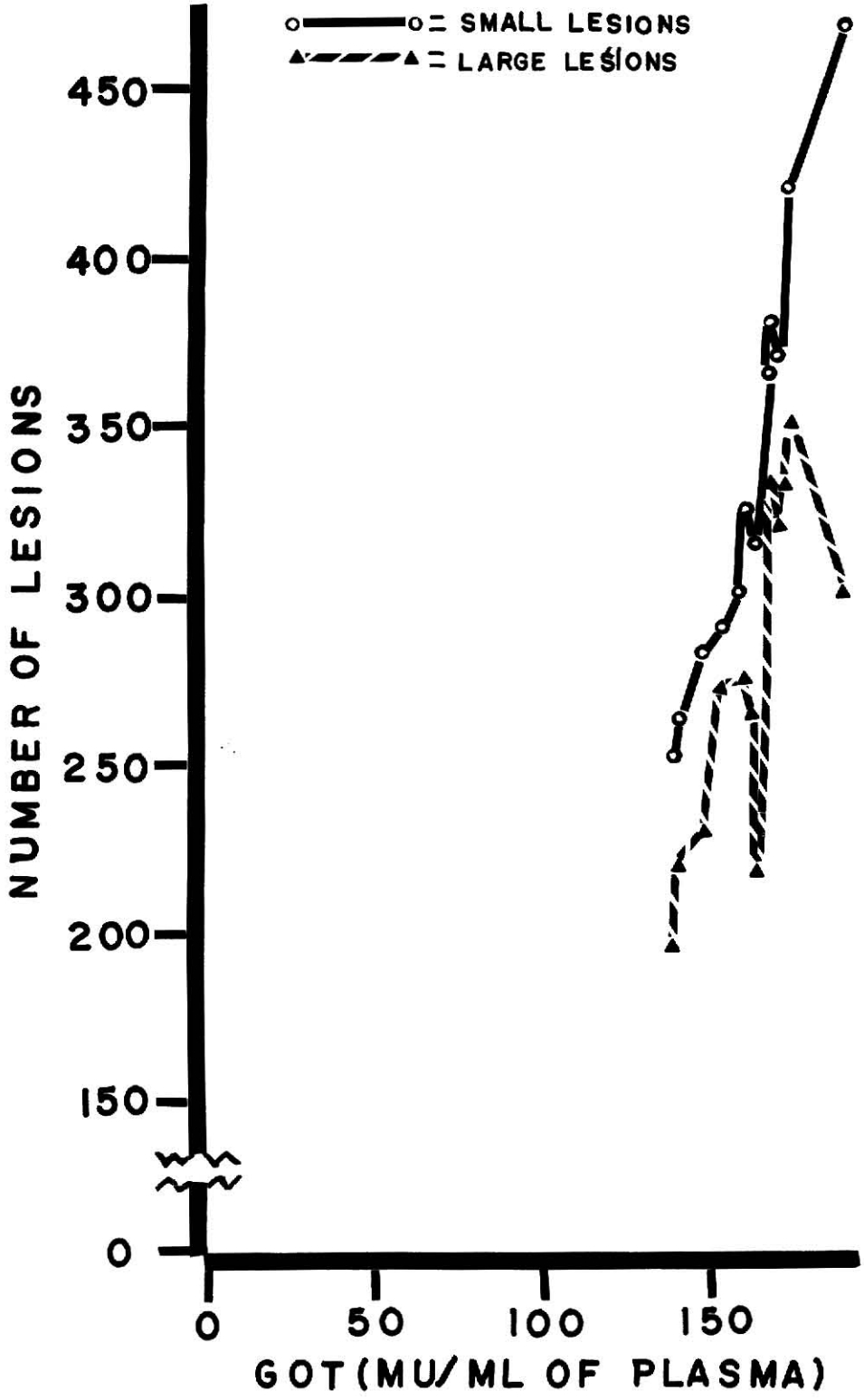
EXPLANATION OF FIG. 10

Figure 10. Relationship between GOT level and the number of lesions. Experiment 1.


SMALL LESIONS
600
550
500
450
400
350
300
250
200
150
100
NUMBER OF LESIONS
0
50
100
150
200
GOT (MU/ML OF PLASMA)

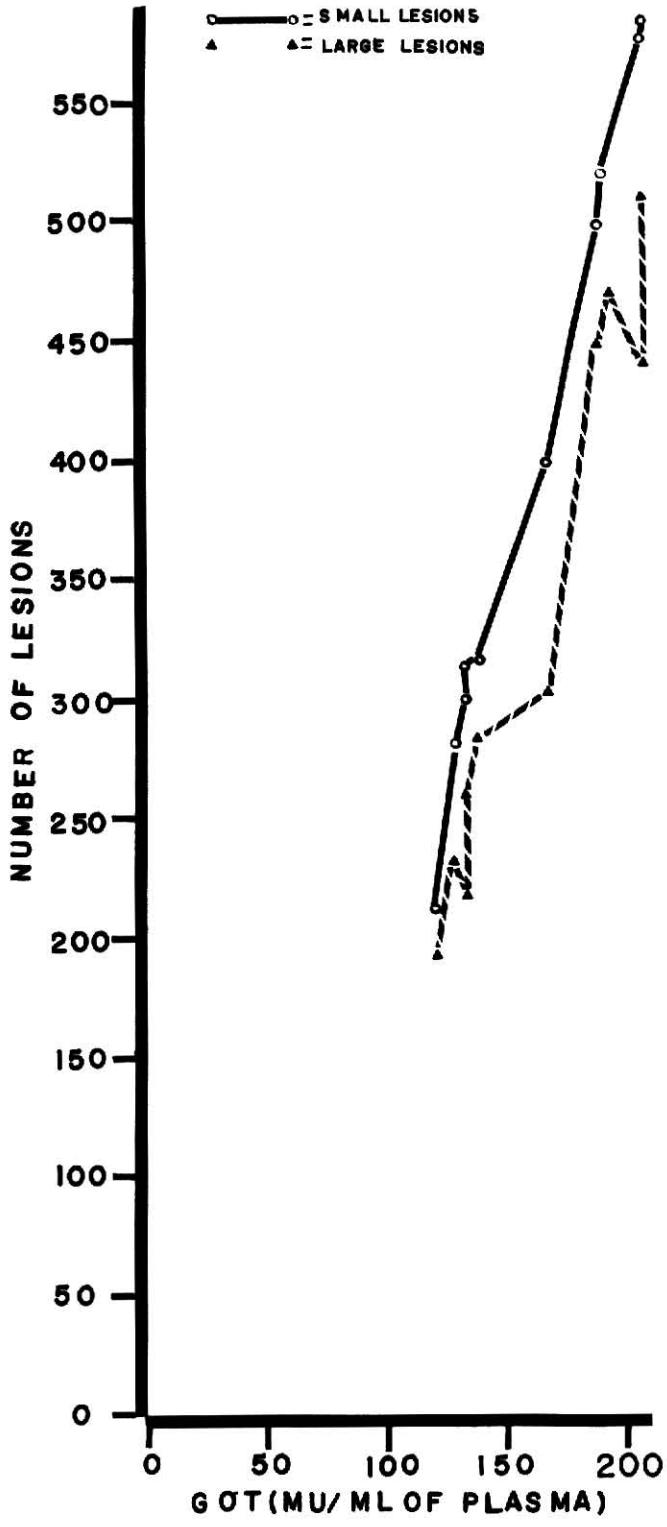
EXPLANATION OF FIG. 11

Figure 11. Relationship between GOT level and the number of lesions. Experiment 2.


o—o = SMALL LESIONS
▲—▲ = LARGE LESIONS
NUMBER OF LESIONS
450
400
350
300
250
200
150
0
0
50
100
150
GOT (MU/ML OF PLASMA)

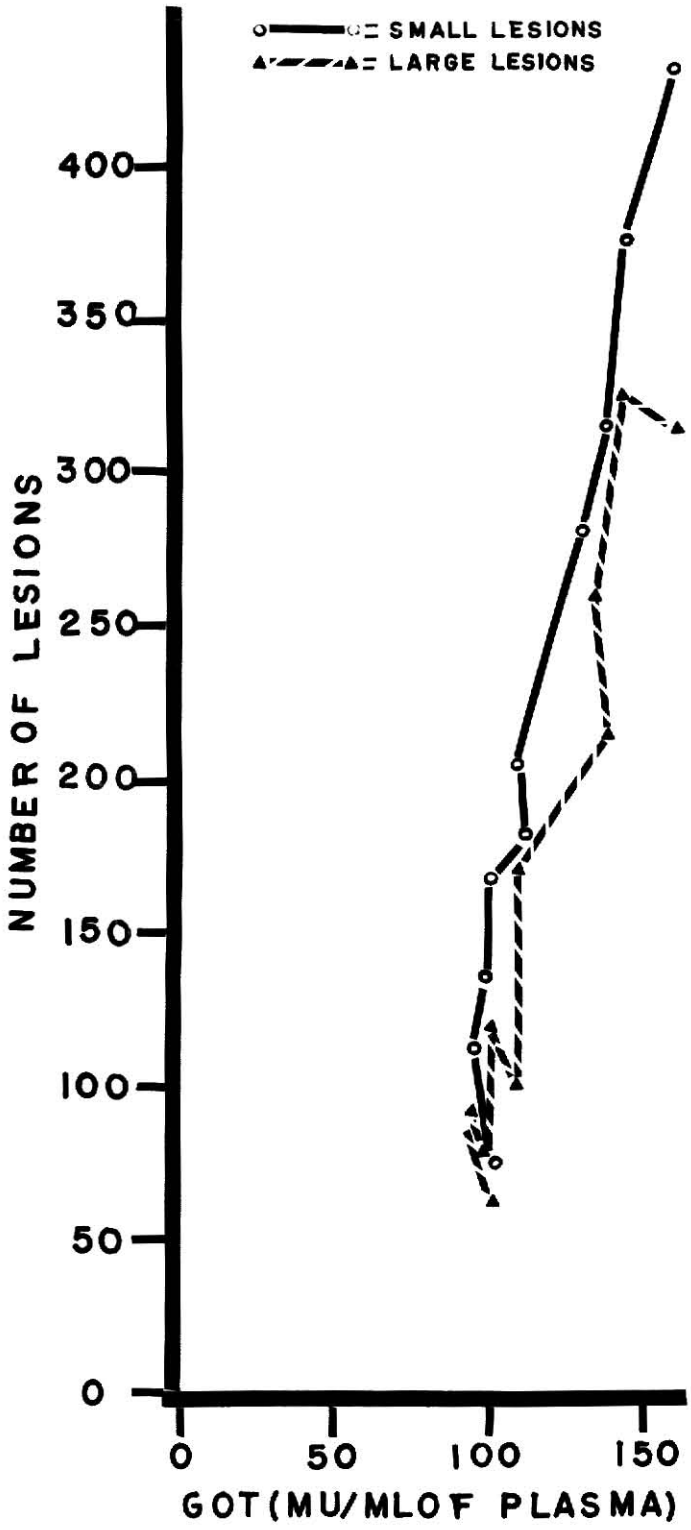
EXPLANATION OF FIG. 12

Figure 12. Relationship between GOT level and the number of lesions in the untreated turkeys. Experiment 3.


SMALL LESIONS
LARGE LESIONS
NUMBER OF LESIONS
550
500
450
400
350
300
250
200
150
100
50
0
0
50
100
150
200
GOT (MU/ML OF PLASMA)

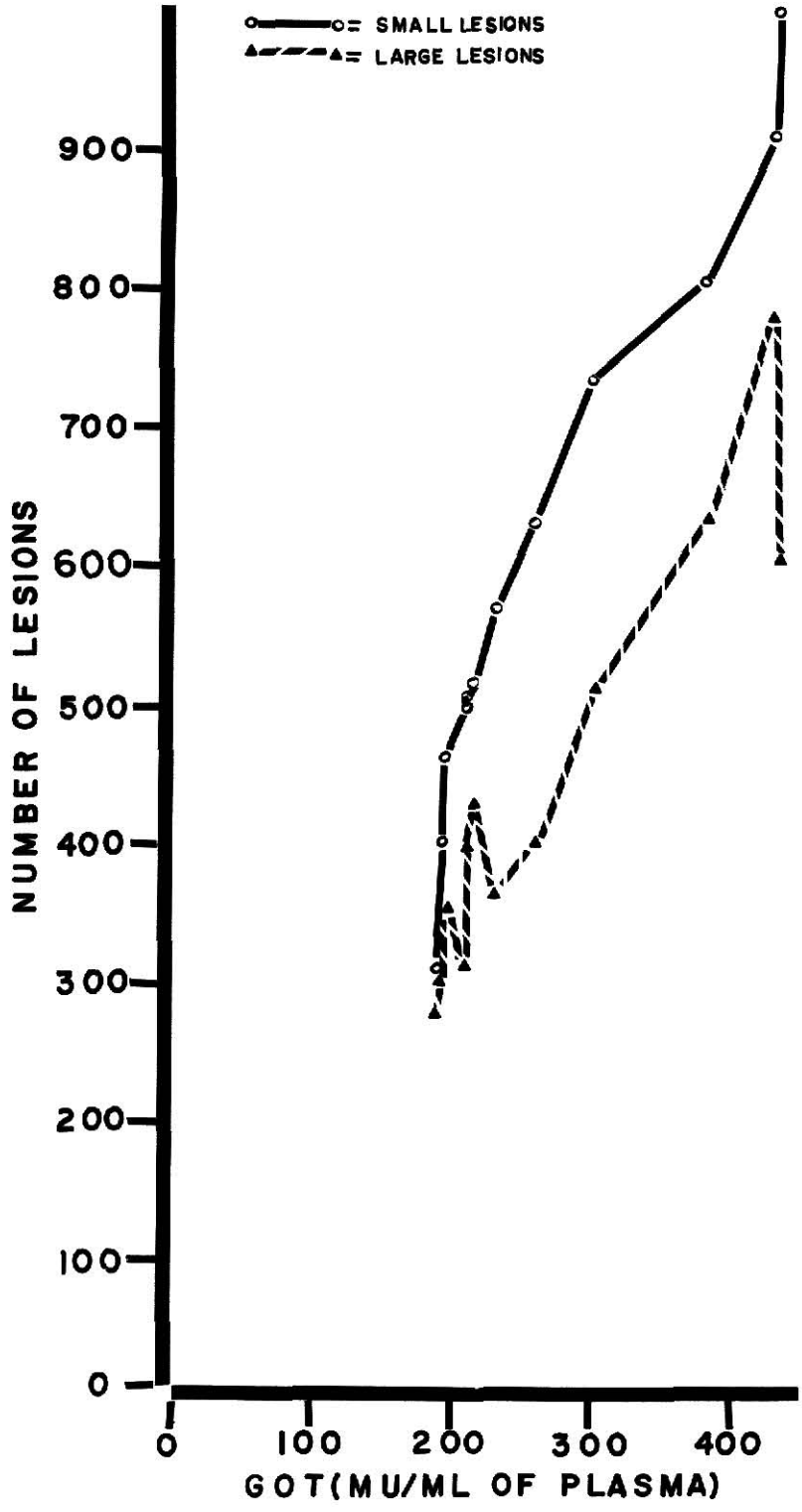
EXPLANATION OF FIG. 13

Figure 13. Relationship between GOT level and the number of lesions in the treated turkeys. Experiment 3.


SMALL LESIONS
LARGE LESIONS
NUMBER OF LESIONS
400
350
300
250
200
150
100
50
0
0
50
100
150
GOT(MU/MLOF PLASMA)

EXPLANATION OF FIG. 14

Figure 14. Relationship between GOT level and the number of lesions. Experiment 4.


SMALL LESIONS
LARGE LESIONS
NUMBER OF LESIONS
900
800
700
600
500
400
300
200
100
0
0
100
200
300
400
GOT(MU/ML OF PLASMA)

DISCUSSION

Glutamic oxalacetic transaminase is one of several enzymes which is highly concentrated in avian livers, therefore, liver damage could cause the elevation of this enzyme in the blood. Data from four experiments showed an elevation of GOT level in birds infected with histomoniasis. This elevation was the result of partial destruction of liver tissues as shown by lesions. All the controls had a normal level of GOT and no liver lesions.

The GOT level in the infected group treated with a histomonastat, Salfuride, was lower than that of the infected untreated group. Likewise, the number of lesions on the liver of the infected treated group were fewer, thus providing evidence that GOT levels show promise as a convenient and accurate method for evaluating the efficacy of drugs as histomonostats. Future studies will include in the experimental design a group of uninfected birds given the trial histomonostat in order to ascertain any effects of the trial drugs per se on GOT levels.

GOT levels in experiment 4 were more than 100 mU/ml of plasma higher than those obtained in experiment 2. This difference in GOT levels could be related to different supply sources for the turkeys or a change in pathogenicity of the histomonads with sub-culturing. In future studies these two factors will be considered in the experimental design. The results were not conclusive with respect to the age of turkeys conditioning susceptibility to histomoniasis even though Kendall (1957), reported that the age of turkeys was not related to susceptibility. However, Desowitz (1951) reported a greater mortality among chickens 21 days old, than among 34-day-old birds. Another study

showed 1-month-old chickens to be more susceptible than 1 or 2 day old or 2-month-old birds (Ohara and Reid, 1960; Ohara and Reid, 1961).

At necropsy, all the infected turkeys showed a variable number of liver lesions. These lesions were scattered over the entire surface of the liver with no predilection for a particular site. The majority of these lesions were a pale white color, some were yellow-green. The borders of the lesions varied from smooth to irregular in outline. Some lesions had crater-like depressions while others were not pitted.

The small lesions were the most numerous. The large lesions are aggregated small lesions. To date researchers have used the number of lesions, without regard to their size, as an index of the severity of histomoniasis in a host. This study has demonstrated that a more sensitive index can be attained if the number of small lesions making up the large lesions are enumerated for the total count.

The literature conclusively supports the fact that GOT is elevated in diseases causing destruction of mammalian liver tissue, however, such knowledge was unknown for avian species until McDougald (1969) showed that GOT was elevated in turkeys having histomoniasis. This study extended McDougald's study by showing that there was a correlation between the number of lesions and the level of GOT in the blood of infected turkeys.

With further study and refinement, the level of GOT in birds having experimentally induced histomoniasis will be useful for screening potential histomonastats and evaluating their efficacy.

SUMMARY

Histomonas meleagridis cultured in vitro was used to infect turkeys at 15 and 30 days of age.

A significant elevation of glutamic oxalacetic transaminase occurred in all infected turkeys 11-days post-infection. Liver damage was demonstrated in all infected birds by the many lesions characteristic of histomoniasis. These lesions were of two types, small and large. The small lesions occurred most frequently; the large lesions were aggregated small lesions.

There was a positive correlation between the amount of GOT and the number of lesions in each infected bird. Whereas the number of both types of lesions correlated ($P<.05$) with an increase in GOT, the number of small lesions more accurately ($P<.01$) expressed the GOT level.

Thirty-day-old turkeys may be less resistant to histomoniasis than are 15-day-old turkeys as shown by higher GOT levels in the former.

Salfuride, a new histomonastat, significantly reduced the level of GOT and the number of lesions during the course of the disease.

This study demonstrated that the GOT level is a good clinical indicator of the course of the disease, and shows promise as a quick and accurate method for use in screening potential histomonastats.

ACKNOWLEDGMENT

The author wishes to acknowledge his major professor, Dr. M. F. Hansen, for his valuable advice and guidance during the course of this study. The author is greatly indebted to Professor C. H. Lockhart for his advice in the preparation of this thesis. The author also wishes to thank Dr. H. A. Kadoum for his criticism and advice.

Special gratitude is also expressed to the fellows in the parasitology group, especially to Dr. R. L. McDougald for his encouragement and advice.

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THE RELATIONSHIP OF PLASMA GLUTAMIC OXALACETIC TRANSAMINASE TO LIVER LESIONS FROM HISTOMONIASIS IN TURKEYS

by

GHASSAN H. AL-KHATEEB

B.S. Baghdad University, Baghdad, 1960

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirement for the degree

MASTER OF SCIENCE

Parasitology

DIVISION OF BIOLOGY

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1970

Dosages of 10,000 organisms of a strain of Histomonas meleagridis were used to infect turkeys 15 and 30 days of age, respectively.

There was a significant elevation of plasma-glutamic oxalacetic transaminase in all infected turkeys 11-days postinfection.

Livers of these infected birds were damaged as evidenced by many lesions characteristic of histomoniasis. These lesions were of two types, small and large. The small lesions occurred most frequently; the large lesions were aggregated small lesions.

This study demonstrated a positive correlation between the amount of GOT and the number of lesions in each infected bird. Whereas the number of both types of lesions expressed an increase in GOT, the number of small lesions more accurately expressed the GOT level.

There was an indication that 30-day-old turkeys may be less resistant to histomoniasis than 15-day-old birds. The GOT level was higher in the former group than in the latter group.

Salfuride, a new histomonastat significantly reduced liver damage as expressed by less elevated levels of GOT and few liver lesions during the course of the disease. The use of GOT level for evaluating the course of the disease and recovery shows promise for a quick and accurate method for use in screening new potential histomonastats.