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SCHOOL OF AVIATION MEDICINE
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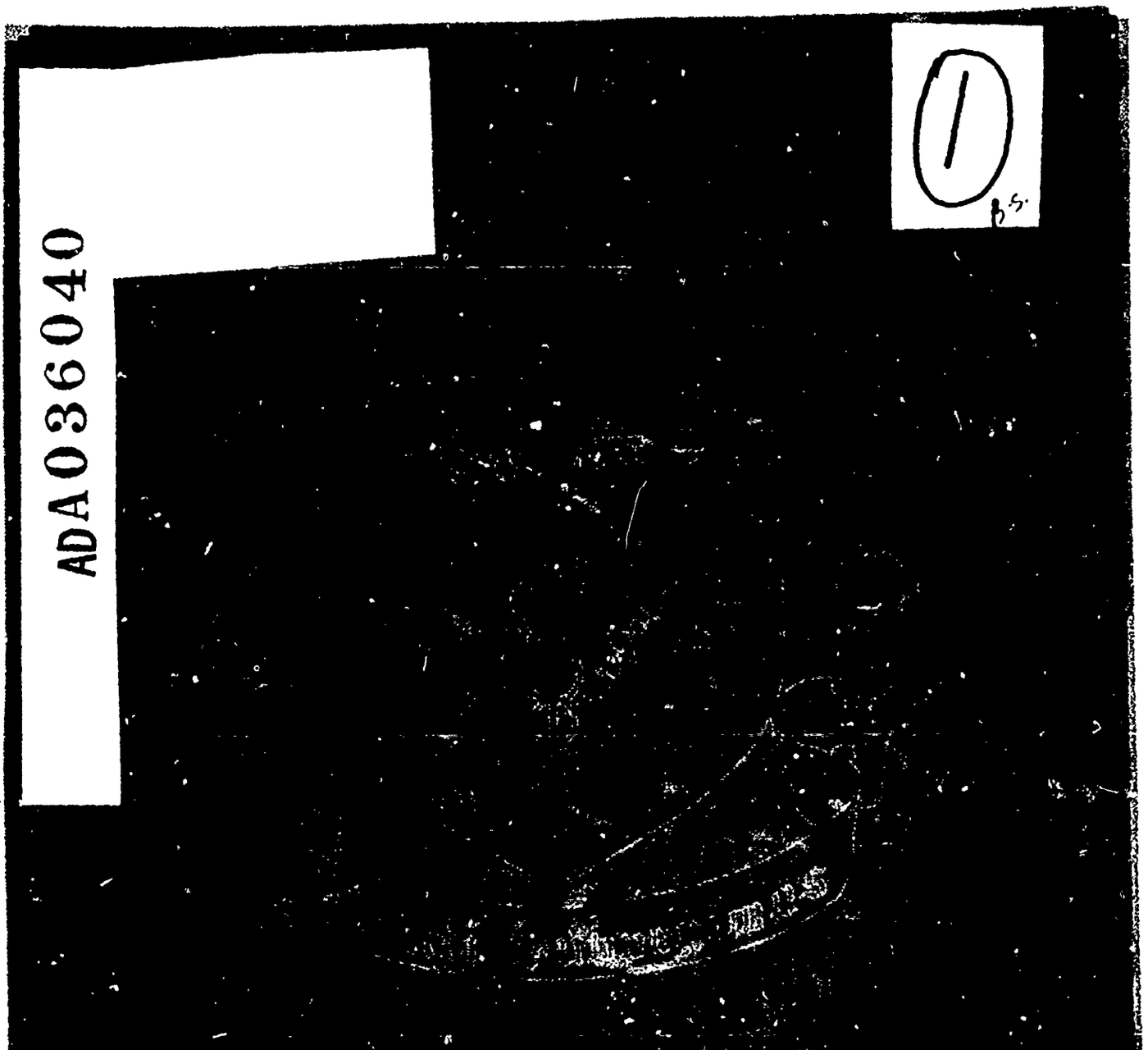
DECEMBER 1958



ENZYME ACTIVITY IN THE SERUM AND COMMON DUCT BILE OF DOGS

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The observations that patients with obstructive jaundice show elevated levels of serum glutamic oxalacetic transaminase and that ligation of the common bile duct in rats results in similar enzyme changes prompted direct investigation of the excretory pathway of the serum enzyme protein moieties. Mongrel dogs, subjected to cholecystectomy and ligation of the common bile duct, were used. Exposure of these animals to carbon tetrachloride vapor, with the expected massive increase in serum glutamic oxalacetic transaminase, failed to result in increased bile levels of the enzyme. Correspondingly, when pure enzyme was administered intravenously to these animals increased serum activity levels failed to induce a corresponding elevated bile activity. These data indicate that if the bile serves as an excretory pathway for serum enzymes, such pathway is minor.

The elevation of the serum enzymes glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in patients with obstructive jaundice (1, 2) suggested that the biliary system might serve as an excretory pathway for enzyme activity. The observation that gallbladder bile contained high enzyme activity (3) and that ligation of the common bile duct in rats was followed by an immediate increase in serum GOT activity which subsided promptly after release of ligation (4) also fitted with this hypothesis. It is now apparent, however, that biliary obstruction in humans is not invariably accompanied by rises in serum GOT (5, 6), and further work by Chinsky et al. (7) with common duct-ligated rats demonstrated immediate serum GOT and GPT rises followed by declining levels in spite of continuing obstruction. In an effort to correlate serum and biliary enzyme levels Pellegrini (8) measured GOT in rabbit gallbladder bile, but found great variability even in identical samples from one individual.

The present study was initiated to determine whether a significant portion of serum enzyme activity is excreted via the biliary system.

Received for publication on 20 August 1958.

METHODS

Animals and surgery

Female mongrel dogs were subjected to cholecystectomy and division of the common bile duct with cannulation of its proximal end.

The internal glass cannula led to an external condom-reservoir via gum rubber tubing. Bile was removed from the reservoir under antiseptic conditions at least daily.

Dogs were fed on lean horse meat to which Tween 80, one teaspoon per feeding, was added. Vitamins A, D, C, B-complex, and K were used as dietary supplements. Antibiotics (terramycin or chloramphenicol) were given daily by mouth. Animals remained in good health for months on this schedule.

Enzyme determinations

1. GOT and GPT were measured by the methods of Karmen et al. (9) and Wroblewski and LaDue (2), respectively.

2. Lactic dehydrogenase (LDH) was measured by the method of Wroblewski and LaDue (10).

3. Common duct bile was dialyzed for 24 hours in the cold against 0.005 molar phosphate buffer, pH 7.4, prior to enzyme analysis. Meister et al. (11) reported that such dialysis produces no loss in GOT activity; the same finding for GPT and LDH was confirmed in this laboratory. Dialysis was required to reduce the intensity of the bile sample blank on the Beckman DU spectrophotometer.

4. Samples of dialyzed common duct bile at room, refrigerator, and freezer temperatures were measured daily over a 2-week period. GOT, GPT, and LDH showed no loss of activity during the first week when stored in icebox or freezer. All samples collected were stored in the refrigerator and analyzed within a week of collection.

5. GOT concentrates were prepared by the method of O'Kane and Gunsalus (12). Pyridoxal phosphate (25 μg.) was added to the test mixture prior to each analysis in experiments involving the GOT concentrate. The LDH used for intravenous administration was obtained commercially.

RESULTS

Enzyme activity in the bile of control animals

Daily enzyme measurements, for periods of 2 to 7 weeks, were performed on the bile of 5 animals under simple maintenance conditions. GOT activity ranged from 0 to 64, GPT from 0 to 23, and LDH from 0 to 105 units/ml./min. Each animal produced approximately 60 to 100 cc. of bile per day. When total daily enzyme output was calculated (units/ml./min. × ml. bile), the variability remained unchanged.

Carbon tetrachloride administration

One dog was exposed to carbon tetrachloride vapor (approximately 200 p.p.m. for 5 hours) and serial measurements of enzyme activity in bile and serum were made for 130 hours postexposure. The results are shown in table I.

TABLE I

Enzyme levels following exposure to carbon tetrachloride vapor

Enzyme	Hours postexposure				
	0	24	36	60	132
Serum					
GOT	15	76	279	194	116
GPT	33	58	259	397	233
LDH	192	100	138	248	153
Bile					
GOT	20	19	13		7
GPT	3	4	5	5	3
LDH	12	27	26	26	

The bile output of the animal represented above remained constant at approximately 90 cc. per day. However, a second dog which received 5 cc. of carbon tetrachloride via gastric tube had an immediate, complete shutdown of bile production. Patency of the fistula was demonstrated by roentgenographic studies This animal has had only a 5-lb. weight loss and very gradually developing icterus for the past three months in spite of total absence of bile production. Serum enzyme levels are presently: GOT 157, GPT 335, and LDH 250.

Enzyme injection

Two dogs were given intravenous injections of GOT concentrate (10 cc., 150,000 units ml./min.). Serum and bile were then serially analyzed for enzyme activity. Results in both animals were similar. Table II presents data from one experiment.

TABLE II

Enzyme levels following injection of GOT concentrate

Time postinjection	GOT serum	GOT bile	GPT serum
0	28	16	23
10 min.	518		
6 hours	303	15	31
24 hours	3[illegible]	13	24
48 hours		23	

Very rapid fall-off in serum LDH activity following intravenous administration of LDH has been reported by Wroblewski and LaDue (10). Accordingly, on two occasions a dog was given intravenous LDH concentrate (1 cc., 800,000 units/ml./min. diluted to 30 cc. with normal saline). Serum and bile were serially analyzed for enzyme activity. The two experiments gave similar results. Typical data are recorded in table III.

TABLE III

Enzyme levels following injection of LDH concentrate

Time postinjection	LDH serum	LDH bile
0	155	14
10 min.	2,280	
5 hours	108	13
24 hours	91	29
48 hours		67

It was thought that the increased activity of the bile LDH should reflect the rapid disappearance of the serum LDH if the biliary system represented a significant excretory pathway for the active enzyme. The same reasoning applied to GOT, although we did not achieve the high serum levels reported by Fleisher and Wakim (13) with the use of GOT concentrates. Therefore, while it is possible to obtain consistent and reproducible enzyme determinations on bile, the hope that such determinations would be useful in the differential diagnosis of jaundice (8) does not appear to be justified.

SUMMARY

1. Total biliary fistulas were surgically created in 5 dogs, and analyses of glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and lactic dehydrogenase (LDH) activities were accomplished on the collected common duct bile.

2. Under basal conditions common duct bile contained between 0-64 units/ml./min. of GOT, 0-23 units/ml./min. of GPT, and 0-24 units/ml./min. of LDH activity.

3. After exposure of one dog to carbon tetrachloride vapor, serum GOT and GPT activity rose to over 10 times control values. No corresponding increase was found in the bile.

4. After intravenous administration of GOT and LDH concentrates, serum levels of these enzymes rose to approximately 15 times control levels. Again, no corresponding increase was found in the bile.

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