COMPARATIVE VALUE OF BROMSULPHALEIN, SERUM PHOSPHATASE, PROTHROMBIN TIME, AND INTRAVENOUS GALACTOSE TOLERANCE TESTS IN DETECTING HEPATIC DAMAGE PRODUCED BY CARBON TETRACHLORIDE

By VICTOR A. DRILL[1] AND A. C. IVY

(From the Department of Physiology, Northwestern University Medical School, Chicago)

(Received for publication August 11, 1943)

Although tests of liver function have been studied for many years, it is only recently that any intensive comparative studies have been made (1 to 3). Liver function studies are performed (*a*) to detect hepatic damage, (*b*) to aid in prognosis and assist in determining any surgical risk, (*c*) to differentiate obstructive from non-obstructive jaundice. The detection of hepatic damage is complicated by the large number of functional activities performed by the liver. As various functions of the liver will be affected to different degrees by a given type of damage, the results of a liver function test will depend upon which function is tested. Thus, while a given test is positive, others may be negative. This has led to the general concept of a *dissociation* of liver functions and liver function tests.

Although tests of hepatic function are used on experimental animals, no experimental comparison has been made of the newer liver function tests. In this investigation, 5 liver function tests were studied in dogs receiving small doses of carbon tetrachloride, twice a week, to ascertain (*a*) the comparative sensitivity of the tests in detecting hepatic damage, (*b*) the constancy, or change in value, of a test as the doses of carbon tetrachloride were continued, (*c*) whether or not *association* or *dissociation* of the liver function tests was present.

METHODS

Adult dogs, weighing 8.4 to 14.6 kgm., were used as experimental animals. Gardner *et al.* (4) have extensively studied the pathological changes produced in the liver of dogs by carbon tetrachloride, and from their data, a dose of 0.5 cc. of carbon tetrachloride per kgm. of body weight was selected. The carbon tetrachloride was mixed with an equal volume of corn oil and administered by stomach tube before feeding. The dogs generally received this dose twice a week (Tables I and II) and the liver function tests were performed at various intervals to ascertain which tests would first detect any hepatic damage. The bromsulphalein method of Rosenthal and White (5) was used, except that 5 mgm. of dye per kgm. of body weight was injected in place of the original 2 mgm. dose. A single blood sample was withdrawn after 30 minutes and the concentration of dye determined, using the standards developed for the 2 mgm. dose (4 mgm. dye per 100 cc. dilute NaOH equivalent to 100 per cent), as it is more difficult to determine the amount of dye with a higher concentration of standards. Thus, with the 5 mgm. dose and the original set of standards, the bromsulphalein retention will range above 100 per cent during liver damage. Normal dogs generally retain 2 to 12 per cent of bromsulphalein at the end of 30 minutes. Any retention of dye above 15 per cent at the end of 30 minutes is regarded as definitely abnormal. The dye concentration was determined in a block comparator, using a blue glass filter. Serum phosphatase was determined according to the method of Bodansky (6). A value above 5 units per 100 cc. of serum is considered abnormal. The one-stage technic of Ziffrin *et al.* (7) was used to determine prothrombin time, and the result compared with control dogs is expressed as per cent of normal. This method was used rather than the more sensitive two-stage technic as the simplicity of the test lends itself to "bedside" usage, making it of interest to compare its sensitivity with the other "laboratory" tests. In the initial part of the study, thromboplastin was prepared from rabbit lung, but later a commercial preparation was used.[2] A prothrombin time of 85 per cent or less was considered definitely abnormal, and values between 86 and 90 per cent, as questionably abnormal As the oral glucose and galactose tolerance tests have proven inadequate, the intravenous galactose tolerance test of Bassett, Althausen, and Coltrin was used (8). After a control blood sample was taken, 1.0 cc. of 50 per cent galactose per kgm. of body weight was injected intravenously, and blood samples taken at 30, 60, and 75 minutes. In initial studies a sample was also taken at 90 minutes, but this did not add any further information and was discontinued. Glucose was removed from the

[1] National Research Council Fellow, 1942–1943. Present address, Department of Pharmacology, Yale University School of Medicine, New Haven, Conn.

[2] Thromboplastin, generously supplied by the Abbott Laboratories.

blood sample by fermentation according to Raymond and Blanco's method (8). Non-fermentable reducing substances were determined in the filtrate by the method of Folin and Wu. The amount of reducing substance in a fasting sample of blood was subtracted, and because of a difference in reducing power, a correction value of 24 per cent was added to the final result. The direct and indirect Van den Bergh tests were performed according to standard procedures.

Five of the dogs were fed the laboratory stock diet consisting of meat scraps, bones, bread, yeast, and cod liver oil. For comparison, and to make the results more reproducible, the second group of 5 dogs was fed a modified form of Cowgill's synthetic casein diet No. III (9), plus a daily supplement of yeast. The results did not indicate any difference with respect to the diet used. All liver function tests were performed at least 16 hours after the previous feeding.

RESULTS

The control determinations and the effect of carbon tetrachloride on the various liver function tests are shown in Tables I and II.

Bromsulphalein retention. This test was the most sensitive of the ones studied. Seven dogs (Nos. 2, 3, 6, 7, 8, 9, 10) developed an abnormal retention of bromsulphalein after 2 doses of carbon tetrachloride were administered. Dogs Nos. 1 and 5 showed an abnormal retention of dye after 3 doses of carbon tetrachloride. The re-

TABLE I

Ability of various liver function tests to detect hepatic damage in dogs receiving carbon tetrachloride

A. Dogs fed stock diet

Days of experiment	Weight	Bromsulphalein retention	Serum phosphatase	Prothrombin time	I. V. galactose tolerance			Comment
					30′	60′	75′	
Dog No. 1								
	kgm.	*per cent*	*units per 100 cc.*	*per cent normal*	*mgm. per 100 cc.*			
	11.3		2.17	100	47	19	6	Control
	11.4	8	3.52	98	58		7	Control
1								CCl_4 admin.
4								CCl_4 admin.
7	11.0	5	2.86	103				
8								CCl_4 admin.
9			6.43					
10	11.4	50	5.99	78	44	13	6	
11								CCl_4 admin.
15								CCl_4 admin.
19								CCl_4 admin.
21	11.2	130	6.23	88	84	55	32	
24				88				CCl_4 admin.
27								CCl_4 admin.
29	11.1	220	9.33	63	75	31	14	
31								CCl_4 admin.
34								CCl_4 admin.
36	10.9	200	10.67	60	78	37	17	
Dog No. 2								
	8.4	5	3.45		37	5	0	Control
	8.2	2	4.58	98	52	16	2	Control
1								CCl_4 admin.
5								CCl_4 admin.
7	10.6		15.91					
8	10.4	125	15.57	94	49	9	5	
9								CCl_4 admin.
13								CCl_4 admin.
17								CCl_4 admin.
19	10.1	140	14.17	90	55	22	5	
22								CCl_4 admin.
25								CCl_4 admin.
27	9.5	250	28.47	94	46	8	5	
29								CCl_4 admin.
32								CCl_4 admin.
34	9.4	230	24.43	90	64	22	8	

TABLE I—*Continued*

Days of experiment	Weight	Bromsulphalein retention	Serum phosphatase	Prothrombin time	I. V. galactose tolerance			Comment
					30′	60′	75′	
Dog. No. 3								
	kgm.	*per cent*	*units per 100 cc.*	*per cent normal*	*mgm. per 100 cc.*			
	10.0	12	1.01	98				Control
	9.5		1.87	107	38	16	7	Control
1								CCl_4 admin.
4								CCl_4 admin.
6	10.0	55	4.03	90	42	22	11	
12								CCl_4 admin.
17								CCl_4 admin.
18	10.5	200	7.31	72	64	38	29	CCl_4 admin.
24								CCl_4 admin.
25				54				
27								CCl_4 admin., died 2 days later in convulsions
Dog. No. 4								
	14.6	5	2.43					Control
	14.8	5	1.10	100	57	17	3	Control
1								CCl_4 admin.
4								CCl_4 admin.
7	14.3	17	0.45		48	14	4	
8								CCl_4 admin.
10	14.8	15	1.19	108				
12								CCl_4 admin.
14	14.5	50	5.25	89	55	32	11	
18								CCl_4 admin.
20	13.6	90	3.01	95				
26								CCl_4 admin.
31								CCl_4 admin.
32	14.5	125	7.33		70	41	24	
38								CCl_4 admin.
39	14.4		11.57	89				
Dog. No. 5								
	9.8	8	3.52	97				Control
	10.1	10	3.18		34	15	2	Control
1								CCl_4 admin.
4								CCl_4 admin.
6	10.2	10	4.18	95	28	12	2	
7								CCl_4 admin.
9	10.0	30	8.28	102	38	18	4	
10								CCl_4 admin.
14								CCl_4 admin.
16	9.8	80	10.06	90	26	11	3	
20								CCl_4 admin.
24								CCl_4 admin.
26	9.6			98	40	16	5	
27								CCl_4 admin.
31								CCl_4 admin.
33	9.9	175	12.84	93	30	15	4	

sults on dog No. 4 were less definite, a slight dye retention being obtained after two doses of CCl_4, only to return to 15 per cent (the upper limit of normal in our series) on the next test, and becoming definitely abnormal after 4 doses of CCl_4. None of the other tests became abnormal before the bromsulphalein test. The retention of bromsulphalein progressively increased as further doses of CCl_4 were administered.

Serum phosphatase. Six dogs (Nos. 1, 2, 5, 6, 8, 9) showed an increase in serum phosphatase above normal at the same time the bromsul-

phalein retention became abnormal. Dogs Nos. 3, 7, and 10 developed a rise in serum phosphatase slightly after the bromsulphalein test indicated hepatic damage, following another dose of CCl_4. In these 9 dogs, the rise in serum phosphatase showed a close correlation with a retention of bromsulphalein. In dog No. 4, however, the rise in serum phosphatase definitely occurred later than the retention of bromsulphalein. The prothrombin time or intravenous galactose tolerance test did not detect any hepatic damage earlier than the serum phosphatase test.

Prothrombin time. The prothrombin time, as determined by the one-stage technic, detected hepatic damage in only one dog (No. 1) as early as did the bromsulphalein test. In the other dogs, the prothrombin time became abnormal after the bromsulphalein and serum phosphatase tests. Four of the dogs (Nos. 2, 4, 5, 6) did not develop an abnormal prothrombin time during the course of the experiment.

Intravenous galactose tolerance. In some cases, this test was difficult to interpret, but, in general, the intravenous galactose tolerance test detected liver damage after the prothrombin time. Four dogs (Nos. 5, 7, 8, 10) did not show any abnormal response. The control values for the intravenous galactose tolerance test varied from dog to dog, and could not be standardized as closely as the bromsulphalein retention, serum phosphatase, or prothrombin time.

The *indirect Van den Bergh* test was performed on 4 dogs, but as it did not show any change from normal during the experiment, it was discontinued.

TABLE II

Ability of various liver function tests to detect hepatic damage in dogs receiving carbon tetrachloride

B. Dogs fed synthetic diet

Days of experiment	Weight	Bromsulphalein retention	Serum phosphatase	Prothrombin time	I. V. galactose tolerance			Comment
					30′	60′	75′	
Dog No. 6								
	kgm.	*per cent*	*units per 100 cc.*	*per cent normal*	*mgm. per 100 cc.*			
	11.3	8	2.67		58	17	8	Control
	11.4	5	3.67	98	48	13	5	Control
1								CCl_4 admin.
4								CCl_4 admin.
7	11.4		14.54					
8	11.4	90	14.40	100	56	17	3	CCl_4 admin.
11	11.3			100	68	23	15	
12								CCl_4 admin.
14	11.3	110	16.78		40	24	14	
15								CCl_4 admin.
18								CCl_4 admin.
20	11.4	175	13.10	87				
26								CCl_4 admin.
31								CCl_4 admin.
32	11.8		11.53		74	32	18	
34	11.6	175	12.35	90				
Dog. No. 7								
	14.1	10	4.50	95	25	10	8	Control
1								CCl_4 admin.
5								CCl_4 admin.
8	13.6	35	4.70	97	27	9	7	CCl_4 admin.
12	13.6		6.80	89	21	8	6	CCl_4 admin.
14	12.7	175	7.42	50	18	10	8	
15								CCl_4 admin.
19								CCl_4 admin.
21	11.6	200	11.32	67		10	1	
23								CCl_4 admin.
26								CCl_4 admin.
28	11.8	250	11.43	67	10	7	4	

TABLE II—*Continued*

Days of experiment	Weight	Bromsulphalein retention	Serum phosphatase	Prothrombin time	I. V. galactose tolerance			Comment
					30′	60′	75′	
Dog. No. 8								
	kgm.	*per cent*	*units per 100 cc.*	*per cent normal*	*mgm. per 100 cc.*			
	11.3	12	4.50					Control
	11.4	10	4.99	98	26	16	4	Control
1								CCl_4 admin.
5								CCl_4 admin.
7	11.1	35	5.62	102	28	11	2	
8								CCl_4 admin.
11	11.2	35	5.16	95	24	11	3	
12								CCl_4 admin.
14	11.4	125	5.01	94	20	6	4	
15								CCl_4 admin.
19								CCl_4 admin.
21	11.6	65	6.50	70	17	11	5	
23								CCl_4 admin.
26								CCl_4 admin.
28	11.4	70	7.14	74	20	4	1	
Dog No. 9								
	10.0	15		104				Control
	10.1	10	2.81	92	53	14	4	Control
1								CCl_4 admin.
4								CCl_4 admin.
5	10.0	25	6.59	90	62	12	7	
7								CCl_4 admin.
9	9.8	50	8.24	85	56	22	8	
10								CCl_4 admin.
14								CCl_4 admin.
16	9.7	100	7.48	71	78	32	15	
17								CCl_4 admin.
21	9.9	80	10.81	78				
24								CCl_4 admin.
26	9.7	130	14.2	59				
28								CCl_4 admin.
32								CCl_4 admin.
34	9.6	200	20.3	50	82	28	12	
Dog No. 10								
	12.8	12	2.84	98	42	18	7	Control
1								CCl_4 admin.
5								CCl_4 admin.
7	12.6	40	3.46	92	32	16	5	
8								CCl_4 admin.
10	12.9	90	8.02	105	39	15	6	
11								CCl_4 admin.
13	13.1			82	49	20	9	
14								CCl_4 admin.
18								CCl_4 admin.
20	12.6	150	12.54	72	36	18	4	
22								CCl_4 admin.
27								CCl_4 admin.
29	13.0				40	12	6	
31								CCl_4 admin.
33	12.6	175	18.38	66	47	17	8	

DISCUSSION

Of the liver function tests studied, the bromsulphalein retention (5 mgm. dose) and serum phosphatase tests were found to be the most sensitive in detecting hepatic damage produced by administering carbon tetrachloride. Next in order of sensitivity was the prothrombin time, as determined by the one-stage technic. The

TABLE III

Summary of liver function tests, comparing the number of doses of carbon tetrachloride after which a given test became abnormal

Dog No.	1	2	3	4	5	6	7	8	9	10
Bromsulphalein retention	3	2	2	2,4	3	2	2	2	2	2
Serum phosphatase	3	2	4	4,7	3	2	3	2	2	3
Prothrombin time	3	—	4	—	—	—	4	6	3	4
I. V. galactose tolerance	6	9?	4	4	—	3	—	—	5	—
Indirect Van den Bergh	—	—				—	—			

least sensitive tests were the intravenous galactose tolerance and serum bilirubin tests. A further comparison of these tests is shown in Table III.

It was to be expected that the determination of serum bilirubin in dogs would be less sensitive than the other tests, as injected bilirubin is excreted faster in the dog than in man (10). The serum of normal dogs does not contain bilirubin. If the doses of CCl_4 were continued longer, serum bilirubin would eventually be present (11).

Although the serum phosphatase test was practically as sensitive as the bromsulphalein test, the rise in serum phosphatase was greater in some dogs than in others, this being especially true of dog No. 8. This difference in the rise in serum phosphatase of dogs receiving CCl_4 has previously been observed (11). Although the serum phosphatase rises above normal, the degree of change does not always parallel the increase in bromsulphalein retention. It has been found that bile fistula dogs develop an abnormal retention of bromsulphalein and a rise in serum phosphatase (12). In the bile fistula dogs, as in the dogs receiving CCl_4, the degree of rise in serum phosphatase also varied. Bile phosphatase was measured in the bile fistula dogs, and it was found that dogs showing a high serum phosphatase excreted *more* bile phosphatase than normal. When the rise in serum phosphatase was not as great, it was found that only a small amount of bile phosphatase was present. It is possible that a similar underlying change in phosphatase metabolism will account for the difference in the rise of serum phosphatase of dogs receiving CCl_4.

Although serum phosphatase has been known to rise in various types of hepatic damage, the rise during obstructive jaundice is higher, and most clinical studies have been directed to using the test in differentiating the two types of jaundice. However, the values during hepatic and obstructive jaundice overlap sufficiently to make the test of doubtful value in differential diagnosis. Experimentally, serum phosphatase has shown a close correlation with the dye retention tests during hepatic damage produced (*a*) by feeding dogs a protein-free diet (13), (*b*) during experimental hyperthyroidism (14), (*c*) in bile fistula dogs (12), and (*d*) in the present study with CCl_4. No extensive clinical reports have been made on the serum phosphatase test compared with other sensitive liver function tests. The experimental results suggest a possible clinical value of this test, which is relatively simple to perform, requiring only one blood sample. Bone diseases, however, must be ruled out.

If the diet is excessively high in carbohydrate or a non-fasting blood sample is used, the serum phosphatase will be increased (15,16). In the present study, however, all blood samples were taken at least 16 hours after the previous feeding.

The prothrombin time, determined by the one-stage technic, was not found to be as sensitive as the bromsulphalein or serum phosphatase tests. In an early report, Ziffren *et al.* (7) obtained good correlation between the one-stage and two-stage technic for prothrombin time in all except one patient with hepatic disease. In a later study, however, they observed that the one-stage technic was satisfactory for following response to vitamin K therapy, but was not found to be as sensitive in detecting liver damage as the two-stage technic, for it measures both the rate of conversion of prothrombin and the concentration of prothrombin (17 to 20). The conversion rate of prothrombin is faster in the dog than in man, although little difference is found in the number of prothrombin units in the plasma (20). Thus, any increase in the rate of conversion of prothrombin may compensate for a deficiency of prothrombin. The one-stage technic will, however, give a practical index of any tendency to bleed.

The liver damage produced can be considered fairly acute and does not entirely correspond with conditions that occur in attempting to assess liver function in human beings. Also, it is not always possible to transfer the results of animal experiments to human beings. This is especially true with studies on jaundice, as dogs do not

develop jaundice to any degree, except with severe intrahepatic damage or complete biliary obstruction. Thus, in the dog, it would be difficult to evaluate accurately the diagnostic importance of various liver function tests in the differential diagnosis of jaundice. It should also be born in mind that the rate of conversion of prothrombin is faster in the dog than in man, which might make this test less sensitive in the dog. Keeping these differences in mind, the dog should still prove to be a valuable test animal in studies of hepatic function.

The liver is known to perform a large number of functional activities. Clinical comparison of liver function tests have shown that in a given case some tests are positive while others may be negative. These positive tests are said to be more sensitive than those giving a negative response. This in turn led to emphasis being placed on a *dissociation* of the various liver functions. The word dissociation generally is taken to mean that the various functions of the liver are separated, or dissociated, so that injury to the liver may interfere with some hepatic functions without affecting others. However, the fact that some tests are negative while others are positive does not furnish adequate proof for the dissociation of liver functions. On the other hand, it would be more remarkable if the various hepatic functions were affected to an equal degree during liver damage. It is more reasonable to suppose that whether one or more tests will detect liver damage will depend on the degree of liver damage. This idea is borne out by the experimental evidence in this paper. The bromsulphalein test detected hepatic damage first, but as further doses of CCl_4 were administered, other liver function tests in turn became abnormal. Thus, it would seem that rather than a dissociation of liver functions, these various hepatic functions are correlated, or *associated*, so that as the degree of damage increases, more of the functions of the liver will become abnormal. Hence, emphasis should not be placed, as it has in the past, on the dissociation of liver functions, but rather on the most sensitive, reliable, test for detecting hepatic damage. This "sensitive" test, first detecting hepatic damage alone, would be followed by other tests becoming abnormal as the degree of damage increases. This idea of liver function tests emphasizes the quantitative *association* of these tests rather than a qualitative *dissociation* of liver function tests.

SUMMARY

1. Ten dogs received 0.5 cc. of carbon tetrachloride per kgm. of body weight, twice a week by stomach tube. The number of doses of carbon tetrachloride required to produce an abnormal liver function was fairly constant. Seven dogs developed an abnormal liver function, which became progressively more abnormal as the administration of CCl_4 was continued, after 2 doses; 2 dogs, after 3 doses; and 1 dog, after 4 doses of carbon tetrachloride.

2. Of the liver function tests studied, the bromsulphalein retention (5 mgm. dose) was the most sensitive in detecting the type of damage produced.

3. The serum phosphatase value rose above normal in all except 1 dog at the same time as, or shortly after, the bromsulphalein retention became abnormal. This test was practically as sensitive as the dye retention test used.

4. The prothrombin time (one-stage technic) was not as sensitive as the serum phosphatase or bromsulphalein tests, but still detected hepatic damage before the intravenous galactose tolerance test. In some dogs, the prothrombin time and galactose test remained normal throughout the period of carbon tetrachloride administration. No change in serum bilirubin was found, when studied in 4 of the dogs.

5. Emphasis is placed on an *association* of hepatic functions and liver function tests, more of which will become abnormal as the degree of damage increases, rather than on a dissociation of liver function tests.

BIBLIOGRAPHY

1. Mateer, J. G., Baltz, J. I., Marion, D. F., and MacMillan, J. M., Liver function tests. J. A. M. A., 1943, 121, 723.
2. White, F. W., Deutsch, E., and Maddock, S., The comparative value of serial hippuric acid excretion, total cholesterol, cholesterol ester and phospholipid tests in diseases of the liver. II. Clinical comparison of the tests. Am. J. Digest. Dis., 1940, 7, 3.
3. Rosenberg, D. H., and Soskin, S., The azorubin S test of liver function; an evaluation, with a comparative study of the bromsulphalein and hippuric acid tests. Ann. Int. Med., 1940, 13, 1644.

4. Gardner, G. H., *et al.*, Studies on the pathological histology of experimental carbon tetrachloride poisoning. Bull. Johns Hopkins Hosp., 1925, **36**, 107.
5. Rosenthal, S. M., and White, E. C. Clinical application of bromsulphalein test for hepatic function. J. A. M. A., 1925, **84**, 1112.
6. Bodansky, A., Notes on the determination of serum inorganic phosphate and serum phosphatase activity. J. Biol. Chem., 1937, **120**, 167.
7. Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P., Control of vitamin K therapy. Compensatory mechanisms at low prothrombin levels. Proc. Soc. Exper. Biol. and Med., 1939, **40**, 595.
8. Bassett, A. M., Althausen, T. L., and Coltrin, G. C., A new galactose test for differentiation of obstructive from parenchymatous jaundice. Am. J. Digest. Dis., 1941, **8**, 432.
9. Drill, V. A., and Hays, H. W., Hyperthyroidism and liver function in relation to B vitamins. Proc. Soc. Exper. Biol. and Med., 1940, **43**, 450.
10. Mills, M. A., and Dragstedt, C. A., Removal of intravenously injected bromsulphalein from the blood stream of the dog. A comparison of the removal of intravenously injected bilirubin and that of bromsulphalein. Arch. Int. Med., 1938, **62**, 216.
11. Freeman, S., Chen, Y. P., and Ivy, A. C., On the cause of the elevation of serum phosphatase in jaundice. J. Biol. Chem., 1938, **124**, 79.
12. Drill, V. A., Annegers, J. H., Snapp, F. E., and Ivy, A. C., Liver function and bile phosphatase in bile fistula dogs. Fed. Proc., 1943, **2**, 9.
13. Hough, V. H., and Freeman, S., The effect of a protein-deficient diet on the serum phosphatase and hepatic dye clearance of dogs. Am. J. Physiol., 1942, **138**, 184.
14. Drill, V. A., and Shaffer, C. B., Serum cholesterol, serum phosphatase, and bromsulphalein retention in experimental hyperthyroidism. Gastroenterology, 1943, **1**, 308.
15. Bodansky, A., Phosphatase studies. VI. Non-osseous origins of serum phosphatase. Its increase after ingestion of carbohydrates. J. Biol. Chem., 1934, **104**, 473.
16. Bodansky, A., Phosphatase studies. VII. Inorganic phosphorus and phosphatase of serum in new born puppies. J. Biol. Chem., 1934, **104**, 717.
17. Ziffren, S. E., Owen, C. A., Warner, E. D., and Peterson, F. R., Hypoprothrombinemia and liver function. Surg., Gynec. and Obst., 1942, **74**, 463.
18. Warner, E. D., Brinkhous, K. M., and Smith, H. P., A quantitative study on blood clotting: prothrombin fluctuations under experimental conditions. Am. J. Physiol., 1936, **114**, 667.
19. Smith, H. P., Warner, E. D., and Brinkhous, K. M., Prothrombin deficiency and the bleeding tendency in liver injury (chloroform intoxication). J. Exper. Med., 1937, **66**, 801.
20. Warner, E. D., Brinkhous, K. M., and Smith, H. P., Plasma prothrombin levels in various vertebrates. Am. J. Physiol., 1939, **125**, 296.