BOVINE LIVER FUNCTION AS AFFECTED BY SHORT CHAIN VOLATILE FATTY ACIDS

by

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ABSTRACT

The relationship between liver function as indicated by the fractional clearance of Bromsulphalein (BSP) from the blood by the liver and the post-hepatic plasma levels of the short chain volatile fatty acids acetate, propionate, and butyrate were studied in the ruminant. Fractional clearance increased after feeding and decreased during fast. Post-hepatic levels of volatile fatty acids have been correlated significantly with fractional clearance under fast conditions but not under normal feeding conditions. The effects of plasma short chain acids on the extent of hepatic perfusion and their relationships to fractional clearance are the subject of this thesis.

CHAPTER 1

INTRODUCTION

Since its initial development in 1924 by Edwin C. White (in 39) Bromsulphalein (BSP) has been utilized as a sensitive indicator of hepatic function in man and animals, especially in the determination of hepatic pathology.

Of primary importance in the determination of hepatic functional modifications is blood flow to the liver and the extent to which that mass is perfused (8, 9, 37).

Ingestion and digestion of a meal by mammals has been shown to increase gastrointestinal and portal blood flow (3, 16, 22, 42, 44). In ruminants the substances causing this increase have been demonstrated to be the rumen levels of the short chain fatty acids acetate, propionate, and butyrate (3, 16, 42, 43).

In the fed, normal, ruminant an unexplained variation in the fractional clearance (K) of BSP has been noted on consecutive days under identical test conditions (23). In monogastric animals no variation was noted on consecutive days (35) since the monogastric, unlike the ruminant, can achieve a relatively basal condition through fasting.

Fractional clearance of BSP has been shown to vary with changes in hepatic blood flow (32, 37). Since the short chain acids which increase portal blood flow are of vital importance to ruminant metabolism (4, 45) and possibly to the extent of liver perfusion (23), their

blood concentrations may alter K. Therefore, as an original premise it is suggested that K may be proportional to the blood levels of the short chain fatty acids acetate, propionate and butyrate.

To test this premise simultaneous determinations of K and the plasma levels of short chain volatile fatty acids were made. Plasma BSP concentration was determined spectrophotometrically and plasma volatile fatty acid concentrations were determined utilizing gas-liquid chromotography.

CHAPTER 2

LITERATURE REVIEW

Liver function in the non-diseased liver has been associated with the rate and level of liver perfusion (9) and experimentally expressed by the ability of the liver to remove the cholephilic dye, Bromsulphalein (BSP), from the blood (12, 24, 25, 32, 38).

Ingelfinger et al. (24) demonstrated that plasma BSP concentration declined in an exponential manner following a single intravenous injection, yielding a straight line when plotted semilogrithmically against time.

Lewis (31, 32) developed the concept of fractional clearance (K) which he defined as the fraction of the total plasma volume cleared of BSP in unit time. Lewis determined K utilizing the following relation:

$$K = C/V = 2.3 (\log P - \log P')/T$$

where: C = clearance (ml/min)
V = total plasma volume (ml)
P and P' = initial and final plasma BSP concentrations (mg/100 ml plasma)
T = elapsed time between samples

Goodman (19) and Lavers et al. (30) have shown that K was equal to the slope of the semilog plot of the disappearance of a single dose of BSP from plasma with time. Goodman obtained a value for clearance (C) by extrapolation of the semilog plot of the regression of plasma BSP concentration (C) to time zero, thus obtaining the distribution

volume (V) of BSP, in effect the plasma volume, utilizing the relation:

$$C = KV$$

Brauer (9) defined hepatic clearance (C) as the volume of plasma cleared of BSP in unit time and noted it was determined by the product of functional liver mass and the clearance per unit mass of actually perfused tissue. He suggested that the functional liver mass was determined by the product of liver mass and the fraction of the hepatic vascular tree actually perfused at any given time. Clearance per unit mass of actively perfused tissue was determined by the product of effective tissue perfusion and extraction efficiency. Brauer considered this product to be an index of the intrinsic functional competence of the hepatic parenchyma.

All experimenters utilizing BSP as a test substance have indicated that the test be performed under basal (fasted, rested) conditions (30, 36).

It was noted that portal and therefore hepatic flow was established by events in the splenic and gastrointestinal beds (20, 40), while distribution of intrahepatic flow was determined by intrahepatic factors (2, 14, 15, 34, 40).

Herrick et al. (21) first observed variations in blood flow rates in various vessels of the dog following feeding. They inferred similar occurrences in the portal vein. Fronek and Stahlgren (18) and Vatner, Franklin, and Van Citters (49) noted increased blood flow in the canine superior mesenteric artery five to fifteen minutes after the

ingestion of feed. Fronek and Stahlgren (18) observed an increase of 133% of prefeeding flow rate in the superior mesenteric artery which was accompanied by an 86% and 74% decrease in flow in the cephalic and iliac arteries, respectively. They suggested that a redistribution of blood occurred during digestion with a preference for the vascular bed of the superior mesenteric artery. Increases in canine total hepatic blood flow post-feeding have been recorded by Greenway and Stark (20). These flow increases were attributed to increased portal flow which remained elevated three to four hours due to intestinal vasodilation.

The fermentation of ingesta by rumen microbial action results in the production of the short chain fatty acids acetate, propionate and butyrate (4, 29, 33, 45). Bergman and Wolff (6) have shown that 30% of the acetate, 50% of the propionate and 90% of the butyrate produced in the rumen were metabolized during absorption into the blood. Cook and Miller (11), Masson and Phillipson (33), and Bergman et al. (4) have demonstrated that the liver metabolized all the propionate and some of the butyrate while acetate was utilized by extrahepatic tissues. Bergman, Roe, and Kon (5) noted that 50 to 60% of the absorbed propionate accounted for 20 to 40% of the glucose turnover depending on the diet. They also determined that the glycogenic amino acids and propionate combined accounted for 90% of the glucose turnover. Katz and Bergman (27) observed that a direct relationship appeared to exist between the total hepatic blood flow and the net hepatic glucose production after feeding since both were observed to increase nearly 50% post-feeding and then

decrease to prefeeding levels. McCuskey (34) established that topical or parenteral β-d-glucose produced insignificant changes in intrahepatic blood vessel diameter or blood flow.

The ingestion of feed by the ruminant and the post-prandial increase in volatile fatty acids has consistantly increased rumen (42, 43) and portal venous blood flow (3, 16, 26). These blood flow changes have been shown to reach a maximum in three to seven hours post-feeding by Bensadoun and Reid (3). Increases in portal flow ranging from 56% to 169% of prefeeding values were demonstrated (3) while fasting produced lower than normal flow rates (3, 26, 42). Katz and Bergman (26) demonstrated that hepatic arterial flow consistantly averaged about 20% of the total hepatic flow in both fed and fasted states. Bensadoun and Reid (3) found the rumen volatile fatty acid levels and portal blood flow trends were similar but were not significantly correlated. They also established that portal volatile fatty acid levels and portal flow rates were not significantly correlated.

Changes in the distribution of liver blood flow and thus in the level of liver perfusion have been noted by Khalil, Wakim, and Mann (28) utilizing quartz-rod illumination and by Daniel and Prichard (15) utilizing serial angiography in the rat, rabbit, cat, and monkey. In the former method large portions of the liver were observed to experience intervals of intermittent circulation. These intervals were irregular and involved up to 75% of the circulation in the basal state. In the serial angiography method two types of flow were noted, diffuse and restricted. In restricted flow the blood was confined

mainly to the hilar region and traversed the liver reaching the inferior vena cava at a faster rate than was noted with diffuse flow. The decreased transit time was possible since blood passed only through sinusoids in the hilar regions. In diffuse flow the entire liver was perfused.

Transhepatic circulation was accelerated following stimulation of the hepatic nerve plexus or by the administration of epinepherine in the rat, rabbit, cat, dog, and monkey by Daniel and Prichard (14). These authors suggested that such alterations in flow were caused by vasconstriction of the smaller vessels of the portal venous tree. Ross and Kurrasch (40) determined that changes in portal flow, in the feline, are due to alterations in intestinal and splenic outflow precipitated by catecholamines rather than to vascular changes in the liver. McCuskey (34) in transillumination studies observed alterations in blood flow distribution in the livers of the rat and rabbit which were caused by the expansion or contraction of sinusoidal sphincter cells under the control of adenosine and/or potassium. McCuskey (34) suggested that the release of adenosine and potassium from the liver parenchyma during glycogenolysis or hepatic cellular metabolism caused dilatation of the sinusoidal sphincters and thus an increase in sinusoidal flow. McCuskey (34) has observed a reduced blood flow through the sinusoids in response to the administration of epinepherine which he suggested caused constriction of the portal venules, hepatic arterioles and central venules.

Brauer et al. (10) utilizing chromic phosphate colloid, whose characteristics of extraction by the liver are similar to those of BSP (9), demonstrated in isolated perfused rat liver preparations that at moderate perfusion rates (less than 3.0 ml blood/gm of tissue/min), as the perfusion rate increased, K increased proportionately. Bovine liver tissue perfusion rates are less than 3.0 ml blood/gm liver/min.

Treacher and Sanson (48) contend that fractional clearance gave no direct indication of the intrinsic ability of the liver to extract BSP from the blood since in any functional state K is dependent upon the supply of blood to the liver and will be directly related to the blood flow to that organ. A decrease in portal flow, as noted during fasting (37), following parturition (48) or surgical procedures (46) reduced K (1, 32) while increases in blood flow increased K (32, 37). Changes in the functional mass of the liver have been shown to correlate positively which changes in K (7, 47).

CHAPTER 3

EXPERIMENTAL PROCEDURE

Fractional Clearance

One gram of Bromsulphalein^{R} (BSP^{R}) manufactured by Hynson, Westcott, and Dunning of Baltimore, Maryland, dissolved in twenty milliliters of distilled water was injected in the right jugular vein of the constrained dairy cow. Elapsed time commenced at the mid-point of the dye injection (t-0). After an elapsed time of four to five minutes to allow for complete mixing of the dye with the blood (12, 13), twenty milliliters of heparinized blood were removed from the left jugular vein. The midpoint of blood removal was noted as elapsed time since dye injection (t-1). Two more twenty milliliter samples were obtained at approximately eight and ten minutes (t-2 and t-3). The entire period of sampling was less than fifteen minutes, within the initial exponential clearance portion of the diphasic BSP plasma elimination curve for the bovine (12, 13, 36).

The plasma samples were centrifuged at 1000 rpm for twenty-five minutes. Dye color was developed in 0.5 milliliters of plasma from each sample by the method of Seligson (in 41). Transmittance (T) of the dye was determined on a Spectronic -20 at 575 mμ and converted to absorbance (A) utilizing the equation:

$$A = -\log T$$

Absorbance was converted to plasma BSP concentration utilizing the slope of an absorbance versus concentration regression curve, calculated by relating known concentrations of BSP in distilled water to their optical densities at 575 mμ, by the following relation:

$$M = P/A$$

where: P = BSP concentration in mg/100 ml
A = absorbance
M = slope

Fractional clearance was determined by the formula of Lewis (32):

$$K = 2.3 (\log P - \log P')/T$$

where: P = BSP plasma concentrations (mg/100 ml)
T = (t-3) - (t-1)

Gas Chromatography

A Micro Tech DSS 200 dual hydrogen flame gas chromatograph was utilized. A six foot, 3/8 inch diameter glass column packed with 20% Tween 80 and 2% phosphoric acid on Chromsorb W as proposed by Erwin, Marco, and Emery (17) was utilized. The column was maintained at an oven temperature of 135 degrees centigrade after curing by gradual increases in temperature, reaching 135 degrees after eighteen hours. Nitrogen carrier gas was maintained at a flow rate of 85 cc per minute. Hydrogen flow to the flame jets was 0.6 cubic feet per hour. Air flow to the detector chamber was 1.2 cubic feet per hour. An empty column with the same nitrogen flow rate as the sensing column was employed for detector balance.

Four milliliters of the second timed plasma samples obtained in the fractional clearance tests was prepared for short chain fatty acid determination by the method of Erwin et al. (17). Four milliliters of plasma was acidified with sixteen milliliters of 0.2 N H_2SO_4 and allowed to stand for ten minutes. Four milliliters of 10% sodium tungstate was then added and the resulting solution vacuum filtered. The supernatant fluid was lyophilized after the addition of 0.25 ml of 3 N NaOH. The lyophilate was dissolved in 0.5 ml of ethyl ether and 0.1 ml of reagent grade HCl was added to convert the sodium salt to the free acid. Micro liter portions of the resulting solution were injected onto the column following a half hour wait. The acids of interest, acetate, propionate, and butyrate, were eluted over a twelve minute period. Their concentrations in grams per liter were determined by peak height comparison with a standard curve constructed at the start of every day.

Experiment 1. The Effects of Time of Feeding on Fractional Clearance

Four normal lactating dairy cows were utilized to determine the within day variations in fractional clearance over a nine hour period under normal herd conditions. Three fractional clearance determinations were performed on each animal throughout the day and comparisons were made between these values and time and the observed eating habits of the animals. This experiment was repeated utilizing the same animals.

Experiment 2. The Effect of Fasting on Fractional Clearance

Two non-gravid, normal dairy cows, near the end of their lactation period, were fasted for 48 hours. Fractional clearance tests were performed during the period of fast and once four hours after the termination of the fast. The fractional clearance values obtained were compared with the levels of short chain acids in the plasma at the time of the test.

Experiment 3. The Effect of Post Hepatic Levels of Plasma Volatile Fatty Acids on Fractional Clearance

Fractional clearance tests were performed on four, fed, normal, lactating dairy cows over a three month period. The values obtained in the determination of fractional clearance and the plasma levels of volatile fatty acids at the time of each determination were compared.

CHAPTER 4

RESULTS

Experiment 1. The Effects of Time of Feeding on Fractional Clearance

Figures 1 and 2 show fractional clearance (K) as a function of time. K was found to vary after feeding in a similar pattern in all animals tested. The average variation in K for both experiments and for all animals was 0.022 with a range of 0.001 to 0.049. The between animal variance for each experiment was found not to be significant after an analysis of variance.

Prior to feeding K was relatively low (Figures 1 and 2). After ingestion of feed K increased and subsequently decreased. Analysis of variance indicated that the increase was not significant. In two animals (695-5Nov and 598-3Nov) K was not obtained prior to feeding.

The total plasma volatile fatty acids (TPVFA) levels determined for each fractional clearance test showed no relationship with K.

Experiment 2. The Effect of Fasting on Fractional Clearance

Figures 3 and 4 indicate the effect of fasting on K and TPVFA.

As the length of fast increased both K and TPVFA levels decreased. Feeding reversed this decline. There was a significant positive correlation ($r = .8495$ for cow 788; $r = .9844$ for cow 119) between K and TPVFA levels for both animals. Table 1 lists the means and standard deviations

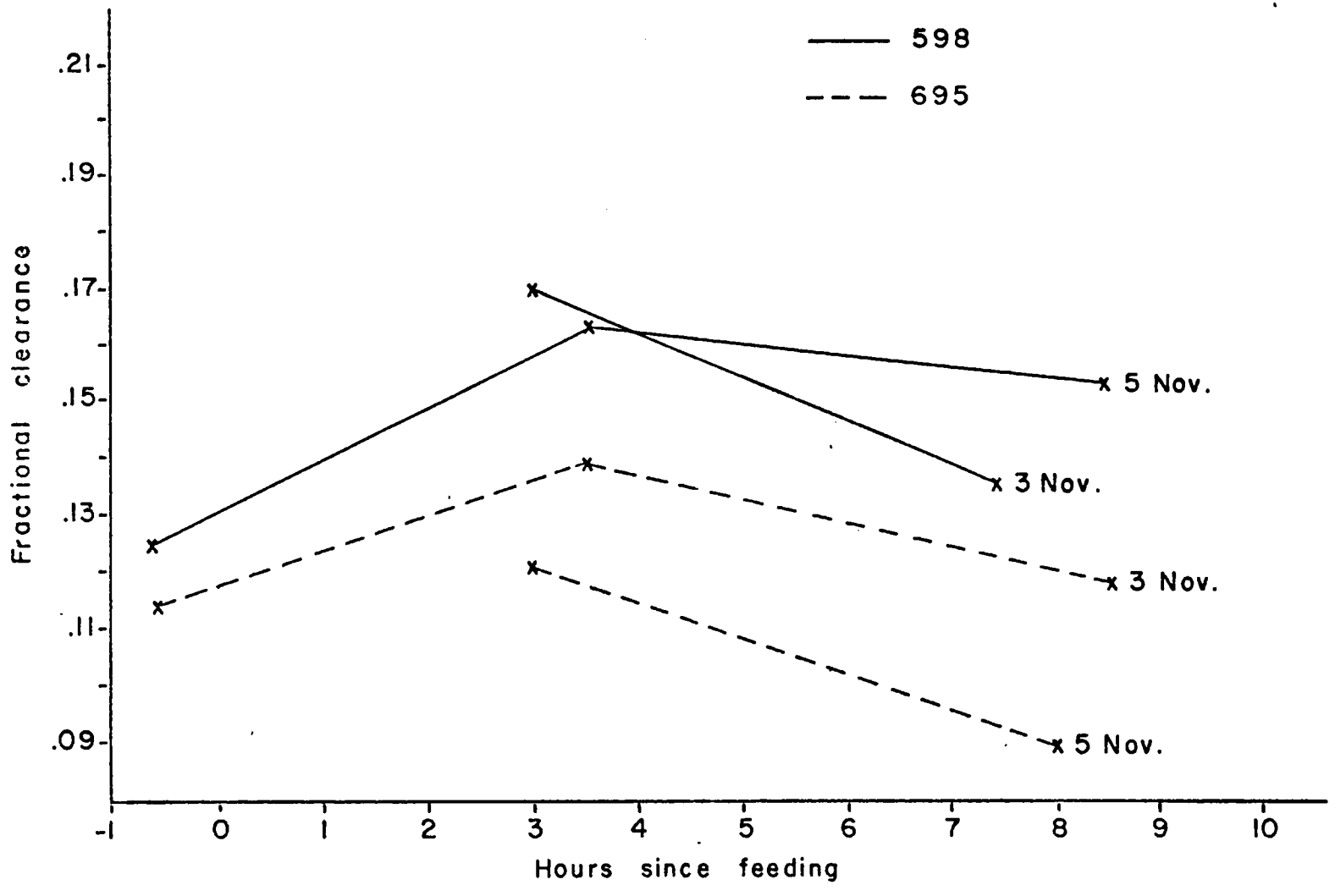

Figure 1. The effect of time of feeding on fractional clearance (Cows 598 and 695).

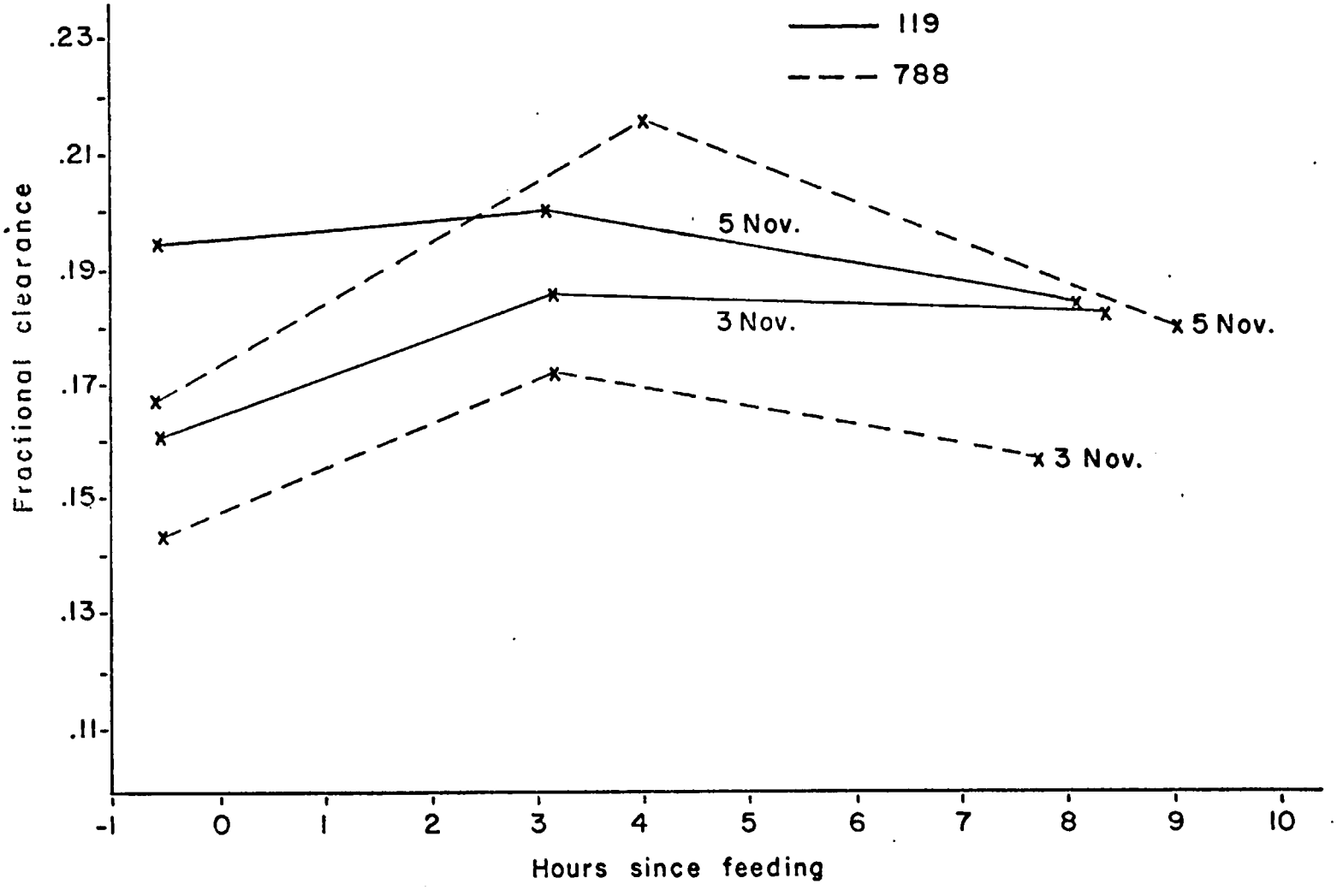

Figure 2. The effect of time of feeding on fractional clearance (Cows 119 and 788).

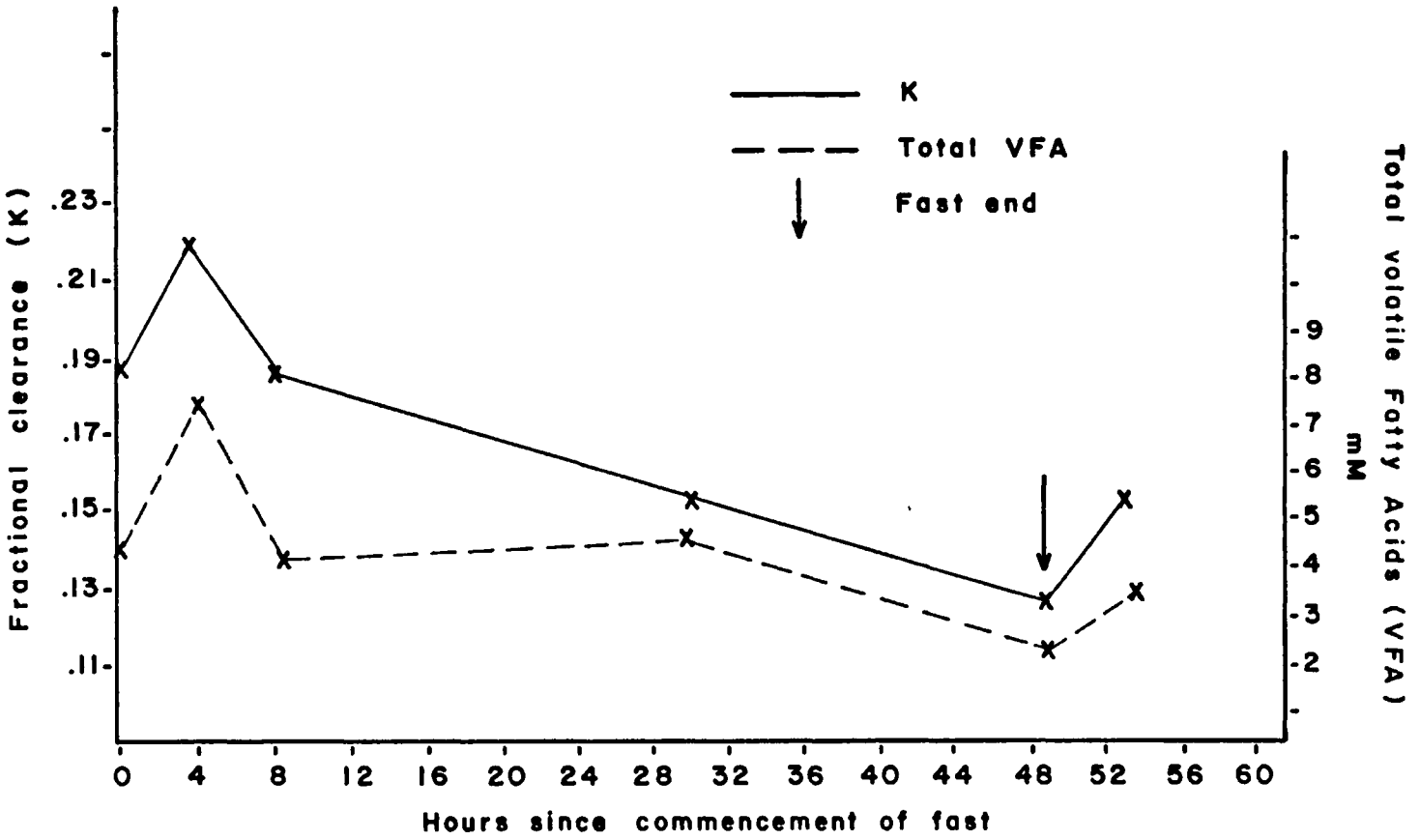

Figure 3. The effect of fasting on fractional clearance (Cow 788).

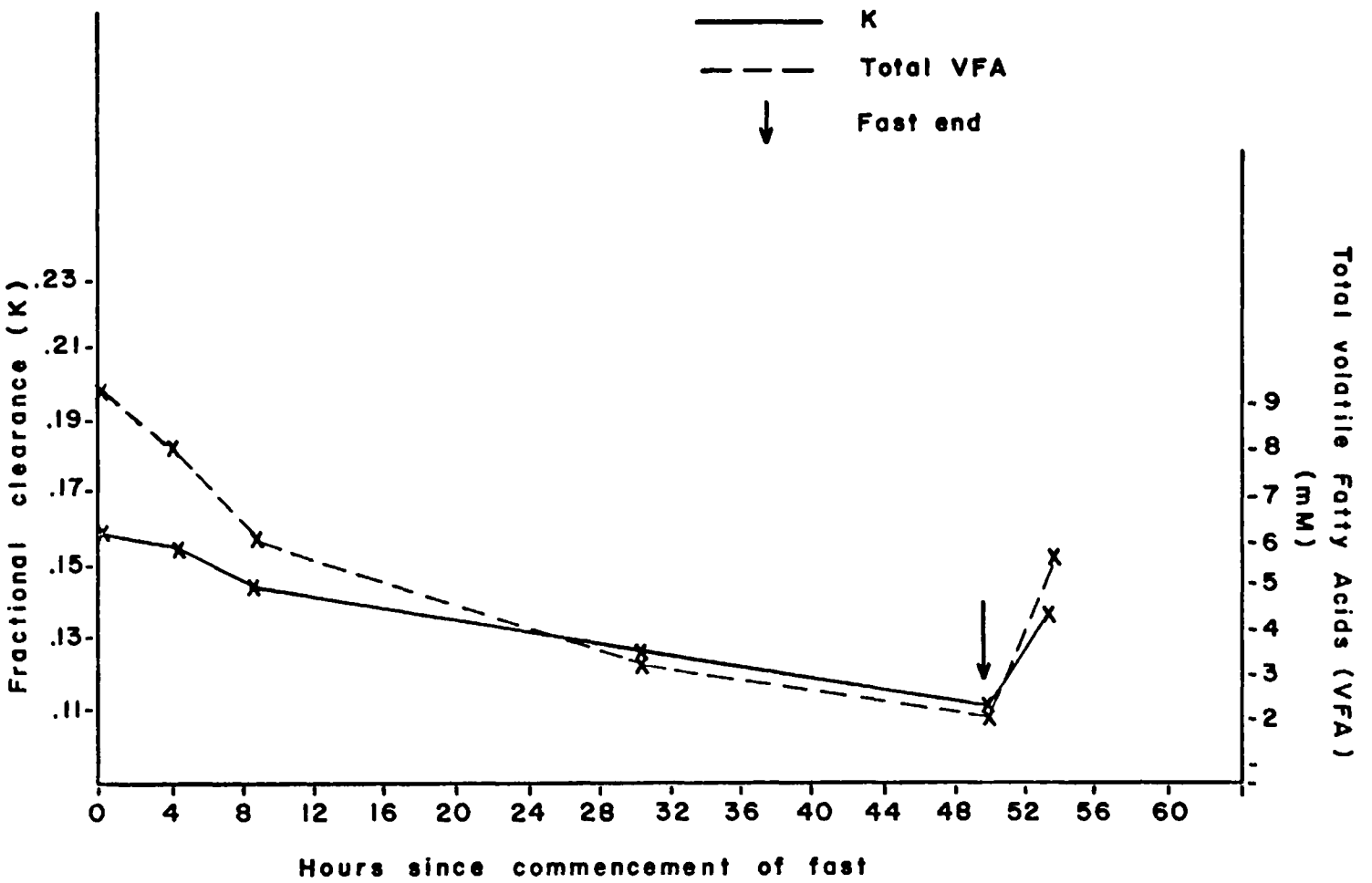

Figure 4. The effect of fasting on fractional clearance (Cow 119).

Table 1. Means and standard deviations of fractional clearance (K) and total plasma volatile fatty acid levels (TPVFA) and the regression coefficient of K versus VFA levels from Experiment 2.

Animal	$\bar{K}$ $\bar{X}\pm\sigma$	Total VFA (mM) $\bar{X}\pm\sigma$	Number of Samples	Regression Coefficient	Significant at .05 Level
788	.174±.030	.428±.193	6	.8495	yes
119	.142±.018	.578±.276	6	.9844	yes

for K and TPVFA levels and the regression coefficient of K versus TPVFA levels for the period of fast of both animals.

Experiment 3. The Effect of Post-Hepatic Levels of Plasma Volatile Fatty Acids on Fractional Clearance

Table 2 lists the means and standard deviation for K and TPVFA for four fed animals sampled a total of seventy-nine times. There was no correlation between K and TPVFA levels in fed animals.

The mean fractional clearance for all BSP tests in Experiments 1 and 3 was $.148 \pm .036$.

Table 2. Means and standard deviations of fractional clearance (K) and volatile fatty acid levels (VFA) and the regression coefficient of K versus VFA levels from Experiment 3.

Animal	K $\bar{X}\pm\sigma$	Total VFA (mM) $\bar{X}\pm\sigma$	Number of Samples	Regression Coefficient	Significant at .05 Level
788	.167±.038	.649±.209	26	.10	No
119	.150±.028	.704±.312	26	.26	No
695	.115±.028	.849±.231	16	.26	No
598	.147±.024	.850±.247	11	.26	No

CHAPTER 5

DISCUSSION

In Experiment 2, both fractional clearance (K) of Bromsulphalein (BSP) and the total plasma volatile fatty acid (TPVFA) levels were observed to decrease as the time of fasting increased. Fasting has been demonstrated to reduce gastrointestinal and portal blood flow in the ruminant (26, 37). Such decreases are attributed to decreases in rumen volatile fatty acids (37). Decreases in liver blood flow are observed to result in decreases in K (37, 46, 48).

McCuskey (34) has suggested a theory for the metabolic regulation of blood flow through the liver based on the observed effects of adenosine and potassium released from cells which are hypoxic because of a high metabolic rate or flycogenolysis. Adenosine and potassium act to contract expanded sinusoidal sphincter cells thus increasing flow through the sinusoid. Increasing levels of propionate and other metabolites in portal blood would result in an increase in the metabolic rate of the parenchyma followed by cellular hypoxia, release of adenosine and potassium into the blood, and relaxation of the sinusoidal sphincter cells. Relaxation of these cells would increase sinusoidal flow and ultimately the extent of liver perfusion. Decreases in TPVFA would result in a decrease in hepatic metabolic rate and thus a reduction in the level of active liver perfusion. The combined effect of decreased portal and hepatic blood flow and reduced levels of hepatic perfusion would be a depressed rate of fractional clearance.

It should be noted that upon ingestion of feed by the fasted animals of Experiment 2, both K and TPVFA levels increased (Table 1). The significant positive correlation of TPVFA levels with K during fasting can be attributed to the decreased production of the short chain acids by rumen microbes. Thus the stimulatory effects of rumen acids on portal and gastrointestinal blood flow were reduced. The main regulatory agent of K in the case of fasting is probably the declining TPVFA levels and the resulting depression of liver perfusion. The results of Experiment 2 suggest that in periods of fast, post-hepatic levels of volatile fatty acids mirror the level of active hepatic perfusion as indicated by K. As indicated by the results of Experiment 3, changing levels of rumen acids and their effect on blood flow may distort any possible TPVFA-K relationship in the fed animal.

In Experiment 3 no correlation was found between numerous determinations of K and post-hepatic TPVFAs at the time of the test. This result must be viewed while keeping in mind the observed and suggested functions of the short chain volatile fatty acids. Rumen short chain acids have been noted to increase gastrointestinal and portal blood flow (3, 16, 26). It has been suggested that plasma volatile fatty acids exert an indirect control over the extent of liver perfusion (34). The total result of increased rumen and plasma short chain volatile acids is an increase in fractional clearance. Even if the effects of rumen and plasma volatile fatty acid levels on K are additive in the fed animal, their separate contributions to K probably do not comprise a constant proportion of the total resulting K. Such

a condition would explain the lack of correlation of K with TPVFA in the fed animal.

In Experiment 1 liver function tests were performed, except in two cases, prior to the ingestion of feed. The increase in K noted three to four hours post-feeing (Figures 1 and 2) indicates an increase in the functional capabilities of the liver. Although the increases noted in K are not statistically significant (probably due to the wide variation in K between animals before and after feeding), K consistently increased following the ingestion of feed, and the mean K was 18% greater after feeding. The increase in K observed is assumed to be the result of increased rumen levels of volatile fatty acids which cause an increase in gastrointestinal and portal blood flow (36, 16, 26). Such increases in liver blood flow have resulted in increased fractional clearance of BSP by the liver (7, 9, 47). The work of McCuskey (34) suggests that increased levels of TPVFA in portal blood acts to increase the extent of liver perfusion and thus increase the rate of fractional clearance of BSP. Thus the post-feeing increase in fractional clearance is probably the combined result of augmented portal flow because of higher concentrations of rumen volatile fatty acids and expanded liver perfusion resulting from the by-products of increased hepatic metabolism.

Because of the continuous production of metabolites by rumen microbes, the liver of the ruminant may be considered to be extensively perfused at any given time. In the fed animal increases in blood

metabolites or in the rate of liver blood flow would thus result in only minor increases in the fractional clearance of BSP.

The mean fractional clearance for all BSP tests in Experiments 1 and 3 was 0.148. This value compares with the mean of 0.151 obtained by Treacher and Sanson (48) for pregnant cows. The mean for K obtained in this study is lower than the mean of 0.230 obtained by Cornelius, Theilen, and Rhode (13) for non-lactating cows and the mean of 0.192 obtained by Mixner and Robertson (36) for lactating cows. Other than the suggestion by Treacher and Sanson (48) that K is lower in lactating than in non-lactating cows there is no apparent reason for the difference.

CHAPTER 6

CONCLUSIONS

Fractional clearance (K) of dye Bromsulphalein (BSP) by the ruminant liver increased following the consumption of feed and decreased during periods of fast. The association of fractional clearance to feed consumption is reportedly due to the stimulating effects of rumen short chain volatile fatty acids on hepatic blood flow and the suggested stimulation of hepatic perfusion by total plasma volatile fatty acid (TPVFA). In fasting dairy cattle the relationship between fractional clearance and post-hepatic levels of plasma volatile fatty acids was shown to be significant. The lack of a significant correlation between K and post-hepatic TPVFA in the fed animal is probably the result of the combined effects of rumen and plasma volatile fatty acids on blood flow and liver perfusion.

LIST OF REFERENCES

1. Anderson, M. N., Norberg, B., and Senning, A. 1956. Studies of Liver Function during Extracorporal Circulation with Low Flow Rate. Surgery 43:397-407.

2. Andrews, W. H. H., and del Rio Lozano, I. 1963. Intrahepatic Vascular Pathways. Quart. J. Exper. Physiol. 48:127-137.

3. Bensadoun, A., and Reid, J. T. 1962. Estimation of Rate of Portal Blood Flow in Ruminants; Effect of Feeding, Fasting and Anesthesia. J. Dairy Sci. 45:540-543.

4. Bergman, E. N., Reid, R. S., Murray, M. G., and Brockway, J. M. 1965. Interconversions and Production of Volatile Fatty Acids in the Sheep Rumen. Biochem. J. 97:53-58.

5. Bergman, E. N., Roe, W. E., and Kon, K. 1966. Quantitative Aspects of Propionate Metabolism and Gluconeogenesis in the Sheep. Am. J. Physiol. 211:793-799.

6. Bergman, E. N., and Wolff, J. E. 1971. Metabolism of Volatile Fatty Acids by Liver and Portal Drained Viscera in Sheep. Am. J. Physiol. 221:586-592.

7. Bollman, J. L. 1953. Hepatic Function Modified by Alteration of Hepatic Blood Flow. Gastroent. 25:532-539.

8. Brauer, R. W. (ed.). 1958. Blood Flow Distribution in the Liver and the Circulatory Control of Liver Function. In Liver Function, pp. 113-127. Am. Inst. Biol. Sci. Monograph No. 4.

9. Brauer, R. W. 1963. Liver Circulation and Function. Physiol. Rev. 43:115-213.

10. Brauer, R. W., Leong, G. F., McElroy, R. F., and Holloway, R. J. 1956. Circulatory Pathways in the Rat Liver as Revealed by P-32 Chromic Phosphate Colloid Uptake in the Isolated Perfused Liver Preparation. Amer. J. Physiol. 184:593-598.

11. Cook, R. M., and Miller, L. D. 1965. Utilization of Fatty Acids in Ruminants. I. Removal of Them from Portal Blood by the Liver. J. Dairy Sci. 48:1339-1345.

12. Cornelius, C. E. 1970. Liver Function. In Clinical Biochemistry of Domestic Animals (Kaneko, J. J., and Cornelius, C. E., eds.), Vol. I, 2nd Edition, pp. 161-223. Academic Press Inc., N.Y.

13. Cornelius, C. E., Theilen, G. S., and Rhode, E. A. 1958. Quantitative Assessment of Bovine Liver Function using the Sulfobromophthalein Sodium Clearance Technique. Amer. J. Vet. Res. 19:560-566.

14. Daniel, P. M., and Prichard, M. M. L. 1951. Effects of Stimulation of the Hepatic Nerves and of Adrenaline upon the Circulation of the Portal Venous Blood within the Liver. J. Physiol. 114:538-548.

15. Daniel, P. M., and Prichard, M. M. L. 1951. Variations in the Circulation of the Portal Venous Blood within the Liver. J. Physiol. 114:521-537.

16. Dobson, A., and Phillipson, A. T. 1963. The Influence of the Contents of the Rumen upon its Blood Supply. J. Physiol. 133:76P-77P.

17. Erwin, E. S., Marco, G. J., and Emery, E. M. 1961. Volatile Fatty Acid Analysis of Blood and Rumen by Gas Chromatography. J. Dairy Sci. 44:1768-1771.

18. Fronek, K., and Stahlgren, L. H. 1968. Systemic and Regional Hemodynamic Changes during Food Intake and Digestion in Non-anesthetized Dogs. Circ. Res. 23:687-692.

19. Goodman, R. D. 1952. Bromsulfalein Clearance: A Quantitative Clinical Test of Liver Function. J. Lab. Clin. Med. 40:531-542.

20. Greenway, C. V., and Stark, R. D. 1971. Hepatic Vascular Bed. Physiol. Rev. 51:23-65.

21. Herrick, J. F., Essex, H. E., Mann, F. C., and Blades, E. J. 1934. The Effect of Digestion on the Blood Flow in Certain Blood Vessels in the Dog. Am. J. Physiol. 108:621-642.

22. Hoffbauer, F. W. 1950. Factors Influencing Pressure in the Portal Vein as Studied in the Intact Animal. Gastroent. 16:194-210.

23. Hunt, S. E., and McCosker, P. J. 1967. Bromsulphthalein Fractional Clearance in Normal Beef Cattle. Clin. Chim. ACTA 18:133-140.

24. Ingelfinger, F. J., Bradley, S. E., Mendeloff, A. I., and Kramer, P. 1948. Studies with Bromsulphthalein. I. Its Disappearance from the Blood after a Single Intravenous Injection. Gastroent. 11:646-654.

25. Jablouski, P., and Owen, J. A. 1969. The Clinical Chemistry of Bromsulfophthalein and Other Cholephilic Dyes. Adv. Clin. Chem. 12:309-386.

26. Katz, M. L., and Bergman, E. N. 1969. Simultaneous Measurements of Hepatic and Portal Venous Blood Flow in the Sheep and Dog. Am. J. Physiol. 216:946-952.

27. Katz, M. L., and Bergman, E. N. 1969. Hepatic and Portal Metabolism of Glucose, Free Fatty Acids, and Ketone Bodies in the Sheep. Am. J. Physiol. 216:953-960.

28. Khalil, L., Wakim, G., and Mann, F. C. 1942. The Intrahepatic Circulation of Blood. Anat. Rec. 82:233-253.

29. Kiddle, P., Marshall, R. A., and Phillipson, A. T. 1951. A Comparison of the Mixtures of Acetic, Propionic and Butyric Acids in the Rumen and in the Blood Leaving the Rumen. J. Physiol. 133:207-217.

30. Lavers, G. D., Cole, W. H., Keeton, R. W., Gephardt, M. C., and Dyniewicz, J. M. 1949. Bromsulfalein Clearance. J. Lab. Clin. Med. 34:965-982.

31. Lewis, A. E. 1948. The Concept of Heaptic Clearance. Am. J. Clin. Path. 18:789-792.

32. Lewis, A. E. 1950. Investigations of Hepatic Function by Clearance Techniques. Am. J. Physiol. 163:54-61.

33. Masson, M. J., and Phillipson, A. T. 1951. The Absorption of Acetate, Propionate, and Butyrate from the Rumen of Sheep. J. Physiol. 113:189-206.

34. McCuskey, R. S. 1966. A Dynamic and Static Study of Hepatic Arterioles and Hepatic Sphincters. Am. J. Anat. 119:455-477.

35. Mendeloff, A. I., Kramer, P., Ingelfinger, F. J., and Bradley, S. E. 1949. Studies with Bromsulfalein. II. Factors Altering its Disappearance from the Blood after a Single Intravenous Injection. Gastroent. 13:222-233.

36. Mixner, J. P., and Robertson, W. F. 1957. Bromsulphthalein Fractional Clearance in Dairy Cattle as a Criterion of Liver Function and the Simultaneous Determination of Volumes of Plasma and Blood. J. Dairy Sci. 40:914-921.

37. Myers, J. D. 1949. Observations on the Excretion of Bromsulphalein. J. Clin. Invest. 28:801-824.

38. Pratt, T. W., Vandlandingham, H. W., Talley, E. E., Nelson, J. M., and Johnson, E. O. 1932. Studies of the Liver Function of Dogs. Am. J. Physiol. 102:148-159.

39. Rosenthal, S. M., and White, E. C. 1925. Studies in Hepatic Function. VI. A. The Pharmacological Behavior of Certain Phthalein Dyes. B. The Value of Selected Phthalein Compounds in the Estimation of Hepatic Function. J. Pharmacol. Exptl. Therap. 24:265-288.

40. Ross, G., and Kurrasch, M. 1969. Adrenergic Responses of the Hepatic Circulation. Am. J. Physiol. 216:1380-1385.

41. Seligson, D., Marino, J., and Dodson, E. 1957. Determination of Sulfobromophthalein in Serum. Clin. Chem. 3:638-642.

42. Sellers, A. F., Stevens, C. E., Dodson, A., and McLeon, F. D. 1964. Arterial Flow to the Ruminant Stomach. Am. J. Physiol. 207:371-377.

43. Sellers, A. F., Stevens, C. E., and Dunlop, R. H. 1966. Factors Influencing Gastric Blood Flow in Normal Cattle. Fed. Proc. 20:242.

44. Shoemaker, W. C., VanItallie, T. B., and Walker, W. F. 1959. Measurement of Hepatic Glucose Output and Hepatic Blood Flow in Response to Glucagon. Am. J. Physiol. 1962:315-318.

45. Sutton, J. D. 1971. Carbohydrate Digestion and Glucose Supply in the Gut of the Ruminant. Proc. Nutr. Soc. 30:243-248.

46. Tagnon, H. J., Robbins, G. F., and Nichols, M. P. 1948. The Effect of Surgical Operations on the Bromsulfalein Retention Test. New Eng. J. Med. 238:556-560.

47. Taleisnik, S. 1955. Liver Mass Determination by Bromsulphalein in Partially Hepatectomized Rabbits. Gastroent. 29:64-70.

48. Treacher, R. J., and Sanson, B. F. 1969. Liver Function in Dairy Cows at Parturition. Res. Vet. Med. 10:461-468.

49. Vatner, S. F., Franklin, D., and Van Citters, R. L. 1970. Mesenteric Vaso-activity Associated with Eating and Digestion in the Conscious Dog. Am. J. Physiol. 219:170-174.

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