Interlobular Distribution of Hepatic Xenobiotic-Metabolizing Enzyme Activities in Cattle, Goats and Sheep

J. A. Wisniewski, D. E. Moody, B. D. Hammock and L. R. Shull

J ANIM SCI 1987, 64:210-215.

INTERLOBULAR DISTRIBUTION OF HEPATIC XENOBIOTIC-METABOLIZING ENZYME ACTIVITIES IN CATTLE, GOATS AND SHEEP[1]

J. A. Wisniewski[2], D. E. Moody[3], B. D. Hammock[3] and L. R. Shull[2]

University of California
Davis 95616

ABSTRACT

Microsomal and cytosolic enzymes that metabolize xenobiotics were measured in composite samples representing entire livers and in samples from three lobes, using livers of cattle, goats and sheep. Within individual species, concentrations of cytochrome P-450 and b_5 and activities of NADPH cytochrome c reductase, aldrin epoxidase, aminopyrine N-demethylase, ethoxycoumarin O-deethylase, microsomal and cytosolic stilbene oxide (epoxide) hydrolase and glutathione S-transferase were not different ($P>.05$) among the various hepatic lobes. Among species, several activities differed ($P<.05$), with cattle livers generally having lower values than sheep or goats.
(Key Words: Metabolism, Toxic Substances, Livers, Ruminants.)

Introduction

The wide variety of synthetic and naturally occurring toxicants to which food-producing animals are exposed can adversely affect animal health and impart hazardous residues to food products consumed by man (Shull and Cheeke, 1983). The fate of many substances in an animal is dependent both quantitatively and qualitatively on the activities of hepatic xenobiotic-metabolizing enzymes. Although inappropriate for ascertaining metabolism of xenobiotics in food animals, various in vitro hepatic systems such as tissue slices, homogenates and subcellular fractions have been valuable in studies of chemical induction (Cook and Wilson, 1970; Ford et al., 1976; Shull et al., 1986) and comparative metabolism (Smith and Watkins, 1984; Smith et al., 1984). Recently, isolated hepatocytes have been employed to ascertain xenobiotic metabolism in cattle (Forsell et al., 1985; Shull et al., 1986), as well as intermediary metabolism in sheep (Clark et al., 1976; Pogson et al., 1983). Studies in laboratory animals have shown that isolated hepatocytes approximate in vivo metabolism better than other in vitro systems (Bridges, 1980; Sirica and Pitot, 1980; Smith and Orrenius, 1984).

In contrast to laboratory animals, only a small portion of the liver, such as the caudate process for preparation of isolated hepatocytes (Forsell et al., 1985), is collected for use in in vitro studies in large animals. However, there are no data available on the uniformity of hepatic xenobiotic-metabolizing enzyme activities in ruminant species. In the present study, we compared the activities of several microsomal and cytosolic enzymes among different lobes of livers collected from cattle, goats and sheep.

Materials and Methods

Animals. Holstein bull calves, approximately 140 kg each and 12 wk of age, were purchased from a local rancher. Goats were Alpine does weighing 40 to 60 kg. Sheep were Suffolk x Finnish ewes weighing 60 to 80 kg. Does and ewes of approximately 2 yr of age were chosen randomly from animals slaughtered at the Cole Animal Facility, University of California, Davis.

Collection and Storage of Livers. All animals were stunned by captive bolt pistol and exsangu-

[1] This research was supported in part by a grant (1 R23FD01 224-01) from the Center for Vet. Med., Food and Drug Administration, Rockville, MD and a graduate research fellowship for J. A. Wisniewski provided by Stauffer Chem. Co. Partial support also was provided by NIEHS Grant ES 02710-06. The authors thank Dr. William Helferich and Mr. Wei-lung Tsai for their technical assistance.

[2] Dept. of Environ. Toxicol. All correspondence should be addressed to Dr. Lee Shull.

[3] Dept. of Entomol. and Environ. Toxicol.

Received February 27, 1986.
Accepted August 28, 1986.

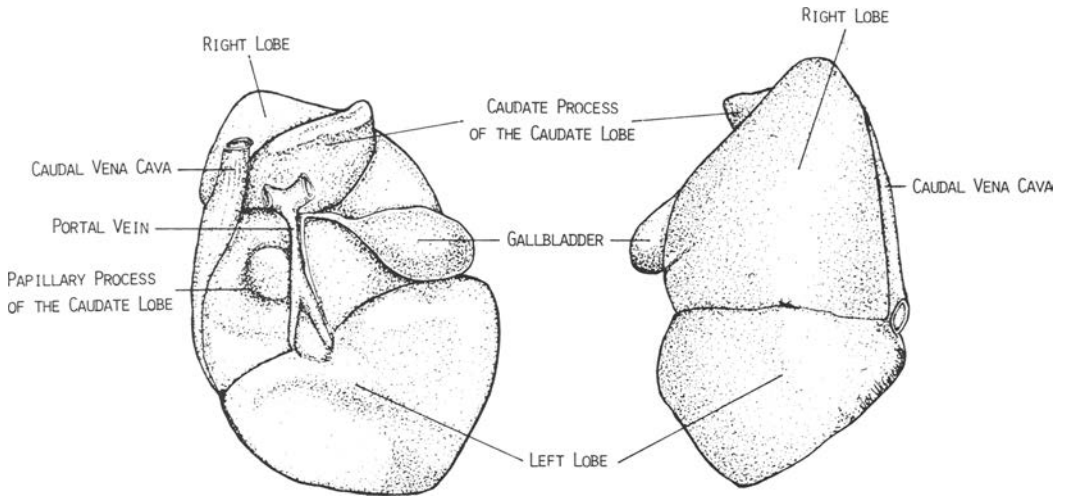

Figure 1. Diagram of the ruminant liver (adapted from Getty, 1975).

inated. Livers were removed within 20 min after exsanguination and immediately chilled in ice-cold 150 mM potassium chloride (KCl) buffer. Each liver (minus gall bladder) was sectioned as follows: 1) the caudate process of the caudate lobe (CP); 2) the right lobe, including the papillary process of the caudate lobe (RL); and 3) the left lobe (LL; figure 1). Each lobe was ground through a hand-crank meat grinder[4], mixed, and a sample weighing about 10 g was removed and frozen by submersion in liquid nitrogen within 30 min after removal from the animal. The remaining ground liver was combined, mixed thoroughly, and a 10-g liver composite (LC) was removed and frozen as described previously. Tissue manipulations were performed in a cold room to maintain the liver temperatures at 0 to 4 C. All samples were stored in liquid N_2 tanks for up to 5 mo. As determined experimentally, freezing of liver in liquid N_2 had no effect on enzyme activities; activities were not different ($P>.05$) when frozen (liquid N_2) and unfrozen (held at 0 to 4 C) tissue derived from livers of the same animal were compared (unpublished data).

Preparation of Microsomes. About 10 g of frozen tissue were slowly thawed in 20 ml of ice-cold homogenization buffer (20 mM Tris-hydrochloride, 1.15% KCl, pH 7.4). Microsomes were prepared as described previously (Shull et al., 1982). Homogenized liver was centrifuged at 16,800 x g for 20 min at 0 to 4 C. The supernatant was centrifuged at 105,000 x g for 60 min, and the cytosol was reserved and frozen at −80 C for cytosolic enzyme measurements. The microsomal pellet was resuspended in 15 ml of .4 M sucrose, 77 mM sodium pyrophosphate (pH 7.5) and recentrifuged as before. The final pellet was resuspended in 150 mM KCl to yield about 30 mg protein/ml. Protein was determined colorimetrically by the biuret method using a bovine serum albumin standard (Gornall et al., 1949).

Cytochrome Assays. Cytochromes P-450 and b_5 were measured spectrophotometrically by the method of Omura and Sato (1964). Protein concentration in 100 mM Tris (pH 7.4) was 1 mg/ml.

Enzyme Assays. NADPH cytochrome c reductase was measured spectrophotometrically (Pederson et al., 1973) using concentrations of .3 mg protein and .257 mM cytochrome c per assay. Aldrin (.55 mM) epoxidation (Krieger et al., 1976), aminopyrine (40 mM) N-demethylation (Anders and Mannering, 1966) and ethoxycoumarin (3.2 mM) O-deethylation (Ullrich and Weber, 1972) were measured as described by Gillette et al. (1986). Microsomal protein concentration was .4 mg/assay and reaction incubation time was 20 min. All monooxygenases, which were run in duplicate, were validated for time and protein linearity in the three species. Microsomal epoxide hydrolase (mEH) and

[4] Food Chopper No. 3. Universal Chopper Div., Union Mfg. Co., Meriden, CT.

glutathione S-transferase (GST) activities toward ^{3}H-cis-stilbene oxide (5×10^{-5} M) and cytosolic epoxide hydrolase (cEH) activities toward ^{3}H-trans-stilbene oxide (5×10^{-5} M) were determined by the method of Gill et al. (1983). The assay for cEH was modified to deplete endogenous glutathione as described by Moody et al. (1986). The specific activity was 6 mCi/mmol for both cis-and trans-stilbene oxide.

Statistical Analysis. Means and standard errors were calculated for three to five individuals of each species. For interlobular comparisons, standard errors (SE) were calculated using data pooled from the three lobes and LC of all animals (n=3,4 or 5) within species, where SE $=\sqrt{MS_E/n}$ (Gill, 1978). Data for each measurement within species were subjected to one-way analysis of variance (ANOVA) and Bartlett's test for heterogeneity of variance. For species comparisons, SE were calculated (Gill, 1978) from pooled liver composite data only, where SE = $s/\sqrt{n}$, and n= total number of cattle, goats and sheep, e.g., n = 13. Data for each measurement among species were subjected to ANOVA, Bartlett's test for heterogeneity of variance, and pairwise comparisons of means using Bonferonni significance levels[5].

Chemicals. Aldrin was purchased from Chem Service, West Chester, PA. Aminopyrine was purchased from Aldrich Chemical Co., Milwaukee, WI. [^{3}H] cis- and [^{3}H] trans-stilbene oxide were prepared by Dr. Bruce Hammock as previously described (Gill et al., 1983). All other reagents were purchased from Sigma Chemical Co., St. Louis, MO or Mallinckrodt, Inc., Paris, KY.

Results and Discussion

Variances for each measurement within each species, e.g., interlobular comparisons, and among the three species were found to be homogenous as determined by Bartlett's test (P>.05). Therefore, a pooled standard error of the mean (SE) is listed for each measurement.

We regarded all liver weights as within normal ranges. Visual appearances of the livers also were considered normal, but microscopic evaluations of liver tissues were not conducted. Lobal sections of cattle liver, as a percentage of whole organ weights, were: CP, 7%; RL, 62%; and LL, 30%. Lobe weights were not recorded for goats or sheep. Values for microsomal protein content are listed in table 1. As determined by ANOVA, no differences were found (P>.05) in microsomal protein content within species or among the three species. Values for cattle and sheep were similar to those reported by Smith and Watkins (1984).

Cytochrome P-450 or b_5 concentrations among the lobes and LC were not different (P>.05). Likewise, NADPH cytochrome c reductase, aldrin epoxidase, aminopyrine N-demethylase, ethoxycoumarin O-deethylase, microsomal and cytosolic stilbene oxide (epoxide) hydrolase and glutathione S-transferase activities did not differ (P>.05) among the lobes within species (table 1).

Comparisons among species were made using the liver composite data only. Several differences (P<.05) were found, as shown in table 1. Cytochrome P-450 concentrations in goats and sheep were about 1.5 times greater than in cattle. However, the trends reported here were in direct contrast to those reported by others. Smith et al. (1984) and Smith and Watkins (1984) found hepatic cytochrome P-450 concentrations to be about .60 nmol/mg protein in cattle, whereas concentrations in sheep were about .40 nmol/mg protein.

Goats had the lowest cytochrome b_5 concentrations, whereas sheep had the highest. Similar cytochrome b_5 values in cattle were reported by Facino et al. (1984).

The NADPH cytochrome c reductase activities were not significantly different among the species (P>.05). Values for cattle are consistent with reported values (Facino et al., 1984).

Although aldrin epoxidase activities in cattle were about 40% that of goats or sheep, the values did not differ (P>.05).

Aminopyrine N-demethylase activities in cattle were less than or equal to those in sheep, which were less than or equal to those in goats (table 1). Cook et al. (1970) reported similar trends, although values for goats were much greater (2.5 times) than for cattle.

Ethoxycoumarin O-deethylation was similar for all three species. Cytosolic epoxide hydrolase activities toward trans-stilbene oxide were similar in cattle and sheep, yet were about 25% higher in goats. Microsomal epoxide hydrolase activities toward cis-stilbene oxide were about twofold greater in goats and sheep than in cattle.

[5] BMDP Statistical Software, Inc., Los Angeles, CA.

TABLE 1. HEPATIC PROTEIN AND CYTOCHROME CONCENTRATIONS AND MICROSOMAL AND CYTOSOLIC ENZYME ACTIVITIES IN CATTLE, GOATS AND SHEEP[a]

Item	Liver lobe[b]	Cattle (n=5)	Goats (n=4)	Sheep (n=4)	SE[c]
Microsomal protein, mg/g liver	LC	18.2[g]	19.7[g]	22.9[g]	.9
	CP	18.2	18.6	24.7	
	RL	19.3	18.5	23.4	
	LL	17.5	18.0	23.0	
	SE[f]	1.4	1.7	1.7	
Cytochrome P-450, nmol/mg protein	LC	.399[g]	.670[h]	.604[h]	.043
	CP	.391	.544	.659	
	RL	.330	.706	.602	
	LL	.363	.634	.626	
	SE	.050	.098	.054	
Cytochrome b_5, nmol/mg protein	LC	.304[gh]	.225[g]	.452[h]	.036
	CP	.253	.212	.456	
	RL	.290	.284	.401	
	LL	.304	.217	.471	
	SE	.048[i]	.039	.036	
NADPH cytochrome c-reductase[d]	LC	102[g]	96[g]	86[g]	7
	CP	109	98	92	
	RL	101	105	84	
	LL	113	102	83	
	SE	14	11	4	
Aldrin epoxidase[d]	LC	.165[g]	.417[g]	.402[g]	.062
	CP	.121	.468	.349	
	RL	.175	.405	.417	
	LL	.163	.383	.384	
	SE	.039[i]	.124	.077	
Aminopyrine N-demethylase[d]	LC	4.55[g]	7.64[h]	5.25[gh]	.50
	CP	3.52	7.58	5.83	
	RL	5.17	6.62	6.36	
	LL	4.32	7.44	5.26	
	SE	.61	1.18	.90	
Ethoxycoumarin 0-deethylase[d]	LC	.570[g]	.604[g]	.553[g]	.076
	CP	.528	.433	.522	
	RL	.562	.525	.596	
	LL	.671	.553	.582	
	SE	.150	.141	.069	
Cytosolic stilbene oxide (epoxide) hydrolase[d]	LC	.96[g]	1.31[h]	1.01[g]	.06
	CP	1.03	1.37	.98	
	RL	.99	1.26	1.04	
	LL	1.00	1.32	.99	
	SE	.09[i]	.05	.09	
Microsomal stilbene oxide (epoxide) hydrolase[d]	LC	18.7[g]	36.0[h]	34.3[h]	2.7
	CP	16.1	34.0	35.3	
	RL	15.7	37.0	33.0	
	LL	16.5	37.0	33.1	
	SE	1.6[i]	3.2	2.5	

(continued)

TABLE 1. (CONTINUED) HEPATIC PROTEIN AND CYTOCHROME CONCENTRATIONS AND MICROSOMAL AND CYTOSOLIC ENZYME ACTIVITIES IN CATTLE, GOATS AND SHEEP[a]

Item	Liver lobe[b]	Cattle (n=5)	Goats (n=4)	Sheep (n=4)	SE[c]
Glutathione s-transferase[de]	LC	3.7[g]	8.6[gh]	13.1[h]	1.6
	CP	3.3	8.5	12.5	
	RL	4.1	9.0	13.1	
	LL	5.4	8.5	14.3	
	SE	2.1[i]	1.5	3.2	

[a]Values are means, where n=3, 4 or 5 animals.

[b]LC = liver composite, CP = caudate process of the caudate lobe, RL = right lobe, LL = left lobe.

[c]Standard errors (SE) were calculated from pooled liver composite data, where SE = $s/\sqrt{n}$, and n=total number of cattle, goats and sheep, e.g. n=13.

[d]Nmol•min^{-1}•mg protein^{-1}.

[e]Substrate is cis-stilbene oxide.

[f]Standard errors (SE) were calculated from pooled data, where SE = $\sqrt{MS_E/n}$, and n = 3,4 or 5 animals.

[g,h]Comparisons among species were made using LC data; means with different superscripts differ ($P<.05$).

[i]N=3

Glutathione S-transferase activities toward cis-stilbene oxide are shown in table 1. Activities in goats were greater than or equal to those in cattle, but were less than or equal to those in sheep.

Discussion

This study was conducted to assess the uniformity of xenobiotic-metabolizing activities in various locations of livers collected from domestic ruminant species. We were particularly concerned whether the caudate process of the caudate lobe (CP) was similar to the rest of the liver; to date, a two-step collagenase perfusion technique using the CP is the best method for preparing viable and functional isolated hepatocytes (Forsell et al.,1985; Shull et al., 1986). The CP as a source of isolated hepatocytes affords several advantages: 1) it avoids the use of voluminous amounts of buffers and collagenase solutions needed to perfuse the whole organ; 2) it allows for a more efficient perfusion because of the minimal cut surface and enclosed capsule; and 3) it provides a large yield of hepatocytes for studying a variety of metabolic activities (Clark et al., 1976; Pogson et al., 1983; Forsell et al., 1985; Shull et al., 1986). The data indicate that the CP, as well as the RL and LL, are not significantly different from the liver composite. In a similar study using rats, no significant differences were found comparing several microsomal monooxygenase activities of freshly isolated hepatocytes from regenerated adult liver with those of the caudate lobe of the parent liver (Guzelian et al., 1977). Although a more thorough examination of phase 1 and phase 2 biotransformations may reveal specific differences among hepatic locations, we feel the activities reported here and by others (Guzelian et al., 1977) are sufficient to support use of the CP. Moreover, liver subsamples collected by biopsy should be appropriate for in vitro studies.

This report also reveals several differences in xenobiotic metabolism among cattle, goats and sheep. In general, cattle livers had the lowest microsomal and cytosolic activities and goat livers had the highest. Smith and Watkins (1984) reported GST activities toward 1-chloro-2,4-dinitrobenzene to be about 2,000 nmol•min^{-1}•mg protein^{-1} in sheep, but only 950 nmol•min^{-1}•mg protein^{-1} in cattle. We report a similar trend in GST activities toward cis-stilbene oxide in cattle and sheep. In some instances, e.g., aldrin epoxidase and ethoxycoumarin O-deethylase, no differences among species were observed. In contrast, O-deethylase activities toward another substrate, ethoxyresorufin, were 10-fold greater in cattle than in

sheep (Smith et al., 1984). Although we are reporting significant species differences, it must be noted that other variables known to influence xenobiotic metabolism, such as age and environment, may be contributing factors. Consequently, the use of some ruminant species as models for others may be inappropriate unless all the factors underlying apparent species differences are identified and controlled.

Literature Cited

Anders, A. M. and G. J. Mannering. 1966. Kinetics of inhibition of the N-demethylation of ethylmorphine by 2-diethylaminoethyl-2,2-diphenylvalerate HCl (SKF-525A) and related compounds. Mol. Pharmacol. 2:319.

Bridges, J. W. 1980. Monooxygenase reactions, glucuronic acid and sulfate conjugation in isolated hepatocytes. Toxicology 18:195.

Clark, M. G., O. W. Filsell and I. G. Jarrett. 1976. Gluconeogenesis in isolated intact lamb liver cells: Effects of glucagon and butyrate. Biochem. J. 156:671.

Cook, R. M. and K. A. Wilson. 1970. Metabolism of xenobiotics in ruminants: Phenobarbital induction of liver microsomal nitrogen demethylase. J. Agr. Food Chem. 18(3):441.

Facino, R. M., M. Carini and C. Genchi. 1984. Impaired in vitro metabolism of the flukicidal agent nitroxynil by hepatic microsomal cytochrome P-450 in bovine fascioliasis. Toxicol. Lett. 20:231.

Ford, E.J.H., O. Araya, R. Rivera and J. Evans. 1976. The effect of phenobarbital, dieldrin, DDT, and benzene hexachloride on hepatic aminopyridine N-demethylase in horses, cattle, and sheep. Toxicol. Appl. Pharmacol. 35:475.

Forsell, J. H., B. W. Jesse and L. R. Shull. 1985. A technique for isolation of bovine hepatocytes. J. Anim. Sci. 60:1597.

Getty, R. 1975. Sisson and Grossman's The Anatomy of the Domestic Animals. pp 908–913. W. B. Saunders Co., Philadelphia, PA.

Gill, J. L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. Iowa State Univ. Press, Ames.

Gill, S. S., K. Ota and B. D. Hammock. 1983. Radiometric assays for mammalian epoxide hydrolases and glutathione S-transferases. Anal. Biochem. 131:273.

Gillette, D. M., R. D. Corey, W. G. Helferich, J. M. McFarland, L. J. Lowenstine, D. E. Moody, B. D. Hammock and L. R. Shull. 1986. Comparative toxicology of tetrachlorobiphenyls in mink and rats. I. Changes in hepatic enzyme activity and smooth endoplasmic reticulum volume. Fund. Appl. Toxicol. (In press).

Gornall, A. C., A. J. Bardwill and M. M. Davis. 1949. The determination of serum protein by means of a biuret reaction. J. Biol. Chem. 177:751.

Guzelian, P. S., D. M. Bissell and U. A. Meyer. 1977. Drug metabolism in adult rat hepatocytes in primary monolayer culture. Gastroenterology 72:1232.

Krieger, R. I., S. I. Gee, J. L. Miller and T. Thongsinthusak. 1976. Monooxygenase catalyzed aldrin epoxidation and dihydroisodrin hydroxylation in monkey liver needle biopsy specimens. Assay and properties. Drug Metab. Dispos. 1(1):28.

Moody, D. E., M. H. Silva and B. D. Hammock. 1986. Epoxide hydrolysis in the cytosol of rat liver, kidney, and testis. Measurement in the presence of glutathione and the effect of dietary clofibrate. Biochem. Pharmacol. 35:2073.

Omura, T. and R. Sato. 1964. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. J. Biol. Chem. 239:2379.

Pederson, T. C., T. A. Beuge and S. D. Aust. 1973. Microsomal electron transport. The role of reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase in liver microsomal peroxidation. J. Biol. Chem. 15:7134.

Pogson, C. I., K.R.F. Elliott, M. A. Lomax, R. A. Munoz-Clares and S. A. Smith. 1983. Variations in hepatic metabolism between species. In: R. A. Harris and N. W. Cornell (Ed.). Isolation, Characterization, and Use of Hepatocytes. Elsevier Science Publishing Co., Inc., New York.

Shull, L. R., M. R. Bleavins, B. A. Olson and R. J. Aulerich. 1982. Polychlorinated biphenyls (Arochlor® 1016 and 1242): Effect of hepatic microsomal mixed function oxidases in mink and ferrets. Arch. Environ. Contam. Toxicol. 11:313.

Shull, L. R. and P. R. Cheeke. 1983. Effects of synthetic and natural toxicants on livestock. J. Anim. Sci. 57 (Suppl. 2):330.

Shull, L. R., D. G. Kirsch, C. L. Lohse, G. P. Carlson, L. A. Doody and J. A. Wisniewski. 1986. Xenobiotic metabolism in suspensions and primary cultures of isolated hepatocytes prepared from the caudate process of bovine liver. Amer. J. Vet. Res. 47:1.

Sirica, A. E. and S. Pitot. 1980. Drug metabolism and effects of carcinogens in cultured hepatic cells. Pharmacol. Rev. 31:205.

Smith, G. S. and J. B. Watkins. 1984. Biotransformations of xenobiotics in tissues of cattle and sheep. Can. J. Anim. Sci. 64 (Suppl.):278.

Smith, G. S., J. B. Watkins, T. N. Thompson, K. Rozman and C. D. Klaassen. 1984. Oxidative and conjugative metabolism of xenobiotics by livers of cattle, sheep, swine and rats. J. Anim. Sci. 58:386.

Smith, M. T. and S. Orrenius. 1984. Studies on drug metabolism and drug toxicity in isolated mammalian cells. In: J. R. Mitchell and M. G. Horning (Ed.). Drug Metabolism and Drug Toxicity. Raven Press, New York.

Ullrich, V. and P. Weber. 1972. The O-dealkylation of 7-ethoxycoumarin by liver microsomes. Hoppe-Seyler's Z. Physiol. Chem. 353:1171.