CHANGES IN SOME BLOOD CONSTITUENTS ASSOCIATED WITH PARTURITION IN HEREFORD HEIFERS FED SOLELY ON RESTRICTED AMOUNTS OF SORGHUM GRAIN

By E. PAYNE, B.Sc.,* J. W. RYLEY, B.V.Sc.,† and R. J. W. GARTNER, B.Sc.‡

SUMMARY

Fifteen Hereford heifers fed solely on restricted quantities of sorghum grain were bled at intervals during the 24-hr period before and after parturition. Blood samples were analysed for glucose, inorganic phosphate, haemoglobin and packed cell volume. Calcium, magnesium, total protein, albumin and globulin were determined on serum.

There was a moderate decrease in blood glucose levels prior to parturition and a significant rise to peak values at or about parturition. Blood inorganic phosphate levels showed a significant decline. No significant changes were evident in blood haemoglobin, packed cell volume, serum calcium and magnesium levels. Total protein and globulin levels in serum decreased significantly when compared with the values obtained some weeks before and after parturition.

The diurnal variations in blood glucose, haemoglobin and packed cell volume were determined on three steers bled at 2-hr intervals throughout a 24-hr period. Two of these steers were fed solely on restricted amounts of sorghum grain; the other received a restricted amount of lucerne chaft. No regular pattern of diurnal variation was evident, although blood glucose levels tended to rise after feeding. The magnitude of changes due to the time of day at which calving took place was considered insufficient to affect conclusions with regard to changes in blood constituents associated with parturition.

I. INTRODUCTION

Investigations of the changes in blood constituents at parturition have been restricted almost entirely to dairy cows. The results vary according to the frequency and proximity of sampling in relation to the actual time of parturition.

Godden and Allcroft (1932), Van Soest and Blosser (1954), Merrill and Smith (1954) and Horrocks and Paterson (1957) have shown that there is a sharp rise in the blood glucose level, often beginning just prior to parturition. This has also been reported in sheep (Snook and Godden 1938; Reid 1960a).

Various workers (Palmer, Cunningham, and Eckles 1930; Godden and Allcroft 1932; Seekles, Sjollema, and van der Kaay 1932; Wilson and Hart 1932; Allcroft and Godden 1934; Duckworth and Godden 1940; Craige *et al.* 1949; Blood and White 1949; Niedermeier, Smith, and Whitehair 1949; Moodie, Marr,

^{*} Chemist, Animal Research Institute, Yeerongpilly

[†] Director of Husbandry Research, Animal Research Institute, Yeerongpilly

[‡] Senior Chemist, Animal Research Institute, Yeerongpilly

and Robertson 1955; Reda and Salem 1955) have reported decreased levels for serum calcium and for blood or plasma inorganic phosphate at parturition. Van Soest and Blosser (1954) obtained samples during a period of some hours immediately before and after parturition and found no significant changes in these constituents in cows showing no clinical symptoms of parturient paresis.

Fewer observations have been made of changes in serum magnesium levels at parturition. Hypermagnesaemia was observed by Seekles, Sjollema, and van de Kaay (1932), Allcroft and Godden (1934) and Niedermeier, Smith and Whitehair (1949), whereas Blood and White (1949) and Moodie, Marr, and Robertson (1955) found no alteration in serum magnesium.

Other blood constituents which have been reported to change at parturition are packed cell volume (P.C.V.) and total serum proteins. Van Soest and Blosser (1954) noted an increase in P.C.V. at the time of parturition and a gradual decline in the period after parturition. Larsen and Kendall (1957) found minimal values for serum proteins at parturition, with only very gradual changes during the five weeks pre- and post-parturition.

It appears that although quite definite conclusions have been reached with regard to changes in serum calcium, blood inorganic phosphate and blood glucose within a few days or weeks of parturition, only limited data are available on the changes occurring within a few hours of parturition. Further, most published findings were obtained with cows fed adequate rations containing roughage.

During the drought feeding experiments of Ryley and Gartner (1962) it was considered desirable to determine changes in blood glucose immediately before and after parturition. These Hereford heifers had been fed for survival solely on sorghum grain during the last three months of pregnancy. Other constituents determined were blood haemoglobin, P.C.V. and inorganic phosphate and serum calcium, magnesium, total protein, albumin and globulin.

To evaluate diurnal variation as a possible influence on the levels found, glucose, haemoglobin and P.C.V. were determined on blood samples drawn over a 24-hr period from three non-pregnant animals of about the same age.

II. MATERIALS AND METHODS

(i) Experimental Animals.—Fifteen of the dehorned Hereford heifers from the experiment of Ryley and Gartner (1962) on the feeding of restricted amounts of crushed sorghum grain in late pregnancy and early lactation were involved. Their mean age at calving was approximately 43 months.

The three animals used in the determination of the diurnal variations of blood constituents were twin Jersey x Australian Illawarra Shorthorn steers and one Hereford steer. Their ages at the time of sampling were 39 and 32 months, respectively.

(ii) Methods of Chemical Analysis.—Analytical methods employed were as follows:— blood glucose, Mendel, Kemp, and Myers (1954); haemoglobin, Donaldson et al. (1951); P.C.V. essentially by the method of Wintrobe (1947), using heparin as the anticoagulant and a relative centrifugal force of 2067 g; blood inorganic phosphate, Moir (1954); serum calcium by a modification of the method of Clark and Collip (1925); serum magnesium by a modification of the method of Holzapfel (1934); total serum protein, albumin and globulin, Gornall, Bardawill, and David (1949). In the determination of blood glucose the blood was added to the trichloracetic acid immediately after the sample was drawn. After centrifuging, an aliquot was either analysed within 6 hr or stored in the deep freeze until analysed.

III. EXPERIMENTAL

Pregnant heifers with a mean stage of gestation of 171 days and in fair condition were allotted to three groups and changed to an all-grain ration over a period of two weeks. They were maintained on grain for 24 weeks, calving taking place between the eleventh and fourteenth weeks of grain feeding. During this period the animals were observed at frequent intervals daily for indications At the first signs of parturition heifers were removed from their respective yards and transferred to individual small holding yards close to the crush used for bleeding. Blood samples were obtained pre- and post-calving. In some cases calving was not observed because parturition was not considered imminent when the afternoon observation was made. These heifers calved during the following night. In others, prolonged calving resulted in a larger number of precalving samples, whereas if calving took place close to midnight fewer postcalving samples were obtained. Of the 28 heifers that calved, satisfactory preand post-calving samples were obtained from 15 animals. The group numbers at this stage and their respective treatments throughout the experiment were as follows:--

	Group	No. of Animals	Crushed Sorghum Grain* (lb/head/day)	Frequency of Feeding				
III II		 5 4 6	6 6 10	Daily Twice-weekly Daily				

^{*} To the sorghum grain was added 1 per cent. of ground limestone

The first post-calving sample was obtained either after the calf had suckled or after 2 hr, whichever was the sooner. Analytical results for periods greater than 48 hr from parturition were taken from the data of Ryley and Gartner (1962).

Samples were analysed for blood glucose, haemoglobin, P.C.V. and inorganic phosphate; serum calcium, magnesium, total protein, albumin and globulin.

The	steers	used to	determine	diurnal	variations	of	blood	constituents	were
housed in	stalls	and fed	daily as fol	llows:	≣				

	Anim	ıal		Body-weight (lb)	Ration	Amount Fed Daily (lb)		
Jersey x A.I.S.	twin		 	782	Sorghum grain*		6	
Jersey x A.I.S.	twin		 	814	Sorghum grain		6	
Hereford	• •		 	626	Lucerne chaff		6	

^{*} The sorghum grain was crushed and 1 per cent, ground limestone added

The animals were fed these rations for six weeks prior to sampling. One blood sample was taken from each animal immediately prior to feeding at 7.50 a.m., then 2 hr after they consumed their feed and subsequently at approximately 2-hr intervals for a 24-hr period. Samples were analysed for blood glucose, haemoglobin and P.C.V.

IV. RESULTS

The changes in blood constituents of the Hereford heifers in terms of the means and their standard errors have been summarized in Table 1 to cover four arbitrary time periods in the 8 hr immediately before and after parturition. Additional data are presented for serum total protein, albumin and globulin in samples collected some weeks before and after parturition. No analysis of data was attempted on a group basis in view of the limited number of animals in each group.

Blood glucose levels showed significant differences among periods (P < 0.01), with a peak at or shortly after parturition. There was also a significant decline in blood inorganic phosphate over the whole 16-hr period (P < 0.05). The serum total protein and globulin levels did not show any significant differences between the periods close to parturition, but the mean values around parturition were significantly lower (P < 0.05 for the total protein, P < 0.01 for the globulin) compared with values obtained some weeks before and after parturition. No other differences were significant, but there was a tendency towards an overall rise in the P.C.V. and serum calcium levels during the 16-hr period.

Data on the changes in blood glucose in individual animals are presented in Table 2. Blood glucose levels in the few days prior to parturition fell to as low as 18 mg per 100 ml. Although differences between groups were not significant on the limited number of animals available, the heifers in Group I tended to show blood glucose levels lower than those of heifers in Groups II and III.

The diurnal variations in blood glucose, haemoglobin and P.C.V. for the three steers are shown in Figure 1. Although there appears to be some variation in the levels during the 24-hr period, no definite conclusions are evident regarding

 ${\color{blue}TABLE~1}$ Means and Their Standard Errors for Analyses of Blood and Serum Obtained from Hereford Heifers Around Parturition

		Means and their Standard Errors* for Periods around Parturition										
Determination	35 to 11 Days Before Parturition	8 to 3 Hours Before Parturition	3 to 0 Hours Before Parturition	0 to 3 Hours After Parturition	3 to 8 Hours After Parturition	55 to 73 Days After Parturition						
Glucose (mg/100 ml)		64·7 ± 4·2	72.3 ± 4.0	92·6 ± 3·4	63·4 ± 5·0							
Haemoglobin (g/100 ml)		12.5 ± 0.4	12.4 ± 0.4	12.9 ± 0.3	13.3 ± 0.5							
P.C.V. (%)		38·8 ± 0·5	39.4 ± 0.5	39·6 ± 0·4	40·3 ± 0·7							
Inorganic phosphate (mg/100 ml) .		4.65 ± 0.2	4.23 ± 0.20	4.10 ± 0.16	3.96 ± 0.25							
Serum calcium (mg/100 ml)		8.97 ± 0.16	9.07 ± 0.15	9.21 ± 0.13	9.37 ± 0.19							
Serum magnesium (mg/100 ml) .		2.65 ± 0.08	2.66 ± 0.07	2.59 ± 0.06	2.53 ± 0.09							
Serum total protein (g/100 ml) .	. 7.06 ± 0.07	6.86 ± 0.07	6.93 ± 0.07	6.79 ± 0.07	6.89 ± 0.09	7.14 ± 0.07						
Serum albumin (g/100 ml)	3.50 ± 0.05	3.56 ± 0.05	3.58 ± 0.05	3.39 ± 0.05	3.56 ± 0.06	3.41 ± 0.05						
Serum globulin (g/100 ml)	3.56 ± 0.08	3.30 ± 0.08	3.35 ± 0.08	3.40 ± 0.08	3.33 ± 0.09	3.73 ± 0.08						
Number of observations	. 15	17	16	16	10	14						

^{*} Standard errors quoted are approximate but exact methods were used in testing differences among periods. Differences among animals have been removed by analysis of variance

 ${\bf TABLE~2}$ Changes in Blood Glucose Associated with Parturition in Hereford Heifers

Group	Blood Glucose (mg/100 ml)														
				Prepar	turition				Post-parturition						
I	42 (25d)*	34 (17d)			21 (10·3)*	47 (7·3)	46 (5·0)	53 (2·5)	37 (3·5)	23 (8·0)	38 (27·0)				50 (60d)
	37 (24d)	38 (16d)					18 (2·7)	47 (0·7)	64 (0·3)	65 (3·0)	30 (9·2)	39 (14·0)	45 (35·0)		39 (61d)
	44 (35d)	45 (27d)				77 (6·0)	70 (3·7)	64 (1·7)	67 (0·7)	58 (2·7)	54 (20·5)				
	22 (12d)	27 (4d)	50 (2d)					32 (5·5)	112 (2·2)	65 (18·0)	67 (24·0)				40 (73d)
	33 (22d)	43 (14d)	29 (5d)					38 (1·5)	66 (1·0)	48 (4·0)	64 (7·0)			27 (2d)	47 (63d)
II	37 (11d)	47 (3d)						57 (1·5)	89 (2·2)	76 (5·2)	58 (8·2)	47 (24·0)			52 (74d)
	34 (22d)	56 (14d)				52 (4·0)	28 (2·0)	48 (0·5)	58 (0·5)	58 (2·5)	69 (12·0)	66 (20·5)			54 (63d)
-	38 (20d)	47 (12d)					86 (6·7)	87 (3·7)	96 (1·7)	73 (12·5)	71 (21·2)				54 (65d)
	41 (21d)	38 (13d)						108 (0·5)	99 (2·0)	62 (8·0)					40 (64d)
III	45 (30d)	37 (22d)					46 (2·5)	33 (0·7)	77 (0·7)	54 (3·0)	51 (4·7)	48 (21·5)			49 (55d)
	43 (21d)	44 (13d)					70 (5·0)	90 (1·5)	55 (3·7)	39 (13·4)	61 (22·0)				26 (64d)
	39 (25d)		52 (11·5)	55 (9·5)	51 (6·2)	25 (4·3)	29 (3·7)	53 (0·7)	65 (1·0)	22 (5·5)	33 (12·5)	43 (30·7)	,		
	31 (25d)	40 (17d)				24 (4·0)	68 (2·0)	68 (1·0)	124 (2·0)	25 (17·7)					39 (60d)
	47 (15d)	51 (7d)					58 (4·5)	89 (2·8)	104 (1·7)	71 (18·0)	43 (24·5)				40 (70d)
	42 (21d)	40 (13d)	38 (4d)				53 (6·5)	65 (3·7)	68 (7·3)	56 (16·0)					54 (64d)

^{*} Figures in parentheses indicate time pre- and post-parturition in hours except where "d" indicates days.

times of peak values. The blood glucose concentration varied over a range of 20 mg per 100 ml and there seemed to be a slight rise after feeding. This may have been compensated to some degree by a haemodilution effect, as indicated by a tendency for haemoglobin and P.C.V. to fall during this period. It is apparent also from Figure 1 that in the steer fed lucerne chaff the blood glucose level was consistently lower than in the steers fed sorghum grain.

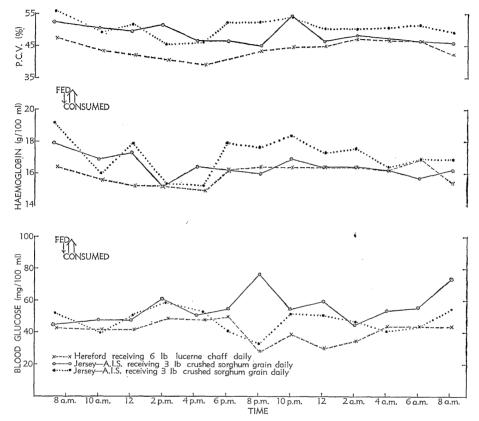


Fig. 1.—Diurnal variation in blood glucose, blood haemoglobin and packed cell volume.

V. DISCUSSION

The general agreement between our results and published data on dairy cattle suggests that the experimental conditions of restricted feeding on all-grain rations had little direct effect on changes in blood constituents associated with parturition. The limited variation in blood glucose, haemoglobin and P.C.V. with time during the 24-hr test period with non-pregnant cattle suggests that the changes in blood constituents are associated directly with parturition rather than with the time of day at which calving takes place. That the diurnal variations in blood constituents would not affect the changes observed overall is also suggested by the fact that calving occurred at various times of day.

The sharp peak in blood glucose at parturition is in agreement with the findings of Van Soest and Blosser (1954) on dairy cattle. A similar rise in sheep (Reid 1960a) has been attributed to the discontinuance of the demand by the foetus on the glucose pool. Although the changes in blood haemoglobin and P.C.V. were not significant, individual results suggest a tendency for increased levels of these constituents at parturition as reported by Van Soest and Blosser (1954).

The decline in blood inorganic phosphate during the period from 8 hr before to 8 hr after parturition, although significant, was not so marked as that described by Blood and White (1949) and Moodie, Marr, and Robertson (1955). The latter workers found that the magnitude of this effect depended on the age of the animal, heifers showing the least fall in this blood constituent. They found also that the lowest values could occur at any time up to 3 days after calving. Our results apply to heifers only and sampling around parturition was restricted usually to 24 hr before and after parturition.

The absence of significant changes in serum calcium may be explained in terms of the age of the animals and the limited number of samples taken more than 24 hr after parturition. Moodie, Marr, and Robertson (1955) found the lowest values in old cows sampled up to three days after parturition. Van Soest and Blosser (1954), who sampled frequently within a few hours of parturition, reported no change in serum calcium in 3–4-year-old cows.

Blood and White (1949) have suggested that the fall in blood inorganic phosphate and serum calcium is a temporary phenomenon associated with the commencement of lactation and that the dairy cow takes a few days to adjust to this continual drain on its resources. In our experiment with beef heifers, milk production at a mean stage of lactation of 68-72 days was less than 0.75 gal per day (Ryley and Gartner 1962), which is considerably less than that expected for dairy cows. This low level of production may also have influenced the magnitude of the changes in these blood constituents.

The absence of any change in serum magnesium is in agreement with the earlier data of Moodie, Marr, and Robertson (1955).

The gradual increase in total serum protein until parturition and the subsequent gradual decline are in agreement with the results of Larsen and Kendall (1957). The minimum values for serum globulin at parturition have been associated with the function of the mammary gland at this time in the production of colostrum which is rich in globulins (Larsen and Kendall 1957; Dixon, Weigle, and Vazquez 1961).

A major reason for determining changes in blood glucose was to examine the possibility of hypoglycaemia just prior to parturition. Reid (1960b) found that with ewes on a low plane of nutrition only those giving birth to twins showed consistent hypoglycaemia. In our experiment the sole ration of 6 lb sorghum grain per head per day was obviously inadequate for maintenance of bodyweight in pregnant Hereford heifers (Ryley and Gartner 1962). The absence of

hypoglycaemia prior to parturition, however, suggests that no marked fall in the level of blood glucose can be expected in pregnant cattle bearing a single foetus and fed solely on restricted amounts of sorghum grain.

The slight rise in blood glucose after feeding in the non-pregnant animals was not nearly so marked as Reid (1959) recorded in pregnant sheep fed sub-optimal amounts of chaff. In the steer fed sub-optimal amounts of lucerne chaff in our experiment it was found that there was a lesser rise than in the steers fed grain. That the blood glucose level was consistently lower in this steer than in the twins fed grain may be accounted for by the fact that the all-grain diet gives rise to a greater amount of propionic acid (unpublished data), which is metabolized by the liver to glucose.

VI. ACKNOWLEDGEMENTS

The authors wish to acknowledge indebtedness to the following officers of the Queensland Department of Agriculture and Stock: Mr. A. W. Beattie for statistical analyses; Mr. A. I. Inkerman, Mr. J. O. Twist and Mr. M. S. O'Bryan for technical assistance.

REFERENCES

- ALLCROFT, W. M., and GODDEN, W. (1934).—Changes in the calcium and magnesium of the serum and in the inorganic phosphorus of the blood of cows at calving and of the calf during early life. *Biochem. J.* 28:1004-7.
- BLOOD, D. C., and WHITE, I. G. (1949).—The effect of parturition on the physiology of dairy cows in relation to milk fever. *Aust. Vet. J.* 25:114-22.
- CLARK, E. P., and COLLIP, J. B. (1925).—A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. *J. Biol. Chem.* 63:461-4.
- Craige, A. H., Johnson, R. B., Blackburn, E. G., and Coffin, J. M. (1949).—Biochemical studies on three cows at parturition including one case of milk fever. *J. Amer. Vet. Med. Assoc.* 114:136-40.
- DIXON, F. J., WEIGLE, W. O., and VAZQUEZ, J. J. (1961).—The metabolism and mammary secretion of serum proteins in the cow. *Lab. Invest.* 10:216-23.
- Donaldson, R., Sisson, R. B., King, E. J., Wootton, I. D. P., and MacFarlane, R. G. (1951).—Determination of haemoglobin. VII. Standardised optical data for absolute estimations. *Lancet* 260:874-81.
- DUCKWORTH, J., and GODDEN, W. (1940).—The partition of serum calcium about the time of parturition in the dairy cow. J. Dairy Res. 11:9-14.
- GODDEN, W., and ALLCROFT, W. M. (1932).—Changes in the composition of cow's blood at time of calving and a comparison of the blood of the calf with that of its dam. *Biochem. J.* 26:1640-6.
- GORNALL, A. G., BARDAWILL, C. J., and DAVID, M. M. (1949).—Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177:751-66.
- HOLZAPFEL, C. R. (1934).—Studies in mineral metabolism. XXVII. Modifications of the methods used at Onderstepoort for the determination of (A) magnesium and calcium; (B) potassium, in grass extracts. Onderstepoort J. Vet. Sci. 2:115-22.

- HORROCKS, D., and PATERSON, J. Y. F. (1957).—Some observations on glucose, ketone bodies and volatile fatty acids in the blood of dairy cattle. *J. Comp. Path.* 67:331-41.
- LARSON, B. L., and KENDALL, K. A. (1957).—Changes in specific blood serum protein levels associated with parturition in the bovine. *J. Dairy Sci.* 40:659-66.
- MENDEL, B., KEMP, A., and MYERS, D. K. (1954).—A colorimetric micro method for determination of glucose. *Biochem. J.* 56:639-46.
- MERRILL, W. G., and SMITH, V. R. (1954).—A comparison of some cellular and chemical constituents of blood at time of parturition and after administration of adreno-corticotrophin. J. Dairy Sci. 37:546-51.
- Moir, K. W. (1954).—The preservation of bovine blood for the determination of inorganic phosphate in the diagnosis of aphosphorosis. *Qd J. Agric. Sci.* 11:143-7.
- MOODIE, E. W., MARR, A., and ROBERTSON, A. (1955).—Serum calcium and magnesium and plasma phosphate levels in normal parturient cows. J. Comp. Path. 65:20-36.
- NIEDERMEIER, R. P., SMITH, V. R., and WHITEHAIR, C. K., (1949).—Parturient paresis. III.

 A study of various blood constituents at parturition in mastectomized cows.

 J. Dairy Sci. 32:927-34.
- PALMER, L. S., CUNNINGHAM, H. S., and ECKLES, C. H. (1930).—Normal variations in the inorganic phosphorus of the blood of dairy cattle. *J. Dairy Sci.* 13:174-95.
- REDA, H., and SALEM, H. (1955).—Variation in the calcium and inorganic phosphorus contents of blood, urine and colostrum of Egyptian buffaloes and cattle associated with parturition. *Indian J. Dairy Sci.* 8:19-25.
- Reid, R. L. (1959).—Studies on the carbohydrate metabolism of sheep. VIII. Hypoglycaemia and hyperketonaemia in undernourished and fasted pregnant ewes. *Aust. J. Agric. Res.* 10:81-96.
- Reid, R. L. (1960a).—Studies on the carbohydrate metabolism of sheep. IX. Metabolic effects of glucose and glycerol in undernourished pregnant ewes and in ewes with pregnancy toxaemia. *Aust. J. Agric. Res.* 11:42-57.
- Reid, R. L. (1960b).—Studies on the carbohydrate metabolism of sheep. X. Further studies on the hypoglycaemia and hyperketonaemia in undernourished pregnant ewes and in ewes with pregnancy toxaemia. Aust. J. Agric. Res. 11:346-63.
- RYLEY, J. W., and GARTNER, R. J. W. (1962).—Drought feeding studies with cattle. 7. The use of sorghum grain as a drought fodder in late pregnancy and early lactation. *Qd J. Agric. Sci.* 19:309-30.
- SEEKLES, L., SJOLLEMA, B., and VAN DER KAAY, F. C. (1932).—Changes in the blood composition and in the tone of the autonomic nervous system of cattle during pregnancy and at the birth period. *Biochem. Z.* 249:424-37.
- SNOOK, L. C., and GODDEN, W. (1938).—The sugar and total ketone content of the blood of ewes and their new-born lambs. *Biochem. J.* 32:2037-9.
- Van Soest, P. J., and Blosser, T. H. (1954).—A detailed study of levels of certain blood constituents in normally calving dairy cows and in dairy cows with parturient paresis. J. Dairy. Sci. 37:185-94.
- WILSON, L. T., and HART, E. B. (1932).—The chemistry of the blood of dairy cows before and after parturition and its relation to milk fever. J. Dairy Sci. 15:116-31.
- WINTROBE, M. M. (1947).—"Clinical Haematology," 2nd Ed. (Lee and Febiger:Philadelphia).