symptoms. It will be noted that in the control curves where no food was given to the patient, the blood sugar level remained essentially unchanged for the 4-hr period. It must be assumed that the amount of exogenous insulin present was the same both in the test period after orange juice, and in the control period. Apparently the rise in blood sugar level caused by the ingestion of glucose stimulated the glucose-disposing mechanisms. When the stimulus produces an exaggerated response the clinical symptoms of hypoglycemic shock appear.

It can be concluded that the too rapid rise in blood sugar concentration can be responsible for the post-prandial hypoglycemias. As a corollary, it may be stated that hypoglycemia can be avoided by dietary regulation directed toward slowing the availability of the carbohydrate. Evidence is being accumulated to show the practical clinical application of these conclusions.

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Blood Glucose and Lactic Acid in Relation to Milk Secretion.†

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The recent *in vitro* synthesis of lactose by Petersen and Shaw[1] from lactic acid and glucose and the demonstration by Graham[2] of the loss of lactic acid from the blood traversing the mammary gland indicates that lactose is synthesized in the gland from lactic acid and glucose of the blood.

Kaufman and Magne[3] found that in lactating animals the mammary venous blood contained less glucose than the jugular blood. Loss of glucose from the blood in passing through the lactating mammary gland has also been shown by Blackwood and Stirling,[4] Lintzel,[5] and Graham, Jones and Kay.[6] The latter workers reported that the amount of glucose taken out of the blood was proportional

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* The data in this paper are from a thesis presented by J. C. Shaw in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, Minnesota Agricultural Experiment Station.

1 Petersen, W. E., and Shaw, J. C., *Science*, 1937, **86**, 398.

2 Graham, W. R., Jr., *J. Biol. Chem.*, 1937, **122**, 1.

3 Kaufman, M., and Magne, H., *Compt. Rend. Acad. Sci. Paris*, 1906, **143**, 779.

to the level of glucose in the arterial blood and to the milk yield per day.

Several workers have demonstrated reduced blood and milk sugar by injection of insulin into cows, goats and sheep. Brown, Petersen and Gortner[7] reported that reducing the blood sugar to 17 mg % resulted in a drop in milk sugar to about one-fourth normal.

It has been much more difficult, however, to increase the lactose by increasing the blood sugar. Whitnah, Riddell and Hodgson[8] obtained only a slight increase in the lactose of milk when the blood sugar was raised to a very high level by pumping glucose solution into the stomachs of lactating cows. Likewise Brown, Petersen and Gortner[7] obtained only a very slight increase in lactose secretion by producing a hyperglycemic condition with intramammary duct injections of isotonic glucose. The sugar content of blood was raised to 300 mg % by Petersen and Boyd[9] by infusion of isotonic solutions of glucose into the external pudic artery without any increase in the lactose of the milk.

In a further study of the relation of blood glucose to milk secretion, a study was made of the relation of the blood glucose absorbed by the mammary gland to the following: time following completion of last milking, arterial blood sugar level, and level of milk production. A further study was made of the use of lactic acid by the mammary gland and the total blood carbohydrate needed for the elaboration of milk sugar.

Arterial and venous blood samples were taken simultaneously from the internal iliac artery or the external pudic artery and the subcutaneous abdominal mammary veins respectively. The method proposed by Graham, Kay and McIntosh[10] was followed in obtaining the arterial blood from the internal iliac. The method of Boyd and Petersen[11] was used in obtaining arterial samples from the same animal on several consecutive days.

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4 Blackwood, J. H., and Stirling, J. D., *Biochem. J.*, 1932, **26**, 357.

5 Lintzel, W., *Z. Zücht., B*, 1934, **29**, 219.

6 Graham, W. R., Jr., Jones, T. S. G., and Kay, H. D., *Proc. Roy. Soc. London*, Series B, 1936, **120**, 330.

7 Brown, W. R., Petersen, W. E., and Gortner, R. A., *J. Dairy Sci.*, 1936, **19**, 147.

8 Whitnah, C. H., Riddell, W. H., and Hodgson, R. E., *J. Dairy Sci.*, 1933, **16**, 347.

9 Petersen, W. E., and Boyd, W. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 537.

10 Graham, W. R., Jr., Kay, H. D., and McIntosh, R. A., *Proc. Roy. Soc. London*, Series B, 1936, **120**, 319.

11 Boyd, W. L., and Petersen, W. E., unpublished data, 1938.

TABLE I.
Glucose in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods.

Cow No.	Glucose level		Arterial Venous Difference, mg %	Remarks
	Arterial, mg %	Venous, mg %		
435	68.0	59.0	— 9.0	2 hr after milking
432	63.6	53.4	—10.2	
446	63.6	58.4	— 5.2	
577	76.0	71.0	— 5.0	
434	68.8	66.4	— 2.4	avg —6.36
435	65.0	56.0	— 9.0	3 hr after milking
241	75.0	62.0	—13.0	
241	62.0	49.0	—13.0	
241	59.4	48.4	—11.0	
435	70.0	63.0	— 7.0	
435	64.0	59.0	— 5.0	
241	81.6	68.4	—13.2	
447	64.0	53.0	—11.0	
419	74.0	71.0	— 3.0	
433	56.0	50.0	— 6.0	avg —9.12
241	73.0	55.0	—18.0	4 hr after milking
241	71.0	60.6	—10.4	
241	74.4	63.0	—11.4	
241	59.6	51.0	— 8.6	
578	52.0	45.6	— 6.4	
26	47.8	39.4	— 8.4	
423	51.2	47.8	— 3.4	
423	78.8	63.0	—15.8	avg —10.30
435	58.0	44.0	—14.0	5 hr after milking
435	65.0	59.4	— 5.6	
241	73.2	64.0	— 9.2	
E-190	70.0	64.0	— 6.0	
435	60.0	56.0	— 4.0	
435	76.4	60.4	—16.0	
443	64.0	52.6	—11.4	
212	59.6	45.8	—13.8	
213	59.6	50.6	— 9.0	avg —9.87
435	64.0	58.4	— 6.0	9 hr after milking
E-190	78.0	71.0	— 7.0	
235	76.0	65.6	—10.4	avg —7.8
435	61.0	56.0	— 5.0	10 hr after milking
241	48.0	33.4	—14.6	
25	74.4	65.4	— 9.0	
A-7	72.0	61.6	—10.4	
586	78.0	63.0	—15.0	avg —10.80
Avg	66.4	57.1	— 9.3	

Lactic acid was determined by West's[12] modification of the von Fürth-Charnass technic.

The method used for blood sugar was essentially that recom-

[12] West, E. S., *J. Biol. Chem.*, 1931, **92**, 483.

mended by Shaffer and Somogyi.[13] The blood filtrates were prepared by the Somogyi[14] method. Sodium citrate was used as an anticoagulant. Considerable precaution was exercised in obtaining the blood samples. In the event that there was apparent excitation of the animal the samples were discarded.

The data for 40 arterio-venous glucose differences are presented in Table I. With the possible exception of the first 2 hours following milking there was no apparent relation between the arterio-venous glucose difference and the period of time following the completion of the last milking. The average arterio-venous blood sugar loss was 9.3 mg %.

A study was made of the relation of the arterio-venous glucose difference to the arterial blood sugar level and to the level of milk production. The results are given in Figs. 1 and 2. In both comparisons the A-V differences are so widely scattered as to hardly justify the plotting of a curve. The same situation prevails in the data presented in Table II in which several experiments were conducted on the same animals over a period of a few weeks. With the same individuals, neither the arterial blood sugar level nor the milk yield was related to the arterio-venous glucose difference.

The glucose and lactic acid concentration of arterial and mammary venous bloods are presented in Table III. There was a loss of lactic acid in 15 of the 17 experiments confirming the work by Graham[2] on lactating goats. The amount of glucose absorbed by

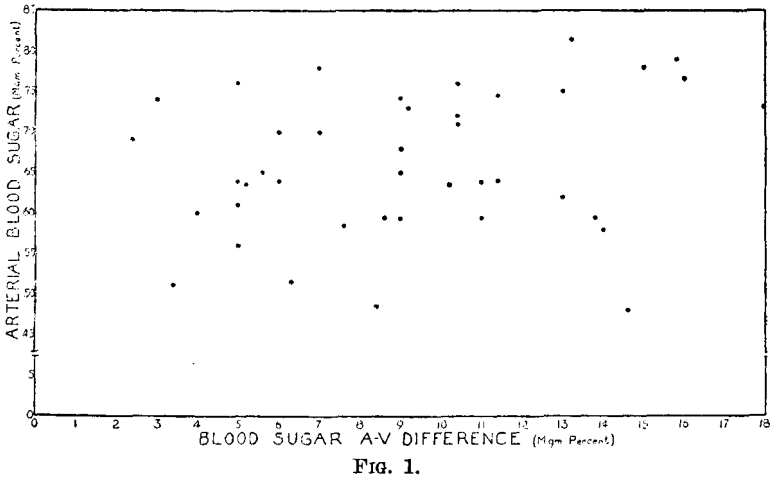

FIG. 1.
Relation of A-V difference to arterial blood sugar level.

[13] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.
[14] Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

TABLE II.
Arterio-Venous Blood Sugar Difference in Relation to Arterial Level of Blood Sugar and to Level of Milk Production.

Cow No. 241		Cow No. 435	
Arterial Blood Sugar Level mg %	A-V Blood Sugar Difference mg %	Milk Yield lb	A-V Blood Sugar Difference mg %
75.0	—13.0	19.6	—5.0
74.4	—11.4	16.6	—5.6
73.2	— 9.2	15.6	—9.0
73.0	—18.0	15.1	—9.0
71.0	—10.4	10.1	—5.0
62.0	—13.0	9.6	—4.0
59.6	— 8.6	8.8	—7.0
59.4	—11.0	8.6	—7.6
48.0	—14.6		

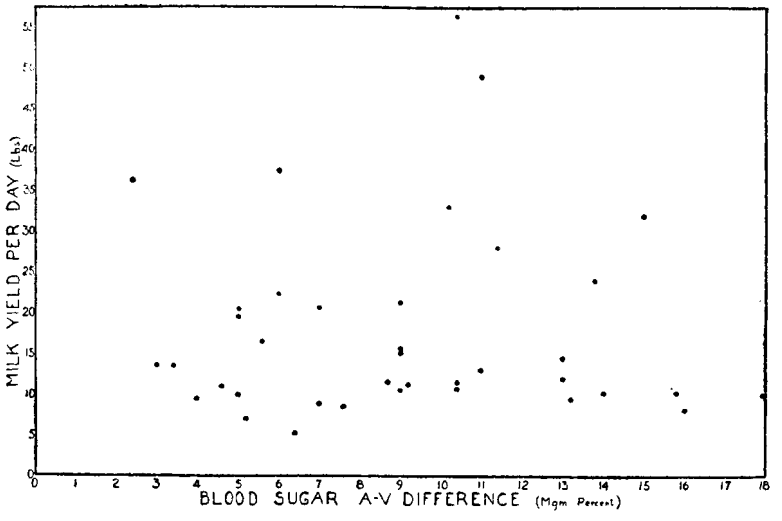

FIG. 2.
Relation of A-V difference to milk yield.

the lactating mammary was usually greater than that of the lactic acid. It will be noted that there is no apparent relationship between the glucose and lactic acid loss at any one time. The same thing will be observed in Graham's data. It is believed that this indicates a storage in the mammary gland of the precursors of lactose. During extreme excitement there is little or no loss in glucose and lactic acid and at times even a higher concentration in the venous blood of both substances.

The volume of blood flow as measured by the ratio of the calcium and fat removed by the gland to the percent of calcium and fat in

TABLE III.
Glucose and Lactic Acid in Arterial and Mammary Venous Blood of Cows.

Glucose			Lactic acid			
Arterial mg %	Venous mg %	Difference mg %	Arterial mg %	Venous mg %	Difference mg %	
63.6	53.4	—10.2	5.72	3.65	— 2.07	
64.0	53.0	—11.0	7.13	8.68	+ 1.55	
74.0	71.0	— 3.0	5.94	3.47	— 2.47	
76.0	71.0	— 5.0	12.97	6.21	— 6.76	
68.8	66.4	— 2.4	7.67	6.58	— 1.09	
56.0	50.0	— 6.0	7.58	3.29	— 4.29	
74.4	63.0	—11.4	9.04	5.30	— 3.74	
74.4	65.4	— 9.0	16.63	6.21	—10.42	
72.0	61.6	—10.4	8.40	3.38	— 5.02	
78.0	63.0	—15.0	3.93	5.48	+ 1.55	
59.6	51.0	— 8.6	19.46	11.69	— 7.77	
52.0	45.6	— 6.4	9.04	5.62	— 3.42	
47.8	39.4	— 8.4	8.45	4.25	— 4.20	
51.2	47.8	— 3.4	6.76	3.79	— 2.97	
78.8	63.0	—15.8	8.59	4.93	— 3.66	
59.6	45.8	—13.8	7.93	5.39	— 2.54	
59.6	50.6	— 9.0	9.04	4.48	— 4.56	
71.2	72.0	+ 0.8	7.64	8.40	+ 0.76	cow* excited
60.6	63.0	+ 2.4	4.69	5.33	+ 0.64	dry cow
		Avg of lactating cows				
65.3	56.5	— 8.8	9.08	5.44	— 3.64	

*Not included in average.

the milk has been shown by Shaw and Petersen[15] to be in the range of 391:1 and 344:1 respectively. The average arterio-venous glucose loss of 9.3 mg % as given in Table I when compared to the average lactose in the milk indicates a ratio of 550 volumes of blood for each unit volume of milk produced. It is, therefore, apparent that the glucose is not removed by the gland in sufficient quantities to account for all of the lactose in the milk. The volume flow of blood per unit volume of milk calculated from the combined average glucose and lactic acid differences as given in Table III and the average lactose percent in milk is about 390 which is in very good agreement with the values calculated from calcium and fat as cited above. Thus the combined glucose and lactic acid loss to the mammary gland appears to be sufficient to account for all of the milk sugar.

This confirms in part the observation by Graham[2] that the blood glucose is not the sole precursor of lactose and that lactic acid is also one of the precursors of lactose. Graham conducted a carbohydrate balance experiment on the mammary gland of a goat. While very good agreement was obtained in the two experiments reported, many more data are essential for conclusive proof.

15 Shaw, J. C., and Petersen, W. E., *Proc. Am. Physiol. Soc.*, 50th Annual Meeting, Baltimore, Maryland, 1938, p. 183.

Conclusions. 1. There was no apparent relation between arterio-venous blood sugar differences and the following: period of time following milking, arterial blood sugar level and level of milk production. 2. Both glucose and lactic acid are absorbed by the lactating mammary gland in considerable quantities. While the glucose arterio-venous loss is not sufficient to account for all of the milk sugar, the combined loss of the two substances to the lactating mammary gland can account for all of the milk lactose.

9945 P

Stimulation of Reproductive Tract of the Infantile Female Mouse by Anuran Anterior Pituitary Substance.*

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Attempts to stimulate the reproductive tract of the infantile female mouse by small doses of anuran anterior pituitary substance have been consistently negative.[1] Even with larger amounts, *e.g.*, 16 to 96 fresh anterior lobes of *Rana pipiens,* averaging 16.2 to 104.0 mg per mouse, the ovaries, uteri and vaginae showed no differences compared with litter mate controls although thyroid and adrenal glands were stimulated to a degree that was significant statistically.[2]

In the present experiments, however, a positive reaction has been secured by approximately doubling the highest dosage of fresh pars anterior previously employed. Infantile female mice, 17 to 21 days old, were used as hosts and *Rana pipiens* as donors of the anterior pituitary substance. The frog anterior lobes were removed and placed in a bottle containing 0.3 cc of 0.9% NaCl packed in ice. As soon as a certain amount of pars anterior substance, judged by number or weight of glands, was secured, it was macerated and then injected into the hind leg of the mouse. Any tissue remaining in the bottle was injected into the other leg in an additional 0.3 cc of salt solution. Equivalent doses of frog brain and smaller amounts

* Aided by a grant from the Rockefeller Foundation.

[1] Lipschütz, A., and Paez, R., *C. R. Soc. Biol.*, 1928, **99**, 693; Martins, Th., *C. R. Soc. Biol.*, 1929, **101**, 957; Zondek, B., *Arch. Gynäk.*, 1931, **144**, 133; *Hormone des Ovariums und des Hypophysenvorderlappens,* 2nd edit., J. Springer, 1935; Magistris, H., *Pflüger's Arch.*, 1932, **230**, 835.

[2] Adams, A. E., and Tukey, G., *Anat. Rec.*, 1938, **71**, in press.