CCLXXXVI. THE METABOLISM OF GALACTOSE.

III. 1. LACTOSE SYNTHESIS FROM (a) A GLUCOSE-GALACTOSE MIXTURE, (b) PHOSPHORIC ESTERS, BY SLICES OF THE ACTIVE MAMMARY GLAND *IN VITRO*.

2. THE EFFECT OF PROLACTIN ON LACTOSE SYNTHESIS BY THE MAMMARY GLAND.

By GORDON ALLISON GRANT.¹

From the Department of Biochemistry, Lister Institute, London.

(Received 30 September 1936.)

I a. LACTOSE SYNTHESIS FROM A GLUCOSE-GALACTOSE MIXTURE BY SLICES OF THE ACTIVE MAMMARY GLAND *IN VITRO*.

SVANBERG [1930] investigated the possibility that a brei made of the lactating mammary gland might synthesize lactose from added glucose or a glucose-galactose mixture. In his experiments toluene was used as a preservative, and the reducing sugars were partitioned into disaccharide and monosaccharide fractions. With one doubtful exception, the results were negative. Michlin & Lewitow [1934] carried out similar experiments and claimed, on the basis of irregular changes in the total reduction of their solutions, to have demonstrated the synthesis of lactose from glucose and galactose mixtures. Grant [1935] determined the extent of lactose synthesis by slices of the active mammary gland immersed in oxygenated physiological salt solutions containing different hexoses. A quantitative micro-method was developed for the estimation of small amounts of lactose, using *S. fragilis* Jörg., as a biological reagent for this sugar. An increased synthesis of lactose was readily obtained with glucose as a substrate, while there was little evidence of such synthesis from fructose, mannose or galactose. In the present communication, the amounts of lactose formed *in vitro*, when glucose or galactose, respectively, is a substrate for slices of lactating tissue, are compared with those obtained from equivalent amounts of glucose-galactose mixtures.

EXPERIMENTAL.

2 ml. portions of an oxygenated physiological salt solution containing glucose (8 mg.), galactose (8 mg.), or a glucose-galactose mixture (4 mg. of each hexose), respectively, were incubated with approximately equal weights of mammary gland slices for 6 hours at 37°, as previously described [Grant, 1935]. At the end of this period the extent of the lactose synthesis was determined in each case; the reducing sugars were estimated by the differential fermentation method, as "fermentable sugar" (glucose, fructose or mannose), galactose and lactose.

¹ Beit Memorial Research Fellow.

There was little evidence of increased utilization of galactose, when glucose-galactose mixtures were used, over the small amount found for galactose alone. The lactose synthesis was scarcely increased above the amount to be expected from the glucose component of the mixture (Table I).

Table I. *Lactose synthesis from glucose, galactose and glucose-galactose mixtures by the active mammary gland, in vitro.*

Wt. of tissue slices mg. (wet wt.)	Substrate	(6 hours, 37°.)		Lactose synthesized*
		Hexose added mg.	Hexose remaining after 6 hrs. mg.	
172	Glucose	8	0.9	3.5
185	Glucose	8	0.4	3.0
160	Galactose	8	5.8	0.47
174	Glucose	4	0	2.1
	Galactose	4	2.8	
103	Glucose	4	0.3	2.3
	Galactose	4	2.5	

* Above that remaining after 6 hours in control experiments without added hexoses, which was 0.88–0.90 mg. lactose per 100 mg. tissue (wet weight).

These findings suggest that the mechanism of lactose synthesis in the lactating parenchyma does not simply involve a direct combination of free glucose and galactose in glucosidic linkage. In this connection, it was shown that a dried acetone-defatted powder of lactating mammary tissue did not contain an active β -galactosidase since it was unable to hydrolyse added lactose over a period of 120 hours (pH 7.3; 37°), but since the same preparation gave negative results for synthesis with glucose, or a glucose-galactose mixture the findings do not rule out the possible significance of this enzyme. Similar results suggesting the absence of an active lactase have been reported by Kleiner & Tauber [1932], using glycerol extracts of the lactating udder, and by Bradley [1912–13].

Experiments with non-living preparations of lactating mammary tissue.

Weinbach [1936] has stated that non-fermentable reducing material was synthesized from added glucose by a dried preparation of lactating glands of rats; in one of his two experiments, the amount of this material formed was in excess of the added glucose, and he believed that this excess came from a lactose-precursor which is non-reducing and only becomes available when the mammary gland preparations are placed in a solution containing glucose. The semi-quantitative method used for the identification of lactose and the absence of data concerning the amounts of lactose originally present in the preparations render an interpretation of the results very difficult.

In the present investigation, an attempt was made to obtain such active non-living preparations. Lactating glands of guinea pigs were rapidly frozen in CO₂ snow, powdered and suspended in physiological salt solution (pH 7.3) and kept in presence or absence of added glucose at 37° for periods of 0, 5 and 10 hours. Portions were removed at stated intervals and the glucose ("fermentable sugar"), galactose and lactose contents were determined in the deproteinized filtrates (Somogyi-Zn(OH)₂ precipitation) after suitable dilution by the method referred to previously. The results are shown in Table II. There was no synthesis of lactose in amounts which would convincingly demonstrate the presence of a non-reducing lactose-precursor in the gland preparations, or the ability of these

Table II. *Changes in sugar content of non-living preparations of lactating mammary tissue when kept at 37°.**

Exp. no.	Amount of mammary gland preparation g. in 10 ml.	Glucose (mg.)			Lactose (mg.)		
		0 hr.	5 hr.	10 hr.	0 hr.	5 hr.	10 hr.
Mammary tissue alone:							
1	2.2	0	1.6	0.6	11.8	10.8	11.5
2	2.5	0.4	2.8	0	5.8	9.2	7.5
3	3.8	0	4.2	4.0	30.2	36.5	35.0
Mammary tissue + added glucose:							
1	2.2	194	192	195	12.8	13.8	13.5
2	2.5	188	199	159	6.8	0	2.5
3	3.8	189	192	184	37.5	38.5	46.3

* Galactose was not present in measurable amounts in either series. Exps. 1, 2 and 3 represent preparations of mammary glands from three different animals; the glands were removed from one to two hours after the last suckling period.

preparations to convert added glucose into lactose. Small amounts of "fermentable sugar" (glucose, fructose or mannose), were produced, but galactose was not present in recognizable amounts. A preparation from mammary tissue by the method used by Meyerhof in preparing active glycolytic extracts from muscle also gave negative results for lactose synthesis from added glucose.

It has been suggested by Helferich [1933] that the process by which a monosaccharide is converted into a disaccharide in the presence of a synthetic catalyst must be regarded as precisely similar to that by which α - and β -glucoses are converted into the two methylglucosides. Thus according to classical theory the synthesis would occur in two steps:

The results obtained in the present investigation are not those one would expect if a rapid direct coupling of glucose and galactose in glucosidic-linkage occurs as part of the mechanism of the lactose synthesis in the mammary gland (reaction 2).

1 b. PHOSPHORIC ESTERS AS SUBSTRATES FOR LACTOSE SYNTHESIS BY SLICES OF THE ACTIVE MAMMARY GLAND *IN VITRO*.

The important role of phosphoric esters in the intermediary carbohydrate metabolism of yeast and muscle has been well established by the extensive researches of Harden, Robison, Meyerhof, von Euler, and their co-workers [see Robison, 1936]. It is possible that these esters may also play a part in the changes taking place during the conversion of glucose into lactose in the lactating mammary gland. It is known that preparations of the active gland produce lactic acid from glucose, galactose, lactose and phosphoric esters and that here, as in yeast and muscle, the phosphorylation taking place is very likely connected with the active glycolysis of the lactating parenchyma [Svanberg, 1930; Barrenscheen & Alders, 1932; Börst, 1932; Brenner, 1932; Folley & Kay, 1935;

Grant, 1935]. No evidence, however, has yet been presented which directly connects the phosphorylation with the synthesis of lactose.

In an attempt to obtain further information on this point, some of the esters which are known to play an important part in the fermentative and glycolytic processes, and which have been shown to be produced during the fermentation of galactose by preparations of adapted yeast [Grant, 1935], have been used as substrates for the synthesis of lactose by slices of the active mammary gland *in vitro*. The following naturally occurring hexosephosphates were employed: glucose-6-phosphate, fructose-6-phosphate, fructose-1:6-diphosphate; the synthetic galactose-6-phosphate which has not been found among the products of galactose fermentation has also been used. In view of the importance of the 3-carbon ester, phosphoglyceric acid, in the later stages of the glycolytic process [Lohmann & Meyerhof, 1934; Meyerhof & Kiessling, 1935], this ester has also been included as a substrate.

EXPERIMENTAL.

The general conditions are those described in Section 1 *a*, suitably altered to enable the sugar analyses, "fermentable sugar" (glucose, fructose and mannose), galactose and lactose, to be made by the differential fermentation method on the ester-free deproteinized tissue filtrates. The neutral sodium salts of the esters used as substrates for the mammary tissue slices were added in equimolar amounts to the glucose used in the comparative experiments, except for the phosphoglyceric acid, for which twice the molar equivalent was employed. The esters remaining at the end of the experimental period were precipitated at pH 8.2 by the addition of barium acetate solution to the alcohol-deproteinized tissue filtrates.

Table III. *Lactose synthesis by slices of the active mammary gland in vitro at 37°. Availability of phosphoric esters as substrates.*

Time hours	Substrate	mMol. ($\times 10^3$)	mg. lactose per 100 mg. tissue (dry wt.)		% substrate decomposed in 6 hours†			
			I*	II	II			
0	Control	—	2.0	1.1	—			
6	Control	—	1.4	3.5	—			
6	Control	—	2.1	2.2	—			
6	Phosphoglycerate	88	2.8	2.4	30			
6	Phosphoglycerate	88	2.3	2.7	43			
6	Phosphoglycerate	88	3.7	3.1	40			
6	Glucose	44	6.5	10.8	92			
6	Glucose	44	7.2	14.7	94			
			III	IV	V	III	IV	V
0	Control	—	—	1.8	—	—	—	—
6	Control	—	3.3	2.7	—	—	—	—
6	Control	—	3.9	2.0	1.9	—	—	—
6	Glucose-6-phosphate	44	1.2	2.1	1.6	54	24	52
6	Galactose-6-phosphate	44	2.4	—	1.0	60	—	63
6	Fructose-6-phosphate	44	1.8	{ 2.1 4.5	1.0	53	{ 40 24	20
6	Fructose-1:6-diphosphate	44	1.5	—	0.8	38	—	15
6	Glucose	44	6.6	11.5	—	83	77	—
6	Glucose	44	—	21.6	5.8	—	85	100

* I, II, III, IV and V represent separate experiments with lactating glands from different animals.

† When the substrate is a phosphoric ester, the value is calculated as the % ester-P set free as inorganic phosphate; when the substrate is glucose the value is calculated as the % decrease in "fermentable sugar".

Table IV. *Action of mammary tissue slices on hexosephosphates.*

Esters originally present in equivalent amounts to the glucose (44×10^{-3} mMol.) i.e. 8 mg. hexose. Exps. III and V are those referred to in Table III.

Substrate	Hexoses present after 6 hours at 37°			
	"Fermentable sugar"*		Galactose	
	Exp. III	Exp. V	Exp. III	Exp. V
Glucose-6-phosphate	0.6	0.3	0	0
Galactose-6-phosphate	0	0	2.7	1.6
Fructose-6-phosphate	1.1	0.3	0	0
Fructose-1:6-diphosphate	1.0	0.8	0	0

* Glucose, fructose or mannose.

Aliquot portions of the ester-free alcoholic solutions were evaporated to dryness at 60° and the aqueous solutions of the residues analysed for "fermentable sugar" (glucose, fructose or mannose), galactose and lactose. The results obtained are summarized in Tables III and IV.

The phosphoric esters were decomposed to varying degrees in the different experiments (15–63 %), as judged by the inorganic phosphate liberated. There was little evidence of an increase in lactose with the esters as substrates, in comparison with that occurring when glucose was employed. Nor was there any accumulation of free galactose, except in the experiments with galactose-6-phosphate, where it arose, presumably, by the hydrolysis of this ester. The varying amounts of free glucose in the glucose-6-phosphate experiments and of free fructose in those with the fructose esters very likely had a similar origin (Table IV). There was no evidence of the synthesis of free hexoses from the phosphoglyceric acid. These negative results are, however, not conclusive since there is always the possibility that the esters were unable to penetrate into the tissue cells.

Effects of fluoride and iodoacetate on lactose synthesis.

Sodium fluoride (0.04 *M*) and sodium iodoacetate (0.02 *M*) almost completely inhibited the utilization of added glucose by the mammary gland slices. The glucose was recovered unchanged to the extent of 93–96 %, in the experiments with sodium fluoride, and 88–92 % in those with sodium iodoacetate; lactose synthesis was stopped, and there was no evidence of the production of free galactose. It is not clear whether the lactose mechanism is inhibited independently, or whether this inhibition is consequent upon the effects of these two compounds upon the glycolytic process.

2. THE EFFECT OF PROLACTIN ON LACTOSE SYNTHESIS BY THE MAMMARY GLAND.

Nelson [1936] has reviewed the numerous researches of the last decade relating to the hormonal control of the mammary gland. It has been possible to correlate the proliferative changes of duct and lobule-alveolar development observed during the growth phase with the action of oestrin, possibly supplemented with progesterone. It is suggested that the accumulation of the products of secretion, and the production of milk, characteristic of the secretory phase, are due to the lactogenic hormone of the anterior lobe of the pituitary. Contradictory evidence

has been reported as to whether the oestrogenic hormones can cause duct and lobule-alveolar development in hypophysectomized animals [Nelson, 1936; Reece *et al.*, 1936]; Gomez & Turner [1936, 1] state that in complete absence of the hypophysis, no growth of the mammary parenchyma is observed. Oestrogenic substances can cause responses in the same mammary gland varying from duct growth and alveolar dilatation to a hyperplasia resulting in traumatic oedema and mammary carcinoma [Collip *et al.*, 1936; Gardner *et al.*, 1935; Burrows, 1935; Pallot, 1936; Lacassagne, 1936]. In contrast to the earlier results obtained with crude extracts of the anterior pituitary, evidence is accumulating that the purified "lactogenic" hormone preparations, while very active in causing proliferation of the crop-gland of the pigeon, do not serve in their lactogenic effect as a complete replacement for the secretion of the animal's own pituitary [Gomez & Turner, 1936; Nelson & Gaunt, 1936, 2].

Evans [1936] found that an alkaline extract of the anterior pituitary caused up to 50 % increase in milk production of lactating cows and goats but had no effect in bringing back into lactation animals which had been allowed to "go dry" for this purpose. De Fremery [1936] obtained only a few drops of milk secretion in virgin goats even though large amounts of prolactin were used; he suggested that prolactin only causes milk secretion in normal quantities after a preliminary action of oestrogenic hormones [see also, Gomez & Turner, 1934; Lyons & Catchpole, 1933].

While prolactin undoubtedly causes the production of variable amounts of a fluid which has the physical appearance of milk, very few investigations so far reported have been concerned with the nature of this induced secretion. For this reason, an investigation was made of the effect of the "lactogenic" hormone on the synthesis of lactose—since this is a specific mechanism of the active mammary gland.

EXPERIMENTAL.

Normal multiparous guinea-pigs were separated from their litters (an equal number of 3 young to each parent) two weeks after the onset of lactation. The involution of the mammary glands was allowed to proceed for several days (stripping the glands twice daily), until only small amounts of lactose-free serous fluid were being produced. At this stage prolactin in aqueous solution was injected subcutaneously, in amounts varying from 2.5 to 16.6 mg. per 100 g. body weight, per day. After a variable period (1 to 3 days), during which the regressing glands secreted small amounts of a cloudy fluid, the secretion of "milk" was reinstated. The milk was collected twice daily in a graduated capillary pipette and washed into 50 % alcohol. After centrifuging, the alcoholic centrifugate and washings were evaporated to dryness on a water-bath at 60°, and the residue was taken up in water to a suitable volume. The lactose content of this aqueous solution was determined by the differential fermentation method [Grant, 1935]. The total protein figures represent the dry weight of the washed precipitate. In some cases, the milk was deproteinized by the Somogyi-Zn(OH)₂ precipitation method, both methods of deproteinization yielding the same lactose value.

Examined under identical conditions in a capillary pipette the "milk" had a yellowish white colour compared with the chalky white colour of the normal milk of the guinea-pig. The microscopic examination revealed numerous fat globules and colostrum corpuscles. The results of the chemical examination are shown in Table V. Lactose was the only sugar present in the "milk" and in abnormally low concentration (0.04–0.24 %). The normal milk of the guinea-pig in early

Table V. *Lactose synthesis, in vivo, reinstated by prolactin in the mammary glands of normal multiparous guinea-pigs, which have just ceased lactation.*

Animal no.	Prolactin injected mg./100 g. per day	No. days	Total milk secretion ml.	Lactose synthesized		Total protein	
				mg.	%	mg.	%
1	3.0*	14	0.24	0.5	0.2	19.6	8
2	3.3†	5	0.11	0.2	0.2	6	5.5
3	3.3†	5	0.07	0.1	0.14	8	11
4	3.3*	6	0.28	0.1	0.04	—	—
	2.5*	3	0.20	0.2	0.1	—	—
	0	9	0.17	0.4	0.24	21	12
5	16.6‡	5	0.16	0.2	0.1	—	—
Normal animals:							
6	0; late lactation 3 hours		0.30	4.6	1.5	15	5
7	0; 1st day lactation 3 hours		0.9—	40.2	4.5	—	—
	0; 2nd day lactation 3 hours		0.9—	49.8	5.5	—	—

Prolactin preparations:

* Prolactin, Allen and Hanbury, Ltd.; 1 g. obtained from approximately 644 g. of fresh anterior pituitary.

† The fraction insoluble in 86% alcohol at pH 6, prepared from frozen fresh anterior pituitaries by the method of Bates & Riddle [1935].

‡ The fraction insoluble at pH 6.4, prepared from acetone-dried anterior pituitary powder by the method of Lyons & Catchpole [1933].

lactation contains 4.5–5.5%, and that of late lactation 1.5% of lactose. The maximum induced milk secretion was 0.08 ml. per day, compared with 0.3 ml. per 3 hours in the final stages of lactation, and over 0.9 ml. per 3 hours on the first and second days of lactation. Though the stimulated mammary glands of the animals were tender, the animals did not undergo significant weight changes during the injection periods.

Individual mammary glands varied greatly in the amounts of milk produced and in the time taken for this to replace the secretion of cloudy serous fluid. A preparation of prolactin (Allen and Hanbury) assaying 1.8 crop-gland units per mg., gave similar results to those recorded in Table V. In doses of 12 mg. per 100 g. body weight per day, injected subcutaneously into female guinea-pigs in the last stage of lactation, this preparation increased the milk secretion from 0.02 to 0.15 ml. per day. Injected for two weeks into animals which had gone completely “dry” it induced only cloudy serous fluid, and in both the secretion and the mammary glands of these animals the sugar was not lactose but glucose (“fermentable sugar”). The preparations of prolactin used were inactive in causing a measurable increase in lactose synthesis from added glucose *in vitro*, but this is not surprising considering the limited effect of these preparations *in vivo*.

DISCUSSION.

Evans [1933], using an alkaline extract of the anterior pituitary, obtained an induced secretion resembling colostrum in its appearance from a virgin goat, but no mention is made of its lactose content. Catchpole *et al.* [1933] reported that the milk secretion induced by prolactin in a virgin heifer had a low lactose (total reduction) and high chloride content. De Jongh & Dingemanse [1931] stated that the milk obtained from the mammary glands of normal male guinea-pigs,

after the cessation of the injection of large amounts of oestrin, possessed a sugar content (total reduction) of 17–21 %, and an “albumin” content of 17–24 % of the total dry residue.

In the present experiments, *in vivo* synthesis of lactose was re-established in the inactive regressing glands of the female guinea-pigs by the stimulus of prolactin, though to a very limited extent. The secretion obtained had the physical appearance of milk but an abnormally low lactose content. The mammary parenchyma of the glands at the time of injection of the prolactin was probably in the intermediate stage of involution, consisting of secreting, regressing and regressed acinar tissue [see de Fano, 1922; Loeb & Hesselberg, 1917]. Apparently these regressing glands must be subjected to additional influences to that afforded by prolactin, before they can again resume either normal milk production or the synthesis of lactose in normal quantities. This is probably not due to a lack of proliferated alveolar tissue but to the need of most of the acinae for additional hormonal stimuli, very likely those of oestrone and progesterone. The results obtained by de Fremery, mentioned above, suggest that such may be the case, and recently Allen & Heckel [1936] found that oestrin prevents the regression of the mammary glands which normally takes place in the pseudo-pregnant animal and also causes a thickening of the glands (when oestrin injections are continued after castration) to a stage characteristic of the preparturient normal female.

The results obtained in the present investigation offer additional evidence in support of the view that the actual process of milk secretion (and of the concomitant lactose synthesis) depends upon the simultaneous action of a number of different hormones, rather than being conditioned solely by the action of prolactin. The methods at present in use for the assay of prolactin depend on glandular proliferation and upon the quantity and physical appearance of the milk and neglect the composition of this induced secretion. Nelson [1936] has called attention to the fact that variations between the pigeon “crop-gland” unit and the mammary gland “lactogenic” unit for rabbits, may be between 15–1 to 100–1, with an average of 40–1. The present findings indicate the importance of determining the chemical composition of the induced secretion in the investigation or assay of the lactogenic property of hormones.

SUMMARY.

1. When glucose-galactose mixtures are employed as substrates for lactating mammary tissue *in vitro* the lactose synthesis is not noticeably increased beyond that for glucose alone. This result is inconsistent with the view that a rapid direct coupling of galactose to glucose in glucosidic-linkage represents the mechanism employed by the active mammary gland in the synthesis of lactose. There was no evidence of an active β -galactosidase in preparations of the lactating gland. Attempts to obtain non-living preparations able to synthesize lactose from glucose, or from a glucose-galactose mixture, were unsuccessful.

2. The following phosphoric esters, glucose-6-phosphate, fructose-6-phosphate, fructose-1:6-diphosphate, galactose-6-phosphate and phosphoglyceric acid did not yield increased synthesis of lactose with slices of lactating mammary gland *in vitro* although the slices were active when glucose was the substrate. Sodium fluoride (0.04 *M*) and sodium iodoacetate (0.02 *M*) inhibit almost completely both glucose utilization and lactose synthesis by slices of lactating tissue.

3. The *in vivo* synthesis of lactose was re-established to a limited extent in the inactive regressing mammary glands of female guinea-pigs by the stimulus

of prolactin. The induced secretion, slight in amount, resembled milk in its physical appearance but possessed an abnormally low lactose content (0.04–0.24 %). The presence and concentration of lactose, a specific product of the lactating mammary parenchyma, indicated to what extent the “lactogenic” stimulus was a physiological one. Prolactin, alone, is unable to reinstate normal milk production in the regressing mammary gland of the female guinea-pig.

I wish to take this opportunity to thank Prof. R. Robison for his interest and advice during this investigation, and to the Lister Institute for providing laboratory facilities. My thanks are also due to Dr Norman Evers, of Messrs Allen and Hanbury, Ltd., for generous supplies of prolactin preparations and to Dr M. Laskowski for the gift of a kilogram of desiccated anterior pituitary powder.

REFERENCES.

- Allen & Heckel (1936). *Science*, **84**, 161.
 Barrenscheen & Alders (1932). *Biochem. Z.* **252**, 97.
 Bates & Riddle (1935). *J. Pharmacol.* **55**, 365.
 Bradley (1912–13). *J. biol. Chem.* **13**, 431.
 Börst (1932). *Hoppe-Seyl. Z.* **212**, 126.
 Brenner (1932). *Hoppe-Seyl. Z.* **212**, 135.
 Burrows (1935). *Brit. J. Surg.* **23**, 191.
 Catchpole, Lyons & Regar (1933). *Proc. Soc. exp. Biol., N.Y.*, **31**, 301.
 Collip, McEuen & Selye (1936). *Amer. J. Physiol.* **116**, Proc. 29.
 Evans (1933). *Proc. Soc. exp. Biol., N.Y.*, **30**, 1370.
 — (1936). *Amer. J. Physiol.* **116**, Proc. 45.
 de Fano (1922). *J. Physiol.* **56**, 469.
 Folley & Kay (1935). *Biochem. J.* **29**, 1837.
 de Fremery (1936). *J. Physiol.* **87**, 50.
 Gardner, Smith & Strong (1935). *Proc. Soc. exp. Biol., N.Y.*, **33**, 148.
 Gomez & Turner (1934). *Bull. Mo. agric. Exp. Sta.* No. 206, 1.
 — (1936, 1). *Proc. Soc. exp. Biol., N.Y.*, **34**, 320.
 — (1936, 2). *Proc. Soc. exp. Biol., N.Y.*, **34**, 404.
 Grant (1935). *Biochem. J.* **29**, 1661, 1905.
 Helferich (1933). *Ergebn. Enzymforsch.* **2**, 74.
 de Jongh & Dingemanse (1931). *Arch. néerl. Physiol.* **16**, 288.
 Kleiner & Tauber (1932). *J. biol. Chem.* **99**, 241.
 Lacassagne (1936). *C.R. Soc. Biol., Paris*, **122**, 183.
 Loeb & Hesselberg (1917). *J. exp. Med.* **25**, 285, 305.
 Lohmann & Meyerhof (1934). *Biochem. Z.* **273**, 60.
 Lyons & Catchpole (1933). *Proc. Soc. exp. Biol. N.Y.*, **31**, 299.
 Meyerhof & Kiessling (1935). *Biochem. Z.* **276**, 239.
 Michlin & Lewitow (1934). *Biochem. Z.* **271**, 448.
 Nelson (1936). *Physiol. Rev.* **16**, 488.
 — & Gaunt (1936). *Proc. Soc. exp. Biol., N.Y.*, **34**, 671.
 Pallot (1936). *Bull. Histol. Tech. micr.* **13**, 90.
 Robison (1936). *Ann. Rev. Biochem.* **5**, 181.
 Reece, Turner & Hill (1936). *Proc. Soc. exp. Biol., N.Y.*, **34**, 204.
 Svanberg (1930). *Hoppe-Seyl. Z.* **188**, 207.
 Weinbach (1936). *J. gen. Physiol.* **19**, 829.