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Anaerobic Respiration in Latex of *Hevea brasiliensis* Substrate and Limiting Factors

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Abstract. The site of anaerobic respiration in the latex is the serum. The main respiratory substrate is fructose. The CO_2 formation in serum is increased by additional fructose on the average about 2.5—3 times. Glucose does not influence CO_2 evolution by serum but slightly increases O_2 consumption.

With respect to sugars, latex serum contains essentially only sucrose and a low amount of raffinose. During the incubation of serum sucrose is hydrolysed, the fructose component is immediately utilized in respiration and glucose accumulates.

The rate of CO_2 formation in latex as influenced by fructose is negatively related to the rubber content of the latex. Latex with a high rubber content reacts only slightly or not at all on additional fructose.

The main limiting factors of latex respiration and sugar utilization are the following:

1. The deficiency of substrate, due to low activity of β-fructofuranosidase.
2. The rate of glucose phosphorylation (D'AUZAC, JACOB 1967).
3. Presumably the low activity of phosphoglucoisomerase.
4. The rubber content of the latex.
5. The concentration of CO_2 in latex; this factor may be important in vivo, in the laticiferous system.

Respiration of fresh latex is predominantly fermentative. The respiratory quotient (RQ) is as a rule higher than 10 (Van den TEMPEL 1954). Pyruvate and phosphoenolpyruvate are the only substrates up to now found to be capable of increasing substantially the CO_2 evolution (BEALING 1964). By contrast sugars such as glucose, galactose and sucrose influenced the CO_2 output only slightly or not at all (Van den TEMPEL 1954, BEALING 1964). The slight conversion of glucose to CO_2 has been determined also radiorespirometrically (D'AUZAC 1965, 1966, BANCHI 1966). As limiting factors of latex respiration the sugar substrates, have thus not been considered but rather the activity of glycolytic enzyme systems (BEALING 1964, D'AUZAC 1965).

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In some plant cells e.g. in pollen tubes, fructose is preferentially incorporated into respiratory pathways (TUPÝ 1962). The reserve sugar is sucrose, which is hydrolysed by pollen β-fructofuranosidase and above all its fructose component is utilized in respiration. An indication for a specificity of fructose utilization in latex can be found in the higher fructokinase activity as compared with glucokinase (D'AUZAC, JACOB 1967). But in addition to sucrose the presence of important amounts of glucose and also of fructose in latex is stated (D'AUZAC and PUJARNISCLE 1959, LOWE 1960).

Material and Methods

Fresh clonal latex refrigerated immediately after its outflow from the tree, or its serum obtained by centrifugation of latex during 30 minutes at 20·000 g has been used.

The intensity of gas exchange was determined by current manometrical techniques using the Warburg apparatus. About 1 g of latex or serum was incubated at 31° under shaking with a 5 cm amplitude and at a rate of 160 strokes/min. The amount of latex used does not practically influence the rate of gas exchange (Van den TEMPEL 1954). The CO_2 evolution was determined by the "direct method" at natural pH of latex (pH $\doteq$ 6, 7). As the rubber content of the different latices varied considerably, all results have been calculated on the weight of serum.

Sugars were analyzed by paper chromatography according to VÍTEK (1964). Serum before chromatography was deproteinized by boiling during 3 min. with 2 volumes of alcohol.

The experiments were made in the rainy season (July, August). All trees used were under normal tapping (twice a week).

Results

In preliminary experiments it was found, that the site of CO_2 formation in latex is the serum (Fig. 1). Most experiments were therefore made with serum isolated by centrifuging.

During the long-termed incubations the microbial activity was inhibited by chloramphenicol. At a concentration of 25 p.p.m., chloramphenicol does not influence the gas exchange of serum, but entirely prevents the sudden increase of aerobic microbial respiration which in its absence occurs after about 3 hours of incubation (Fig. 1), that is about 4 hours after tapping.

The rate of CO_2 liberation by serum is greatly increased by fructose. After its exhaustion the rate of CO_2 formation passes immediately on the level of the control serum (Fig. 2). The oxygen consumption in the presence of fructose is decreased. The increase of CO_2 output and the decrease of

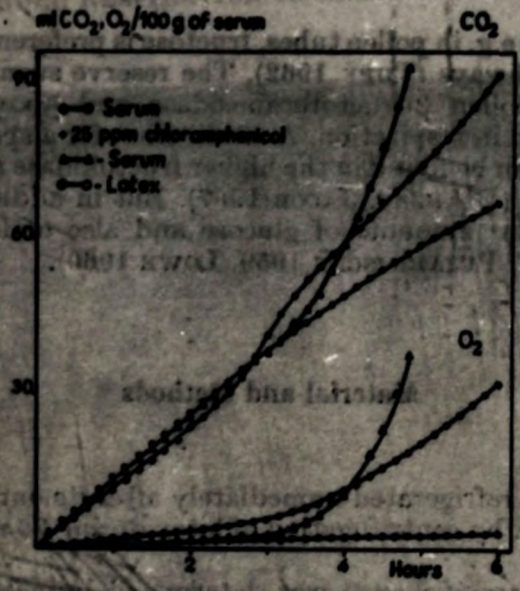

Fig. 1. Gas exchange in latex and serum during six hours of incubation. Effect of chloramphenicol.

O_2 consumption by fructose is further demonstrated for four different latex samples by Table 1.

The addition of glucose or sucrose does not influence the CO_2 formation in the serum during 3 hours of incubation. In a further experiment these sugars were added to the serum after 330 min. of incubation, when as a result of sugar

Table 1

Effect of fructose on the gas exchange (ml per 100 g of serum per hour) in serum of the clone GT 1 within 2 hours of incubation. Final concentration of additional fructose was 7×10^{-3}M

Exp.	CO_2		%	O_2		%
	Mannitol	Fructose		Mannitol	Fructose	
1	13·0	28·0	223	1·1	0·7	64
2	18·2	45·0	247	0·4	0·2	50
3	10·7	46·4	434	0·5	0·2	40
4	21·5	44·5	207	0·8	0·7	87
Mean	15·8	41·0	280	0·7	0·45	60
	$\overline{RQ}$ Mannitol = 22			$\overline{RQ}$ Fructose = 91		

substrate exhaustion, the CO_2 evolution had remarkably decreased (Table 2). In accordance with preceeding experiments, fructose strongly increased CO_2 output. The addition of sucrose approximately restored the initial rate of CO_2 formation. Glucose is almost without influence on CO_2 evolution but increases the O_2 consumption. Glucose utilization in respiration is at least 3 times slower than that of fructose, i.e. assuming that all CO_2 in the sample with glucose originated from glucose.

Table 2

Changes in the rate of CO_2 evolution and O_2 consumption in serum (clone GT 1) during 7 hours of incubation and the effect of additional sugars. The volume of 0·2 ml of sugar solutions was added after 330 min. of incubation to 1 ml of serum to a final concentration of 5×10^{-2} M of added sugar. The serum contained 25 ppm of chloramphenicol. The effect of sugars is expressed in ml CO_2, O_2 per hour and per 100 g of serum as well as in percent with regard to the first three hours of incubation (linear phase)

No.	First 3 hours (lin. phase) = 100%			1 hour before sugar addition			+ $5 \cdot 10^{-2}$ M of sugar	100 min. after sugar addition				
								ml			%	
	CO_2	O_2	RQ	CO_2	O_2	RQ		CO_2	O_2	RQ	CO_2	O_2
1	17·1	0·5	34·2	8·8	0·2	44·0	0	2·1	0·4	7·7	18	80
2	17·0	0·4	42·5	9·0	0·6	15·0	Mannitol	2·9	0·3	9·7	17	75
3	18·3	0·3	61·0	9·3	0·5	18·6	Glucose	5·5	1·7	3·2	30	567
4	17·1	0·4	42·7	8·1	0·5	16·2	Fructose	44·2	0	—	258	0
5	18·1	0·3	60·3	8·2	0·5	16·4	Sucrose	20·7	0·1	207·0	114	33
6	18·8	0·5	37·6	7·5	0·7	10·7	Galactose	11·0	0·4	27·5	58	80
Mean	17·7	0·4	46·4	8·5	0·5	20·1	—	—	—	—	—	—

During this long-termed incubation the RQ decreases. Its value is increased by fructose, but decreased by glucose.

Only a large quantity of sucrose and a smaller amount of raffinose were chromatographically found in the serum of this experiment before incubation.

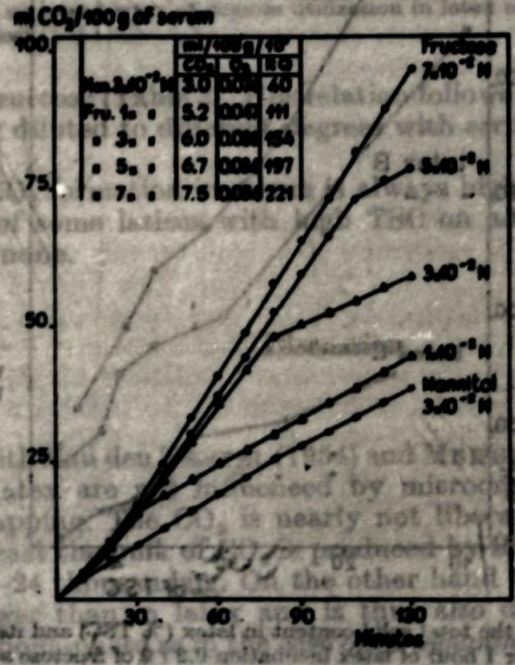

Fig. 2. Effect of different fructose concentrations on gas exchange in the serum of clone GT 1. The fructose was added about 15 min. before the beginning of measurements. RQ of each sample is calculated from the means of CO_2 evolution and O_2 absorption per 10 min. within the time before the sudden decrease of CO_2 evolution.

Table 3

Comparison of the effect of additional fructose on the rate of CO_2 formation in serum and latices with different total solid contents. The various latices and sera originated from the clone GT 1 of the same field

	TSC %	ml CO_2/1 hour/100 g of serum		% of increase
		Without Fructose	Fructose 5×10^{-2} M	
Latex a	42·01	12·4	17·8	129
b	40·32	13·5	20·0	149
c	39·62	8·0	11·6	147
d	38·09	22·6	35·0	155
Serum (Mean of 4 experiments)	—	15·6	41·0	263

After seven hours of incubation sucrose disappeared, the amount of raffinose lowered whereas substantial amounts of glucose appeared but not of fructose (Fig. 3). Gradual changes in sugar content of serum during incubation are demonstrated in a further experiment (Fig. 4).

In fresh serum we never found free fructose (< 10 µg per 1 ml of serum).

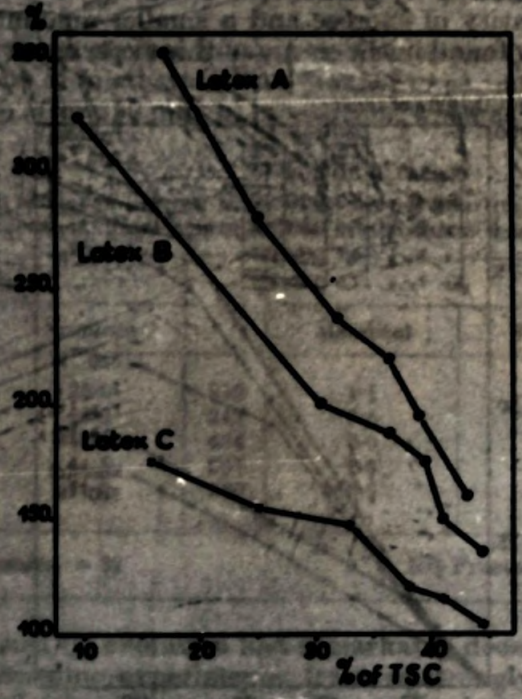

Fig. 5. Relation between the total solid content in latex (% TSC) and its response on additional fructose (%). After 1 hour of latex incubation 0.2 ml of fructose solution was added from the side arm to a concentration of $5 \cdot 10^{-2}$ M and the gas exchange was followed during a further 60 min. The percent of CO_2 evolution increase was calculated from the volume of CO_2 liberated per weight of serum. All latices originated from the clone PR 107, latex A and B from the same, latex C from another field.

Serum from latex of various clones (GT 1, PR 107, PB 86), from trees of different ages and tapped to different intensities have been analysed, as well as the alcoholic extracts obtained directly from these latices. The content of sucrose in serum is on the average about 10 mg per ml.

The CO_2 evolution from latex is less influenced by fructose than that from corresponding serum. The comparison of the response of different latices of the same clone to additional fructose has shown a negative relation between the total solids content of the latex (TSC*) and the increase of CO_2

Fig. 6. The main supposed ways of sucrose utilization in latex and their limiting factors (interrupted lines).

evolution by fructose (Table 3). This relation follows also from the experiment in which latex diluted to different degrees with serum of the same latex was used (Fig. 5).

While the CO_2 formation in serum is always highly increased by fructose, the response of some latices with high TSC on additional fructose is only very small or none.

Discussion

In accord with Van den Tempel (1954) and Maasdam (1956) the respiratory processes in latex are not influenced by microorganisms until about four hours after tapping. The CO_2 is nearly not liberated by lutoids or rubber globules. At least the bulk of CO_2 is produced by serum isolated by centrifugation at 20—24 thousands g. On the other hand O_2 consumption in serum is always slower than in latex and is thus also dependent on the lutoids and/or encutehouc-phase as in the case of *Strophanthus* latex (Maasdam 1956).

* The rubber content (DRC) of *Hevea* latex varies from between 20 and 50%. Its relation to the total solid content is about DRC = TSC − 3.

The striking increase of CO_2 formation and the decrease of O_2 consumption in serum so influenced by fructose, where RQ reaches values of about 150 shows a definitely anaerobic utilization of fructose in respiration. On the other hand glucose, the presence of which in serum decreases the RQ values, is at least partly respired aerobically. This follows also from the decrease of RQ during long-termed incubation, where the fructose liberated from sucrose is exhausted and glucose accumulates. If the respiration of latex proceeds by common metabolic pathways, the fructose would be utilized in anaerobic glycolisis and glucose would be also incorporated in the pentose phosphate cycle.

The first step of hexose incorporation in respiratory pathways is their phosphorylation. The differentiation between glucose and fructose takes place in this phase already, on the basis of different activities of their hexokinases. Fructose is about 1·2—2·8 times more rapidly phosphorylated than glucose (D'Auzac, Jacob 1967). But there is about a 3—4 times greater difference in their utilization in respiration than in the rates of glucose-6-phosphate and fructose-6-phosphate formation. The fermentative catabolism of fructose and the at least partly oxidative catabolism of glucose shows that the further limit in glucose utilization in anaerobic glycolisis may be low activity of phosphoglucoisomerase. But it is also possible to speculate on the presence of 1-phosphofructoaldolase and of fructokinase which forms fructose-1-phosphate as it has been found e.g. in the liver.

With regard to the high activity of the fermentative respiratory system, free fructose does not occur in latex and most latices react positively to additional fructose. The limiting factor of anaerobic glycolysis is thus the substrat, let us say the rate of its liberation by β-fructofuranosidase from sucrose or raffinose. On the other hand, with respect to the accumulation of glucose during incubation, the aerobic respiratory system would be rather limited by the activity of its enzymes.

The degree of CO_2 formation increase by fructose is negatively related to the rubber content in latex. If we keep in mind, that pyruvate is a direct or indirect precursor of acetyl-CoA, the starting compound for rubber biosynthesis, the inhibition of anaerobic glycolysis by the rubber may be considered as an endproduct-inhibition. This represents a further possible limiting factor of fermentative respiration which may be effective in trees of low tapping intensity i.e. with latices of high rubber content.

While analysing the sugars in serum after incubation we have found, that the decrease of their content in Warburg flasks containing KOH is always somewhat greater than in flasks without KOH. It follows, that the high concentration of CO_2 inhibits also the latex respiration. This factor is probably effective in vivo, in latex vessels, where the CO_2 output is limited.

The main supposed ways of sucrose utilization in latex and their limiting factors are summarized in Fig. 6.

A text with more experimental details is available at the Institut des Recherches sur le Caoutchouc au Cambodge, B.P. 11, Kompong-Cham (Cambodge).

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Anaerobní dýchání latexu je vázáno na sérum. Hlavním dýchacím substrátem je fruktosa. Výdej CO_2 sérem je zvyšován fruktosou v průměru přibližně 2,5 až 3krát. Glukosa tvorbu CO_2 v séru neovlivňuje, zvyšuje mírně spotřebu O_2.

Z cukru je v latexu přítomna v podstatě pouze sacharosa a menší množství rafinosy. Během inkubace séra je sacharosa hydrolysována, fruktosová složka okamžitě prodýchána a hromadí se glukosa.

Zrychlení výdeje CO_2 latexem vlivem fruktosy je nepřímo závislé na množství kaučuku v latexu. Latex s vysokým obsahem kaučuku na fruktosu reaguje jen nepatrně nebo vůbec ne.

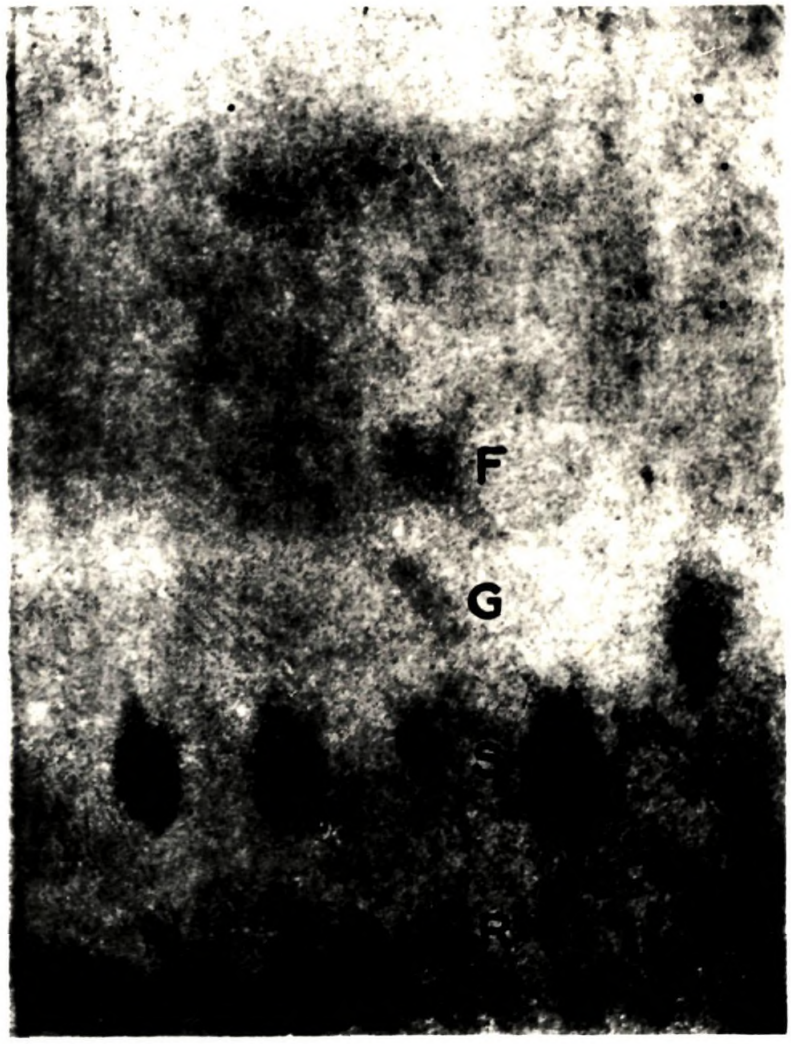
Hlavní limitující faktory dýchání latexu a využívání cukrů jsou (viz Fig. 6):

1. Nedostatek substrátu daný nízkou aktivitou β-fruktofuranosidasy.
2. Rychlost fosforylace glukosy (D'Auzac, Jacob 1967).
3. Pravděpodobně nízká aktivita fosfoglukoisomerasy.
4. Přítomnost kaučuku v latexu.
5. Koncentrace CO_2 v latexu; tento činitel se patrně uplatňuje *in vivo*, v míšeninách.

Я. Тупы, В. Л. Рисинг, Институт исследования каучука, Хум де Хуп, Камбоджа: Анаэробное дыхание латекса *Hevea brasiliensis*. Субстрат и лимитирующие факторы. — Biol. Plant. 10 : 72—80, 1968.

Анаэробное дыхание латекса связано с сывороткой. Основным субстратом дыхания является глюкоза. Выделение углекислоты сывороткой увеличивается действием фруктозы в среднем приблизительно 2,5—3 раза. Глюкоза не влияет на образование углекислоты в сыворотке, слегка повышает потребление кислорода.

Из сахаров латекс содержит в основном лишь сахарозу а также рафинозу в меньшом количестве. При инкубации сыворотки сахароза подвергается гидролизу, фруктозная компонента сразу же расходуется на дыхание и накапливается глюкоза.



3. Sugars in serum of various Hevea clones. The alcoholic extracts corresponding to 50 µl of serum along with 25 µg of standard sugars were chromatographed. F — fructose, G — glucose, S — sucrose, R — raffinose.

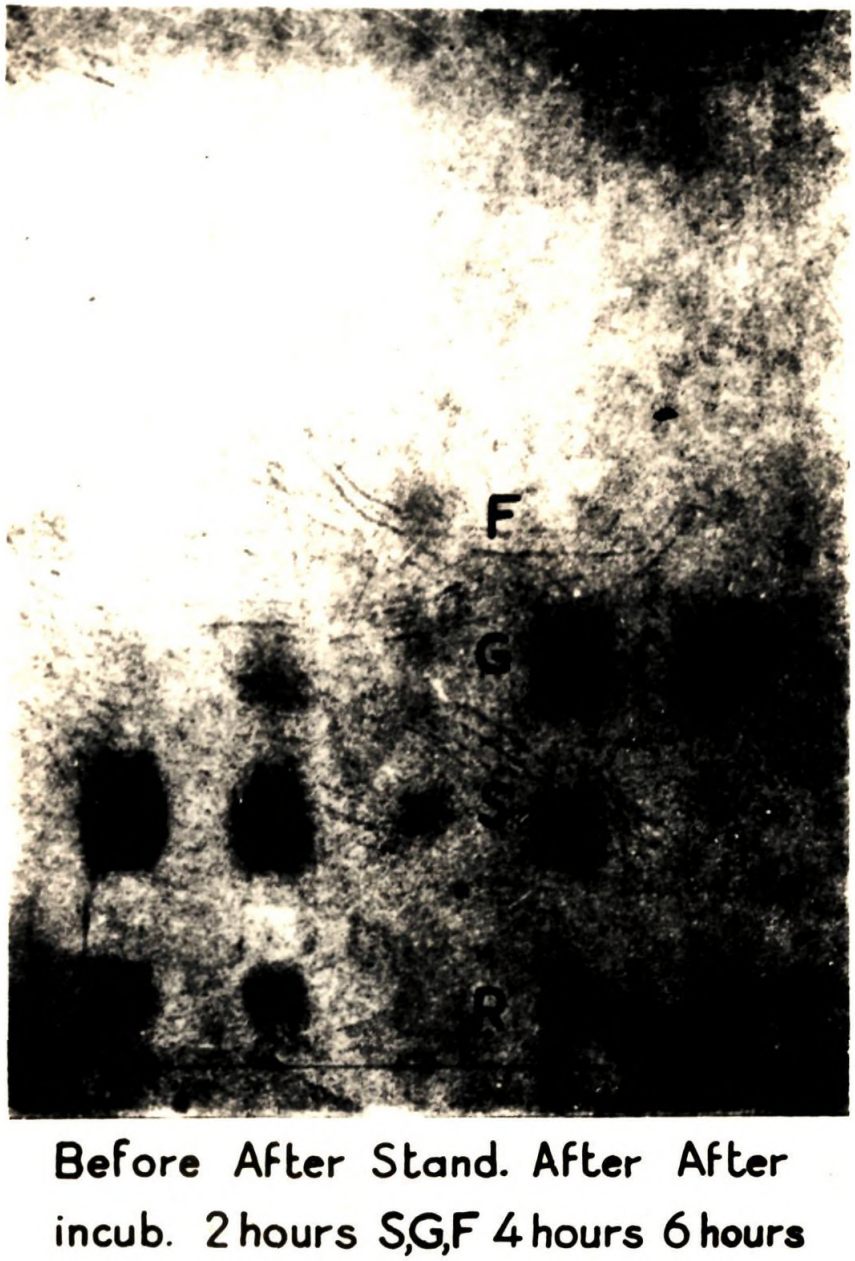


Fig. 4. Changes in the sugar level of serum, clone GT 1, during incubation. For further explanations see Fig. 3.

Ускорение выделения углекислоты под влиянием фруктозы обратно пропорционально содержанию каучука в латексе. Латекс с высоким содержанием каучука на фруктозу реагирует лишь незначительно или совсем не реагирует.

Основными для дыхания латекса и для использования сахаров лимитирующими факторами являются следующие: (см. рис. 6):

1. Недостаток субстрата, что является следствием пониженной активности β-фруктофуранозидазы.
2. Скорость фосфорилирования глюкозы. (D'Auzac, Jacob 1967).
3. Вероятно низкая активность фосфоглюкоизомеразы.
4. Наличие каучука в латексе.
5. Концентрация углекислоты в латексе: данный фактор вероятно играет роль *in vivo* в млечных сосудах.

Ускорение выделения углекислоты под влиянием фруктозы обратно пропорционально содержанию изучуна в латексе. Латекс с высоким содержанием изучуна на фруктозу реагирует лишь незначительно или совсем не реагирует.

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