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Control of Glycogen Metabolism in the Developing Fetal Lung

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Summary

The control of glycogen metabolism was studied in the lung of the developing rat or rabbit fetus by means of hormonal suppression (*in utero* decapitation, or thyroidectomy, or drug administration) and hormone administration to intact or hormone-deprived fetuses. In the rat fetus, *in utero* decapitation plus maternal adrenalectomy did not impair lung glycogen accumulation between days 17.5 and 19.5. On the contrary, glycogen breakdown, which occurs after day 20.5 in the rat and day 27 in the rabbit, was impaired, at least partly, by decapitation of the 17.5-day-old rat fetus and of the 23-day-old rabbit fetus. On the average, on day 21.5 the glycogen content of the lungs of the decapitated rat fetus was 323 ± 33 mg/g proteins *versus* 195 ± 14 mg/g proteins in the lungs of their controls. Thyroid hormone deprivation did not reproduce the effect of decapitation. Phosphorylase activity, which increases near term, still increased after decapitation; however, on the average, it was reduced by approximately 25%. Cortisol injection to decapitated fetuses restored normal glycogen breakdown without restoring phosphorylase activity. Growth hormone administered to decapitated fetuses restored a normal phosphorylase activity, but the lung glycogen content remained higher than in controls.

These results led us to search for a second mechanism of glycogen degradation. Lysosomal amyloglucosidase, which increased by 40% through days 17.5 and 21.5 in the rat, was unaffected by either hormone deprivation or by cortisol administration. Autophagic activity, estimated by β-N-acetyl-glucosaminidase (NAGase) that was liberated by homogeneisation and osmotic shock, was reduced by hormone deprivation (in fetal rat lung, free NAGase after homogeneisation: 27.2 ± 0.6% of total activity in decapitates *versus* 35.1 ± 1.3% in controls; free NAGase after osmotic shock: 54.9 ± 1.1% of total activity in decapitates *versus* 60.2 ± 1.5% in controls). Cortisol administration restored the autophagic activity of the tissue. In intact rat fetuses, growth hormone induced an anticipated rise of phosphorylase activity (16.8 ± 1.2 U/g proteins in 19.5-day-old treated rat fetuses *versus* 9.7 ± 0.6 U/g proteins in controls). Cortisol increased autophagic activity. It is concluded that: (1) glycogen accumulation in the fetal lung is probably not dependent upon fetal hormones, (2) fetal (and possibly maternal) hormones influence glycogen breakdown, (3) the rise of phosphorylase activity observed at the end of pregnancy partially occurs in pituitary deprived fetuses but it is stimulated by growth hormone which may be necessary for a complete development of the enzyme activity and (4) the effects of corticosteroids on glycogenolysis might be exerted through the control of autophagy.

Speculation

The present observations suggest that phosphorylase is insufficient by itself to fulfill glycogen breakdown in fetal lung and that hydrolysis in lysosomal structures is necessary for glycogen utilization and lung maturation. Taking into account the lysosome-related nature of lamellar bodies, the presence of hydrolases in these structures and their spatial association with glycogen areas, the assumption of a link between glycogen utilization and phospholipid synthesis in the developing lung is reinforced.

In the developing lung of mammalian fetuses, glycogen accumulates, mostly in epithelial cells, during a definite period of gestation, and disappears thereafter (46). Although not directly demonstrated, a relationship between surfactant elaboration and utilization of glycogen stores has been postulated by several authors on the basis of ultrastructural (10, 34) or biochemical (36) studies. Despite this probable relationship only a few studies deal with the control of glycogen metabolism in the fetal lung.

Corticosteroids have been reported to stimulate glycogen deposition in the mouse fetal lung *in vitro* at early developmental stages (1). It is unknown whether or not endogenous corticosteroids control glycogen storage in lungs as they do in the liver (27, 31). Later in gestation, corticosteroids (7, 20, 35, 39) and thyroxin (7), both *in vivo* and *in vitro*, and theophyllin *in vitro* (37) decrease lung glycogen contents. On the other hand, insulin prevents lung glycogenolysis *in vitro* (37). Decapitation *in utero* of rat fetuses diminishes lung glycogen breakdown before birth (5, 6). Because decapitation *in utero* almost totally suppresses several fetal endogenous hormones (31), it can be assumed that endogenous hormones exert a control upon lung glycogenolysis. The nature and the mode of action of the implicated hormone(s) remains to be established. The activity of glycogen phosphorylase in the fetal lung increases sharply near term in rat and rabbit (36, 9). Therefore a possible role of hormone(s) in the development of phosphorylase activity should be investigated.

The aim of the present work was to study these different points. Fetal hormone deprivation was produced by decapitation *in utero*, thyroidectomy *in utero* or drug administration. Decapitation not only suppresses hormones, but one can assume that an effect results from hormone deprivation when it is reversed by hormone replacement. Therefore, hormone injections were done as an attempt to compensate the effects of decapitation. They were also used to induce anticipated changes in intact fetuses. Glycogen contents, activities of glycogen synthase and phosphorylase were determined first in the lung of normal fetuses and later after various treatments. It appeared that some of the results could be accounted for only by an additional mechanism for glycogen breakdown. Hence, acid amyloglucosidase activity was studied in fetal lung and an estimation of autophagic activity was made. Most of the data were obtained from rats, but some experiments were carried out on rabbits.

MATERIALS AND METHODS

ANIMALS

Wistar albino rats and Fauves de Bourgogne rabbits, allowed food and water *ad libitum*, were used. Female rats were mated overnight and pregnant rats were recognized 14 days later by palpation. Time of ovulation and fertilization (approximately 1

a.m.), was considered as the beginning of gestation (31). In rabbits, time of mating was considered as time zero.

SURGICAL PROCEDURE

Surgery was performed under ether anesthesia in rats and sodium pentobarbital (30 mg/kg body weight) in rabbits. *In utero* decapitation was performed according to Jost (26).

Recently, some authors use the word anencephaly as an equivalent for decapitation (6, 7). This might be confusing because as a rule, anencephals have an anterior pituitary (encephalectomy is the nearest experimental duplication of anencephaly, see 28, 31).

In utero thyroidectomy was done on rabbit fetuses by the method of Jost (29). The thyroid gland was removed with part of the trachea, and the skin was sutured.

In some instances, maternal adrenalectomy was done in rats by the dorsal way. The rats received saline to drink.

Fetal lungs were removed as rapidly as possible, immediately frozen in liquid nitrogen, and stored for several days at −25°C until assayed. Biochemical determinations were always performed on an entire lung. Usually glycogen determination was done on the left lung and enzymic assays on the right lung.

HORMONES AND DRUGS

Hormones were administered to the fetuses under the skin of the back through the uterine wall and fetal membranes.

Cortisol acetate in microcrystalline suspension (Roussel, Paris, France), rat growth hormone (NIAMDD, Bethesda, Md., USA) and L-3-3'-5 triiodothyronine (Sigma, St. Louis, Mo., USA) dissolved in saline were used.

1-methyl-imidazole 2-thiol was purchased from Fluka (Switzerland). It was given to pregnant rats in drinking water at the concentration of 0.1%.

BIOCHEMICAL PROCEDURES

Glycogen contents of the lung tissue was determined by the method of Chan and Exton (11). Homogenates were made in water and 100 µl were dropped on Whatman 3 MM chromatography paper (3 x 2 cm). The papers were washed successively: 3 times (10 min each) in 66% ethanol, one time (10 min) in acetone, and dried. Glycogen trapped in the paper was hydrolysed by amylo α-1.4, α 1.6 glucosidase (Boehringer, Mannheim, Germany) in acetate buffer pH 4.5 and glucose measured with glucose oxidase (Perid-method, Boehringer). Oyster glycogen (Calbiochem, San Diego, Ca., USA) was used as reference.

Proteins were measured using the modification by Schacterle and Pollack (45) of the method of Lowry *et al.*

Active and total glycogen synthase (EC 2.4.1.11.) were determined by incorporation of U-[^{14}C] from UDP-U-[^{14}C]-glucose into glycogen according to DeWulf and Hers (16). The incorporated radioactivity was measured after adsorption of an aliquot of incubation mixture on filter paper (Whatman 31 ET). The activity was expressed as µmoles of glucose incorporated into glycogen per min per g proteins *i.e.*, Units/g proteins.

Phosphorylase *a* activity (EC 2.4.1.1.) was assayed in the direction of glycogen synthesis by the method of Hue *et al.* (24). Inorganic phosphate liberated from glucose-1-phosphate (Calbiochem) by the reaction was determined according to Fiske and Subbarow (18). The activity was expressed in µmoles inorganic phosphate released per min per g proteins, *i.e.*, Units/g proteins. Total phosphorylase activity was not determined.

Lysosomal acid amyloglucosidase (EC 3.2.1.20) was determined by the speed of *p*-nitrophenyl glucoside hydrolysis (Sigma) in the presence of triton × 100 (Sigma) according to the technique of Torres and Olavarría (49). The activity was expressed as nmoles of *p*-nitrophenol released per min per g proteins, *i.e.*, milliunits.

Autophagic activity of lung tissue was indirectly estimated in fresh tissue by the technique of Deter and DeDuve (14). The method is based on the fact that formation of autophagic vacuoles is accompanied by an increased lysosomal fragility (4). It consists in the measurement of the fraction (%) of a marker enzyme of lysosomes, β-N-acetyl-glucosaminidase or NAGase (EC 3.2.1.30) liberated by tissue homogeneisation (mechanical shock, standardized procedure: 10 strokes with a glass teflon homogenizer, in ice) or by a 30 min osmotic shock (dilution by 2 with water), total activity of the enzyme being determined after lysosome destruction by a detergent (Triton × 100). The higher the autophagic activity, the higher the % of liberated enzyme. NAGase was preferred to acid amyloglucosidase for that estimation because its higher rate of activity allows detection of smaller variations of lysosomal fragility.

STATISTICAL METHODS

The mean of the experimental data is accompanied with S.E. The comparison of means was done using either "Student" *t* test for unpaired values, or a non-parametric test for small series (52).

RESULTS

NORMAL PATTERN OF GLYCOGEN STORAGE AND BREAKDOWN IN THE LUNG OF RAT AND RABBIT FETUSES

The pattern of glycogen storage and breakdown in the strains used in this study is indicated in Fig. 1.

In the rat, glycogen concentration in the lung increases until day 19.5 and decreases thereafter, but due to growth, the total glycogen content of the whole organ increases until day 20.5. In other words, net glycogen deposition continues until day 20.5, after which time the total amount of glycogen decreases. A similar

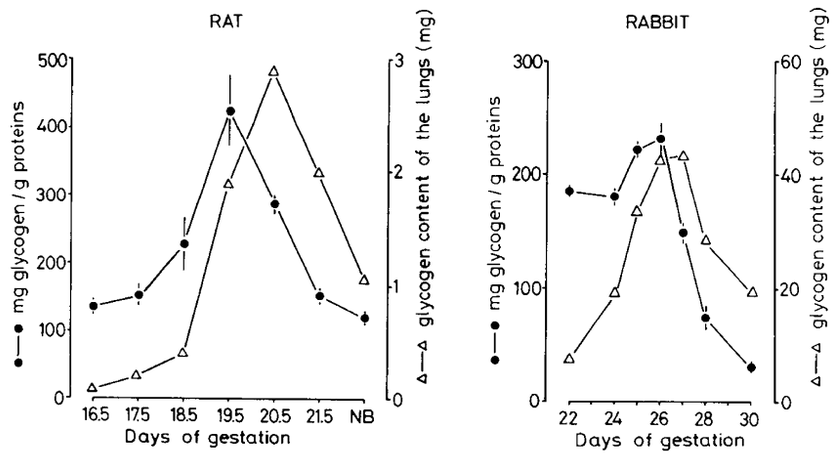

Fig. 1. Evolution of glycogen concentration and whole organ glycogen content in the lung of rat and rabbit fetuses in late gestation. Mean ± S.E. on 10–21 determinations per stage.

Table 1. *Evolution of glycogen synthase, phosphorylase a and acid amyloglucosidase activities in the lung of the rat fetus during the last days of gestation*

Stage of gestation (days)	16.5	17.5	18.5	19.5	20.5	21.5	Newborn
Glycogen synthase *a* (U/g proteins)	0.37 ± 0.05[1] (9)	0.74 ± 0.07 (15)	0.65 ± 0.03 (14)	0.75 ± 0.05 (15)	0.84 ± 0.09 (16)	0.53 ± 0.05 (15)	
Glycogen synthase *a* + *b* (U/g proteins)	1.19 ± 0.07 (9)	1.56 ± 0.08 (15)	1.92 ± 0.09 (14)	2.90 ± 0.15 (15)	2.77 ± 0.16 (16)	3.05 ± 0.21 (15)	
Phosphorylase *a* (U/g proteins)	2.5 ± 0.9 (11)	4.2 ± 1.5 (9)	12.8 ± 2.2 (14)	14.1 ± 1.2 (15)	30.6 ± 1.7 (15)	37.0 ± 2.2 (15)	17.2 ± 1.3 (6)
Acid amyloglucosidase (milliunits)		258 ± 20 (10)	290 ± 25 (10)	315 ± 13 (11)	309 ± 8 (12)	342 ± 14 (12)	

[1] Mean ± S.E. of the number of determinations indicated in parentheses.

pattern is observed in rabbits. The maximal concentration is observed on day 26, but the total glycogen deposition continues until day 27 and breakdown occurs afterwards.

Development of glycogen synthase and phosphorylase *a* activity in the lung of the rat fetus is shown in Table 1. Glycogen synthase *a* increases between day 16.5 and day 17.5. It remains practically constant for 4 days and decreases between day 20.5 and day 21.5. Total synthase activity remains constant between day 20.5 and day 21.5 (Table 1). This indicates that the fall of active synthase corresponds to an inactivation of the enzyme, possibly because of the sharp rise of phosphorylase activity. In the liver, phosphorylase *a* has been shown to be an inhibitor of synthase phosphatase (48). The increase of phosphorylase *a* activity is constant from day 16.5 to term.

LUNG GLYCOGEN ACCUMULATION AND BREAKDOWN IN DECAPITATED RAT AND RABBIT FETUSES

Rat fetuses. To determine if endogenous corticosteroids are necessary for lung glycogen accumulation as they are for accumulation of glycogen in the fetal liver (31) and if hormones are implicated in lung glycogen breakdown, pregnant rats were adrenalectomized on day 14.5 of pregnancy. Two to four fetuses per litter were decapitated on day 17.5. Fetal lungs were removed either on day 19.5, the stage of maximal glycogen concentration, or on day 21.5, when glycogen degradation is obvious. Because *in utero* decapitation causes fluid retention inside alveoli and ducts of the lungs (30), it was not possible to relate glycogen contents to the wet weight of tissue. Inasmuch as protein concentration appears to be the least modified parameter in lung cells (6), glycogen concentration was expressed per g lung proteins.

On day 19.5, there was no significant difference between decapitated and littermate intact fetuses in lung glycogen concentration (Table 2). The increase in glycogen content, occurring in lungs between days 17.5 and 19.5, was the same in decapitated fetuses as in controls, despite the reduction in circulating corticosteroids induced by *in utero* decapitation plus maternal adrenalectomy (40).

By contrast, the lung of the decapitates on day 21.5 contained more glycogen than that of controls (Table 2), which means that the decrease in glycogen concentration which normally occurs between days 19 and 21 was not as pronounced in decapitates as in controls.

In another set of experiments, fetuses of nonadrenalectomized mother animals were decapitated on days 17.5, 18.5 or 19.5 and studied on day 21.5. As in the previous group of adrenalectomized animals, there was more glycogen on day 21.5 in the lungs of the fetuses decapitated on day 17.5 than in those of their littermate controls (Fig. 2*a*). It appears that the presence or the absence of the maternal adrenals did not change the lung glycogen content of the decapitates during the period under study. In both the adrenalectomized and the nonadrenalectomized mothers, the values for glycogen concentration in decapitated fetuses were widely scattered, varying from very high concentrations to normal concentrations. In fetuses decapitated on day 18.5 or 19.5, glycogen breakdown was normal in almost all the fetuses. There was no difference in the average between control and decapitated fetuses on day 21.5 (data not shown). Since glycogenolysis is much less pronounced in fetuses decapitated on day 17.5 than in those decapitated on days 18.5 or 19.5, a critical stage seems to exist between days 17.5 and 18.5 for the mechanism of control of glycogen breakdown occurring after day 19.5.

Table 2. *Effects of in utero decapitation of the 17.5-day-old rat fetus of adrenalectomized mother upon lung glycogen concentration on days 19.5 and 21.5 of gestation (mg glycogen/g proteins)*

Gestational day	19.5 days	21.5 days
Decapitates	313 ± 18[4,5] (9)[2]	226 ± 24[1] (10)[3]
Controls	365 ± 24 (12)	148 ± 15 (16)

[1] Significant difference with controls for $P < 0.01$.

[2] From 5 different litters.

[3] From 8 different litters.

[4] Mean values ± S.E. for the number of determinations indicated in parentheses.

[5] Not significant.

Rabbit fetuses. Rabbit fetuses of intact mothers were decapitated on days 22, 23 and 24. On day 28, the lungs of fetuses that had been decapitated on day 22 or 23 contained more glycogen than those of their littermate controls (119.2 ± 13.2 mg/g proteins, $n = 9$, *versus* 56.1 ± 7.7 mg/g proteins, $n = 19$; $P < 0.001$). On the contrary, glycogen breakdown occurred normally in fetuses decapitated on day 24 (59.3 ± 7.9 mg/g proteins, $n = 7$, *versus* 49. 8 ± 6.6 mg/g proteins, $n = 10$). A critical period, therefore, seems also to exist in that species, between 23 and 24 days.

In conclusion, *in utero* decapitation did not prevent glycogen accumulation in the lung of the rat or rabbit fetus, but it impaired later glycogen breakdown when it was performed before day 18 in rats or day 24 in rabbits.

LUNG GLYCOGEN BREAKDOWN IN THYROID HORMONE-DEPRIVED FETUSES

Rabbit fetuses were thyroidectomized on day 23, a time when decapitation impairs lung glycogen regression. On day 28, there was no difference between thyroidectomized and control fetuses for lung glycogen content (65.3 ± 14.0 mg/g proteins in thyrex fetuses, $n = 8$, *versus* 62.5 ± 3.8 mg/g proteins in controls, $n = 19$).

Pregnant rats were given 1-methyl-imidazole-2-thiol (methimazole) continuously from day 16.5 until day 21.5 to depress the thyroid function of mothers and fetuses. Controls were fetuses of mothers bred at the same time and given tap water. Methimazole has been shown to depress thyroid function in rats at a much lower dosage than we administered (38). This treatment decreased slightly the maximal concentration of glycogen reached on day 19.5 (methimazole treated: 340 ± 10 mg/g proteins; controls: 384 ± 21 mg/g proteins, $n = 15$) but did not impair glycogen breakdown between days 19.5 and 21.5 (on day 21.5, methimazole treated: 105 ± 5 mg/g proteins, controls: 140 ± 4 mg/g proteins, $n = 15$).

Normal thyroid function does not seem to be necessary for lung

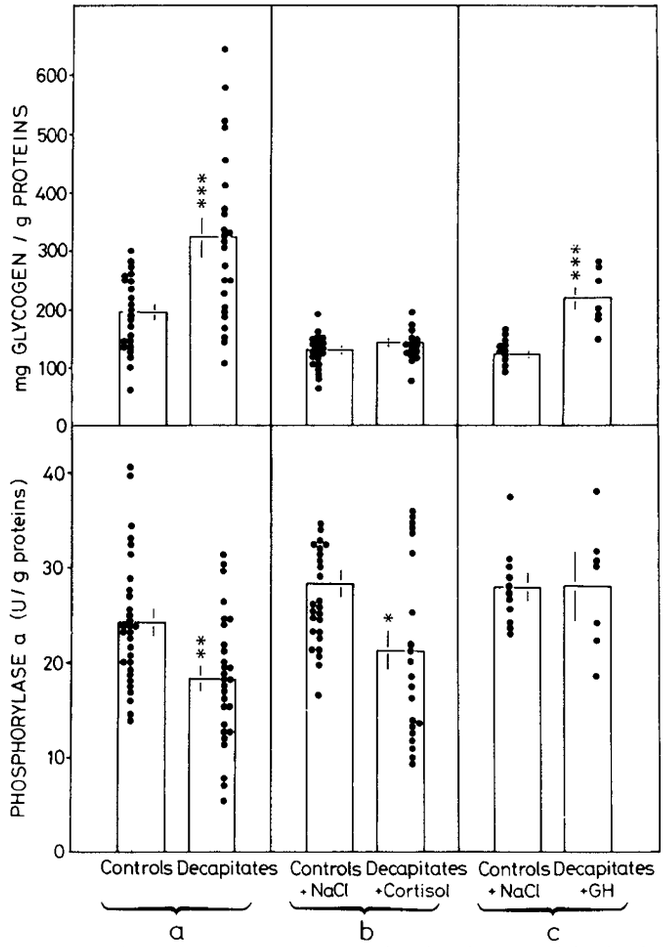

Fig. 2. Glycogen concentration and phosphorylase *a* activity in the lungs of: *a*, 21.5-day-old rat fetuses decapitated on day 17.5 and their littermate intact controls (nonadrenalectomized mothers); *b*, 21.5-day-old rat fetuses decapitated on day 17.5, given 0.25 mg cortisol at the same time, and their saline-injected littermate controls: *c*, 21.5-day-old fetuses decapitated on day 17.5, given 0.1 mg rat growth hormone at the same time, and their saline-injected littermate controls. Significant difference between treated fetuses and controls for: $*P < 0.05$; $**P < 0.005$; $***P < 0.001$.

glycogen breakdown in rat or rabbit fetuses. The effects of decapitation cannot be accounted for by impairment of thyroid function.

PHOSPHORYLASE *a* ACTIVITY IN THE LUNG OF DECAPITATED RAT FETUSES

Active phosphorylase was assayed on day 21.5 in the lung of rat fetuses decapitated on days 17.5, 18.5 or 19.5.

In fetuses decapitated on day 17.5, the enzyme activity was 25% lower on day 21.5 than in controls, although it had increased over the level already attained on the day of decapitation (compare Fig. 2*a* and Table 1). The difference between decapitates and controls was significant ($P < 0.005$) but the individual values were widely scattered, many decapitates presenting activities in the range of those of controls. The same difference was observed between decapitated fetuses of adrenalectomized mothers and their controls (data not shown).

In fetuses decapitated on day 18.5 or 19.5, lung phosphorylase activity was normal on the average although a few fetuses had a low activity (data not shown).

THE EFFECTS OF GIVING HORMONES TO DECAPITATED RAT FETUSES UPON GLYCOGEN CONTENT AND PHOSPHORYLASE ACTIVITY IN THE LUNG

Effect of cortisol on decapitated fetuses. Two to three rat fetuses were decapitated on day 17.5 in one uterine horn, and received simultaneously 0.25 mg of cortisol acetate. In the other uterine horn, control fetuses received the same volume of saline.

On day 21.5, phosphorylase *a* activity was similar in the lung of injected and noninjected decapitated fetuses with large individual variations (Fig. 2*a* and *b*). On the other hand, the cortisol injection diminished lung glycogen, which had become identical to that of controls, in decapitates with normal as well as low phosphorylase activity (Fig. 2*b*). Thus, the cortisol injection restored a normal decrease of glycogen without modifying phosphorylase activity.

Effect of growth hormone (GH) on decapitated fetuses. Rat fetuses were decapitated on day 17.5 and given 0.1 mg of rat GH. Because of the mortality of the treated decapitates and of the low amount of hormone available, only seven fetuses were obtained in this group. On day 21.5, phosphorylase *a* activity was identical in the hormone treated decapitates and in controls, but the glycogen concentration in the lungs of the GH treated decapitates was not as reduced as in controls (Fig. 2*c*).

In conclusion, it appeared that a high activity of phosphorylase *a* is neither necessary for normal glycogen breakdown nor sufficient by itself to induce normal glycogen degradation in the lung. These results can be accounted for if another mechanism for glycogen breakdown exists in fetal lung and if corticosteroids stimulate that mechanism. The presence of the lysosomal hydrolase of glycogen, acid amyloglucosidase was searched for in fetal lung. Moreover, autophagic activity was also studied.

LYSOSOMAL ACID AMYLOGLUCOSIDASE AND AUTOPHAGIC ACTIVITY IN THE LUNG OF NORMAL AND DECAPITATED FETUSES

Rats. Measurable activity of acid amyloglucosidase is present in the lung of the rat fetus as soon as day 17.5 of pregnancy. This lysosomal enzyme increases regularly between this stage of pregnancy and term by about 40% (Table 1).

Autophagic activity of the tissue was estimated through the release of NAGase by homogeneisation or by a 30 min osmotic shock. Total NAGase increased slightly between day 18.5 (6.0 ± 0.3 mU, $n = 9$) and day 21.5 (7.0 ± 0.3 mU, $n = 7$). Both homogeneisation and osmotic shock liberated a little more activity on day 21.5 than on day 18.5. On day 18.5, homogeneisation liberated $23.7 \pm 2.2\%$ of total activity and osmotic shock $50.5 \pm 2.0\%$. On day 21.5, homogeneisation liberated $32.4 \pm 1.4\%$ of total activity and osmotic shock $57.4 \pm 0.7\%$. The differences between days 18.5 and 21.5 are small but significant ($P < 0.05$) and can reflect an increased autophagic activity.

In rat fetuses deprived of corticosteroids (decapitation on day 17.5 and maternal adrenalectomy), lung acid amyloglucosidase on day 21.5 was a little higher than in their littermate controls (566 ± 47 Units/g proteins in five decapitates *versus* 459 ± 22 Units/g proteins in five controls). Total NAGase was not modified but the activity liberated by tissue homogeneisation or by a 30 min osmotic shock was lower in the lungs of decapitated fetuses than in controls (Table 3). On the average, the difference was small but the % of free enzyme was always lower in decapitated fetuses than in littermate controls. This could reflect a lesser lysosomal fragility and a lower autophagic activity in the lungs of decapitated fetuses.

Rabbits. Similar results were observed in lungs from rabbit fetuses decapitated on day 23 and removed on day 28 (the pregnant rabbits were not adrenalectomized). Amyloglucosidase activity was identical in decapitated fetuses (242 ± 21 Units/g proteins, $n = 5$) and in controls (242 ± 23 Units/g proteins $n = 7$). Free NAGase activity after homogeneisation or osmotic shock was lower in decapitated fetuses than in controls (Table 3). When cortisol (125 mg) was given to the rabbit fetuses at the time of decapitation, normal fragility of lysosomes seemed restored (Table 3).

ATTEMPTS TO ANTICIPATE THE DEVELOPMENT OF PHOSPHORYLASE AND AUTOPHAGIC ACTIVITY BY HORMONE TREATMENTS

Phosphorylase a. On day 18.5, rat fetuses received either 0.25 mg of cortisol, or 10 μg of triiodothyronine, or 10 μg to 100 μg of rat growth hormone (RGH). Controls received saline.

Table 3. *Effect of in utero decapitation of rat and rabbit fetuses and of cortisol injection (1.25 mg) to decapitated rabbit fetuses upon autophagic activity in fetal lung as estimated by liberation of NAGase activity. Pregnant rats were adrenalectomized when 14.5 days of pregnancy. Pregnant rabbits were not adrenalectomized. Rat fetuses were decapitated on day 17.5 and harvested on day 21.5. Rabbit fetuses were decapitated on day 22 and harvested on day 28*[5]

	Rat fetuses		Rabbit fetuses			
	Decapitates	Controls	Decapitates	Controls	Cortisol-treated Decapitates	Controls
Total NAGase activity (milli-units)	10.1 ± 1.5[4] (7)	9.6 ± 1.3 (10)	10.9 ± 0.5[4] (11)	10.7 ± 0.4 (12)	12.1 ± 1.2[4] (9)	11.5 ± 0.9 (11)
Free activity after homogeneisation (% of total activity)	27.2 ± 0.6[3] (7)	35.1 ± 1.3 (10)	28.1 ± 1.4[2] (11)	34.8 ± 1.7 (12)	38.1 ± 2.4[4] (9)	33.1 ± 2.1 (11)
Free activity after osmotic shock (% of total activity)	54.9 ± 1.1[2] (7)	60.2 ± 1.5 (10)	45.2 ± 1.7[1] (11)	49.9 ± 1.2 (12)	51.6 ± 2.5[4] (9)	49.7 ± 2.4 (11)

[1] $P < 0.005$.
[2] $P < 0.01$.
[3] $P < 0.001$.
[4] Not significant.
[5] Mean values ± S.E. for the number of determinations indicated in parentheses.

On day 19.5, phosphorylase activity was increased only in the fetuses given RGH (16.8 ± 1.2 U/g proteins, $n = 25$ in GH-treated fetuses, *versus* 9.7 ± 0.6 U/g proteins, $n = 31$ in controls). The mean value per litter was always higher for GH-treated fetuses than for their controls and two treated fetuses presented a phosphorylase activity (33 U/g proteins) on day 19.5 as high as it is normally in 21.5-day-old fetuses.

Amyloglucosidase and autophagic activity. Rat fetuses were given 0.25 mg cortisol on day 19.5. On day 21.5 their lung amyloglucosidase activity (349 ± 5 milliunits, $n = 6$) was a little less than that of littermate controls injected with saline (394 ± 12 milliunits, $n = 6$).

Autophagic activity was estimated in the lung of 19.5-day-old rat fetuses that had been given 0.25 mg of cortisol on day 17.5. Total NAGase activity was not modified (7.3 ± 0.3 milliunits in 17 injected fetuses, *versus* 6.7 ± 0.1 milliunits in 18 controls, nonsignificant difference). More activity was liberated by homogeneisation or osmotic shock in cortisol treated fetuses than in their littermate controls. Activity was higher in the latter than in fetuses of the same age of intact mothers, possibly because of transfer of corticosteroids from injected fetuses or from stressed mother animals (Fig. 3).

RGH (20 μg) or triiodothyronine (10 μg) given on day 17.5 had no effect upon the % of liberated NAGase activity measured on day 19.5 (data not shown).

DISCUSSION

HORMONES AND GLYCOGEN DEPOSITION IN FETAL LUNG

Decapitation *in utero* of rat fetuses plus maternal adrenalectomy did not prevent glycogen deposition in the fetal lung between days 17.5 and 19.5. The situation appears very different from that encountered in the fetal liver, since in similarly treated fetuses, liver glycogen storage is prevented (27, 31).

The apparent independence of glycogen accumulation in the lung from corticosteroids is consistent with the fact that synthase activity increases in fetal rat lung between days 16.5 and 17.5 (Table 1) whereas blood corticosterone concentration increases only between days 17.5 and 18.5 (12, 17). On the contrary, the rise of synthase activity in the liver is induced by fetal corticosteroids. It takes place between days 17.5 and 20.5 (15) and is prevented by decapitation plus maternal adrenalectomy and restored by corticosteroid administration (25, 41).

Alescio and Dani (1) reported that corticosteroids increase glycogen content in the 11-day-old mouse fetal lung bud *in vitro*. However, corticosteroids are not likely to be abundant in fetal blood at that early stage.

As a whole, glycogen deposition in the fetal lung does not appear to be controlled by the fetal pituitary or adrenal glands.

HORMONES AND GLYCOGEN BREAKDOWN IN FETAL LUNG

Contrary to glycogen deposition, glycogen breakdown in the fetal lung appears to be hormone dependent.

The fact that glycogen breakdown was impaired in rat fetuses decapitated on day 17.5 and not in those decapitated on days 18.5 or 19.5 might depend upon an early corticosteroid controlled process for the following reasons: (1) a normal glycogen breakdown could be obtained in decapitated fetuses that had been given cortisol on day 17.5 (GH produced a subnormal glycogen breakdown), (2) during normal development *in vivo*, there are relatively low concentrations of corticosteroids in fetal blood on day 17.5, which increase two fold between days 17.5 and 18.5 (12), (3) thyroid hormone blood concentration also increases at this age (19) but thyroid hormones do not seem to be involved (no effect of thyroidectomy on rabbit fetuses, no effect of methimazole on rats), (4) GH is undetectable in the fetal blood before day 19.5 (42), and (5) blood insulin (21) and blood glucagon (21) increase mostly between days 19.5 and 20.5. Catecholamines remain extremely low in the fetal adrenals until day 19.5 (44).

Corticosteroids from the fetus itself must be implicated because decapitation had the same effects on fetuses of either adrenalectomized or nonadrenalectomized mothers.

The hypothesis that the different effects of decapitating the rat fetuses on either day 17.5 or on day 18.5 are due to the absence of corticosteroids on day 18.5 in rats decapitated on day 17.5 should be verified by measuring blood corticosterone on day 18.5 in fetuses decapitated the previous day. In later stages, corticosteroids circulating in the maternal blood reach the fetus (17, 40). In fetuses decapitated on day 18.5, blood corticosterone is reduced at term only if the mother is adrenalectomized (40).

Finally, it should be mentioned that in rabbit fetuses, the volume of the adrenal cortex remains very underdeveloped in fetuses decapitated on day 22 or 23. On day 24, the cortex attains a larger degree of development (28).

As a whole, it is likely that corticosteroids represent the main factor controlling glycogen breakdown in the fetal lung (57).

According to Blackburn *et al.* (7), thyroxin (2–8 μg/fetus) injected into the fetus on day 18.5 induced a decrease of lung glycogen content. One may wonder whether this effect could be of a pharmacologic nature since endogenous thyroid hormones do not seem necessary for lung glycogen breakdown.

The increase of phosphorylase activity normally occurring in late gestation was partly impaired by decapitation of the rat fetus on day 17.5. Only active phosphorylase was determined; therefore, it is unknown whether the difference in active phosphorylase reflects a difference in total phosphorylase, *i.e.*, in the synthesis of the enzyme, or an inactivation of it. The difference is probably not a consequence of surgically induced stress in control fetuses and absence of stress in decapitates because (1) phosphorylase

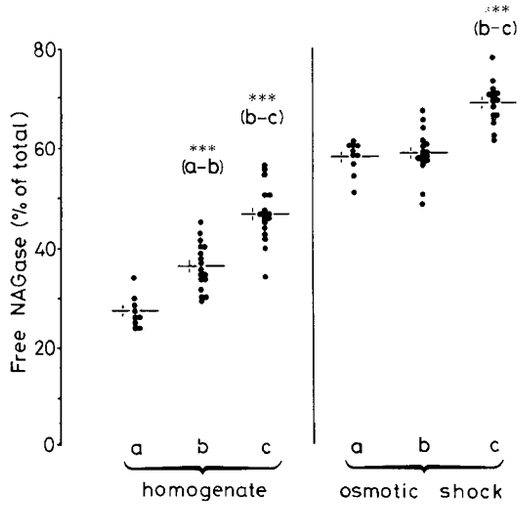

Fig. 3. Effect of cortisol upon autophagic activity in the lung of the fetus as estimated by liberation of β-N-acetyl-glucosaminidase (NAGase) activity after homogeneisation of tissue and a 30 min osmotic shock. Points represent individual data and horizontal bars represent mean values with S.E. *a*, 19.5-day-old fetuses of nonoperated mothers. *b*, 19.5-day-old fetuses injected with saline on day 17.5 (same litters than fetuses of group *c*). *c*, 19.5-day-old fetuses injected with cortisol (0.25 mg) on day 17.5. ***Significant difference for $P < 0.001$.

activity is not higher in littermate controls of the decapitates when comparing these animals with fetuses of the same age of intact mother animals (compare Table 1 and Fig. 3*a*) and (2) cortisol did not restore normal phosphorylase activity in the decapitates.

The part that the deficit of phosphorylase activity plays in the impairment of lung glycogenolysis is uncertain since this deficit is weak, on the average, and since many decapitates exhibited a phosphorylase activity in the range of the activities of controls. Thus, it appears that although glycogen breakdown in decapitates was impaired, the active form of phosphorylase increased to a subnormal value. However, it must be recalled that the activity of phosphorylase *a* determined *in vitro* does not necessarily reflect glycogenolysis *in vivo*. For instance, in the liver of the rat fetus, glycogen deposition occurs in the last days of gestation in the presence of an elevated phosphorylase activity (15).

Growth hormone, which was able to both restore normal phosphorylase *a* in decapitates and induce an anticipated rise of phosphorylase activity, appears to be a physiologic stimulating factor for phosphorylase increase. Its effects are likely due to synthesis rather than activation of the enzyme since in some rats given GH on day 18.5, phosphorylase was as high on day 19.5 as it is normally on day 21.5. According to Maniscalco *et al.* (36), active phosphorylase is higher on day 21.5, than is total phosphorylase on day 19.5. The role of GH in phosphorylase development is consistent with the fact that a rise of plasma GH occurs in the vicinity of lung glycogen breakdown in various species including the rat (42), the rabbit (32), the sheep (22) and man (33). However, the increase of phosphorylase in the lung of the rat fetus begins before GH appears in fetal blood. Some increase of phosphorylase activity occurred in the decapitates although their blood contained no growth hormone on day 21.5 (43). Growth hormone might stimulate, even if it does not induce, phosphorylase development. Growth hormone probably stimulates also phosphorylase development in the liver (50).

Acid amyloglucosidase activity in the fetal lung appears to be hormone independent. It increases slightly in late gestation but is already high at earlier stages when glycogen accumulates. Therefore, it seems to be unrelated to the shift from glycogen storage to breakdown. Instead, this shift seems related to an increase of autophagic activity of lung tissue. The increase of the fraction of NAGase liberated by homogeneisation and osmotic shock, which was observed in the rat fetus between days 18.5 and 21.5, may appear modest. However, if it represents only 7–8% of total NAGase activity, it is actually equal to one-third of the activity liberated by homogeneisation on day 18.5. This increase is on the same order as that reported by Deter and DeDuve (14) in adult rat liver after exposure to glucagon. It can be assumed that such a difference really reflects an increased lysosomal fragility. Contrary to amyloglucosidase activity, autophagic activity seems to be influenced by corticosteroids: cortisol compensated the effects of decapitation on both glycogenolysis and on lysosome fragility. It also induced an increase of lysosome fragility in intact fetuses, which may reflect an anticipated increase of autophagic activity. It can be assumed that corticosteroids exert their effects on glycogen breakdown in the fetal lung through the control of autophagic activity (57).

As a whole, glycogen breakdown in the fetal lung appears to be performed both by phosphorolysis and hydrolysis, but the present study does not bring informations about the relative part of each mechanism.

In some of the fetuses decapitated on day 17.5 and given cortisol, the phosphorylase activity was very low while glycogen contents had decreased as much as in controls. It may be supposed that in these cases, glycogen breakdown can be accounted for by lysosomal hydrolysis, which compensated the deficit of phosphorylase activity. The involvement of lysosomal degradation is also suggested by the fact that not only do corticosteroids appear to control autophagy but they are likely to be the most important hormone controlling glycogen breakdown. Villee (51) observed liberation of free glucose by fetal human lung *in vitro*. Since the fetal lung has very low glucose-6-phosphatase activity (8, 47), this could reflect hydrolysis of glycogen by the tissue rather than phosphorolysis plus hydrolysis of glucose-6-phosphate. The assumption that glycogenolysis occurs through an autophagic process would be confirmed by the visualisation of glycogenosomes by electron-microscopy at the end of gestation. Autophagic vacuoles (secondary lysosomes) including portions of cytoplasm have been described in type II cells of the rabbit fetal lung (34). But it could not be determined if glycogen could be seen inside these vacuoles. Glycogen particles have been shown to be associated with lamellar bodies in late gestation of the rabbit (34). Since these organelles contain hydrolase activities (23) it is possible that they perform glycogenolysis and provide substrates for synthesizing activities. In a general manner, tissue autolysis seems to be related to maturational processes (13). According to Ashford and Porter (2), lysosomes represent portions of the cytoplasm set aside for hydrolysis, to provide breakdown products for use in a reoriented physiology. This is precisely what happens in the fetal lung at the time of glycogen degradation and phospholipid synthesis. Taking into account the abundance of lysosome-related structures in the fetal lung (34) and the putative lysosomal origin of lamellar bodies where surfactant accumulates (3, 23, 34), the present data strengthens the assumption of a metabolic link between glycogen breakdown and the synthesis of phospholipids of surfactant.

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57. The action of corticosteroids could be indirect, possibly through their inducing effect on the maturation of adrenal medulla [Roffi, J.: Influence des corticosteroids sur la synthèse d'adrénaline chez le foetus et le nouveau-né de rat et de lapin, J. Physiol., Paris, *60:* 455 (1968)]. This would explain why the critical period for the effects of decapitating the fetuses takes place earlier than the final effect of corticosteroids, *i.e.*, glycogen breakdown.

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