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Zoologia. — *Lactate pyruvate ratio in white rat foetuses with respect to their intrauterine position* (*). Nota (**) di ROBERTO COLOMBO, ALBAROSA DUZIONI e CLAUDIA LANDONI, presentata dal Socio S. RANZI.

RIASSUNTO. — Da notizie trovate in letteratura e da nostri dati sperimentali, è stato possibile mettere in evidenza l'influenza dell'ambiente periembrionale sulle caratteristiche dello sviluppo di diversi cicli metabolici in feti ed embrioni di ratto.

Finora si è riusciti ad evidenziare l'esistenza di due zone uterine in cui, a causa del diverso apporto ematico, i feti si sviluppano secondo meccanismi metabolici alquanto differenti:

1) La *zona paraovarica* (estremità tubale del corno uterino) in cui i feti si sviluppano ricorrendo alla glicolisi anaerobia; infatti accumulano notevoli quantità di piruvato sotto inibizione della LDH ad opera dell'ossamato di potassio, mostrano una bassa attività di trasporto elettronico mitocondriale e sono scarsamente influenzati da anossie materne e da ischemie sperimentali.

2) La *zona paravaginale* (estremità vaginale del corno uterino) in cui i feti, trovando una più alta pO_2, possono incominciare a sviluppare meccanismi metabolici tipicamente ossidativi, cioè un più basso accumulo di piruvato sotto inibizione della LDH da parte dell'ossamato di potassio, una più alta attività di trasporto elettronico mitocondriale, un elevato grado di risposta ad anossie materne e a condizioni sperimentali ischemiche.

Col proposito di verificare ulteriormente questo fatto, sono state saggiate le concentrazioni di piruvato e lattato in feti impiantati nei sopraccitati distretti uterini.

In our researches aimed at establishing the influence of the periembryonic environment on metabolic mechanisms worked out by the embryo for its own development, we have pointed out in each uterine horn of the bipartite rat uterus two zones, respectively named paraovarian (tubal end) and paravaginal (vaginal end), where foetuses develop according to rather different metabolic mechanisms.

In the paraovarian zone, chronically lacking oxygen owing to the poor utero-placental blood supply, foetuses develop utilizing a strictly anaerobic metabolism.

In fact, they show a marked accumulation of pyruvate under LDH inhibition by potassium oxamate (Colombo, R. and Giavini E., 1973).

Furthermore, in mitochondrial fractions obtained from homogenates of foetuses implanted in the aforesaid uterine zone, a minor dehydrogenase activity was evidenced in respect to brothers implanted in lower positions (Colombo R. and Giavini E., 1975).

Other Authors (Woollam D. H. M. and Millen J. W., 1962) have stated that, in the mouse, paraovarian foetuses are the least sensitive to maternal anoxia.

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(**) Pervenuta all'Accademia il 28 ottobre 1977.

Others (Barr M. and Brent R. L., 1970), have found that, by clogging experimentally the two arteries irrigating the uterus, the foetal mortality is always restricted to the uterine paravaginal district.

These data suggested the existence of the above-mentioned uterine districts: an ischemic (paraovarian) presenting sub-optimal development conditions and a second (paravaginal) abundantly irrigated district, with good periembryonic environmental conditions.

In order to further verify the metabolic differences characterizing the two districts, the pyruvate and lactate concentration has been tested in foetuses implanted in the two uterine districts considered.

Twenty pregnant females of the white rat (Sprague-Dawley stock) were used. They were killed at the 15th day of gestation. The pregnant uterus was evidenced by abdominal incision and then the foetuses present in each uterine horn were counted subdividing them into two groups, corresponding to the different uterine districts considered; later, foetuses taken from the amnion were frozen in a dry-ice/acetone mixture and settled in different basins according to their uterine implantation site.

Foetuses of both uterine districts were then homogenized, separately for each single female, directly in $HClO_4$.

The homogenate obtained was centrifuged at 3.000 g for 10'.

The floating material was then removed and subjected to the spectrophotometric determinations.

The Hohorst method for determination of lactate (from Bergmeyer, 1965) was followed. For the determination of pyruvate the Bucher, Czok, Lamprecht and Latzko method (from Bergemeyer, 1965) was used.

RESULTS

The results obtained have been schematized in the following Table, including values of pyruvate and lactate levels found in both categories of foetuses considered and also values of lactate/pyruvate ratio for foetuses coming from the two different uterine zones.

TABLE

Data relating to white rat foetuses at the 15th day of gestation.

(mean ± S.E.M. with *n* in brackets)

Uterine zone	µmoles lactate /gr. tiss./h	µmoles pyruvate /gr. tiss/h	L/P ratio
Paraovarian	10,048 ± 0,448 (*) (18)	0,254 ± 0,009 (**) (18)	39,5
Paravaginal	6,641 ± 0,400 (*) (20)	0,321 ± 0,013 (**) (20)	20,6

(*) Data with highly significant difference ($p < 0{,}01$).
(**) Data with statistically significant difference ($p < 0{,}05$).

Discussion

The anaerobic condition of the paraovarian district is also evidenced by data obtained from this series of tests.

In fact, in foetuses implanted towards the tubal end (paraovarian) of the uterine horn, a greater amount of lactate and a smaller amount of pyruvate was found in respect to the levels of two metabolites considered found in foetuses implanted towards the vaginal end of the same uterine horn.

The fact that also in the paravaginal district rather marked concentrations of lactate have been found may be explained on the basis of studies carried out by Berger and Hommes (1971) on mitochondria of foetal cells.

These Authors have established that, even in aerobic conditions, mitochondria of embryonic cells fail to oxydate all the pyruvate produced by glycolysis; only 10-15% of the pyruvate is oxydated while the part is reduced to lactate.

From the results obtained it might be supposed that, with relation to a higher uterine blood supply, i.e. in more favourable periembryonic environmental conditions, foetuses of uterine paravaginal zone show better developed and functional mitochondrial apparatus than brothers implanted in ischemic uterine districts; this fact is supported by data previously established (Colombo R. and Giavini E., 1975) and stated in our introduction, concerning the mitochondrial dehydrogenase activity in foetuses implanted in different uterine districts.

It may useful to remark that in foetal mitochondria the oxydative capacity is increasing considerably during the last period of gestation and the first phases of new-born life (Hommes F. A. and Richters A. R., 1969; Gregson N. A. and Williams P. L., 1969; Jakovcic S. and Cow., 1971).

This should be mainly ascribed to an increase in number of mitochondria, reaching the utmost value towards the 15th day after birth.

In relation to the increase in number of mitochondria, there is also a growth of enzymes of the Krebs cycle (Hommes F. A. and Cow., 1969, 1971).

In this connection, particular importance is acquired by the pyruvic-dehydrogenase complex, as an enzyme taking part in Acetyl-CoA production.

It has been observed that its activity approximately trebles during the period three days before the birth onwards (Knowles S. E. and Ballard F. J., 1974).

Berger and Hommes (1974) have also found, on embryonic hepatic cells, that uncoupling substances like FCCP (Carbonylcyanide *p*-trifluoromethoxyphenylhydrazone) and valinomycin have a considerable influence on the value of the lactate/pyruvate ratio which from rates of about 7.5 reaches, because of the action of said substances, an approximate value of 70.

The values of the lactate/pyruvate ratio we have ascertained in foetuses inhabiting the different uterine zones, seem to suggest that mitochondria coming from paraovarian foetuses show a poor activity, with respect to py-

ruvate oxidation, practically behaving like mitochondria poisoned by an uncoupler.

We feel therefore entitled to assume that also for the pyruvic-dehydrogenase complex, as has been established for that of succinic-dehydrogenase (Colombo R. and Giavini E., 1975), there exists a different development rate in foetuses implanted in uterine sites with different periembryonic environmental conditions.

In the light of data supplied by the literature or verified by our laboratory tests we have reached the conclusion that, owing to the scarce utero-placental blood irrigation, foetuses of the paraovarian district, in order to produce the energy necessary to their development, should elaborate a strictly anaerobic metabolism; this fact is clearly pointed out by the high value of the lactate/pyruvate ratio they show (see Table) as well as from preceding considerations arising from studies on foetal mitochondrial apparatus carried out by Colombo and Giavini (1975), by Knowles and Ballard (1974) and by Berger and Hommes (1974).

On the contrary, foetuses implanted in paravaginal districts are able to work out a less strict anaerobic metabolism; in the latter the pyruvate produced can partly find an outlet in the mitochondrial apparatus which, thanks to the favourable periembryonic environment, is fairly well developed.

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