Metabolic alterations induced by tumour growth during the perinatal phase

Alteraciones metabólicas inducidas por el crecimiento tumoral durante la fase perinatal

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ABSTRACT

The implantation of a fast-growing tumour is associated with severe metabolic complications in the host, leading to cachexia and finally death. In contrast, the implantation of the fast-growing Yoshida AH-130 ascites hepatoma to pregnant rats at term did not result in any changes in fetal growth, the availability of amino acids for the fetus being unchanged by tumour burden. Similarly, the implantation of the fast-growing rat Walker 256 carcinosarcoma resulted in very little changes in lipid metabolism during lactation, the metabolism of the mammary gland being basically unaffected by the presence of the tumour. It may be suggested that during the perinatal phase the mother can cope with some of the metabolic disturbances induced by tumours this being able to protect either the fetus or the newborn.

Key Words:Tumour growth. Pregnancy. Lactation. Cytokines.

RESUMEN

La implantación de un tumor de rápido crecimiento se asocia con importantes complicaciones metabólicas en el huésped, que conducen a la caquexia y finalmente a la muerte. En cambio, la implantación del hepatoma ascítico de rápido crecimiento Yoshida AH-130 a ratas gestantes a término no ocasionó cambio alguno en el crecimiento fetal, manteniéndose inalterada la disponibilidad de aminoácidos por parte del feto. De forma parecida, la implantación en rata del carcinosarcoma de rápido crecimiento Walker 256 dió lugar a cambios muy pequeños en el metabolismo lipídico durante la lactancia, manteniéndose prácticamente inalterado el metabolismo de la glándula mamaria pese a la presencia del tumor. Se sugiere que durante la fase perinatal, la madre puede enfrentarse con algunas de las alteraciones metabólicas inducidas por los tumores, siendo capaz de proteger al feto o al neonato.

Palabras clave: Crecimiento tumoral. Gestación. Lactación. Citoquinas.

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INTRODUCTION

A tumour can indeed be considered as a parasite that feeds itself at the expense of its host. This results in a very great weight loss which leads to host cachexia (1) (Table 1). Although it is known that tumours can obtain substrates and energy necessary for their synthesis from the host at a significant energy cost, it is still not quite clear whether the cachexia can be fully accounted for by inadequate energy intake and increased energy expenditure. This could occur as a result of the energy cost of tumour development, uncoupling of oxidative phosphorylation or increased futile cycle activity (2). There is still quite a controversy regarding the preferred substrates to maintain tumour growth and the control of their utilization by cancer cells. In fact, tumor cells can cope with virtually any substrate: glucose (3,4), lipids (5,6) or amino acids (3,4,7), although their relative importance will vary according to the type of tumour and even the state of development of the tumour.

Table 1.—Main factors involved in cancer cachexia

Anorexia
Reduced food intake
Increased vomiting due to tumour or treatment
Mecanical obstruction and/or malabsorption
Metabolic changes
Increased hepatic gluconeogenesis
Increased muscle protein degradation
Increased lipolysis
Increased uncoupling of oxidative phosphorylation
Altered hormonal milieu

The tumour changes the host's metabolism

In order to grow, the tumour requires metabolites that must be supplied by the host. When the tumour's demand become greater than the host can deal with (especially when the energy input is reduced as a consequence of diminished food intake), host weight loss and ultimately malnutrition and death occur (Table 1). The appearance of the tumour affects the metabolism of the host at two different levels. First, it produces changes in the metabolism and hormonal environment of the host as a result of the successful competition of the tumour with the normal tissues for important metabolites, both building blocks and energetic substrates and trophic factors. Secondly, it influences some host tissues by decreasing differentiation, changing their enzyme characteristics, their sensitivity to hormones and disturbing the negative feed-back systems which co-ordinate the activities of central and peripheral endocrine

glands. Although the tumour cells act by draining host metabolites continuously (and thus place the host in a kind of «accelerated starved state»), the metabolic response which is generated is quite different from that which arises in starvation. In this sense, the decrease in oxygen consumption and carbon dioxide production normally seen in starvation does not occur in the host (8). In the first stages of starvation, fat mobilization is enhanced by low levels of circulating insulin and increased sympathetic nerve stimulation of adipose tissue cells (9). These metabolic events are followed by muscle proteolysis and enhanced amino acid disposal, hepatic gluconeogenesis and urea production (9). The metabolic adaptations to starvation are accompanied by the ability of the tissues to adapt themselves to the utilization of both fatty acids and ketone bodies (9). The host adaptations after the implantation of the tumour do not follow the same pattern although they share some of these trends. The recycling of lactate generated by the tumour occurs together with an increase in the utilization of ketone bodies by tumour cells (10). In a way, the metabolic adaptations of the host share some of the responses to both anorexia and starvation, thus placing the animal in a very distinctive metabolic environment.

Tumour cell metabolism

It was already in 1930 that Warburg (11), pointed out that all tumours have a high rate of aerobic lactate production from glucose and that they do not show a decreased glycolytic rate at relatively high oxygen concentrations; in other words, the Pasteur effect is extremely small. However, a marked Crabtree effect is often seen in tumour tissues. Supplying tumour cells with glucose results in an inhibition of oxygen consumption which magnifies the dependence on glucose for energy. Other cellular types do not normally show this effect since they maintain respiration from other substrates even in the presence of glucose. Although nowadays we know that the high glycolytic capacity is neither an underlying factor in the normal to neoplastic transformation process, nor even an universal characteristic of tumours (12), it is a very distinctive behaviour in a large number of tumours.

Concerning nitrogen metabolism, tumours act as metabolic traps (13), especially concerning their ability to act as a nitrogen sink where amino acids are retained and used for both oxidation and protein synthesis. In a way, the excessive uptake of certain amino acids (one of the most characteristic ones being glutamine) by tumour tissue results in a decrease of availability of amino acids for muscle and could thus partially explain the impairment in protein synthesis (14,15). In tumours, this metabolic process is increased probably because of increases in the enzymes catalysing for the synthesis of

purines and pyrimidines (16). In addition, in the tumour cells, there is a re-arrangement of the protein-synthesizing machinery (17) and the population of membrane-bound ribosomes (specialized in the synthesis of extracellular proteins such as hormones) is reduced, thus indicating a relative loss of secretory function versus proliferation.

Studies involving the Ehrlich ascites tumour cells indicate that practically all esterified fatty acids present in these cells are derived from *de novo* synthesis (18). This suggests that, at least in some tumours, lipogenesis is a very important pathway within the cancer cell, as important as any other source of fatty acids supplied by the host to the tumour. Although fatty acids are used by the tumour predominantly for oxidation with a clear energetic fate, they can also be used for the synthesis of both membrane cholesterol and phospholipids. In hepatoma cells there seems to be an abnormal cholesterol metabolism which results in a lack of feedback inhibition of its synthesis by dietary cholesterol (19). This is correlated with the content of cholesterol in tumour cell mitochondria which is very high (20). It seems likely, therefore, that altered membrane-dependent properties of tumour mitochondria are related to both membrane lipid and protein composition (21).

Metabolic changes in the host

The tumour is always ahead of the rest of the tissues in its competition for circulating glucose. There is, however, an important aspect to be taken into consideration. Not all the tumour-bearing animal models necessarily develop hypoglycaemia. To account for these findings one has to take into consideration that the host responds with virtually all its potential metabolic machinery to fight for glucose homeostasis through the activation of both glycogenolysis and gluconeogenesis. Although the former pathway may only play a minor role in maintaining normoglycaemia in the host, it is possible that the depletion of both liver and muscle glycogen would act by triggering the main compensatory mechanism, gluconeogenesis. This metabolic pathway is also necessary for the recycling of the lactate generated by the tumour (12).

Fat depletion in cancer seems to be almost directly related to the increasing tumour mass, thus progressively contributing to the sustaining of both the normal cell types and the tumour. The huge lipid mobilization leads to a hyperlipidaemia as the concentration of both triglycerides (mainly associated with VLDL) and fatty acids proceeds. Important decreases in LPL activity in adipose tissue (22-24) contribute to this phenomenon.

In the host the nitrogen balance (protein synthesis versus protein degradation) is disturbed since there is a net flux of nitrogen from normal tissues to the neoplastic ones as protein degradation exceeds synthesis. This is particularly

the case of skeletal muscle, its waste contributing to host's cachexia (25). The results of our group have shown that the tumour increases the fractional protein degradation rate in skeletal muscle (26) through the activation of ATP-dependent proteolysis (27,28).

Tumour growth during pregnancy

Pregnancy is a physiological state characterized by increased food intake and important metabolic changes involving carbohydrate, lipid and protein metabolism. These adaptations are essential to sustain exponential fetal growth at the end of gestation. Especially relevant are the changes associated with increased protein degradation in skeletal muscle (29) and decreased ureogenic capacity of the pregnant rat (30), both allowing for a nitrogen-saving mechanism which is essential to sustain the high nitrogen demand of the growing fetus. Indeed, growth involves the accretion of a net quantity of protein, underlining the importance of amino acids as nutrients in fetal life. In this way the umbilical uptake of amino acids is the primary nutritional supply line for these compounds during fetal life. However, the role of amino acids in fetal metabolism is not restricted to the sole process of tissue protein accretion, since the quantity of amino acids supplied to the fetus exceeds by far the rate of accretion of tissue protein (31,32). The majority of amino acids (except glutamate and aspartate) are transferred to the fetus in large amounts where they are oxidized to carbon dioxide (33). This is supported by the high rates of urea synthesis present in the fetus of most species (34).

Very few studies have related pathological situations with alterations in amino acid transport to the fetus. A number of studies have attempted to demonstrate an effect of maternal ethanol ingestion upon fetal uptake of amino acids (35). In spite of the methodological problems involved in this kind of study, the different data suggest an alteration in the placental uptake of amino acids from then maternal circulation during ethanol ingestion. Chronic fetal hyperglycaemia does not seem to have any effects upon alanine transport to the fetus (36).

To some extent, the growth and metabolic characteristics of the fetus share important similarities with those of a fast-growing tumour. Both tissues consume appreciable amounts of glucose and amino acids for both oxidation (conversion of glucose to lactate) and protein synthesis involved in growth in the fetus and in tumour mass. The control of glucose utilization by the rat fetus is closely related to its insulin levels (36), whereas in the case of most tumours, glucose utilization appears to be unaffected by insulin and is solely dependent on the glucose availability (37). A similar situation may exist for the utilization of amino acids. Consequently, competition may occur between the fetus and the tumour and the interactions may affect their growth.

Fetal growth and placental amino acid transport

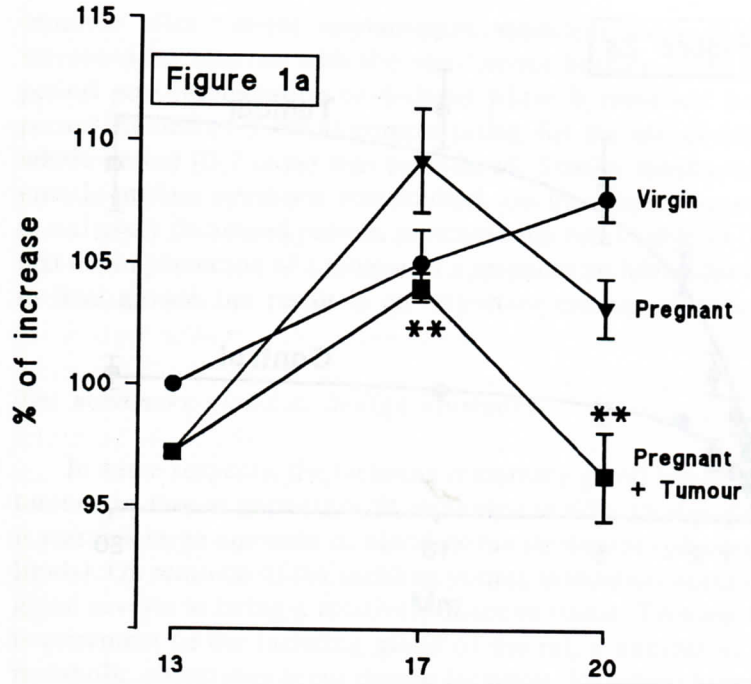
To some extent the fetus can be compared with a fast-growing tumour since their growth rates are exponential, both being highly dependent on glucose and amino acids. Bearing this in mind, we have therefore compared the effect of a rapidly growing tumour, the AH-130 Yoshida ascites hepatoma, implanted during gestation so that the tumour is growing exponentially concomitantly with the exponentially growing fetus. In order to accomplish this objective, we studied the impact of tumour growth on amino acid transport to the fetus. Previous results derived from the implantation of the Walker 256 carcinosarcoma to late pregnant rats suggested that tumour burden impairs neonatal development (38). In contrast with these observations, we have seen that the implantation of the fast-growing Yoshida AH-130 ascites hepatoma results in normal fetal growth coexisting with a normal tumour growth (Figure 1). This is related to an enhanced uptake of fetal amino acids as measured by the *in vivo* accumulation of radioactive amino acid analogues (39) and experiments using reconstituted placental membrane vesicles (40) (Figure 2).

Maternal protein turnover

During the early stages of rat gestation, tissue anabolism is manifested by increased accretion of adipose tissue (41), liver glycogen (42), and lean tissue mass (43). In the final days of a 3-week pregnancy, insulin resistance emerges, and exaggerated tissue catabolism supervenes, particularly during periods of fasting. Adipose tissue turnover is accelerated (41), liver glycogen content falls (42), and lean body mass is markedly reduced (43).

There is a physiological purpose for the onset of a catabolic phase in this pre-partum period, since mobilization of stored nutrients guarantees their flux to rapidly growing fetuses whose nutrient demands rise near term. Hyperphagia and nitrogen retention contribute to the positive nitrogen balance common in gestation (44). At late gestation, protein turnover accelerates while amino acid catabolism decreases, both processes contributing to an enhanced amino acid availability to the growing fetus (45). These events are remarkably similar to what is observed during the course of normal human pregnancy (46). The shift from an early anabolic period to a late catabolic stage is believed to be caused primarily by the changing hormonal status of advancing pregnancy.

Since no changes were observed in fetal growth following the implantation of the tumour, we decided to study the effects upon maternal protein metabolism. Tumour-bearing pregnant rats showed an accelerated muscle protein degradation which resulted in decreases in both gasctrocnemius and soleus weight and protein content. While very slight changes were observed in liver protein



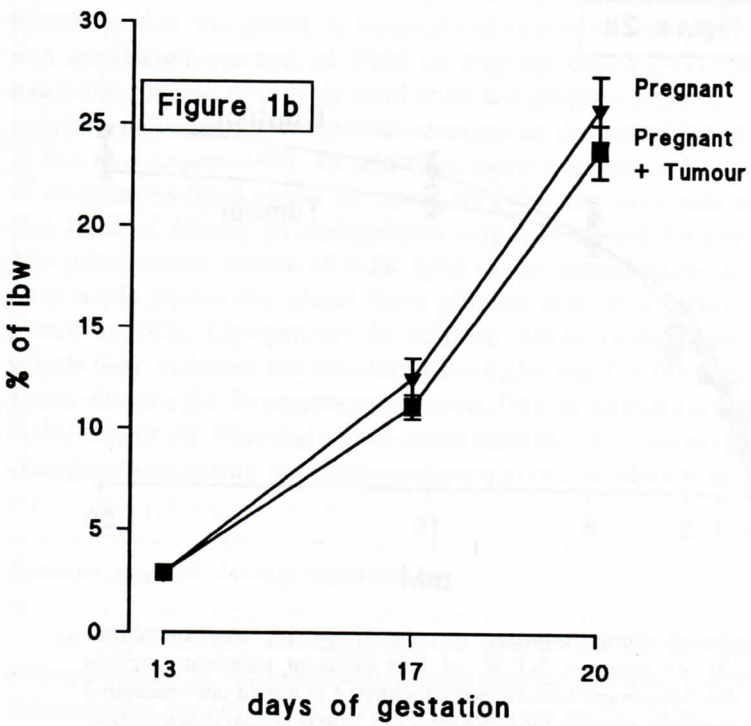
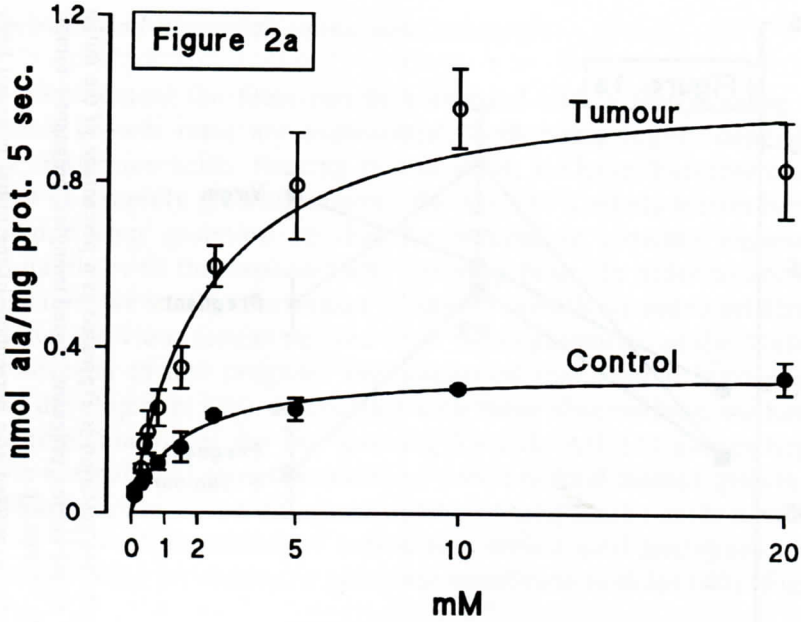


Fig. 1a.—Maternal body weight (excluding the fetoplacental unit and tumor) during late gestation is expressed as a percentage of the initial body weight and referred to virgin rats. Data are means ± S.E.M. (n=5-7). Virgin: closed circles; pregnant: closed triangles; pregnant + tumor: closed squares. Statistical significance of the results (tumor-bearers vs pregnant): ** $P<0.01$.

Fig. 1b.—Increase in the feto-placental unit weight in both groups of pregnant rats, expressed as a percentage of the initial maternal body weight.



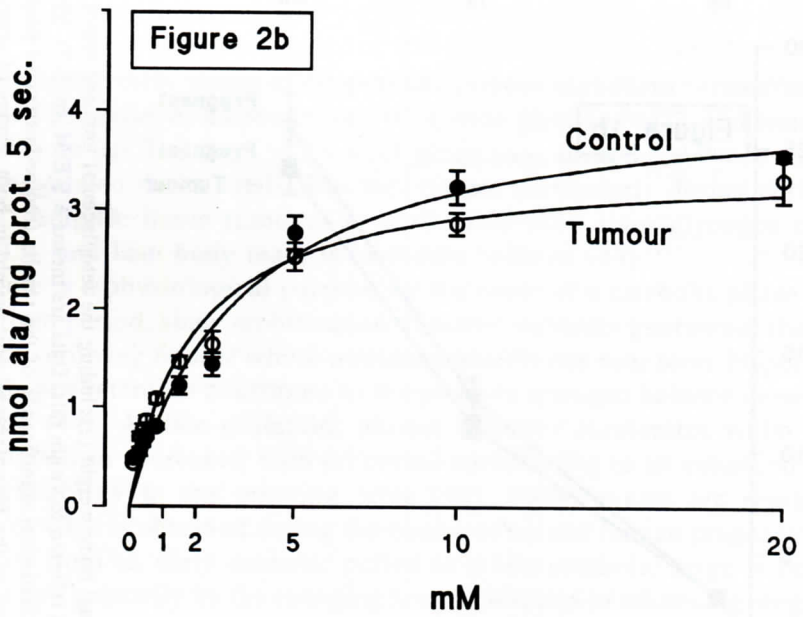


Fig. 2.—Kinetics of alanine transport in reconstituted placental membrane vesicles. Results are means ± S.E.M. of four different membrane vesicle preparations (40). Membrane vesicles were incubated in a medium containing different amino acid concentrations ranging from 0.1 to 20 mM in final concentration. Figure 2a: Na^+-independent uptake. Figure 2b: Na^+-dependent uptake.

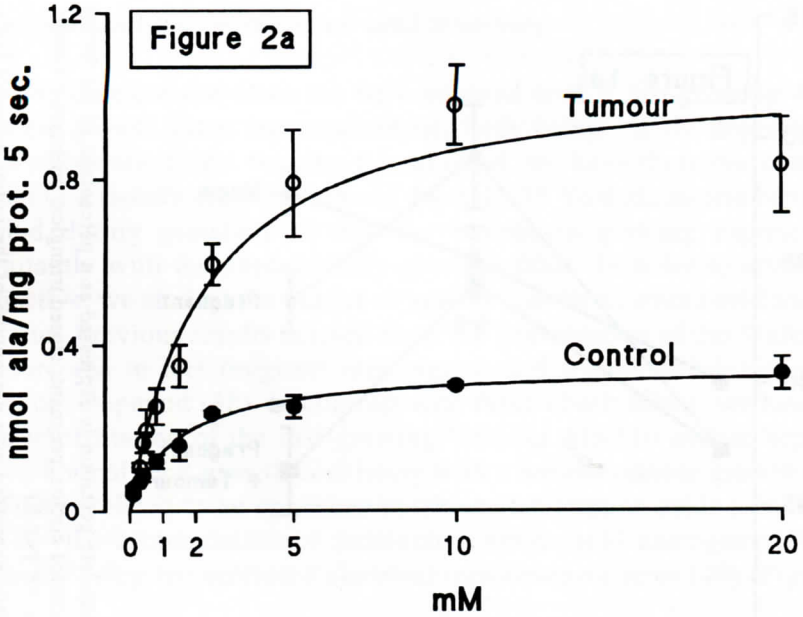
turnover after tumour implantation, muscle protein degradation was very increased (in relation with the non-tumour-bearing pregnant rats) in the first period post-implantation (0-4 days) while it remained lower in the second period studied (4-7 days) compensating for the initial differences when the whole period (0-7 days) was considered. Similar results were observed when muscle protein synthesis was studied. On the whole, tumour growth resulted in a slightly decreased protein accumulation rate (Table 3). The results suggest that the implantation of a tumour in a pregnant rat have little or no consequences in fetal growth but result in an important muscle waste in the mother.

The mammary gland as benign «tumour»

In some respects, the lactating mammary gland can be viewed as a benign tumour in that at parturition it increases rapidly in size and during lactation it extracts large amounts of blood-borne substrates (glucose, amino acids and lipids). On removal of the suckling young, involution occurs and the mammary gland reverts to being a relatively inactive tissue. To meet the large substrate requirement of the lactating gland of the rat, a number of physiological and metabolic alterations occur during lactation, including hyperphagia, increased blood flow to the gland, increased extraction of triacylglycerol by the gland and decreased storage of lipid in adipose tissue (47). The re-direction of available plasma triacylglycerol from the adipose tissue to the lactating gland is partly achieved by reciprocal changes in the activities of lipoprotein lipase in the two tissues (48). In addition, there appears to be decreased oxidation of exogenous lipid in the lactating rat (49); the net result of these changes is that lipid of dietary or endogenous origin is spared for secretion in the milk. The other major source of milk lipid is the considerable *de novo* synthesis of fatty acids within the gland from glucose and, to a lesser extent, lactate and pyruvate (50). Lipogenesis in adipose tissue is decreased during lactation which may increase the availability of glucose for the mammary tissue (51). Thus, despite the hyperphagia, the net flux in adipose tissue of lactating rats is in favour of triacylglycerol mobilization. A comparison of some of the changes in lactating and tumour-bearing rats is shown in Table 2.

Tumour growth during lactation

In view of the similarity in the alterations of the lipid metabolism of adipose tissue in lactation and during tumour growth, it seemed of interest to examine the possible interactions of the two in the same animal. Both tissues consume appreciable amounts of glucose and amino acids for the synthesis of



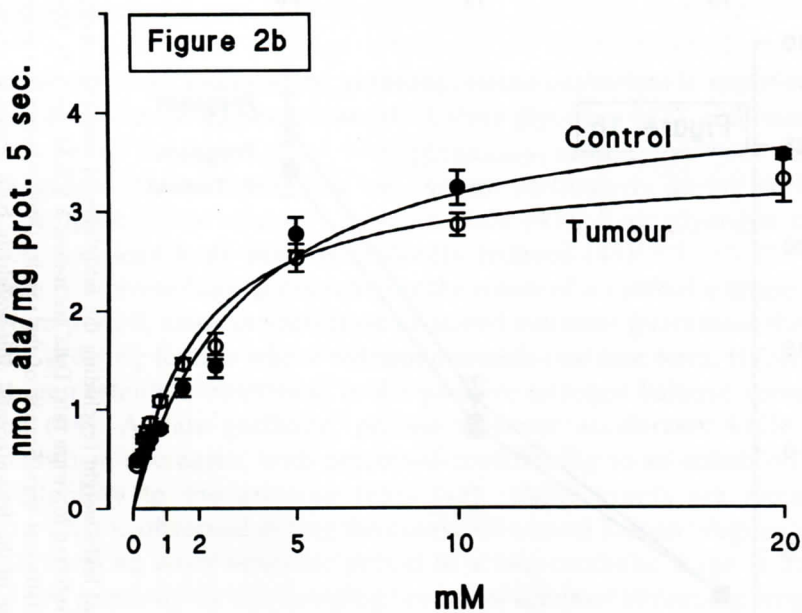


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Table 2.—Comparison between tumour-bearing and lactating rats

	Lactating	*Tumour-bearing*
Proliferative mass (tumour or mammary gland as a % of carcass weight)	6-10%	2.5-10%
Food intake	increased	unchanged or decreased
Plasma insulin	decreased	decreased
Circulating triacylglycerols	increased	increased
Adipose tissue		
mass	decreased	decreased
LPL activity	decreased	decreased
lipogenesis	decreased	decreased
lipolysis	increased	increased

Table 3.—Fractional tissue protein turnover rates in pregnant rats

Liver	K_d		K_{sN}		K_a	
	0-4	*4-7*	*0-4*	*4-7*	*0-4*	*4-7*
Virgin	25.7	10.5	27.6	9.9	2.2	–0.4
Pregnant	22.7	9.5	25.9	8.6	1.8	–0.7
Pregnant + tumor	21.4	12.6	23.8	9.9	1.3	–3.6

Gastrocnemius	K_d			K_s			K_a		
	0-4	*4-7*	*0-7*	*0-4*	*4-7*	*0-7*	*0-4*	*4-7*	*0-7*
Virgin	1.67	2.47	2.01	2.34	3.08	2.65	0.56	0.58	0.57
Pregnant	1.81	8.73	4.77	2.81	6.34	4.32	1.08	–3.67	–0.96
Pregnant + tumor	8.01	1.32	5.15	6.59	0.84	4.13	–2.43	–0.51	–1.61

Rates of protein synthesis (K_s), degradation (K_d) and accumulation (K_a) are expressed as percent per day, and assessed on the 0-4, 4-7 (liver and gastrocnemius) and 0-7 day intervals (gastrocnemius).

the macronutrients of milk (lactose, lipid and protein) in mammary gland and for the energy (conversion of glucose to lactate) and protein needed for growth in the tumour. The control of glucose utilization by rat mammary tissue appears to be in part regulated by insulin (51) and is solely dependent on the glucose availability (37). A similar situation may exist for the utilization of plasma amino acids. Consequently, competition may occur between the gland and the tumour and the interactions may affect either the function of the former or the growth of the later.

The implantation of a fast-growing tumour (Walker 256 carcinosarcoma) to lactating rats did not result in changes in the efficiency of the lactating process since the rate of pup growth was similar than in the non-tumour-

bearing lactating controls (52). The presence of the tumour did not result in any changes in circulating triacylglycerols or LPL activity in either mammary gland or adipose tissue, whereas it promoted important changes in virgin, non-lactating animals (52). In addition, the presence of the tumour did not alter the tissular fate of an oral triolein load in the lactating rats whereas it was altered in the virgin rats. Interestingly, the presence of the tumour did not influence mammary gland or hepatic lipogenesis in either the virgin or the lactating rats (52). The overall conclusion of this study is that the presence of the tumour has little impact on lipid metabolism in the lactating rat whereas it promotes important changes in the non-lactating animals.

The role of cytokines

It was in the last century that Coley (53) introduced the idea that tumour regression in human cancer patients could be accomplished by challenging them with bacterial toxins. Much later, in 1985 Old identified a protein, in the serum of endotoxin-treated rabbits, that was responsible for the haemorrhagic necrosis of tumours (54). It was then named as tumour necrosis factor (TNF) and later on as TNF-a after the discovery of lymphotoxin or TNF-b. It was more or less coincidentally that Kawakami and Cerami (55) identified a molecule responsible for the wasting syndrome seen in many chronic diseases (such as cancer or chronic infection). This molecule was named cachectin, since it was responsible for the induction of cachexia, and later on proved to be identical to TNF-a (56). TNF is sinthesized, mainly by macrophages in response to invasive stimuli, as a 26 kDa membrane-bound precursor that is cleaved proteolytically to a mature 17 kDa form with the prosequence polypeptide remaining associated to the membrane (57). The peptide is bioactive as a 51 kDa trimer, which can be recognized by two distinct receptors, TNFR1 or p55 (55 kDa) and TNFR2 or p75 (75 kDa). TNF is a pleiotropic factor that exherts a variety of effects such as growth promotion, growth inhibition, angiogenesis, cytotoxicity, inflammation, and immunomodulation (see (58) for review).

We investigated the effects of both TNF-a and IL-1, another cytokine that has been involved in controlling the metabolic adaptations to tumour growth (59), on lipid metabolism in virgin and lactating rats (60). The administration of these cytokines resulted in a decrease in intestinal lipid absorption, a decrease in the *in vivo* oxidation of an exogenous lipid load and an increase in the circulating triacylglycerols. These changes affected both virgin and lactating rats suggesting that, although they may mediate some responses associated with tumour growth, other factors/mediators must also be involved to explain the effects of tumour growth upon the mammary gland during lactation.

Concluding remarks and future research: a role for TNF in pregnancy?

In addition to its role as a cytotoxic compound for tumour cells, TNF has to be considered a pleiotropic factor that could have a very important role in gestation. Indeed, the results of many investigations have revealed that the cytokine is widely found through maternal, placental and fetal tissues. TNF seems to have a clear role in menstruation and early pregnancy maintenance as well as in parturition.

One of the few drawbacks encountered with this interpretation is that none of the receptor protein knockout models studied has been associated with an abnormal pregnancy performance. Further research will have to concentrate on this point and try to clarify why the models studied show no changes in pregnancy performance. The use of double knockouts (for instance for TNFR1 and IL-6) may provide a good tool to clarify this point. Another interesting aspect that will no doubt attract a considerable amount of future research will be that of the complex interaction of TNF with other cytokines to maintain a local cytokine balance responsible, at least in part, of the homeostatic mechanism that enables the interaction of the mother with the feto-placental unit. In close relation, a better understanding of the interactions of TNF with maternal hormones will also be necessary.

TNF has been detected in human milk. This is a very interesting point that needs further investigation and clarification. Is it really a mechanism to protect the newborn? What other functions does TNF have for the newborn? Is TNF an essential molecule to regulate lipid metabolism in the mammary gland? Those are intriguing questions that may give clues as to the role of TNF during the perinatal phase.

Finally, future research will have to concentrate on regulatory aspects of TNF synthesis and release. The pattern of both TNF messenger and protein during gestation has to be strictly regulated to insure correct pregnancy outcome. The same applies for the two different TNF receptors, how is their synthesis regulated in different tissues? Altogether the future research on this field will provide very relevant information for both the understanding of the physiological mechanisms associated with pregnancy and for the design of therapeutic strategies for complicated pregnancies.

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