The surface coat of the 8th day pseudopregnant rats reacted like at metestrus whereas at day 17th, the cell coat resembled that seen in proestrus and estrus.

Methylation decreased alcianophilia, which was restored incompletely by saponification. This indicated the presence of both sulfate and carboxyl groups in cell surface components with a preponderance of sulfate groups as indicated by the metachromasia with azure A, pH 1.0, and persisting alcianophilia at pH 1.0 which withstood the effect of 1.0 *M* $MgCl_2$. The alcianophilia was reduced by methylation at 60 °C and partially restored by saponification. Similar results were noted with the colloidal iron and the AB-safranin procedure and the azure A pH 1, which showed metachromasia. With the PA-*p*-diamine, the fimbriated end was negative whereas in the ampulla and the isthmus, the glycocalyx stained a deep brown. With this procedure in the uterine horns, the coat was grayish brown (Table II).

Electron microscopy of the uterus at metestrus revealed a surface coat made up of filamentous and globular structures measuring up to 550 nm which were distinctly stained by ruthenium red and which appeared surrounding the microvilli (Figures 13 and 14).

Our data seemed to indicate that the lumenal cell coat is a differentiation of the lumenal plasma membrane of the epithelial cells of the accessory organs of the rat female genital tract with peculiar histochemical ultrastructural characteristics. Some of the qualitative and semi-quantitative changes observed by histochemical techniques suggested that the hormones of the ovaries may control the changes noted[2, 3, 17, 18].

The present results are in keeping with previous data on the glycocalyces of the male accessory organs of the rat, suggesting that they were under endocrine control. Bilateral orchidectomy was followed by a significant decrease of the content of sialic acid of the glycocalyx of rat epididymis[19]. The content of sialic acid of homogenates of whole epididymis of castrated rats was significantly lower than in the intact control rats[20].

It has been suggested that estrogens might control the synthesis of mucins in the fallopian tubes of the rabbit, whereas progesterone would be required for the releasing of the mucous secretion[21]. The secretion of the oviduct is increased by estrogen administration[22]. This seemed supported by the observation of cyclic variation of PAS positive substances in mouse uterus[4] and of the changes in the size of the Golgi body induced by estrogens[17, 18]. This corresponded with data showing that the Golgi body was larger in estrus[23].

The functional role of the complex carbohydrates of cell surfaces is unknown. It has been proposed that complex carbohydrates of the lumenal surface coat of certain epithelial were released into the corresponding biological fluids[19]. It can be proposed that glycocalyx components of the oviduct might be required for the nutrition of the egg. In the uterus, substances of the lumenal cell surface might have a role during implantation.

It can be concluded that surface coat materials characterized in this study are chemically heterogeneous. The alcian blue positive substances were of early appearance and varied little in the present experimental conditions, whereas PAS material appeared at or near puberty and showed changes in these various conditions[24].

Summary. The lumenal surface coat of the rat oviducts and uterine horns have been histochemically characterized at prepuberty, estrous cycle, castration, hormone replacement and pseudopregnancy. Under the EM, the coat was made up of filamentous and globular structures. Histochemical variations suggested that coat components are under endocrine control.

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Properties of Glutamine Aminohydrolases in Subcellular Fractions of Liver of Tumour Bearing Mice

The intracellular localization of glutamine aminohydrolase in tumours and host tissues has been found to be different[1] from that reported earlier in normal tissues[2–4]. It has also been indicated[1] that there may be a shift in the site of synthesis of glutaminase from mitochondria to the supernatant fraction of the host liver and kidney, due to the presence of tumour in the body of the animal. This led us to investigate the time and exact location of the shift of the enzyme in the liver after the transplantation of tumour into the animals. An attempt has also been made to see if the enzyme obtained from the two sources are in any way different from each other.

Materials and methods. Ehrlich ascites cells (EAC) were maintained in our laboratory by serial i.p. transplantation in Swiss mice. Liver from both normal and EAC-bearing mice were taken out, washed and then homogenized in 0.25 *M* sucrose (1:10 w/v) in cold using Potter Elvehzem homogenizer. Cell fractionation was done according to the method of de Duve et al[5]. The incubation mixtures for the total homogenate and mitochondrial fractions were 0.1 *M* NaH_2PO_4 (pH 7.4), 0.25 *M* *Tris* buffer (pH 7.4), 0.04 *M* glutamine and 0.1 ml of tissue fraction and that for microsomal and supernatant fractions were 0.2 *M* NaH_2PO_4 (pH 8.6), 0.25 *M* *Tris* (pH 8.6), 0.1 *M* glutamine and 0.1 ml of the cell fractions in a total volume of 3.0 ml. All incubations were carried out at 37 °C for 20 min and ammonia produced was estimated

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Intracellular distribution of glutamine aminohydrolase in liver of normal and EAC-bearing mice

Tissues	Homogenate	Nucleus	Mitochondria	Lysosome	Microsome	Supernatant
Normal liver	59.4	0.0	48.6 (82%)	4.8 (18%)	0.0	0.0
Liver of EAC-bearing mice	330	0.0	63.0 (19%)	10.8 (3%)	132.0 (40%)	114 (34%)

Results expressed as µmoles ammonia produced/mg protein/h and are the average of 4 experiments (% activity of the initial homogenate given).

according to BRAGANCA et al.[6]. All values were corrected for the necessary substrate and enzyme blanks. Protein estimations were carried out according to the method of LOWRY et al[7].

Results. The supernatant fraction of the host liver after centrifugation at 12,000 g^1 was further fractionated and glutamine aminohydrolase was found to be present in all the subcellular fractions, viz. mitochondria, lysosome, microsome and soluble supernatant fraction of the host liver. The host liver microsomal fraction contained the highest percentage of activity (40%) followed by the soluble supernatant fraction (34%) of the total activity of homogenate. Whereas most of the activity in normal liver was found to be mostly in the heavy mitochondrial fraction, only 18% of the activity was in lysosomal (light mitochondria) fraction (Table).

Glutaminase activity was estimated in each fraction of the liver tissue beginning from the day of transplantation and continued till the maximum development of the tumour in the body, i.e. 10 days. Figure 1 shows that 4th day after transplantation, the enzyme appeared both in microsomal and supernatant fractions. Gradually, the activity increased in microsomal and supernatant fractions until 10th day, but the activity in mitochondria remained more or less constant. The activity in the supernatant attained maximum on 8th day, after that it remained constant.

Having obtained the above data, an attempt was made to characterize the enzymes present in each fraction. The results showed that, like normal liver mitochondria, the PO_4 concentration for all the fractions of host liver was 0.2 *M* (Figure 2). The enzyme in normal and host liver mitochondria showed a plateau at 0.04 *M* glutamine; whereas the microsomal and soluble fractions showed no plateau, even at 0.1 *M* glutamine concentration. The optimal pH for the enzyme in liver mitochondria of EAC-bearing mice was found to be 7.6, which was similar to the normal liver mitochondria. But for the enzyme of microsomal and soluble fractions, the pH optima was 8.6 (Figure 4)

Discussion. The results presented here indicate that glutaminase in the microsomal and soluble supernatant fractions of liver, which appears after 4th day of transplantation indicates a definite effect of the tumour on the host tissues as regards glutaminase. It seems from the results that the mitochondrial enzymes in both normal and host liver have similar properties and show most of the characters of 'liver type' of HOROWITZ et al.[8]. On the

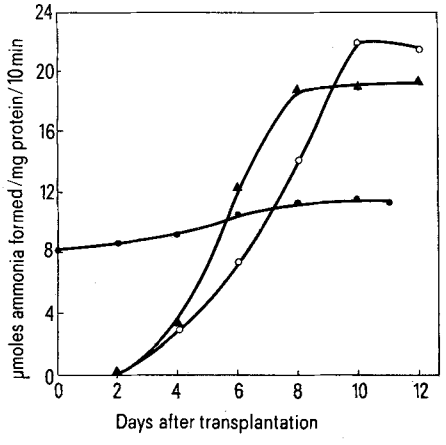

Fig. 1. Effect of transplantation on glutaminase activity of liver cell fractions. ●—● mitochondria; ○—○ microsome; ▲—▲ supernatant.

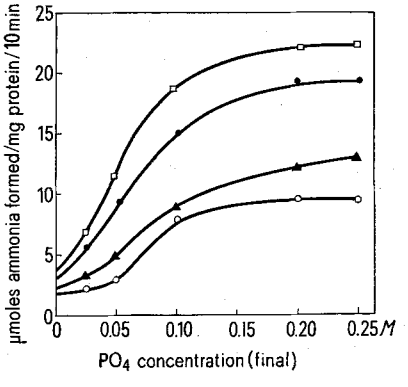

Fig. 2. Phosphate requirements for glutaminase activities of subcellular fractions of normal and host liver. ○—○ Normal liver mitochondria; △—△ EAC liver mitochondria; □—□ EAC liver microsome; ●—● EAC liver supernatant.

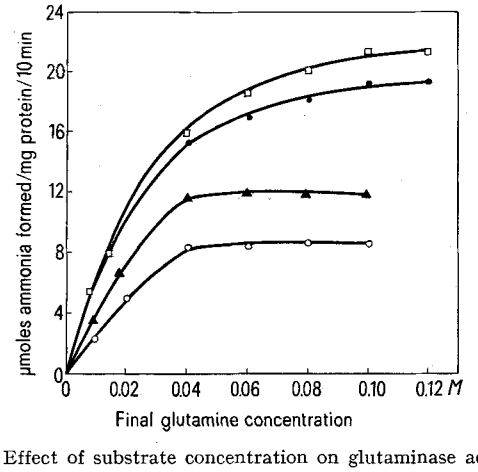

Fig. 3. Effect of substrate concentration on glutaminase activities of subcellular fractions of liver. ○—○ Normal liver mitochondria; △—△ EAC liver mitochondria; □—□ EAC liver microsome; ●—● EAC liver supernatant.

other hand, the microsomal and soluble supernatant glutaminases possess the properties of 'kidney type' glutaminase[8]. The enzyme activity has also been found to be proportional to the rate of growth of tumour[9,10].

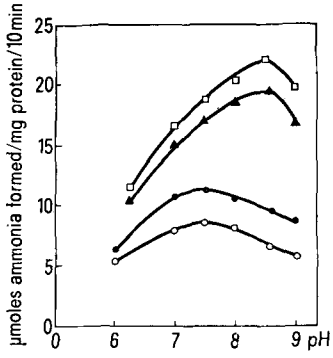

Fig. 4. PH optima curves of glutaminases of different fractions of mouse liver. ○—○ Normal liver mitochondria; ●—● EAC liver mitochondria; □—□ EAC liver microsome; △—△ EAC liver supernatant.

Summary. Glutamine aminohydrolase is found to be present in microsomal and soluble supernatant in liver of EAC-bearing mice. Enzymes obtaimed from these two sources were characterized and found to behave differently from the mitochondrial glutaminase of both normal and tumour-bearing mice.

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Analyse expérimentale de l'expression de la mutation léthale récessive («ulcère») chez l'embryon de *Pleurodeles waltlii* (Amphibien Urodèle)

Experimental Studies of the Expression of a Lethal Recessive Mutation ('ulcère') in *Pleurodeles waltlii* embryo (Amphibia Urodela)

La mutation «ulcère» (*u*), mutation léthale récessive du Pleurodèle identifiée par SIGNORET et al.[1] se manifeste au moment de l'éclosion et avant la première prise de nourriture. Elle est caractérisée par une croissance générale diminuée et un arrêt du développement du bourgeon du membre antérieur, par des branchies faiblement ramifiées et un ulcère ventral correspondant à une éversion de l'intestin et de la paroi abdominale. Au niveau histologique elle est caractérisée par la persistance dans tous les types cellulaires de plaquettes vitellines et celles-ci sont particulièrement abondantes au niveau du tube digestif dont l'histodifférenciation est retardée. Le cytoplasme apparaît vacuolisé dans le foie et le pronéphros et les fibrilles musculaires sont dissociées.

Dans le but de rechercher si la mutation «ulcère» s'exprime de façon autonome ou non nous avons effectué des expériences du type de celles mises en oeuvre chez l'Axolotl par HUMPHREY[2,3] au cours de l'étude de plusieurs mutations et chez le Pleurodèle par GOUNON et COLLENOT[4] au cours de l'analyse de l'expression de la mutation «léthal-mitotique».

Méthode expérimentale. Nous avons réalisé plusieurs types d'opérations sur des embryons au stade du bourgeon caudal (stades 22 à 24 de la table chronologique[5]): 1. des associations en parabiose ou en télobiose, 2. des associations en chimères hémiparties selon la technique de HOUILLON[6], 3. des greffes hétérotopiques d'ébauches d'organes, 4. des greffes hétérotopiques de parties antérieures et postérieures d'embryons, la section transversale étant effectuée au niveau du champ du membre antérieur.

Ces opérations ont été réalisées dans la solution physiologique de STEINBERG[7]. Les séries d'associations en parabiose, télobiose et chimères hémiparties étaient mises en oeuvre avec des embryons appartenant à une même ponte issue de parents hétérozygotes de telle sorte que sur l'ensemble des opérations réalisées 6/16 (37,5%) d'entre elles représentent des combinaisons entre un embryon normal et un embryon léthal. Les greffes hétérotopiques d'ébauches d'organes et de parties antérieures et postérieures d'embryons ont été effectuées sur le flanc, les embryons donneurs provenaient de pontes issues de parents hétérozygotes et les embryons receveurs appartenaient à des pontes standard; ainsi

Opérations	Nombre d'opérations réalisées	Nombre de combinaisons léthal-normal obtenues et pourcentage correspondant
Associations en parabiose	92	30 (32,6%)
Associations en télobiose	86	31 (37,2%)
Chimères hémiparties	95	29 (30,5%)
Greffes tête et abdomen	71	22 (31%)
Greffes d'ébauches		
Œil	19	5 (26,3%)
Membre antérieur	27	6 (22,2%)
Queue	32	9 (28,1%)

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