tor superficialis muscle of the pectoral fin were fixed in Bouin fluid. Paraffin sections were stained by hematoxylin-eosine. Discoloration by H_2O_2 has been used as a specific histochemical test for melanine.

Results. The amplitudes of the ESR signals, expressed in arbitrary units, are given in the Table. The intensity observed in the white muscle is too low to be significant. But the cardiac and the red skeletal muscles show signals which are easily distinguished from the background fluctuations. In the case of the adductor and abductor muscles of the pectoral fin, few determinations (not reported in the Table) have also been made on the internal parts which do not appear to contain myoglobin; the spectra did not differ from that of the white muscle. The amplitude of the ESR signals parallels, therefore, the intensity of the colour of the muscle and, according to a cross-section examination of the abductor muscle of the pectoral fin, the decrease in the fibre size. The figure obtained in the case of the red muscle of the lateral line is, however, much higher than that corresponding to the other red muscles.

Histological sections of the red muscle of the lateral line and of the abductor superficialis muscle of the pectoral fin have been compared. As shown in the Figure, the former contains pigmented cells. They are usually situated along blood capillaries but also occur along conjunctive tracts. These cells contain melanine granules which are discolored by H_2O_2. On the other hand, we did not observe any melanocytes in the abductor superficialis muscle. The high amplitude signal obtained for the red muscle of the lateral line is thus due to the fact that it contains melanine granules. It is interesting to note that in amphibians the presence of this pigment has been observed in the heart and various skeletal muscles[9]. In fish muscle, its localization thus seems to be much more restricted.

In conclusion, determinations of ESR signals together with histological observations have led us to note a peculiarity of the fish red muscle of the lateral line. This muscle differs from the other fish red muscles in the presence of some melanocytes located along its blood capillaries and conjunctive tracts. The other red skeletal muscles and the cardiac muscle give signals of about the same intensity which are definitely higher than the very low signal obtained for the white muscle. The parallelism usually observed between mitochondrial content and ESR signal intensity in various tissues appears valid also in the case of the two types of skeletal muscle[10].

Résumé. Les mesures de résonance paramagnétique électronique effectuées jusqu'à présent sur le muscle n'ont pas tenu compte de la différentiation de ce tissu en muscles blancs et rouges. Nous avons comparé les signaux du muscle blanc, du muscle cardiaque et de divers muscles rouges de carpe. Le muscle blanc présente une très faible intensité; le myocarde et différents muscles rouges donnent des valeurs plus élevées en accord avec leur plus grande teneur en mitochondries. Le muscle rouge de la ligne latérale se distingue toutefois des autres muscles rouges par un signal environ 2 × plus intense dû, comme le montrent des examens histologiques, à la présence dans ce cas de mélanocytes situés le long de faisceaux conjonctifs et de capillaires sanguins.

G. HAMOIR[11], R. MARECHAL[12] and R. BASSLEER[13]

Laboratory of Biology, Faculty of Sciences, University of Liège, B–4000 Liège (Belgium); Department of Atomic and Molecular Physics, Sart-Tilman, University of Liège; and Institute of Histology, Faculty of Medicine, University of Liège (Belgium), 28 June 1972.

[9] N. MILLOTT and W. G. LYNN, Nature, Lond. *209*, 99 (1966).

[10] The authors wish to thank Dr. Q. BONE who drew their attention to the occurrence of melanine in amphibian muscles and Prof. A. VAN DE VORST for advice on ESR spectroscopy.

[11] Laboratory of Biology, Faculty of Sciences, University of Liège, Belgium.

[12] Department of Atomic and Molecular Physics, Sart-Tilman, University of Liège, Belgium.

[13] Institute of Histology, Faculty of Medicine, University of Liège, Belgium.

Enzymatic Hydroxylation of Anthranilamide in Rat Liver

A new metabolic pathway from anthranilic acid, one of the tryptophan metabolites, to conjugates of 5-OH-anthranilamide and 3-OH-anthranilamide was suggested to be present by the authors.

Anthranilamide has been isolated and identified as a new metabolite of anthranilic acid in our laboratory[1,2] and when anthranilamide was injected to the rat, two main metabolites supposed to be conjugates of 5-OH-anthranilamide and 3-OH-anthranilamide[3], could be also observed in organs such as liver and kidney as well as in urine. Considering the above discoveries, it is of interest to study the hydroxylation and the conjugate formation of the anthranilamide.

It has been reported by KASHIWAMATA et al.[4] that an enzyme which is capable of hydroxylating anthranilic acid to 5-OH-anthranilic acid is present in the microsome of rabbit liver, but the hydroxylation activity in that of rat liver was found to be negligible[5].

An *Achromobacter* sp. has been shown be able to oxidize anthranilic acid via 5-OH-anthranilic acid by LADD[6], and CAIN[7] also reported that in *Nocardia opaca*, 5-OH-anthranilic acid was an metabolite of anthranilic acid.

The hydroxyl-derivatives of anthranilamide has been observed in the urine of rabbit by BRAY et al[8] and in both liver and urine of rat by SUTAMIHARDJA et al.[3], but the enzymatic hydroxylation in vitro could not be demonstrated until now.

[1] T. M. SUTAMIHARDJA, A. ISHIKURA, J. NAITO and I. ISHIGURO, Chem. pharm. Bull., Tokyo, submitted (1971).

[2] T. M. SUTAMIHARDJA, J. NAITO and I. ISHIGURO, Chem. pharm. Bull., Tokyo, submitted (1972).

[3] T. M. SUTAMIHARDJA, I. ISHIGURO and T. SHIOTANI, Abstract of the Meeting of Japanese Biochemical Society, in Sendai, Japan, October 1971; Seikagaku *43*, 762 (1971).

[4] S. KASHIWAMATA, N. NAKASHIMA and Y. KOTAKE, Biochim. biophys. Acta *113*, 244 (1966).

[5] S. KASHIWAMATA, Seikagaku *36*, 317 (1964).

[6] J. N. LADD, Nature, Lond. *194*, 1100 (1962).

[7] R. B. CAIN, Antonie van Leeuwenhoek *34*, 417 (1968).

[8] H. G. BRAY, H. J. LAKE, F. C. NEALE, W. V. THORPE and P. B. WOOD, Biochem. J. *42*, 434 (1948).

Table I. Intracellular localization of anthranilamide-5-hydroxylase activity

Fraction	5-OH-anthranilamide formed (calculated as 5-OH-anthranilic acid; nmole)	Protein (mg/ml)
Homogenate	14.29	22.34
Nuclear and mitochondrial fraction	1.49	13.73
Supernatant I (at 10,000 × *g*)	21.43	13.73
Microsomal fraction	0.01	4.14
Supernatant II (at 109,000 × *g*)	0.00	9.22

Table II. Activity of the recombined preparation of the microsomal and supernatant II fraction which hydroxylate anthranilamide to 5-OH-anthranilamide

Fraction	5-OH-anthranilamide formed (calculated as 5-OH-anthranilic acid; nmole)
Supernatant I	38.69
Microsomal fraction	0.01
Supernatant II	0.00
Microsomal fraction + Supernatant II	30.04

In this communication, subcellular localization of an enzyme which catalyzes the hydroxylation of anthranilamide to 5-OH-anthranilamide in the rat liver is discussed. From the results described as follows, the enzyme is localized in the microsomal fraction, and it requires NADPH and some other factors in the soluble fraction for the activity.

Material and methods. The enzyme preparation was obtained from the liver of albino rats of Wistar strain, weighing 200–250 g. An ether anesthesized rat underwent laparotomy, and after portal perfusion with physiological saline solution, the liver was immediately removed and stored at –20°C. The frozen liver was homogenized for 2 min with 9 volumes of 0.14 *M* KCl by using a homogenizer. Subcellular fractionation of the liver homogenate was prepared according to the method of SONE and HAGIHARA[9].

The enzyme reaction mixture contained 100 μmoles of potassium phosphate buffer (pH 7.5), 0.3 ml of enzyme solution, 45 μmoles of anthranilamide, 8 μmoles of nicotinamide, 2.5 μmoles of $MgCl_2$, 0.5 μmole NADPH, 4 μmoles of glucose-6-phosphate, 0.4 unit of glucose-6-phosphate dehydrogenase, and after addition of water, the final volume was 2.0 ml. The reaction mixture was incubated for 1 h at 37°C in air, and the product, 5-OH-anthranilamide, was measured.

The 5-OH-anthranilamide was determined by the indophenol reaction, which was modified for the assay of 5-OH-anthranilic acid by KASHIWAMATA[4].

Results and discussion. The identification of the reaction products were carried out by paper chromatography

[9] N. SONE and B. HAGIHARA, J. Biochem., Tokyo *56*, 151 (1961).

Table III. Effect of heat and dialyzed treatment on the enzyme activity

Fraction	5-OH-anthranilamide formed (calculated as 5-OH-anthranilic acid; nmole)
Supernatant I	10.71
Microsomal fraction	0.01
Supernatant II (sup. II)	0.00
Heated microsomal fraction (10 min) + sup. II	0.01
Microsomal fraction + dialyzed sup. II	13.26

Table IV. Specificity of the enzyme to various substrates

Substrate	Enzyme activity (calculated as 5-OH-anthranilic acid; nmole product formed)
Anthranilamide	35.46
Kynurenine	0.00
o-Aminoacetophenone	1.35
Anthranilic acid	0.00

using the Toyo-roshi No. 51 filter paper and a solvent of n-butanol: acetic acid: water (4:1:1). The fluorescent spots were identified by comparison with the authentic compound of 5-OH-anthranilamide isolated from anthranilamide-injected rat urine in our laboratory. In the enzyme as described in the method, beside the unmetabolized anthranilamide (B), a spot of Rf 0.50 (A) could be detected, which corresponded to 5-OH-anthranilamide (Figure). In the absence of NADPH, 5-OH-anthranilamide could not be observed.

As shown in Table I, it is obvious that the enzyme activity is mainly present in the supernatant I fraction which consists of the microsomal and soluble fractions of the rat liver. Neither the mitorchondrial nor microsomal fractions could hydroxylate anthranilamide to 5-OH-anthranilamide.

KASHIWAMATA et al.[4] have reported that the microsomal fraction of rabbit liver could hydroxylate anthranilic acid to 5-OH-anthranilicacid; however, in our experiments, the microsomal fraction of the rat liver alone could not hydroxylate anthranilamide to 5-OH-anthranilamide (Table II). If the supernatant II and the microsomal fraction were recombined, the hydroxylating activity was recovered by 90% of the supernatant I containing microsomal and soluble fraction.

As shown in Table III, the recombined preparation of the heated microsomal fraction and the supernatant II could not hydroxylate anthranilamide, but if only the supernatant II was heated, the recombines preparation with the microsomal fraction could still hydroxylate anthranilamide. It suggests that some other factors in the soluble fraction are necessary for the hydroxylation of anthranilamide to 5-OH-anthranilamide by microsomal fraction. The recombined microsomal fraction with the dialyzed supernatant II, as shown in Table III, there could be found no decreasing in the enzyme activity.

Since L-kynurenine, anthranilic acid, and *o*-aminoacetophenone were not hydroxylated (Table IV), the hydroxylation was specific for anthranilamide. In the mitochondrial fraction, as reported by OKAMOTO et al.[10], it could hydroxylate kynurenine to O–3H-kynurenine, but this mitochondrial system could not hydroxylate anthranilamide to 3-OH-anthranilamide or 5-OH-anthranilamide.

The details of the kinetic studies on the hydroxylation of anthranilamide to 5-OH-anthranilamide by the anthranilamide-5-hydroxylase found in the microsomal fraction will be reported further elsewhere.

Zusammenfassung. Das als Anthranilamid-5-Hydroxylase bezeichnete Enzym, das von Anthranilamid zu 5-OH-Anthranilamid hydroxyliert wird, wurde in den Mikrosomen von Rattenleberzellen lokalisiert.

T. M. SUTAMIHARDJA, A. ISHIKURA, Y. NAGAMURA and I. ISHIGURO

Department of Biochemistry, Gifu College of Pharmacy, Mitahora, Gifu (Japan), 15 May 1972.

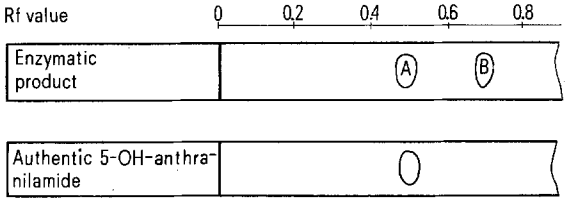

Fig. 1. Paper chromatography of enzymatic product and authentic 5-OH-anthranilamide.

[10] H. OKAMOTO, T. YAMAMOTO, M. NOZAKI and O. HAYAISHI, Biochem. Biophys. Res. Commun., *26*, 309 (1967).

Abnormal RNA Metabolism in Male Genital Tract of Tailless Mouse (T/*t*)

In previous experiments, secretion and excretion of epididymis and ductus deferens were investigated in C_{57}BL mouse using H^3-uridine or H^3-orotic acid. By mean of histochemical and chemical techniques, it was proved that one of the components of the secretion consists of RNP and more specifically RNA. The turn-over of these molecules was investigated[1].

Recent experiments showed striking differences of uridine turn-over between mouse strains, especially in tailless T/*t*. These strains well known to be a balanced lethal system[2] were developed as an inbred stock in our laboratory[3]. An abnormal r-RNA metabolism was previously reported in mouse spleen of lines heterozygous for t_{12}[4] and thought to be related to an anomaly of the nucleolar organizer, from which it was inferred that the chromosome which contains the *t* loci is also nucleolus organizing. Cytological observations failed to confirm this inference (unpublished personal data).

However, owing to deep variations of fertility from line to line in tailless mice, we decided to investigate some particularities of RNA metabolism in the genital system.

Material and methods. Two strains of 6-month-old tailless mice T/t_6 (from Harwell) and T/t_{12} (from Columbia) are utilized in the present experiments. Comparative experiments were realized with 6–7-month-old C_{57}BR mice as controls. Mice were injected i.p. with 0.5 ml 5-^{3}H-uridine (25 µCi per animal; specific activity: 6 Ci/m*M*) or ^{3}H-orotic-acid (25 µCi per animal; specific activity: 17 Ci/m*M*).

The animals were sacrificed at different times after injection. For measuring the radioactivity, epididymides were isolated after sectioning at the end of the ductus efferens and at the beginning of the ductus deferens. Two experiments were successively performed. In the first experiment, designed to measure the uptake of the whole organ, one epididymis was grinded in 0.5 ml hyamine-

[1] J. MOUTSCHEN, Experientia, *28*, 893 (1972).
[2] P. CHESLEY and L. C. DUNN, Symp. quant. Biol. *21*, 187 (1956).
[3] J. MOUTSCHEN, Archs Zool. exp. gén. *112*, 361 (1971).
[4] J. KLEIN and K. RAŠKA JR, Proc. of the 12th Int. Congr. of Genetics, Tokyo (1968), vol. 1, p. 149.