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.

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

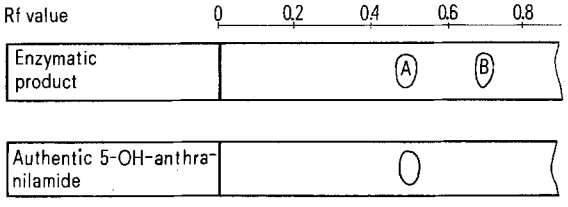

Fig. 1. Paper chromatography of enzymatic product and authentic 5-OH-anthranilamide.

[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.

hydroxide then transferred in toluol for scintillation counts. After perfusion of the other organ, saline extraction (0.14 *M* NaCl, centrifugation 5000 rpm/30 min at 0 °C), the activity of the supernatant was measured by liquid scintillation (Packard Tricarb counter). The scintillation medium contained 60 g naphtalene, 35 g PPO and 25 mg POPOP dissolved in 500 ml dioxane (for scintillation).

In the second experiment designed to measure excretion, only the activity of the supernatant was measured after extraction by procedure mentioned above.

Results. The uptake of ^{3}H-uridine by the whole organ is compared in both normal-tail (C_{57}BR) and tailless (T/t_6). No significant difference of uptake could be detected (Figure 1). The activity of the supernatant remained all the time lower in the tailless strain (Figure 2).

From this experiment, it can be concluded that even if the uptake of ^{3}H-uridine by the organ is not modified in the tailless strain, the secretion of the compound from epithelium to the lumen is much slower in the tailless strain.

In the second experiment designed to investigate the excretion processes from epididymis during a long period of time, the release of activity was compared in the supernatant of 2 strains (T/t_6; T/t_{12}) with the normal-tail animal (Figure 3). In T/t_6 strain, the level of activity remains fairly constant during a 4-day-period. After reaching a plateau, the activity decreases. The release of activity in the control is almost exponential, which confirms previous results[1]. On the other hand, the release of activity in the T/t_{12} strain was found to be intermediary between the control and the T/t_6 strain. With T/t_{12} the initial level of activity was significantly lower than the control but higher than T/t_6. Owing to the small number of T/t_{12} animals available, this experiment should be confirmed.

Discussion and conclusions. It was found that the uptake of uridine by the T/t_6 epididymis is not different from the control which should be compared with previous results[1]. On the other hand, some data show clearly that the secretion is slower in the tailless strain. This is also probably the case in the strain T/t_{12}. It was also shown that the excretion processes are slower in the tailless strain (T/t_6) since for a period of 4 days there is an equilibrium between the secretion and the excretion processes.

Some questions remain unsolved: 1. What kind of RNA molecules are secreted or excreted? In spleen, abnormal ribosomal-RNA was demonstrated in some tailless strains[4]. The present results suggest that part of the RNA detected is of this kind since pink-bodies, proved to contain RNA, are resistant to histological manipulations[1]. 2. If the holocrine nature of the secretion is taken for granted, is it not logical to postulate that a part of the secretion also contains RNAs of lower molecular weight.

However, the present experiments do not produce any evidence on the kind of metabolism which is decreased in the tailless strain. New investigations attempting to re-

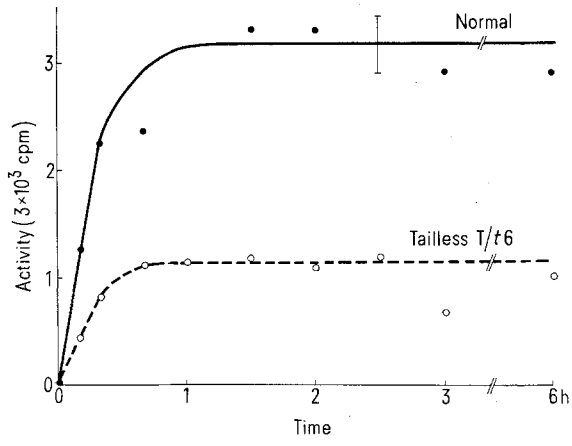

Fig. 2. Activity (cpm) of the supernatant of epididymis (mean of 10 organs for each point) at different times (h) after injection of ^{3}H-uridine.

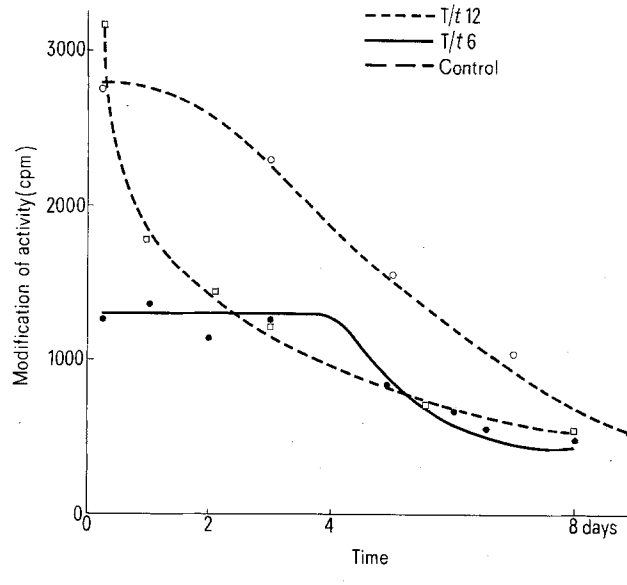

Fig. 3. Modification of the activity (cpm) of the supernatant of epididymis (mean of 10 organs for each point at different times: days) after injection of ^{3}H-orotic acid.

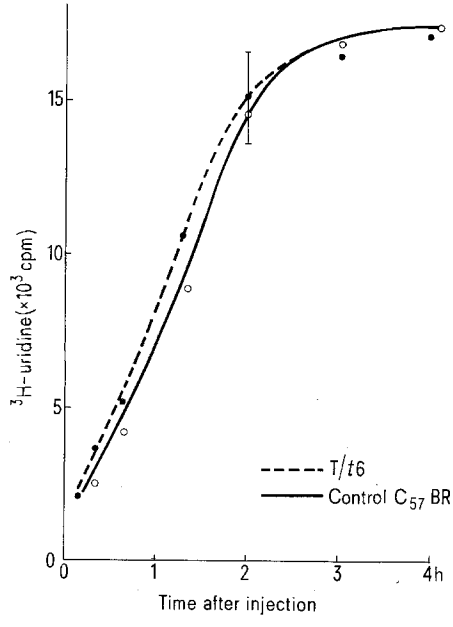

Fig. 1. Uptake of ^{3}H-uridine (cpm) by whole epididymis (mean of 10 organs for each point) of tailless (T/t_6) and control C_{57}BR.

[5] Recherche réalisée sous les auspices du Fonds de la Recherche Fondamentale Collective, Belgique.

late the abnormal RNA metabolism with the modified sterility of tailless strains are in progress[5].

Résumé. Le métabolisme du RNA de l'épididyme a été étudié dans une souche de souris anoure, T/t_6. La courbe d'absorption de la ^{3}H-uridine n'est pas différente de celle du témoin C_{57}BL. On a cependant démontré que les processus de sécrétion et d'excrétion sont beaucoup plus lents dans la souche anoure T/t_6. Ces résultats ont été partiellement confirmés avec une autre souche anoure: T/t_{12}.

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In vitro Contraction of Embryonic Skin Produced by Prostaglandins

Contraction of small skin wounds, until recently, had been thought to involve recoiling or shortening of collagen and elastic fibers[1]. However, the present knowledge suggests that collagen filaments are not contractile and do not shorten except under unusual conditions. PEACOCK and VAN WINKLE[1] state that the role of collagen fibers in wound contraction is a passive one.

There is a growing number of reports which indicate that fibroblasts contract[2–4] and the implication is that contraction individually and collectively among a mutually adherring population of fibroblasts helps to pull peripheral normal tissue about the wound margin towards the center of the wound as cell migration and proliferation ensue.

The proposed phenomenon of fibroblastic contractions is related to an intracellular fibrillar system, similar to that in smooth muscle[2,4] or similar to a primitive type peripheral microfilamentous network as observed in axons[5], and is sensitive to a variety of inflammatory mediators[3].

Certain observations of the results of culturing embryonic skin with prostaglandins[6] prompted a thorough investigation of a possible contraction phenomenon. Back skin of six and one-half-to seven-day-old chick embryos was removed, dissected into bilateral pairs and cultured on stainless steel rafts. The full description of the procedure has been published elsewhere[7]. One piece of the bilateral pair was treated, in-vitro, with 50 µg/ml of crystallized prostaglandin B_1, B_2, E_1, or F1α. The other piece of each pair served as control. After the skin was flattened on the grid the number of mesh windows which were completely covered by the explant were counted, and this procedure was repeated at 3 or 5 days, whenever harvesting of the tissues took place.

Wilder's reticulum stain demonstrated anchor filaments, and some tissues were injected with ^{3}H-thymidine and the wound margins especially examined for presence of label. Other tissues were studied by electron microscopy[8] for evidence of active contractile phenomena, both extra- and intracellular.

The response of the skin in culture is virtually the same for treatment with PGB_1, PGB_2 and PGE_1. PGF1α produces no visible differences from the control explants. The effect of explant size reduction has been observed ever since we began using crystallized PGB_1 ($XPGB_1$). This result has been unequivocated, having been observed in more than 1,000 treated cultures.

Control explants increase whole skin growth by 50% at 3 days incubation, and downfeather organs develop during that time (Figure 1). The treated explants fail to sustain

[1] E. E. PEACOCK and W. VAN WINKLE, *Surgery and Biology of Wound Repair* (W. B. Saunders Co., Philadelphia 1970).
[2] G. GABBIANI, G. B. RYAN and G. MAJNO, Experientia *27*, 549 (1971).
[3] G. MAJNO, F. GABBIANI, B. J. HIRSCHEL, G. B. RYAN and P. R. STATKOV, Science *173*, 548 (1971).
[4] G. GABBIANI and G. MAJNO, Am. J. Path. *66*, 131 (1972).
[5] N. K. WESSELS, B. S. SPOONER, J. F. ASH, M. O. BRADLEY, M. A. LUDUENA, E. L. TAYLOR, J. T. WRENN and K. M. YAMADA, Science *171*, 135 (1971).
[6] C. W. KISCHER, *Immunopathology of Inflammation* (Eds. B. K. FORSCHER and J. C. HOUCK; Excerpta Medica, Amsterdam 1971), p. 197.
[7] C. W. KISCHER, Devl Biol. *16*, 203 (1967).
[8] Moody Memorial Electron Microscopy Laboratory, Department of Pathology.

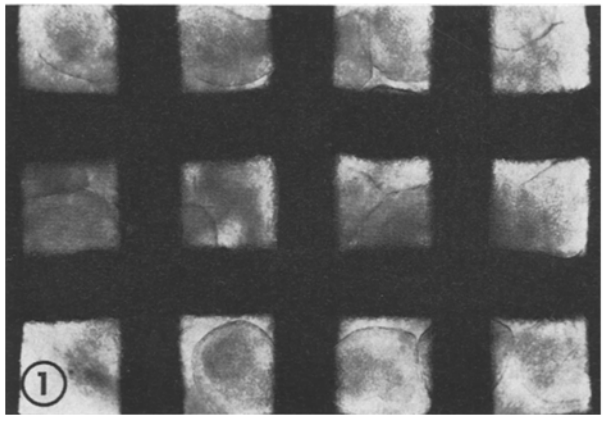

Fig. 1. An explant of chick embryo skin grown 3 days still fixed to culture grid. Down feathers visible on surface. ×60.

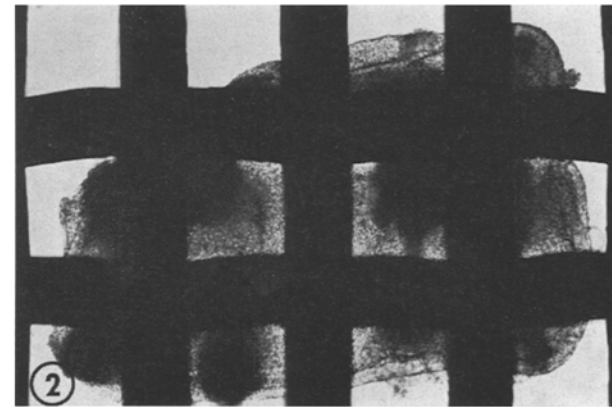

Fig. 2. An explant of chick embryo skin grown 3 days with 50 µg/ml of $XPGB_1$. Compare area of explant with Figure 1. ×60.