mettre en relief d'importantes variations phénotypiques au niveau des protéines simples, des lipoprotéines et des glycoprotéines sériques de ces 3 espèces de truites.

L'association de ces méthodologies pourraient s'avérer utile dans des travaux de génétique utilisant ces différences au niveau des protéines simples et complexes comme marqueurs.

Nous nous proposons d'étudier la nature biochimique des différences entre les protéines sériques d'une espèce à l'autre[13].

Summary. It is shown that there are important differences among the serum proteins of 3 different species of trout. We stress that these genetic variations concern the lipoproteins as well as the glycoproteins. Furthermore, 3 serum proteins of *Esox lucius* have antigenic determinants common with the serum proteins of *S. gairdneri*; they are an α_2-lipoprotein, an α_2-glycoprotein and a γ-globulin.

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Centre de Biomédecine, Université Laval, Québec (Canada), 7 avril 1967.

[13] Travail présenté au Congrès de la North-East Wild Life Conference, Québec 1967 et subventionné par le Conseil national des recherches (Ottawa).

The Acetylation of Tryptophan Metabolites by Rapid and Slow Acetylators of Sulfamethazine

N-acetyl-kynurenine (NA-K) and N-acetyl-3-hydroxy-kynurenine (NA-OHK) were identified in rat urine following tryptophan ingestion by Dalgliesh et al.[1]. NA-K was demonstrated in the urine of other species, including man, following tryptophan ingestion, by Brown and Price[2]. A simple paper chromatographic method for demonstrating kynurenine (K), 3-hydroxy-kynurenine (OHK), NA-K and NA-OHK in human urine following tryptophan ingestion was devised by Westall[3].

There is a Mendelian polymorphism in man for the acetylation of sulfamethazine, isoniazid and hydrallazine (Evans and White[4]). Other compounds such as sulfanilamide (Peters et al.[5]) and PAS (Motulsky and Steinmann[6]), though extensively acetylated in man, are monomorphically acetylated, their acetylation apparently not being influenced by the polymorphism.

It was, therefore, of interest to determine whether the acetylation of tryptophan metabolites in man was subject to the action of the acetylation polymorphism.

Materials. Experimental subjects: Volunteer subjects were healthy medical students and laboratory staff.

Sulfamethazine was kindly donated by I.C.I. Pharmaceutical Division, Wilmslow, Cheshire, U.K.[7]. Ammonium sulfamate was British Drug Houses Reagent Grade. L-Tryptophan[8] and 3-hydroxy-DL-kynurenine were purchased from Koch-Light Laboratories, Colnbrook, Bucks, U.K. DL-Kynurenine from Fluka AG Chemische Fabrik, Buchs, SG, Switzerland. Chromatography paper was Whatman No. 1 10 × 10 inch, 'A' pattern. Other chemicals were analytical grade reagents purchased from British Drug Houses.

Methods. Sulfamethazine test on experimental subjects: Fasting subjects were observed to swallow 160 mg pure sulfamethazine powder per kg metabolically active mass (MAM = weight$^{0.7}$) with a little water. 2 h following drug ingestion subjects were allowed tea and toast. All urine passed during 8 h following drug ingestion was collected. The urine volume was measured and a portion stored at − 20 °C to await analysis

Estimation of sulfamethazine concentration in urine: Free and total sulfamethazine were estimated by the Bratton-Marshall procedure[9]. Unknowns were processed in duplicate and a range of standards at 2, 4, 6, 8 and 10 μg sulfamethazine per ml, together with suitable blanks, were processed in duplicate simultaneously.

Determination of acetylator phenotype: The subjects who received a tryptophan load test some weeks following the sulfamethazine test formed part of a much larger series of healthy subjects who had undertaken the latter procedure. It will be seen from Figure 1 and from the Table that these 18 subjects are a representative sample of the population.

L-Tryptophan test on experimental subjects: 18 subjects who had been phenotyped by the sulfamethazine test, were utilized for this portion of the study. Subjects were fasted from 22.00 h on the previous evening, and on the test morning were observed to swallow 5.0 g L-tryptophan with a little water. The same dosage was given to each subject regardless of weight. A light meal of tea and toast was allowed 2 h following L-tryptophan ingestion. Urine was collected for 6 h following the ingestion of the amino-acid. The urine was mixed, the volume recorded and a portion placed at − 20 °C to await further processing.

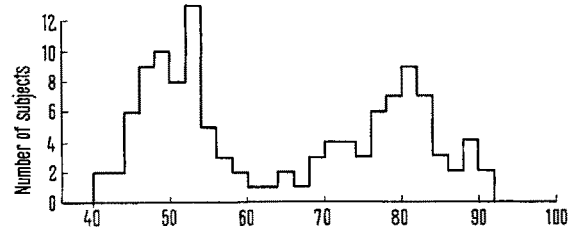

Fig. 1. The percentage of urinary sulfmethazine acetylated in 119 healthy subjects.

[1] C. E. Dalgliesh, W. E. Knox and A. Neuberger, Nature *168*, 20 (1951).
[2] P. R. Brown and J. M. Price, J. biol. Chem. *219*, 985 (1965).
[3] R. G. Westall, Personal communication (1965).
[4] D. A. P. Evans and T. A. White, J. Lab. clin. Med. *63*, 394 (1964).
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[6] A. G. Motulsky and L. Steinmann, J. clin. Invest. *41*, 1387 (1962).
[7] T. A. White and D. A. P. Evans, Clin. Pharmac. Ther., in press (1967).
[8] W. I. Austad, J. R. Clamp and R. G. Westall, Nature, *207*, 757 (1965).
[9] H. Varley, in *Practical Clinical Biochemistry* (Heinemann, London, First Edition, 1954).

Paper chromatography of urine: 50 µl of each urine specimen were spotted on a corner of a chromatography paper. Three papers were prepared in this way from each urine specimen. Ascending chromatography was carried out for 5 h in a mixture of *n*-butanol-glacial acetic acid-water (12:3:5). After thorough drying overnight, ascending chromatography was carried out for 1 h in 20% aqueous potassium chloride solution, the direction of solvent flow being at an angle of 90° to the direction of flow of the first solvent. After drying, the papers were inspected in UV-light and gave a pattern as shown in Figure 2.

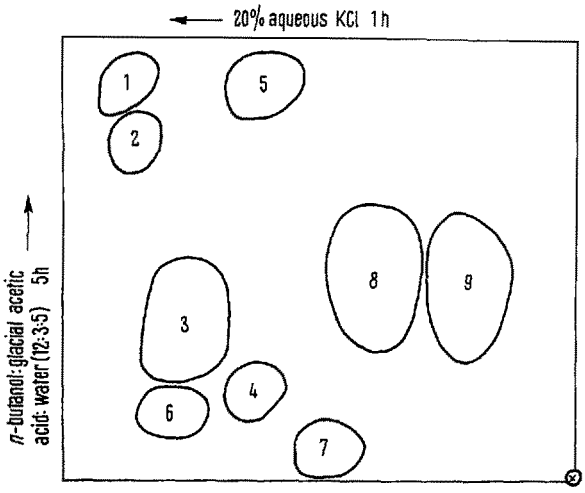

Fig. 2. The paper chromatogram of human urine following a loading dose of L-tryptophan. The spots which all fluoresce under UV-light have been identified as follows: (1) N-acetyl kynurenine, (2) N-acetyl 3-hydroxy kynurenine, (3) Kynurenine, (4) 3-Hydroxy kynurenine, (5) Salicyluric acid, (6) 3-Hydroxy kynurenine sulphate, (7) Xanthurenic acid glucuronide, (8) Kynurenic acid, (9) Xanthurenic acid. The untreated urine sample of 50 µl was applied at the point *X* (method of WESTALL[3]).

The percentage of sulfamethazine acetylated and the percentage of L-Kynurenine acetylated in the urines of experimental subjects

Experimental subject	Sex	% of sulfamethazine acetylated (x)	% of L-kynurenine and L-3-hydroxy-kynurenine acetylated (y)
CO (first)	M	68.1	83.8
(second)			58.0
JFP	M	82.9	57.4
RIP	M	57.7	61.5
AWS	M	53.0	73.2
JL	F	55.3	49.6
CN	M	60.5	65.6
CC	M	78.0	42.1
A Be	F	53.7	44.5
JC	M	82.9	56.3
MK	M	91.4	60.2
A Bu	F	54.0	80.7
PA	M	52.2	45.6
RH	M	81.4	42.8
DB	M	83.5	38.3
ALJ	M	64.4	61.6
MG	M	80.9	57.6
DJ	M	92.0	63.5
CI	M	82.9	55.7

The computed regression of y upon x is $y = 70.354 - 0.188x$. Standard deviation of scatter of points about the regression line, Sr = 11.67 units of y. Standard error of regression coefficient = 0.196. The mean value of y for subject Co was used to compute the regression.

Elution and assay of chromatographed metabolites: Preliminary experiments had shown that the recovery of DL-kynurenine from paper following chromatography as described above was quantitative up to 20 µg in one spot. The spots for K and OHK tended to overlap and were, therefore, cut out as one spot and eluted with 4.0 ml of water. Similarly the spots of NA-K and NA-OHK overlapped; and these were cut out as one spot and eluted with 4.0 ml water. Three chromatography papers were thus identically processed. The optical densities of these eluates were measured at 230 nm in a Beckman DU spectrophotometer. It is assumed that all 4 compounds concerned had the same extinction coefficient. The percentage of K and OHK acetylated was computed as

$$\frac{\text{OD (NA-K + NA-OHK)}}{\text{OD (K + OH-K) + OD (NA-K + NA-OHK)}} \cdot$$

Close agreement was found between triplicates and the mean value taken for further computations. Statistical methods were standard (BAILEY[10]).

Results. The results are given in the Table. The computed regression of the percentage of K and OHK acetylated upon the percentage of sulfamethazine acetylated, yields a slope that is not significantly different to the horizontal.

Discussion. It will be noted that there is a wide variation in the percentage of K and OHK acetylated between different subjects from 38–83%. It will also be seen that the one subject (C.O.) who received a loading dose of L-tryptophan on 2 different occasions gave 2 widely divergent results for the percentage of K and OHK acetylated. This was quite different to the result obtained when a sulfamethazine test was repeated, in which case there was a high degree of replicability (WHITE and EVANS[7]).

The results of this experiment suggest that the tryptophan metabolites K and OHK are not polymorphically acetylated but are monomorphically acetylated in the same manner as sulfanilamide (PETERS[5]).

As Figure 2 indicates most subjects showed a well-marked salicyluric acid spot and this is in keeping with the finding of AUSTAD et al.[8] that this substance is a metabolite of tryptophan in man.

Zusammenfassung. Es wurden an gesunden Versuchspersonen Belastungsversuche mit Sulfamethazin und Tryptophan ausgeführt und die Ausscheidung der azetylierten Metaboliten im Harn verfolgt. Bei den Tryptophanmetaboliten Kynurenin und Hydroxykynurenin war – im Gegensatz zur Azetylierung von Sulfamethazin – kein Polymorphismus zu beobachten.

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[10] N. T. J. BAILEY, in *Statistical Methods in Biology* (English Universities Press, London, 1959).