excreated radioactivity was derived from the degradation of A–B*, the urine collected from the first day period was dialyzed against 0.2*M* ammonium carbonate. Data are given in Table II. The specific radioactivity of the solution in the membrane was equal to the dialysate. This reveals that a decomposition was occurred in the A–B*. To ascertain how much of the excreated radioactive material was unchanged biotin or processed biotin activity, excess avidin was added to an aliquot of the urine which was then dialyzed. This experiment indicated that 92% of the total radioactivity contained in the urine was in the form of avidin-combinable.

In another way of approach, radioavidin-biotin complex (A*–B) was prepared by the labelling of avidin part of avidin-biotin complex with ^{131}ICl followed the technique of BALE et al.[11]. The free radioiodide was removed by DEAE Sephadex and the pure A*–B solution (42 µc) was injected i.p. or i.v. into male rats. The rats were then scanned with Magnascanner III (Picker X-Ray Corp., Cleveland, Ohio) at 40 min, 2 h, 8 h, and 24 h after the injections. Figure 1 shows that A*–B was decomposed and the radioiodine was started to be deposited in thyroid gland at 40 min after the i.p. injection. At the end of 24 h almost all of the radioactivity was uptaken by the gland. The experiment of i.v. injection gave similar result, as shown in Figure 2, except that the radioactivity uptaken by the thyroid was faster than the experiment of i.p. injection.

Further studies on the in vitro dissociation of avidin-biotin complex in minced or sliced tissues are now under investigation. The preliminary results showed that marked degradation of the complex occurred in the presence of liver tissue and less with kidney, when they were incubated with medium 199 (NIH) at 37 °C under an atmosphere of 95% O_2 – 5% CO_2[12].

Zusammenfassung. Es wurde mittels des mit ^{14}C markierten Biotins und mit ^{131}J markierten Avidins gezeigt, dass der Avidin-Biotin-Komplex, i.p. oder i.v. in Ratten injiziert, dissoziert war.

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The in vivo and in vitro Formation of 2-Amino-3-Hydroxyacetophenone from 2-Aminoacetophenone

It was reported by DALGLIESH[1] that 2-amino-3-hydroxyacetophenone-*O*-sulfate was excreted in small amounts in some normal human urines, and in appreciably larger amounts in certain pathological urines. ALLEN et al.[2] demonstrated the possibility of 2-amino-3-hydroxyacetophenone (AHA) being the cause of cancer of the bladder in man. Recently, we have isolated and identified AHA from the urine of rats, and 2-aminoacetophenone (AA) and AHA from the urine of hens, following ingestion of tryptophan[3]. However, the biosynthetic pathway of these compounds remains obscure. It is assumed that AHA is produced either by the degradation of 3-hydroxykynurenine or by the direct hydroxylation of AA, which may be formed from kynurenine.

This communication describes the isolation and identification of AHA from the urine of rats following ingestion of AA, and the formation of AHA from AA by microsomes isolated from rat liver.

Isolation and identification of AHA. Male Wister albino rats, weighing 100–150 g, received daily for 4 days 15 mg of AA per kg of body wt. by i.p. injection. Three 24-h-urine collections (200 ml) were pooled and filtered. The filtrate was adjusted to pH 3 with acetic acid and shaken with ether-ethanol (3:1). The organic phase was evaporated to dryness under nitrogen. The residue was dissolved in 20 ml of 1*N* HCl and hydrolyzed at 80 °C for 1 h under nitrogen. The solution was adjusted to pH 3 with NaOH and treated with ethyl ether. The ether extract was washed with 0.1% Na_2CO_3 solution, dried over sodium sulfate, anhydrous and concentrated under a stream of nitrogen gas. The sticky dark brown residue was dissolved in a small volume of dried ether and applied to a column (1 × 9 cm) of silicic acid, which was then eluted by 30 ml of dried peroxide-free ether. The eluate was evaporated to dryness under nitrogen. The residue was dissolved in a small volume of ethanol and partially purified by paper chromatography. Whatman No. 3 MM papers were used with the solvent system of MASON and BERG[4] containing 1 ml of glacial acetic acid per 100 ml of the solvent. *n*-Butanol-acetic acid-water (4:1:1) was also used. The fluorescent area corresponding to AHA on the chromatograms was cut out and treated with methanol-ether (1:2) to extract the fluorescent material. The extract from pa[illegible]r strips was

Chromatographic properties of a reaction [illegible]oduct with authentic 2-amino-3-hydroxyacetophenone

	Product	Synthesized
Paper chromatography[a]		
Mason-Berg with 1% acetic acid, Rf	0.91	0.91
n-Butanol-acetic acid-water (4:1:1 by volume), Rf	0.88	0.88
Thin layer chromatography		
Ethylacetate-isopropanol-28% ammonia water (9:6:4 by volume), Rf	0.88	0.88
Chloroform-ethylacetate-formic acid (60:40:1 by volume), Rf	0.72	0.72
Fluorescence at 3650 Å	Greenish blue	Greenish blue
DSA[b]	Pink orange	Pink orange
Ekman's reagent	Purple	Purple
Ehrlich's reagent	Pink orange	Pink orange
Absorption maxima at pH 7.0 (nm)	233, 270, 378	233, 270, 378

[a] Whatman No. 3 MM filter paper. [b] Diazotized sulfanilic acid.

concentrated in vacuo. The residue was further purified by thin-layer chromatography. Thin-layers of silica gel G (Merck, Darmstadt, Germany) were prepared on glass plates. The solvent systems of ethyl acetate-isopropanol-28% ammonia water (9:6:4) and chloroform-ethyl acetate-formic acid (60:40:1) were used. The details of thin-layer chromatography have already been described[5,6]. The isolated material was pure on both thin-layer and paper chromatogram. The Rf values in paper and thin-layer chromatography, colour reactions of Ekman's reagent[7], DSA[7] and Ehrlich's reagent[8], and UV-absorption spectra were all identical between the isolated compound and authentic AHA (Table). When the isolated compound was quantitatively converted to 3-methoxy-2-trifluoroacetylamino acetophenone with diazomethane and trifluoroacetic anhydride, and analyzed by gaschromatography according to the method described before[9], the retention time of the derivative of the isolated compound was identical with that of the derivative of the authentic sample.

The formation of AHA from AA by rat liver microsomes. For the isolation and identification of AHA, incubation was carried out as follows: microsomes, prepared according to the method of SCHNEIDER and HAGEBOON[10], equivalent to 10 g wet weight of rat liver were incubated aerobically at 37 °C for 2 h in 0.25 mmole of *Tris*-HCl buffer at pH 8.0 containing 6 μmoles of NADPH, 20 μmoles of nicotinamide and 20 μmoles of AA. The final volume was 20 ml. The reaction was terminated by the addition of 4 ml of 20% perchloric acid. The precipitate was separated by centrifugation. The supernatant was treated by the same procedure as that for the isolation of AHA described above. A spot corresponding to AHA appeared on thin-layer and paper chromatograms. The compound was identified as AHA using a similar technique to that described in the Table. The amount of AHA formed was found to be 0.65 μmole when it was determined according to the previous report[11]. In the above system, the amount of AHA increased linearly for 2 h incubation. In the absence of NADPH, AHA was not formed. The omission of nicotinamide caused 2% inhibition. When employing either microsomes boiled for 1 min or other subcellular fractions (nuclei, mitochondria and the 100,000 × *g* supernatant fraction which were prepared by SCHNEIDER and HAGEBOON[10]) as enzyme sources, the formation of AHA was not observed.

Zusammenfassung. Nach Verabreichung von 2-Aminoacetophenon an Ratten wurde 2-Amino-3-hydroxyacetophenon aus dem Urin isoliert und identifiziert. In-vitro-Untersuchungen haben gezeigt, dass Lebermikrosomen 2-Aminoacetophenon zu 2-Amino-3-hydroxyacetophenon hydroxylieren.

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Oxygen Consumption and $^{14}CO_2$ Production in Tissue of Rats with Chronic and Acute Selenite Poisoning

Although cases of selenium poisoning have been reported[1-3], little is known of its influence upon the metabolic processes of the animal. Histopathological examinations have shown alteration in liver and kidney of animals treated with toxic quantities of selenite[4] thus suggesting a study in the two organs of the biochemical changes occurring during toxicity.

The aim of this work was to study the respiratory differences in liver and kidney minces between 2 groups of rats with chronic and acute selenium toxicity respectively.

Materials and methods. 30 albino rats, 6–8 weeks old, and with the average weight of 140 g were used in the experiment. Half of them were fed a laboratory standard diet supplemented with sodium selenite (20 mg Na_2SeO_3/kg food). They were kept in individual cages, and food and water were given ad libitum. The food consumption of each animal was measured weekly to estimate the Na_2SeO_3 intake. From this group 4 rats each time were killed 30, 58 and 96 days after the beginning of the experiment.

The rest of the animals were fed a normal laboratory standard diet during the whole experiment. From this second group 4 rats each time were given Na_2SeO_3 orally at a rate of 18.0, 36.5 and 68.0 μg/g body wt. respectively 20 h before being killed. These quantities represent approximately $^1/_4$, $^1/_2$ and the equivalent amount of Na_2SeO_3 intake over 30 days of rats fed a selenite-enriched diet.

The control group also contained 4 rats; they were tested together with the animals of group 2.

The oxygen uptake of kidney and liver minces was measured by Warburg manometry at 37 °C for 2 h of incubation. Approximately the same amount of tissue from each animal was utilized. The incubation medium was Krebs Ringer phosphate solution[5]. For each tissue, half of the Warburg flasks contained also D-glucose

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