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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(0.68% of the final volume of the incubation medium) with 1 μc of uniformly labelled D-glucose ^{14}C added from the sidearm just prior to oxygen uptake measurements.

The final volume of the incubation medium was 2.8 ml. The samples were then treated as reported by BLENDERMANN and FRIEDMAN[6]. The precipitated $Ba^{14}CO_3$ was filtred, weighed and then suspended in a phosphor formed by 7 volumes of toluene containing PPO (0.4%) and POPOP (0.01%) and 6 volumes of Triton X 100.

The samples were counted in a Packard Tri-Carb liquid scintillation spectrometer model 3320.

Results. Table I shows the oxygen consumption of liver and kidney minces from rats fed on the Na_2SeO_3 enriched diet for up to 96 days. There was a significant increase ($P < 0.01$) for the livers of the animals receiving the highest amount of Na_2SeO_3 (group 3) as compared with the controls both with and without the addition of glucose to the incubatium medium. No significant difference was detected between the kidneys of the 4 groups incubated without added glucose. However, when kidneys were incubated with glucose, their oxygen consumption was significantly greater in group 2 ($P < 0.05$) than in the controls.

Table II shows the oxygen consumption of liver and kidney minces from rats given Na_2SeO_3 in one dose. When the livers of the Na_2SeO_3-treated animals were incubated without added glucose only those in group 1 showed a significantly higher ($P < 0.01$) oxygen consumption than the controls but with added glucose both group 1 ($P < 0.05$) and group 3 ($P < 0.01$) showed a significantly greater oxygen consumption than the controls. For kidney minces there were no significant dif-

Table I. Chronic selenium toxicity in rats

	Controls Normal diet	Group 1 70 μg Na_2SeO_3/g body wt. in 30 days	Group 2 110 μg Na_2SeO_3/g body wt. in 58 days	Group 3 180 μg Na_2SeO_3/g body wt. in 96 days
Liver				
Without glucose	0.48 ± 0.03	0.53 ± 0.03	0.52 ± 0.03	0.71 ± 0.09
With glucose	0.52 ± 0.10	0.56 ± 0.02	0.54 ± 0.03	0.89 ± 0.17
Kidney				
Without glucose	1.11 ± 0.14	1.20 ± 0.15	1.18 ± 0.09	1.02 ± 0.16
With glucose	0.99 ± 0.09	1.12 ± 0.11	1.23 ± 0.04	1.21 ± 0.18

Oxygen consumption (μl/h/mg wet tissue) of tissue minces (mean ± S.D.). Each value is the mean of results from 4 animals.

Table II. Acute selenium toxicity in rats

	Controls Normal diet	Group 1 18.0 μg Na_2SeO_3/g body wt.	Group 2 36.5 μg Na_2SeO_3/g body wt.	Group 3 68.0 μg Na_2SeO_3/g body wt.
Liver				
Without glucose	0.48 ± 0.03	0.57 ± 0.03	0.53 ± 0.09	0.52 ± 0.05
With glucose	0.52 ± 0.10	0.68 ± 0.01	0.59 ± 0.07	0.97 ± 0.01
Kidney				
Without glucose	1.11 ± 0.14	1.09 ± 0.14	1.15 ± 0.11	1.01 ± 0.08
With glucose	0.99 ± 0.09	1.15 ± 0.10	1.14 ± 0.04	1.03 ± 0.05

Oxygen consumption (μl/h/mg wet tissue) of tissue minces (mean ± S.D.). Each value is the mean of results from 4 animals.

Table III. Effect of Na_2SeO_3 on production of $Ba^{14}CO_3$ from ^{14}C-glucose by rat liver and kidney minces (cpm/mg $BaCO_3$/g wet tissue)

	Controls Normal diet	Group 1 70 μg of Na_2SeO_3/g body wt. in 30 days	Group 2 110 μg of Na_2SeO_3/g body wt. in 58 days	Group 3 180 μg of Na_2SeO_3/g body wt. in 96 days
Chronic toxicity				
Liver	1168 ± 113	263 ± 68	523 ± 151	284 ± 132
Kidney	4040 ± 419	2863 ± 697	3102 ± 481	575 ± 107
Acute toxicity				
	Controls Normal diet	18.0 μg of Na_2SeO_3/g body wt.	36.5 μg of Na_2SeO_3/g body wt.	68.0 μg of Na_2SeO_3/g body wt.
Liver	1168 ± 113	304 ± 84	158 ± 98	19 ± 4
Kidney	4040 ± 419	1161 ± 576	774 ± 294	164 ± 38

Mean ± S.D. Each value is the mean of results from 4 animals.

ferences, except with added glucose when groups 1 and 2 were higher ($P < 0.05$) than the controls.

In Table III the effect of Na_2SeO_3 administration on the $Ba^{14}CO_3$, when uniformly labelled ^{14}C-glucose was added to the incubation medium is shown. In both chronic and acute toxicity and in both tissues studied a statistically significant decrease ($P < 0.01$) in $Ba^{14}CO_3$ production was found at all levels of selenite administered.

The decrease was greater in amount in the kidney than in the liver and also in acute toxicity than in chronic toxicity. It is interesting to note the much greater sensitivity shown by the selenite in acute than in chronic toxicity.

Discussion. From Table I it appears, on the whole, that increase in the oxygen consumption noticeable in the 2 organs is not related to the presence in the incubation medium of exogenous glucose. At this stage it is unexplained why there is an increase of O_2 consumption in kidney minces of group 2. On the other hand, increasing quantities of selenite decreased $^{14}CO_2$ production from the tissue minces. A decrease in the utilization of glucose and an increase in that of fatty acids could explain these results. In fact a decrease in the formation of $^{14}CO_2$ due to an increase in the utilization of non-radioactive glucose, thus producing a greater amount of non-radioactive CO_2 with a decrease in the specific activity of $Ba^{14}CO_3$, seems not to be the cause, since from group 1 the decrease in the production of radioactive carbon dioxide is not accompanied by a proportional increase in the respiratory activity. Selenite could stimulate fatty acid degradation, thus lowering the specific activity of $^{14}CO_2$.

This experiment has shown that, except for some unexplained erratic results, both chronic and acute selenium toxicity have negligible effects on the oxygen uptake of rat liver and kidney minces, with or without added glucose, but that in both types of toxicity the aerobic production of $^{14}CO_2$ from D-^{14}C-glucose by these minces is markedly diminished.

Further experiments with labelled fatty acids could demonstrate an increased catabolism of these substances induced by selenite[7].

Riassunto. Nessuna influenza sull'attività respiratoria di cellule di fegati e reni di topi è stata notata quando gli animali sono stati trattati con somministrazioni acute o croniche di Na_2SeO_3. La diminuzione della produzione di $^{14}CO_2$, quando i tessuti degli animali trattati con Na_2SeO_3 sono stati incubati con ^{14}C glucosio, suggerisce la possibilità di un aumento dell'attività catabolica dei lipidi.

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Activity of 6-Phosphogluconate Dehydrogenase in the Preimplantation Mouse and Rabbit Embryo

The glucose 6-phosphate dehydrogenase (G6PD) activity in the preimplantation mouse embryo during the first few days of development is very high[1], yet the ratio of carbon dioxide formed from carbon one of glucose compared to carbon six of glucose is approximately one throughout the preimplantation period[2]. In contrast, although total glucose utilization in the rabbit embryo is about three times higher than in the mouse embryo, the G6PD activity in the rabbit embryo is only about $^1/_5$ the level found in the mouse embryo[3], and the ratio of CO_2 from carbon one to carbon 6 of glucose is about 9 during the first 3 days of development in the rabbit[4,5]. This evidence suggests that G6PD is not acting as a regulator of pentose shunt activity in a similar manner in the early mouse and rabbit embryo. Therefore, the activity of 6-phosphogluconate dehydrogenase (6PGD) was measured to see if the total activities of this enzyme more accurately reflected the activity of the pentose shunt in the embryos.

The methods for obtaining the embryos and handling the embryos have been previously described[1,5]. Briefly, the embryos were obtained by flushing the reproductive tracts of the animals at specific times after ovulation. The embryos were washed free of debris and other cells and stored at $-70\,^\circ$C in 6×60 mm tubes. The freezing liberates the enzyme. The 6PGD activity was assayed by measuring the fluorescence of NADPH produced during a 60 min incubation at 37 °C. Details of the assay techniques have been described[3]. The reaction mixture consisted of 100 µl of 50 m*M Tris* buffer (pH 7.8), containing NADP (1.0 m*M*), 6-phosphogluconate (1.0 m*M*), magnesium chloride (10 m*M*), EDTA (1.0 m*M*), and crystalline bovine serum albumin (0.1%). The NADPH formed was determined fluorometrically following treatment with alkali[6,7].

The results of the determinations on both mouse and rabbit embryos for the entire preimplantation period are shown in the Table. The 6PGD activity is 10–20 times higher in the rabbit embryo than in the mouse embryo during the first 3 days of development. In fact, the 6PGD activity in the rabbit embryo is about the same as the G6PD activity in this species throughout the preimplantation period. Both enzymes are level during the first 3 days of development in the rabbit and then begin to increase in activity on day 4 with formation and expansion of the blastocyst. However, there is considerable tissue mass increase associated with blastocyst development in the rabbit, and therefore both 6PGD and G6PD decrease in specific activity during the preimplantation period (Table).

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