Naunyn-Schmiedeberg's Arch. Pharmacol. 311, 273–284 (1980)

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Transfer across Mucosal Epithelium, Tissue Content and Metabolic Fate of ^{125}I-(Ipodate-Sodium) on Isolated Everted Segments of Rat Small Intestine*

B. Komp and W. Forth

Institut für Pharmakologie und Toxikologie der Ruhr-Universität Bochum, Im Lottental, D-4630 Bochum 1, Federal Republic of Germany

Summary. 1. Transfer and tissue content of ^{125}I-radioactivity was measured after administration of ^{125}I-(ipodate-sodium) to everted rat jejunal segments.

2. After having administered 10^{-5} M ^{125}I-(ipodate-sodium) on both sides of the everted sacs the S/M ratio of the concentration of ^{125}I-radioactivity was 1.5 in jejunal segments and 2.3 in ileal segments. The tissue content was nearly equal for both segments. According to the apparent partition coefficient for ipodate-sodium at pH 7, the ^{125}I-radioactivity is accumulated in the tissue about 10-fold.

3. Lowering of the temperature of the incubation medium from 37° C to 15° C prevents the building up of a concentration gradient between the serosal and the mucosal side on either jejunal and ileal segments whereas the tissue content of ^{125}I-radioactivity was nearly unchanged.

4. With increasing concentrations ($1.6 \cdot 10^{-6} - 9.6 \cdot 10^{-4}$ M) of ^{125}I-(ipodate-sodium) administered on the mucosal side the transfer and the tissue content of ^{125}I-radioactivity were decreased. This appears to be a toxic effect since in jejunal segments also the S/M ratio for the concentration of glucose decreases.

5. The analysis of the ^{125}I-radioactivity in the serosal fluid of jejunal segments showed that the bulk of the ^{125}I-radioactivity was present in the aqueous phase and only 33% as the unchanged ipodate-sodium in the organic phase. 10% of the ^{125}I-radioactivity must be attributed to inorganic iodine. The concentration of ^{125}I-(ipodate-sodium) administered in the mucosal fluid only was $3.2 \cdot 10^{-6}$ M. At lower temperature (7° C) the bulk of the ^{125}I-radioactivity in the serosal fluid was found in the organic phase, i.e. as unchanged ipodate-sodium.

6. After the incubation of the aqueous phase with β-glucuronidase or NaOH about 97% of the ^{125}I-radioactivity could be extracted into the organic phase. This means that the bulk of the ^{125}I-radioactivity in the aqueous phase is present as a conjugate, e.g. ester glucuronide of the unchanged ipodate.

7. Apparently, the process of the conjugation of ipodate-sodium in the mucosal cells is involved in the transfer of the ^{125}I-radioactivity across the mucosal epithelium.

Key words: ^{125}I-(ipodate-sodium) – Glucuronidation in the mucosal epithelium – Transfer across mucosal epithelium – Tissue content – Metabolic conversion of ipodate-sodium during absorption.

Send offprint requests to W. Forth, Pharmakologisches Institut der Ludwig-Maximilians-Universität, Nussbaumstrasse 26, D-8000 München 2, Federal Republic of Germany

* The results were presented as doctor's thesis of B. Komp (1978), Johann Wolfgang Goethe-Universität, Frankfurt/Main, FRG, as well as at the spring meeting of the DPhG 1976 and 1977 (Komp, 1976, 1977)

Introduction

Mucosal epithelium cells are able to conjugate drugs and foreign compounds during absorption with glucuronic and sulfuric acid (for ref. see Hartiala, 1973; Josting et al., 1976; Wollenberg and Ulrich, 1979). Radioopaque substances of the type of triiodinated phenylalkanoates which are used for cholecystography are metabolized mainly into conjugation products (for ref. see McChesney, 1971). Therefore, it is necessary to investigate the production of conjugates during the absorption process in the mucosal epithelium cells especially of those agents which are commonly administered orally for cholecystography (Rosenstrauch, 1971), e.g. ipodate-sodium (ipodate-Na).

Materials and Methods

^{125}I-(Ipodate-Na). The inactive carrier as well as the ^{125}I-labelled compound were obtained from Schering AG., Berlin (West)[1].

1 We thank Dr. Ulrich Speck, Department Biodynamik, Pharma-Forschung, Schering AG., Berlin (West)

0028-1298/80/0311/0273/$02.40

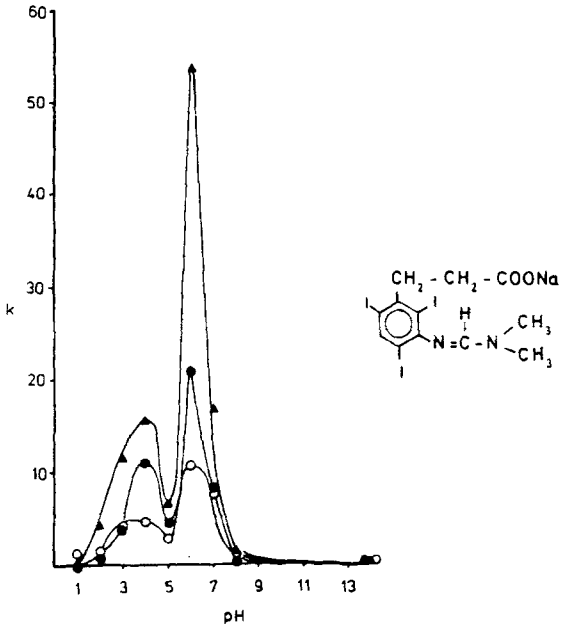

Fig. 1. Apparent partition coefficient k of ^{125}I-(ipodate-Na) at different pH. Buffers: pH 1, HCl/KCl (0.2 M); pH 2, citrate/HCl (0.1 M); pH 3 – 6, citrate/NaOH (0.1 M); pH 7, phosphate (0.15 M); pH 8, borate/HCl (0.2 M/0.1). ▲ Ether; ○ chloroform; ● benzene

The apparent partition coefficient (k) of ipodate-Na was determined at different pH values between buffer-solutions, ether, chloroform and benzene (see Fig. 1). ^{125}I-(ipodate-Na) was used; for details see measurement of radioactivity. The highest value for k (55) was obtained with ether at pH 6. Therefore, the extraction of ^{125}I-(ipodate-Na) from biological material was carried out routinely with organic solvents at pH 6.

Animals. Male Wistar rats (200 – 250 g body weight, W. Gassner, Sulzfeld, FRG) were used. The animals were fed with Höveler Standarddiät (Höveler Kraftfutterwerke, Langenfeld- Immigrath). Food was withdrawn 20 h before the experiment; the animals had always free access to water.

Preparation of Everted Sacs from Rat Small Intestine. The preparation of everted sacs was carried out according to the method of Wilson and Wiseman (1954). The sacs were prepared in a length of 5 cm either distal from the flexura duodenojejunalis (jejunal segments) or proximal from the valvula ileoceocalis (ileal segments). The sacs were filled with 0.7 ml (serosal fluid) and incubated in 15 ml of Tyrode solution (mucosal fluid).

Composition of the Tyrode solution: NaCl 137, KCl 2.7, $CaCl_2 \cdot 6H_2O$ 1.4, $MgCl_2 \cdot 6H_2O$ 0.5, $NaHCO_3$ 12, NaH_2PO_4 0.4 and glucose 15 mM. The incubation fluid was saturated with a gas mixture of 95% O_2 and 5% CO_2.

At the end of the experiment the segments were washed three times in isotonic saline and blotted carefully on filter paper. The serosal fluid was collected and measured volumetrically. Afterwards the wet weight of the intestinal tissue was determined.

Preparation of Perfused Rat Jejunal Segments. In some experiments in vitro perfused rat jejunal segments were used which were prepared according to the method of Fisher and Parsons (1949), modified by Rummel and Stupp (1960). Two adjacent jejunal segments of 10 cm length were prepared in ether anesthesia beginning with the flexura duodenojejunalis. The true length of the segments was measured at the end of the experiment: 10.1 cm ($\bar{x}$) ± 0.4 ($s_{\bar{x}}$); $N = 37$. The absorbed fluid amounted to 2.1 ml ($s_{\bar{x}} = \pm 0.1$; $N = 37$), i.e. 0.21 ml/cm jejunal segments. The concentration of glucose in the absorbate was 45.9 mM ($s_{\bar{x}} = \pm 2.7$; $N = 20$).

On the mucosal side the intestinal segments were perfused by 50 ml Tyrode solution: NaCl 137, KCl 2.7, $CaCl_2 \cdot 6H_2O$ 1.4, $MgCl_2 \cdot 6H_2O$ 0.5, $NaHCO_3$ 12, NaH_2PO_4 0.4 and glucose 15 mM. The perfusion fluid was saturated with a gas mixture of 95% O_2 and 5% CO_2. The temperature of the perfusion fluid was kept at 37° C. Perfusion volume/min: 10 ml; perfusion pressure: 25 cm H_2O.

At the end of the experiment the volume of the fluid absorbed (absorbate) was measured volumetrically. The jejunal segments were blotted gently on filter paper and weighed (w.w. of the tissue). The absorbate and the tissue was analyzed as described below.

As far as the functional and the morphological integrity of the intestinal segments is concerned see Forth and Rummel (1975); Pfleger (1975).

^{125}I-(ipodate-Na) was mixed with carrier ipodate-Na and administered in 0.5 ml Tyrode solution to the incubation and perfusion fluids. The final concentrations in the incubation and perfusion fluids are given in the figures and tables.

Analyses

^{125}I-(Ipodate-Na) in Fluids. 0.5 ml of the fluid sample was mixed with 1 ml of phosphate buffer, pH 6 (0.15 M). This mixture was extracted with 2.5 ml benzene and twice with 1.5 ml ether. The ^{125}I-radioactivity of aliquots of the organic phase was measured by aid of a γ-Spectrometer (Packard, Auto-Gammaspectrometer 3002).

In order to determine inorganic iodine the aqueous phase was acidified with 50 µl HNO_3, 10%, and mixed with 10 mcl NaI solution, 10% as well as 100 µl $AgNO_3$ solution, 20%. The precipitate was washed and its ^{125}I-radioactivity was measured. After having solubilized the precipitate in saturated KCN solution the further analysis on thin layer plates was carried out. As reference ^{125}I (Na I) precipitated with $AgNO_3$ and resolubilized in KCN was used.

After the precipitation of inorganic iodine the ^{125}I-radioactivity in the residual fluid aqueous phase was measured by aid of a γ-spectrometer. The cpm of both phases as well as of the precipitate were summarized and the percent fraction of the total ^{125}I-radioactivity was calculated for both phases as well as for the precipitate.

Tissue Analysis. The tissue was homogenized in 2 ml phosphate buffer pH 6 (0.15 M) in a Potter-Elvehjem homogenizer. The homogenate was rinsed with 3 ml phosphate buffer pH 6 into a centrifuge tube.

After two extractions with 2 ml ether the aqueous and the organic phase was separated. The tissue was extracted again twice with 3 ml phosphate buffer pH 6 and ether (2 · 2 ml) as described above and the phases were collected. The volume of the aqueous and the organic phase was determined. The ^{125}I-radioactivity was measured in 0.5 ml of the ether phase and the total amount of ^{125}I-radioactivity was calculated.

The aqueous phase was divided into two samples. To each sample 100 µl HNO_3, 10 µl NaJ, 10% and 500 µl $AgNO_3$, 20%, were added. The precipitate was washed and the ^{125}I-radioactivity was measured in both samples and summarized. The calculation of the percent fraction of the ^{125}I-radioactivity of both phases as well as in the precipitate was carried out as described above.

Recovery. By this procedure 99.9% of the ^{125}I-(ipodate-Na)-radioactivity could be recovered from the incubation fluid ($s_{\bar{x}} \pm 0.02$, $N = 5$) and 99.6% from the intestinal tissue ($s_{\bar{x}} = \pm 0.06$, $N = 5$).

Metabolites in the Aqueous Phase. After the ether extraction 1.5 ml of the aqueous phase was incubated with 3 U β-glucuronidase (E.C. 3.2.1.3.1) from Escherichia coli (Boehringer, Mannheim, FRG) at pH 4.5 for 30 min. The sample was extracted at pH 6 with 1.5 ml ether and the ^{125}I-radioactivity in the ether was measured. Since

Table 1. Thin layer chromatography systems for the analysis of ^{125}I-(ipodate-Na) in the organic phase

Stationary phase	Silica gel G[a]	Silica gel G[a]
Solvent system[b]	benzene 6, acetic acid 4, ethylacetate 4, methanol 3	*n*-butanol 8, acetic acid 2, H_2O 2
R_f ^{125}I-(ipodate-Na)	0.7	0.9

[a] Precoated aluminium sheets, 0.2 mm layer thickness, 20 × 20 cm, Merck AG, Darmstadt, Nr. 5553
[b] All chemicals used were p.a. grade; Merck AG, Darmstadt, FRG

Table 2. Thin layer chromatography systems for the analysis of the ^{125}I-radioactivity in the $AgNO_3$ precipitate

Stationary phase	Silica gel G[a]	Silica gel G[a]	Cellulose[b]
Solvent systems[c]	Isopropanol 80, NH_3 (25%) 20	Ethylacetate 55, isopropanol 35, NH_3 (25%) 20	
R_f value of ^{125}I-(NaI)	0.5	0.38	0.2

[a] Precoated aluminium sheets, 0.2 mm layer thickness; 20 × 20 cm, Merck AG, Darmstadt, Nr. 5563
[b] Precoated aluminium sheets, 0.1 mm layer thickness; 20 × 20 cm, Merck AG, Darmstadt, Nr. 5552
[c] All chemicals used were p.a. grade, Merck AG, Darmstadt, FRG

apparently the activity of the enzyme was stopped in the presence of free ipodate-Na this procedure was repeated several times in intervals of 15 min.

In a second experiment 1.5 ml of the aqueous phase were incubated with 0.2 ml NaOH (1 M) at 20° C. The ^{125}I-radioactivity was extracted into ether and was calculated as percent fraction of the total radioactivity found in the aqueous phase.

Thin Layer Chromatography. The ^{125}I-radioactivity in the organic phase was analyzed on thin-layer plates in two different solvent systems (see Tab. 1). Table 1 contains also the R_f-values of the reference ^{125}I-(ipodate-Na).

The stationary phase as well as the solvent systems used for the analysis of the $AgNO_3$ precipitates is summarized in Table 2. The R_f-values of Table 2 show the apparent identity between ^{125}I-(NaI) and the ^{125}I-radioactivity in the $AgNO_3$ precipitates solubilized in saturated KCN.

The thin-layer plates were developped in the dark at 4° C. After the development the plates were divided into 1 cm tracks. The precoated silica gel or cellulose of the tracks was scraped off and the ^{125}I-radioactivity of the scraped-off powder was measured by aid of a γ-spectrometer.

Glucose was determined photometrically in the mucosal and the serosal fluid by aid of GOD-Perid®, Test-Kombination (Boehringer, Mannheim, FRG).

Results

Transfer Across the Mucosal Epithelium and Tissue Content of ^{125}I after the Administration of ^{125}I-(Ipodate-Na) into the Mucosal Fluid of Everted Sacs of Jejunal and Ileal Segments in vitro of Rats

Transfer Depending on Increasing Concentrations of ^{125}I-(Ipodate-Na)

^{125}I-(ipodate-Na) was administered in increasing concentrations to the mucosal fluid of jejunal and ileal everted sacs. The concentration of the ^{125}I-radioactivity in the mucosal fluid decreased during the experimental period by about 15%.

The amount of ^{125}I-radioactivity transferred across the mucosal epithelium which has been calculated from the concentration of the ^{125}I-(ipodate-Na) and the fluid volume on the serosal side is given in Fig. 2. The data of the normal fluid volume and of the concentration of ^{125}I-(ipodate-Na) are omitted here.

In *jejunal* segments the amount of ^{125}I-radioactivity transferred remains unchanged over a wide range ($1.6 \cdot 10^{-6} - 3.3 \cdot 10^{-5}$ M). With higher concentrations ($3.2 \cdot 10^{-4} - 9.6 \cdot 10^{-4}$ M) the amount of ^{125}I-radioactivity transferred decreases sharply.

With *ileal* segments similar results were obtained (see Fig. 2). Also with ileal segments in higher concentrations the amount of ^{125}I-radioactivity transferred decreases sharply.

The concentration of ^{125}I-radioactivity[2] in the serosal fluid of jejunal segments equaled that in the mucosal fluid or, with ileal segments, was 20–25% higher than that in the mucosal fluid at the beginning of the experiments (Komp, 1978). This holds true up to a concentration of $3.3 \cdot 10^{-5}$ M. With higher concentrations of ^{125}I-(ipodate-Na) the concentration of ^{125}I-radioactivity in the serosal fluid decreased. At the highest concentration ($9.6 \cdot 10^{-4}$ M) the concentration of the ^{125}I-radioactivity in the serosal fluid amounted to less than half of that in the mucosal fluid.

2 For the sake of simplicity the figures of the concentration of the ^{125}I-radioactivity are not given in this paper and the authors refer to the doctor's thesis of Komp (1978)

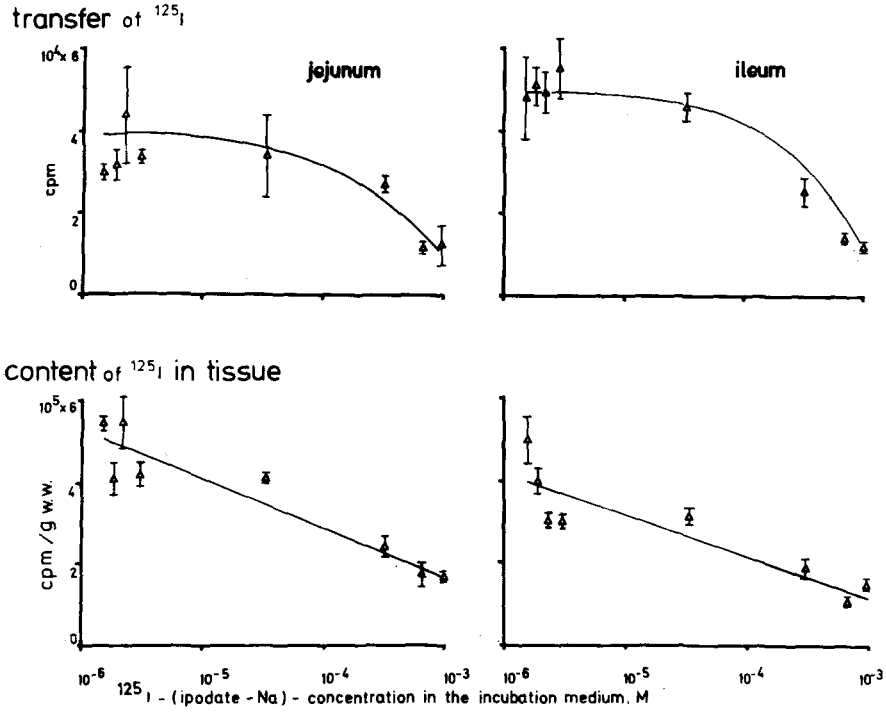

Fig. 2
Transfer and content in tissue of ^{125}I-radioactivity on everted jejunal and ileal segments after the administration of increasing concentrations of ^{125}I-(ipodate-Na). Methods according to Wilson and Wiseman (1954). Incubation medium: tyrode solution. Time: 2 h, temperature: 37° C. ^{125}I-(ipodate-Na) was added only to the mucosal fluid; $3.2 \cdot 10^{-6}$ M $\hat{=}$ 50,000 cpm/ml. Each symbol represents $\bar{x} \pm s_{\bar{x}}$ of 3–8 everted sacs

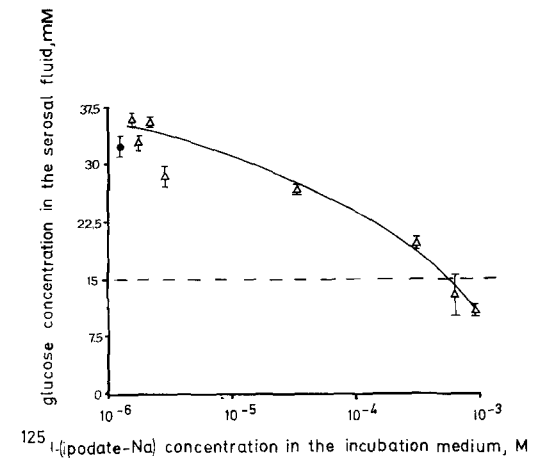

Fig. 3. The influence of ^{125}I-(ipodate-Na) on the uphill transfer of glucose on everted jejunal segments. Method according to Wilson and Wiseman (1954). Incubation medium: tyrode solution, glucose concentration 15 mM. ^{125}I-(ipodate-Na) was administered at the mucosal side only (see Fig. 2). Time: 2 h, temperature: 37° C. Each symbol represents the mean $\bar{x} \pm s_{\bar{x}}$ of 3–8 everted sacs. ● Controls without ipodate-Na. ▲ Results in the presence of ipodate-Na

With increasing concentrations of ^{125}I-(ipodate-Na) the concentration of glucose in the serosal fluid decreased (Fig. 3). From $3.1 \cdot 10^{-6}$ M ^{125}I-(ipodate-Na) on wards the concentration of glucose in the serosal fluid is statistically significantly lower. Beyond of $6.4 \cdot 10^{-4}$ M ^{125}I-(ipodate-Na) no concentration gradient for glucose was established between the serosal and mucosal fluid. Therefore, in the following only concentrations lower than 10^{-5} M ^{125}I-(ipodate-Na) were used. Normally, at the end of the experiment the ratio of the concentration of glucose in the serosal and the mucosal fluid is 2.

Tissue Content of ^{125}I-Radioactivity

The content of ^{125}I-radioactivity in the intestinal tissue decreased with increasing concentrations of ^{125}I-(ipodate-Na) administered in the mucosal fluid (Fig. 2). There is apparently no difference in the tissue content of ^{125}I-radioactivity between jejunal and ileal segments.

Transfer and Tissue Content of ^{125}I After the Administration of ^{125}I-(Ipodate-Na) Into the Incubation Fluid of Jejunal and Ileal Segments; Time Dependency

In order to find out whether there is a preferential direction of the transfer ^{125}I-(ipodate-Na) was administered at the mucosal and the serosal side of the everted sacs in a concentration of 10^{-5} M (see Fig. 4). During the experimental period the concentration of ^{125}I-radioactivity was measured in either the mucosal or serosal fluid.

In jejunal segments after decrease the initial concentration of the ^{125}I-radioactivity at the serosal side is

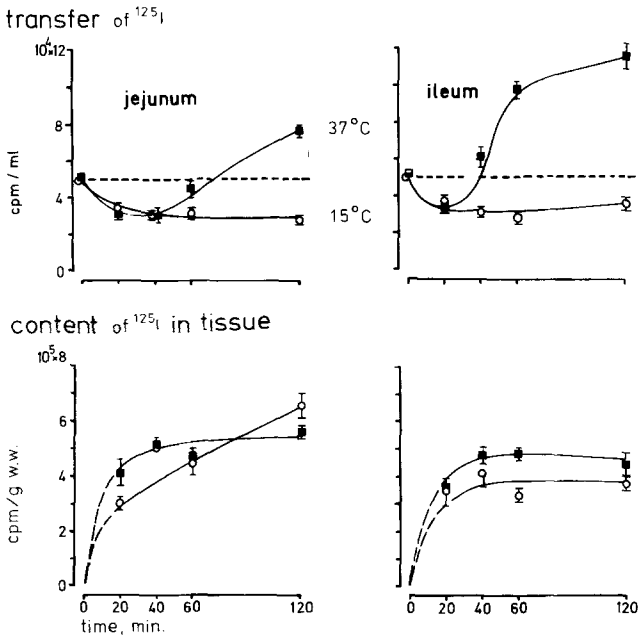

Fig. 4. Transfer and content in tissue of ^{125}I-radioactivity on everted jejunal and ileal segments after the administration of ^{125}I-(ipodate-Na); dependency on time and temperature. Method according to Wilson and Wiseman (1954). Incubation medium: tyrode solution; ^{125}I-(ipodate-Na) was added to the mucosal and the serosal fluid at the beginning of the experiment. Concentration: 10^{-5} M $\hat{=}$ 50,000 cpm/ml. This concentration of the ^{125}I-radioactivity is represented by the broken line. Each symbol gives the mean $\bar{x} \pm s_{\bar{x}}$ of 3–6 everted sacs. ■ 37° C; ○ 15° C

reached again, 1 h after the beginning of the experiments, at the end of the experiment the concentration is about 50% higher than at the beginning of the experiment. The tissue content of ^{125}I-radioactivity increases rapidly with time and the final values are reached between 40 and 60 min after the beginning of the experiment. Apparently the decrease of the concentration of the serosal fluid must be attributed to the rapid uptake of the radioactivity into the tissue (Komp, 1978).

In ileal segments the transfer of ^{125}I-radioactivity is apparently more rapid than that in jejunal segments. After a short decrease there is again a sharp increase of the concentration of ^{125}I-radioactivity in the serosal fluid. At the end of the experiments the concentrations of the ^{125}I-radioactivity is more than doubled as compared with that at the beginning of the experiments.

Also in ileal segments there is a rapid uptake of ^{125}I-radioactivity into the tissue. After 40 min the final values are reached. Again the initial decrease of ^{125}I-radioactivity at the serosal side must be attributed to the rapid uptake into the tissue.

The most important information of this experiment is that apparently ^{125}I-radioactivity is transferred preferentially into the serosal fluid after the administration of ^{125}I-(ipodate-Na) on both sides of the segments.

Dependency on Temperature of Transfer and Tissue Content of ^{125}I-Radioactivity

Everted segments of jejunum and ileum were incubated also at 15° C. The ^{125}I-(ipodate-Na) was administered in a concentration of 10^{-5} M on either side of the everted sac, i.e. in the mucosal and the serosal fluid. The results of these experiments are summarized in Fig. 4.

At 15° C apparently both jejunal and ileal segments lost their ability to establish a concentration gradient for the radioactivity at the end of the experimental period. The tissue content is not very much influenced by temperature. The time course of the uptake of the ^{125}I-radioactivity is at least in jejunal segments slower at lower temperatures as compared to 37° C (Komp, 1978).

The Metabolic Fate of ^{125}I-(Ipodate-Na) During Transfer Across the Mucosal Epithelium

When having administered ^{125}-(ipodate-Na) $3.2 \cdot 10^{-6}$ M at the mucosal side only, at the end of the experiment 33% of the ^{125}I-radioactivity could be extracted from serosal fluid into the organic phase (Fig. 5). The greater part remained in the aqueous phase (57%). About 10% of the ^{125}I-radioactivity was precipitated by $AgNO_3$ and, hence, had to be attributed to inorganic iodine. The R_f-value of the precipitated ^{125}I-radioactivity dissolved in KCN corresponded with that of ^{125}I-(NaI).

In the intestinal tissue only 4% of the entire ^{125}I-radioactivity was found in the aqueous phase (Fig. 5). 1% of the total radioactivity was precipitated by $AgNO_3$. 95% of the ^{125}I-radioactivity was unchanged ^{125}I-(ipodate-Na). This holds true for the experiments in which the intestinal segments were incubated at 37° C.

When having lowered the incubation temperature to 7° C the transfer of ^{125}I-radioactivity in the mucosal fluid was reduced by more than 60%; the content of ^{125}I-radioactivity in the intestinal tissue remained unchanged.

In contrary to the experiments at 37° C most of the ^{125}I-radioactivity at the serosal side was unchanged ^{125}I-(ipodate-Na) (87%). Only 13% of the entire ^{125}I-radioactivity remained in the aqueous phase; about 6% of the ^{125}I-radioactivity in the aqueous phase could be precipitated by $AgNO_3$. In the intestinal tissue the bulk of the radioactivity was unchanged ^{125}I-(ipodate-Na); only a minute amount (0.3%) remained in the aqueous phase or could be precipitated by $AgNO_3$.

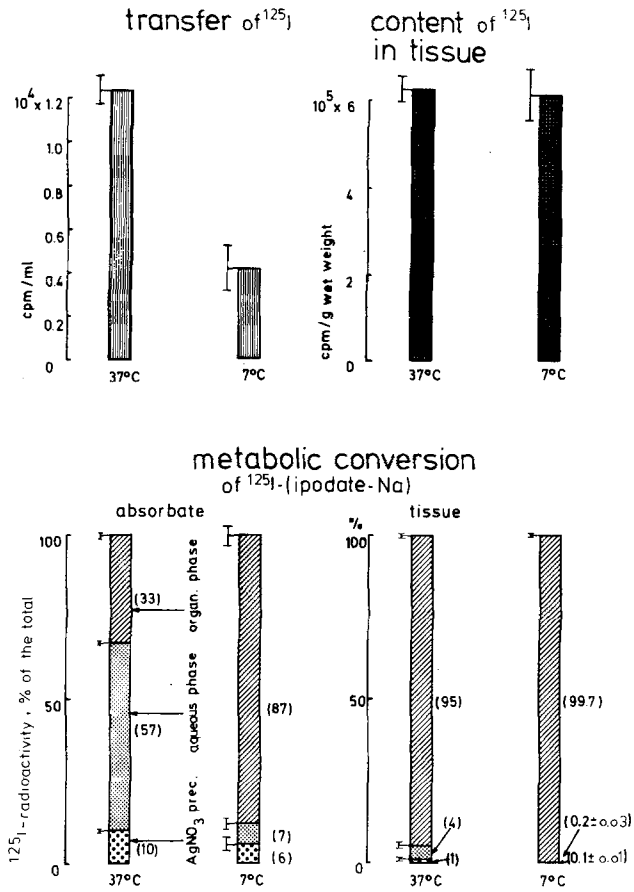

Fig. 5. The metabolic conversion of ^{125}I-(ipodate-Na) at different incubation temperatures on everted jejunal segments in vitro of rats. Method according to Wilson and Wiseman (1954). Incubation medium: tyrode solution; ^{125}I-(ipodate-Na) was added in the mucosal fluid only, $3.2 \cdot 10^{-6}$ M ≙ 100,000 cpm/ml. Time: 2 h. The height of the bars represents the mean of 5–7 experiments $(\bar{x}) \pm s_{\bar{x}}$. The figures in brackets give the percent fraction of the total ^{125}I-radioactivity

Analysis of the ^{125}I-Radioactivity in the Aqueous Phase

Apparently, during the transfer of ^{125}I-(ipodate-Na) a polar hydrophilic metabolite was synthetized. Since it is well-known that drugs can be conjugated during transfer in the mucosal epithelium of the rat intestine (Hartiala, 1973; Josting et al., 1976; Wollenberg and Ulrich, 1979), the aqueous phase was incubated with β-glucuronidase. This procedure was repeated ten times and within nearly three hours about 97% of the total radioactivity of the aqueous phase could be extracted by ether.

Similar results were obtained by alkaline hydrolysis of the aqueous phase by adding NaOH 1 M; 97.4% of the ^{125}I-radioactivity in the aqueous phase could be extracted after the alkaline hydrolysis. Hydrolysis was nearly complete after 45 min and only three additions of 0.2 ml NaOH 1 M. From these results the conclusion can be drawn that the main hydrophilic metabolite in the serosal fluid is a conjugate e.g. of glucuronic acid; less than 3% of the total radioactivity remained in the aqueous phase.

Transfer, Tissue Content and Metabolic Conversion of ^{125}I-(Ipodate-Na) Administered in Increasing Concentrations at the Mucosal Side of Jejunal Segments

The following experiments with increasing concentrations of ^{125}I-(ipodate-Na) were carried out on isolated jejunal segments of rats which were perfused with Tyrode solution on the mucosal side.

From the experiments on everted jejunal segments it was known that concentrations of ^{125}I-(ipodate-Na) above of 10^{-4} M inhibit the transport capacity of the mucosal epithelium. Therefore, a concentration range of ^{125}I-(ipodate-Na) between $3.2 \cdot 10^{-6} - 10^{-3}$ was chosen. ^{125}I-(ipodate-Na) was administered in the perfusion fluid at the mucosal side only. The results of this experiment are summarized in Fig. 6.

From Fig. 6 it can be taken that ipodate-Na in a concentration of 10^{-3} M inhibits fluid absorption by nearly 75%. The concentration of ^{125}I-radioactivity in the absorbate is decreased by 75%, the amount of ^{125}I-radioactivity absorbed by about 93%.

The content of ^{125}I-radioactivity in the tissue decreases sharply when the concentration of ^{125}I-(ipodate-Na) is elevated from $3.2 \cdot 10^{-6}$ up to 10^{-5} M. Then, in a wide range of concentrations from $10^{-5} - 10^{-3}$ M the ^{125}I-content in tissue remains remarkably constant. No interpretation can be given for the difference of the tissue content of ^{125}I between the concentrations of ^{125}I-(ipodate-Na) of $3.2 \cdot 10^{-6}$ and the higher ones used in these experiments.

After having administered ^{125}I-(ipodate-Na) in $3.2 \cdot 10^{-6}$ and 10^{-4} M the bulk of radioactivity in the absorbate appears in the aqueous phase, indicating that it belongs to (a) hydrophilic metabolite(s). The fraction of the ^{125}I-radioactivity attributable to the unchanged ^{125}I-(ipodate-Na) amounts to 19–28% (Fig. 7). The fraction of the inorganic ^{125}I-radioactivity precipitated by $AgNO_3$ amounts to 5–8%. After having increased the ^{125}I-(ipodate-Na) concentration in the perfusion fluid to 10^{-3} only minute amounts of the ^{125}I-radioactivity (2%) appear in the aqueous phase. 27% of the ^{125}I-radioactivity must be attributed to the unchanged parent compound ^{125}I-(ipodate-Na). About 1% of the entire radioactivity in the absorbate must be attributed to inorganic ^{125}I precipitated by $AgNO_3$.

In the mucosal tissue normally the bulk of the radioactivity must be attributed to the unchanged ^{125}I-(ipodate-Na). Apparently the hydrophilic conjugates leave the mucosal cells rapidly; only 4–9% of the total ^{125}I-radioactivity were found in the aqueous phase

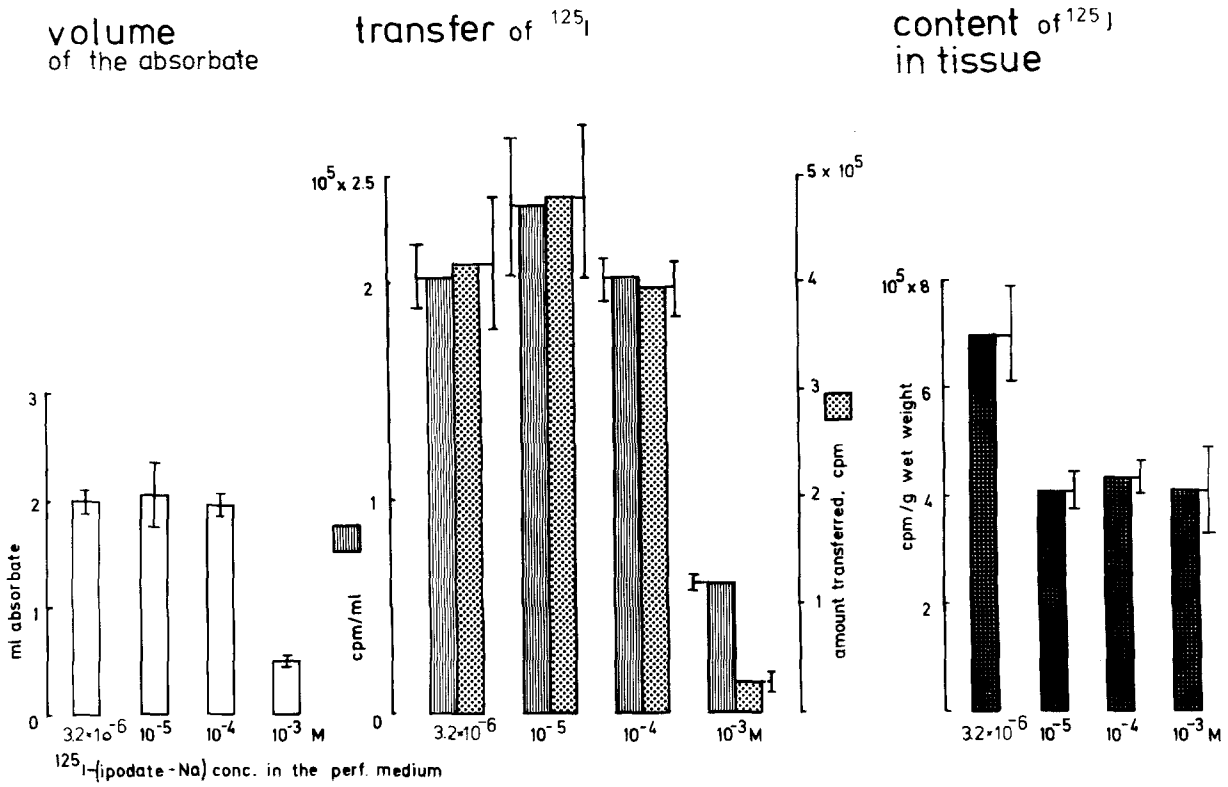

Fig. 6. Transfer and tissue content of ^{125}I-radioactivity on isolated non-blood perfused jejunal segments in vitro of rats after the administration of ^{125}I-(ipodate-Na) in increasing concentrations on the mucosal side. Method according to Fisher and Parsons (1949) modified by Rummel and Stupp (1960). Time: 2 h, temperature: 37° C. The columns represent $\bar{x} \pm s_{\bar{x}}$; $N = 3-8$. The initial concentration of the ^{125}I-radioactivity was ≙100,000 cpm/ml

when having administered ^{125}I-(ipodate-Na) $3.2 \cdot 10^{-6}$ and 10^{-4} M. After having increased the concentration of ^{125}I-(ipodate-Na) up to 10^{-3} M the fraction of the ^{125}I-radioactivity in tissue fell to 1.5 %. 98 % of the total ^{125}I-radioactivity were present in the organic phase and about 0.5 % could be precipitated by $AgNO_3$.

It should be added here that also on isolated jejunal segments prepared according to Fisher and Parsons (1949) as was described for everted jejunal segments in vitro lowering of the temperature of the perfusion fluid (15° C) decreased the fraction of ^{125}I-radioactivity in the aqueous phase of the absorbate. In tissue, however, no change of the percent fraction of the total ^{125}I-radioactivity in the aqueous and the organic phase was measured (Komp, 1978). Possibly, the temperature was not low enough, since with everted segments temperatures as low as 7° C were used (see above).

The Movement of the Hydrophilic Metabolites of ^{125}I-(Ipodate-Na) from the Tissue to the Mucosal and Serosal Side of the Jejunal Segments

In order to find out whether there is a preferential direction of the movement of the hydrophilic metabolites of ^{125}I-(ipodate-Na) the concentration of the ^{125}I-radioactivity in the aqueous phase of the perfusion fluid, the absorbate and in the cellular fluid of the tissue was calculated from the results presented already (cf. Fig. 6). The amount of the ^{125}I-radioactivity linked to the hydrophilic metabolites were calculated from the ^{125}I-radioactivity in the aqueous phase and the fluid volume present at the mucosal and the serosal side of the segments. The percent fraction of the ^{125}I-radioactivity in the aqueous phase of the perfusion fluid, the absorbate and in the tissue are summarized in the Table 3.

From Fig. 8a it can be taken that after administration of ^{125}I-(ipodate-Na) below of 10^{-4} M apparently the concentration of the ^{125}I-radioactivity present as hydrophilic metabolites of ipodate in the absorbate at the serosal side is 3.5 – 4 times higher than that in the cellular fluid whereas in the perfusion fluid the concentration of the ^{125}I-radioactivity present as hydrophylic metabolites of ipodate is very low. Above of 10^{-3} M the concentration of the radioactivity in the aqueous phase of the absorbate, the cellular fluid and the perfusion fluid are nearly equal.

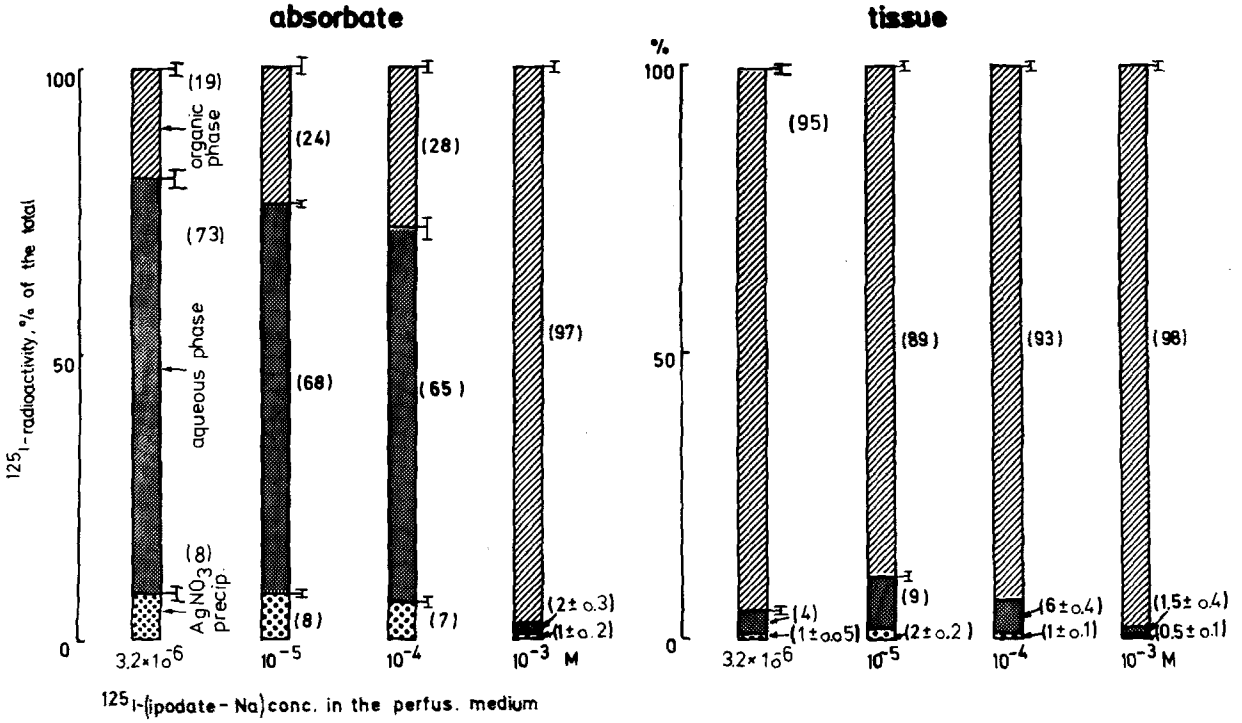

Fig. 7. Metabolic fate of ^{125}I-(ipodate-Na) administered in increasing concentrations to the mucosal side of jejunal segments in vitro during the transfer across the intestinal wall. The results are derived from the same experiments as shown in Fig. 6. The ^{125}I-radioactivity was extracted from the absorbate and the homogenized intestinal tissue at pH 6 (site rate-NaOH-buffer) into an aqueous and an organic phase (benzene, ether). Inorganic iodide was precipitated from the aqueous phase by $AgNO_3$ in an acidic medium. $\bar{x} \pm s_{\bar{x}}$; $N = 3-8$

When having calculated the amounts of the ^{125}I-radioactivity present as hydrophilic metabolites moved to the mucosal as well to the serosal side it appears that at least in lower concentrations of ^{125}I-(ipodate-Na) ($3.2 \cdot 10^{-6}$ and 10^{-5} M) the hydrophilic metabolites are transferred in equal amounts into both directions, into the serosal and into the mucosal medium without any preference (cf. Fig. 8b). The decrease of the amount of ^{125}I-radioactivity in the aqueous phase in the perfusion fluid as well as in the absorbate after having administered ^{125}I-(ipodate-Na) in higher concentrations (10^{-4} and 10^{-3} M) is apparently caused by the inhibition of the cellular metabolism by which also the capacity of the mucosal tissue to form hydrophilic metabolites as well as to absorb water is diminished by ipodate-Na itself. Since in the presence of the highest concentration of ipodate-Na (10^{-3} M) especially the movement of the fluid absorbed across the mucosal epithelium leaving mainly through the drainage by lymph and blood vessels which are the preferential way for the fluid from the mucosal to the serosal side of non blood-perfused isolated intestinal segments in vitro (Lee, 1963), it is not surprising that also the ^{125}I-radioactivity in the aqueous phase measured at the serosal side is much less than that in the perfusion fluid.

Table 3. Percent fraction of the ^{125}I-radioactivity in the aqueous phase of the perfusion fluid, the absorbate and the jejunal tissue. The ^{125}I-concentration in the perfusion fluid at the beginning of the experiment was $\hat{=}$ 100,000 cpm/ml for each concentration of ipodate-Na. At the end of the experiments ^{125}I-concentration was diminished by approximately 10 %. ($\bar{x} \pm s_{\bar{x}}$; $N = 3-8$). At the beginning of the experiments the percent fraction of ^{125}I-radioactivity in the aqueous phase of the extracts of the perfusion fluid was 0.7 ± 0.06 ($N = 67$)

^{125}I-(ipodate-Na)	^{125}I-radioactivity in the aqueous phase: (percent fraction of the total ^{125}I-radioactivity)		
	Perfusion fluid	Absorbate	Jejunal tissue
$3.2 \cdot 10^{-6}$	7 ± 1.2	73 ± 2	4 ± 0.4
10^{-5}	8.5 ± 2.2	68 ± 0.5	9 ± 0.5
10^{-4}	2.5 ± 0.2	65 ± 1.9	6 ± 0.4
10^{-3}	0.5 ± 0.05	2 ± 0.3	1.5 ± 0.4

Discussion

From the physicochemical properties the conclusion could be drawn that in the physiological pH-range of 6–7 ipodate-Na is highly lipid soluble and, hence, well adapted for the penetration through biological mem-

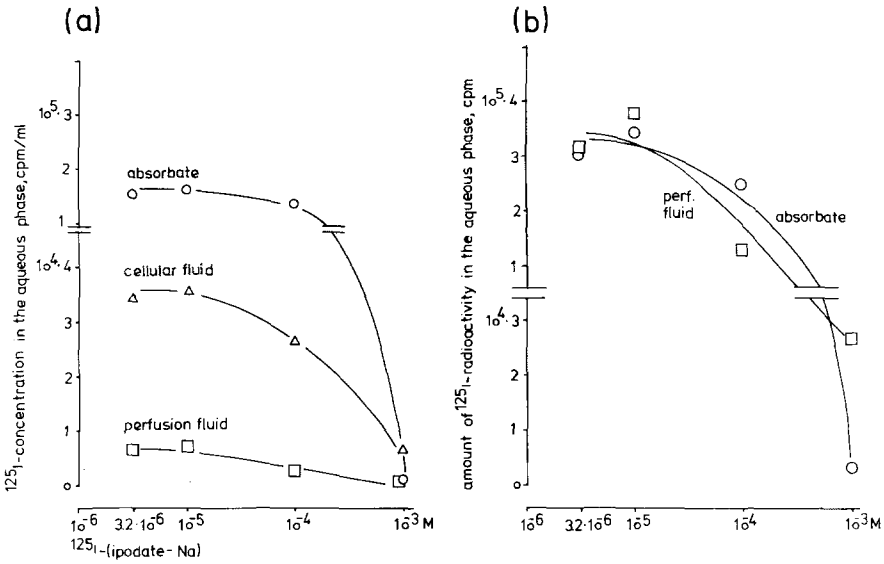

Fig. 8
The concentration of the ^{125}I-radioactivity linked to hydrophilic constituents, the perfusion fluid, the absorbate and the cellular fluid (**a**) as well as the total amount of ^{125}I-radioactivity present in the aqueous phase and the perfusion fluid (**b**) at the end of the experiment. The data are calculated from the results shown in Fig. 6. For the calculation of the ^{125}I-concentration in the cellular fluid the following assumptions were made; (1) extracellular space: 20% of the w.w. of the jejunal segments (Nell et al., 1976). (2) Tissue dry weight: 18% of the w.w. of the jejunal segments (Forth, 1971). For the sake of simplicity only $\bar{x}$ is given: $s_{\bar{x}}$ of each value is less than 10% of $\bar{x}$. The percent fraction of the ^{125}I-radioactivity in the aqueous phase of the perfusion fluid, the absorbate and the jejunal tissue is summarized in Table 3

brane systems. A good absorption of ipodate-Na is not only known from the use as radioopaque substance after oral administration (for lit. see Sperber and Sperber, 1971). The good absorption of ipodate-Na was also measured on tied-off jejunal segments in situ of anesthetized rats in vivo (Komp, 1978).

Surprisingly, in the results presented here a preferential direction from M→S was observed for the transfer of ^{125}I-(ipodate-Na). This preferentially directed transfer depends on the intact cellular metabolism. Lowering of the temperature in the incubation medium to 15° C results in a decrease of the transfer of ^{125}I-(ipodate-Na) whereas the tissue content did not differ very much from that obtained with segments incubated at 37° C. Inspite of this observation it did not seem very likely that the rat intestine may transport ^{125}I-(ipodate-Na) actively using cellular energy and a special transport system. When having administered ^{125}I-(ipodate-Na) in increasing concentrations the transfer did not show a saturation type kinetic. The decreasing transfer and even the decreasing content in intestinal tissue of ^{125}I-(ipodate-Na) with increasing concentrations is apparently the consequence of a toxic effect. This effect may be due to the well-known protein binding properties of triiodinated phenylalkanoates (Knoefel, 1971). It appears likely that cellular proteins involved in the transport system for glucose or the transport Na-K-ATPase which is involved in the sodium transport and, hence, the epithelial water transfer (Diamond, 1971), may bind ipodate-Na and become thus unable to exert their biological functions. An inhibition of the reabsorption of sodium and, hence, of water in the tubules of the kidneys may be the cause for the well-known nephrotoxic effect of triiodinated phenylalakaonates (Mudge, 1971). They inhibit also blood clotting by binding to the plasma proteins involved in the blood clotting mechanism (Schulze and Kaps, 1977). A similar binding to the protein and, thus, inhibition of the efficacy of the enzyme may be involved in the effect of ipodate-Na set free by β-glucuronidase which had to be administered repeteadly to the incubation medium in order to obtain a complete splitting of the conjugates.

The key for the understanding of the preferentially directed transfer of ipodate-Na is the observation of the conjugation of the compound during the transfer across the mucosal epithelium. The capacity of the mucosal tissue for glucuronidation of drugs in foreign compounds is well-known (Hartiala, 1973; Josting et al., 1976; Wollenberg and Ullrich, 1979). So it appears to be highly probable that ^{125}I-(ipodate-Na) is rapidly taken up by the mucosal epithelium according to its partition coefficient in favour of organic solvents in the physiological pH-range 6–7. In the mucosal epithelial cells ^{125}I-(ipodate-Na) is conjugated most probably with glucuronic acid and, at least in part, with sulfuric acid. It cannot be excluded that the β-glucuronidase contains impurities of sulphatase activity. No attempt was made to determine glucuronic acid and sulfuric acid conjugates of ipodate-Na separately. Since the conjugates could be split by exposure to an alkaline medium the conjugates are possibly esters.

Most probably the conjugates were produced with the unchanged ipodate. In rats a considerable amount

of the metabolites of ipodate-Na found in bile and urine had a free aromatic aminogroup, apparently after splitting of the dimethylformamido-group (Harwart et al., 1959). Ipodate as well as (after the splitting of the dimethylformamido-group) the resulting 3-amino-2,4,6-trijodophenyl-propionic acid are highly lipid soluble. Both compounds are extractable into organic solvents. However, in two solvent systems no additional spot containing ^{125}I-radioactivity was observed beside that of the mother compound. Therefore, it is highly probable that splitting of the dimethylformamide group from the ipodate-Na does not play a major role in the mucosal tissue.

The conjugates apparently leave the epithelial cells rapidly. The content of ^{125}I-radioactivity in the aqueous phase of the intestinal tissue is remarkably low. On the basis of the presented results it cannot be decided whether there exists a special transport system for conjugates out of the cells or whether the conjugates leave according to the concentration gradient merely by diffusion and the partition coefficient.

The decreased capacity of the jejunal segments in vitro to conjugate ipodate-Na can be caused by either a limited capacity of the tissue to conjugate or by a toxic effect of ipodate-Na. A toxic effect of ipodate-Na is apparent by the inhibition of absorption of fluid and the uphill transport of glucose. Highly possibly, the same mechanism, e.g. the inhibition of enzymes by the well-known capacity of ipodate-Na to bind proteins, probably inhibits the production of conjugates of ipodate-Na in the intestinal tissue.

The results presented here can be taken as being indicative for the existence of a transport system for conjugates produced in the mucosal cells which depends on cellular energy. It is well-known that other epithelial structures, for instance in the biliary system (for lit. see Smith, 1971) and in the epithelial cells of the tubules in the kidney (for lit. see Weiner, 1973) have transport systems for conjugates with glucuronic acid. It may be pointed out here that apparently the isolated, non-blood perfused jejunal segment in vitro offers a simple preparation for the investigation of such transport systems.

Considerable amounts of the conjugates leave the mucosal cell also across the mucosal membrane into the gastrointestinal lumen. As can be taken from Fig. 8b at the end of the experiment the amount of ^{125}I-radioactivity in the aqueous phase of the perfusion fluid equals that in the absorbate. This observation which needs confirmation by further experiments would be of some biological significance since it means that no preference of the transfer of the conjugates to neither side of the mucosal epithelium exists.

Summing up the transfer mechanism of ipodate-Na can be described as given in the scheme of Fig. 9. Ipodate-Na is taken up into the mucosal tissue according to its partition coefficient at pH 7. In the mucosal cells in lower concentrations ($< 10^{-4}$ M) (glucuronic acid) conjugates of ipodate-Na are produced; the parent compound may be transferred to a minor extent. The conjugation products are transported out of the cells and/or leave the cells according to the concentration gradient by simple diffusion. The decrease of the percent fraction of the (glucuronic acid) conjugates of the total amount of ipodate transferred in favor or the transfer of the unchanged mother compound observed after the administration of higher concentrations of ipodate-Na may be the consequence of a limited capacity of the mucosal epithelium for conjugation. In addition, however, in higher concentrations ($> 10^{-4}$ M) ipodate-Na inhibits not only the absorption of water and uphill transport of glucose but also the conjugation mechanism.

Finally, the attention may be drawn to the formation of inorganic iodine during the transfer of ^{125}I-radioactivity from ^{125}I-(ipodate-Na) across the mucosal epithelium. After having administered ^{125}I-(ipodate-Na) $3.2 \cdot 10^{-6}$ M, 10% of the ^{125}I-radioactivity which passed the mucosal epithelium must be attributed to inorganic iodine. This is a greater amount than is referred to hitherto in literature. Up to now less than 1% of the iodine content of ipodate was assumed to be split in the organism (for ref. see McChesney, 1971). However, under therapeutical conditions the dose of ipodate-Na is 3 g and more. This dose swallowed into the gastric fluid of about 1 – 2 l would result a concentration of $2.5 \cdot 10^{-3}$ M. In order to evaluate the capacity of the mucosal epithelium to splitting inorganic iodine from ipodate-Na during the transfer process it was necessary to control the metabolic conversion of this compound also in higher concentrations.

Apparently the formation of inorganic iodide decreased with increasing concentrations of ^{125}I-(ipodate-Na). This may be the result of a metabolic deiodination of the mother compound since it is well-known that rats are able to deiodinate the mother compound of radiocontrast agents, i.e. 2,3,5-triiodobenzoic acid (Barker et al., 1967; Moy and Ebert, 1967; Gutenmann et al., 1967). It must be added here that no ^{125}I-radioactivity could be detected in the perfusion fluid at the beginning of the experiment. This means that the mother compound was free of detectable contaminations with inorganic iodide. In addition it must be mentioned that inorganic iodide is moved across the mucosal epithelium just in the reverse direction i.e. preferentially from the serosal to the mucosal side of the isolated intestinal segments in vitro (Komp, 1978). The higher amount of inorganic iodide in the perfusion fluid at the end of the experiment as

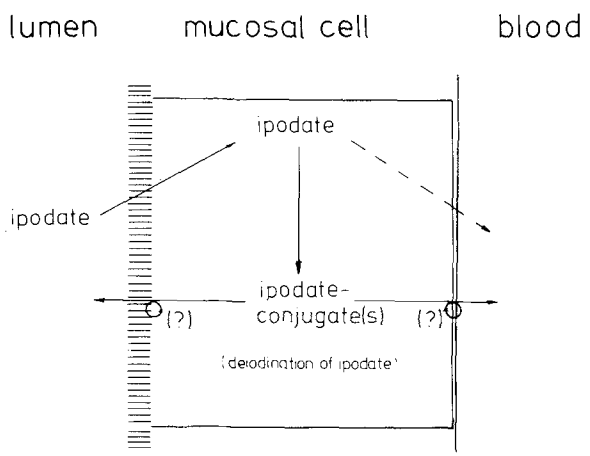

Fig. 9. Scheme of the transfer of ^{125}I-(ipodate-Na) across the intestinal wall of non-blood perfused jejunal segments in vitro of rats

compared with that in the absorbate may be the consequence of that preferential movement of I-ions to the mucosal side.

The formation of inorganic iodide from organic bound iodine in radioopaque substances must be deliberated on the basis of the results presented here de novo. In lower concentrations ($< 10^{-4}$ M) 7–10% of the ^{125}I-radioactivity was found as inorganic iodide precipitated by $AgNO_3$. In higher concentrations ($> 10^{-4}$ M) the amount of ^{125}I-radioactivity attributable to inorganic iodide falls to about 1%. In the literature the deiodination of radiocontrast agents is well-known (see McChesney, 1971). However, it is difficult to find reliable quantitative data. For rat and man the deiodination of ioglycamid is proven (Langecker et al., 1964). As was mentioned above for the mother compound of the entire series of radiocontrast agents, 2,3,5-triiodobenzoic acid deiodination was found in the rat by several authors (see above). If the amount of 1% deiodination found on isolated jejunal segments in vitro is representative also for the intestine in vivo a rather high amount of inorganic iodide is produced already during the process of absorption. When assessing the formation of inorganic iodide in the organism after the administration of organic bound iodine in radiocontrast agents it appears somewhat peculiar to refer to the concentration of inorganic iodide in urine only as is often made in literature (for ref. see McChesney, 1971). It is well-known that inorganic iodide is taken up into the thyroidal tissue rapidly and nearly completely. Assuming a splitting of 1% of the iodine-content of ipodate-Na as inorganic iodide during the absorption process then between 9–18 mg of inorganic iodide will be set free after the administration of 3–6 g of ipodate-Na which is the usual dose for diagnostic measures. The daily intake of inorganic iodide is recommended with 5 mcg. The amount of inorganic iodide possibly produced only during the absorption process is already the 1800–3600-fold of this amount.

Deiodination of radiocontrast agents is a serious problem in diagnostic procedures. This can be taken from the observation that it is impossible within at least 10 days up to several weeks and even months after the administration of a radiocontrast agent to obtain reliable data on the thyroidal function by testing the radio-iodine uptake by the thyroidal tissue (for ref. see McChesney, 1971). Therefore, it appears to be worthwhile to investigate experimentally the deiodination in the living organism in order to obtain quantitative data on this process as well as the information of the preferential locations for the deiodination processes of radiocontrast agents within the body.

References

Barker, W. M., Thompson, D. J., Ware, J. H.: Studies on the metabolism of 2,3,5-triiodobenzoic acid (TIBA). Fed. Proc. Fed. Am. Soc. Exp. Biol. **26**, 568 (1967)

Diamond, J. M.: Standing gradient model of fluid transport in epithelia. Fed. Proc. Fed. Am. Soc. Exp. Biol. **30**, 6–13 (1971)

Fisher, R. B., Parsons, D. S.: A preparation of surviving rat small intestine for the study of absorption. J. Physiol. (Lond.) **110**, 26–46 (1949)

Forth, W.: Resorption von Eisen und chemisch verwandten Metallen in vitro und in vivo; die Spezifität eines eisenbindenden Systems in der Mucosa des Jejunums von Ratten. In: Frontiers of nuclear medicine (W. Horst, Hrsg.), p. 83. Berlin, Heidelberg, New York: Springer 1971

Forth, W., Rummel, W.: Activation and inhibition of intestinal absorption by drugs. In: IEPT, Sect. 39b. Pharmacology of intestinal absorption: gastrointestinal absorption of drugs (Forth, W., Rummel, W., eds.), p. 171. Oxford: Pergamon Press 1975

Gutenmann, W. H., Bache, C. A., Lisk, D. J.: Fate of the plant regulator 2,3,5-triiodobenzoic acid (TIBA) in the bovine. J. Agric. Food Chem. **15**, 600–604 (1967)

Hartiala, K.: Metabolism of hormones, drugs and other substances by the gut. Physiol. Rev. **53**, 496–534 (1973)

Harwart, A., Kimbel, K. H., Langecker, H., Willenbrink, J.: β-(3-Dimethylamino - methylenamino - 2,4,6 - trijodphenyl) - propionsäure als Gallekontrastmittel. Naunyn-Schmiedeberg's Arch. Exp. Path. Pharmak. **237**, 186–193 (1959)

Josting, D., Winne, D., Bock, K. W.: Glucuronidation of paracetamol, morphine and 1-naphthol in the rat intestinal loop. Biochem. Pharmacol. **25**, 613–616 (1976)

Knoefel, P. K.: Binding of iodinated radiocontrast agents to the plasma proteins. In: Radiocontrast agents; IEPT, Sect. 76, Vol. I and II (P. K. Knoefel, ed.), pp. 133–145. Oxford: Pergamon Press 1971

Komp, B.: Intestinal absorption of ^{125}J-sodium-ipodate in rats. Naunyn-Schmiedeberg's Arch. Pharmacol. **293**, R44 (1976)

Komp, B.: Formation of glucuronides of ^{125}J-Na-Ipodate during the transfer across the isolated jejunal and ileal segments in vitro of rats. Naunyn-Schmiedeberg's Arch. Pharmacol. **297**, R2 (1977)

Komp, B.: Intestinale Resorption und metabolische Veränderung während des Resorptionsvorganges von ^{125}J-(Ipodat-Na) im Jejunum der Ratte. Inaugural-Dissertation; Fachbereich

Biochemie und Pharmazie, Johann Wolfgang Goethe-Universität, Frankfurt/Main 1978

Langecker, H., Harwart, A., Kolb, K.-H., Kramer, M.: Diglycolsäure-di-(3-Carboxy-2,4,4-trijodanilid) (Joglycamid), ein Kontrastmittel für intravenöse Cholangiographie. Naunyn-Schmiedeberg's Arch. Exp. Path. Pharmak. **247**, 493–508, (1964)

Lee, J. S.: Role of mesenteric lymphatic system in water absorption from rat intestine in vitro. Am. J. Physiol. **204**, 92–96 (1963)

McChesney, E. W.: The biotransformation of iodinated radiocontrast agents. In: Radiocontrast agents, IEPT Sect. 76, Vol. I and II (P. K. Knoefel, ed.), pp. 147–163. Oxford: Pergamon Press 1971

Moy, P., Ebert, A. G.: Studies on the metabolism of 2,3,5-triiodobenzoic acid. Fedn. Proc. Fedn. Am. Soc. Exp. Biol. **26**, 567 (1967)

Mudge, G. H.: Uricosuric action of cholecystographic agents. N. Engl. J. Med. **284**, 929–993 (1971)

Nell, G., Forth, W., Rummel, W., Wanitschke, R.: Pathway of sodium moving from blood to intestinal lumen under the influence of oxyphenisation and deoxycholate. Naunyn-Schmiedeberg's Arch. Pharmacol. **293**, 31–37 (1976)

Pfleger, K.: Methods for the investigation in animals. In: Pharmacology of intestinal absorption: Gastrointestinal absorption of drugs; IEPT, Sect. 39B, Vol. 1a, 2 (W. Forth, W. Rummel, eds.), pp. 809–811. Oxford: Pergamon Press 1975

Rosenstrauch, L. S.: Radiocontrast of the gall, bladder and the biliary ducts. In: Radiocontrast agents, IEPT, Sect. 76, Vol. I and II (P. K. Knoefel, ed.), pp. 237–260. Oxford: Pergamon Press 1971

Rummel, W., Stupp, F. H.: Der Einfluß von Kalium und Calcium auf die Salz-, Glukose- und Wasserresorption des isolierten Dünndarmes. Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol. **240**, 79–92 (1960)

Schulze, D., Kaps, H. P.: Gerinnungshemmende Wirkungen trijodierter Röntgenkontrastmittel. Arzneim. Forsch. (Drug. Res.) **27**, 972–975 (1977)

Smith, R. L.: Excretion of drugs in bile. In: Concepts in biochemical pharmacology. Part 1; Handbook of exper. pharmacol. XXVIII/1, (B. B. Broedie, J. R. Gilette, eds.), pp. 354–389. Berlin, Heidelberg, New York: Springer 1971

Sperber, I., Sperber, G.: Hepatic excretion of radiocontrast agents. In: Radiocontrast agents, IEPT, Sect. 76, Vol. I and II (P. K. Knoefel, ed.), pp. 165–235. Oxford: Pergamon Press 1971

Weiner, J. M.: Transport of weak acids and bases. In: Handbook of physiology, Sect. 8, renal physiology (I. Orloff, R. W. Berliner, S. R. Geiger, eds.), pp. 521–594. Washington, D. C.: American Physiological Soc. 1973

Wilson, T. H., Wiseman, G.: The use of sacs of everted small intestine for the study transferrence of substance from the mucosal to the serosal surface. J. Physiol. (Lond.) **123**, 116–125 (1954)

Wollenberg, P., Ullrich, V.: In vitro vasculary perfused small intestine of the mouse for the investigation of absorption and biotransformation of foreign compounds. Naunyn-Schmiedeberg's Arch. Pharmacol. **307**, R3 (1979)

Received June 22/Accepted December 11, 1979