dem dritten Tag nach der Infektion täglich gegeben, verhindert sie die Ausbildung eines lokalen Amoebenabszesses. Ausführliche Publikationen über tierexperimentelle, klinische und chemische Arbeiten erscheinen demnächst.

Summary. 5-[nitro-thiazolyl-(2)]-2-oxo-tetrahydroimidazole was found to possess schistosomicidal and amoebicidal properties. In mice this substance exhibited a curative effect in experimental infections with *S. mansoni* and *S. japonicum*. Preliminary clinical trials indicated that the compound is effective and well tolerated in the treatment of vesical bilharziasis.

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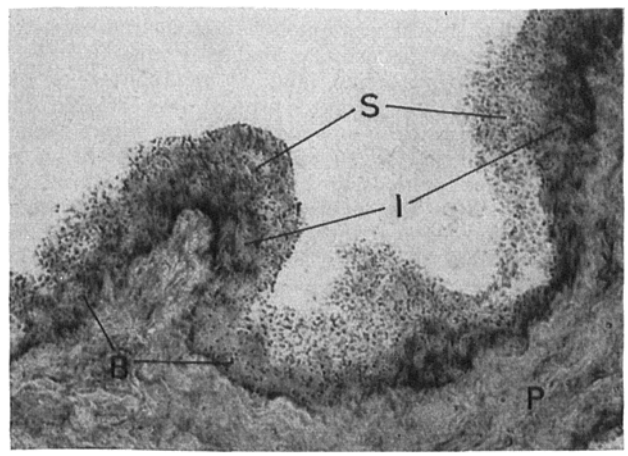
The Characteristic Appearance of Label in the Urinary Bladder Epithelium Following Injection of D,L-Tryptophan-H^3 in Mice

After injection of labelled tryptophan, considerable amounts of labels were reported to appear in the urine, since a variety of incompletely oxidized products were always produced in the metabolism of tryptophan (HENDERSON et al.[1,2], GREENBERG[3]). By means of radioautography we have recently observed that characteristically strong radioactivity appeared in the urinary bladder epithelium following injection of DL-tryptophan-H^3 in mice and have added some experiments about the origin and chemical nature of the labels.

Adult male mice were injected intraperitoneally with D,L-tryptophan-H^3 (generally labelled, 5 μc/g body weight, specific activity of 836 mc/m*M*, New England Corporation). The animals were sacrificed under ether narcosis 3, 5, 15 and 30 min and 1, 2, 4 and 24 h after the injection. The excised urinary bladders were fixed in 10% neutral formalin, embedded in paraffin, and cut into 10 μ thick sections. Following deparaffinization, both unstained sections and those stained with Harris hematoxylin and eosin were coated with Kodak AR 10 by the stripping method. After exposure for 2 weeeks, 1 and 2 months, the slides were developed and fixed. The unstained sections were stained with Giemsa solution after development and fixation. A grain count was made in the 3 types of epithelial cells, namely, layers of surface cells, intermediate cells and basal cells (WALKER[4]); lamina propria and tunica muscularis and numbers of grains were recorded as grains per 1000 μ^2.

Autoradiographic results showed that the surface cell layer contained moderate amounts of silver grains already 5 min after injection, maximal deposition of grains after 15 min (Figure 1), followed by a slight decrease from 2 h, moderate amounts being demonstrable after 24 h (Table I). The amorphous substance within the bladder cavity showed the marked deposition of silver grains. The layers of intermediate and basal cells of the epithelium revealed a moderate number of grains, which was always smaller in number than the surface cells each time, and showed similar but somewhat delayed increase in number as compared to the surface cell layer (Table I). This finding seems to suggest that some amounts of labels might move from the surface to the basal cell layer and further to the propria.

In order to investigate whether the label is derived from the urine or is secreted from the epithelium independent of the urine, the following experiment was performed. The mice, in which both ureters had been ligated just before the injection, were given the same dose of labelled tryptophan subcutaneously and were sacrificed after 10, 15 and 30 min. Complemental experiments already showed that subcutaneous injection of labelled tryptophan in the normal mice also produced marked deposition of label in the bladder epithelium similar to the intraperitoneal injection. As is clearly seen from Table II, the surface cell layer did not show any characteristic deposition of label as observed in the previous experiment, and contained rather less grains than the intermediate



Autoradiographic picture of the epithelium of the urinary bladder of the mouse injected with D,L-tryptophan-H^3 (5 μc/g body weight) 15 min before sacrifice. Large amounts of silver grains can be observed in the surface cell layers (S), smaller amounts in the intermediate (I), and basal (B) layers, and propria (P). × 470.

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Table I. Grain counts over the epithelium (surface, intermediate and basal cell layers), propria and muscularis after intraperitoneal injection of D,L-tryptophan (5 μc/g). The number of grains represents mean numbers per area of 1000 μ^2. Treatment included 'non' and 'TCA'; 'non' means non-treatment (normal ordinary section) and 'TCA' cold trichloracetic acid extraction before film coating. Exposure time 1 month

Time after injection	5 min		10 min		15 min		30 min		1 h	2 h	24 h
Treatment	non	TCA	non	TCA	non	TCA	non	TCA	non	non	non
Epithelium: surface cell	51	29	106	27	130	89	106	26	120	90	52
intermediate cell	30	39	94	31	57	58	78	27	61	83	44
basal cell	28	27	71	29	27	39	68	17	42	72	40
Propria	22	34	69	30	97	17	112	24	122	98	50
Muscularis	22	27	34	23	40	16	91	24	70	64	46

Table II. Grain counts over the epithelium, propria and muscularis of mice, in which both ureters were ligated and same doses of labelled tryptophan were injected subcutaneously. Exposure 1 month

Time after injection	10 min	15 min		30 min	
Treatment	non	non	TCA	non	TCA
Epithelium: surface cell	20	31	28	33	33
intermediate cell	32	37	28	48	38
basal cell	30	51	33	44	33
Propria	24	33	28	21	24
Muscularis	18	37	30	11	11

and basal cells and lamina propria. This observation seems to indicate that the labels at least within the surface cells might be ascribed to the labels contained in the urine.

Next, we wished to examine whether the radioactivity in the tissues of the urinary bladder is due to free or loosely bound tryptophan (and its metabolites) or the tryptophan synthesized into protein. We treated the formalin-fixed, paraffin-embedded sections with 5% cold trichloracetic acid for 10 min, with the intention of removing free or loosely bound tryptophan and its metabolites from the labels incorporated into protein. The result showed that the grains of the soft amorphous substance and of the surface cell layer decreased markedly in number by the extraction of cold trichloracetic acid (Table I). Accordingly, it is clear that moderate amounts of labels could remain in the state of free or loosely bound tryptophan and its metabolites in the tissue sections different from the current view of the radioautographic technique (WARSHAWSKY et al.[5]) and they were mainly demonstrable, especially in the surface cell layer. This might be ascribed to the fact that the epithelium of the bladder (especially surface cells) showed large amounts of mucopolysaccharides and alkaline phosphatase (MENDE et al.[6]). On the other hand, the labels appeared after ligation of both ureters did not show any appreciable changes after cold trichloracetic acid extraction (Table II), a finding which indicates the label to be incorporated into the newly formed protein.

Our observations that tryptophan and some of its metabolites may be adsorbed or absorbed in the surface cells of the urinary bladder epithelium from the urine, and remain there for some time, seem to be of some importance, because some tryptophan metabolites were reported to be concerned with the production of bladder cancer (BOYLAND et al.[7,8], BROWN et al.[9,10]). Therefore, it seems highly necessary to make the chemical identification of the labelled compounds which appeared especially in the surface cell layer of the epithelium by the biochemical method and further to elucidate the subcellular localization of the labels by the electronmicroscopic radioautography.

Zusammenfassung. Nach subkutaner oder intraperitonealer Injektion von D,L-Tryptophan-H^3 wurde radioautographisch eine charakteristische, starke Radioaktivität in den oberflächlichen Epithelzellen der Mausharnblase gefunden. Nach Unterbindung der beiden Ureter vor der Injektion unterblieb die meiste Radioaktivität. Die Resultate scheinen darauf hinzuweisen, dass die Radioaktivität auf dem im Harn ausgeschiedenen markierten Tryptophan oder auf seinen Abbauprodukten, die während einiger Zeit in oberflächlichen Epithelzellen adsorbiert und gespeichert wurden, beruht.

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