Oviducts in juvenile hormone-induced supernumerary larvae. Last instar larvae treated with juvenile hormone at day 0 undergo another (6th) larval molt leading to a supernumerary, giant larvae. The genital tracts of these larvae were dissected at day 1 and at day 10 (fig. 1). At day 1 the oviducts have definitely a larval form with an index L/W of 64 (fig. 2). At day 10 the index is 30, indicating an incomplete transformation. It is noteworthy that these animals have synthesized a new cuticle, but are unable to shed the old one. The new cuticle exhibits some adult characteristics. Its deposition testifies to the build-up of an ecdysteroid titer in supernumerary larvae. The incomplete oviductal transformation may be caused by persisting exogenous juvenile hormone, a (slight) activity of the corpora allata, a suboptimal ecdysteroid titer, a combination of these or still other factors.

The experiments have demonstrated unequivocally that transformation of the oviducts is not strictly linked to a developmental stage, i.e. 5th larval instar, but is regulated by specific hormonal signals: It is evoked by high ecdysteroid titer, but requires the absence of juvenile hormone. If these conditions are created prematurely (by precocene), or if they are postponed (by juvenile hormone treatment), oviductal transformation can be shifted to earlier or later stages in development. This is comparable with the hormonal regulation of larval-adult transformation of other tissues and organs[10].

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Binding of (^{125}I) triiodothyronine to human peripheral leukocytes and its internalization

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Summary. Ultrastructural autoradiography showed high specific binding of (^{125}I) triiodothyronine, as confirmed by a competition test, to plasma membranes, nuclei and mitochondria of human peripheral leukocytes. A high level of binding was also noted on the granulocytes' granules, especially in eosinophils.
Key words. Triiodothyronine; binding sites; ultrastructural autoradiography.

The binding of triiodothyronine (T_3) has been biochemically demonstrated in plasma membrane[1], cytosol[2], mitochondria[3] and nuclei[4], usually of rat hepatocytes. Autoradiographic studies confirmed T_3 localization in the nuclei and mitochondria, but not on the plasma membrane; clear-cut results regarding T_3 binding to different organelles were not obtained, probably due to differences in the methods and cell types used (e.g. hepatocytes[5-7], lymphocytes[8], lung alveolar cells[9], posterior hypophysis and median eminence cells[10]). We have used ultrastructural autoradiography to investigate T_3 binding and its intracellular distribution in human peripheral leukocytes, cells which can be easily obtained without damaging manipulations.

Material and methods. Human leukocytes from heparinized peripheral blood of euthyroid donors were obtained after 60 min of sedimentation at room temperature and 3 washings in Eagle's minimal essential medium (MEM) for 10 min at 200 × g. Two parallel sets of samples containing 15×10^6 leukocytes were incubated for 30 and 60 min at 37 °C in 1 ml either of MEM with 10^5 Bq (^{125}I) T_3 (sp. act. 44 TBq/g) or under the same conditions with a 50–100-fold excess of unlabeled T_3. Lymphocytes isolated according to Bøyum[11] were incubated under the same conditions as the leukocytes; control lymphocytes with a 200-fold excess of unlabeled T_3. All samples were washed 3 times for 10 min at 200 × g in MEM cooled to 4 °C, and fixed in 4% paraformaldehyde. Ultrathin sections from pellets embedded in Epon-Araldite were prepared by standard methods. The stability of T_3 binding was proved in a special set of samples. Autoradiograms were prepared after Williams[12a] using Ilford L4 emulsion, and developed physically and chemically. 103 experimental and 93 control autoradiograms of isolated lymphocytes were evaluated morphometrically by the circle method[12b] at a final magnification of 25,000 times.

Results. Autoradiograms of leukocytes exhibited binding on the plasma membrane (PM), mitochondria (M), tubulovesicular structures (TV), granules (G), lysosomes (L) and nuclei (N). In the eosinophilic granulocytes, a high level of binding appeared on eosinophilic granules (EG) (fig. 1). Control leukocytes displayed lower labeling than experimental cells.

In isolated lymphocytes, the binding was analogous to that of the leukocytes, i.e. over the plasma membrane, the cytoplasm and the nuclei (fig. 2). Morphometric analysis of the samples after 60 min of incubation proved non-random distribution of grains and specific binding in mitochondria, plasma membrane and nuclei. The intensity of the labeling of cell structures was as follows: M > PM > N > C/N border > other cytoplasmic structures (C).

Control lymphocytes displayed a lower labeling and morphometric analysis revealed another sequence of labeling intensity: C > M > C/N border > N > PM (table).

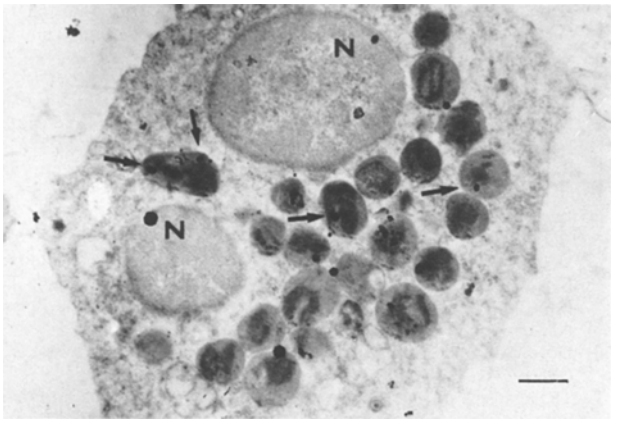

Figure 1. Human eosinophilic granulocyte incubated 30 min with (^{125}I)T_3. Physical developing leads to a reduction of metallic silver around the point of radioactive activation in emulsion and formation of electron dense grains. Label predominantly on eosinophilic granules (arrows) and nucleus (N); bar 1 μm.

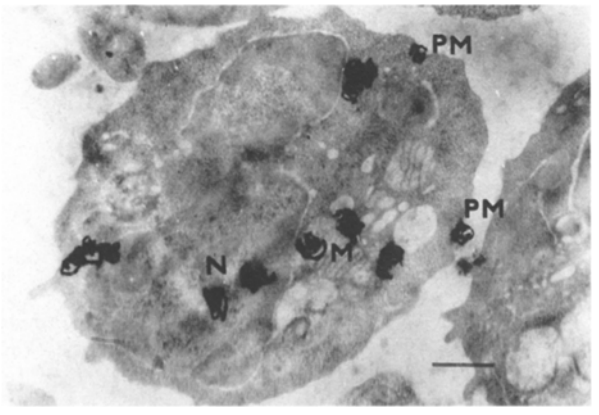

Figure 2. Human peripheral lymphocyte incubated 60 min with (^{125}I)T_3. Chemical developing leads to a reduction of silver crystals = large and characteristic grains. Label, which can be easily distinguished and is suitable for circle analysis on plasma membrane (PM), mitochondria (M), nucleus (N) and other structures; bar 1 μm.

Discussion. Controversial findings regarding T_3 binding in different cell structures can be avoided by the use of intact living cells for autoradiography. Dispersed hepatocytes have been successfully used[6], but enzymatic dispersion of the tissue could have destroyed the plasma membrane receptors. In human leukocytes from peripheral blood, we obtained isolated cells with minimal interference. In these cells, we were able to prove T_3 binding on the plasma membrane and thus add support to previous biochemical findings in other cell types. We saw specific T_3 binding on the eosinophilic granules of granulocytes, which had not been observed previously. We tried also to strenghten our results morphometrically, an approach hitherto not attempted in comparable experiments. We chose to use lymphocytes, because these cells had already been biochemically analyzed and a comparison of autoradiographic and biochemical approaches was possible. However, we are aware that the number of grains available for this analysis lies near the lowest values permitted. The difference observed between cells without and with competition points to the importance of binding on the plasma membrane (decreased by competition by 44%), mitochondria (38%), and nuclei (33%). The order of binding on different cellular structures with competition may indicate the capacity of the binding. We suppose that these findings indicate that binding to the plasma membrane may play a role in T_3 internalization.

Analysis of grain distribution in autoradiographs of human peripheral lymphocytes incubated 60 min with (^{125}I)T_3 (a), and with (^{125}I)T_3 and 200-fold excess of unlabeled T_3 (b)

Items	% of real grains		% of hypothetical grains		X^2		SA	
	a	b	a	b	a	b	a	b
N	30.50	20.25	18.92	19.69	56.79	0.36	1.61	1.08[c]
C	10.50	26.82	10.35	12.05	0.02	51.96	1.01	2.22
M	9.50	7.67	3.66	4.72	74.49	5.27	2.59	1.62[c]
N/C border	15.38	13.58	13.45	10.21	2.20	3.21	1.14	1.33
PM	21.63	14.63	13.26	16.12	42.18	0.39	1.63	0.91[c]
Ery, platelets	9.50	12.54	10.96	12.36	1.56	0.01	0.87	1.01
Extracellular	3.00	3.48	29.40	24.85	189.65	52.70	0.10	0.14
Total X^2					366.89	113.90		

a) 800 real grains counted, 3605 hypothetical grains calculated; b) 287 real grains counted, 3220 hypothetical grains calculated; C, cytoplasm without mitochondria and plasma membrane; M, mitochondria; N, nucleus; PM, plasma membrane; Ery, erythrocytes; SA, specific activity (% of real grains/% of hypothetical grains); [c], 0.001 > p (Student's t-test and f-test used), grain distribution highly non-random and differences between a and b significant on 0.1% level.

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