findings suggest that the reticuloendothelial system is involved in the endotoxin-induced termination of immunologic unresponsiveness. Unlike endotoxin, however, zirconium does not cause such a termination even though it produced splenic hyperplasia indicative of phagocyte proliferation. The ethyl stearate decreased the average weight of the spleens (10–12 spleens) to a near-normal level and there was no evidence that ethyl stearate was cytotoxic, judging from trypan blue tests for viability of the peritoneal phagocytes.

In earlier work by one of us (S.M.) it was shown that endotoxin could cause increased resistance to challenge with viable *D. pneumoniae* even when the mice were immunologically paralyzed to the capsular antigen[7]. Two interpretations were possible: (a) the resistance was independent of the immunologic state, and did not, therefore, require antibody for expression, and (b) the observed effect was due to a conversion of the paralytic state to the immune state by the endotoxin. The present experiments cannot decide between the 2 alternatives, since resistance to *D. pneumoniae* may also involve antigens other than the capsular polysaccharide antigen[8]. But the data do extend the findings of BROOKE, who observed termination by endotoxin in similarly paralyzed animals. Unlike the findings by that author, the titers in the present experiments are high, and suggest an active synthesis of antibody rather than a release from antibody-forming or carrying cells by a cyto-allergic mechanism. Further, they show that a blockade of the phagocytic cells of the peritoneal cavity decreases the capacity of endotoxin to produce the conversion. They also suggest that the mechanism by which the effect is brought about is through the antigenicity of endotoxin, since the similarly acting zirconium did not cause the conversion[9].

Zusammenfassung. Immuntolerante Mäuse können durch Endotoxin in immunologisch reaktive Tiere verwandelt werden. Eine zu wiederholten Malen verabreichte Substanz (Äthylstearat) hemmt die Umwandlung.

S. MARGHERITA and R. A. PATNODE

Department of Microbiology, University of Oklahoma Medical School, Oklahoma City (Oklahoma, USA), 23 October 1967.

S. MARGHERITA and H. FRIEDMAN, J. Bact. *89*, 277 (1965).
[8] C. A. NEEPER and C. V. SEASTONE, J. Immun. *91*, 378 (1963).
[9] This work was supported by PHS Training Grant No. 5T1AI162-05.

Two Different Iodinating Systems in Isolated Thyroid Cells

The cellular site of iodide-binding to thyroglobulin in the thyroid has been a much discussed topic between biochemists and morphologists[1]. Arguments have been presented for its location in the follicular lumen[2–4], in the thyroid cell[5–7] and at the cell colloid interface[4–8]. NUNEZ et al.[9] have suggested the existence of 2 iodination sites: 1 in the follicular lumen for the iodination of preformed molecules, the other in the thyroid cell for the iodination of newly synthesized molecules. In this communication we report evidence of the existence of 2 iodinating systems in isolated thyroid cells.

Suspensions of isolated sheep and horse thyroid cells were prepared by means of an adaptation of the continuous flow trypsinization technique of TONG[6–10]. The incubation procedure, and the collection of isolated cells, after the incubation, have been described previously[10]. The duration of the incubation was 2 h for the measurements of iodide uptake and iodide organification, and 6 h for the measurements of ^{125}I iodide incorporation in the iodoamino acids of proteins. For the latter experiments, the incubation medium contained ^{125}I iodide 4 μM (specific activity 750 C/M). Iodide trapping was evaluated by the C/M ratio, where C and M are the radioactivities of ^{131}I iodide in identical volumes of cells and medium[6–10]. At $10^{-7} M$, the C/M ratios were about 10 for horse cells and about 40 for sheep cells. Iodide organification was measured by the incorporation of ^{131}I iodide in the twice washed 10% trichloracetic acid precipitate of the cells. Incorporation of ^{125}I iodide into iodothyronines was calculated from the radioactivity of the cell homogenate and from the relative radioactivities of the iodothyronine spots on chromatograms of the cells hydrolysates (chromatographic systems: tertiary isopentanol-NH_4OH 2.5 N, and n-butanol-ethanol-NH_4OH 0.5 N (5:1:2))[10]. When results of separate experiments were pooled, the means, the standard deviation of the mean, and the Student's t-values were calculated from the common logarithms of these results. In this case, results are expressed as antilogarithms of the means and of the means $\pm$ the standard deviation of the mean[11]. Thyrotropin (thytropar) and twice crystallized bovine liver catalase were obtained respectively from Armour (Kankakee, USA) and Sigma (Saint Louis, USA).

$NaClO_4$ 2 mM completely inhibited the trapping of iodide by isolated thyroid cells at all the concentrations of iodide which were used (C/M = 0.8). The iodide organification which proceeds in the presence of perchlorate may therefore be called 'organification independent' of iodide trapping while the organification which is suppressed by $NaClO_4$ may be called 'organification dependent' on iodide trapping. Above concentrations of iodide of $10^{-6} M$ for horse thyroid cells and of $10^{-7} M$ for sheep thyroid cells, the addition of $NaClO_4$ did not depress iodide organification; i.e. the organification of iodide was independent of iodide trapping; below these concentrations the organification of iodide was partially inhibited by $NaClO_4$, i.e. partially dependent on iodide uptake.

'Dependent' and 'independent' ^{131}I iodide organification have been studied in horse isolated thyroid cells for

[1] L. J. DEGROOT, New Engl. J. Med. *272*, 243 (1965).
[2] N. J. NADLER and C. P. LEBLOND, Brookhaven Symp. Biol. *7*, 40 (1955).
[3] S. H. WOLLMAN and I. WODINSKY, Endocrinology *56*, 9 (1955).
[4] O. STEIN and J. GROSS, Endocrinology *75*, 787 (1964).
[5] L. J. DEGROOT and E. CARVALHO, J. biol. Chem. *235*, 1390 (1960).
[6] W. TONG, P. KERKOF and I. L. CHAIKOFF, Biochim. biophys. Acta *60*, 1 (1962).
[7] R. PITT-RIVERS, J. S. F. NIVEN and M. R. YOUNG, Biochem. J. *90*, 205 (1964).
[8] I. PASTAN, Endocrinology *68*, 924 (1961).
[9] J. NUNEZ, J. MAUCHAMP, V. MACCHIA and J. ROCHE, Biochim. biophys. Acta *107*, 247 (1965).
[10] F. RODESCH and J. E. DUMONT, Expl Cell Res. *47*, 386 (1967).
[11] J. E. DUMONT, Bull. Soc. Chim. biol. *46*, 1131 (1964).

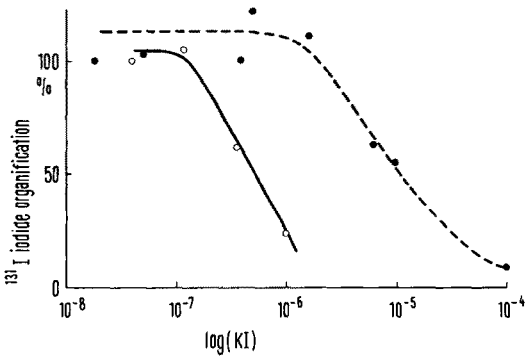
Action of TSH and catalase on ^{131}I iodide organification by isolated sheep thyroid cells

	Iodide organification			
	Total A KI: $5.10^{-8}M$	'Independent of iodide trapping' extracellular B KI: $5.10^{-8}M$ $NaClO_4$ $2.10^{-3}M$	'Dependent on iodide trapping' intracellular C = A − B	Extracellular with high levels of KI KI: $2.6.10^{-5}M$
Control	106,500 ± 2,500	42,600 ± 1,400	63,900	11,200 ± 1,200
TSH (10 mu/ml)	174,000 ± 4,000	68,900 ± 5,100	105,100	22,200 ± 1,100
Catalase (0.2 mg/ml)	90,500 ± 500	13,800 ± 500	76,700	2,100 ± 1,800

Results are measured in cpm in $PB^{131}I$/flask (2 ml) and expressed as means ± range of the duplicates (2). $PB^{131}I$: protein bound radio ^{131}I iodine.

increasing concentrations of iodide in the medium (Figure). 'Dependent ^{131}I iodide organification' was depressed above $10^{-7}M$, whereas 'independent ^{131}I iodide organification' was depressed above $10^{-6}M$, indicating that the 2 organification systems were saturated for different levels of iodide concentration in the medium. As radioiodide trapping decreased only above the concentration of $2.10^{-5}M$, the depression of ^{131}I iodide organification reflects the saturation of the organification systems and not of the trapping mechanism. These experiments show, therefore, that in isolated cells, 2 different organification systems, with different saturating levels of medium iodide concentration, exist. As for low concentrations of iodide, 'independent organification' was of the same order of magnitude as 'dependent organification', simple diffusion of iodide to a common iodinating system cannot account for 'dependent organification'.

The simplest explanation for the existence of the 2 organification systems, was that the 'dependent organification' would be intracellular, while the 'independent organification' would be extracellular. In such a case, one might expect catalase, an enzyme inhibiting organification[1-5], to depress the extracellular 'independent organification', but not the intracellular 'dependent organification'. The experiments confirmed this expectation (Table). However, the data do not permit any conclusion on the true saturating levels of the intracellular iodinating system, as the intracellular concentration of iodide was higher than the concentration of the medium.



Effect of iodide concentration in the medium on iodide organification by isolated horse thyroid cells. Results are expressed in % of ^{131}I iodide organification at $1.8 \times 10^{-8}M$ KI. o——o ^{131}I iodide organification suppressed by $2 \times 10^{-3}M$ $NaClO_4$ = 'organification dependent on iodide trapping' (intracellular). ●---● ^{131}I iodide organification in the presence of $2 \times 10^{-3}M$ $NaClO_4$ = 'organification independent of iodide trapping' (extracellular).

'Extracellular iodide organification' by isolated cells could be artefactual. For instance, it could be accounted for by broken cells with accessible intracellular iodide organification systems. Thyrotropin (TSH) stimulated the organification of ^{125}I iodide by isolated sheep thyroid cells in the presence of $NaClO_4$ 2 mM and iodide 4 μM, i.e. under conditions in which only 'extracellular organification' takes place. The effect was maximal at a concentration of 1 mU/ml of TSH: 150% of the control (142–159), $P < 0.001$. Analysis of the protein bound radioactivity of the cells evidenced ^{125}I thyroxine (15.5%) and ^{125}I triiodothyronine (3.4%) in stimulated and unstimulated cells. The stimulation of ^{125}I iodide incorporation into the iodothyronines of the proteins was of the same order of magnitude as the total stimulation of iodide organification. Moreover, when 'intracellular' and 'extracellular' organification were allowed to proceed, both systems were stimulated to the same extent by TSH (Table). Homogenates of thyroid bind iodide to proteins. However, this organification is not stimulated by TSH[1]. Furthermore, unfortified homogenates do not incorporate iodide into the iodothyronines of proteins[1-5]. These experiments therefore argue strongly against the hypothesis that the 'extracellular' organification might be due to broken cells.

The existence of 2 organification sites in isolated thyroid cells suggests a similar organification in vivo. Studies on thyroid subcellular preparations may allow the identification of 2 organification systems with different characteristics. These results were confirmed with purified TSH (15 U/mg; gift of P.G. Condliffe)[12,13].

Résumé. Deux systèmes distincts d'iodination ont été mis en évidence dans des préparations de cellules isolées de thyroide. Les résultats expérimentaux obtenus suggèrent une localisation intracellulaire à l'un des systèmes et extracellulaire à l'autre.

F. Rodesch[14], A. Jortay and J. E. Dumont

Department of Obstetrics, Bordet Institute and Laboratory of Nuclear Medicine, School of Medicine, University of Bruxelles, and Department of Biology, Euratom, Bruxelles (Belgium), 28 September 1967.

[12] Acknowledgments. The authors wish to thank Mr. A. Melis for his technical assistance and Miss Ch. Borrey for the preparation of the manuscript.

[13] Work realized under contract Euratom/Universities of Brussels and Pisa No. 026-63-4 BIAC (contribution No. 337 of Euratom Biology Division) and supported by grant No. FRFC 22 of the Fonds del a recherche fondamentale collective.

[14] Aspirant of the Fonds national de la recherche scientifique.