of the yolk synthesized inside the ooplasma is initiated by follicular cells itself and another part comes from the hemolymph. In *Hydrophilus olivaceus,* the nucleolar origin of RNA and its stable nature suggest that this is rRNA which enters into the formation of yolk bodies, as described earlier for the ovary of *Musca* by Bier[4].

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IgG purification to measure the level of an iodinated thyroglobulin peptide, the 3,5,3′,5′ tetraiodo-l-tyrosyl-l-tyrosine in human serum[1]

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Summary. Antibodies reacting with 3,5,3′,5′ tetraiodo-l-tyrosyl-l-tyrosine (I_2Tyr-I_2Tyr) were elicited in rabbits by immunization with an oxidized yeast conjugate coupled with I_2Tyr-I_2Tyr. Ion-exchange chromatography was used to purify immunoglobulins, in order to improve the specificity in measurement of I_2Tyr-I_2Tyr level in patient serum. IgG binding capacity versus I_2Tyr-I_2Tyr was considerably increased after immunoglobulin purification.
Key words. Thyroglobulin, iodinated; IgG purification; tetraiodo-l-tyrosyl-l-tyrosine.

I_2Tyr-I_2Tyr has been isolated from bovine thyroglobulin tryptic digests[3] and identified in thyroglobulin from other animals. Thyroglobulin, and some of the products of its proteolytic cleavage, are known to enter the circulating blood in some pathological thyroid diseases. Therefore, evidence was needed to determine whether or not I_2Tyr-I_2Tyr was actually present in the blood of euthyroidian and dysthyroidian subjects. To this end, we needed a precise and sensitive measurement for the presence of this compound in human serum and developed a technique of radioimmunoassay of I_2Tyr-I_2Tyr. The first attempts to produce I_2Tyr-I_2Tyr antibodies were carried out using oxidized yeast coupled with I_2Tyr-I_2Tyr, and were carried out in rabbits. This hapten-carrier immunogen was produced by the technique described by Bernard et al.[4]. After periodate oxidation, the yeast cell wall produces reactive aldehyde groups that react with side-chain amino group of I_2Tyr-I_2Tyr. The coupling is stabilized, and nonreactant aldehydes are blocked by reduction with sodium borohydride. The antisera thus obtained showed that this iodinated fraction of thyroglobulin is a normal constituant of human serum at a very low concentration. In order to determine its biological and physiological significance in human serum, it is necessary to purify the antisera. So the fractionation of immunoglobulin classes was performed to improve the measurement.

Materials and methods. I_2Tyr-I_2Tyr was synthesized and labeled with ^{125}iodine with a sp. act. of 1200–1500 μCi/μg at INSERM U 71 by the technique described by Maurizis et al.[5]. The yeast *Saccharomyces cerevisiae* was supplied by Gallier Laboratories (Paris, France). Before oxidation, the yeast was washed with a 0.05 M phosphate-buffered saline (PBS) (pH 7.2) containing 0.1 M NaCl, then with a 0.2 M HCl glycine buffer (pH 2.5), and finally with PBS.

For coupling I_2Tyr-I_2Tyr to oxidized yeast, 0.5 g yeast was oxidized in 500 ml 1 N HCl containing 0.1 M sodium-periodate ($NaIO_4$) (Merck). The reaction medium was stirred for one hour at room temperature. The yeast was then centrifuged at 2000 × g, extensively washed with PBS, centrifuged and resuspended with a Potter-Elvehjem homogenizer in 10 ml of absolute ethanol. 20 mg I_2Tyr-I_2Tyr was incubated for 20 h with 0.5 g oxidized yeast in 10 ml of absolute ethanol. The coupled yeast was centrifuged at 2000 × g, washed with 0.1 N NH_4OH to eliminate the unbound I_2Tyr-I_2Tyr, washed with H_2O and then with 0.04 M barbital buffer (pH 8.4) and resuspended with a Potter-Elvehjem homogenizer in 500 ml of the same buffer. Reduction with 1% sodium borohydride ($NABH_4$) (Sigma) was carried out under magnetic stirring in 500 ml barbital buffer (pH 8.4), for one hour at room temperature. The yeast was centrifuged, extensively washed in PBS and resuspended with a Potter-Elvehjem homogenizer in 100 ml of the same buffer. The amount of I_2Tyr-I_2Tyr bound to yeast, after coupling, was 9.3 mg/g yeast[4].

Immunization. 10 New Zealand male rabbits were immunized. They received an i.v. injection of 5 mg of yeast coupled to I_2Tyr-I_2Tyr suspended in 1 ml PBS daily, for 5 consecutive days. The 5-day immunization cycle was carried out twice with a week-long rest period between each cycle. 1 month later, the rabbits received two booster injections of 5 mg yeast: an i.m. injection followed by an i.v. injection 6 h later. 1 week after the last injection, blood samples were taken.

Immunoglobulin fractionation was performed using DEAE-Sephacel (Pharmacia). Immunoglobulin fractionation[6] with DEAE-Sephacel, which separates IgG, IgA, and IgM classes was preferred to antibody affinity purification which separates only the IgG class from other serum proteins. 3 ml of rabbit serum previously dialyzed against PBS was eluted from DEAE-Sephacel with 30 ml of 0.0175 M phosphate buffer (pH 6.8), 40 ml of 0.08 M phosphate buffer (pH 6.6) and 40 ml of 0.3 M phosphate buffer (pH 6.5). The OD was measured at 280 nm in each eluted fraction. The top of each peak was concentrated to OD 280 nm = 10.

Binding capacity measurement. 5×10^{-12} g of $^{125}I_2$Tyr-$^{125}I_2$Tyr (about 45,000 dpm) dissolved in 0.1 ml of 0.05 M phosphate

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buffer (pH 7) was incubated in hemolysis tubes with 0.1 ml anti-I_2Tyr-I_2Tyr antiserum, or 0.1 ml of immunoglobulins purified on DEAE-Sephacel, at dilutions varying from 1:10 to 1:5000, and 0.1 ml of 0.05 M phosphate buffer (pH 7) containing 5% ligand-free rabbit serum, in order to eliminate any I_2Tyr-I_2Tyr present in the normal rabbit serum. This ligand-free serum was prepared as follows: 1 ml rabbit serum was added to 200 mg cationic resin: DOWEX 50 × 2 (BIORAD) in 4 ml of 0.05 M phosphate buffer (pH 7). This mixture was stirred for 3 min and centrifuged at 2000 × g for 5 min. The ligand-free rabbit serum was essential for quantitative antigen-antibody association. It could not be successfully used if the protein concentration of the reaction medium was too low. In such cases the reaction medium was cooled to 4 °C for 30 min and the free hapten separated from the bound by adding 500 µl of a suspension containing 5 mg charcoal Norit A (Serva) and 50 µg dextran T_{10}/ml (Serva). The suspension was centrifuged for 10 min at 2000 × g. Radioactivity of the supernatant containing the bound hapten was measured by a NaI (Tl) crystal gamma counter. The protein concentration of each fraction was measured according to Lowry et al.[7]. The bound hapten was computed per g of protein.

Affinity constants using Scatchard's method with whole I_2Tyr-I_2Tyr antisera and purified immunoglobulins were measured at dilutions (1:500 to 1:5000) such that 50% of a hapten labeled with 1200–1500 µCi/µg would be bound to the antibodies. The reaction medium contained 5–440 × 10^{-12} g of $^{125}I_2$Tyr-$^{125}I_2$Tyr, and a sample of whole antiserum or purified immunoglobulin dilutions dissolved in 400 µl of a 0.05 M PBS buffer containing 8% ligand-free rabbit serum prepared as previously described. The assay was carried out in triplicate. The tubes were incubated for 90 min in a 37 °C stirred water bath, then cooled to 4 °C in ice. Bound and free hapten fractions were separated as described above. The same incubations were carried out simultaneously without antisera to measure the amount of labeled hapten nonspecifically bound to serum proteins. The bound/free ratio was plotted as a function of the hapten concentration according to Scatchard's method[8]. The slope of the graph gave the affinity constant (K_a). Figure 1 shows the Scatchard[8] analysis of the purified immunoglobulins eluted in the first fraction on DEAE-Sephacel, from the L_{10} antiserum.

Table 1. Binding capacity of whole antisera and of purified immunoglobulins to I_2Tyr-I_2Tyr after ion exchange chromatography on DEAE Sephacel of antisera from rabbits L_1, L_3, L_4 and L_{10}

Rabbits	Fractions	Binding capacity in g I_2Tyr-I_2Tyr/g protein
L_1	Whole antiserum	0.56×10^{-10}
	Fraction 1	1.2×10^{-6}
	Fraction 2	3.4×10^{-7}
	Fraction 3	1.2×10^{-8}
L_3	Whole antiserum	0.93×10^{-11}
	Fraction 1	1.15×10^{-5}
	Fraction 2	3×10^{-6}
	Fraction 3	5.3×10^{-7}
L_4	Whole antiserum	0.80×10^{-11}
	Fraction 1	1.5×10^{-6}
	Fraction 2	2×10^{-7}
	Fraction 3	2×10^{-8}
L_{10}	Whole antiserum	0.68×10^{-7}
	Fraction 1	3.1×10^{-5}
	Fraction 2	3.7×10^{-6}
	Fraction 3	7.9×10^{-8}

Table 2. Affinity constants (Scatchard's method) of antisera and their purified immunoglobulins from rabbits L_1, L_3, L_4 and L_{10}

Rabbits	Fractions	Dilution	Affinity constant K_a (M^{-1})
L_1	Whole antiserum	1:1000	1.2×10^{12}
	Fraction 1	1:100	2×10^{12}
	Fraction 2	1:100	1.3×10^{12}
L_3	Whole antiserum	1:500	2.87×10^{13}
	Fraction 1	1:500	1.54×10^{13}
	Fraction 2	1:500	2.12×10^{13}
L_4	Whole antiserum	1:1000	9.2×10^{10}
	Fraction 1	1:10	1.04×10^{12}
	Fraction 2	1:10	5.12×10^{12}
L_{10}	Whole antiserum	1:5000	6.2×10^{13}
	Fraction 1	1:500	1.22×10^{13}
	Fraction 2	1:1000	1×10^{13}

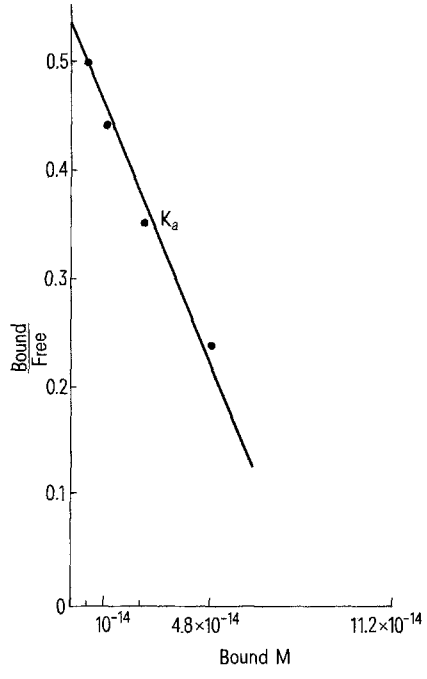

Figure 1. Scatchard's analysis in fraction 1 of purified immunoglobulins eluted on DEAE-Sephacel, from L_{10} antiserum. The bound/free ratio was plotted as a function of the hapten concentration. The slope of the graph gave the affinity constant.

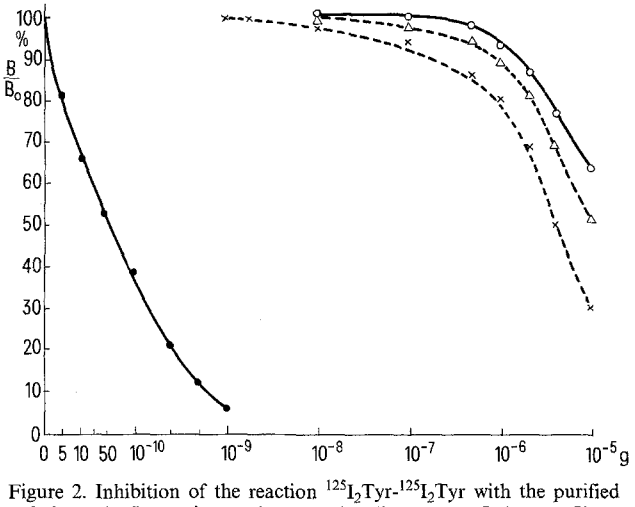

Figure 2. Inhibition of the reaction $^{125}I_2$Tyr-$^{125}I_2$Tyr with the purified IgG from the L_{10} antiserum by: ●, I_2Tyr-I_2Tyr; ×, I_2Tyr; △, ITyr; ○, T_4 or T_3.

Cross reactivity of purified IgG from anti-I_2Tyr-I_2Tyr antisera. 5×10^{-12} g I_2Tyr-I_2Tyr in 0.1 ml of 0.05 M phosphate buffer (pH 7) was incubated in hemolysis tubes with 0.1 ml of purified IgG from L_{10} antisera at the dilution 1:5000, 0.1 ml of 0.05 M phosphate buffer (pH 7) containing 5% ligand-free rabbit serum, and 0.1 ml of 0.05 M phosphate buffer (pH 7) containing 5×10^{-10} to 5×10^{-8} g of thyroxine (T_4), 3,5,3′ triodo-l-tyrosine (T_3), 3-iodo-l-tyrosine (ITyr) or 3,5-diiodo-l-tyrosine (I_2Tyr). The incubation and the separation of the free antigen from the bound antigen were performed as described previously.

Results. Binding capacity was measured on whole antisera. Four rabbits (L_1, L_3, L_4 and L_{10}) gave significant amounts of anti (I_2Tyr-I_2Tyr) antibodies. The immunoglobulins were separated by elution on DEAE-Sephacel from these four whole antisera. Binding capacity was measured for each class of immunoglobulin. The values given in table 1 are expressed in g of I_2Tyr-I_2Tyr per g protein. The binding capacity for the whole antisera ranges from 0.93×10^{-11} for the rabbit L_3 to 0.68×10^{-7} for the rabbit L_{10}. The difference reaches 10^4. The 1st fraction obtained from DEAE-Sephacel with each antiserum shows the best binding capacity for I_2Tyr-I_2Tyr. The 2nd fraction has a weaker binding capacity. The 3rd fraction does not bind specifically to the I_2Tyr-I_2Tyr. Table 2 shows the affinity contants (K_a) for different antisera, which range from 9.2×10^{10} (M^{-1}) for rabbit L_4 to 6.2×10^{13} (M^{-1}) for L_{10}. The difference is about 3×10^3 (fig. 1). Cross-reactivity with other iodinated amino acids was measured. The specificity of the antibodies was determined by measuring the displacement of the binding of the $^{125}I_2$Tyr-$^{125}I_2$Tyr to the immunoglobulins by nonlabeled I_2Tyr-I_2Tyr, I_2Tyr, T_4, T_3 and ITyr. Results are expressed in figure 2. So, more (10^5) I_2Tyr had to be added and more (10^6) ITyr, T_4 or T_3 than the nonlabeled dipeptide to reach the same inhibition level.

Discussion. The difference in the binding capacity in g of I_2Tyr-I_2Tyr per g protein reaches 10^4, between the four whole antisera tested. This corresponds to the heterogeneity obtained in the response when rabbits are immunized. However, the purification of immunoglobulins by ion exchange chromatography on DEAE-Sephacel allows a considerable increase in the binding capacity of IgG to I_2Tyr-I_2Tyr. These results are obtained for the four antisera. L_3 and L_{10} antisera have the best binding capacity in the IgG fraction, about 10^{-5} of I_2Tyr-I_2Tyr per g protein. The second fraction obtained on DEAE-Sephacel contains IgG, IgA and serum albumin. The third fraction contains the IgM. These two last fractions have a lower binding capacity with I_2Tyr-I_2Tyr than the first fraction which corresponds to most of the eluted IgG. The affinity constants obtained for whole antisera and for the first and second immunoglobulin peaks eluted on DEAE-Sephacel were identical. The whole antisera affinity constants seem to correspond to the IgG eluted in peak 1, and, secondly, to the IgG eluted in peak 2. An affinity constant from the third peak could not be determined because the eluted IgM had too weak a binding capacity with I_2Tyr-I_2Tyr. The antisera obtained by use of this original carrier were very specific for the I_2Tyr-I_2Tyr and the purification of immunoglobulins improves their specificity. Displacement studies using iodothyronines and iodotyrosines have not shown cross-reactivity that would be incompatible with the dosing of this hapten in biological media, where this compound is present in low concentrations, as opposed to the I_2Tyr, ITyr and iodothyronines.

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Lectin receptors in snail proteogalactans. II. *Archachatina marginata* and *Achatina achatina* (preliminary report)

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Summary. The lectin receptor sites on the proteogalactans from the albumin glands of West African land snails, *Archachatina marginata* and *Achatina achatina* have been studied by precipitin reactions using the agar-gel double diffusion technique with various lectins. The proteogalactans from both snails have predominantly terminal β-D-galactose structures; but they show characteristic differences in the topographical features at the surfaces of the carbohydrate structures presumed to be compatible with the combining site for these lectins.

Key words. Snail, land; *Archachatina marginata; Achatina achatina;* albumin gland; lectin receptors; proteogalactans.

Different receptors for heterophile lectins of plant, invertebrate and microbial origin have been identified on the proteogalactans extracted from the albumin glands of the snail *Achatina fulica*[1-3]. Most of these lectins are galactose specific[4]. This paper reports preliminary investigations on the proteogalactans from other related snail species, *Archachatina marginata* and *Achatina achatina.*

The proteogalactans were extracted from the albumin gland tissue with saline and 4 M guanidinium hydrochloride, then fractionated as described earlier[5]. The results of agarose double-diffusion experiments with lectins and the semi-purified proteogalactans are shown in the table.

Strong precipitin lines were formed by the lectins from *Bauhinia purpurea* alba seeds and peanut, against the proteogalactans from both snail species. The precipitin lines exhibited close identity indicating similar combining sites for these lectins. Whereas, the disaccharide D-Gal β(1→3) D-GalNAc has been considered to be the dominant receptor structure for the peanut lectin[6], the structure, D-Gal β(1→3) D-GalNAc β(1→3) D-Gal was proposed for the *Bauhinia purpurea* lectin[7].