The Influence of Iodine-Labelling on the Non-Specific Fixation of Immunoglobulins to Tissues

Immunoglobulin affinity for tissues is often measured in immunopathological studies. The specific antibody activity may be difficult to distinguish from a non-specific background, in which several physico-chemical factors play a role[1]. It is known[2] that trace-iodination does not affect antibody function if no more than 2 iodine atoms per molecule are substituted. In the present paper, we study whether the process of labelling, as in radioautography and other techniques, alters the immunoglobulins (Ig) so that their non-specific affinity for tissues would be increased.

Seven samples of Ig from 7 normal donors were iodized, each by 4 different methods simultaneously. The 4 preparations from the same sample were then studied to see if they showed the same non-specific affinity. This property was determined by measuring the proportion of labelled Ig able to adhere to a lyophilisate of homologous liver. In complementary experiments, synthetic resins were substituted for the liver powder; in these experiments, the non-specific affinity could also be determined by an elution method without previous labelling.

Methods. Tissue preparation. Normal human liver, obtained from the Department of Surgery, was homogenized and lyophilized.

Immunoglobulin preparation. 2 ml serum samples were obtained from 7 normal human donors. The Ig were isolated from each sample by Rivanol treatment[3]. Each batch of Ig was controlled by immunoelectrophoresis and its concentration measured by microkjeldahl.

Iodination. Each of the 7 batches of Ig was labelled simultaneously by the 4 following techniques. In each technique, 1 mg of protein was labelled in the cold; unbound iodine was removed by dialysis; free iodine remaining after dialysis was determined by $ZnSO_4$ precipitation and in no case was more than 2% of the total radioactivity found. The yield, as well as the number of I atoms/molecule, was calculated. In *technique 1* (modified after [4]), a mixture containing the 1^{125}, KI 0.001 mEq, HCl 0.4 mEq and $NaNO_2$ 0.01 mEq was added to the Ig. After 5 sec the solution was brought to a pH of 8 by addition of NaOH 1*N*. *Technique 2*[2] is also a nitrite method, 0.005 mEq $NaNO_2$ was mixed to HCl, KI and 1^{125}. This mixture was neutralized by NaOH in borate buffer pH 8 and added to a borate buffer solution containing the Ig. In *technique 3*[5], a KI_3 solution containing the 1^{125} was added to the Ig dissolved in carbonate buffer pH 10. In *technique 4*[6], the mixture of protein and radioactive iodine was oxidized with low amounts of chloramine T. The reaction was stopped with sodium metabisulphite.

Determination of non-specific affinity. From each preparation of labelled Ig 7 samples of progressive quantities (1–300 µg) were prepared and incubated with 50 mg liver powder for 30 min at 37 °C. After cold centrifugation, the supernatant was separated and the bottom washed 3 times with buffer. The supernatants recovered after washing were added to the first ones. The activity of both precipitate and supernatant was measured. The activity of the precipitate, expressed as percentage of the whole activity, gives the proportion of fixed Ig.

Fixation of synthetic resins and elution. The experiment was repeated as previously, but by substituting resins for the liver powder. 7 incubations were made on each of the following substances: Amberlite IRA 400, Dowex 1, Dowex 50, CM-cellulose and polystyrene-sulphonate. The latter, which was found to fix labelled Ig in the same proportion as liver powder, was used for the complementary experiment, in which the fixation was measured by an elution method without previous labelling. In these experiments, the non-labelled immunoglobulin, still fixed to the resin after washing, was eluted either by a hypertonic solution of 2*N* NaCl or by shifting the pH to 9.0[1]. The amount of Ig was determined by microkjeldahl in the luates and in the supernatants and the proportion of fixed Ig established as previously.

Results. The Table shows that the values of non-specific affinity were very similar from one batch of Ig to another, provided the same iodination technique was used. On the contrary, if we compare with each other the means obtained using the 4 different techniques, it is clear that iodine labelling may modify the amount of globulin fixed to the substrate. This value is low and similar with the

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Non-specific affinity of 7 batches of Ig, when iodized by 4 different techniques

Batch No.	Technique 1		Technique 2		Technique 3		Technique 4	
	Nb I at/mol	% fixed	Nb I at/mol	% fixed	Nb at/mol	% fixed	Chlor. T (µg)	% fixed
1	1.5	8.7	7.0	3.9	4	2.5	50	2.5
2	0.2	15.5	5.1	2.3	6	2.1	100	1.5
3	11.2	7.6	4.7	4.1	5	0.9	400	2.4
4	2.3	7.8	3.4	7.6	7	1.8	400	1.4
5	16.0	14.7	2.3	6.0	20	1.8	400	2.1
6	2.9	13.0	2.2	7.3	20	2.9	400	1.3
7	2.3	9.9	2.0	3.5	3	2.5	800	1.8
$\bar{x}$		10.9		4.9		2.1		1.9
σ		3.23		2.03		0.66		0.48
ε		1.22		0.77		0.24		0.18

This affinity is expressed as the proportion of Ig fixed to a biological substrate after incubation and washing. For each technique, the means and the standard error of the means are calculated.

techniques 3 and 4, but significantly higher with technique 2 (4.9%) and especially with technique 1 (10.9%). Techniques 1 and 2 have in common the same oxidizing agent: $NaNO_2$. Moreover, in technique 1 (which leads to the highest fixation) the Ig is for a while dissolved in an acid solution.

The hypothesis that this might be the cause of such an increase was substantiated by the following experiment. 4 batches of Ig were preincubated for 48 h at pH 7.3 (control), 6.0, 5.0 and 4.0 and then neutralized. Their affinity for tissues was found then to be increased respectively 2.3, 7.0 and 31.0% for the 3 acidity degrees studied.

In the 3 experiments where unlabelled Ig was incubated with the polystyrene-sulfonate, it was possible to elute a quantity corresponding respectively, on an average, to 1.0, 2.7 and 3.0% of the whole. The results were the same with both methods of elution.

In techniques using KI as a carrier, the number of I atoms per molecule was calculated. The Table shows the absence of correlation between this number and the affinity of the labelled Ig. In a complementary experiment, 6 labellings were performed simultaneously on one batch of Ig using progressive quantities of carrier so that different numbers of I atoms (from 0.01–60) could be introduced into the molecule; those 6 preparations behaved in the same manner as far as non-specific affinity to tissue was concerned.

Discussion. It is clear that some techniques of iodination alter the immunoglobulin so that it adheres more to the tissues. This was not the case with the techniques 3 and 4 used here. These methods did not require denaturing reagents. The fixation of 2% obtained after labelling with these 2 methods may well represent the true value of this non-specific affinity, not only because the same result was found with these 2 methods, but also because the same result was reached if non-labelled protein was incubated. Nevertheless, this last point could only be established on a substitute for the biological substrate since elution from the liver powder would have been impossible.

The non-specific affinity was increased after labelling with techniques 1 and 2, because the globulin was denatured by one of the reagents. The $NaNO_2$ must be responsible in both cases, and the acid milieu in technique 1. In most techniques used nowadays iodination is performed in neutral or alkaline solution; nevertheless, this last observation is important since Ig preparations may be treated by acid solutions in circumstances other than labelling, for instance purification by $(NH_4)_2SO_4$ or other substances, and then used in autoimmunity research.

Contrary to what has been observed for the antibody function[2], the property studied here was not influenced by the number of iodine atoms per molecule.

Résumé. On recherche si le marquage au radio-iode peut dénaturer les immunoglobulines au point d'en modifier la fixation non spécifique aux tissus (affinité non spécifique). Des 4 techniques de marquage essayées, 2 ne modifient pas cette propriété, tandis que les 2 autres augmentent nettement la proportion d'immunoglobulines fixées.

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Turnover of Lymphocytes in Rat Peritoneal Fluid

Peritoneal fluid contains a large and varied population of cells including (at least in the mouse) some with hemopoietic stem-cell capability[1]. In the rat, small lymphocytes constitute approximately 60% of all cells present in peritoneal fluid[2–4]. Although the turnover of peritoneal macrophages has been studied by several workers[5–8], there is little information concerning the movement of small lymphocytes into and out of the peritoneal cavity of unstimulated animals. The present study provides a preliminary characterization of lymphocyte kinetics in the peritoneal fluid.

Methods. Young male albino rats (175–250 g) received a single i.v. injection of tritiated thymidine (^{3}HTdR). The animals were anesthetized with ether and the isotope (0.5 μCi/g, specific activity 1.9 Ci/mmole) was injected in a volume of 0.15 ml into the exposed right saphenous vein. To check the i.p. availability of ^{3}HTdR injected i.v., a separate group of rats received 1.0 ml of the isotope i.p. 20 min prior to sacrifice.

Rats in groups of 2 or 3 were sacrificed by cervical dislocation and cells were collected by rinsing the peritoneal cavity with 50 ml of cold saline containing heparin. The resulting fluids were pooled and centrifuged and the cell pellet resuspended in a few drops of rat serum and smeared. Autoradiographs were prepared with Kodak NTB-2 emulsion, exposed for 6 weeks, developed, and stained with Giemsa; 5000 cells were counted for each point.

Many peritoneal leucocytes are difficult to classify unequivocally[2]. In the present study, cells were scored as 'small lymphocytes' (<10 μ diameter) or large mononuclear cells (10–20 μ diameter, appearance similar to the 'macrophages' of glycogen-induced exudates[5]); sizing was done with an eyepiece reticle. Mast cells, eosinophils, and neutrophils, which together constitute 30% of the population, were not enumerated.

Results and discussion. Only 1% of the peritoneal cells is labeled 20 min after i.v. or i.p. injection of ^{3}HTdR (Figure). Of these labeled cells, 92% are large mononuclear cells. The proportion of labeled peritoneal cells

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