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

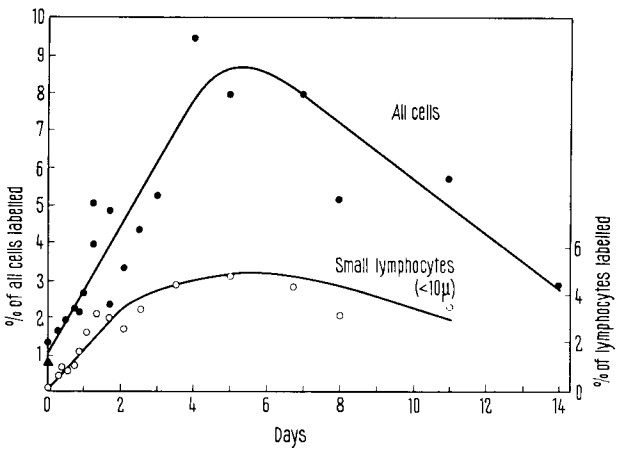
[1] L. J. COLE, Am. J. Physiol. *204*, 265 (1963).
[2] A. KANTHACK and W. HARDY, J. Physiol. *17*, 81 (1894–95).
[3] J. PADAWER and A. GORDON, Anat. Rec. *124*, 209 (1956).
[4] J. W. HARRIS and T. R. NOONAN, Int. J. Radiat. Biol. *13*, 183 (1967).
[5] A. VOLKMAN, J. exp. Med. *124*, 241 (1966).
[6] A. VOLKMAN and J. L. GOWANS, Br. J. exp. Path. *46*, 50 (1965).
[7] J. W. GOODMAN, Blood *23*, 18 (1964).
[8] R. VAN FURTH and Z. A. COHN, J. exp. Med. *128*, 415 (1968).

increases at a rate of 1.9% per day, reaching a maximum of 8.5% by the 4th to 6th day. This increase in the proportion of labeled cells is attributable to both large mononuclear cells and small lymphocytes. The latter increase from 0.1% at zero time to 4.9% by the 4th day.

Peritoneal large mononuclear cells are heavily labeled 20 min after injection of ^{3}HTdR (median grain count 49, range 20–60), remain at this level through the 4th day (44 grains/cell), then fall to 13 grains/cell (range 7–34) by the 14th day. Small lymphocytes in both blood and peritoneal fluid are also heavily labeled on the 1st day (median grain count 35 for peritoneal lymphocytes, 50 for blood lymphocytes) but the range is similar in both cases (20–60) and the means are not statistically different.

These data are consistent with the view that the labeled small lymphocytes which appear in peritoneal fluid originate in the blood. Although some lymphocytes might originate from division of large mononuclear cells within the peritoneum, the grain counts and, particularly, the paucity of mitotic figures in the large mononuclear cell population indicate that this source is not quantitatively important.

The peritoneal cavity of a 200 g rat contains approximately 12.5×10^6 cells of which 60% (7.6×10^6) are small lymphocytes[4]. The proportion of labeled small lymphocytes increases at a rate of 1.7% per day during the first 2 days (Figure), so (neglecting exit of labeled cells) about 6.5×10^4 labeled lymphocytes join the population each day, or 2.7×10^3 labeled cells/h. Since less than 2% of the small lymphocytes in blood are labeled during this interval[6], the total influx of small lymphocytes (labeled plus unlabeled) into the peritoneal fluid may be as much as 50-fold greater, or 1.4×10^5 cells/h. Since the total number of peritoneal lymphocytes remains constant, the same number of cells must leave the peritoneal fluid (or be destroyed within it) as enter.



Percent of labeled cells in rat peritoneal fluid after a single i.v. injection of ^{3}H-TdR. Each point was obtained by counting 5000 cells from a pooled sample obtained from 2 or 3 rats. The labeling after a single i.p. injection of ^{3}H-TdR is also shown (▲). Points on the lymphocyte curve represent a 3-point moving average calculated from the actual data.

If we ignore the movement of cells out of the population and assume that peritoneal small lymphocytes are homogenous with respect to residence-time within the peritoneal fluid, the turnover time (time for complete replacement) for these cells is $7.6 \times 10^6/(6.5 \times 10^4)(50) = 23.4$ days. However, many lymphocyte compartments contain a mixture of short-lived and long-lived cells and if this is also true for the peritoneal fluid the calculated turnover time is merely the mean for 2 (or more) populations. Obviously, the calculated value cannot be taken too seriously until this matter can be resolved, since the calculation will be greatly influenced by the presence of even a small number of very short-lived or very long-lived cells[9].

Zusammenfassung. Zellkinetische Untersuchungen an Ratten nach Injektion von H^3-Thymidin ergaben eine rasche Markierung der grossen mononukleären Zellen innerhalb von 20 Min und eine langsamere Markierung der kleinen Lymphozyten, für welche eine Turnover-Zeit von 23,4 Tagen errechnet wurde. Stündlich gelangen ca. 10^5 kleine Lymphozyten in die Bauchhöhlenflüssigkeit.

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Calf Thymus Fractions: Enhancement and Suppression of Immunocompetent Cells in Neonatal Mice

The functional significance of the thymus has been an enigma for many years. Recent studies have indicated that the thymus may function, at least in part, as a source of humoral substances necessary for development of lymphoid tissue and immunological integrity[1]. Earlier studies indicated that thymus extracts may induce lympho cytopoesis and increased lymphoid tissue size[2,3]. More recent reports have indicated that thymus extracts can also prevent wasting disease and fatal virus infections in thymectomized mice[4–6].

A fraction derived from calf thymus, termed thymosin, was prepared by Goldstein, Slater and White[7] in 1966. This factor could stimulate lymphoid tissue hyperplasia and DNA, RNA and protein synthesis in vivo. In 1967, Hand, Castor and Luckey[8] prepared a basic protein from calf thymus extracts. This substance stimulated lymphocytopoesis and lymphoid tissue hyperplasia in mice when administered in microgram quantities. Law and Agnew[9], and Law, Goldstein and White[10] reported that thymosin could restore immunologic reactivity of spleen cells of thymectomized mice. Recently, Trainin, Small and Globerson[11] demonstrated in an in vitro system that a thymus extract could restore immunocompetence to spleen cells from thymectomized mice. However, relatively large amounts of their substance were necessary. We now describe the effects of purified