

may be argued that the lymphocytes with complement receptors represent only a part of the total population of B-lymphocytes.<sup>14</sup> However, the effect of A.T.G. was identical when either human complement or guinea-pig complement was used for EAC-rosette formation. With human complement the proportion of lymphocytes forming immune rosettes is significantly lower than with guinea-pig complement, suggesting that lymphocyte receptor for the two complements may not always be shared.

This work was supported by grants from the Medical Research Council of Canada (MT859).

—We are, etc.,

NADIR R. FARID  
ROBERT E. MUNRO  
VAS V. ROW  
ROBERT VOLPE

Endocrinology Research Laboratory,  
The Wellesley Hospital,  
Toronto, Ontario

- Bach, J. F., and Antoine, B., *Nature*, 1968, **217**, 658.
- Munro, A., et al., *British Medical Journal*, 1971, **3**, 271.
- Bewick, M., Ogg, C. S., Parsons, V., Snowdon, S. A., and Manuel, L., *British Medical Journal*, 1972, **3**, 491.
- Bach, J. F., and Dardenne, M., *Transplantation Proceedings*, 1972, **4**, 345.
- Biozzi, G., Stiffel, C., Mouton, D., Bouthillier, Y., and Decreusefond, C., *Transplantation Proceedings*, 1972, **4**, 339.
- Jondal, M., Holm, G., and Wigzell, H., *Journal of Experimental Medicine*, 1972, **136**, 207.
- Yata, J., Tsukimoto, I., and Tachibana, T., *Clinical and Experimental Immunology*, 1973, **14**, 319.
- Farid, N. R., Munro, R. E., Row, V. V., and Volpe, R., *New England Journal of Medicine*, 1973, **288**, 1313.
- Wybran, J., and Fudenberg, H. M., *New England Journal of Medicine*, 1973, **288**, 1072.
- Farid, N. R., Munro, R. E., Row, V. V., and Volpe, R., *New England Journal of Medicine*, 1973, **289**, 1111.
- Bianco, C., Patrick, R., and Nussenzeig, V., *Journal of Experimental Medicine*, 1970, **132**, 702.
- Hamburger, J., *Proceedings of the Royal Society of Medicine*, 1972, **65**, 1051.
- Raff, M. C., and Owen, J. J. T., in *Advances in Experimental Medicine and Biology* Vol. 12, p. 11, ed. K. Lindahl-Kiessling, G. Alm, and M. C. Hanna jun. New York, Plenum Press, 1971.

### Rosette Inhibition Test and Cell-mediated Immunity

SIR,—Dr. Helen M. Chapel and Dr. J. R. Batchelor (17 November, p. 385), reporting the results obtained with the rosette inhibition test carried out on lymphocytes from patients with severe burns, have shown that the minimum inhibitory concentration (M.I.C.) of antilymphocyte globulin is not related to the immunosuppression which follows a severe burn but to the ratio of sodium to potassium excreted in the urine. They conclude that the test is not a direct measure of cell-mediated immunological reactivity and may be affected by other factors, such as the increased production of adrenal steroids which is known to occur in this condition.

Since this test has been successfully used to monitor the degree of immunosuppression and to predict allograft rejection in transplanted patients,<sup>1,3</sup> the point raised by the authors deserves further consideration. We have used the test to follow the progress of 28 patients who had received a renal allograft, making two or three determinations per week from the day of transplantation for at least two months. Munro's method<sup>1</sup> with the modification introduced by Jondal<sup>4</sup> to increase the number of rosettes, was used. The M.I.C. was significantly increased in 26 out

of 27 separate determinations made two to five days before rejection became clinically evident and in only 21 out of 260 determinations after which no clinical evidence of rejection developed.<sup>5</sup>

In order to check the possibility of interference with the rosette inhibition test by factors that can affect the urinary sodium:potassium ratio we have studied 12 patients, all receiving prednisone and azathioprine treatment, in whom there had been an immediate resumption of urinary flow after transplantation. No correlation was found between variations in the M.I.C. and in the urinary sodium:potassium ratio ( $r=0.11$ ,  $n=130$ ,  $P>0.1$ ). These findings support the view that the test can be successfully applied to predict renal allograft rejection which, as is well known, is mediated by cellular immunity.

The difference between our findings and those reported by Drs. Chapel and Batchelor may perhaps be explained by the differences in the method used. The modification introduced by Jondal,<sup>4</sup> which permits highly accurate marking of the T-cells, has been used also by Farid *et al.*<sup>6</sup> who showed a close correlation between the MIC of antilymphocyte globulin and lymphocyte sensitization to thyroid antigens in Graves's disease and Hashimoto's thyroiditis. This suggests that the variation in the proportion of marked T-cells may be critical.—We are, etc.,

A. CANTALUPPI  
G. FIORELLI

Istituto di Patologia Medica

C. PONTICELLI

Istituto di Urologia,  
Sezione di Nefropatologia,  
Universita di Milano,  
Milan, Italy

- Munro, A., et al., *British Medical Journal*, 1971, **3**, 271.
- Cullum, P. A., et al., *British Medical Journal*, 1972, **2**, 71.
- Bewick, M., Ogg, C. S., Parsons, V., Snowdon, S. A., and Manuel, L., *British Medical Journal*, 1972, **3**, 491.
- Jondal, M., Holm, G., and Wigzell, H., *Journal of Experimental Medicine*, 1972, **136**, 207.
- Fiorelli, G., et al., Communication to the 6th Congress of the Organ Transplantation Society, Varese, 12-15 September 1973.
- Farid, N. R., Munro, R. E., Row, V. V., and Volpe, R., *New England Journal of Medicine*, 1973, **289**, 1111.

### Thyopac-5 Test

SIR,—The practice of modifying serum thyroxine values to allow for variations in thyroxine-binding protein concentrations is well established.<sup>1</sup> We have compared the diagnostic accuracy of a new Radiochemical Centre kit (Thyopac-5), which provides a

means of determining both a serum thyroxine concentration and a normalized thyroxine ratio<sup>2</sup> with those of other widely used tests. The tests considered were: in vivo—<sup>131</sup>I neck uptakes (2-hour and 24-hour), <sup>131</sup>I neck uptake: thigh ratios (2-hour and 24-hour); in vitro—serum protein-bound iodine, serum thyroxine (Thyopac-4, Thyopac-5), triiodothyronine binding capacity (Thyopac-3), normalized thyroxine ratio (E.T.R. (Mallinckrodt), Thyopac-5), free thyroxine index (Thyopac-4 value  $\times$  100/Thyopac-3 value).

Tests were performed on 300 patients (242 female and 58 male) ranging in age from 2 days to 84 years. Ninety-six investigations were performed using the Thyopac-5 test and over 200 by each of the other methods. Follow-up information regarding the patients showed 35 (11.7%) to be hypothyroid, 165 (55.0%) euthyroid, and 92 (30.7%) hyperthyroid, with eight remaining equivocal. The percentages of patients correctly differentiated by each method at the hypothyroid-euthyroid and hyperthyroid-euthyroid borders are given in the table. Patients who were pregnant, taking a drug known to affect any of the tests, aged less than one year, or who had been previously treated for thyrotoxicosis with radiiodine were excluded from the selected group. The two normalized thyroxine ratio kits were of equivalent reliability, their test values being related by the equation: Thyopac-5 value = 0.65 (E.T.R. value) + 0.39.

All the tests, with the exception of the 24-hour <sup>131</sup>I neck uptake: thigh ratio, were more reliable for the diagnosis of hyperthyroidism than for hypothyroidism. Of the in vitro methods the normalized thyroxine ratio tests were the most reliable and the least affected by abnormal serum protein levels. The diagnostic accuracies of these tests were comparable with that of the best in vivo test, the 24-hour <sup>131</sup>I neck uptake. The in vitro tests offer the advantage of greater convenience to both hospital staff and patients and do not involve the administration of radioisotopes to the patients. We have demonstrated the usefulness of the normalized thyroxine ratio tests and found the additional serum thyroxine figure available as part of the Thyopac-5 test to be a valuable feature of the kit.—We are, etc.,

J. G. HARDY  
G. M. NEWBLE

Regional Radiotherapy Centre,  
Essex County Hospital,  
Colchester

- Clark, F., and Horn, D. B., *Journal of Clinical Endocrinology*, 1965, **25**, 39.
- Ashkar, F. S., and Bezjian, A. A., *Journal of the American Medical Association*, 1972, **221**, 1483.

Test	Patients Correctly Diagnosed (%)			
	Hypothyroid-euthyroid Border		Hyperthyroid-euthyroid Border	
	All patients	Selected patients	All patients	Selected patients
2-hour <sup>131</sup> I neck uptake .. .. .	76	76	91	92
24-hour <sup>131</sup> I neck uptake .. .. .	80	82	90	94
2-hour <sup>131</sup> I neck uptake: thigh ratio .. .. .	70	73	88	91
24-hour <sup>131</sup> I neck uptake: thigh ratio .. .. .	73	76	*	*
Serum protein-bound iodine .. .. .	76	77	88	91
Serum thyroxine .. .. .	70	70	86	89
Triiodothyronine binding capacity .. .. .	68	70	84	90
Normalized thyroxine ratio .. .. .	78	83	91	92
Free thyroxine index .. .. .	66	67	89	93

\*The 24-hour <sup>131</sup>I neck uptake: thigh ratio was of no diagnostic value at this border.