Reduced Bactericidal Capacity of Polymorphonuclear Leucocytes in Nephrotic Syndrome and Effect of Steroid Therapy

Patients with nephrotic syndrome have an increased susceptibility to infections, many of which run a fulminant course. The mechanisms underlying such processes are not clear, but probably they touch more than one facet of immunocompetence. We have looked at the intracellular bactericidal capacity of polymorphonuclear leucocytes (PMNs) of children suffering from nephrosis, and evaluated the effect of corticosteriod therapy on such function.

Methods. The diagnosis of nephrotic syndrome was based on generalized edema, massive proteinuria and hypoalbuminemia. The bacterial test was essentially as described by QUIE et al.[1] using surface counting of *Staphylococcus aureus* colonies on nutrient agar plates. The bactericidal capacity of PMNs was calculated by the ratio : number of viable intracellular bacteria at 140 min/number at 20 min of the culture. The test was also done on nephrotic patients receiving prenisolone in a dose of 60 mg/m^2 body surface area, per day.

Results and discussion. There was a marked reduction in intracellular bacterial killing by PMNs of nephrotic children as shown in the Table ($P < 0.001$). The exact cause for this defect is not clear. There are at least 4 possible mechanisms for killing organisms inside macro- and microphages: Lysosomal phagocytin system involving arginine rich cationic proteins, hydrogen peroxide system, muramidase activity and hydrolases. In nephrotic syndrome, changes in protein metabolism (loss through proteinuria, reduced intake due to anorexia, increased catabolism, and impaired absorption due to edema of the gut wall) might reduce the availability of amino acids, thereby adversely affecting the synthesis of lysosomal enzymes. This simulates the state of malnutrition in which bactericidal capacity of PMNs is significantly impaired.[2]

The values in nephrotics receiving prednisolone overlapped those of patients not on such a therapy. The log mean for the former was, however, significantly lower ($0.05 > P > 0.02$). The administration of glucocorticosteroids suppresses inflammatory response and increases susceptibility to infection. On macrophages, the chief effect is a diminished influx of such cells from the blood to the site of inflammation.[3] The suppressed reduction of nitroblue tetrazolium by PMNs of patients receiving steroids[4] and the in vitro inhibition by hydrocortisone of NADH oxidase[5] are apparently incongruous with our data. The dichotomy between reduction of nitroblue tetrazolium and bactericidal capacity is well known in children with chronic granulomatous disease[6,7]. The improved bacterial killing by microphages of nephrotics on steroid therapy could be due to an actual or impending amelioration in the underlying renal disease and its consequences on protein metabolism, or it might be the result of stabilization of lysosomal membranes inside the cells[8].

Zusammenfassung. Es wird der Nachweis erbracht, dass die bakterizide Wirkung der polymorph-kernigen Leukozyten bei Patienten mit nephrotischem Syndrom vermindert ist und dass Prednisolon einen günstigen Einfluss auf die Bakterizidie polymorph-kerniger Leukozyten beim nephrotischen Syndrom hat.

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Intracellular bacterial killing by PMNs expressed as the ratio-number of viable intracellular bacteria at 140 min/number at 20 min of the culture

Group	Number	Bactericidal capacity		
		Range	Geometric mean	S.D.
Healthy subjects	24	0.02–0.21	0.07	0.03
Nephrotic syndrome	12	0.10–0.54	0.33	0.11
Nephrotics receiving prednisolone	16	0.03–0.64	0.25	0.12

1 P. G. QUIE, J. G. WHITE, B. HOLMES and R. A. GOOD, J. clin. Invest. *46*, 668 (1967).
2 V. SETH and R. K. CHANDRA, Archs Dis. Childh. *47*, 282 (1972).
3 J. THOMPSON and R. VAN FURTH, J. exp. Med. *131*, 429 (1970).
4 J. H. CHRETIEN and V. F. GARAGUSI, Experientia *27*, 1343 (1971).
5 G. L. MANDELL, W. RUBIN and E. W. HOOK, J. clin. Invest. *49*, 1381 (1970).
6 E. N. THOMPSON, R. K. CHANDRA, W. A. COPE and J. F. SOOTHILL, Lancet *1*, 799 (1969).
7 R. K. CHANDRA, W. A. COPE and J. F. SOOTHILL, Lancet *2*, 71 (1969).
8 G. WEISSMANN, Fedn Proc. *23*, 1038 (1964).

Effect of Serum from Tumor-Bearing Mice on the in vitro Migration of Thymocytes

The growth of a transplanted syngeneic tumor is known to induce a marked atrophy of the thymus[1–3]. Bilateral adrenalectomy reduces this phenomenon[4] and corticosteroid hormones are thus probably involved. Yet, another factor must operate since the total number of circulating lymphocytes increases during the course of tumor development[2]. It can be postulated that an increased migration of the thymic lymphocytes to the periphery might also account for the depletion of the thymus in this situation. One could further imagine that some changes in the properties of the serum might mediate the phenomenon.

The present work was undertaken in order to examine this last possibility. We have studied the migration of normal mouse thymus cells out of capillary tubes onto glass in culture chambers containing diluted syngeneic serum[5]. This technique allowed us to compare the effect

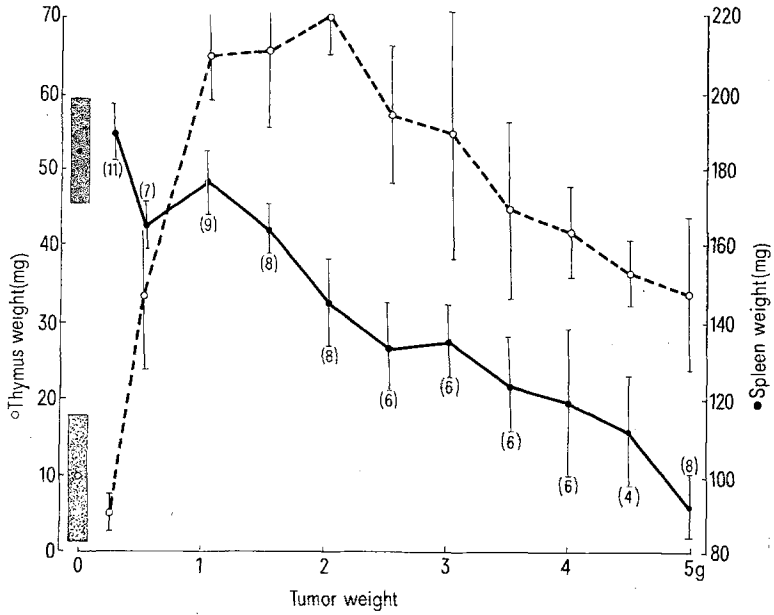

Figure 1. Weight (mean ± S.E.) of thymus (●) and spleen (○) as a function of tumor-growth. The number of TP8 bearing Swiss B mice studied for each point is indicated in parentheses. The shaded bars represent the ranges (mean ± 2 S.D.) of thymus and spleen weight in 60 normal 2 months-old, Swiss B female mice.

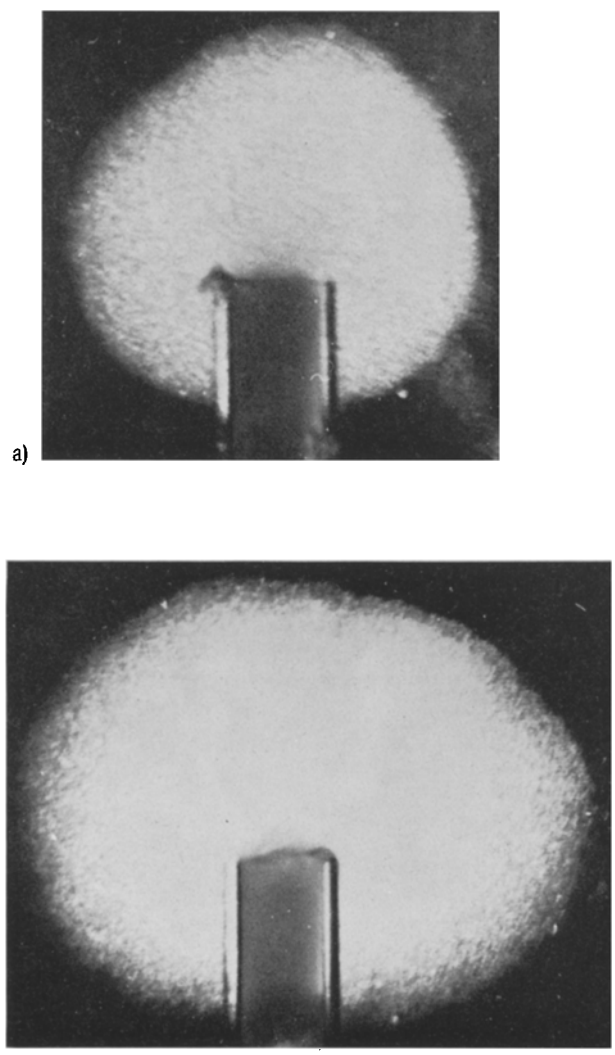

Figure 2. Normal Swiss B mice thymocytes migrations as observed at 36 h. a) In 10% normal Swiss B mouse serum. b) In 10% serum from a Swiss B mouse bearing a 4 g TP8 sarcoma.

on thymocytes migration, of the sera from normal mice or from individuals grafted with a chemically-induced syngeneic tumor.

Material and methods. Two-month-old female mice of the inbred strain Swiss B were used in all experiments. The tumor TP8 was induced by methylcholanthrene and maintained by serial transplantation on syngeneic hosts[6]. 1 mm^3 of this tumor was grafted subcutaneously into the back of the mouse. The sera from tumor-bearing and control mice were collected under aseptic conditions and stored at −20°C until used. For the migration tests, the sera were diluted 1/10 in RPMI 1640 Medium supplemented with antibiotics.

Capillary tubes were filled with a suspension in serum-free culture medium of normal mouse thymocytes (10^8 cells/ml), sealed at one end with melted paraffin and centrifuged at 220 *g* for 10 min. The tubes were cut at the cell-fluid interface and fastened in Mackaness-type chambers (2 tubes per chamber). Each chamber was filled with the diluted serum under test. The chambers were incubated for 36 h at 37°C and the areas of migration measured by planimetry. The results were expressed as percentage of migration according to the formula:

$$\text{Percent migration increase} = \frac{\text{Mean migration with serum tested}}{\text{Mean migration with control serum}} \times 100 - 100$$

The normal range of migration was computed by studying the migration of 40 control capillary tubes maintained in identical conditions with normal serum. The standard deviation was found to be 6%, so that at a significance level of 0.01, the confidence interval is

[1] A. M. El Hassan and A. E. Stuart, Br. J. Cancer *19*, 343 (1965).
[2] A. J. Edwards, M. R. Summer, G. F. Rowland and C. M. Hurd, J. natn. Cancer Inst. *47*, 301 (1971).
[3] A. J. Edwards, G. F. Rowland, M. R. Summer and C. M. Hurd, J. natn. Cancer Inst. *47*, 313 (1971).
[4] G. Simu, V. Toma, D. Nestor and M. S. Rosculet, Oncology *22*, 36 (1968).
[5] M. George and J. H. Vaughan, Proc. Soc. exp. Biol. Med. *111*, 514 (1962).
[6] D. Oth and C. Burg Folia biol. *16*, 374 (1970).

± 16%. Thus, we considered as significant, a response giving more than a 16% increase in migration.

Results and discussion. As depicted by Figure 1, TP8 induces during its growth in Swiss B mice, the thymic alteration as well as the splenomegaly already reported by others for various transplanted syngeneic tumors[1-3,7].

Figure 2 shows the results of a typical experiment where we have compared the migration of thymocytes from normal mice in the presence of serum from mice bearing this tumor b) to the migration of the same thymocytes in the presence of serum from healthy control mice a). The mean migration area (± S.E.) for 10 capillary tubes was found to be 972 ± 25 (arbitrary units) in b) and 540 ± 12 in a), which corresponds to a 80% increase of the thymocyte migration in the serum from the cancerous mouse as compared to the migration in normal serum.

As represented by Figure 3, this thymocyte-migration-increasing effect of the serum from mice bearing TP8 depends on the weight of the tumor. The percent migration increase reaches the threshold of 16% when the tumor weighs 1 g, and then rises progressively to a maximal mean value of 55% when the tumor weighs 4 g. Subsequently, the effect seems to fall slightly. We obtained similar results using another methylcholanthrene-induced tumor.

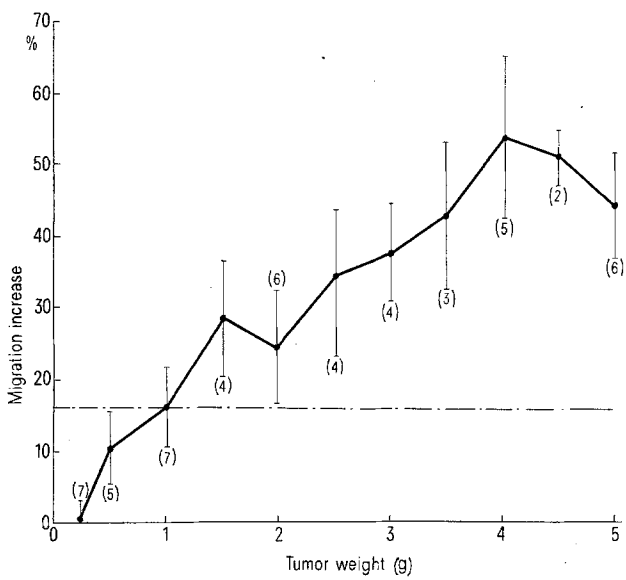

Figure 3. Percent increase of the migration of normal Swiss B thymocytes in $^1/_{10}$ diluted serum from TP8-bearing Swiss B mice as a function of tumor-weight. Each point represents the mean (± S.E.) for the number of experiments indicated in parentheses. Every experiment included at least 6 capillary tubes in the serum under test and 6 capillary tubes in control normal serum. The dotted line gives the upper limit (2.6 S.D.) of the range for 40 capillary tubes maintained in normal serum.

This action of the serum from tumor-bearing mice on the migration of thymocytes might be interpreted in 2 opposite ways: either as a true stimulation or as the suppression of an inhibition. There could exist either a thymocyte-stimulatory substance in the serum from tumor-bearing mice, or the reverse, an inhibitory one in the normal serum which would disappear when the tumor is flourishing. We have evidence in favour of this second hypothesis. We found that normal mouse serum contains some factor inhibiting the in vitro migration of syngeneic or allogeneic thymocytes. This factor is stable for at least 1 month at −20°C and can be readily destroyed by heating to 56°C for 30 min[8]. Its amount in the serum could substantially decrease when the tumor has reached a critical size, but this remains to be established.

Although the physiological meaning of these changes in the properties of the serum is still obscure, the comparison of Figure 3 with Figure 1 reveals a striking parallelism between the evolution of the action of the serum on the in vitro migration of thymocytes and the behavior of the thymus. The thymus begins to involute when the increase of in vitro migration begins to rise significantly. Moreover, the onset of this phenomenon seems to coincide with the peak of splenomegaly. It would probably be a simple view to consider the capillary tube system as an exact model of what occurs in the organism. Nevertheless, this increased motility of thymocytes in the sera from our tumor-bearing mice might be of importance in the mechanism of progressive involution of the thymus during tumor development[9,10].

Résumé. La migration in vitro, hors de tubes capillaires, des thymocytes de souris est plus importante en présence du sérum de souris porteuses d'une tumeur syngénique transplantée chimio-induite qu'en présence de sérum normal. Ce phénomène, qui est fonction du poids de la tumeur, pourrait être en rapport avec l'involution thymique accompagnant la croissance tumorale.

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27 April 1972.

[7] M. F. A. WOODRUFF and M. O. SYMES, Br. J. Cancer *16*, 120 (1962).
[8] F. DUMONT, D. SABOLOVIC and C. BURG, submitted for publication.
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Stilboestrol-Induced Depression of the Antibody Response

Involution of the thymus is a well established consequence of administration of natural or synthetic oestrogenic compounds to experimental animals[1]. Such treatment is also known to induce a wasting syndrome in neonatal animals[2], similar to the post-thymectomy wasting[3], which appears to be partly due to increased susceptibility to infection of such animals[4]. Although administration of oestrogens has been reported to induce lymphopoenia in adult animals[5], their effects on peripheral lymphoid tissues are rather variable[1,6,7]. Reports concerning effects of oestrogens on immunity have also been somewhat contradictory. Resistance to infection in adult animals is generally increased[8], but antibody responses have been found to be unaffected[9], increased[10] or decreased[11].

In the present work we report results obtained on the humoral antibody response to sheep erythrocytes in mice and rats pretreated with stilboestrol (diethylstilboestrol).