## The Role of Submandibular Glands on Extrarenal Erythropoietin Production

The kidney is the main organ controlling erythropoietin (EP) production. This subject has recently been reviewed[1].

MIRAND and PRENTICE[2] first demonstrated the production of erythropoietic factor in hypoxic anephric rats. It has been observed that serum EP produced in hypoxic anephric rats was immunologically indistinguishable from renal EP[3,4]. Nevertheless, no conclusive evidence on the site of production has been reported.

Otherwise, different facts related to salivary glands, such as: Isolation of several humoral factors[5–8] including renin[9]; hormonal control on submaxillary glands[10,11]; resemblance of ultrastructure of duct cells of submaxillary glands with tubule cells of the kidney[12], among others, led us to think of the possibility of those glands as a site of extrarenal EP production.

*Material and methods.* Male Wistar rats were used. Young rats were 30–33 days old and adult rats $2^1/_2$ months old. Removal of kidneys and submandibular glands were performed under ether anesthesia. The adrenals were left intact.

The production of EP was stimulated either by hypoxia in an altitude chamber at 22,000 ft or by hypoxia and anemia. The latter was obtained by cardiac puncture withdrawing 2 ml of blood per 100 g of body weight, replacing the same volume with normal rat serum injected in the femoral vein.

The erythropoeitic activity of the rat plasma was measured as the percent of $^{59}$Fe incorporation in total circulating red blood cells, in CFW/ep ex-hypoxic mice,

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Table I. Erythropoietic activity of ex-hypoxic mice injected with 1 ml of plasma from young rats after different treatments

| Group | Treatment | 48 h $^{59}$Fe incorporation | $P$ |
|---|---|---|---|
| A | Normal | 0.48 ± 0.06[a] (7) | NS |
| B | 5 h hypoxia | 20.81 ± 1.92 (7) | versus C < 0.001 |
| C | Nephrectomy + 5 h hypoxia | 9.02 ± 1.38 (6) | versus D < 0.001 |
| D | Nephrectomy + submandibularectomy + 5 h hypoxia | 1.66 ± 0.18 (7) | versus E < 0.001 |
| E | Saline controls | 0.40 ± 0.08 (6) | – |
| A | Normal | 0.45 ± 0.06 (7) | NS |
| B | 5 h hypoxia | 21.92 ± 2.14 (8) | versus C < 0.001 |
| C | Nephrectomy + 5 h hypoxia | 9.87 ± 1.38 (7) | versus D < 0.001 |
| D | Nephrectomy + submandibularectomy + 5 h hypoxia | 2.69 ± 0.15 (7) | versus E < 0.001 |
| E | Saline controls | 0.36 ± 0.05 (6) | – |

[a] Standard error of the mean. $P$, statistical significance. NS, not significant. Number of mice in parenthesis.

Table II. Erythropoietic activity of ex-hypoxic mice injected with plasma from adult rats after different treatments

| Group | Treatment | 48 h $^{59}$Fe incorporation | $P$ |
|---|---|---|---|
| A | Normal | 0.44 ± 0.14 (10) | NS |
| B | Saline Controls | 0.40 ± 0.07 (6) | – |
| A | 5 h hypoxia | 12.87 ± 1.02 (7) | versus B, C, D < 0.001 |
| B | Nephrectomy + 5 h hypoxia | 0.41 ± 0.04 (10) | NS |
| C | Nephrectomy + submandibul-arectomy + 5 h hypoxya | 0.33 ± 0.04 (11) | NS |
| D | Saline controls | 0.33 ± 0.03 (6) | – |
| A | Anemia + 7 h hypoxia[a] | 36.38 ± 2.19 (8) | versus B < 0.001 |
| B | Nephrectomy + anemia + 7 h hypoxia | 2.93 ± 0.51 (8) | versus C < 0.02 |
| C | Nephrectomy + submandibularectomy + anemia + 7 h hypoxia | 1.20 ± 0.17 (6) | versus D < 0.001 |
| D | Saline controls | 0.44 ± 0.11 (6) | – |
| A | Anemia + 7 h hypoxia[a] | 36.20 ± 3.75 (8) | versus B < 0.001 |
| B | Nephrectomy + anemia + 7 h hypoxia | 3.30 ± 0.98 (9) | versus C < 0.05 |
| C | Nephrectomy + submandibularectomy + anemia + 7 h hypoxia | 1.00 ± 0.17 (9) | versus D < 0.02 |
| D | Saline controls | 0.36 ± 0.14 (6) | – |

Figures and symbols represent the same as in Table I. [a] Only 0.5 ml of plasma per mouse was injected. All other groups received 1 ml of plasma.

as described elsewhere[13]. Pools of plasma were made from rats similarly treated. The total dose was divided into 2 halves, and were injected s.c. 24 h apart. First injection was made on 7th day of the post hypoxia period.

*Results and discussion.* Table I shows the results of the erythropoietic activity of young rats and Table II of the adult rats. The 48 h $^{59}$Fe uptake of mice injected with plasma of normal rats did not differ from saline injected controls. The highest erythropoetic activity was, as expected, found in the plasma of normal stimulated rats (kidney present).

The most important and new finding is the marked reduction in extrarenal EP titers when submandibular glands were removed: The average $^{59}$Fe uptakes were 1/4 in the case of young rats and 1/3 in the adults, compared to anephric rats but with submandibular glands left intact. Since the relationship between $^{59}$Fe incorporation and EP titers is logarithmic, it means that a several time reduction on EP production after removal of those salivary glands had occurred.

Therefore, a reasonable interpretation would be that submandibular glands are a site of extrarenal EP production, either directly or through a mechanism similar to the renal erythropoietic factor (REF) described by GORDON et al.[14]. In the present study, we are not able to differentiate the mechanism of the hormone production[15].

*Resumen.* La extirpación de las glándulas submandibulares en ratas machos nefrectomizadas produjo una marcada disminución en la actividad eritropoyética del plasma, medida por la incorporación de $^{59}$Fe en ratones policitémicos. Este hallazgo es compatible con la formación de eritropoyetina extra renal en esas glándulas salivares.

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## Enhancement of 'Memory Cell' Pool by Polyanions in Mice

It has been shown that polyanions, (PA) enhance the primary immune response to sheep red blood cells (SRBC) in mice[1,2]. Experiments reported previously[3] concerning the effects of polyanions on the secondary response to SRBC in mice indicated that PA may enhance not only the number of antibody forming cells, but possibly also the pool of memory cells. For instance, mice primed with an optimal dose of antigen and injected with dextran sulfate (DS) at the time of priming, give a slightly but significantly higher secondary response than animals primed with antigen alone[3]. On the other hand, it has also been reported that both anti-SRBC IgG and anti-SRBC IgM injected together with SRBC enhance the ImR to SRBC[4], and that the mechanism of stimulation of the ImR by antibodies might be based on specific accumulation of the antigen in the spleen[4]. Since mice primed with SRBC and injected with DS at the time of priming showed enhanced anti-SRBC titers at the time of antigen challenge in comparison to mice primed with antigen alone[3], it could not be decided whether enhancement of the secondary ImR in the DS pretreated mice was due to an acceleration of the antigen uptake by an antibody-mediated mechanism suggested by DENNERT et al[4] or to an increase in the number of 'memory cells' in lymphoid tissues.

The present experiment was designed to determine wheter polyanions really influence the memory cell pool in mice primed with SRBC.

Groups of C57 B1/6J, mice, 6 animals per group, 8–10 weeks old, were injected either with polyanion (dextran sulfate, molecular weight $5 \times 10^5$, 1 mg/mouse

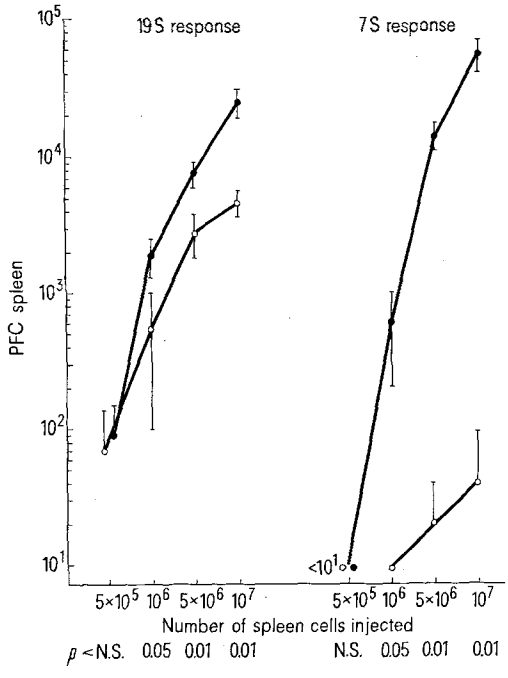


Fig. 1. PFC response to SRBC in irradiated C 57 B1/6J mice injected with graded numbers of spleen cells derived either from dextran sulfate and SRBC primed (●) or from SRBC primed syngeneic mice (○). PFC response assayed 7 days after cell transfer and antigen inoculation. Each point represents average values obtained in 6–8 mice. The standard errors are shown by vertical bars. N.S. = not significant.

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