Thymus and spleen relative weights and hemagglutination titers to SRBC in sham operated (SH), submandibularectomized (SMX) and gonadectomized (GX) mice

Organ	(1) SH	(2) SMX	(3) GX	(4) SMXGX	Significance at $p = 0.05$ or less
Series 1 (30 days)					
Thymus	0.2213 (11)	0.2500 (10)	0.3590 (10)	0.4494 (12)	4 > 3 > 2.1
% b.wt	0.0470	0.0447	0.0528	0.0587	
Spleen	0.2372 (11)	0.2298 (10)	0.3242 (10)	0.3341 (12)	4.3 > 1.2
% b.wt	0.0264	0.0323	0.0630	0.0477	
Series 2 (33 days)					
Thymus	0.1612 (8)	0.1672 (11)	0.3030 (10)	0.3171 (12)	4.3 > 2.1
% b.wt	0.0354	0.0281	0.0536	0.0900	
Spleen	0.2967 (8)	0.3099 (11)	0.4123 (10)	0.4200 (12)	4.3 > 2.1
% b.wt	0.0352	0.0370	0.0827	0.0944	
Hemagglutin	2.294 (10)	2.340 (11)	2.906 (9)	2.532 (11)	3 > 4.2.1
titer $\log_{10}$	0.346	0.527	0.368	0.250	
Series 3 (40 days)					
Thymus	0.1224 (14)	0.1554 (12)	0.2482 (11)	0.2534 (11)	4.3 > 2.1
% b.wt	0.0307	0.0294	0.0275	0.0454	
Spleen	0.2844 (14)	0.2734 (12)	0.3245 (11)	0.3051 (11)	None
% b.wt	0.0476	0.0593	0.0748	0.0596	
Series 4 (42 days)					
Thymus	0.1059 (12)	0.1141 (13)	0.2422 (9)	0.2514 (8)	4.3 > 2.1
% b.wt	0.0293	0.0229	0.0438	0.0302	
Spleen	0.3921 (10)	0.2810 (13)	0.2996 (9)	0.2915 (9)	1 > 2.3.4
% b.wt	0.1046	0.0631	0.0751	0.0492	

Values given are means ± SD; ( ) indicates number of mice per group; days are intervals between surgery and sacrifice. Series 2 mice were immunized with SRBC 5 days before sacrifice.

constant throughout the Series. Significant splenic hyperplasia was inconsistently observed in castrated mice through the Series. Significant thymic hyperplasia did not occur in submandibularectomized mice in any series. Hemagglutination titers were similar in SH, SMX, and SMXGX mice, but elevated in GX animals of Series 2.

Discussion. In general, submandibular-sublingualectomy did not result in significant thymic hyperplasia, either when the operation was done alone or with castration. We cannot confirm reports of thymic hyperplasia 28–40 days after submandibularectomy[2,3]. However, our operation consisted of extirpation of both submandibular and sublingual glands rather than only submandibular removal as done by other authors.

Neither hemagglutination titers nor spleen relative weights were significantly different for immunized SH and SMX mice (Series 2, table). Mice submandibularectomized 8 weeks previously are reported to have a significantly low plaque forming cell response to SRBC[5]. Although our time interval is shorter, our results are not consistent with this observation. In summary, our data do not suggest an influence of the submandibular-sublingual glands on thymus and spleen weights or SRBC antibody response in the mouse.

1 Acknowledgment. This study was aided by NIDR grant 1F32 DE05305-01 to D. Dean.
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Blood oxygen affinity in large white pig

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Summary. Large white pig and human blood oxygen affinities are different due to different primary structures of hemoglobin. Empirical equations are reported to predict the oxygen partial pressure at half-saturation of hemoglobin (p50) from known values for pH, pCO_2 and 2,3-diphosphoglycerate, with an accuracy of ± 0.82 torr.

Although the large white pig is frequently used in physiological studies, little is known at present about the oxygen carrying properties of its blood. The primary structure of pig hemoglobin differs from that of human HbA in the exchange of 22 amino acids for each chain[1,2]. Differences in the oxygen affinity between humans and pigs should be expected, because the changes involve the site of the reaction of hemoglobin with 2,3-diphosphoglycerate (2,3-DPG).

The aim of this work is to investigate the simultaneous

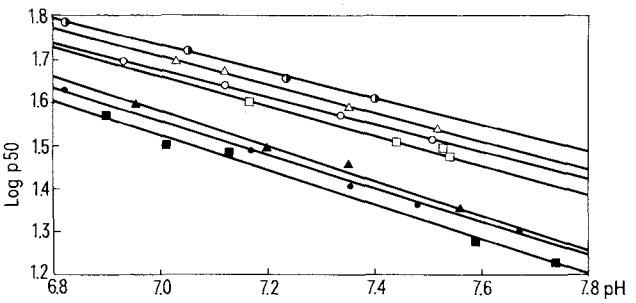

Figure 1. The Bohr effect, determined at 3 [2,3-DPG]/[Hb] ratios (1.08, 2.38, and 2.80, which are labeled with filled, open, and half-filled symbols, respectively), and at 3 pCO_2 (90, 45, and 20 torr, which are labeled with triangles, circles, and squares, respectively). The regression coefficients range from 0.986 to 0.999.

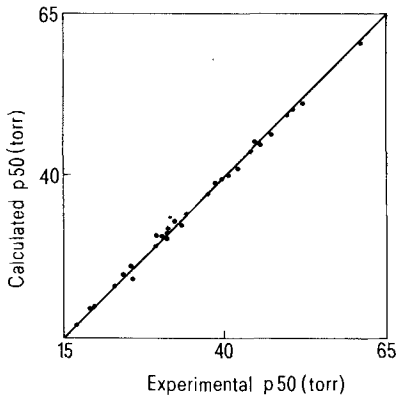

Figure 2. Correlation between the experimental and the calculated p50-values. The regression equation is: $y = 0.781 + 0.978$ x, $n = 32$, $r = 0.997$, and the theoretical line only is shown.

effects of H^+, CO_2, and 2,3-DPG on the p50 (the pO_2 at which hemoglobin is half-saturated with oxygen), which defines the position of the oxygen equilibrium curve, and thus is an index of the blood oxygen affinity. Although pig blood oxygen affinity has already been investigated[3-5], the simultaneous dependence of p50 on pH, pCO_2, and the concentration of 2,3-DPG is at present unknown.

Materials and methods. Blood was withdrawn without anesthesia from the jugular vein of 12 selected, 3 months old, male pigs, and was immediately heparinized and analyzed. Cellulose acetate electrophoresis was carried out using the Zip Zone kit from Helena Laboratories (Beaumont, Texas 77740)[6]. Total hemoglobin concentration was determined by the Drabkin method, using an extinction coefficient of 11.05 mM^{-1} cm^{-1} at 540 nm; 2,3-DPG was assayed using kits from Boehringer Mannheim (FRG). Hematocrit and the red blood cell count (RBC) were determined by standard laboratory procedures.

The p50-value was measured at 37 °C with a method[7,8] independent of the optical properties of hemoglobin, and suitable for nonhuman blood samples, provided that data analysis is modified as explained.

Results and discussion. Red cells are smaller (table), and the concentration of 2,3-DPG is larger in pigs than in humans, according to previously reported data[3-5]. The hemoglobin electrophoretic mobility was identical for all the samples and similar to that of human HbA.

Pig and human blood Hill coefficients were similar (2.64 ± 0.07, $n = 5$; vs 2.68 ± 0.07, $n = 24$, respectively), but the calculation of p50[7] was nevertheless changed appropriately, for greater accuracy. 32 p50-values were determined (fig. 1) under several preset[7] conditions of pH (range 6.8–7.8), pCO_2 (range 21–90 torr), and [2,3-DPG]/[Hb] molar ratio (range 1.0–2.8). To increase 2,3-DPG, red cells were incubated for 3 h at 37 °C with isotonic inositol:pyruvate:phosphate in the ratio 10:15:20[9], while 2,3-DPG was depleted from red cells by incubation with isotonic NaCl for 40 h, without detectable formation of methemoglobin.

Hematologic data in large white pig blood

	Mean	SD	n
Weight (kg)	19.1	1.1	7
[Hb] (g/dl)	10.61	0.7	12
[2,3-DPG] (mmole/l blood)	3.66	0.43	12
Hematocrit	31.7	2.4	7
RBC ($\times 10^{12}$/l)	6.37	0.34	7
[2,3-DPG]/[Hb] (mole/mole)	2.22	0.17	12
MCV (fl)	49.73	2.30	7
MCHC (g/dl)	33.3	1.85	7

The log p50 vs pH plot, i.e., the Bohr effect (fig. 1), was always linear (-0.35 ± 0.03, mean $\pm$ SD). Increasing pCO_2 and decreasing [2,3-DPG]/[Hb] increase the Bohr factor slightly. The equations to predict p50-value from known values for pH, pCO_2, and the [2,3-DPG]/[Hb] ratio, were obtained using the described procedure[7]:

$$\log p50_{(pH\,7.0)} = 1.4034 + 6.80 \cdot 10^{-4} * pCO_2 + (0.1060 - 5.7 \cdot 10^{-5} * pCO_2) * G$$

$$\log p50_{(pH\,7.6)} = 1.0913 + 2.09 \cdot 10^{-3} * pCO_2 + (0.1543 - 6.5 \cdot 10^{-4} * pCO_2) * G$$

$$\log p50_{(pH)} = [(pH - 7.0) * (\log p50_{(pH\,7.6)} - \log p50_{(pH\,7.0)})]/0.6 + \log p50_{(pH\,7.0)}$$

where $G = [2,3\text{-DPG}]/[Hb]$. The equations are valid at 37 °C within the investigated ranges of the variables, for a hemoglobin with electrophoretic mobility similar to that of human HbA. The average difference between the experimental and the calculated values is 0.004 ± 0.82 torr, and the correlation is shown in figure 2.

Since fetal and adult pig hemoglobins are functionally similar[5], and the 2,3-DPG concentration found by other workers in fetal and adult blood is well encompassed in the range of variation in which the equations are valid, we believe that this procedure may provide a powerful tool in predicting the oxygen transport in adults and in newborns, as well as the oxygen exchange across the placenta.

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