Table IV. Attenuation coefficients μ_o ($cm^2\ g^{-1}$), number of electrons per g *Ne* and average atomic number $\bar{Z}$ of bones in man

Person	661.6 keV		59.57 keV		28.5 keV	
	μ_o	*Ne*	μ_o	$\bar{Z}$	μ_o	$\bar{Z}$
	Corpus femoris					
H	0.0899	3.509×10^{23}	0.3610	$13.7_4 \pm 0.05$	1.461	$12.7_3 \pm 0.03$
I	0.0884	3.451	0.3465	13.5_2	1.559	13.1_1
K	0.0827	3.228	0.3460	14.1_2	1.537	13.3_5
Mean				13.8		13.1
	Caput femoris					
H	0.0895	3.494	0.2771	11.1_5	1.052	11.3_2
I	0.0875	3.416	0.2665	10.9_5	1.007	11.2_4
K	0.0890	3.474	0.2865	11.5_7	1.027	11.2_5
Mean				11.2		11.3
	Trochanter major					
H	0.0904	3.529	0.3003	11.9_1	1.218	11.9_1
I	0.0888	3.466	0.3019	12.1_5	1.055	11.3_7
K	0.0873	3.408	0.2807	11.5_6	1.142	11.7_8
Mean				11.9		11.7
	Ribs					
I	0.0893	3.486	0.3246	12.7_8	1.355	12.4_3
K	0.0875	3.416	0.3031	12.3_2	1.351	12.4_9
Mean				12.6		12.5
	Squama frontalis					
I	0.0882	3.443	0.3290	13.0_6	1.474	12.8_5
K	0.0826	3.224	0.3313	13.7_1	1.535	13.3_5
Mean				13.4		13.1

The variation of 0.05 or 0.03 on $\bar{Z}$ corresponds to an average error of 0.7% on the attenuation coefficient per electron μ_o/Ne.

28.5 keV. (With the values of HUBBEL, $\bar{Z} = 7.38$ and 7.44 respectively.)

The measured tissues were taken immediately after the death of the subjects and put into the Plexiglas cylinders mentioned above. The bones were crushed. An example of the experimental data for one tissue (striated muscle) is given in Table II. The summarized results of Tables III and IV show that $\bar{Z}$ varies from 6 to 8 for the soft tissues and from 11 to 14 for the bones[7]. The high and variable values of $\bar{Z}$ for the bones suggest the possibility of an in-vivo determination of calcium and phosphorus[8].

Résumé. Le coefficient d'atténuation massique μ_o des tissus humains pour 662, 60 et 28,5 keV est mesuré dans une géométrie à faisceau étroit, avec un détecteur NaI(Tl) mince, pourvu d'une électronique à fenêtre. Le nombre *Ne* d'électrons par g est déterminé avec une précision de $\leqslant$1,5% (S.D.). A partir des valeurs de μ_o mesurées à 60 et 28,5 keV, avec une précision de $\leqslant$1,7% (S.D.), on détermine un $\bar{Z}$ moyen qui varie de 6 à 8 pour les tissus mous et de 11 à 14 pour les os. On montre que le $\bar{Z}$ moyen de l'eau est très voisin de celui des tissus mous, la graisse exceptée.

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[7] The radiation of ^{125}I not being monoenergetic, the measured attenuation coefficient slightly varies with the thickness of the absorber. The result may be, for the high Z, an error of about 2% on μ_o whose consequence will bring down the values of $\bar{Z}$ for the bones.

[8] Acknowledgments are due to the Fund for Scientific Research, University of Zürich, which financed the electronic equipment and the NaI(Tl)-Crystal, and to the Institute of Pathological Anatomy of the University Zürich (Prof. Dr. med. E. Ühlinger and Prof. Dr. med. C. Hedinger) for supplying us with tissues.

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Inability of Specific Antibodies to Discriminate Between Frog and Tadpole Haemoglobins in Mixture

Rana catesbeiana tadpole (larval) and frog (adult) haemoglobins have different electrophoretic mobility and amino acid composition[1,2]. Both are relatively potent immunogens, and sera of rabbits immunized against tadpole or frog haemoglobins have been shown to be specific and without any significant degree of cross reactivity[3,4]. The following results show that rabbit antisera against tadpole or frog haemoglobins cannot discriminate between these two antigens when *R. catesbeiana* larval and adult haemolysates are mixed

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together. This finding is compatible with the formation of complexes between tadpole and frog haemoglobins in vitro.

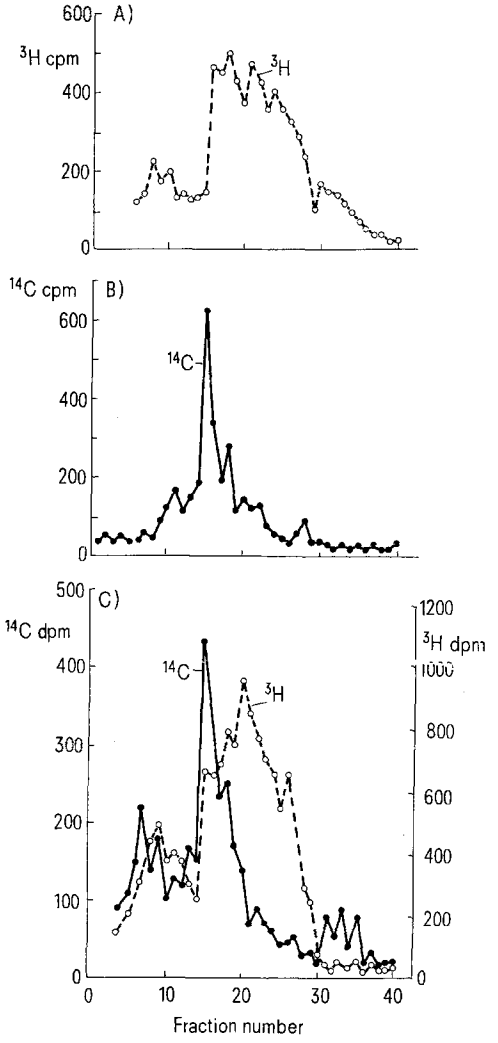
The source of *R. catesbeiana* tadpoles and frogs, and the methods of blood collection and separation of the various haemoglobin fractions by polyacrylamide gel electrophoresis, have been described[5]. Rabbits were immunized against the major tadpole and frog haemoglobin components as outlined by MANIATIS and INGRAM[3]. 3 to 5 injections, each containing 0.3–1.0 mg of the immunogen, were given. 1 week after the last injection the animals were bled and the antisera obtained were characterized by immunodiffusion, immunoprecipitation and immunofluorescence as described in reference[4].

Tadpole and frog red cells were labelled by incubation for 16 h at 29°C in Amphibian Culture Medium (Grand Island Biological Company) with either ^{14}C amino acids mixture (NEC-445) or tritiated L-valine and DL-lysine (Schwarz Bio-Research). After incubation the cells were chilled, washed in Ringer's solution and lysed in *tris*-glycine buffer (pH 8.3) by freezing and thawing. The haemoglobin was converted into cyanmethaemoglobin and the stroma and ribosomes were sedimented by centrifugation at 105,000 *g* for 90 min. Roughly equal amounts of the supernatant frog (^{14}C) and tadpole (^{3}H) haemoglobin solution were mixed together, dialyzed overnight against *tris*-glycine buffer (pH 8.3) supplemented with KCN and kept frozen until used.

Immunoprecipitation experiments between the haemoglobin solutions and the antisera were carried out in the presence of antibody excess as determined by standard immunoprecipitation curves[4]. Between 2–3 µg haemoglobin equivalent to 100–200 ^{14}C (frog haemoglobin) cpm and 300–500 ^{3}H (tapole haemoglobin) cpm were treated with either 0.4 ml anti tadpole haemoglobin antiserum or 0.1 ml anti frog haemoglobin antiserum. The mixtures were left overnight at 4°C. The immunoprecipitated material was washed twice in the cold, dissolved in formic acid and transferred to vials for determination of radioactivity.

The results of a representative experiment are shown in the Table. Treatment of labelled tadpole haemolysates with antisera against tadpole haemoglobin resulted in the precipitation of 38% of the radioactivity of the antigen; when frog haemolysates were treated with antisera against frog haemoglobin, 91% of the antigen was precipitated. The amount of the radioactivity precipitated from tadpole haemolysates by anti frog haemoglobin antisera, and from frog haemolysates by anti tadpole haemoglobin antisera, did not exceed 7%. This apparent specificity of the antisera could not be demonstrated when the antigens (^{14}C frog haemolysates and tritiated tadpole haemolysates) were mixed together. When larval and adult heamoglobins were both present in the reaction mixture, antisera against frog haemoglobin precipitated 96% of the ^{14}C (frog haemoglobin) counts and 46% of the ^{3}H (tadpole haemoglobin) counts. Similarly, antisera against tadpole haemoglobin precipitated 43% of the ^{3}H (tadpole haemoglobin) counts and 27% of the ^{14}C (frog haemoglobin) counts. Repeated experiments with higher amounts of antigen and antisera yielded similar results.

In order to confirm that the precipitin reaction involved indeed globin, and not some other non-haemoglobin red cell protein, the immunoprecipitates were further characterized by polyacrilamide gel electrophoresis. The immunoprecipitated material was dissolved in deionized 8 *M* urea, possible disulfide bonds were reduced by the addition of mercaptoethanol, thiol groups were blocked by alkylation with iodacetamide and the dissociated protein subunits were resolved by polyacrylamide gel electrophoresis in 8 *M* urea. After electrophoresis the gels were fractionated and counted as detailed in reference[5]. The results of the elctrophoretic separation and alkylated immunoprecipitated globin, depicted in the Figure, show that the immunoprecipitate obtained by treatment of a mixture of ^{14}C frog haemolysate and tritiated tadpole haemolysate with antiserum against tadpole haemoglobin, consisted of both tadpole and frog globin chains. It may be conluded, therefore, that specific antisera against tadpole and frog haemoglobins cannot discriminate between these two antigens when both of them are present in the reaction mixture.



← Electrophoresis of immunoprecipitated reduced and alkylated tadpole (*Rana catesbeiana*) and frog haemoglobin in alkaline 8 *M* urea polyacrylamide gels. The methods of preparation of the antisera, and of reduction and alkylation of the immunoprecipitation and the conditions for electrophoresis are detailed in references[4] and [5]. A) Tritiated tadpole haemolysates treated with antisera against tadpole haemoglobin. B) ^{14}C frog haemolysates, treated with antisera against frog haemoglobin. C) A mixture of tritiated tadpole haemolysates and ^{14}C frog haemolysates treated with antisera against tadpole haemoglobin.

[5] J. BENBASSAT, J. Cell Sci., in press.

Immunoprecipitation of tadpole (*Rana catesbeiana*) and frog labelled haemolysates

Antigen	TCA precipitable (cpm)		Antiserum	Immunoprecipitated (cpm)	
	^{14}C	^{3}H		^{14}C	^{3}H
Tadpole haemolysate (^{3}H)		648 (100%)	Anti-T	—	246 (38%)
			Anti-F	—	20 (3%)
			Control	—	18 (3%)
Frog haemolysate (^{14}C)	191 (100%)		Anti-T	10 (5%)	—
			Anti-F	174 (91%)	—
			Control	6 (3%)	—
Tadpole (^{3}H) and frog (^{14}C) haemolysates	100 (100%)	286 (100%)	Anti-T	27 (27%)	123 (43%)
			Anti-F	96 (96%)	132 (46%)
			Control	7 (7%)	16 (6%)

Anti-T and anti-F refer to antisera of rabbits immunized with the major components of tadpole and frog haemoglobins, respectively. The control consisted of serum of a non-immunized rabbit.

A conceivable explanation for this finding could be that roughly half of the amount of tadpole or frog haemoglobins in the mixture are present in the form of tadpole-frog haemoglobin complexes or hybrids. Haemoglobin is known to undergo dissociation in acid or alkaline solutions, concentrated salt media[6], and even at neutral pH and low ionic strength[7]. Hybridization i.e., recombination of subunits belonging to different mammalian[8] or amphibian[2] haemoglobins, has been reported to occur only when mixtures of haemoglobins were exposed to a pH near 4.7 or 11.0 and subsequently neutralized. The experiments reported in this paper were carried out at pH 8.3 and at low ionic strength. If valid, the conclusion that *R. catesbeiana* tadpole and frog haemoglobins form hybrids or complexes under these conditions, would suggest possible differences in the association-dissociation properties of haemoglobins from different species.

Résumé. Des anticorps spécifiques de l'hémoglobine de la grenouille et têtard de *Rana catesbeiana* ne peuvent pas être distingués lorsque tous deux sont présents dans la mixture de la réaction. Cette observation peut être expliquée par la formation d'hybrides entre les hémoglobines adultes et larvaires de *Rana catesbeiana*.

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Inhibition by Testosterone of Immune Reactivity and of Lymphoid Regeneration in Irradiated and Marrow Reconstituted Mice

It is well known that testosterone given to the chick embryo prevents the development of lymphoid tissues in the bursa of Fabricius and inhibits the antibody formation[1,2]. The present investigation was undertaken to determine the effect of testosterone on the antibody response in mammals.

Materials and methods. Male C3H/He mice of 8 weeks old received 850 rad whole body γ-irradiation from a ^{60}Co source and were then injected i.v. with 36×10^6 syngeneic bone marrow cells within 3 h. The animals were divided into 4 groups. The 1st group of animals was not treated with testosterone. 10 mg of testosterone in 0.2 ml aquous suspension (Enarmon, Teikokuzoki Pharm. Co., Tokyo; a mixture of isotonic saline, 17β-hydroxy-androst-4-en-3-one, arabic gum and thimerosal) was given s.c. 7 times to the 2nd group at 4 h intervals; and to the 3rd group at 24 h intervals from the day of marrow reconstitution. The 4th group animals were injected also 7 times at 24 h intervals from the 6th day after marrow reconstitution. The animals in all groups received i.v. 2×10^8 sheep red blood cells 30 days after marrow reconstitution and were sacrificed 5 days after antigen challenge. Assays of haemolysin were carried out, employing microtitration equipment. The plaque forming cells (PFC) were enumerated according to the method of Cunningham and Szenberg[3]. The rosette forming cells (RFC) were calculated using the method of Haskill et al.[4]. The sections of the thymus, spleen and mesenteric lymph node were stained with methyl green pyronin.

Results. The results are shown in the Table. The involution of the thymus was striking in testosterone-treated animals. The reduction of the spleen weight was not so remarkable. The fall of the PFC response was particularly conspicuous in group 3. The haemolysin titer also fell markedly. The fall of the RFC response was not so dramatic as that of the PFC response. The histo-

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