percent ($P < .01$) and glucose uptake by 60 percent ($P < .01$) of basal levels. On the other hand, glycogen consumption was increased by 45 percent ($P < .01$) and oxygen consumption by 29 percent ($P < .1$) above basal activity. These changes were associated with a 37 percent fall in platelet ATP over 1 hour (Table 1). The decreased glycolysis and the associated increase in glycogenolysis suggest that aspirin inhibits glycolysis at a point between extracellular glucose and glucose-6-phosphate. Preliminary studies have failed to reveal an effect of aspirin on platelet hexokinase assayed in vitro, which suggests that aspirin suppresses glucose transport. The increase in oxygen uptake may be a consequence of the impaired glycolysis and fall in ATP levels or may be due to an uncoupling of oxidative phosphorylation (*4*). The mechanism by which aspirin produces its effects on platelets is uncertain. Aspirin has been shown to acetylate numerous human proteins (*11, 12*) and it is possible that it inhibits ADP release and glucose transport by acetylation of membrane proteins.

Collagen produced a 19 percent increase in lactate production ($P < .05$) and a 34 percent increase in glucose uptake ($P < .05$), but no effect on glycogen utilization or oxygen uptake (Table 1). In addition, collagen produced release of ADP and redistribution of particle-bound hexokinase into the cytoplasm (*10*). The effect on both lactate production and release of ADP was markedly reduced when the collagen preparation was heated to 55°C or incubated with collagenase (0.1 mg/ml; 37°C) to the point where it just failed to produce visible aggregation. In a series of four experiments, heat denaturation reduced the increase in lactate production from 35 to 6 percent and release of ADP from 1.7 to 0.1 μmole per 10^{11} platelets. Collagenase treatment reduced the increase in lactate production to 4 percent and the release of ADP to 0.1 μmole per 10^{11} platelets. This suggests that the effects on both platelet aggregation and glycolysis are caused by a common property of the collagen fiber.

Aspirin completely inhibited ADP release but did not prevent the effects of collagen on glycolysis and particle-bound hexokinase. This suggests that aspirin does not suppress the overall interaction of platelets with collagen but that it interferes with certain steps in the platelet collagen reaction. Collagen is thought to produce a generalized increase in platelet membrane permeability (*13*) and it may be that it overcomes the inhibitory effect of aspirin on glucose transport by such a mechanism. On the other hand, an increase in membrane permeability would not necessarily cause release of ADP because it has been shown that only the ADP present in granules and not that in cytoplasm is released by collagen (*14*).

It thus appears that aspirin acts on human platelets in at least two ways: by inhibiting release of ADP and inhibiting glucose transport. The inhibition of ADP release is unlikely to be a direct result of the decreased glycolysis and energy production.

J. C. G. DOERY
J. HIRSH
G. C. DE GRUCHY
University of Melbourne,
Department of Medicine,
St. Vincent's Hospital,
Melbourne, Australia

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Tadpole Antibodies against Frog Hemoglobin and Their Effect on Development

Abstract. Rana catesbeiana *tadpoles immunized with the main hemoglobin component of the adult frog of the same species produced precipitating and agglutinating antibodies against the immunogen. After natural metamorphosis, the immunized froglets have as their only major hemoglobin a protein immunologically and electrophoretically different from the major hemoglobin of control froglets.*

During the metamorphosis of *Rana catesbeiana* tadpoles the larval hemoglobin is replaced by adult hemoglobin, a protein with a different peptide chain constitution (*1, 2*); rabbit antibodies against either hemoglobin do not cross-react with the other (*3, 4*). It has now been demonstrated that tadpoles form precipitating antibodies when their own adult hemoglobin is used as the immunogen. These animals are particularly likely to show this phenomenon because the larval stages are immunologically competent (*5*) and because the major frog hemoglobin was not detectable immunologically in tadpoles before metamorphosis (*4*).

Larval and adult *R. catesbeiana* were obtained from the Connecticut Valley Biological Supply Co. Tadpoles were immunized with the dimer of the major frog hemoglobin, which had been fractionated by gel filtration on Sephadex G-100 (*2*). The dimer (5 μg) was emulsified in complete Freund's adjuvant and injected subcutaneously into the backs of tadpoles before metamorphosis. One month later a booster injection of the same amount was given intraperitoneally without adjuvant. Two months after the first injection, serum was obtained by decapitation of three immunized tadpoles and pooled and concentrated to one-fifth of the original volume by lyophilization. The concentrated antiserum tested against the immunogen by double diffusion in agar gave a single precipitin line. Since the animals do not come from an inbred strain, we tried to obtain evidence against the presence in the immunogen of an unusual hemoglobin variant. The concentrated tadpole antiserum was tested against hemoglobins from frogs supplied by three different companies. A single precipitin line developed in all cases (Fig. 1A), whereas with concen-

trated serum from nonimmunized control tadpoles there was no precipitin line. Tadpole antiserum was also tested against the immunogen by the passive agglutination method with barium sulfate particles (*6*). The antiserums tested gave a positive result up to a 1:24 dilution.

Six of the 64 tadpoles immunized with frog hemoglobin underwent natural metamorphosis during the summer (25 died during that period, and 20 were used for testing). About 2 or 3 weeks after the completion of metamorphosis (tail resorption), the serum of these froglets did not contain detectable antibodies to frog hemoglobin.

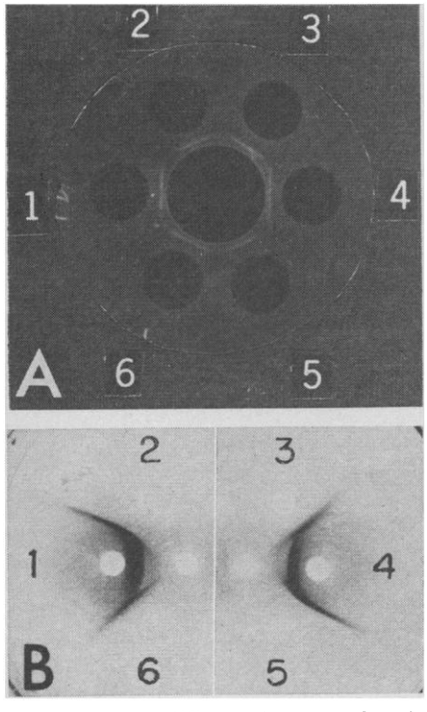

Fig. 1. Immunodiffusion in agar of antiserums against frog hemoglobins. (A) Tadpole antiserum. In each pair there is a single, unstained precipitin line. The center well contained pooled serums from three immunized tadpoles; the serums had been concentrated to one-fifth of the original volume. Well 1 contained frog hemoglobin solution, fractionated on Sephadex G-100 (0.1*M* NaCl, 0.1*M* tris-HCl, *p*H 8.2). This was used as immunogen. Well 2 contained unfractionated frog hemoglobin (Connecticut Valley Biological Supply). Wells 3 and 4 contained unfractionated frog hemoglobin (Carolina Biological Supply). Wells 5 and 6 contained unfractionated frog hemoglobin (Lemberger). (B) Rabbit antiserum. Precipitin lines showing spurs; the precipitate was stained with Ponceau red. The center wells contained hemoglobin from two immunized froglets. Wells 1 and 4 contained rabbit antiserum against the major frog hemoglobin. Wells 2 and 5 contained unfractionated hemoglobin from a control froglet. Wells 3 and 6 contained immunogen.

The animals were killed and their hemoglobins were tested by double diffusion with rabbit antiserum against the major frog hemoglobin. As controls we used two froglets immunized as tadpoles with either hemocyanin or bacteriophage φX174. In the double diffusion test the hemoglobins of froglets immunized as tadpoles against their own adult hemoglobin (Fig. 1B) showed a reaction of partial identity with both frog and control froglet hemoglobins. There was a spur to the precipitin lines which stained with the benzidine reagent for heme proteins. Control froglet and adult frog hemoglobin showed a reaction of identity against the same antiserum.

Control and experimental hemoglobins were also compared by disc electrophoresis on split polyacrylamide gels (Fig. 2A). The hemoglobins of the two control froglets migrated identically with normal frog hemoglobin. By contrast, the hemoglobins of the six froglets previously immunized against frog hemoglobin gave a pattern different from that of the control and the tadpole pattern. Clearly the main hemoglobin component of the immune tadpoles migrated more slowly than that of the controls. Furthermore, the normal main hemoglobin was absent in the experimental animals.

Since hemoglobin of the adult bullfrog polymerizes in vitro (*7*) and since the hemoglobin of the immunized froglets also polymerizes [as judged by the multiple bands of the electrophoretic pattern (Fig. 2B-1)] the observed electrophoretic difference might have been due to different states of polymerization. To test this hypothesis, we reduced and alkylated the hemoglobins; under these conditions frog hemoglobin depolymerizes to the four-chain molecule (*7*). The electrophoretic patterns of the reduced and alkylated hemoglobins are shown in Fig. 2B. The difference between the hemoglobins of control and of previously immunized experimental animals is still visible; therefore it is not simply due to a different state of polymerization. It remains to be shown that the peptide chains of the new hemoglobin are indeed different in their primary structure.

It is unlikely that the observed differences are due to genetic polymorphism of the amphibian hemoglobins, because all six immunized froglets did contain the new hemoglobin while none of the controls did. Also, we have not noticed this new hemoglobin in any of the adult frogs that we bled. In three

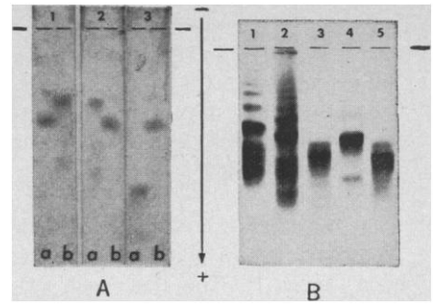

Fig. 2. Polyacrylamide discontinuous gel electrophoresis at *p*H 10.3, 0°C (*2*). (A) Split gels, unstained. Gels 1a and 2b contained hemoglobin from a control froglet; gels 1b and 2a contained hemoglobin from two immunized froglets; gel 3a contained tadpole hemoglobin; gel 3b contained adult frog hemoglobin. (B) Whole gels, stained with benzidine. Gel 1 contained hemoglobin (large quantity applied) from an immunized froglet and shows multiple, slowly migrating bands of polymerized hemoglobin. Gel 2 contained hemoglobin (large quantity applied) from an adult frog; this hemoglobin is also polymerized. Gel 3 contained reduced and alkylated hemoglobin from a control froglet. Gel 4 contained reduced and alkylated hemoglobin from an immunized froglet. Gel 5 contained reduced and alkylated adult frog hemoglobin. For reduction and alkylation, the samples were left in 0.1*M* mercaptoethanol at *p*H 8.65 for 2 hours at 37°C and then in 0.2*M* thrice recrystallized iodoacetamide for 15 minutes. Since the sulfhydryl groups are negatively charged at the *p*H at which the electrophoresis was carried out, alkylation of these groups with iodoacetamide alters the mobility of the hemoglobin components.

instances in which pooled samples from three to five animals have been subjected to electrophoresis, a variant hemoglobin comprising 20 percent or more of the sample would have been easily detected.

Hemoglobin-containing cells can be recognized by antibodies directed against purified hemoglobin; we have found that washed frog red cells are specifically agglutinated by a rabbit antiserum prepared against the frog hemoglobin. The reaction is inhibited by prior absorption of the antiserum with frog hemoglobin purified successively by crystallization, gel filtration, and gel electrophoresis. This control indicates that the rabbit antibody recognizes hemoglobin and not stromal material. The agglutination reaction is also specific for the frog red cells, but not for the tadpole's, even though the latter had been suspended in a solution of frog hemoglobin and then washed.

The above-mentioned experiments

show (i) that larval *R. catesbeiana* can be made to produce antibodies against their own major frog hemoglobin and (ii) that the hemoglobin of the postmetamorphic animals developed from immunized tadpoles is different from the usual hemoglobin of the adult frog.

There are several possible ways by which the immunization of the tadpoles might have altered the froglet hemoglobin. First, since the animals do not come from an inbred strain, it is conceivable that the only immunized animals able to go through metamorphosis were those with the variant hemoglobin. However, as mentioned above, it seems unlikely that such a variant exists. The presence of antibodies in a developing animal against certain allotypic specificities of that animal may lead to persistent changes of the phenotype of these animals. This has been shown for the rabbit and mouse immunoglobulins (*8*). A similar situation may have occurred in our experiments. The presence in the tadpoles of antibodies directed against their own adult hemoglobin at a time when a new adult erythropoietic cell line was developing (*9*) may have eliminated this adult cell line. Such conditions might favor another cell line which in the normal animal is either present in small numbers or completely absent. These results suggest the potential use of antibodies in altering the course of development by a simple and specific method.

GEORGE M. MANIATIS
LISA A. STEINER
VERNON M. INGRAM
Department of Biology, Massachusetts Institute of Technology, Cambridge 02139

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Uncus and Amygdala Lesions: Effects on Social Behavior in the Free-Ranging Rhesus Monkey

Abstract. *The effect of uncinectomy on the social behavior of free-ranging rhesus monkeys was observed on Cayo Santiago Island. Operated subjects showed social indifference, failed to display appropriate aggressive and submissive gestures, were expelled from their social group, and eventually died. Two juvenile subjects with the lesion restricted to amygdala survived and have rejoined their social group. Size of lesion and age at operation were major factors in determining the degree of behavioral change.*

While considerable data have accumulated on the behavior of subhuman primates after circumscribed brain lesions, these observations have been limited to the caged animal. In order to extend the scope of our studies of brain behavior to include a long-neglected but vitally important dimension of behavioral observations, we have begun to examine the effects of circumscribed brain lesions on the social behavior of subhuman primates under free-ranging conditions. Several studies of the effects of uncus and amygdala lesions on the social behavior of related species that display different temperaments and social behavior in the cages, as well as under free-ranging conditions, are now under way. In two studies the effects of amygdalectomy on social interaction in groups of laboratory housed macaques have been examined. Rosvold, Mirsky, and Pribram (*1*) have reported that amygdalectomized rhesus monkeys fall in dominance in a group situation but that in a paired situation the operated animal may become dominant. Mirsky (*2*) has noted that after amygdalectomy these monkeys become less competitive for food in a group situation, lose rank in the group hierarchy, and are less fearful of man. The following is a preliminary report on one of our first attempts to study the effects of a brain ablation on behavior in free-ranging monkeys. We chose to begin with observing the effects of amygdaloid lesions on the rhesus monkeys of Cayo Santiago Island (*3*).

The subjects for the study were members of group E of the rhesus colony of Cayo Santiago, a 238-acre (96-hectare) island off the coast of Puerto Rico. Group E and six other social bands (a total population of almost 900 animals) are maintained with Purina Chow dispensed at six hog-feeders located around the island. Within each group, a clearly defined social structure regulates daily life; similarly, a definite rank order characterizes relations between groups. Social status, both within and between groups, determines access to food, space, and, to a degree, reproductive partners. Only a few adult males live in isolation from existing groups. Group cohesion appears to be maintained by familial ties and by patterns of feeding, grooming, and reproduction. Relationships between individuals are expressed and maintained by facial, postural, and vocal communication (*4*, *5*). All animals were tattooed on chest and thigh, easily identified, and not too perturbed by human observers after the first few weeks of contact.

In this setting, the 85 members of group E were observed by Dicks from early birth season (March) to early mating season (August) of 1968. The observer attempted to distribute a maximum of 25 hours of observation per week over all members of the group. Patterns of association, grooming, and aggression, and reproductive and feeding behavior were of primary interest.

At the end of 2 months of observation, when the social habits of the subjects began to emerge from the data, two young adult males (5 and 6 years old, hereafter called 5AU and 6AU) were selected for operation and trapped. Here, as in all cases, care was taken to select one animal from intermediate dominance levels of each age group. Amygdaloid nuclei and uncinate cortex were removed bilaterally, aseptically, under Nembutal anesthesia.

After the operation these two animals were kept together in a large enclosure [approximately 20 by 20 feet (6 by 6 m)]. Both remained relatively inactive, sitting in what appeared as a withdrawn state but with their eyes open. However, both responded appropriately to animals threatening through the wire walls of the enclosure. In the only observed violent encounter, 6AU defeated the previously dominant, younger, and smaller 5AU. The younger monkey tended to follow and sit close to the older. Neither began normal feeding until the doors of the food bin were propped open by the observer; before operation, these animals fed only after another monkey had opened the doors.