tive serological properties in the stem cells entering the thymus may be inhibited. Inhibition may be due to either loss of the inductive capacity of the involuting thymus, or refractoriness of the stem cells to the inducing capacity of the thymus. Boyse *et al.* (*20*) have recently observed that expression of the TL antigen could be suppressed in the thymus of neonatal and adult mice by antibodies against the TL antigen; evidence was presented that this phenomenon was not due merely to masking of the antigenic sites by blocking antibodies; experiments in vitro showed that treatment of TL-positive cells with actinomycin D abolishes their capacity to modulate to TL-negative cells. Thus it seems that treatment with TL antibody triggers an active metabolic process leading to loss of the TL antigen.

Recent evidence is that administration of cortisol may inhibit synthesis of purine nucleotide and protein in the thymus, and that it may impair the ability of thymic ribosomes to incorporate amino acids (*21*). The changes in TL antigenicity and in sensitivity to guinea pig serum, observed by us, were very abrupt and complete within 1 day or 2. Since the expression of TL antigenicity and guinea pig serum–sensitivity in the thymus seems to involve an inductive process, the abrupt "switch off" and "switch on" of thymus-distinctive properties may reflect an effect on the genetic regulatory mechanisms of thymus cells.

While the antigenic changes observed in the involuting thymus could reflect inhibition of inductive processes, it now seems entirely possible that they could be accounted for by selection of a cell population lacking thymus-distinctive serological properties. Further information is necessary to determine which of these two mechanisms causes the changes.

M. SCHLESINGER
V. K. GOLAKAI

Department of Experimental Medicine and Cancer Research, Hebrew University–Hadassah Medical School, Jerusalem, Israel

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Heme and Globin Synthesis Control: Observations in vivo in Beta Thalassemia

Abstract. *After administration of glycine-2-^{14}C to a patient with thalassemia, the specific activities of heme and globin of F hemoglobin were consistently higher than those of hemoglobin A. After reaching a maximum, the ratio of the specific activity of heme to that of globin remained constant within each hemoglobin. Explanations considered include dilution by preformed subunits, differential turnover of hemoglobins, and possibly more than one heme-synthesizing pool.*

The biosynthetic relation between heme and globin is a problem of current interest, and the heritable abnormalities of hemoglobin synthesis, such as the thalassemias, provide an opportunity to obtain insight into this relation. The thalassemia mutations are considered to interfere with the rate of synthesis of globin chains, and there is no evidence of structural abnormality in the hemoglobins (*1*). Our study is concerned with the relation between the heme and globin moieties of hemoglobins A and F.

A splenectomized patient with β-thalassemia intermedia, who was producing a mixture of hemoglobins, A (56 percent), F (44 percent), and A_2 (1.1 percent), was given a single dose of 150 μc of glycine-2-^{14}C intravenously for the purpose of studying the labeling of hemoglobins A and F in the circulating red cells and the labeling of various pyrrole compounds excreted in the urine and feces (*2*). Washed red cells were hemolyzed, and the stroma was removed by toluene treatment and centrifugation. The hemolyzates were stored at −20°C for a few days and were then dialyzed overnight against phosphate buffer, *p*H 6.0 (0.05*M* sodium phosphate; 0.05*M* NaCl; 0.005*M* NaCN). Hemoglobins A and F were separated on a carboxymethyl Sephadex C-50 column (*3*). The phosphate buffer was also used to equilibrate the column and to elute hemoglobin F. To elute hemoglobins A and A_2, phosphate buffer (*p*H 7.0, in 0.1*M* NaCl) was used. The separation of the fractions was checked by starch-gel electrophoresis. The small amount of A_2 hemoglobin was included with the A fraction. The hemoglobin solutions were concentrated by pressure dialysis, and heme and globin were prepared from each (*4*).

Measured samples of solutions prepared from crystalline hemin were placed on a planchet in a thin layer, and their ^{14}C activity was measured in a gas-flow counter. Some activity was evident at 24 hours after the pulse labeling (Fig. 1), at which time the activity of the heme from hemoglobin F was already greater than that of the heme from hemoglobin A. This difference persisted throughout the period of study, the ratio of specific activities of heme (F/A) ranging from 1.4 to 2.1 (mean 1.7). The ratio taken from the smoothed curves is highest at day 1, then falls steadily, but after 18 days remains at approximately 1.5.

Globin was dried to constant weight, and ^{14}C activity was measured in thick samples in the gas-flow counter, and also by liquid scintillation counting (Fig. 2). Some radioactivity was present at 24 hours, and the activity of globin from hemoglobin F was consistently greater than that of globin from hemoglobin A. The ratio of specific activities (F/A) was very similar to that for heme, ranging from 1.3 to 2.5 (mean 1.7); but the ratio taken from the smoothed curves was lower at day 1 (1.3), reached its highest value of 2.4 at day 8, and subsequently fell somewhat before leveling off at about 1.5, also after day 18.

Appropriate corrections for self-absorption and quenching were applied so that the specific activities obtained by different counting methods could be directly compared. The two α- and two β- or γ-chains of hemoglobins A or F incorporate 40 glycine residues for each molecule (*5*). The corresponding four heme units incorporate 32 glycine residues per molecule of hemoglobin. On this theoretical basis, a ratio between specific activities of ^{14}C-glycine in heme and globin can be calculated, so that if the source of their glycine is a single uniformly labeled pool, the ratio will be unity. Ratios calculated from observed specific activities in normal subjects and in thalassemia are less, ranging from 0.5 to 0.8 (*6*). In our study, the ratios for hemoglobins A and F were approximately the same. After reaching a plateau on day 3, they remained constant at a mean value of 0.7 for the duration of this study.

Studies of hemoglobin formation in vitro have tended to focus on differences in rates of synthesis of peptide chains. Scant attention has been paid to differences in the rates of synthesis of the attached heme groups, which might provide a clue to the integration of the processes, especially since heme is an essential stimulus to globin synthesis; it is not entirely clear at what point it acts, although it may stabilize the polyribosome-peptide complex (*7*).

In β-thalassemia there is decreased incorporation by reticulocyte ribosomes of amino acids into hemoglobin A in vitro as compared to hemoglobin F; and it has been suggested that the reduction is due to a decrease or a defect in messenger RNA for A hemoglobin (*8*). In experiments in vitro where individual chains have been isolated, in β-thalassemia the specific activity of α-chains from A hemoglobin is usually greater than that of β-chains, whereas the opposite is found in control material (*9*). Huehns and McLoughlin, however, have demonstrated the presence of free α-chains in vitro in β-thalassemia; when these free α-chains are carefully separated, the specific activity of combined α-chains from A hemoglobin is always less than that of β-chains (*10*). They suggest that this discrepancy may be due to interchange occurring in vitro between free and combined α-chains, particularly those of A hemoglobin (*11*).

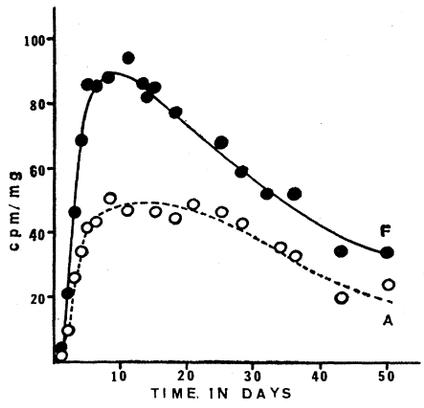

Fig. 1. Specific activity of heme (gas-flow counting) of hemoglobin F (●——●) and hemoglobin A (○- - -○).

We have here shown that, in vivo, specific activity of globin (and heme) of F hemoglobin is nearly twice as great as that of A hemoglobin. This result differs from the findings of Gabuzda *et al.* (*12*) who compared specific activities in vivo of whole hemoglobins A, A_2, and F in β-thalassemia and found that hemoglobin F had initially lower specific activity and subsequently higher specific activity than A. However, these authors did not separate heme and globin.

Our finding, that the specific activities of the hemes of hemoglobins A and F differ from each other and parallel those of the corresponding globins, is somewhat surprising, since it might have been supposed that a single heme pool would contribute heme of uniform specific activity indiscriminately to α-, β-, and γ-chains. Several possibilities must be considered in explaining the difference in the labeling of the hemes and the parallelism with their respective globins:

1) If there is a single heme pool so that the initial specific activities of the hemes of A and F hemoglobin are the same, the difference might arise early in the overall process by unequal dilution by pre-formed subunits. Since α-chains are common to the two hemoglobins and if they are equally labeled in both, it would be necessary to postulate that a pool of preformed β-chains with associated heme diluted the labeling of hemoglobin A. There is no direct evidence of a β-chain pool, but excess free α-chains occur in β-thalassemia (*10*, *13*) and an alternative explanation, that free α-chains might dilute activity by interchanging to a greater extent with hemoglobin A α-chains than with hemoglobin F α-chains, is suggested by the recent observations of Huehns and McLoughlin (*11*).

2) Again, if there is a single heme pool with equal initial specific activities for hemes A and F, the observed difference might arise by different turnover rates of the two hemoglobins. Since there is heterogeneous distribution of hemoglobin F in the red cells, this would imply that cells rich in F survived longer than those rich in A, or a process of differential destruction, for which there is some evidence (*12*). In this case, however, the ratio F/A should increase with time, whereas in our data, this ratio tends to decrease.

3) A third possibility compatible with our data would be the presence of more than one heme pool. Separate pools might be associated specifically with each heme-protein; such a system could facilitate coordinated synthesis of heme and each individual protein moiety. Coordinate syntheses of heme and the apoenzyme of tryptophan pyrrolase in rat liver have recently been reported (*14*). Furthermore, from studies in the tetrapyrrole synthesizing strains of *Rhodopseudomonas spheroides*, Lascelles has suggested the possibility of two distinct δ-aminolevulinic acid synthetases (the rate-limiting enzyme in heme formation), one directed towards the magnesium branch (chlorophyll) and one toward the heme pathway, and each specifically con-

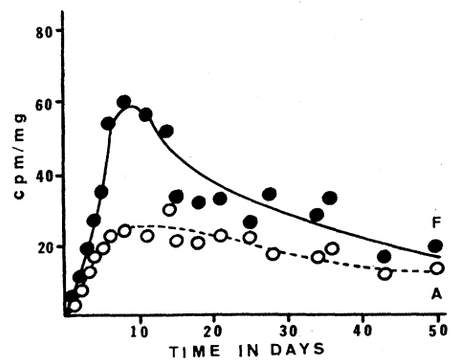

Fig. 2. Specific activity of globin (liquid scintillation counting) of hemoglobin F (●——●) and hemoglobin A (○- - -○).

trolled by its end product (*15*). Our speculation would imply several heme-synthesizing systems each, perhaps under separate genetic control, geared to α-, β-, or γ-chains.

MARTHA KREIMER-BIRNBAUM
ROBIN M. BANNERMAN
Department of Medicine, State University of New York at Buffalo, and *Buffalo General Hospital, 100 High Street, Buffalo 14203*

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Purified Staphylococcal Alpha Toxin: Effect on Epithelial Ion Transport

Abstract. *Purified staphylococcal alpha toxin, when added to the serosal bathing medium of the isolated toad bladder, causes a rapid fall in short-circuit current and transepithelial potential difference. It has no effect when added to the mucosal bathing medium. Oxygen consumption by suspensions of minced bladder tissue is stimulated by the toxin. These effects are neutralized by staphylococcal antitoxin.*

Purified staphylococcal α-toxin (hereafter referred to as toxin) is a heat-labile protein (molecular weight, 44,000) that produces hemolysis, dermatonecrosis, and death in animals (*1*, *2*). Studies of the action of the toxin on rabbit erythrocytes and cultures of kidney cells have indicated that the primary site of action may be the cell membrane. The very rapid release of potassium from erythrocytes prior to lysis, followed by loss of hemoglobin during lysis, suggests that a membrane "leak" occurs. Small molecules escape initially; large ones are lost as the leak increases in size (*3*). Tissue culture cells incubated with toxin release amino acids first and proteins later (*4*). This toxin appears to be one of several substances capable of causing lysis in a variety of membrane-bounded structures such as erythrocytes, platelets, lysosomes, bacterial protoplasts, and *Mycoplasma* (*5*). The purpose of our study was to determine the effect of purified staphylococcal α-toxin on transport phenomena and oxygen consumption in the isolated toad bladder.

Purified staphylococcal α-toxin was prepared by a modification of the method of Madoff and Weinstein (*1*). The final preparation was frozen in borate buffer (0.3*M*, *p*H 8.6) and stored in 0.5-ml portions at −60°C. The specific activity of the toxin was 30 to 60 hemolytic units (HU) per microgram of protein. Analysis by the Ouchterlony double-diffusion technique with Burroughs-Wellcome antitoxin to staphylococcus (1 ml neutralized 100,000 HU of purified α-toxin) revealed a single band with toxin containing 35,000 HU/ml; concentrations of 70,000 HU/ml, and less potent preparations stored at −60°C for 2 to 3 months, produced a faint second band.

Short-circuit current (SCC) in microamperes (μa), and spontaneous transepithelial potential difference (PD) in millivolts (mv) across the isolated toad bladder (*Bufo marinus*) were determined by the method of Leaf, Anderson, and Page (*6*). Each half of a single bladder was mounted between two sections of a lucite chamber with cross-sectional area of 7.07 cm^2. The bladder tissue was bathed on each side by 20 ml of toad Ringer solution, *p*H 7.35 (*6*). Only bladders with which the current fluctuated over a range of no more than ± 10 μa for 20 minutes or showed a continuous rise during this period were used. Bladders exhibiting a potential difference of less than 15 mv or an electrical resistance of less than 150 ohm (calculated as the quotient of PD and SCC) were discarded.

In initial studies, 500 μl of purified toxin (40,000 HU/ml) was added to the serosal bathing medium of one hemibladder. The same quantity of toxin, inactivated by heating at 56°C for 30 minutes, was similarly applied to the control. A rapid decline in both SCC and PD was observed after the addition of toxin in all of ten experiments. The heated material produced no significant change. These experiments were then repeated, with toxin neutralized by antitoxin to exclude the possibility of a nonspecific effect. Six studies were carried out with 150 μl of α-toxin (6000 HU). One set of bladders was treated with toxin alone and another with the same quantity previously incubated with a fully neutralizing dose of staphylococcal antitoxin (75 μl). In order to maintain equal volumes in all the preparations, 75 μl of Ringer solution was added to the unneutralized toxin in three experiments, and 75 μl of normal horse serum in three others. An abrupt but small rise in short-circuit current occurred within 1 to 2 minutes after treatment with toxin in half the experiments; this was followed by a rapid fall in all experiments to less than 10 μa in 30 to 45 minutes. During the same time interval, PD declined steadily. The control hemibladders remained unchanged (Fig. 1). The difference in

Table 1. Effect of staphylococcal alpha toxin and a toxin-antitoxin mixture on oxygen consumption (microliters per minute) by minced toad-bladder tissue. Numbers in parentheses indicate the number of samples.

Test substance	Oxygen consumption (Mean ± S.E.M.) *	*P* †
Toxin (17)	6.5 ± .19	<.001
Toxin + antitoxin (7)	4.5 ± .28	>.9
Antitoxin (8)	4.5 ± .20	>.9
Borate buffer (11)	4.4 ± .20	>.8
Tris buffer (15)	4.8 ± .15	

* S.E.M. = Standard error of the mean. † Each *P* value represents comparison by *t*-test to the control (tris buffer alone).