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Factor VIII Antihemophilic Globulin*)

General Review

Alfredo Pavlovsky**)

The study of hemophilia is closely bound to the investigations on coagulation, so much so, that although the disease does not create a social problem, it has been the subject of innumerable papers, existing several centers specialized in its study and foundations to sponsor the researches.

Studies on the clotting defect in this disease have been responsible for real advances in the knowledge of hemostasis. Among the several problems that comprise the study of hemophilia (genetic, clinical, therapeutical) the etiological factor occupies a prominent place.

With the object of deepening and revising the knowledge on this subject a Symposium on Factor VIII (Antihemophilic globulin) has been organized, to take place in August in the Instituto de Investigaciones Hematologicas, Buenos Aires, with the participation of some outstanding hematologists with vast experience in the matter.

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*) **Synonyms:** (In accordance with the International Committee for Standardization of Nomenclature of the Blood Clotting Factors).
Factor VIII (Koller)
Antihemophilic Globulin (Patek and Taylor)
Antihemophilic Globulin A (Cramer)
A. H. F. Antihemophilic Factor (Brinkhous)
P. T. F. Plasma Thromboplastic Factor (Ratnoff)
P. T. F. A. Plasma Thromboplastic Factor A (Aggeler)
T. P. C. Thromboplastic Plasma Component (Shinowara)
Facteur Antihemophilique A (Soulier)
Thromboplastinogen (Quick)
Prothrombokinase (Feissly)
Platelet Co-factor (Johnson)
Plasmokinin (Laki)
Thrombokatalysin (Lenggenhager).

**) Instituto de Investigaciones Hematologicas de la Academia Nacional de Medicina. Melo 3081. Buenos Aires. Argentina.

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The present paper is a preamble to the contributions. In it a short review on the present problems, and a summary of the work performed by our team during the lapse of 27 years, have been made.

Schmidt in 1893 (43) suggested that the defect of coagulation in hemophilia was due to a deficiency of thromboplastin. This induced Manteuffel (43) to investigate what zymoplastic substances shortened the clotting time of hemophilic blood.

* * *

Since then, slow but sure progress in the individualization of this factor has been made, trying to find out its origin, nature, location in the process of coagulation, and its later destiny. Intense efforts have been made to purify it, enabling its use for therapy. As Quick (66) pointed out (1942), several authors have supported Schmidt's theory.

In 1935, Quick, Stanley-Brown and Bancroft (69) proved that prothrombin time was normal in quality and quantity in hemophilic blood, which was deficient in thromboplastin. This was later confirmed by Brinkhous (1939) (17).

Addis' work (1911) (1), precipitation of a globulin of normal plasma, was the precursor of the important investigations of the school of Minot (46), in Boston (Patek and Taylor etc. [49—50]), and those of Bendien and van Creveld (5) in Holland which impelled to isolate and study the properties of the antihemophilic globulin (as it was called by Patek and Taylor).

Later two types of hemophilia existed, and the deficiency of antihemophilic globulin described by the latter authors corresponded only to one of the varieties.

In 1944, Castex, Pavlovsky and Simonetti (22), observed that the blood of some hemophiliacs corrected the defect of coagulation of other hemophiliacs. Pavlovsky et al. (60) later, proved that the antihemophilic globulin of patients with thrombopenic purpura, congenital fibrinopenia and some hemophiliacs, had the same coagulant activity as the normals.

Subsequently, in 1947 (6, 21), this data was confirmed, although no satisfactory explanation could be found.

In 1950, (58, 21) the authors found that the correction observed with the blood of two hemophiliacs, was due to the deficiency of different factors (one of them adsorbed by barium sulphate), which permitted the separation of hemophiliacs into two groups. In the same year, Koller (37a) concluded that the mutual correction might be produced by the deficiency of two different clotting factors. Schulman and Smith (1952) presented a similar observation (72).

Soulier and Larrieu in 1952 (81), classified those factors A and B. Also in 1952 Aggeler and col. (2), and Biggs et al. (10) separated these two groups as different entities: P.T.C. deficiency the first, and Christmas disease the latter. In 1953 Cramer (25) et al. proposed to call the deficiency of factor VIII hemophilia A and the defect of Factor IX hemophilia B. Pavlovsky (52) 1954, believes that at present there is not enough proof to separate them as different diseases but as varieties of the same process.

Origin and Metabolism of Factor VIII (AHG)

The best studies to determine the site of origin of Factor VIII are from the school of Brinkhous (18) (experiences performed in dogs). Neither the liver poisoning with ($CHCl_3$) carbon tetrachloride, the administration of Dicoumarol, the total X radiation, the splenectomy (Gross et al. [30]), the pancreatectomy, nor the renal insufficiency, could diminish the level of AHG.

Pool and Spaet (65) confirmed that dl-ethionine produces depression of Factor VIII in rats and great damage to the bone marrow, for which reason they consider it its place of origin. According to Graham (28), this would be due to the inhibition by dl-ethionine of the incorporation of methionine into the molecule of the AHG blocking its production. This author considers that this factor is originated in several places of the body. Brinkhous and Penick (20) observed that the injury produced by cold in dogs, is followed by a real decrease of Factor VIII, which does not occur with fibrinogen and prothrombin.

Langdell et al. (39) reported, that when AHG is injected in hemophilic dogs, it decreases after 4 hours to half its quantity. These results were also corroborated when the globulin was injected into normal dogs, seemingly demonstrating that in the hemophiliacs there would not be a greater consumption of this factor than in the normals. When massive doses of this factor were injected increasing it to twice the quantity of the normals, the levels were only maintained for 18 hours.

Purification of Factor VIII

After the work of Addis (1), Patek and Taylor (50) and Bendien and van Creveld (5) succeeded in isolating from normal plasma the globulin capable of correcting the hemophilic coagulation.

From then on many techniques have been proposed to obtain that factor in a purer form. Soulier (79a) postulates the ideal prerequisites to obtain a fraction with antihemophilic activity.

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1st. Small volume.

2nd. High yield.

3rd. Easy vehiclelization and application.

4th. Satisfactory stability.

In the International Symposium on Hemophilia, New York 1956 (19), the subject was extensively treated (Warner et al. [88], Winterstein and col. [89], Soulier [79a]).

The percentage of AHG of human plasma and of plasmas of other species were determined, human 1, ovine 2.5, canine 5, bovine 2—12, porcine 7.5—10, equine 0.6.

According to the agent used in the fractioning the following techniques may be considered:

1. Isoelectric precipitation (Patek and Stetson [49], Bendien and van Creveld [5], Alexander and Landwehr [4] etc.).
2. Salting-out (Spaet and Kinsell [84], Biggs, Evelyn and Richards [12], Bidwell [8]).
3. Precipitation by organic solvents (Cohn [23], Kekwick [36], Kekwick and Wolf [37]).
4. Adsorption and elution (Lorand [40]).
5. Combined methods (Shinowara [76], Quick [67], Blombäck [14], Seegers and Landaburu [74] etc.).

To criticize these techniques the postulates of Soulier (79a) must be considered. In this respect, the separation of fibrinogen that accompanies regularly the antihemophilic activity, and that attempts against its application "in vivo" due to its high viscosity, has been tried without success by thermic (56° C) or chemic (thrombin) coagulation of fibrinogen, or by selective absorption. One possibility to obtain AHG fibrinogen-free is starting from fibrinogenopenic or afibrinogenic plasma (Pavlovsky and Simonetti [60]).

Soulier (80), using plasma of a congenital fibrinogenopenia, proved that the precipitation of the AHG changes in the absence of fibrinogen. We have recently confirmed this fact (16). We also noted that the antihemophilic globulin changes its conditions of precipitation in absence of fibrinogen and that its fractionation is obtained by adsorption or coprecipitation with other proteins, without specificity. Referring to the techniques of adsorption, Soulier (79) observed that Bentonite can adsorb the fibrinogen at a concentration of 5%, leaving 50% antihemophilic activity in the supernatant. This activity may be adsorbed on its turn at a concentration of Bentonite of 10%.

We have to mention specially the techniques to give AHG in purified fractions. A valid criterium that seems the best prognosis of the activity "in vivo" is given "in vitro" by two analytical techniques.

1. Correction of the prothrombin consumption of a hemophilic plasma by the aggregate of progressive dilutions of AHG.

2. Use of AHG as adsorbed plasma in the thromboplastin generation test of Biggs and Douglas (9).

In a study of this last technique to be presented at the next Symposium on Factor VIII, Simonetti et al. (76) proved that when that factor was purified at a greater degree it seemed to be deprived of aiding conditions or factors. This would induce erroneously the evaluation of its efficiency. Due to this error the more impure fractions could be taken as the more active (Patek and Taylor) in comparison to the more pure ones (Blombäck [14]). A better evaluation of the action "in vivo" and at the same time a better comparison with the results of the correction of prothrombin consumption of hemophilic plasma "in vitro", is obtained by the dilution of the purified fraction with $Al(OH)_3$ adsorbed 1 : 5 diluted hemophilic plasma instead of buffer.

We have also studied the action of Factor V, prothrombin and thrombin discarding these factors as the ones provided by hemophilic plasma used as a diluent.

From the comparative study of the techniques to prepare AHG we have arrived at the following conclusions:

Better concentration and recovery: Winterstein (89) (Patek and Taylor modified), Blombäck (14), Keckwick (36).

Greater purification: Blombäck and Kekwick.

Better storage and stability to freezing and thawing: Blombäck.

Properties of Factor VIII

Taylor et al. (85, 24), in 1945—1946, found that the protein picture and the distribution of the amino-acids in the hemophilic plasma is the same as in the normal. The electrophoretic study did not show any differences between both plasmas.

However this factor has been identified from the beginning as a globulin, hence its name. At present it has been termed very wisely, Factor VIII. It still remains to be stated, whether it is really a globulin or if the latter is the carrier of the factor.

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With electrophoretic study, it has been possible to establish that Factor VIII corresponds to a β_2 globulin. Spaet (84) and Wagner (88) also found Factor VIII activity in the albumin fraction.

Alexander (3) points out its instability in normal plasma, in presence of small quantities of thrombin. Hence extreme precautions that should be carried out whilst drawing and handling plasma. The ultraviolet adsorption spectrum shows a typical peak at the 280 mμ (Wagner and Thelin [88]. Porcine material). When heated for 5 minutes at 56° C, 90% of the activity is destroyed.

Wagner et al. (88) remarked that the chemical properties of the unique fraction of this globulin are still unknown, for which reason more homogenous fractions should be obtained.

Stability: It is very unstable. Its maximum stability would be at pH 6.8 (Bidwell [8]). If Factor VIII is well obtained with ACD solution at 4° C it maintains according to Brinkhous (18) its properties during a week. Recently Wolf (90) studied the temperature and pH stability of human AHG in plasma and in a concentrate, finding that in the latter form it is less stable. Penick and Brinkhous (62) suggested that the inactivation could be due to fibrinolysin. This does not seem to be corroborated by Brakman et al. (16). Bergsagel and Biggs (7a) reported that the AHG is gradually inactivated while it is combined with the inhibitor of Factor B, favouring at the same time the activation of Factor IX.

Adsorption: It is adsorbed with norit, kaolin (Lorand [40]) treated with a buffer of phosphate pH 5.9, and by Seitz filter. It is not adsorbed with $Al(OH)_3$, $BaSO_4$, $Ca_3(PO_4)_2$, Berkefeld filter, nor with Bentonite 4‰, but it is adsorbed at 20‰ (Soulier [79]). It does not dialyse.

Precipitation: In plasma: 14.5% Na_2SO_4; 40—50% $(NH_4)_2SO_4$.

pH: 5.9—6.4 in diluted plasma; at saturation in diluted plasma with CO_2.

This factor is in Cohn's fraction I and III and only in small traces in the fraction IV (Taylor and col. [85]).

Dosage of Factor VIII

At first to evaluate the activity of the AHG, its effect on the clotting time of hemophilic blood was tested; but when it was observed that some hemophiliacs had a normal clotting time, it was necessary to apply to more specific methods, which we will mention by order of their sensitiveness.

Clotting time: sensitive only when the AHG is below 5% (according to Langdell [39] 1%).

Howell's Time: is more sensitive if platelet-free (Verstraete [86]). It only has a relative value.

Heparin Tolerance Test: it is not specific, but useful (Soulier [79]).

Prothrombin Consumption: detects a deficiency of 20%, although it is not specific. According to Langdell (39) 1%.

Partial Thromboplastin Time: (Langdell-Warner-Brinkhous [38]) using a known hemophilic plasma as substrate, (it does not allow to differentiate the "A" from the "B") detects a deficiency of 15%. Modified by Graham (29) the two hemophilias can be individualized.

Thromboplastin Generation Test: (Biggs and Douglas [9]).

Specific quantitative assay of AHG: (Pitney [63], Quick [68]).

Undoubtedly, the thromboplastin generation test (11), constitutes a great advance, not merely to classify the hemophilias but to measure the AHG; only when the patient has a high dosage of anticoagulants, this technique is not useful. In those cases, the techniques proposed by Soulier and Larrieu (81) or Bergna and Pavlovsky (6) may be used. The latter consists in precipitating the AHG in the diluted plasma of the hemophiliac in study, and to test it with known hemophilic plasmas. It was not possible for us (7) to use the AHG isolated by our method with the addition of Factor V, in Pitney's technique, since the generation was not sufficient, even starting with normal plasmas. We think, as we pointed out before (76), that when precipitating the AHG, it is deprived of another factor necessary for the coagulation, because we obtain a better generation using adsorbed hemophilic plasma instead of Factor V.

Pitney's (63) modifications of the thromboplastin generation test, to test quantitatively the AHG, allows better determination the degree of deficiency of that factor. Subsequently, different modifications to that technique have been proposed, with the object of simplifying it and of obtaining more constant results (Winterstein and col. [89], Pool and Robinson [64], Verstraete [86] etc.). Bergna et al. (7) shall present a report on the importance of these modifications.

Role of Factor VIII in Hemostasis

Until the knowledge of the process of coagulation is completed (discovery of new factors, intermediate phases, importance of the contact factor), the initial scheme of Morawitz is continually modified. At the same time the location of Factor VIII, which acts in the first stage of coagulation has varied its position.

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With the object of having a point of reference in our discussions, we present the following diagram (57), the result of the opinion of several authors who consider the problem at present.

The process is sketched admitting that the initiation corresponds to the activation of Hageman Factor by contact (Ratnoff, Margolis [70], Margolis [44]; Soulier, Wartelle and Ménacheé [82]).

Hageman + glass

↙

Activated Hageman + PTA + Ca^{++}

↙

Third Thromboplastic Factor + IX + VIII + Platelets

| Stuart Factor

↓ Factor V

Plasmatic Thromboplastin (Biggs et al. [11])

or

Prothrombinase (Owren [48])

Other workers studying the rôle of Stuart Factor, place it before the platelet factor (Hougie [33], Fisch and Duckert [27]).

Seegers (73) has faced the problem of coagulation from another point of view, for which reason it is difficult to adapt it in this scheme, however his valuable contributions are attentively followed and greatly appreciated. We consider that to interpret his concepts we should place ourselves in the standpoint of the author.

The normal tissue thromboplastin corrects "in vitro" the hemophilic alteration, and the hemophilic tissue thromboplastin behaves similar to the normal. This was demonstrated by Lowenburg (41) in 1918, and was confirmed by us 55—57. In that work we pointed out that to explain the presence of those components in the hemophiliac's tissue, and the lack of them in their plasma, we would either have to suppose that there exists an endothelial barrier impeding the entrance of these factors into the circulation, or that specific inhibitors inactivate them in the blood. Brinkhous and Penick (20) consider that the tissue thromboplastin does not substitute in every sense the AHG. Until now it was neither possible to explain the rôle of the inhibitors in hemophilic coagulation with exactness, nor to prove Graham (29a) the importance that Tocantins (85a), Pavlovsky (55), Seegers (55), Verstraete (86) and Hecht (31, 32) ascribe to them in the process of hemophilic coagulation. The latter author identifies the anticoagulant with sphingosine.

Disease Caused by the Deficiency of Factor VIII

Classic hemophilia (hemophilia A) is the result of the deficiency of Factor VIII. Recent works have dealt with this process (Brinkhous [17], Blombäck [14], Graham [28], Quick [67], Biggs and Macfarlane [13], Pavlovsky [53], Verstraete [86]).

It is accepted that the seriousness of the process is in connection with the degree of deficiency of this factor.

Several problems still remain to be solved, in the first place, the relation with hemophilia B. There have been demonstrated cases with simultaneous lack of both factors in different proportions (Soulier and Larrieu [81], Pavlovsky [55], Bergna and Pavlovsky [7], Marc Verstraete [87], Sjölin [77]). This shows the difficulty of considering both processes as different diseases. Sjölin (78) has studied a group of "A" patients whose defect can be corrected with adsorbed serum.

It is interesting to connect the clinical evolution of the hemophiliacs and their accidents, with the deficiency of Factor VIII and the vascular alterations (fragility and atony). The deficiency of Factor VIII might promote at the same time vascular alterations. Nevertheless the deficiency of Factor VIII is constant, whilst the vascular alterations usually present themselves in the form of crises' and are less evident after the puberal period (61), when the prognosis of the illness improves.

Jacques (34) observed experimentally, that the deficiency of a clotting factor may exist without accidents; however, in association with stress it provokes hemorrhages. Jorpes et al. (35, 47) managed to isolate together with the AHG, a factor capable of correcting the vascular alterations in Willebrand's disease. This factor can also be isolated from hemophiliacs A lacking Factor VIII. These facts show that there are factors closely related to clotting factors, that would act independently on the vessels (54).

These vascular factors would be more closely related to the deficiencies of factors acting in the first stages of coagulation, like in hemophilia, and not to those acting in the last phases, as in congenital fibrinopenia.

Deficiency of Factor VIII in Women

In a recent paper we have revised (59) the casuistic reports on female hemophilia, adding two cases of our own. These two patients have the same quantitative deficiency Factor VIII as their fathers, from whom they inherited the disease.

The difficulty lies in the evaluation of the deficiency in carriers.

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Verstraete (86), modifying the thromboplastin generation test, studied the correcting action of diluted plasmas of carriers and normals, on hemophilic plasmas. He observed evident differences, although these were not absolute. Some normal women may present the same deficiencies. Bergna and Pavlovsky (7) using Pitney's modified method, obtained similar results.

Utilization of Factor VIII in Therapy

We have already pointed out what a problem it means to maintain Factor VIII in the hemophiliacs at useful levels, to keep them from bleeding. Between 1000 to 1500 cc of plasma should be transfused once or twice a day, to keep an aproximate level of 30% of AHG (Biggs and Macfarlane [13]). Langdell et al. (39) remark that occassionally the hemorrhages are controlled only by increasing the level of AHG to 5%.

We have also indicated the technical difficulties that present themselves whilst preparing a pure and concentrated fraction. That is why, now-a-days, it is more practical to inject fresh, frozen or lyophilized plasma drawn under optimal conditions. Nevertheless, it is interesting to obtain this fraction purified for use in extreme cases, and to study its real nature to be able to replace it by synthetic products or other natural substances more easely obtained.

A criterium to evaluate the action of the injection of AHG is to observe its effect on hemophilic blood.

To normalize the:

Clotting time: the AHG has to be increased to 1—5% (Blombäck [14])

Prothrombin consumption: the AHG has to be increased to 2—10%

Thromboelastogram: the AHG has to be risen to 10%

Thromboplastin generation test: 1500 cc. of plasma have to be injected to rise the AHG 30%. The patient is then within the limits of safety (13).

According to Langdell (39), to increase the level of AHG 1%, 1 cc. of plasma per kilo of weight needs to be injected. However, when the patient has a circulating anticoagulant, all of these calculations fail. Moreover on some occasions the vascular alterations (atony and fragility) may constitute themselves in the predisposing cause of certain bleeding crises. This would explain why the hemophiliacs can remain long periods without bleeding, especially after the puberal period in spite of not having improved their coagulation, which, actualy, may sometimes become worse due to an increase of the circulating anticoagulants. Langdell (39) remarked that there are some hemophiliacs without AHG, who do not bleed.

Biggs and Macfarlane (13) observed that the injection of 500 cc. of plasma to a hemophiliac, did not increase his AHG; in spite of that, the patient improved rapidly.

We have already mentioned the experiences of Jacques (34) and Jorpes (35), in which they seem to demonstrate the importance of the vascular factor in the onset and control of the bleeding crises.

It is well known that the activity of the AHG disappears quickly. Langdell (39) noted that it has a medium life of 4 hours.

As it is so difficult to obtain enough quantity of AHG of human origin, Macfarlane, Biggs and Bidwell (42) proposed to obtain it from bovine plasma with Bidwell's (8) technique.

With the injection of this globulin, they attained in the hemophiliacs a level of 30%; but they advise, considering its antigenic power, not to follow the injection after a continued period of 10—14 days, avoiding to give it for a second period. Due to this inconvenience, we believe it is necessary to have more experience before deciding on its usefulness. For this reason Deutsch (26) remarks that human preparations are preferable.

It is possible to increase the power of the AHG by the addition of platelet extracts, as suggested by Seegers (56). This potentiation action seems to be due to a concentration of plasmatic factors in the plasmatic atmosphere of platelets (Bounameaux [15]) as if hemophilic platelets do not exert this action (56).

In our experiments, following Seegers' suggestions, we observed in vitro that good results were obtained to correct the clotting time and prothrombin consumption, with the addition of platelet concentrates to the AHG. With the injection of platelet concentrates alone, only a slight shortening of the clotting time was observed, without improving the prothrombin consumption or the thromboplastin generation test.

As a conclusions of our studies (4a), we can say that the transfusion of total blood during severe bleeding crises, improves the state of shock providing at the same time AHG, but when an increase of the coagulant action is wished it is convenient to add the latter factor concentrated.

With the injection of 500 cc. of fresh plasma, we could not normalize the generation of thromboplastin in hemophiliacs "A", although we obtained satisfactory hemostatic effects, if the injection was daily repeated during the crisis.

Periodic transfusions for prophylactical purposes do not seem a solution for the treatment of hemophiliacs, since although they produce an improvement in the capacity to work and in the number of hemorrhagic accidents, these can not be completely avoided, even during the periods of treatment (Alexander and Landwehr [4], Alexander [3], Schulman [71], Spaet,

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Aggeler and Richards [83]). There also exists the danger of provoking the onset of thrombopenic purpuras (Schulman [71]), or the appearance of anticoagulants.

We believe that an AHG supplying all the conditions demanded by Soulier (79a), has not yet been prepared; that is why up to the present moment, the transfusion of fresh total blood or plasma are used more, since while they increase the level of AHG they also improve the secondary vascular alterations. The latter disturbances can be corrected by smaller quantities of blood than the ones needed to normalize coagulation. It still remains to be proved what substances are capable of acting on the vessels.

It has been mentioned already, that the transfusions in spite of not correcting the hemophilic coagulation (the thromboplastin generation test is very seldom normalized), have a beneficial effect on the hemorrhagic accidents, and that the induction of refractory states and even the increase of circulating anticoagulants seem to deteriorate with repeated transfusions. We feel that the causes of these states should be investigated. Perhaps, they are connected with the ones that impede the normal synthesis of AHG. The origin of the vascular alterations in these patients should also be studied.

Summary

After an historical introduction the author reviews to properties of Factor VIII, its origin and metabolism, methods for its isolation and determination, its rôle in hemostasis and its therapeutic use in cases of hemophilia A and vascular hemophilia A.

Résumé

Après une introduction historique l'auteur passe en revue les propriétés du facteur VIII, son origine et son métabolisme, les méthodes utilisées pour son isolement et son dosage, son rôle dans l'hémostase et son utilisation thérapeutique dans les cas de déficience (hémophilie A et hémophilie vasculaire A).

Zusammenfassung

Nach einer historischen Einleitung stellt der Autor die Eigenschaften des Faktor VIII, seine Herkunft und seinen Stoffwechsel, die Methoden, die für seine

Isolierung und seine Bestimmung verwendet werden, seine Rolle in der Blutstillung und seine therapeutische Verwendung bei der Hämophilie A und bei der vaskulären Hämophilie A zusammen.

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