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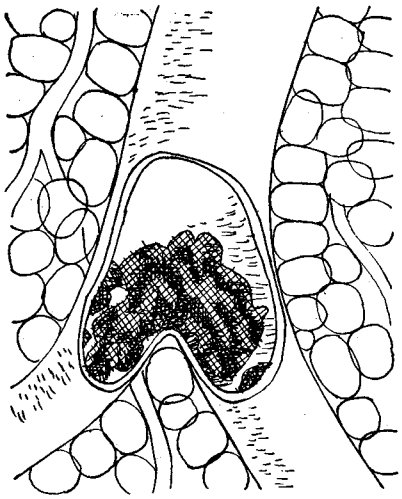

Current Status of Clot Dissolution Therapy

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Clot dissolution using fibrinolytic agents is a promising approach to the treatment of thromboembolic disease, particularly peripheral venous and arterial occlusion. In their present state of development, these agents are not recommended for general use in thrombosis or embolism in the cerebral or coronary circulations. Dosage units are still not standardized. The commercially available "fibrinolysins" are, in reality, combinations of plasmin (or fibrinolysin) and an activator of plasminogen (or profibrinolysin). Physicians must know the limitations of these preparations and the factors that alter dosage requirements.

THE THREAT of venous and arterial thromboembolism is a constant companion of virtually every practicing physician. The sudden formation of an intravascular clot can injure or destroy any tissue, any organ. It is not surprising, therefore, that thromboembolism has been the object of intensive clinical and laboratory study during this century. Nor is it surprising that much of this study has centered upon efforts to devise effective means to treat thrombotic disorders. Recently, a new therapeutic approach to thrombotic disease —clot dissolution using fibrinolytic agents—has come upon the scene. During the last several years, a number of investigators have demonstrated conclusively that fibrinolytic agents can dissolve susceptible intravascular thrombi.

The Concept

The concept which underlies fibrinolytic therapy is disarmingly simple. It is based on the fact that mammalian blood contains an enzyme system capable of dissolving fibrin, the protein which forms the matrix of blood clots. This enzyme system is outlined in *Figure 1. Plasminogen* (or profibrinolysin) is an inactive precursor sub-

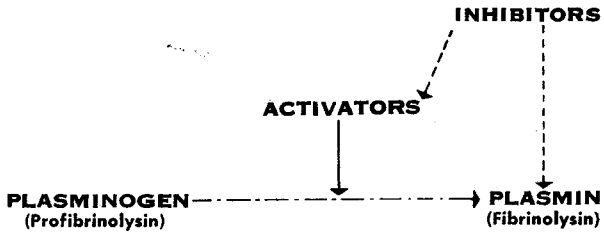

FIGURE 1. *The intrinsic fibrinolytic system present in the circulating blood.*

stance or proenzyme which can be converted to an active proteolytic enzyme *plasmin* (or fibrinolysin) by a number of agents which serve as *activators*. While plasmin can attack many proteins, it exhibits an adsorptive affinity for fibrin. As in any system associated with coagulation, the plasmin system is subject to *inhibition*.

Available Agents

Inspection of this intrinsic fibrinolytic mechanism indicates several methods which might be used to promote dissolution of thrombi in vivo. First, activators of endogenous plasminogen might be administered. Second, plasminogen itself might be isolated from human blood, activated in vitro and readministered as plasmin. Third, one might attempt to reduce plasma inhibitor levels. Finally, several of these approaches might be combined. Agents of each of these types are currently under investigation (*Table 1*). This discussion will be concerned only with the "leading contenders."

ACTIVATORS

Urokinase (UK) is obtained from human urine. It is a direct activator which does not require interaction with any other substance to convert plasminogen to plasmin. While animal studies have been promising, only recently has this material been purified enough to permit trials in man.

Streptokinase (SK), the most widely known activator of plasminogen, is isolated from filtrates of streptococcal cultures. To activate plasminogen, SK must first combine with some component of human plasma. There is evidence that this component (proactivator) may be plasminogen itself. Thus, SK action involves two steps (*Figure 2*). First, SK combines with proactivator (?plasminogen) to form activator complex; then, this activator converts plasminogen to plasmin.

SK itself has several disadvantages in terms of general clinical application. Despite extensive effort, even the most highly purified SK is not consistently free of pyrogenicity. Furthermore,

TABLE 1.

Available Thrombolytic Agents

ACTIVATORS
Streptokinase
Urokinase
ACTIVATOR-PLASMIN COMPOUNDS
SK-Plasminogen mixtures
UK-Plasminogen mixtures
PLASMIN
Spontaneously activated plasminogen
Chloroform-activated plasminogen
UK-activated plasminogen
OTHER
Nicotinic acid
Thrombin E
Bacterial pyrogens

TABLE 2.

	"Fibrinolysin" A	*"Fibrinolysin" B*	*Ratio A/B*
LABELED POTENCY:			
"Fibrinolytic units"	1,000,000.0	50,000.0	20.0/1
CASEIN ASSAY*:			
Fibrinolysin units	31.4	23.8	1.3/1
Activator units	7,621.0	6,120.0	1.2/1

* *Modified from Remmert-Cohen*

Comparison of labeled "fibrinolytic units" per vial of the two commercially available SK-plasminogen compounds with results of casein assays in our laboratory. Note the predominance of activator activity. Also note the lack of correspondence between arbitrary "fibrinolytic units" and caseinolytic units.

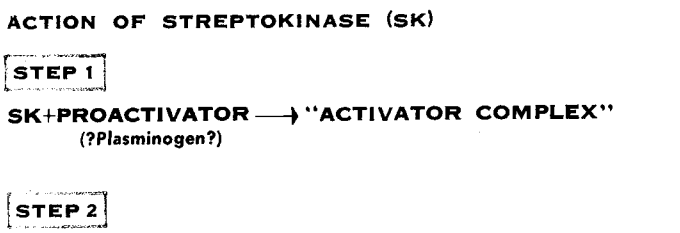

FIGURE 2. *The steps by which streptokinase (SK) activates plasminogen into plasmin.*

streptococcal infection is so ubiquitous that inhibitors to SK exist in most people. The level of inhibition varies so widely that the dose of SK necessary to achieve a given pharmacologic effect must be determined for each patient individually. The unquestioned *antigenicity* of free SK also raises questions regarding its effectivity and safety. For example, administration of SK results in a rise in plasma inhibitory capacity sufficient to render retreatment impractical for six months or more. Finally, the long-term toxic consequences of infusing and reinfusing an antigenic material into man provide concern.

ACTIVATOR-PLASMIN COMPOUNDS

Since urokinase-plasminogen mixtures have so recently become available, only mixtures of streptokinase with human plasminogen will be discussed here. These compounds are of the greatest contemporary interest since both fibrinolytic materials released for clinical use (Actase® and Thrombolysin®) fall into this category. As mentioned previously, the interaction between human plasminogen and SK produces an activator complex which can convert profibrinolysin to fibrinolysin. By manipulating the ratio of SK to plasminogen, and by changing other features of the reaction, an end-product which ranges from virtually pure activator complex to virtually pure plasmin can be obtained.

Plasmin and activator complex are different biochemical, physiologic and immunologic entities. Therefore, the potency of any SK-plasminogen compound should be defined in terms of both its true fibrinolysin content *and* its plasminogen-activator activity. Since this has not been the case in the past, some words of caution are in order:

Beware the term "fibrinolytic unit." Each pharmaceutical house and many investigative groups have defined separate fibrinolytic units. None are equivalent. One unit may represent five, 10 or 20 times more activity than another, as indicated in *Table 2*.

Beware the term "fibrinolysin." The two "fibrinolysin" preparations now available commercially are, as we have indicated, combinations of plasminogen-activator activity and true plasmin. Their activator potency is many times their fibrinolysin content (*Table 2*). There are preparations of virtually pure fibrinolysin which may become available for general use. It is essential that true fibrinolysin, activator-fibrinolysin compounds and pure activator compounds be differentiated, for each material has its own particular toxic and pharmacologic characteristics.

The diversity of preparations with thrombolytic capabilities has made selection of standard assays difficult and various assays have been used. Currently, two major types of assay are in general use: fibrinolytic and caseinolytic. Fibrinolytic assays (*Figure 3*) measure the potency of an agent in terms of the speed of clot lysis. Such assays have suffered from their lack of ability to distinguish between activator and true plasmin activity.

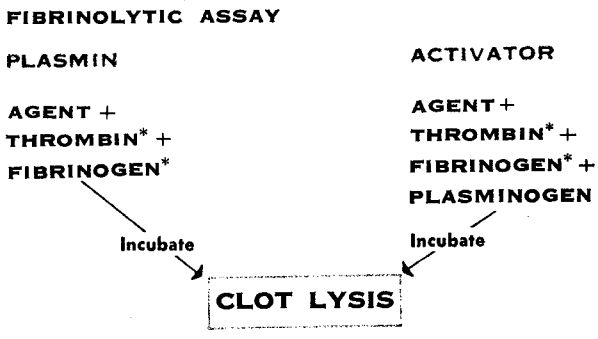

FIGURE 3. *The scheme of the fibrinolytic assay for measuring the potency of a drug in terms of the speed of clot lysis.*

Caseinolytic assays (*Figure 4*) measure potency in terms of casein digested per unit time. While they are cumbersome and subject to theoretical objections, their ability to distinguish activator from plasmin activity has been helpful. The labeling of available agents in "casein activator" and "casein plasmin" units, as well as in arbitrary fibrinolytic units, has been a step in the right direction. The problem of standardization, however, is still not resolved. When evaluating thrombolytic agents, physicians should be aware of these problems and be informed as to the true significance of the words and numbers appearing on the labels of any such agents.

PLASMIN

Pure plasmin, activated spontaneously or with small amounts of urokinase, is now available for study. Current thinking about such compounds is divided into two camps because of a controversy over the nature and function of its inhibitors. All workers agree that normal plasma contains a considerable amount of "antiplasmin" activity. As shown in *Figure 5*, those who subscribe to concept A contend that the combination of plasmin and antiplasmin in vivo is a firm one. Therefore, enough plasmin must be given to bind all circulating antiplasmin before any is free to attack the blood clot. Accordingly, a detectable blood level of plasmin must be reached and, in doing so, there is risk of destroying other clotting factors.

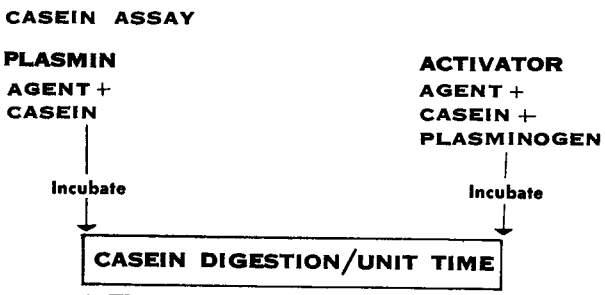

FIGURE 4. *The scheme of the caseinolytic assay for measuring the potency of a drug in terms of casein digested per unit time.*

Others in this field support concept B (*Figure 5*); namely, that the plasmin-antiplasmin combination is largely dissociable in the presence of fibrin. They hold that fibrin, because of its high affinity for plasmin, competes very successfully with antiplasmin for plasmin. This means that antiplasmin serves merely as a *carrier* of plasmin in the blood, so that plasmin cannot attack other clotting factors but retains the ability to attach to and dissolve fibrin. Furthermore, detectable "free" plasmin need not be present in the blood. Investigations are under way in several laboratories to discover which of these two concepts is more correct.

Clinical Considerations

Having reviewed basic theories, concepts and laboratory observations, it is time to discuss the problems surrounding clinical application of the two fibrinolytic agents which are now available.

TOXICITY

Pyrogenicity has been the outstanding toxic feature of these SK-plasminogen compounds in the past. However, constant improvement in the components used in these agents has significantly reduced the incidence and severity of febrile reactions. In our view, pyrogenicity no longer limits the clinical use of these compounds (*Table 3*).

Hemorrhage is the second major toxic risk. This risk, unlike pyrogenicity, depends upon the dosage. With the dosage schedules we have used to date, our group has not encountered hemorrhage attributable to these agents in more than 500 patients. This experience includes patients who have received doses of 1,000,000 "fibrinolytic units" per hour ("fibrinolysin" A units in *Table 2*) for four hours. At the present time, we cannot recommend doses above this level unless

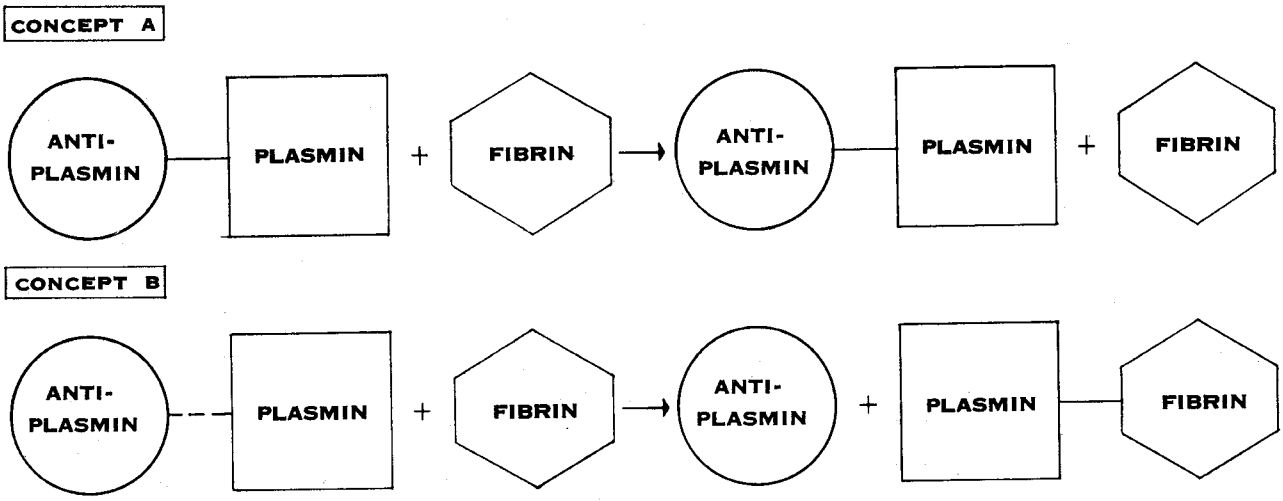

FIGURE 5. *Two concepts (A and B) regarding the mechanism of action of plasmin inhibitors or antiplasmin.*

special facilities for monitoring coagulation changes are available. Studies to define the upper ranges of safe dosage are currently underway in our laboratory and others.

What are the best signals that the coagulation mechanism has become unduly disturbed? Recent observations indicate that the risk of hemorrhage correlates best with the appearance of so-called *antithrombin* activity in the blood. This antithrombin activity, apparently due to digestion products of fibrinogen and fibrin, may appear without marked depression of the fibrinogen level or other clotting factors although it does prolong the one-stage prothrombin time. At the present time, serial measurements of the prothrombin time and, where available, the antithrombin activity and the fibrinogen level will permit satisfactory detection of hemorrhagic risk. In the dosage ranges mentioned previously, such risk is minimal. Furthermore, we now have an effective inhibitor of fibrinolytic activity in the compound epsilon amino caproic acid (EACA). EACA promptly inhibits both activator and plasmin activity in the blood. (It is the vitamin K, or pro-

TABLE 3.

Period	February 1957–December 1957	January 1958–December 1960	August 1960–February 1961	April 1961–February 1962
*Dose range**	30–90	75–100	100–300	500–3,000
Patients	72	42	71	44
Temperature elevation				
0°–1° F.	56%	52%	40.0%	63.8%
1°–2° F.	15%	19%	28.6%	29.5%
2°–3° F.	13%	22%	14.3%	2.2%
3°–4° F.	13%	2%	10.0%	4.5%
> 4° F.	4%	5%	7.1%	0.0%
Chills, nausea	~25%	~33%	8%	2.2%

* *In thousands DCGH units*

Experience with pyrogenicity of one SK-plasminogen preparation (Actase) in successive trials since 1957. All doses were given intravenously over a two-hour period. Note diminution in pyrogenicity in most recent material despite increased dosage employed.

tamine, of fibrinolytic therapy.) Lysine serves a similar purpose.

Other toxic responses to these compounds have been infrequent. Perhaps the most striking of these has been the appearance of severe epigastric pain several hours after infusion. The etiology of this rare but dramatic response is unclear. It is not associated with fever and usually responds promptly to analgesics. Close clinical and laboratory observation has disclosed no residual abnormality.

Other toxic responses have included occasional urticarial rashes and one instance of bona fide serum sickness. To our knowledge, true anaphylaxis has not yet been recorded, although it is likely that such a response will eventually occur.

FACTORS INFLUENCING DOSAGE

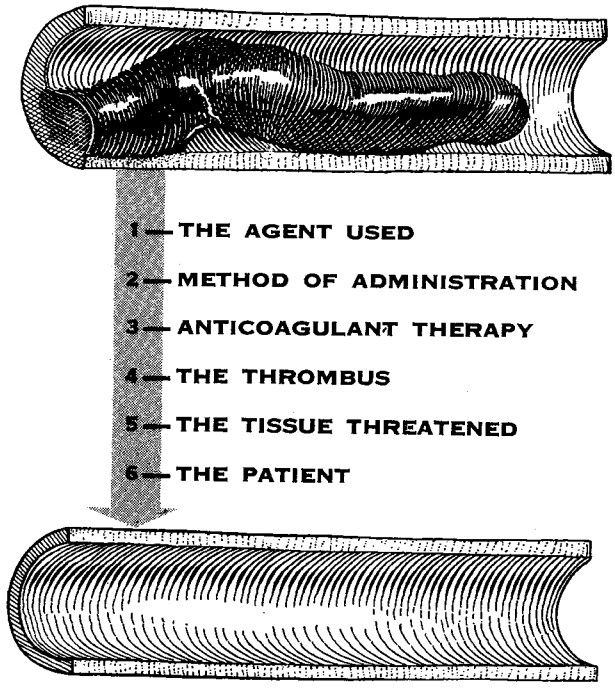

FIGURE 6. *The criteria for dosage in clot dis ion therapy involve many factors, some of which are unk*

DOSAGE

Obviously, proper dosage is a requisite for achieving optimum therapeutic effect. Unfortunately, no practical and reliable laboratory methods are yet available for predicting the dosage regimen necessary to achieve thrombolysis in a given patient. The difficulty in defining ideal dosage criteria reflects the fact that this dosage is the resultant of a complex equation involving many unknowns (*Figure 6*). The *composition and structure* of the thrombus is one such unknown. It has been demonstrated that susceptibility of a clot to lysis is inversely related to the fibrinogen, lipid and platelet content of the clot. Therefore, with identical dosage schedules, thrombolysis may succeed in lysing a clot with low fibrinogen, lipid or platelet content and fail to dissolve one rich in any of these components.

The *age of the thrombus* is a very critical factor, and one which is difficult to assess clinically. Susceptibility to dissolution begins to decrease shortly after clot formation occurs. It progressively declines until, after three to seven days, endothelialization or organization renders the clot totally resistant to fibrinolytic attack.

The dosage requirement is also affected by the *method of administration* chosen. Should the agent be given as close to the thrombus as possible, or systemically by any peripheral vein? Should it be given as a single, large injection, or as multiple small injections or as one prolonged infusion? If given as one infusion, at what rate and for how long a period? Our own views on these questions are discussed at the end of this section, but the fact is that definitive answers to them do not yet exist.

Whether or not concomitant *anticoagulant therapy* is given also influences the selection of fibrinolytic dosage. Anticoagulant therapy here refers only to the use of heparin. We have advocated concomitant heparin therapy for several reasons: to prevent rethrombosis after clot disso-

lution is achieved; to protect patients with nonthrombotic occlusive disease, and to prevent interval extension of the thrombus if initial thrombolytic dosage is inadequate. Clinical and laboratory evidence also indicates that true heparin-fibrinolytic synergism exists, that is, clot dissolution can be achieved with smaller dosages of fibrinolytic agent in the presence of heparin. Studies are currently under way in our laboratory to identify the reasons for this synergism. While we advocate concomitant heparin therapy, a word of caution is in order. Obviously, heparin can increase hemorrhagic risk if used with high fibrinolytic dosage regimens. When doubt exists, it is wise to sacrifice synergism for safety by starting heparin *after* fibrinolytic therapy.

The *tissue threatened* by the thrombus also influences dosage decisions. In peripheral venous thrombosis, thrombolysis within 24 hours will be attended by little permanent injury. In peripheral arterial thrombosis, the limb may survive with minimal defect if the vascular supply is restored within a few hours. The same cannot be said of arterial occlusion in the heart, the brain or the eye where *rapid* dissolution of critically located thrombi is essential if structure and function are to be preserved. Unfortunately, we cannot expect the relationship between dosage and speed of thrombus dissolution to be linear. Our experience suggests that, beyond a certain point, increase in dose per unit time will not speed dissolution but will merely provide additional toxic risk.

The last variable in the equation for proper dosage is the *patient.* Among the many characteristics of the patient which may influence dosage are the presence of any disorders which limit or contraindicate the use of fibrinolytic drugs, the level of naturally occurring or induced inhibitors and the status of the patient's coagulation apparatus.

The apparent complexity of the equation for dosage selection does not make such selection an impossible task. The situation is no less complex with many widely used therapeutic agents. We recommend dosage regimens which we know are safe, can be simply monitored and offer a reasonable prospect of therapeutic response. Rather than suggesting general use of the maximum-tolerated dose to achieve maximum response at this time, we suggest a practical middle-ground of well-tolerated dosage with recognition that often this is not an optimum therapeutic dose. As larger doses are proved safe and more effective, they will be suggested. But at each step, we follow the dictum "do no harm" while attempting to help.

What, then, is a good current procedure for the determination of a dosage regimen? These remarks apply only to treatment of peripheral venous and arterial thrombosis, since, in our view, these are the only disorders in which thrombolytic therapy should be generally used at the present time.

We carry out a "predicted dose test," using the patient's plasma and serial dilutions of the agent to be used. The amount of the agent necessary to achieve complete lysis of 1 cc. of the patient's clotted plasma, in 20 minutes, times the calculated plasma volume is the "predicted dose." Just what this test—or any variant—predicts, is subject to question. We use it as a rough guide to the total composite inhibitory activity in plasma. If this predicted dose is less than 3,000,000 fibrinolytic units, (units of "fibrinolysin" A in *Table 2*) we give the entire amount. If it is above 3,000,000 units, total daily dosage is limited to 50 to 75 per cent of the predicted dose. Heparin (1 mg. per 1 kg.) is given just before fibrinolytic infusion.

In venous thromboembolism, the fibrinolytic drug is given via any convenient peripheral vein. Twenty per cent of the dose is given as a rapid, priming injection, followed by 20 per cent per hour for four hours. We follow, as a minimum, the one-stage prothrombin time hourly as a

safety check. If this exceeds twice the baseline value, infusion is slowed; if three times, it is discontinued. After infusion, a fibrinogen level is obtained. If the initial course of therapy is not successful, the same regimen is repeated the next day.

In peripheral arterial thrombosis, we prefer direct installation into the affected vessel. Our experience indicates that direct injection permits thrombolysis at dosage levels below those necessary with intravenous therapy. One-third of the total dose is rapidly injected into the affected vessel. If there is no response within 30 to 60 minutes, the remainder is given via a polyethylene catheter during the next three hours. Further dosage depends on the clinical response and the coagulation changes induced. If direct injection cannot be achieved, the same intravenous regimen as in venous thrombosis is used. Where prompt surgical intervention is feasible, thrombolytic therapy is often used as preparation is made for surgery. If complete response is not achieved, surgery is then carried out, followed by further thrombolytic treatment. Such medical-surgical cooperation appears to offer advantages by reducing both pre- and postoperative thrombotic extension.

Comment

There is no longer any question that various fibrinolytic agents—activators, activator-plasmin compounds and plasmin—can dissolve susceptible intravascular thrombi. However, this review has indicated that many problems must be resolved before these agents can be used with optimum expectation of therapeutic success and minimum risk. Furthermore, the fibrinolytic agents now available for clinical use cannot be regarded as ideal. Viewed in proper perspective, the current status of the fibrinolytic agents compares with that of the anticoagulant drugs in the early stages of their development. The controversy which still surrounds the efficacy and proper dosage of anticoagulant drugs after two decades of use gives us a glimpse of what lies ahead for the fibrinolytic agents. Those of us interested in these agents hope that our efforts may spare thrombolytic therapy such a prolonged period of uncertainty and debate. At this point, we feel such agents do have a place in the treatment of peripheral venous and arterial occlusion. We recommend against their general use in other thrombotic disorders until further experience is accumulated.

A coupon for ordering a bibliography accompanying this article may be found adjacent to the Index to Advertisers.

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