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The Effects of Anesthetics on Blood Coagulation in Vitro

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In the discussion on the nature of cell stimulation * several authors, *e.g.* Heilbrunn [1,2], Kraus and Fuchs [3], and Runnström and coworkers [4,5], have suggested that the typical changes of protoplasm viscosity are related to the natural coagulation of blood. (*Cf.* also works on the connection between the latter phenomenon and *rigor mortis* [6,7,8], and Kühne's monograph on contractility [9].) Some of the above authors (*vide* especially Heilbrunn [1] and Runnström [5]) have claimed that these changes of protoplasm viscosity constitute a universal and fundamental feature of cell stimulation.

Another common property of all living cells is their ability to alter their responsiveness or automatic activity under the influence of a number of agents, anesthetics (or narcotics), most of which are lipophilic chemicals. When administered in medium doses **, anesthetics produce anesthesia (narcosis) in the cells, *i.e.* "a physiological condition in which the normal responsiveness or automatic activity of the living system — organism, tissue, or cell — is temporarily decreased or abolished" (Lillie [10b]). With large doses of anesthetics these effects become irreversible, *i.e.* they lead to death. Further, it is a well established fact that anesthetics, when applied in small subanesthetic doses, increase the responsiveness of cells (Winterstein [11, pp. 13–20]; Seelich [12]). This can be observed as an increased degree of contraction of a muscle or as a decreased stimulation threshold (*cf.* Graham [13], Blume [14]). The excitability increasing effect of anesthetics on nerves has been studied by Thor [15] and Blume (*l.c.*).

If there is any analogy or homology between the increase of plasma viscosity after stimulation and in blood coagulation, the influence of anesthetics on the latter must be of great theoretical interest. The present paper

* It is not easy to define generally the concept of stimulation, but at present we ascribe to the word the response elicited in a living system when some factor (natural or otherwise) in its environment is suddenly changed more than a certain amount, the stimulation threshold. This response is an activity change which is specific for the living system in question: a muscle, for instance, contracts whatever the nature of the environmental change (stimulus). (*Cf.* Heilbrunn [1], chap. 35 and Lillie [10a]).

** In this general discussion we prefer the term "dose" to "concentration", as physical agents too (*e.g.*, heat, cold) may produce a state of anesthesia.

is to be regarded as a preliminary investigation of the effects of anesthetics, typical local anesthetics as well as general narcotics, on the coagulation process. — The chief difference between general and local anesthetics lies in their mode and site of application, conditioned by physical properties (volatility, solubility, diffusivity) of the molecules. However, the fact that typical general anesthetics such as ethanol (Gallego [16]) and ether (Lorente de Nó [17, p. 356]) act through a lowering of the membrane potential of the nerve, whereas typical, local anesthetics, *e.g.* procaine (Bennett *et al.* [18]) and cocaine (Lorente de Nó [17, p. 115]) do not lower this potential, seems to be a fundamental difference in the mode of action which has to be explained in any stimulation-narcosis theory. That is the reason why we distinguish between the two types of substances.

In this introduction the main features of the process of blood coagulation, in so far as they are known, must be briefly recapitulated (*cf.* Astrup [19], Seegers [20], and handbooks in enzymology). Two phases of the process can be recognized: (1) From injured cells a thrombokinase is liberated, which in the presence of Ca ions activates the blood protein, prothrombin, to thrombin. As thrombin is formed, it autocatalyzes the formation of more thrombin from prothrombin; for this autocatalysis Ca ions and a globulin, Ac-globulin, are necessary. (2) The thrombin thus formed catalyzes the transformation of the blood protein, fibrinogen, to insoluble fibrin, which clots. For this part of the process Ca ions are not necessary.

Several factors contained in the blood regulate the rapidity of the clotting, *e.g.* the polysaccharide acid, heparin, and several proteins [20]. Of great interest from the standpoint of a possible relation between cell stimulation and blood coagulation is the possibility of extracting thrombokinases (or substances with thromboplastic activity) from all kinds of cells, many of which under normal circumstances never come in contact with blood, *e.g.* semen [7,21], yeast [7,22,23], protozoa [7]. In experiments which will be published in another context, we have found thromboplastic activity in human semen, yeast, *Vicia faba* roots, and in *Drosophila melanogaster*. All these activities have a more or less specific ability to initiate or accelerate the coagulation process *in vitro*.

I. EFFECTS OF ANESTHETICS IN MEDIUM CONCENTRATIONS

In all experiments horse blood was used, which was drawn into a sodium citrate solution to a final concentration of 0.75 per cent of $Na_3C_6H_5O_7 \cdot 2H_2O$. The blood cells were centrifuged off immediately after the drawing at 1 000 *g* for 45 min. Thromboplastic substances were extracted from different specimens according to the very simple methods of Lehmann [24, *cf.* 24a] and Quick [25]. In the experiments presented here, extracts from hare brain and from horse sciatic nerve were used. As regards the mode of preparation, see Table 1 and the legends to the figures. The coagulation times of different mixtures were determined according to the methods mentioned [24,25], at 37° C. In the first experiments (Fig. 1) a mixture of 0.25 ml each of thrombokinase solution,

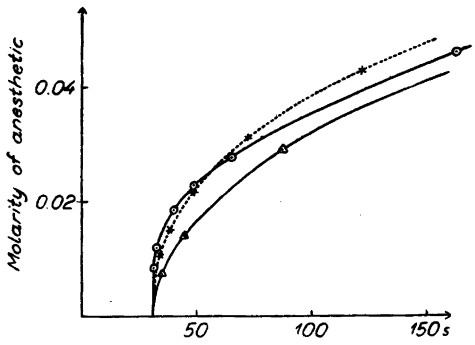

Fig. 1. The influence of xylocaine (×), N-diethylaminoacetanilide (⊙), and procaine (△) on the coagulation time of blood. Thrombokinase II (cf. Table 1).

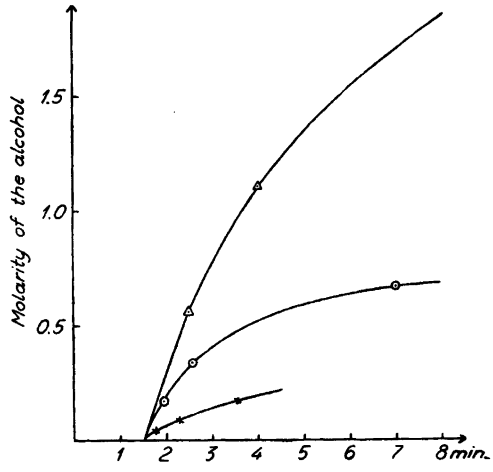

Fig. 2. The influence of n-alkanols on the coagulation time of blood. ×: 1-butanol, ⊙: 1-propanol, △: ethanol. Thrombokinase I (cf. Table 1).

anesthetic (both in saline), and citrate plasma was left to reach the temperature of the thermostat. Then 0.25 ml of 1.0 per cent $CaCl_2$ at 37° C. was added and the stop watch set. In later experimental series the amounts of component solutions were 0.15, 0.10, 0.10, and 0.10 ml respectively. The clotting times were noted in seconds. The pH of all component solutions was adjusted to 7.4.

From Figs. 1 and 2 it will be seen that when medium (*i.e.* approximately anesthetic) concentrations of anesthetics are applied — in the diagrams the concentration of the anesthetic is given in moles per liter of reaction mixture — the coagulation times are markedly prolonged: Some factor accelerating the coagulation process is inhibited by the anesthetics. Further, it can be observed that the activities of the alkanols in this respect (Fig. 2) parallel their lipid solubilities (as in narcosis; *cf.* Meyer and Hemmi [26]) or surface activities (Traube's rule; *cf.* Seelich [12]).

In order to determine the point of attack of the anesthetics, their effect was studied separately on each of the two phases of the clotting process. A fibrinogen-free prothrombin was prepared by acid precipitation of diluted plasma and a prothrombin-free fibrinogen by precipitation with three-fifths saturated NaCl, both according to Eagle and Harris [27]. If mixtures of a thromboplastic substance, prothrombin, and $CaCl_2$ are incubated for different times before the addition of fibrinogen, we get the *clotting time-incubation time* curve shown in Fig. 3 (a). In this case, of course, the clotting time has been

determined from the moment when the fibrinogen was added. If we now let the incubation, *i.e.* prothrombin activation, occur in the presence of an anesthetic (in this case, procaine) we get markedly prolonged clotting times (curve b). On the other hand, an addition of the same amount of anesthetic together with the fibrinogen is without any effect.

Although the action of the anesthetic can thus be localized to the first phase of the coagulation process, our experiments have not yet proved definitely which of the factors determining this phase is inhibited. However, the lipophilic nature of the anesthetics, and especially the fact that the inhibition by alkanols parallels the lipophilic properties of the latter, makes it most probable that the thromboplastic substances, which are typical lipo-proteins (Chargaff and coworkers [28]), are the points of attack. Considering the glyco-albumin nature of prothrombin [20] and the somewhat similar properties of Ac-globulin, it seems unlikely that these substances are inhibited. Further, it was demonstrated above that the thrombin is not inactivated; it is therefore unlikely that its chemically related precursor becomes inactivated. Compare this too with the fact that fat solvent anesthetics do not inhibit the precipitin reaction [29].

We have not been able to find any simple stoichiometric relation between thrombokinase (T) and anesthetic (A)

$$\frac{(TA)}{(T)\ (A)} = k_1,\ (k_1 = \text{const.}) \tag{1}$$

where (TA) is the concentration of inactivated thrombokinase and (T) is the concentration of free thrombokinase, both in units equivalent to the anesthetic. If we determine the relation between *clotting time* and *anesthetic concentration* at some concentrations of T so dilute that the activity of T may be considered proportional to its concentration, and determine (T) by comparison with clotting times obtained by dilution of the same thrombokinase solution, we get the $(A)_o$, (T) curves shown in Fig. 4; $(A)_o = (A) + (TA)$. If equation (1) be correct, these curves should be represented by

$$\frac{(1-x)\ [1 + k_1(T)_o x]}{k_1 x} = (A)_o \tag{2}$$

where $(T)_o$ is the total concentration of T in units equivalent to $(A)_o$, and x is the relative concentration of non-inactivated T, *i.e.*, $x = (T)/(T)_o$. Equation (2) fits any single curve, when $(T)_o$ is taken small enough; at greater thrombokinase concentrations the curves are often concave, contrary to the hypothe-

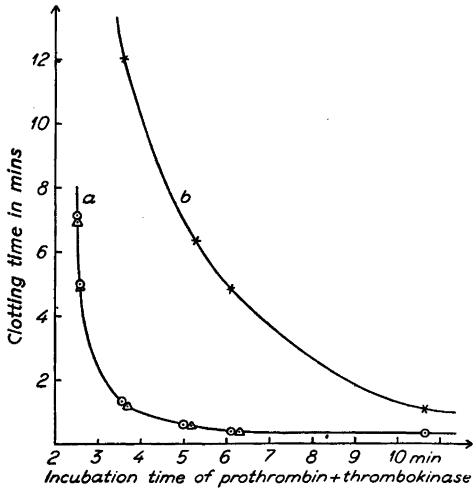

Fig. 3. Effect of procaine (0.03 mol/l of reaction mixture) on fibrin formation (△) and on prothrombin activation (×). No anesthetic: ⊙. Cf. text. Thrombokinase II (Table 1).

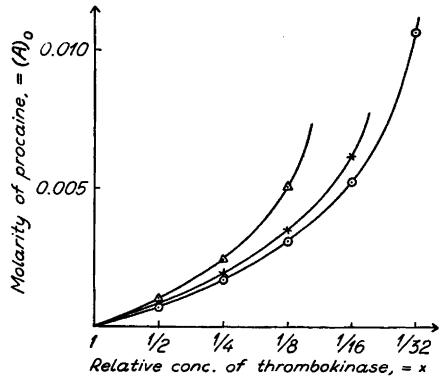

Fig. 4. Calculated decrease of thrombokinase concentration as a function of amount of procaine added (cf. text). Thrombokinase I (Table 1), dilution 1/400 (⊙), 1/800 (×), 1/1600 (△).

sis. But the k_1 value calculated from one curve is not valid at another thrombokinase concentration, $(T)_o$. As will already be seen from Fig. 4, the anesthetic is relatively more effective at higher concentrations of T, which would be impossible if the whole thing were a simple inactivation represented by $T + A \rightleftharpoons TA$, *i.e.* equation (1). Fig. 4 shows the effect of procaine; similar results are obtained with butanol.

These findings would be hard to explain if we had used pure thrombokinase preparations, because all the substances from the blood exerting an influence on the process were present in constant concentrations throughout the experiments. Differences in pH between individual experiments, due to the degree of dilution of the thrombokinase or anesthetic, could possibly have an effect in this direction, (a) if a weak base anesthetic [30] was used, or (b) if some part of the coagulation process was strongly dependent on the pH level. A pH effect can, however, be excluded because (a) *e.g.* butanol behaves similarly to the weak base anesthetic, procaine, and (b) the pH was constant to within a few hundredths in every series of experiments with one anesthetic. The most probable explanation of the complicated effect described here would be the presence in the thrombokinase solution and (or) the blood plasma of contaminating substances, one or several of which are inhibitors of the throm-

bokinase. These substances, which we will call antithrombokinase, I, can be bound or inactivated, respectively, by anesthetics, A. In point of fact, a lipid antithrombokinase can be isolated from brain tissue (Tocantins *et al.*[31]; *cf.* Hecht [32]), blood plasma, and other tissues (Overman *et al.*[33]; Chargaff [28b]). We therefore suggest hypothetically that the following reactions determine the actual concentration of thrombokinase, (T), in the presence of an anesthetic:

$$I + T \rightleftharpoons TI \quad (TI)/(I)(T) = k_2 \tag{3}$$
$$T + A \rightleftharpoons TA \tag{1}$$
$$I + A \rightleftharpoons IA \quad (IA)/(I)(A) = k_3 \tag{4}$$

II. ACTION OF ANESTHETICS IN LOW CONCENTRATIONS

If the antithrombokinase, I (*cf.* above), were inactivated more readily than the thrombokinase, it would be possible to find some (low) concentrations of anesthetics at which the amount of active thrombokinase would be greater than in the unanesthetized standard.* This is also the case with both the general anesthetics, butanol and chloroform, and procaine. Each of these substances in low concentrations gives a somewhat *shorter* coagulation time than the control. Fig. 5 shows the influence of butanol on two different diluted thrombokinase preparations. (*Cf.* the concentrations given in Fig. 2.) Chloroform produces a similar shortening of the coagulation time at about 0.1 mmoles per liter (0.8 mmoles per liter is retarding). Procaine has a slight accelerating effect at concentrations of about 0.75 mmoles per liter (*cf.* the retarding concentrations in Fig. 1).

The well-known ability of very strong thrombokinase solutions to give a longer coagulation time than the somewhat more diluted solutions [34,31] was also found to be valid for our preparations (Fig. 6). The accelerating effect of low concentration anesthetics on the coagulation process does not, however, constitute inhibition of the retardation occurring in very concentrated thrombokinase solutions. This is proved by the following facts: (a) The short coagulation time of point 2 (Fig. 6) cannot be reached if we add the anesthetic to the thrombokinase concentration of point 1; (b) the accelerating effect of low concentration anesthetics also occurs if we add the anesthetic to throm-

* A derivation of the equations, (1, 3, 4) above, preliminarily disregarding the formation of TIA, at the limit $(A)_0 = 0$ gives a positive value of $d(T)/d(A)_0$, *i.e.*, an increasing amount of T on the first addition of anesthetic, if

$$\frac{k_3}{k_1} > \frac{(I)}{(T) + 1/k_2}$$

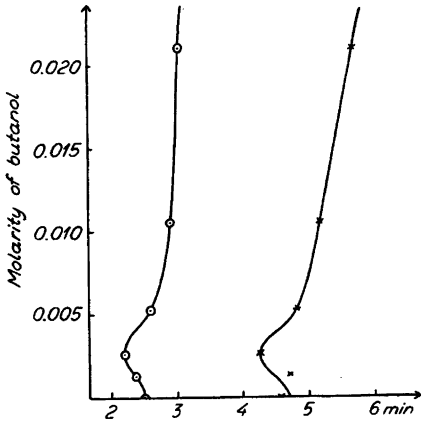

Fig. 5. Effect of low concentrations of butanol on the clotting time. Thrombokinase I (Table 1), dilution 1/1600 (⊙) and 1/3200 (×).

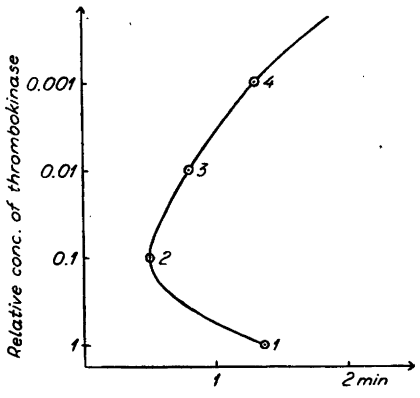

Fig. 6. Effect of dilution of a high concentration of thrombokinase (I, Table 1, not centrifuged) on the clotting time.

bokinase of concentrations 3 and 4 (Fig. 6). In such cases the accelerating effect of procaine is not always demonstrable — at all events it is only very slight — whereas that of butanol is evident (Fig. 5). If the inhibition of thrombokinase by antithrombokinase follows the simple relation (3), it can be calculated that the concentration of free thrombokinase cannot decrease with increasing concentrations of a T + I preparation (*cf.* Tocantins *et al.*[31]). (It must be pointed out, however, that these equations are only a rough working hypothesis at the present stage of the investigation. Already the fact that concentration must be used instead of activity makes them imperfect.)

In this connection it should be mentioned that the general anesthetics (butanol and chloroform were investigated here) have the ability without addition of any thromboplastic substance to initiate the coagulation process in the presence of Ca ions (*cf.* Nolf[35]; Scheuring[36]). This effect, which occurs at high concentrations of the anesthetics, is not found in the case of procaine. The latter substance only retards the slow coagulation of recalcified plasma, at about the same concentrations which inhibit the rapid clotting in the presence of thrombokinase. The coagulation initiating effect of chloroform is the most obvious, and perhaps of a special nature[35-37]; but this effect of both chloroform and butanol is very dependent on the state of the plasma (origin, time and temperature of aging). Thus it will be seen that the effects of anesthetics on blood coagulation are rather complicated.

III. DISCUSSION

Earlier knowledge of the effects of anesthetics on blood coagulation mostly consists in reports of changes in bleeding time in surgical cases under different conditions of anesthesia. The facts known are highly contradictory, which can well be understood from the complicacy demonstrated by our experiments *in vitro*. (*Cf.* the review by Adriani [38].) For instance, barbiturates prolong or shorten the coagulation time (Aykut [39]); ether narcosis shortens it, whereas procaine has no effect (Levy *et al.*[40]). Ether and chloroform increase the rate of inactivation of thrombin (Pálos [41]).

If we regard the hypothesis of an analogy or homology between blood coagulation and cell stimulation, it is of interest to compare the minimum concentrations for narcosis with the relative amount of thrombokinase inactivated at these concentrations (if our assumption that thrombokinase is inhibited by anesthetics is correct). It is noteworthy that a fairly high percentage is really inactivated at these concentrations (Table 1). Before it can be decided whether the thrombokinase extracted from different tissues is the substance inhibited in anesthesia of the same tissue, the following conditions must be fulfilled: (a) It must be proved conclusively that the thrombokinase is the substance inhibited in the case of blood coagulation; (b) the thrombokinase must be extracted from the tissue used for anesthesia investigations. (For unknown

Table 1.

Anesthetic	Minimum concentration for narcosis of frog nerve moles/l	Calculated decrease of the thrombokinase conc., in per cent, at anesthetic conc. of column 2	Source and method of thrombokinase preparation
Ethanol	1.45 [55]		
	0.6 – 1 [14]		
	1 – 2 [16]		
	Average: 1	93	Thrombokinase I. Dried hare brain. 1 g/20 ml of saline; centrifuged for 5 min at 1 000 *g*. Diluted 1 000 – 3 000 times.
1-Propanol	0.3 [55]	85	
1-Butanol	0.094 [55]	65	
Procaine	0.0125 [50]	95 – 98	
		83	Thrombokinase II. Dried ischiatic nerve from horse. 1.2 g/20 ml of saline; decanted after 12 hrs at 0° C. Not diluted.
Xylocaine	0.0038 [30]	25	
N-Diethylamino acetanilide	0.0095 [30]	25 – 40	

reasons our attempts to make a thrombokinase from frog nerve yielded only rather weak preparations, not suitable for experiments); (c) the blood coagulation and tissue anesthesia must be investigated at the same temperature. However, the possible role of the thromboplastic substances in cell stimulation and anesthesia must be taken into recount.

From a more general point of view the present investigation strongly supports the hypothetically proposed relationship between cell stimulation and blood coagulation. It is of interest to review some data on essential similarities and relations between the two processes:

1. Both involve a remarkable increase in the viscosity of plasma.

2. Both processes are checked and promoted, respectively, by similar concentrations of fat solvent anesthetics.

3. Both processes require Ca ions, and are therefore inhibited by other cat-ions [42,43] (*e.g.* the anesthetics, K^+ and Mg^{2+} [44]). Ca ions in a too high concentration [45] also inhibit both processes.

4. Heparin-like substances appear in connection with cell stimulation [46].

5. An important fact in this connection is the general occurrence in cells of thromboplastic substances (*cf.* p. 342 above).

6. All the substances necessary in the blood coagulation can be extracted from, *e.g.*, muscle [3].

7. Injury substances from animals promote both blood clotting and stimulation [*Cf.* 1, p. 561]. Histamine promotes blood clotting *in vivo* [47]; it has an antiheparin effect, demonstrated *in vitro* [48].

8. Substances formed in blood plasma during the coagulation process may stimulate muscles [9] and nerves [51] and cause vascoconstriction [49] (*cf.* the hypertensinase activity of the substances in question [51]). These substances can also promote cell division [52,53].

SUMMARY

1. Local as well as general anesthetics prolong the clotting time of blood when they are added to the plasma-thrombokinase mixture in *medium* (anesthetic) concentrations.

2. The prothrombin activation, not the fibrin formation, is inhibited.

3. The inhibition through *n*-alkanols runs parallel to the lipid solubilities of the latter. Therefore it is suggested that the lipoprotein, thrombokinase, is the factor inhibited.

4. Superimposed on the inhibition by anesthetics there seems to be an activation of some factor participating in the thrombin formation. This probably is due to the inactivation of an antithrombokinase, which contami-

nates the thrombokinase and (or) the blood. In *low* (subanesthetic, stimulating) concentrations, anesthetics shorten the coagulation time of blood.

5. *High* concentrations of anesthetics promote the coagulation of blood without any addition of thrombokinase (observed in the cases of *n*-butanol and chloroform).

6. Data are related to observations of the effects of anesthesia on bleeding time. They are also compared with known similarities between blood coagulation and the protoplasm coagulation which occurs after stimulation.

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