EXPERIMENTAL STUDIES OF THE BREAKDOWN OF EPONTOL:
DETERMINATION OF PROPANIDID IN HUMAN SERUM

BY
A. Doenicke, I. Krumey, J. Kugler and J. Klempa

SUMMARY
The concentration of propanidid in human serum was determined and was found to depend on the rate of injection. None was found 25 minutes after injection over a 20-second period. Propanidid passed the placental barrier, the umbilical vein concentration depending on the dose injected. Serum cholinesterase was inhibited by propanidid both in vivo and in vitro; the red-cell esterase was also inhibited. The concentration in umbilical vein blood was highest in mothers with reduced cholinesterase activity. The duration of anaesthesia appears to be influenced by serum cholinesterase activity. In vitro, enzymatic hydrolysis in human serum was slow but occurred rapidly in purified concentrated cholinesterase. When suxamethonium was injected after Epontol the period of apnoea was increased and this is ascribed to enzyme inhibition. The e.e.g. was useful in determining the duration of anaesthesia. Prolongation of anaesthesia in subjects with low enzyme activity corresponded with the e.e.g. findings. Propanidid is bound to plasma proteins and it is recommended that a dose of 3–4 mg/kg of Epontol is sufficient for induction of anaesthesia in high-risk patients.

In the first publications on the pharmacology of the short-acting anaesthetic Epontol, which contains the active drug propanidid and the solubilizing agent Cremophor-EL, the very rapid breakdown of propanidid to an acid without anaesthetic effect was ascribed to splitting of the ester linkage. These views were, however, based on animal experiments (Duhm et al., 1965) and in-vitro studies (Wirth and Hoffmeister, 1965; Pütter, 1965). Propanidid (fig. 1) is an oil which is sparingly soluble in water. The solubilizing agent initially used, Cremophor-EL (polyoxyethylated castor oil), consisted of 16 per cent glycerin-polyglycol-ethers and polyglycolin (hydrophylic part) and about 84 per cent of a hydrophobic portion, the major part of which consisted of esters of ricinoleic acid and glycerin-polyglycol-ethers.

The preparation of Epontol currently available contains in 100 ml of solution the hydrophobic part of Cremophor-EL as a solubilizing agent, i.e. "polyoxyethylated castor oil ELB (Tensid)" in a quantity of 16 g, with propanidid 5 g and NaCl 0.7 g. The molecular weight of propanidid is 337. Scholtan and Lie (1966) reported that the molecular weight of Tensid is 3,000 at 20°C and 3,170 at 37°C. In a solution of Tensid in water (instead of alcohol), only a minor proportion of the substance is present in the form of individual molecules. The major proportion of the substance aggregates in the form of micelles. Tensid essentially enhances the solubility of propanidid in water. This improved solubility comes about by the incorporation of propanidid into the micelles of the Tensid. In the clear Epontol solution, propanidid is not distributed in the form of a molecular dispersion but is present in colloidal solution containing the active substance propanidid which can be chemically determined in the serum.

In addition to this research by Scholtan and Lie, the results reported by Kurz (1966) stimulated our interest in the following problems.

(1) What is the time-course of the concentration of propanidid in human serum? Does the serum concentration of the drug depend on the speed at which it is injected?

(2) Is there a relationship between the serum propanidid concentration and the depth of sleep as recorded by electroencephalography?
Does the drug pass through the placental barrier and reach the foetus?

(4) Does the serum cholinesterase play an essential role in the enzymatic splitting of propanidid?

(5) What effect has propanidid on the action of suxamethonium?

METHODS

EXPERIMENTAL

The concentration of propanidid in human serum was determined by the method of Kurz (1966) which is based on the principle of extraction with a heptane phase.

The following solutions are needed:

(a) For serum cholinesterase inhibition N/1 KF (1.4 g KF/25 ml H₂O).

A serum concentration of 0.01 mol should be obtained.

(b) n-Heptane (purified with aluminium oxide—aluminium oxide 100 g to heptane 400 ml).

(c) NaCl, analytical grade. Technique: 4 ml serum (+KF)+3.75 g NaCl+12 ml n-heptane, shaken for 30 minutes, centrifuged.

The heptane phase is measured against double distilled water at 281 m\(\mu\) in a Beckman-Du spectrophotometer.

To prevent errors due to inhibition of serum cholinesterase (SChE) in healthy human volunteers after injection of Epontol, blood samples taken from the vein were immediately placed into iced water. After the test period, i.e. 45 minutes after beginning the injection of Epontol (7 mg/kg body weight), the blood samples were centrifuged and the activity of the enzyme determined by the spectrophotometric method of Kalow and Lindsay (1955). The dibucaine number (Kalow and Genest, 1957) was determined to exclude genetic enzyme variants.

In-vitro tests to determine the I₅₀ were carried out with propanidid concentrations increasing from \(6 \times 10^{-7}\) up to \(10^{-3}\). The purpose was to have different initial serum cholinesterase activities in the individual sera. The final concentration using the method of Kalow and Lindsay (1955) would amount to a quarter of the initial concentration.

The same test order was maintained for the fractions of high-tension electrophoresis showing serum cholinesterase activity (alpha-2-beta-globulins) and for a purified 3,000-fold concentrated serum cholinesterase (Haupt et al., 1966) (Behring-Werke).

Enzymatic splitting of propanidid was investigated in vitro as follows:

12 ml test material, e.g. serum with a defined serum cholinesterase activity, is divided into 6 portions. Incubation with a known molar concentration of Epontol.

After incubation for 2, 6, 10, 20, 30 or 40 minutes the corresponding sample is mixed with a serum cholinesterase inhibitor (KF), transferred from the water bath of 37°C into iced water and the propanidid concentration assayed by the stated method.

The test procedure is the same with the purified concentrated serum cholinesterase, whereas cholinesterase-free serum and albumin solution do not need addition of the inhibitor KF.

Carrier-free high-tension electrophoresis after protein separation into 48 fractions enables serum cholinesterase to be demonstrated in the alpha-2-
Experimental studies of the breakdown of Epontol

After the 

beta-globulin region. The fractions containing cholinesterase were used for inhibition tests and studies of enzymatic splitting of propanidid.

Electroencephalograph recordings were made with 12-channel Hellige apparatus, with a paper speed of 7.5 mm/sec. While the e.e.g. was recorded on 8 channels, the other channels were used to record, at the same time, the oculogram (electrodes being applied near the temporal palpebral fissures), the electrocardiogram (lead I), respiration (using a mechanoreceptor under a belt around the chest), and the mass movement of the body (using a sensitive myotonograph, Philips).

The depth of anaesthesia and the tendency to sleep were assessed by visual evaluation of the e.e.g. curves. For every 40-second period the depth of sleep was estimated according to a modification of the classification of Loomis, Harvey and Hobart (1938). The values therefore represent the averages of every 40-second period (shorter variations of sleep depth are neglected).

They were continually recorded in an ordinate system and a graph was constructed. The various stages were described in detail by Doenicke et al. (1966).

Clinical

Serum concentration of propanidid.

Ten healthy people, aged 20–24 years, volunteered for the clinical tests to determine propanidid concentrations and cholinesterase activity in the serum.

Anaesthesia with Epontol (7 mg/kg body weight) was carried out in the morning. Every volunteer received two anaesthetics for which the drug was injected at different speeds. The interval between the two anaesthetics was at least 4 weeks. After applying the e.e.g. electrodes, recording pulse and blood pressure, a Gordh cannula of 1.8 mm diameter was inserted into a forearm vein of the arm not intended for injection of the anaesthetic, and a blood sample was obtained for determination of the control value and serum cholinesterase activity. Atropine was not given. Epontol, 5 per cent, was injected through a Gordh needle at least 1.3 mm wide into the other extended arm using a distal arm vein or a vein of the back of the hand. The injection was made over 5 seconds or 20 seconds.

Two blood samples each of 8 ml were taken after 1, 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, 25, 30 and 45 minutes. The blood was immediately placed into iced water. Cholinesterase inhibitor had previously been put into the tubes taking blood for propanidid assays.

Before, during anaesthesia, and for the next hour the e.e.g. was recorded, and the pulse and blood pressures were checked at the times of blood sampling.

Placental passage of propanidid.

The propanidid concentration following Epontol anaesthesia was determined in obstetrical patients after delivery of the child's head. Before anaesthesia an initial serum cholinesterase and control sample of blood was taken. As soon as possible after the injection (20 seconds) maternal and foetal samples were obtained and concentrations determined as described.

Effect of propanidid on the action of suxamethonium.

To study the question of prolongation of the action of suxamethonium after Epontol the following test order was chosen. To exclude reduced serum cholinesterase values or genetic variants (atypical esterase), the pre-anaesthetic serum cholinesterase activity of selected patients was determined (method of Kalow and Genest, 1957).

Premedication consisted of atropine only, given intravenously 15 minutes pre-anasthetically. Injection of Epontol 7 mg/kg body weight in 5–8 seconds was followed immediately by the injection of suxamethonium (7 mg/10 kg body weight). Artificial pulmonary ventilation was started immediately after the onset of complete muscle relaxation. The trachea was intubated. In these tests we did not wait until the onset of the hyperventilation phase which is regularly associated with Epontol but which usually occurs only 10–20 seconds after the completion of injection.

Apnoea following suxamethonium was measured with a stopwatch until the return of the first respiratory movements. Ventilation was controlled manually, hyperventilation being avoided. The anaesthetic mixture consisted of nitrous oxide with oxygen (2:1) and halothane 0.5–1.5 vol. per cent according to requirements. After the return of normal spontaneous respiration, a second similar dose of suxamethonium was given and the period of apnoea recorded as before.
### Table IA
Propanidid serum concentration after Epontol (7 mg/kg); injection speed 5 seconds.
Single values of propanidid concentrations in human serum.

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### Table IB
Propanidid serum concentration after Epontol (7 mg/kg); injection speed 20 seconds.
Single values of propanidid concentrations in human serum.

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<th>SCHE a.u.1000/3 min at 26°C</th>
<th>In maternal blood after</th>
<th>In umbilical blood after</th>
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RESULTS

Serum concentrations of propanidid.

When the injection was made over a period of 5 seconds no concentration was found after 15 minutes except 0.8 µg/ml in patient No. 4 (table IA).

The magnitude of the concentration differs between individuals. Comparing the mean serum concentration after rapid and slow injection (table IB) there was a significant difference from the 2nd minute onwards (fig. 2). After injection over 5 seconds no serum concentration was found 15 minutes from the onset of anaesthesia, while after the slow 20-second injection zero level was not reached until the 25th minute (table IA, B; fig. 2).

Passage across placental barrier.

In obstetrical patients who had been given a brief Epontol anaesthetic at the time of delivery of the child's head the serum propanidid concentrations were similar to the values obtained in the volunteers (table II, fig. 3).

The propanidid concentration in umbilical vein blood, following placental transfer, was lower. As far as these preliminary studies allow a conclusion to be drawn, it appears that, in the 3rd minute after the start of injecting a dose averaging 7.18 mg/kg body weight, a concentration balance occurs. At that time the average concentration was 4.1 µg/ml in the maternal venous blood and 4.6 µg/ml in the umbilical blood.

The propanidid concentration in the umbilical vein blood appeared to depend on the initial serum cholinesterase activity. In patient No. 9 in whom a reduced serum cholinesterase activity of 75 Δu was measured, the highest concentrations were found (12.9 µg/ml in the 2nd minute and 11.9 µg/ml in the 3rd minute after beginning the injection).

With an average Epontol dose of 3.54 mg/kg, which is sufficient for brief anaesthesia in obstetrics (reduced serum cholinesterase activity), the propanidid concentrations in the umbilical vein blood are distinctly lower and in some of the tests no propanidid was found. Here again the highest propanidid concentration (3.9 µg/ml) was found in the 2nd minute and in this case a reduced serum cholinesterase activity of 64 Δu was measured.

Role of serum cholinesterase.

Serum cholinesterase activity was inhibited more after rapid injection (5 seconds) than after 20-second injection of Epontol. The individual
serum cholinesterase initial values were rated as 100 per cent and the percentage of inhibition calculated; comparison of the average curves after slow and rapid Epontol injection reveals a clear
difference in enzyme inhibition. Three minutes after the start the 5-second injection was followed by, on average, 12 per cent more inhibition of serum cholinesterase activity.

Statistical examination calculation showed that the difference was significant only in the 3rd minute. At the 15th minute (fig. 4) only 94 per cent of the initial value (ten subjects, see tables IA, IB) had been regained after the 5-second injection, but the full value had been regained after the 20-second injection.

**Effect of Epontol on duration of apnoea after suxamethonium.**

In 31 unselected patients aged 14–77 years apnoea was on average 3 minutes 44 seconds longer after Epontol with suxamethonium than after suxamethonium alone (fig. 5). None of the patients possessed an atypical cholinesterase and in only one case was the enzyme activity reduced. The patient with the lower values was a man of 74 years whose serum cholinesterase activity was determined as 69 Δu and the dibucaine number as 65. This case might be an intermediary type but further genetic examination, to include consanguinous relatives, was not possible. The enzymatic values may explain the prolonged apnoeic
Inhibition of serum cholinesterase in vivo which is stronger after injection in 5 seconds (interrupted line) than after a 20-second injection period (continuous line). Data from same patients as in tables IA, IB, and fig. 2.

Electroencephalography.

Figure 6 illustrates the e.e.g. changes noted in a 20-year-old male volunteer. High slow activity was observed within a few seconds; this ceased by the 3rd minute. In the 4th minute he was already awake and able to open his eyes. The curves of average depth of sleep and serum concentration (fig. 7A, B) show that hypnotic concentrations were eliminated from the serum more quickly after rapid than after slow injection. Normal depth of sleep did not, however, return earlier in the e.e.g. of healthy volunteers; the records did not show significant differences between slow and rapid injection.

Certain remarkable features were noted in a subject with reduced cholinesterase activity in the serum (72 Δu). These were prolonged persistence of high rapid activity and slower subsidence of beta-activity. The analytical curves with Schwarzer as well as Tönnes* analyzers (fig. 8A, B) show this clearly; in both tests this subject only woke up in the 7th minute.

* We are grateful to Messrs. Schwarzer and Messrs. Tönnes for placing the apparatus at our disposal.

Continuous e.e.g. recording combined with determination of the propanidid serum concentration enables the question of the acute tolerance of the drug to be clarified. Both after rapid injection and slow injection the volunteers awoke in the 5th minute, although the serum propanidid concentration was 3.5 μg/ml after rapid and 8.7 μg/ml after slow injection.

An attempt has been made to clarify the findings in volunteers and patients by means of in-vitro experiments. An important prerequisite for the investigation of the in-vitro hydrolysis appeared to be the clarification of the question of what factors determine the 50 per cent inhibition of the activity of serum cholinesterase by propanidid, in particular of the question of whether the inhibition of the enzyme depends on: (1) the starting material (whether normal serum, fractionated serum (fractions α and β), or purified and enriched enzyme); and (2) the level of the enzyme activity.

A 50 per cent inhibition (I₅₀) of the cholinesterase activity by the substrate propanidid occurred at 5 x 10⁻⁶ molar concentration as well as in human serum of normal cholinesterase activity (80 to 130 Δu/ml) and also in purified

---

**Fig. 4**

Pseudo-cholinesterase activity after Epontol (7 mg/kg)

**Fig. 5**

Duration of apnoea after Epontol and suxamethonium

Duration of apnoea after suxamethonium

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**Fig. 6**

Duration of apnoea after Epontol followed by administration of suxamethonium (upper part). Normal apnoeic periods after suxamethonium (lower part). Total case material 31 patients.
FIG. 6

The electroencephalogram of a 20-year-old male volunteer after Epontol 500 mg. In the 30th second the record shows the onset of high slow activity (delta waves) which occurred within a few seconds and lasted for about 70 seconds. In the 4th minute the man opened his eyes.

↑ ↓ = opening eyes and closing lids.
EXPERIMENTAL STUDIES OF THE BREAKDOWN OF EPONTOL

Curves showing average depth of sleep (interrupted curve) and serum concentration of propanidid (continuous curve). Averages of 10 test persons each, after injection in 5 seconds.

Curves showing average depth of sleep (interrupted curve) and serum concentration of propanidid (continuous curve). Averages of 10 test persons each, after injection in 20 seconds.

Serum cholinesterase enriched from 28 to 168 $\Delta u$/ml (fig. 9).

The extent to which the protein content plays a role in the enzyme inhibition may be illustrated by the different values for $I_{50}$ between a normally high serum cholinesterase activity (e.g., 132 $\Delta u$) in the presence of an albumin content of 50 per cent and a patient serum with low cholinesterase activity (35 $\Delta u$) with a haematocrit of 26 per cent and an albumin content of 32 per cent. This permits the conclusion that in human serum, too, propionidid is bound in part to proteins. It should be noted in passing that the solution-enhancing agent Cremophor EL does not inhibit the activity of serum cholinesterase.

Additional in-vitro investigations were carried out to clarify the dependence of the breakdown of the drug on the character of the starting material, such as human serum of various levels of enzyme activity, and purified and enriched cholinesterase at various substrate concentrations. The studies with purified serum cholinesterase revealed that the enzymatic cleavage of propionidid depends primarily on the level of the initial activity. Thus, purified and enriched cholinesterase activities at 28 to 50 $\Delta u$/ml (corresponding clearly to in-vivo values of pathological import) were not able to effect a propionidid cleavage at a concentration of $5 \times 10^{-4}$ mol (=$I_{50}$) to more than a slight extent (fig. 10A). On the other hand, hydrolysis was very rapid at higher levels of enrichment of the purified cholinesterase (fig. 10C; 135 and 168 $\Delta u$). When propionidid was incubated with human serum, the resulting enzymatic hydrolysis was substantially less pronounced and substantially slower (fig. 10A, B, D). When propionidid at concentrations of from $1 \times 10^{-5}$ to $1 \times 10^{-3}$ was incubated in pure albumin solution or in serum fractions containing no cholinesterase (albumin or $\gamma$ globulin), there was no cleavage of it (fig. 11). The inhibition ($I_{50}$) of the acetylcholinesterase-activity (erythrocyte-esterase) by the substrate propionidid occurred at $1 \times 10^{-4}$ mol concentration.

DISCUSSION

Epontol has been in general clinical use since 1965. Anaesthetists are interested in its very short anaesthetic action but also pharmacologically in its nature as a propylester of phenylacetate. Another ester, the ester of succinic acid (suxamethonium), was introduced with great success into anaesthesia in 1949 and still arouses scientific discussion because exact physico-chemical data and chemical demonstration methods are still lacking.
Prolonged anaesthetic (about 2 minutes longer than normal value, viz. fig. 7A, B) in a subject with reduced serum cholinesterase activity at repeated examinations: 9.2.67 and 19.4.67; 72 A. The Epontol dosage was in each case 7 mg/kg body weight, 20 sec injection time.

(A) Left, upper traces: combined frequency analysis and amplitude integration by Schwarzer analyzer. Lower curve: sleep depth.

(B) Right, upper traces: e.e.g. and interval analysis by Tönnes analyzer. The distribution of dots (ordinate 0–30 c.p.s.) corresponds to the frequency of e.e.g. waves. Analyzer adjusted to high sensitivity for rapid activity. Lower curve: sleep depth.
According to Wirth and Hoffmeister (1965) the short-lived anaesthetic action of propanidid is supposed to be largely connected with rapid ester cleavage. On the basis of experimental studies on the whole animal (rat) they concluded that serum cholinesterase, reported to be inhibited by paraoxon, did not play an appreciable role in the breakdown of propanidid. Doenicke and colleagues (1965) administered 0.5 and 1.0 mg doses of neostigmine for the purpose of in-vivo inhibition of cholinesterase but did not obtain a prolongation of anaesthesia, although inhibition had been measured in vitro. These findings appeared as a discrepancy in the interpretation of the results. Both teams derived from their observations the view that serum cholinesterase is not an important factor in the enzymatic splitting of propanidid.

The present findings (fig. 4) of enzyme inhibition are in clear contrast to previous conclusions by Wirth and Hoffmeister (1965) and Doenicke et al. (1965). The first authors, when determining the duration of anaesthesia with or without previous administration of paraoxon (a specific inhibitor of serum cholinesterase), did not take into account the fact that the animal species used (rat, mouse) possess little or no serum cholinesterase activity (Goedde, Doenicke and Altland, 1967; Haupt et al., 1966). There could not therefore be a difference in anaesthetic period between treated and untreated animals.

Inhibition of serum cholinesterase in vivo by 0.5 and 1.0 mg doses of neostigmine is not only too weak, but the degrees of inhibition observed were also not correct, so that again in this case no significant prolongation of the anaesthetic effect could be expected. In our previous studies (1965) values of esterase inhibition were measured which were lower than the actual in-vivo values, because the blood samples had been allowed to stand at room temperature for prolonged periods.

The observations on patients in whom a significantly prolonged apnoea occurred when Epontol was followed by suxamethonium gave rise to doubts about the correctness of the findings described in 1965 (fig. 5). With an improved test order it has now been possible to demonstrate also in vivo an enzyme inhibition after Epontol. The in-vivo enzyme inhibition appears to be related to the speed at which the anaesthetic is injected. Three minutes after the start of the injection, serum cholinesterase activity is on average 12 per cent more inhibited when the Epontol was injected in 5 seconds than after slower injection (fig. 4).

In-vitro determination of the $I_{50}$ regularly shows inhibition at $5 \times 10^{-4}$ molar, both in purified concentrated cholinesterase and in human serum with normal enzyme activity. The different $I_{50}$ in patient sera with a lower protein content (albumin fraction = 32 per cent; serum cholinesterase = 35) is noteworthy. In this case $I_{50}$ occurs at the lower concentrations $1 \times 10^{-4}$ and $5 \times 10^{-5}$ (fig. 9).

These findings may indicate a relationship between the rate of inactivation and the rate of binding of the drug to plasma protein. A lower protein content means more affinity for the enzyme and more blockade of serum cholinesterase. The investigations by Scholtan and Lie (1966) substantiate our assumption. These authors found that in a protein solution containing albumin 4 g/100 ml and additionally gamma globulin 3 g/100 ml (serum model solution) the proportion of free propanidid is 61 per cent.
FIG. 10A, B, C

FIG. 10D

FIG. 11
Serum cholinesterase-free eluate (VAP apparatus) + Epontol. No enzymatic splitting of Epontol in pure albumin solution and in cholinesterase-free serum fractions.
The interaction of albumin and propanidid follows the law of mass action; an albumin molecule possesses for the binding of propanidid \( n \) binding groups of equal affinity; \( n \) must be larger than 10. The results of these authors and of our own inhibition tests in vitro are fully consistent with the clinical observations. For patients in poor general condition (low protein content) Epontol \( 3 \text{ mg/kg body weight} \) is a sufficient anaesthetic dose; with \( 7 \text{ mg/kg anaesthesia is prolonged several minutes.} \) It is also interesting that albumin is only able to bind propanidid but not the Tensid.

Although for suxamethonium no chemical demonstration method is as yet available, Kurz (1966) in animal experiments achieved the determination of propanidid in plasma as well as in tissue. His method is based on extraction with \( n \)-heptane. This solvent also extracts propanidid incorporated in micelles, which is significant in view of the colloidal chemical properties of the active substance reported subsequently.

With Kurz's method, which he kindly placed at our disposal, we were able to determine the propanidid concentrations in human serum and umbilical vein blood shown in tables I and II and in figures 2 and 3. It has, however, to be taken into account that in determination by the Kurz method the heptane phase carries not only the free but also the protein-bound portion of the drug. In the tests with purified concentrated esterase (fig. 10c, d) neither albumin nor gammaglobulin is present.

The differences in serum concentrations after different injection speeds should be considered in more detail. Rapid injection (5 sec) of about 500 mg Epontol (fig. 2; table I) yields a higher initial concentration of propanidid. The peak concentration occurs with the short injection time in the first minute and amounts to 14.9 \( \mu \text{g/ml} \), while after the longer injection time (20 sec) it is only found in the second minute and reaches 14.3 \( \mu \text{g/ml} \). The higher initial concentration results in stronger enzyme inhibition which was proved by our in-vivo investigations (fig. 4) as well as by the in-vitro tests (fig. 9). Stronger enzyme inhibition and higher initial concentration after rapid injection provide for more rapid passage of the blood/brain barrier. Deeper anaesthetic stages might therefore be expected after this form of injection and were confirmed by clinical observations as well as by continuous e.g. controls (fig. 7a, b). On the other hand, enzymatic splitting of propanidid is more rapid at a higher concentration, according to the in-vitro results (fig. 10a); at \( 10^{-3} \) and \( 5 \times 10^{-4} \) molar concentration, corresponding in vivo to an injection dose of 430 mg and 190 mg respectively, about 50 per cent Epontol is broken down in vitro in the first 20 minutes.

In the serum, the drug is more quickly broken down after the short "one shot" injection; at a higher concentration, comparable to the molar concentration \( 10^{-3} \) or \( 5 \times 10^{-4} \) used in vitro (fig. 10b), the total concentration decreases much more (fig. 7a). Passage from the c.s.f. to the blood begins earlier and anaesthesia is terminated by distribution to other organs well supplied with blood. Consequently, anaesthesia is of shorter duration when injection is made in 5 seconds.

The factor of a protein binding has been neglected in the discussion so far. Kurz (1966) reported that Epontol is bound to protein to the extent of 70.09 per cent. In vivo, this certainly plays a minor role, due to the rapid enzymatic degradation. In laboratory tests with human serum (fig. 10a, b) it is the only factor to which the low breakdown rate of Epontol can be ascribed, since the determinations by the method of Kurz include the protein-bound propanidid; only free, unbound drug can be hydrolyzed. The actual degradation rate is seen from the comparison with purified concentrated esterase (fig. 10c) where no protein binding is possible because of the absence of both albumin and gammaglobulin.

The short-lived persistence of propanidid in the serum can only be explained by the results of Pütter (1965) and Scholtan and Lie (1966), the animal experiments with propanidid \(^{14} \text{C} \) of Duhm and associates (1965), the physico-chemical data reported by Kurz (1966), and the in-vitro and in-vivo studies reported here.

Duhm and associates (1965) and Kurz (1966) also believed that distribution in the body probably was a factor. Kurz (1966) reporting physico-chemical data of some anaesthetics reported the lipid solubility as: heptane/H.O 1.702 for Epontol, 1.026 for thiopentone; protein binding/plasma 73.09 for Epontol and 91.67 for thiopentone. Considering these figures it appears
certain that, apart from enzymatic hydrolysis of the drug, there must be redistribution into other organs which are well supplied with blood. Kurz observed in animal experiments passage of propanidid into the musculature within 4 minutes of administration. The rapid passage through the placental barrier also suggests the possibility of rapid distribution in the body.

These results have practical significance only if there is a deficiency of the enzymes which degrade propanidid or an abnormally low protein content. In such cases the anaesthetic action of Epontol will last a few minutes longer. Anaesthesia is terminated, as with thiopentone (Price, 1960), by redistribution in the body.

The present studies as well as previous publications by Duhm and associates (1965), Scholtan and Lie (1966), and also Kurz (1966), do not support Hoffmeister's view (1967) that retention due to linkage with plasma protein would not be of practical significance for the termination of anaesthetic action.

ACKNOWLEDGEMENT
This work has been supported by the Deutsche Forschungsgemeinschaft.

REFERENCES


ETUDES EXPERIMENTALES DE LA CHUTE DE L'EPONTOL: DETERMINATION DU PROPAVIDIDE DANS LE SERUM HUMAIN

SOMMAIRE
On a déterminé la concentration du propanidide dans le sérum humain et l'on a trouvé en rapport de l'injection. On n'avait rien trouvé 25 minutes après une injection s'étendant sur une période de plus de 20 secondes. Le propanidide avait traversé la barrière placentaire, la concentration dans la veine ombilicale était dépendante de la dose injectée. La cholinesterase du sérum était inhibée par le propanidide à la fois in vivo et in vitro, ainsi l'estérase du globe rouge était inhibée. La concentration dans le sang de la veine ombilicale était la plus forte chez les mères avec une activité réduite de la cholinesterase. La durée de l'anesthésie semble être influencée par l'activité de la cholinesterase du sérum. In vitro, l'hydrolyse enzymatique dans le sérum humain était lente, mais se produisait rapidement dans la cholinesterase purifiée et concentrée. Lorsque du suxaméthonium était injecté après l'Epontol, la période de l'apnée était augmentée, ce que l'on peut rapporter à l'inhibition enzymatique. On a trouvé que l'électrocardiographe était utile dans la détermination de la durée de l'anesthésie. La prolongation de l'anesthésie chez des sujets montrant une basses activité enzymatique était en correspondance avec les résultats électrocardiographiques. Le propanidide est lié aux protéines du plasma et l'on estime qu'une dose d'Epontol de 3-4 mg/kg suffit pour produire une anesthésie chez des malades se trouvant en grand péril.

*Propanidide=Ester propylique de l'acide acétique [4-(diethyl-carbamoyl) méthoxy]-3-méthoxyphényl.
EXPERIMENTELLE UNTERSUCHUNGEN ZUM
ABBAU VON EPONTOL—NACHWEIS VON
PROPANIDID IM MENSCHLICHEN SERUM

ZUSAMMENFASSUNG
Die Serumkonzentration von Propanidid wurde
bestimmt; es konnte festgestellt werden, dass sie von
der Schnelligkeit der Injektion abhängt. Bei einer
Injektionszeitspanne von 20 Sekunden fand man 25
Minuten nach der Injektion keine Propanididkonzentra-
tion mehr im Serum. Propanidid kreuzt die
Placenta-Schranke, die Konzentration in der V.
umbilica hängt von der injizierten Dosis ab. Serum-
cholinesterase wurde sowohl in vivo als auch in vitro
durch Propanidid gehemmt. Die Erythrozytenesterase
wurde in vitro gehemmt. Die Konzentration in der V.
umbilica war am höchsten bei Müttern mit vermin-
derter Cholinesterase-Aktivität. In vitro war die
enzymatische Hydrolyse im menschlichen Serum
langsamer, sie trat aber sehr schnell in gereinigter kon-
zentrierter Cholinesterase auf. Wurde Suxamethonium
nach Epontol injiziert, so nahm die apnoeische Periode
zu; diese Tatsache wird der Enzymhemmung zuge-
schrieben. Das EEG erwies sich für die Bestimmung
der Anaesthesie-Dauer als nützlich. Eine Verlängerung
der Anaesthesiezeit bei Patienten mit niedriger Enzym-
Aktivität stand in Korrelation mit den EEG-Befunden.
Propanidid ist an Plasmaproteine gebunden. Es wird
vorgeschlagen, dass bei Patienten in reduziertem
Allgemeinzustand 3–4 mg Epontol/kg Körpergewicht
zur Einleitung einer Anaesthesie bewirkt werden.

REGISTRARS' PRIZE (ANAESTHETICS)

Applications are invited by the Royal Society of Medicine, Section of Anaesthetics,
for a prize of £50 provided by Messrs. May & Baker Ltd., for a paper written by a
medical practitioner of Senior Registrar or Registrar status holding an appointment in
anaesthesia in a department or hospital, or in the armed forces of the Commonwealth
or of the Republics of South Africa or Eire. Fellowship of the Royal Society of Medicine
is not necessary for entry. The subject will be of the author's choice, but must be
connected with anaesthesia. All papers for the 1969 award must be submitted in tripli-
cate by January 1, 1969.

Further details and rules of the prize can be obtained from the Assistant Secretary,
Royal Society of Medicine, 1 Wimpole Street, London, W.1.

A further prize of £25 may be awarded on the recommendation of the judges.