From: The Dept. of Pharmacology, Karolinska Institutet (L.-O. B.), Kungl. Farmaceutiska Institutet (F. S.), and the Dept. of Oral Surgery, Tandläkarhögskolan, Stockholm, Sweden (E. Å.).

EXPERIMENTAL AND CLINICAL STUDIES ON THE SYNERGISM AND ANTAGONISM OF ORAL ANALGESICS

by

LARS-OLOF BORÉUS
FINN SANDBERG
ERIK ÅGREN

Among the additions of recent years to the drugs of the antipyretic-analgesic group are salicylamide and N-acetyl-p-aminophenol.

Salicylamide is resorbed rapidly from the gastrointestinal canal without being decomposed there, and is excreted in the urine, for the most part linked to glucuronic acid. *Hart* (1947) demonstrated in animal experiments that the analgesic effect of salicylamide is about six times that of acetylsalicylic acid and three times that of phenazone. Its good antipyretic and antirheumatic effect was demonstrated by *Wegman* (1950) and has since been confirmed by other investigators (*Harthon* and *Sigroth,* 1952). In therapeutic doses, salicylamide rarely causes stomach trouble, and in other respects as well it is tolerated better than acetylsalicylic acid.

N-acetyl-p-aminophenol is the chief metabolite of acetophenetidin (phenacetin), and according to *Brodie* and *Axelrod* (1949) it is responsible for the good analgesic and antipyretic effect of the latter. Since N-acetyl-p-aminophenol has lower toxicity and gives a considerably lower methemoglobin formation than acetophenetidin, it has definite therapeutic advantages over acetophenetidin.

The object of the present investigation was to study by human experiments and clinically how the analgesic effect of the mixture of salicylamide and N-acetyl-p-aminophenol (in the proportions 1 : 1) may be increased by adding other substances, such as barbiturates and morphine derivatives.

The starting point was an investigation (*Boréus* and *Sandberg*, 1953) showing, on the basis of animal experiments, that a synergism exists between the analgesic effects of salicylamide and N-acetyl-p-aminophenol, and that diallymal increases the analgesic effect of a mixture of these substances. As pointed out earlier (*Boréus* and *Sandberg*, 1955), the quantitative results obtained by animal algesimetric methods are not directly applicable to man. It therefore seemed reasonable to extend our investigations of the substances in question to experimental and clinical pain in human subjects.

EXPERIMENTAL

Experimental pain.

The following two methods, based on different principles were used:

I. *Electrical stimulation of tooth pulp.*

The experiments were carried out on 194 untrained, healthy female and male subjects; their age ranged from 21 to 28 years. Each subject was used for one experiment only. The type of stimulator described by *Björn* (1947), among others, was used. This method has not previously been applied for evaluating analgesics, but only for local anaesthetics and general anaesthetics, *i.e.* nitrous oxide (*Persson*, 1951). The threshold determinations were carried out on the maxillary central incisors. The scale value of the current needed for the first distinct painful sensation was read off on the potentiometer dial. The determinations were repeated until approximately the same value was obtained at least three times in succession. The first reading was disregarded, and the mean of the following readings was taken as the threshold value of the dental pulp. The analgesic action is reflected by an increase in the threshold value. Threshold determinations were made immediately before and one and two hours after drug administration.

II. *Radiant heat stimulation of the skin.*

The *D'Amour-Smith* (1941) modification of the original *Hardy-Wolff-Goodell* (1940) method was applied for our experiments on untrained test subjects. These were simultaneously used for tooth pulp algesimetry (see above). The forehead of the test subject is exposed to heat stimulus of constant intensity and varying exposure time *(reaction time)*. The end point of stimulation is reached when the test subject begins to recognize a sensation of pain. The determinations were repeated five times, using different areas of the forehead. The first reading was disregarded, and the mean of the following ones was taken as the threshold value of the skin. The analgesic effect is reflected in a prolonged reaction time. Threshold determinations were made immediately before and one, two and three hours after drug administration.

In some experiments in this study, radiant heat stimulation was applied to the rat. The principle is the following. A shaved area of the rat's back is exposed to heat radiation of constant intensity until a definite response is elicited. The criterion of the response is a rapid twitching of the skin, distinctly evident over the irradiated zone. The interval elapsing between the beginning of the heat stimulus and the response of the rat is denoted as the reaction time. The analgesic effect is reflected in a prolonged reaction time.

For further details regarding the experimental methods, see *Boréus* and *Sandberg* (1955).

Clinical pain.

The patient material comprised 166 persons (male and female) 19—70 years of age, suffering from various complaints related to dental surgery.

With respect to the nature of the pain, the material was divided into two groups:

Group I: Patients with postoperative pain (apicoectomy, removal of impacted teeth, surgical treatment of cysts of the oral cavity, oral surgery for prosthesis, etc.).

Group II: Patients with other kinds of pain (dry socket, pericoronitis, dento-alveolar abscesses, acute cellulitis of the face, etc.).

The patients in group I were as a rule premedicated with 0.1 g of nembutal 45 minutes before the operation. All patients were treated ambulatorily and given the test substance (mixtures 6 and 7) or placebo in the form of tablets of identity unknown to the test subjects. No other treatment against the pain was given during the observation period. At the onset of the pain, 2 tablets were given with plenty of water. The 3-hour-period after each administration constituted one observation. Each patient thus provided one or more observations.

Each patient was instructed to fill in a form, stating: 1) name, age, and sex; 2) degree of pain (severe, moderate, slight); 3) latency period; 4) degree of analgesic effect (good, moderate, poor, none); 5) duration; 6) side effects (drowsiness, stimulation, other, none).

The operation performed or the diagnosis was then filled in from the case-sheet.

From a clinical point of view, good or moderate effect of an analgesic may be regarded as satisfactory, whereas poor or no effect is unsatisfactory. It is therefore warranted to combine the two former degrees into one group and the two latter into one. These two groups were used in the statistical analysis of the therapeutic effect.

STATISTICS

Experimental pain.

The analgesic effect was computed as the post-medication deviation from the pre-medication threshold value as follows. In each test subject, X_0 denotes the pre-medication threshold value, X_1, X_2 and X_3 the first, second and third post-medication threshold value, respectively. A value, Y, of the analgesic effect for each test subject is derived from the following formula:

$$Y = X_1 + X_2 + X_3 - 3X_0$$

Y represents the response of each subject, and might be called "the area under the curve". On the basis of the Y values for the test subjects with the *same* medication, the mean and the standard error of the mean were calculated for each drug. The significance of the analgesic effect was tested by t-analysis.

The means of X_0, X_1, X_2 and X_3 were computed for each drug treatment and were symbolized by $\overline{X}_0$, $\overline{X}_1$, $\overline{X}_2$ and $\overline{X}_3$. In the Figures $\overline{X}_0$ was put in the origo. The deviations of $\overline{X}_1$, $\overline{X}_2$, $\overline{X}_3$ from $\overline{X}_0$ were plotted in the Figures to visualize the variation in the analgesic effect of various drugs during the post-medication period.

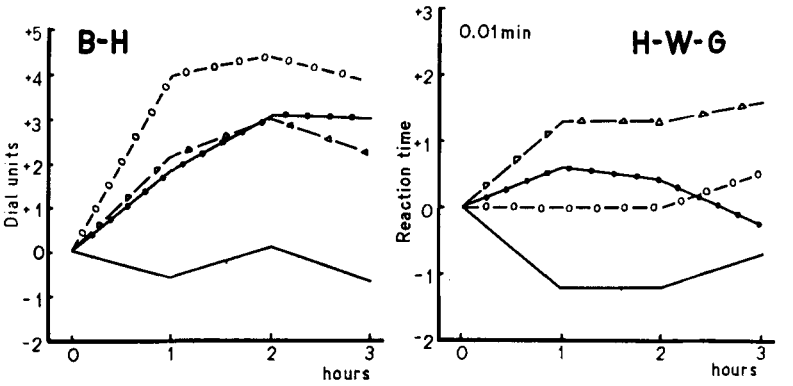


Fig. 1. The mean analgesic effect tested by the B-H-method (electrical stimulation of the tooth pulp) and the H-W-G-method (radiant heat stimulation of the skin) in man produced by the oral administration of 2.5 tablets of

—△—△—△— *Mixture 1:* N-acetyl-p-aminophenol 0.15 g, salicylamide 0.15 g, amobarbital N.N.R. (pentymal Ph. S. XI) 0.075 g, codeine phosphate 0.02 g, per tablet — (18 subjects),

—○—○—○— *Mixture 2:* N-acetyl-p-aminophenol 0.15 g, salicylamide 0.15 g, diallyl-barbituric acid 0.075 g, codeine phosphate 0.02 g, per tablet — (21 subjects),

—•—•—•—• *Mixture 3:* N-acetyl-p-aminophenol 0.15 g, salicylamide 0.15 g, diallyl-barbituric acid 0.075 g, codeine phosphate 0.02 g, caffeine 0.075 g, per tablet — (19 subjects),

———————— Controls — (19 subjects).

Clinical pain.

The statistical significance of a single value for the therapeutic effect of a drug treatment on clinical pain is computed from the formula:

$$p \pm \sqrt{\frac{p(100-p)}{n}}$$

where p = percentage of pain relief of the treated patients,
n = total number of treated patients.

t-analysis was used to test the significance of the therapeutic effect of a drug treatment and the difference between two treatments.

RESULTS AND DISCUSSION

To the mixture of salicylamide and N-acetyl-p-aminophenol (in the proportions 1:1) were added codeine phosphate and a barbiturate (for doses and number of test subjects, see Fig. 1). This mixture is denoted as mixture 1, and its analgesic effect, determined simultaneously by two fundamentally different methods on the same test subjects, is seen from Fig. 1. The effect was probable according to the B-H-method ($P = 0.05$—0.02), and significant according to the H-W-G-method ($P < 0.001$).

How is the analgesic effect modified, if the pentymal is replaced by the same dose of another barbiturate, such as diallymal (mixture 2)? The answer to this question is given by Fig. 1, which shows, unexpectedly, that the analgesic effect is somewhat increased according to the B-H-method ($P = 0.05$), but lowered according to the H-W-G-method ($P = 0.02$—0.01). These results are in agreement with the earlier findings of *Boréus* and *Sandberg* (1955) that the degree of analgesic effect of a certain drug varies with the type of experimental pain.

How is the analgesic effect modified, if caffeine is added to mixture 2 (giving the drug called mixture 3)? It is seen from Fig. 1 that the effect is lowered according to the B-H-method ($P = 0.05$—0.02), whereas determination according to the H-W-G-method shows no change in the total effect ($P = 0.7$—0.5). In earlier work based on animal experiments (*Boréus* and *Sandberg*, 1953), caffeine was found to be antagonistic to the analgesic action of N-acetyl-p-aminophenol. There are thus several experimental corroborations of the antianalgesic effect of caffeine.

In addition to salicylamide and N-acetyl-p-aminophenol, the analgesic effects of which have previously been studied in the rat, mixture 2 contains diallymal and codeine in the proportions 3.75:1. It was therefore interesting to investigate whether any synergism between diallymal and codeine in the proportions used could be found with the same method applied to the rat.

That such is the case appears clearly from the curves of Fig. 2, in which all the effects are significant ($P = 0.01$—0.001 and $P < 0.001$) when tested with the t-analysis.

In the experimental series recorded in Fig. 1, it was possible to study how the analgesic effect varied when pentymal was replaced by diallymal in a mixture of salicylamide, N-acetyl-p-

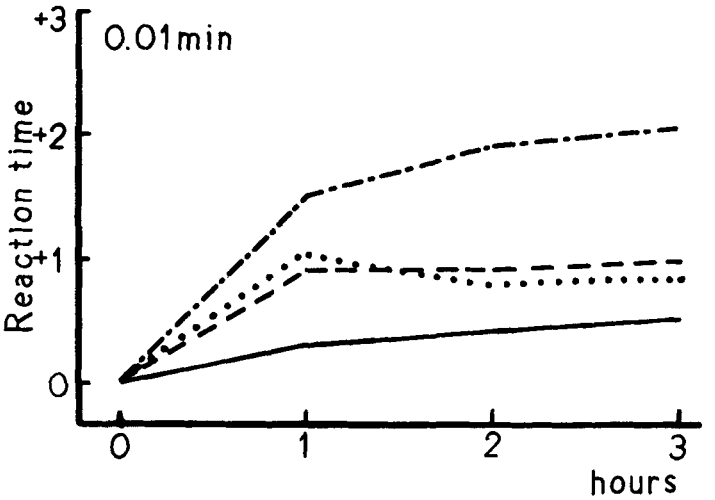


Fig. 2. The mean analgesic effect tested by the rat method (radiant heat stimulation of the skin) produced by the oral administration of

— — — — Codeine phosphate (10 mg/kg) — (40 rats),

. . . . . Diallyl-barbituric acid (40 mg/kg) — (38 rats),

—.—.—.— Codeine phosphate + diallyl-barbituric acid (10 + 40 mg/kg) — (40 rats),

———— Controls — (38 rats).

aminophenol, and codeine (cf. mixtures 1 and 2). The results of a similar experiment are recorded in Fig. 3, which shows the variations in the analgesic effect when diallymal is replaced by the same dose of a new barbiturate, a dialkyl-barbituric acid, in a mixture of salicylamide, N-acetyl-p-aminophenol, and caffeine (cf. mixtures 4 and 5). The former series thus contains codeine, whereas the latter contains caffeine. It appears from Fig. 3 that, in the doses used, the new barbiturate lowered the analgesic effect, as determined by both methods. There are de-

finite quantitative and qualitative differences between the barbiturates. It is thus not irrelevant which barbiturate in a given dose is used in a combined analgesic.

It was, however, found in the aforementioned human experiments that the combined analgesics used (mixtures 1—5) produced a more or less pronounced hypnotic side effect. In the case of mixture 4, this was confirmed by the clinical study, which showed a good analgesic effect but too strong a hypnotic effect

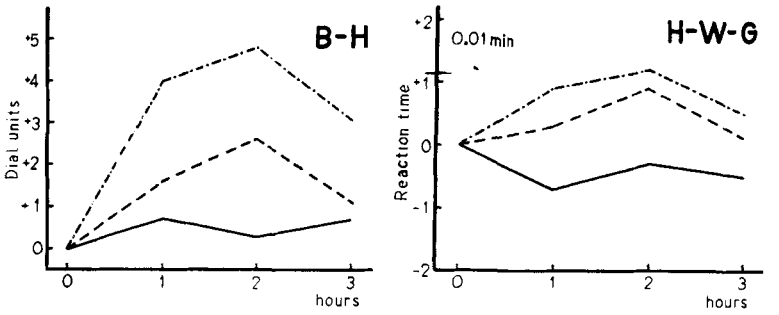


Fig. 3. The mean analgesic effect tested by the B-H-method (electrical stimulation of the tooth pulp) and the H-W-G-method (radiant heat stimulation of the skin) in man produced by the oral administration of 2.5 tablets of

—·—·—·—·— *Mixture 4:* N-acetyl-p-aminophenol 0.15 g, salicylamide 0.15 g, diallyl-barbituric acid 0.075 g, caffeine 0.075 g, per tablet — (24 subjects),

— — — — *Mixture 5:* N-acetyl-p-aminophenol 0.15 g, salicylamide 0.15 g, dialkyl-barbituric acid 0.075 g, caffeine 0.075 g, per tablet — (18 subjects),

———— Controls — (24 subjects).

to make the mixture suitable as a day-time analgesic. The barbiturate evidently increases the hypnotic effect of the mixture of salicylamide and N-acetyl-p-aminophenol. That this mixture *per se* produces a hypnotic effect appears from an experimental investigation in animals by *Berger* (1954). Consequently, it was natural to reduce the amount of barbiturate used. Fig. 4 shows the results of a series of experiments with the rat method, in which the amount of diallymal was reduced by half (cf. mixtures 4 and 6), and in which codeine was added to the mixture

with the smaller diallymal dose (cf. mixtures 6 and 7). As is seen from Fig. 4, the analgesic effect was lowered when the diallymal dose was halved ($P < 0.001$), and the analgesic effect of the latter mixture could not be increased by the addition of codeine ($P = 0.7—0.5$).

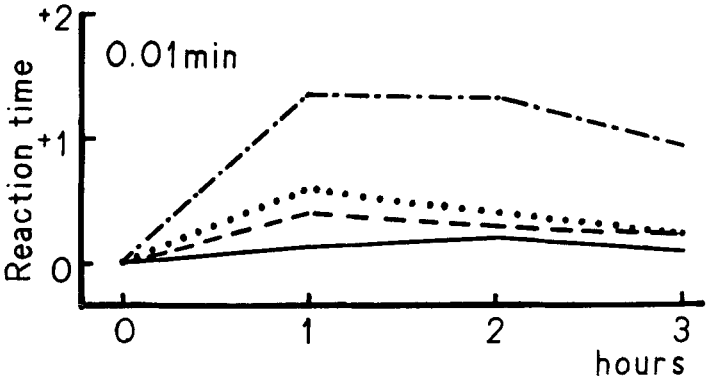


Fig. 4. The mean analgesic effect tested by the rat method (radiant heat stimulation of the skin) produced by the oral administration of 0.33 tablet/kg of

—•—•—•— *Mixture 4:* N-acetyl-p-aminophenol 0.15 g, salicylamide 0.15 g, diallyl-barbituric acid 0.075 g, caffeine 0.075 g, per tablet — (36 rats),

• • • • • *Mixture 6:* N-acetyl-p-aminophenol 0.15 g, salicylamide 0.15 g, diallyl-barbituric acid 0.04 g, caffeine 0.075 g, per tablet — (36 rats),

— — — — *Mixture 7:* N-acetyl-p-aminophenol 0.15 g, salicylamide 0.15 g, diallyl-barbituric acid 0.04 g, caffeine 0.075 g, codeine phosphate 0.015 g, per tablet — (34 rats),

———— Controls — (34 rats).

It appears from this experiment and the preceding one that the amount and kind of barbiturate is of importance for the analgesic effect of a combined analgesic. It must be emphasized that the quantitative results of determinations of the analgesic effect obtained in animal experiments are not directly applicable to human beings. Consequently, in order to obtain a good analgesic action without too marked a hypnotic side effect the dose of barbiturate in a combined analgesic must be determined by human experiments and clinical tests.

Fig. 5 gives the results of tests in man, using both methods, of mixture 6 and a combined analgesic much used in Sweden (mixture 8, see legend to Fig. 5) as the standard. The preparations were administered in the usual therapeutic doses. It is seen from the figure that mixture 6 was superior as to the analgesic effect ($P = 0.01$—0.001 for the difference between them, tested by t-analysis).

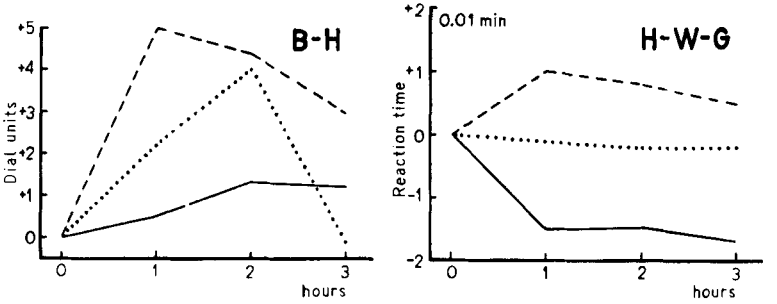


Fig. 5. The mean analgesic effect tested by the B-H-method (electrical stimulation of the tooth pulp) and the H-W-G-method (radiant heat stimulation of the skin) in man produced by the oral administration of 2 tablets of

— — — — *Mixture 6:* N-acetyl-p-aminophenol 0.15 g, salicylamide 0.15 g, diallyl-barbituric acid 0.04 g, caffeine 0.075 g, per tablet — (17 subjects),

..... *Mixture 8:* isopropylantipyrine 0.15 g, acetophenetidin 0.25 g, 3-3-diethyl-2.4-dioxo-tetrahydropyridine 0.05 g, caffeine 0.05 g, per tablet — (18 subjects),

———— Controls — (16 subjects).

The tests with experimental pain thus established that mixture 6 had a good analgesic effect but not excessive hypnotic effect. It was therefore considered warranted to test this mixture on clinical pain. In similarity to the animal experiments recorded in Fig. 4, this clinical investigation was organized as a comparison between mixtures 6, 7 and a placebo.

The results are recorded in the following tables:

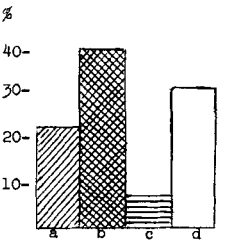
MIXTURE 6.

Dose: 2 tablets for each observation.

Postoperative pain.

Number of subjects: 45
» of observations: 96.

	Pain relief		No effect		Side effects
	good	moderate	poor	none	
Severe pain	8	10	6	2	
Moderate pain	38	9	5	2	
Slight pain	14	2	—	—	
	62.5 %	21.9 %	11.5 %	4.1 %	
	84.4 %		15.6 %		

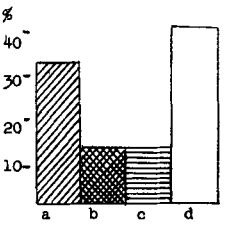


a = stimulation
b = drowsiness
c = other
d = none

Pain of other origin.

Number of subjects: 15.
» of observations: 28.

	Pain relief		No effect		Side effects
	good	moderate	poor	none	
Severe pain	9	—	—	—	
Moderate pain	10	2	—	—	
Slight pain	5	2	—	—	
	85.7 %	14.3 %	0 %	0 %	
	100 %		0 %		



a = stimulation
b = drowsiness
c = other
d = none

MIXTURE 7.

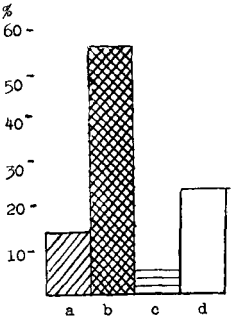
Dose: 2 tablets for each observation.

Postoperative pain.

Number of subjects: 54
» of observations: 123.

	Pain relief		No effect	
	good	moderate	poor	none
Severe pain	17	10	1	2
Moderate pain	45	10	1	1
Slight pain	32	4	—	—
	76.4 %	19.5 %	1.6 %	2.4 %
	95.9 %		4.1 %	

Side effects



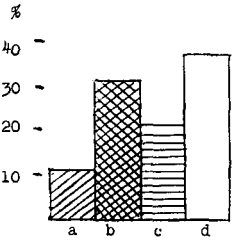
a = stimulation
b = drowsiness
c = other
d = none

Pain of other origin.

Number of subjects: 19
» of observations: 54.

	Pain relief		No effect	
	good	moderate	poor	none
Severe pain	18	1	2	1
Moderate pain	20	6	2	—
Slight pain	4	—	—	—
	77.8 %	13.0 %	7.4 %	1.8 %
	90.8 %		9.2 %	

Side effects



a = stimulation
b = drowsiness
c = other
d = none

PLACEBO.

Dose: 2 tablets for each observation.

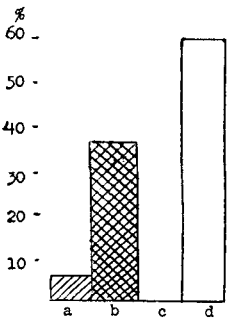
Postoperative pain.

Number of subjects: 17

» of observations: 31.

	Pain relief		No effect	
	good	moderate	poor	none
Severe pain	—	1	2	3
Moderate pain	5	3	1	7
Slight pain	6	1	—	2
	35.5 %	16.1 %	9.7 %	38.7 %
	51.6 %		48.4 %	

Side effects



a = stimulation
b = drowsiness
c = other
d = none

It is seen from the tables that the placebo group is smaller than the other groups; this was due to the difficulty of entirely disregarding humanitarian considerations where the patients were concerned. For the same reason, the number of cases in the placebo group under the heading of other pains is so small that they were not recorded in tabular form. The statistical analysis was therefore performed on the group of postoperative pain, and it shows that mixtures 6 and 7 both had a significant therapeutic effect ($P < 0.001$ in both cases) and that mixture 7 had a greater therapeutic effect than mixture 6 ($P = 0.01—0.001$). Codeine (the addition in mixture 7) thus increases the analgesic effect in man, which was not the case in the rat (cf. Fig. 4).

Of the side effects, drowsiness is that of greatest interest. When mixture 6 was used, 40 % of the patients in the postoperative group exhibited this effect. The corresponding figure for mixture 7 is 56 %, and for the placebo group 35 %.

It is thus evident that mixture 6 was associated with a lower incidence of drowsiness than mixture 7 in this investigation, whereas the latter mixture produced a better analgesic effect. When used as a day-time analgesic, mixture 6[1] is therefore to be preferred. When used as a night analgesic, on the other hand, the hypnotic effect is an advantage, and mixture 7 would thus be preferable. Mixture 2[1], which — in comparison with mixture 7 — has a larger diallymal dose and contains no caffeine, is still better from this point of view.

The latency period in the use of mixture 6 varied between 10 and 80 minutes and was mostly 10—15 minutes. The duration varied between ½ and 8 hours and was mostly 3—4 hours.

In medication with mixture 7, the latency period varied between 15 and 120 minutes and was mostly 15—20 minutes. The duration varied between 1½ and 8 hours and was mostly — as in the case of mixture 6 — 3—4 hours.

Summarizing the results of the clinical tests, both mixtures 6 and 7 may be said to have shown a satisfactory analgesic effect, latency period, and duration. The side effects were varying, but their nature and degree were not such as to render the mixtures in question unsuitable in clinical practice.

SUMMARY

Experimental investigations were made in the rat and in man by two different methods (electrical stimulation of tooth pulp and radiant heat stimulation of the skin), in order to ascertain how the analgesic effect of salicylamide + N-acetyl-p-aminophenol (1 : 1) is modified by the addition of barbiturates, codeine, and caffeine (seven different mixtures).

Three of the best mixtures were tested in a clinical series, and two of them (mixtures 6 and 7) proved to have satisfactory properties as analgesics.

[1] Mixtures 6 and 2 will be put on the market under the names of "Desalgon" and "Desalgon forte", respectively, by AB Pharmacia, Uppsala.

RÉSUMÉ

ÉTUDES EXPÉRIMENTALES ET CLINIQUES SUR LE SYNERGISME ET L'ANTAGONISME DES ANALGÉSIQUES PAR VOIE ORALE

On a étudié, par des expériences faites sur des rats et sur des hommes avec deux méthodes différentes (stimulation électrique de la pulpe dentaire et stimulation de la peau par rayonnement de chaleur), comment l'effet analgésique d'amide de salicyl + N-acétyl-p-aminophénol (1: 1) est modifié par addition de barbiturate, de codéine, et de caféine (7 compositions différentes).

On a essayé trois des meilleures compositions sur des sujets cliniques, et deux ont montré des qualités analgésiques satisfaisantes.

ZUSAMMENFASSUNG

EXPERIMENTELLE UND KLINISCHE UNTERSUCHUNGEN ÜBER DEN SYNERGISMUS UND ANTAGONISMUS DER ORALEN ANALGETIKA

Durch Versuche mit Ratten und Menschen mit zwei verschiedenen Methoden (elektrische Stimulation der Zahnpulpa und Wärmestrahlungsstimulation der Haut) ist untersucht worden, wie die analgetische Wirkung von Salicylamid + N-acetyl-p-aminophenol (1: 1) durch Zusatz von Barbiturat, Kodein und Koffein (7 verschiedene Kompositionen) verändert wird.

Drei der besten Kompositionen wurden klinisch durchprüft, wobei zwei von diesen befriedigende Eigenschaften als Analgetika aufwiesen.

We are indebted to AB Pharmacia, Uppsala, for financial support of this investigation.

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Addresses:

Boréus
Farmakologiska Institutionen
Karolinska Institutet
Stockholm 60
Sweden

Sandberg
Kungl. Farmaceutiska Institutet
Stockholm Va
Sweden

Ågren
Kungl. Tandläkarhögskolan
Stockholm
Sweden