Table II. Effect of active principles in extracts

Organisms	Ability to inhibit growth of bacteria					
	I	II	III	IV	V	B[a]
Rf[b]	0.20	0.30	0.45	0.62	0.85	0.56
Lactobacillus plantarum	+	+	−	+	−	
Leuconostoc mesenteriodes	+	+	−	+	−	
Streptococcus faecalis	+	+	−	+	−	−

[a] B is the fluorescent biliary excetory product. [b] Rf in isopropanol-formic acid-water (2:5:5 v/v).

The effects of the plant extracts on other bacterial species must be studied in vitro and in vivo to have an idea of the possible effect of the bark on human gut flora.

Résumé. Les extraits de l'écorce de *Saccoglotis gabonensis* Urban empêchent l'accroissement de la bactérie du vin de palme (une boisson alcoolique) et du *Streptococcus faecalis* isolé de l'intestin. Les effets métaboliques des extraits de composés fluorescents de cette écorce sur les flores gastro-intestinales sont discutés.

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PRO LABORATORIO

The Use of Diazepan as Premedication in Pentobarbital Anaesthesia in Guinea-Pigs

It is known how difficult it is to obtain a good anaesthetic plane in the guinea-pig, necessary to perform short or long surgical prodecures, when pentobarbital, chloralose or urethane are used[1–10].

With pentobarbital the results are unpredictable; high doses are usually lethal, due to marked depression of the respiratory centers; on the other hand, low doses might be inadequate to produce deep anaesthesia; supplementary doses, administered intraperitoneally are not under control; in some cases even after a 2nd or 3rd dose, the animal is not in the surgical plane, whereas in others, 1 supplementary dose may kill the animal.

The use of adjuvants has permitted to reduce the dose of the most commonly used anaesthetics. Maykut[1] has combined pethidine (meperidine) and pentobarbital; the potentiation produced made it possible to reduce the dose of pentobarbital. Chloral hydrate has also been used with pentobarbital; however, supplementary doses of chloral hydrate were usually needed when anaesthesia was not deep enough after 20 min[2]. Dolowy and Hesse[3] have used chlorpromazine as premedication in doses of 25 mg/kg followed by pentobarbital 30 mg/kg. Other authors[4] have associated pentobarbital and ethyl alcohol without achieving the desired potentiation.

The effect of diazepan as premedication followed by anaesthesia with pentobarbital was investigated in guinea-pigs.

Materials and methods. Guinea-pigs of either sex, weighing 250–900 g were used. The control group received only pentobarbital i.p., and the study group was firstly

[1] M. O. Maykut, Can. Anaesth. Soc. J. *2*, 161 (1958).
[2] E. S. Valenstein, J. exp. Analysis Behav. *4*, 6 (1961).
[3] W. Dolowy and A. L. Hesse, Illinois Vet. *3*, 112 (1960).
[4] E. U. Kinsey, J. Am. Pharm. Ass. *29*, 342 (1940).
[5] E. B. Carmichael and L. C. Posey, Proc. Soc. exp. Biol. Med. *30*, 1329 (1933).
[6] E. B. Carmichael and L. C. Posey, Anesth. Analg. curr. Res. *16*, 156 (1937).
[7] E. B. Carmichael and L. C. Posey, Anesth. Analg. curr. Res. *16*, 199 (1937).
[8] E. B. Carmichael, Anaesthesia *8*, 589 (1947).
[9] R. M. Hoar, Fedn Proc. *28*, 4 (1969).
[10] R. Kato, E. Chiesara and Vassanelli. Biochem. Pharmac. *12*, 357 (1965).

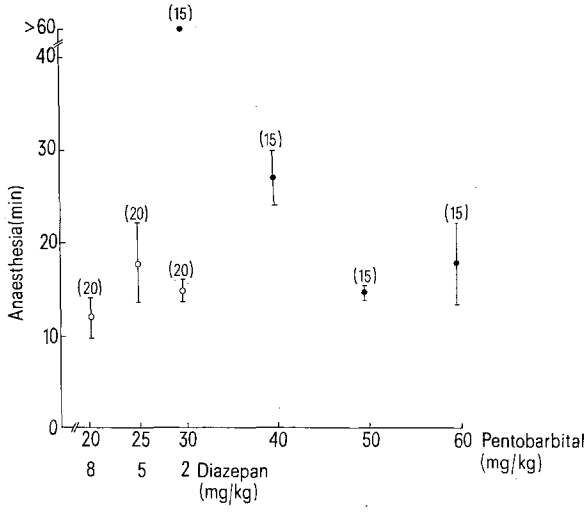

Fig. 1. Beginning of the surgical anaesthetic plane in guinea-pigs. Full circles, pentobarbital i.p.; open circles, diazepan i.m. followed 30 min later with pentobarbital i.p. In brackets, number of animals; vertical bars, standard deviation.

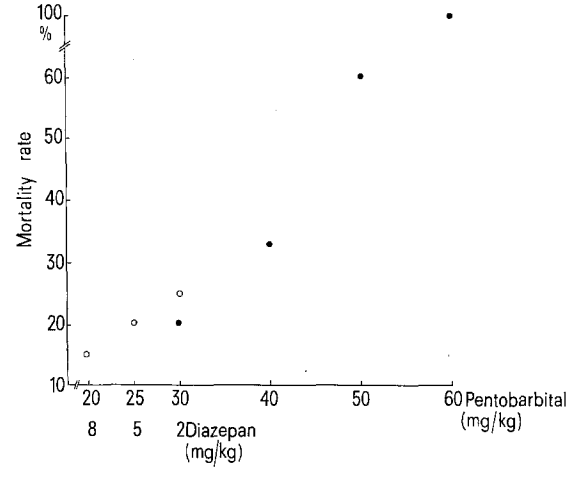

Fig. 2. Mortality rate (in percentage) of guinea-pigs. During and after the 1st h. Full circles, pentobarbital i.p.; open circles, diazepan i.m. followed 30 min later by pentobarbital i.p.

injected i.m. with diazepan and 30 min later received an i.p. injection of pentobarbital. The control group was divided in 4 subgroups of 15 guinea-pigs each, and each subgroup received 60, 50, 40 or 30 mg/kg pentobarbital. The study group was divided in 3 subgroups of 20 animals each; the 1st subgroup received 2 mg/kg diazepan and 30 mg/kg pentobarbital, the 2nd 5 mg/kg diazepan and 25 mg/kg pentobarbital and the 3rd 8 mg/kg and 20 mg/kg respectively.

The animals were carefully observed during 1 h and survival was evaluated after 16 h. The inability to stand on its legs was taken as first sign of anaesthesia, and it was considered that the animals were in surgical plane when they were deeply asleep and there was not withdrawal of the limb on application of a severe painful stimulus (forceful pinching of the toes).

Drugs used were: diazepan (Valium Roche) and sodium pentobarbital (Nembutal, Abbot).

Results. Control group. In the subgroup of guinea-pigs which received 60 mg/kg pentobarbital, the ability to stand on its legs was lost at 5.2 ± 1.2 min (mean ± standard deviation). The surgical plane was reached at 17.1 ± 11.0 min, and all the animals died within the 1st h. With 50 mg/kg, the guinea-pigs could not stand on their legs at 10.0 ± 6.2 min; at 14.7 ± 1.2 min, 53% were in the surgical plane, but the rest did not reach a deep anaesthetic plane within the 1st h; mortality in this subgroup was 60%: 40% during the 1st h, and 20% afterwards; 2 animals died inspite of being in a light plane of anaesthesia. The guinea-pigs which received 40 mg/kg pentobarbital fell off their legs at 10.6 ± 5.3 min; in 40% surgical anaesthesia began at 27.5 ± 7.1 min, the rest maintained the nociceptive reflex; 1 of those died after the 1st h, as well as another animal that was deeply anaesthetized; during the 1st h mortality was 20% and total death rate was 33%. With the dose of 30 mg/kg, 66% of the animals could not stand on their legs at 25.0 ± 11.0 min, the rest did not show any sign of anaesthesia; no animal in this subgroup reached the surgical plane, however 20% died, all after the 1st h.

Study group. The guinea-pigs which received 2 mg/kg diazepan and 30 mg/kg pentobarbital, could not stand on their legs at 3.0 ± 0.4 min; surgical anaesthesia was observed at 15.5 ± 2.7 min in 95% of the animals and lasted over 1 h; only 1 guinea-pig presented the nociceptive reflex; total mortality was 25%, 20% during the 1st h. When 5 mg/kg diazepan was used followed by 25 mg/kg pentobarbital, the guinea-pigs fell off their legs at 5.8 ± 2.8 min and 90% reached the surgical plane at 18.3 ± 9.4 min; the rest did not show deep anaesthesia; during the 1st h 10% of the animals died, and another 10% died afterwards. With 8 mg/kg diazepan and 20 mg/kg pentobarbital, the animals could not stand on their legs at 2.5 ± 0.6 min; 90% lost the nociceptive reflex at 13.3 ± 5.0 min, but 10% responded to painful stimuli; total mortality was 15%, 5% during the 1st h.

The association of 8 mg/kg diazepan and 20 mg/kg pentobarbital produced an earlier beginning of the anaesthesia (Figure 1) and the lowest mortality rate (Figure 2). The use of diazepan as adjuvant of the pentobarbital anaesthesia produces a longer surgically useful plane than that reported with chlorpromazine[3].

Resumen. Se estudia el uso de diazepán como premedicación en la anestesia con pentobarbital en cobayos. Los resultados se comparan con el uso de pentobarbital solo. Se observa que el plano de anestesia útil quirúrgicamente comienza antes y dura más. La premedicación con diazepán permite reducir las dosis de pentobarbital.

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PRO EXPERIMENTIS

A Direct Progesterone Radioimmunoassay

A radioimmunoassay for plasma progesterone without chromatographic purification has been developed. Other methods[1–4] have been reported previously which require some type of chromatography for purification of the extract and some are not as specific or as sensitive as the method described herein. A direct specific radioimmunoassay for progesterone was reported recently[5] in which high levels of progesterone in female rhesus monkeys were measured. However, the sensitivity of the method was unsatisfactory for measuring progesterone levels in humans. Other investigators have reported their preparations of antisera for different steroids conjugated to protein at different positions[6,7].

A simple radioimmunoassay method is described below in which ether extraction is the only purification step. This procedure can be used to assay a large number of samples with high sensitivity and specificity.

Materials and methods. Disposable glassware rinsed with a solution of methanol: methylene chloride 1:1 was used. The Al_2O_3 microcolumns and their purification have been previously described[8].

Preparation of the 11-BSA conjugate and production of antisera. The 11α-hydroxyprogesterone hemisuccinate was prepared by refluxing a mixture of 11α-hydroxyprogesterone and succinic anhydride for 15 h. The resulting precipitate was isolated and purified. Elemental analysis, IR-spectra and melting point confirmed the structure. The progesterone 11-albumin conjugate was prepared by the methodof ERLANGER[9].

Plasma extraction and radioimmunoassay. Plasma samples (0.05 ml from women in the luteal phase, 0.25 ml from women in the follicular phase, and 0.50 ml from men)

[1] A. RIONDEL, J. F. TAIT, S. A. S. TAIT, M. GUT and B. LITTLE, J. clin. Endocr. *25*, 229 (1965).
[2] B. L. RUBIN, M. MARALIT and J. H. KINARD, J. clin. Endocr. *31*, 511 (1970).
[3] G. E. ABRAHAM, R. S. SWERDLOFF, D. TULCHINSKY and W. D. ODELL, J. clin. endocr. Metab. *32*, 619 (1971).
[4] S. FURUYAMA and C. A. NUGENT, Steroids *17*, 663 (1971).
[5] J. M. SPIELER, R. L. WEBB, R. J. SALDARINI and J. A. COPPOLA, Steroids *19*, 751 (1972).
[6] H. R. LINDNER, E. PEREL, A. FRIEDLANDER and A. ZEITLIN, Steroids *19*, 357 (1972).
[7] A. R. MIDGLEY JR. and G. D. NISWENDER, Symposium presented at Karolinska on Research Methods in Reproductive Endocrinology, March 23–25, 1970.
[8] D. MAYES and C. A. NUGENT, J. clin. Endocr. *28*, 1169 (1968).
[9] B. F. ERLANGER, F. BOREK, S. M. BEISER and S. LIEBERMAN, J. biol. Chem. *234*, 1092 (1959).