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)

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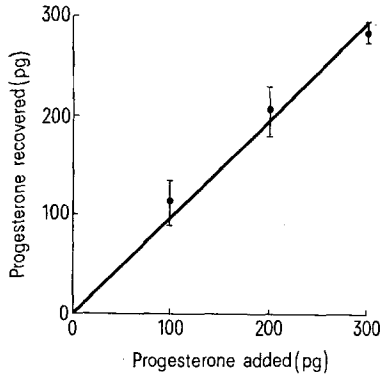

Fig. 1. Recovery of progesterone added to 0.1 ml of water (range). Correlation coefficient = 0.997 ± 0.056 (SE), $y = 1.05x - 7.96$; p 0.001.

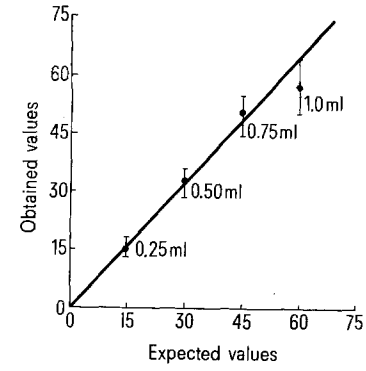

Fig. 2. Accuracy study. Values of progesterone expected and found when increasing aliquots of male plasma were assayed (range). Correlation coefficient = 0.990 ± 0.098 (SE), $y = 1.04x - 2.63$; p 0.005.

containing 12,500 dpm of 1,2-H³-progesterone were extracted with 10 volumes of anhydrous ether (Baker). The extracts were dried and dissolved in 3.4 ml of hexane. Column chromatography was used only for comparisons. The extract was mixed well and divided into three 1 ml portions (about 3,000 dpm each). Two aliquots were used for radioimmunoassay and 1 aliquot was used to check the recovery. For the standard curve, 8 standard solutions containing 0, 10, 25, 50, 75, 100, 200 and 500 pg of progesterone per 0.1 ml of absolute ethanol and 2,500 dpm of 1,2-H³-progesterone were assayed in triplicate. A semi-automatic air blowing decive[10] and a vacuum oven were used to dry the 1.0 ml volumes of hexane in the assay tubes. The standard and the sample tubes with diluted antiserum (1:3500) were incubated overnight at 4 °C. Cold saturated ammonium sulfate was used to separate free from bound progesterone. After centrifugation, the tubes were counted for 5 min in a scintillation counter (Nuclear Chicago).

Results and discussion. The recovery of labeled progesterone improved from 84.8 ± 8.8 (SD)% to 88.9 ± 10.3 (SD)% when column chromatography was omitted. The inter and intra assay precision was measured by assaying a pooled sample (female plasma, follicular phase, 93.3 ng/100 ml) the same day and on different days. On 6 duplicate determinations assayed on the same day, the coefficient of variation was 4.8%. On 24 determinations assayed on different days, the coefficient of variation was 10.9%.

The accuracy was checked by adding known amounts of progesterone to distilled water and calculating recovery. Results are shown in Figure 1. Increasing aliquots of pooled male plasma were assayed in triplicate to further check the accuracy of the method. Figure 2 shows the results.

At the 95% confidence limit, 25 pg was significantly different from 0 pg in every standard curve. The coefficient of variation of each standard curve point assayed in triplicate was always less than 6%. When columns were used, blank values (double distilled water) were 2.8 ± 1.1 pg per assay tube. When columns were omitted, blank values were always zero.

The specificity of our antibody was tested by its direct incubation with 27 other steroids. Percent cross-reaction was calculated as described by ABRAHAM[11], and compared to 27 steroids values previously reported by other authors[3–6].

LEYENDECKER et al.[12] reported difficulties with high blank values due to dried down solvents and materials used for extraction and chromatography of serum samples By the omission of microcolumns and care in separating the ether from the lower layer (water from blanks or plasma samples) the problem of eliminating positive water blank values was solved.

Our antibody binds progesterone with an affinity of about 1,000 times stronger than those present in the blood in significant concentrations such as cholesterol, cortisol, estradiol, pregnanediol, etc. The steroids 11α-hydroxyprogesterone (55%) and 4-pregnene, 3,11,20-trione (52%) displayed considerable cross-reaction, but this was expected since the protein molecule is attached at the 11 position of the hapten. However, these steroids are not present endogenously. The two other steroids that cross reacted to some extent, 5α- and 5β-pregnane, 3,20 dione (6.5% and 4.8%) are also not believed to be present in the blood[11]. Of the remaining naturally occurring steroids, deoxycorticosterone (2.3%), corticosterone (0.8%), and 17α-hydroxyprogesterone showed minor cross reaction. Other steroids tested showed no cross reaction (< 0.1%). The results show the high specificity of the antibody produced, allowing the assay of samples without column separation of the other steroids present in the ether extract with progesterone.

Résumé. Exposé d'une méthode de radioimmunoassai du progestérone dans le plasma sans purification chromatographique. Le 11-hémisuccinate de la progestérone a été joint au sérum bovin de l'albumine et injecté aux lapins pour stimuler la production d'anticorps. Des échantillons de plasma humain, extraits avec de l'éther, sont ajoutés à des quantités données de 1,2-H³-progestérone. Les résultats avec ou sans chromatographie sont comparables. La méthode est précise et très sensible pour mesurer les niveaux peu élevés de progestérone humaine.

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