for the metabolism of PCPE. If this is true, our experiments raise the questions as to whether the substance excreted in the urine and characterized as "pregnanediol" by the Klopper method is actually 5β-pregnan-3α, 20 α-diol or alternatively, whether the "pregnanediol" measured originates from PCPE or arises from endogenous sources under the influence of PCPE. To answer these points, the metabolism of isotopically labeled PCPE must be studied in animals as well as in humans.

Summary. Progesterone and its 3-cyclopentyl enol ether (PCPE) have been compared in a battery of biological tests. Orally or intraperitoneally administered PCPE was respectively 1/80th and 1/20th as active as subcutaneously injected progesterone in the Clauberg test. Oral doses of PCPE, 10-20 times those of subcutaneous progesterone were unable to maintain pregnancy in ovariectomized rats. Three to four times as much "pregnanediol" was detected in the urine of rabbits following oral administration of progesterone than a comparable dose of PCPE. Orally or intraperitoneally administered PCPE at doses 20-40 times those of effective doses of progesterone failed to potentiate the anesthetic effect of a subhypnotic dose of sodium hexobarbital. These data demonstrate that PCPE and progesterone have a markedly different biological profile and cast serious doubt on the possibility that biologically active amounts of PCPE are converted to and/or metabolized in the same way as progesterone, as claimed by others. Our data strongly support the view that if any transformation of PCPE to progesterone occurs, the likeliest site of this conversion is the gut.

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An Inexpensive and Simple Method for Preparing Chromosome Spreads.*† (29948)

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The extent of recent literature in cytogenetics conceals the fact that relatively few laboratories perform chromosome analyses as a routine procedure, and even fewer analyze populations of a size desirable for extraction of statistically valid information. A major deterrent is probably cost of time and money; and a minor one, possibly, the feeling that tissue culture techniques require highly specialized equipment and skill. In fact, the basic technique is quite straightforward and fully amenable, without loss of reliability, to simplification and economy. The method de-

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† The following materials have been found satisfactory: Preservative free heparin, Abbott Laboratories, North Chicago, Ill. Minimum Essential Medium (Eagle) for spinner cultures with added glutamine, Grand Island Biological Co., Grand Island, N. Y. Evacuated glass tubes, 100 x 16 mm with pink nontoxic rubber stoppers, Becton Dickinson & Co., Rutherford, N. J.

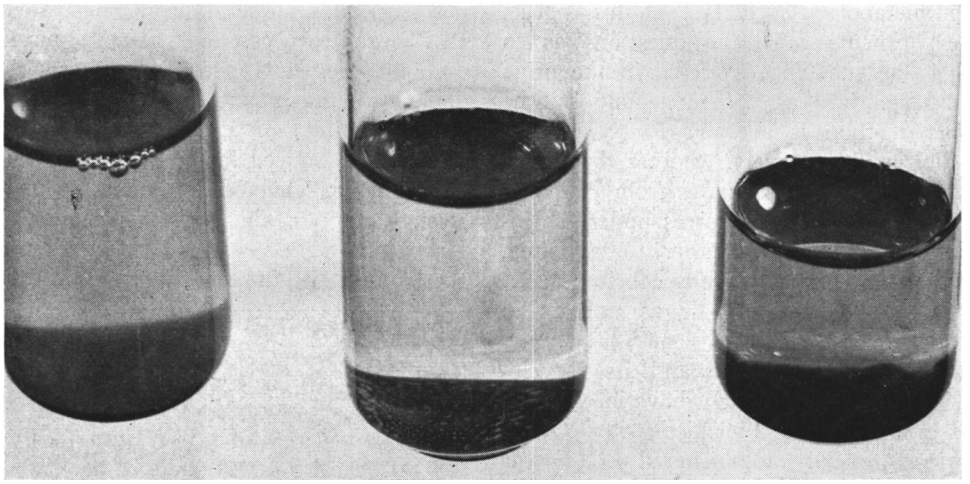

FIG. 1. Cultures (left) soon after inoculation, (center) after 3 days incubation at 37°C showing colonies of white cells growing on a base of sedimented red cells, and (right) after fixation in Carnoy's fluid.

scribed here permits efficient study of large numbers of individual specimens with a minimum of effort, materials and equipment.

Reagents. 1. Phytohaemagglutinin (PHA).

a. Wash red kidney beans (Phaseolus vulgaris) in water.

b. Grind in a pestle and extract overnight at 4°C in normal saline using 10 ml per gram of bean.

c. Centrifuge to clear the supernate, sterilize by filtration and store frozen.

2. Medium.

a. 80% Minimum Essential Medium Eagle (in its spinner formulation and with added glutamine) and 20% pooled human serum (outdated bank blood does very well).

b. Add 50 units per ml of preservative free heparin and 0.2 to 0.5 ml of PHA per 100 ml. The amount of PHA varies with the strain of Phaseolus used and the concentration of the extract and should be ascertained for each batch.

c. Dispense the medium with needle and syringe in 2 ml lots into nontoxic rubber stoppered, evacuated test tubes, and replace the vacuum with room or alveolar air using a needle and plastic tube containing cotton (those found in transfusion sets are quite satisfactory). Store frozen.

Preparation of chromosome spreads. 1. Inoculate each tube with 0.2 ml of venipuncture blood, or 10 drops from a skin prick, and incubate for 2-4 days until igloo-shaped colonies of white cells can be seen rising upon the sedimented red cells (Fig. 1). 2. Add 1 drop of a 25 mg/ml solution of colcemide 2 to 4 hours before harvesting and at harvest remove most of the supernatant medium with a Pasteur pipette, being careful not to suck away the white cells. 3. Suspend cells in 5 ml of 0.5% (50-60 milliosmolar) trisodium citrate for 20 minutes, centrifuge at 300 rpm for 5 minutes and remove the supernatant, again without disturbing the sediment. 4. Fix by wetting down the sides of the tube with freshly prepared Carnoy's fluid (2 parts absolute ethyl alcohol and 1 part glacial acetic acid) until the swollen and fragile cells lie undisturbed under 1 to 2 ml of fixative. 5. When the hemoglobin turns brown, remove half the fixative, replace with fresh Carnoy's, and leave overnight at 4°C. 6. Remove as much fixative as possible and gently suspend the cells in 1 ml of fresh fixative. 7. Place 3 drops of the suspension on a clean slide brimming with 20% ethyl alcohol, and blaze dry by passing through a flame. 8. Stain in 2% safranine or any basic dye.

Note that the balanced salt solution contains no calcium or magnesium: this aids dispersion of dividing cells during swelling and fixing. No antibiotics are needed if ordinary bacteriologic precautions are taken when inoculating. The heparin ensures collection of

skin prick blood without clotting. The gaseous phase is not disturbed by removal of the stopper when collecting finger prick blood and in any case is more important in maintaining the pH of unused media than that of inoculated cultures, which are rapidly conditioned upon addition of the whole blood.

The inoculated culture may be left up to 48 hours at 4°C before incubating. Storage at room temperature before incubation slows but does not halt cell division so that harvesting should take place 2 to 4 days after inoculating rather than after incubation at 37°C. During the 20 minutes of treatment with citrate, the swollen cells sediment by gravity and the subsequent centrifugation is meant only to pack them slightly in order to ease the removal of hypotonic fluid without disturbance. Prolonging the centrifugation will not increase the yield of cells. Cloudiness in the supernate can be disregarded. If necessary the cells may be left in fixative for days before preparation of slides.

The virtues of this method are, first, the use of a minimum of materials and equipment. Most of the latter is already in standard hospital use and only one tube is used throughout. Second, the method of collection and culture is flexible, being equally suitable for infants, children and adults, as well as for experimental animals, both in and out of hospital, times of inoculation and incubation being adjusted to suit circumstances. Third, the combination of economy and flexibility means that the fundamental information to be gained from population, as opposed to case, studies should now be available without crippling expense, especially if viewed in combination with computer techniques for karyotypic analysis(1).

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Beta-Hydrazinopropionitriles: A New Series of Osteolathyrogenic Agents for the Rat.* (29949)

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In the course of screening a variety of different types of compounds for osteolathyrogenic activity in the weanling rat, we found that β-hydrazinopropionitrile (BHPN), 2-cyanoethylhydrazine, caused lathyritic skeletal changes similar to those produced by β-aminopropionitrile (BAPN). In an attempt to relate specific details of structure to lathyrogenicity, we have also studied the lathyrogenic actions of a few methyl-substituted derivatives of BHPN.

Methods. Milled Rockland Rat Stock Diet served as the basal diet. To this were added varying amounts of the BHPN under test. Male Holtzman rats weighing 55 to 65 g were fed these diets and examined after varying periods for skeletal abnormalities and aortic damage. In addition, hydroxyproline levels were determined in saline-soluble and insoluble collagen fractions of sponge biopsies from adult rats which were fed these substances and pair-fed with normal controls. For the latter determinations, polyvinyl surgical sponges were implanted subcutaneously as previously described(1). In some instances the animals were pre-fed the experimental diets one day before sponge implantation and sacrificed at the indicated intervals; in more recent trials sponges were implanted into normal rats, experimental diets were started 19 days after implantation and the animals were sacrificed 10 days later. Similar results were obtained by both methods. After the sponges were removed, they were frozen solid. They were then homogenized in 2 ml of water containing one drop of octanol-1 by means of a high-speed Virtis "45" homogenizer using 3 micro blades on a micro aerosol-free assembly

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