tained on chow or horse meat were injected twice daily with Armour's‡ growth hormone or ACTH. A total of 1 mg per rat per day was given for 14 days, after which the rats were sacrificed for a determination of liver xanthine oxidase. The results are shown in the first two experiments of Table II. Neither the growth hormone nor the ACTH had any effect on liver xanthine oxidase. The endogenous respiration of the livers from the rats treated with growth hormone or ACTH was the same as that for the controls. Horse meat gave significantly higher levels of activity than chow. The growth hormone experiment was repeated in female albino rats, and the results, Expt. 3 of Table II, again showed no effect. Methylene blue added to the Warburg flasks gave the same average 1.54 increase in xanthine oxidase activity in the rats treated with growth hormone as was obtained in the controls.

‡ We are indebted to Dr. I. M. Bunding of Armour and Co., for the ACTH and growth hormone.

Summary. The Qo_2 of a liver slice, in contrast to an homogenate, was relatively independent of the xanthine oxidase activity of the liver, since the latter could be varied widely by dietary procedures without affecting the former.

Thyroxine increased the liver slice Qo_2 of rats fed stock chow, a purified 21% casein diet, or a purified 8% casein diet; no hyperthyroidal exhaustion of the liver Qo_2 could be produced.

Thyroxine had no effect on liver xanthine oxidase when rats were fed a purified 21% casein diet, but tended to give higher values with chow-fed adult rats. When administered with a low protein diet, thyroxine prevented the marked depletion of liver xanthine oxidase and gave results more characteristic of starvation than of a low protein diet.

Growth hormone and ACTH had no effect on liver xanthine oxidase.

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Effect of Particle Size of Suspensions of Estrogen Crystals on Duration of Estrus. (17750)

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The implantation of pellets of all hormonal steroids has become a common practice both in the laboratory and in the clinic since the demonstration by Deanesly and Parkes(1,2) that dry crystals or compressed pellets of androgens and estrogens when implanted, acted like a reservoir of the steroid and produced a prolonged physiological effect. Between solutions of steroids in water or oil and the pellets weighing several milligrams, lies a region of particle size which has been little investigated in a systematic manner. Richards, Spruth and Russell(3) showed that a single injection of 500 μg (5,000 I. U.) of an aqueous suspension of small crystals of estrone that would pass a 26 gauge needle, resulting in an average duration of estrus 15-23.5 days in four different groups of castrate rats. The same amount of estrone in oil solution resulted in estrus lasting only 2 days. Recently this report has been confirmed by Simond *et al.*(4) but only high dosage levels of 5,000 to 20,000 I.U. were used.

1. Deanesly, R., and Parkes, A. S., *Proc. Royal Soc. B*, 1937, v124, 279.

2. Deanesly, R., and Parkes, A. S., *Lancet*, 1938, v235, 606.

3. Richards, R. K., Spruth, H. C., and Russell, M. K., PROC. SOC. EXP. BIOL. AND MED., 1941, v48, 400.

4. Simond, A., Lindquist, K. M., Tendick, F. H., and Rowe, L. W., *J. Am. Pharm. Assn.*, 1950, v39, 52.

Miescher, Gasche and Frey(5) have studied the duration of effectiveness of crystalline suspensions of differently sized particles of α estradiol, α estradiol dipropionate, α estradiol monobenzoate and estrone but used only the one dosage level of 500 mcg. They also carried out some studies with progesterone. Meier,Gasche and Frey(6) conducted similar studies using desoxycorticosterone and found the duration of effectiveness to be proportional to dosage up to a single injection of 40 mg. They found crystalline suspensions to be about 10 times as effective as oil solutions. Prolongation of estrogenic activity in rats is also well established for certain esters of estradiol even though administered in oil solution(7). Because the clinical use of aqueous suspensions of estrogens has become extensive, further experimental studies on the quantitative aspects of dosage and particle size seem to be indicated.

We have studied the effect of variable amounts of estrone of certain particle sizes on duration of physiological activity using both estrus in adult castrate rats and uterine weight in castrate immature female rats as indicators.

Materials and methods. The estrogen used was a crystalline ketonic preparation from pregnant mares' urine consisting mainly of estrone. The crystals were screened through standard 60, 100, 200 and 325 mesh screens. Particles that passed through a 60 mesh screen and not through a 100 mesh screen were designated as 60 mesh size. Those which passed through a 100 mesh and not a 200 mesh screen were designated as 100 mesh size, and so on. A corn oil solution of the same crystalline estrogen was also prepared. The crystals were suspended in a medium containing 0.5% sodium carboxymethylcellulose (CMC)* and 0.125% polyoxyalkylene sorbitan oleate (Tween-80).† The CMC makes the solutions sufficiently viscous to prevent rapid settling of the crystals. The Tween-80 helps to wet the steroid crystals so that they suspend readily. Adult ovariectomized rats, which had been primed with estrogens, and which were in condition to use for a routine estrogen assay were used in most of the tests.

The experimental procedure was to give subcutaneously, using a 22 gauge needle, a single injection of 0.1 to 0.5 ml of the aqueous estrogen suspension under test. Vaginal smears were taken, except on Saturday and Sunday. In order to provide continuous figures on the chart, half of each group was started on a Friday and the other half on a Monday so that all days would be covered by a portion of the rats. As a result, 4 days out of each 7 are represented by only half as many rats as the other 3 days. This gives rise to an increase in percentage estrus at some points along the curve. This increase is due to the use of averaged values from the two different groups and is not due to some of the rats going out of estrus and then coming back into estrus again. One series of experiments was carried out using rats ovariectomized when they were 21 days of age. After a recovery period of 2 weeks, these animals were injected without priming. Smears were taken at intervals. Groups of 4 rats were killed and the uteri excised and weighed.

Experimental. Duration of Estrus with Oil Solutions of Estrone. The cellular changes in the vagina which produce what is known as an estrus smear, require a period of about 36 hours to develop even with high dosage of estrogens. Hence, no rats were in estrus one day after injection. Using standard bioassay procedures, an oil or aqueous solution containing about 15 I.U. of estrone will produce estrus in 50% of a group of adult castrate rats about 36-48 hours after injection. Approximately 50 I.U. (5 mcg) will produce estrus in 100% of the animals. The duration of estrus in both cases is only about 24 hours or until 3 days after the initial injection.

As a basis for comparison between oil solutions and suspensions there are plotted in Fig. 2 and 3 the averaged data obtained with several groups of rats when given a single injection of an oil solution containing 10,000 I.U. (1 mg) of the crystals. About

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6. Meier, R., Gasche, P., and Frey, H., *Schweiz. Med. Woch.,* 1946, v76, 107.

7. Pedersen-Bjergaard, K., and Tonnesen, M., *Acta Endocrinologica,* 1948, v1, 350.

* Hercules Powder Co., Wilmington, Del.

† Atlas Powder Co., Wilmington, Del.

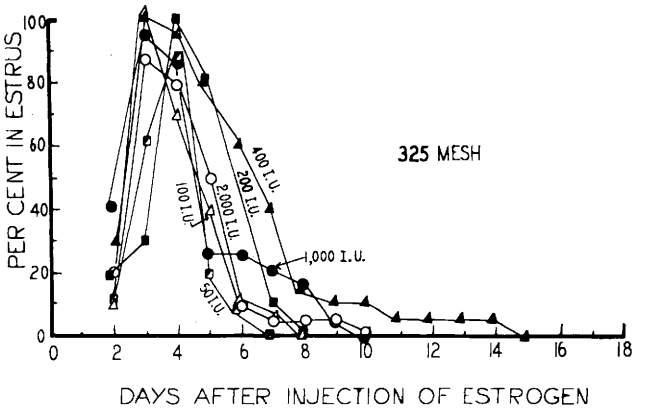

FIG. 1.

Duration of estrus in adult ovariectomized rats as a function of the dosage of estrogens. A single injection of an aqueous suspension of crystals of 325 mesh size was given. 20 rats were used on each dosage level.

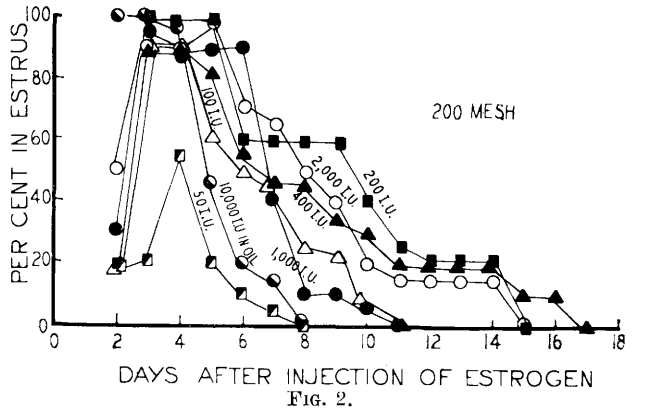

FIG. 2.

Duration of estrus in adult ovariectomized rats as a function of the dosage of estrogens. A single injection of an aqueous suspension of crystals of 200 mesh size was given. 20 rats were used on each dosage level.

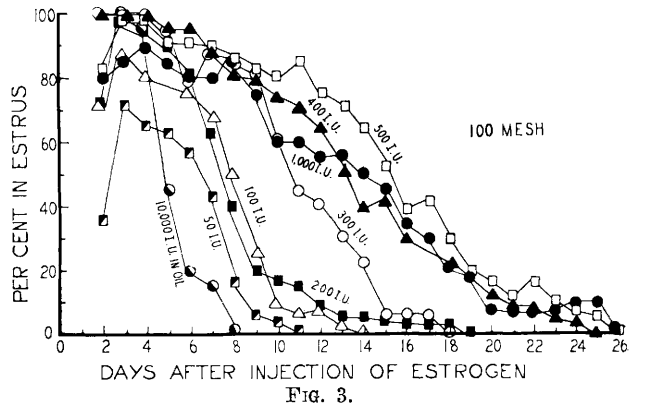

FIG. 3.

Duration of estrus in adult ovariectomized rats as a function of the dosage of estrogens. A single injection of an aqueous suspension of crystals of 100 mesh size was given. 60 rats were used on each dosage level.

50% of the rats were still in estrus on the fifth day, or for a period of time which is only two days longer than with 50 I.U. in oil.

Effect of Variable Dosage on the Duration of Activity of Aqueous Suspensions. The effect of increasing the dosage of aqueous suspensions of estrogens of different particle size is shown in Fig. 1, 2 and 3. Several interesting points are demonstrated in these figures.

1) In most cases in Fig. 1, 2 and 3 it is seen that, with suspensions, many of the rats do not go into estrus until after 3-4 days especially at the lower dosage levels. This is unlike the uniform 36 to 48 hour period to reach estrus which is found after injection of oil solutions. This indicates that the solubility of the crystalline particles is such that in some rats a threshold concentration in the blood is not reached until 24-48 hours after injection, *i.e.*, about 36 hours before a positive estrus smear was found. This threshold value was never reached in the case of some rats especially those receiving only 50 I.U. or 100 I.U. of the crystals.

2) With particles of 100 mesh size (Fig. 3) the duration of estrus increases with increasing dosage up to a dosage of 400 or 500 I.U. Above these concentrations the duration of estrus was not lengthened appreciably by increasing the dosage. Unplotted data on an additional group of 20 rats receiving 2,000 I.U. showed results similar to those plotted for 400 I.U.

3) It can be seen in Fig. 3 that 50 I. U. (5 mcg) injected as a suspension of 100 mesh particles was more effective in most of the rats than 10,000 I. U. (1,000 mcg) injected in an oil solution. This indicates an increase of the order of 200 times in the efficiency of utilization of the estrogen in the case of the suspension relative to an oil solution.

4) With 200 mesh particles (Fig. 2) usually a shorter duration of estrus was found than with 100 mesh particles. A dosage of 200 I.U. of 200 mesh crystals was found to be as effective as 2,000 I.U. of the same but was superior to 100 I.U. and 50 I.U. A dosage of 100 I.U. of particles of 200 mesh size was found to be more effective than 10,000 I. U. in oil.

5) With the 325 mesh particles (Fig. 1) the duration of effectiveness was considerably reduced from that found with larger particles and did not differ much with respect to dosage levels. Again, as little as 50-100 I.U. of the suspensions were found to be as effective as 10,000 I.U. in oil. However, 2,000 I.U. of the suspension were no more effective. This indicates the important role that particle size plays in controlling the duration of activity with these suspensions. Particle size was more important than the total dosage in determining the duration of action above a dosage level of 100 units.

Effect of Particle Size on Duration of Estrus. Data on the effectiveness of suspensions of different particle size at a selected dosage level upon the duration of estrus is presented graphically in Fig. 4. The greater duration of estrus as the particle size increases is obvious.

In Table I is shown some of the pertinent data relative to the particle size obtained with screens of the mesh size used. There is a better correlation between the duration of estrus and the linear size of the sieve opening than with the square or the cube of the sieve opening. Since estrone crystallizes as flat plates it is probable that the proportionality found is actually due to the smallest dimension of the crystals which is the thickness of the plates. The thickness of the particles could not be measured but it is probably proportional to the mesh size.

Note that single particles of 100 mesh size might weigh 1 to 3 μg and of 60 mesh size might weigh 10-15 μg. Hence only a few particles are required to make 100 I.U.

Effect of Particle Size on the Duration of Estrus in Immature Ovariectomized Rats. Estrus smears show only that sufficient estrogen is present to produce a positive vaginal smear and in no way indicate how much estrogen is present in excess as uterine weight would. Ovariectomized immature rats were therefore, given single injections of suspensions as indicated in Fig. 5 and were killed in groups of four. The uteri were excised and weighed. As with the estrus smears in the adult castrate rats it is apparent that 100 mesh particles have a longer duration of effect than 325 mesh particles. The duration of effectiveness of the 100 mesh particles is 14 days, a

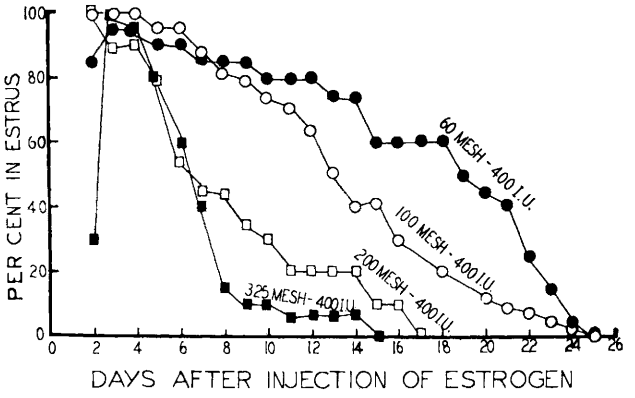

FIG. 4.

Duration of estrus in adult ovariectomized rats as a function of particle size at a dosage level of 400 I.U. (40 mcg). 40 rats were used in the 60 mesh-400 I.U. experiment. The other curves also appear in Fig. 1, 2 and 3.

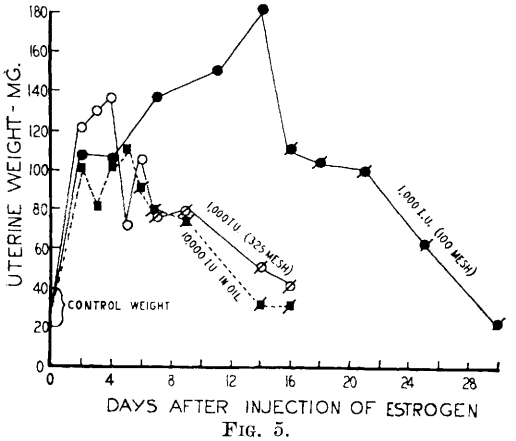

FIG. 5.

Uterine weight increase in immature ovariectomized rats as a function of time after a single injection of estrogen of different particle sizes. A bar through the point indicates all rats out of estrus. Each point represents average obtained on 4 rats.

period which agrees with the data obtained with estrus smears on adult ovariectomized rats. The duration of effectiveness of the oil solution (10,000 I.U.) and the 325 mesh particles (1,000 I.U.) is approximately 5-6 days, a period which is also similar to that obtained in the adult ovariectomized rats. The superiority of the larger sized particles is again obvious and the increase of effectiveness of 10-100 times is also confirmed.

The uterine weight appeared to increase as long as an effective concentration of estrogen was present. There appeared to be a rapid decrease in uterine weight immediately after the estrogen stimulus ceased. Concomitant with this sudden drop in weight, negative estrus smears were obtained. Atrophy of the anestrus uterus then proceeded more slowly during a period of about 2 weeks back to a control weight level.

Summary. The duration of effectiveness of a single injection of aqueous suspensions of a crystalline ketonic estrogen preparation of 60, 100, 200 and 325 mesh particle size was

TABLE I.

Showing the Correlation Between Particle Size and Duration of Estrus.

Sieve No. (mesh/inch)	Sieve opening (μ)	Area of sieve opening (μ)^2	Max. wt of particles* (μg)	Duration of estrus† (days)
60	250	62,500	15.6	19
100	149	22,208	3.3	14
200	74	5,476	0.4	8
325	44	1,936	0.08	5–6

* Wt calculated on basis of a cube of the dimensions of the sieve opening and a density of 1.

† Approximately the day on which 50% of the group of rats were out of estrus with 400 to 2,000 I.U.

compared with that of an oil solution of the same preparation. The dosages of the suspensions were varied from 50 I.U. to 2,000 I.U. Uterine weight or estrus smear were used as the criteria of effectiveness of the estrogens. 800 rats were used in the vaginal smear studies.

With the larger sized particles (60 and 100 mesh) 100 I.U. were as effective as 10,000 I.U. in oil solution. When the dosage level of 400 I.U. was used, estrus was maintained in 50% of the rats for 19 days with 60 mesh particles, 14 days with 100 mesh particles, 8 days with 200 mesh particles and 5-6 days with 325 mesh particles. Further increase in dosage levels up to 2,000 I.U. did not increase the duration of the estrus. This indicates the primary importance of particle size with this estrogen in controlling the duration of its effectiveness.

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Metabolism of Various 8-Aminoquinolines in Rhesus Monkey.* (17751)

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The following observations on metabolic transformations of various 8-aminoquinoline derivatives were an unexpected outgrowth of routine studies on the subacute toxicity of 2-methoxy -8- (3-aminopropylamino) -quinoline (CN 1100) for the rhesus monkey. These studies showed that, as compared with some 200 other 8-aminoquinolines which had been examined previously(1,2), CN 1100 had remarkably low toxicity, producing no untoward reactions when administered orally in repeated daily doses as large as 384 mg base per kg body weight.† Since the concentrations of this quinoline in the plasma were unusually low, even at doses as large as the above, and since less than 0.5% of the administered drug could be recovered from the urine, the question was raised whether the lack of toxicity of CN 1100 merely reflected poor absorption from the gastrointestinal tract. This possibility led to a study of the toxicity of CN 1100 administered parenterally. It was found that the drug had little toxicity when given intramuscularly in single doses as large as 48 mg per kg, suggesting that the low toxicity observed previously was a real characteristic of CN 1100 rather than an artifact resulting from defective absorption.

As part of the above study on parenteral toxicity, measurements were made of the rate of disappearance of CN 1100 from the plasma. The 8-aminoquinoline concentrations were determined by the method of Brodie(3) which employs primary extraction of the quinoline with an organic solvent, followed by re-extraction and coupling of the drug with diazotized sulfanilic acid to yield a colored complex. In anticipation of the results to be obtained by this procedure, one of us (HBH) added diazotized sulfanilic acid directly to diluted samples of plasma and noted the development of colors which appeared far more intense than those obtained later in the analysis of the ethylene dichloride extracts. This impression was confirmed by quantitative studies which showed clearly that the plasma of monkeys receiving CN 1100 contained considerable quantities of an aqueous alkali-soluble, ethylene dichloride-insolu-

* This study was supported in part by grants-in-aid from the National Foundation for Infantile Paralysis and from the Division of Research Grants and Fellowships, National Institutes of Health, United States Public Health Service.

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2. Unpublished observations, this laboratory.

† Limitations in the supply of CN 1100 precluded studies at higher doses.