On the other hand, the present experiments do not favor any one hypothesis for the mechanism of vascular clamping: a) hypoxia b) acidosis c) accumulation of toxic metabolities e) formation of new breakdown products under extreme conditions. They do suggest that the clamping procedure has a primary metabolic effect, since reduction of metabolic needs is protective.

Summary. Vascular clamping of the pregnant rat uterus results in congenital malformations, fetal growth retardation, and embryonic death. Varying the temperature of the clamped uterus can modify the effects of the clamping procedure. Two and one-half hours of clamping the 8 day pregnant uterus maintained at 35°C resulted in fetal mortality of 100%. With the same clamping procedure and a uterine temperature of 4°C the fetal mortality was 16.7%. Weight reduction and malformation rates were also reduced by lowering the temperature of the clamped horn. These results substantiate the concept that uterine vascular clamping results in congenital malformations *via* a disturbance in some metabolic pathway. They also show that the uterine vascular clamping can be utilized to isolate one uterine horn for extended periods for the study of fetal drug effects and maternal fetal relationships.

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Received September 6, 1966. P.S.E.B.M., 1967, v124.

Comparison of the Effect of Hypothalamic and Pituitary Implants of Estrogen and Testosterone on Reproductive System and Adrenal of Female Rats. (31718)

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In an earlier report(1), we were able to demonstrate that implants of either testosterone or estradiol in the median eminence (ME) could decrease the weights of the accessory sex organs in the male rat. These same implants frequently evoked adrenal enlargement and the implants of estradiol produced an enlargement of the anterior pituitary gland. Some of these effects could also be obtained by implantation of the steroids in the anterior pituitary itself or in the mammillary bodies, but implants in other hypothalamic areas were usually ineffective. The conclusion was drawn that these gonadal steroids acted locally in the hypothalamo-hypophyseal region to inhibit gonadotrophin secretion and to stimulate the release of ACTH. It appeared of interest to determine if similar relationships existed in the female, and the present report describes the results of experiments in which implants of estradiol or testosterone were located in various hypothalamic areas or in the pituitary gland of female rats.

Methods. The experimental animals in this study were female, Sherman rats which weighed 220-300 g. The animals were divided into 3 groups. The first was a group of normal controls sacrificed at 4 stages of the cycle. The other 2 groups of rats were implanted in various loci of the hypothalamo-pituitary region with either gonadal steroids or cholesterol to serve as a control on the possible non-specific effects of the implantation procedure and the implants themselves. For each group implanted with testosterone or estradiol-17B, a group of animals from the same colony was implanted on the same day

with cholesterol at the same locus.

The implants of testosterone were prepared by tamping small quantities of crystalline hormones or cholesterol into 20 gauge stainless steel tubing. A thin film of saturated solution of sucrose was then applied to the tip of the tube, which on drying provided a protective layer around the crystalline material. The desired dose of estradiol was achieved by mixing equal parts of crystalline cholesterol with the hormone. The mixture was brought carefully to its melting point, and the 27 gauge tubing used in this case was dipped into the molten mixture. Any hormone adhering to the outside of the tube was removed under direct vision with a disecting microscope. This was accomplished by first scraping visible material off the tubing with a spatula and then washing the outside of the tube with absolute alcohol.

While lightly anesthetized with ether, the rat was mounted in a Krieg-Johnson stereotaxic instrument, and single midline or bilateral implants were located in the appropriate position and fastened in place by means of dental cement and special screws which were attached to the skull. Body weight of the animal was recorded at this time. Each rat was caged separately in a constant temperature room (23-24°) and fed Purina© chow *ad libitum*. The lights were on from 7 A.M—7 P.M.

Prior to the implantation, the estrous cycle was followed in each rat for at least 3 consecutive cycles and only those which had normal cycles were included. After implantation the cycle was followed until sacrifice. If the rats were cycling, they were sacrificed on the second day of diestrus between 10 A.M.-4 P.M. Only those rats which had not lost weight were used.

The base of the brain and pituitary gland was carefully inspected at autopsy and only those rats in which the exact spot of implantation could be located were included. Weights of anterior pituitary (A pit), adrenals (Adr), ovaries (Ov), uterus (Ut), and thyroid (Thy) were determined after squeezing to remove secretions and blotting to remove excess moisture. The animals were carefully inspected for mammary gland changes. Target glands were examined histologically after staining with hematoxylin and eosin.

Results. Normal controls. Cyclic variations in organ weight were observed (Table I). Uterine weight reached a maximum at proestrus and was at a minimum on the first day of diestrus. This difference in weight was significant statistically ($P<.01$). Anterior pituitary weight was minimal on the first day of diestrus ($P<.02$), but this change may be a reflection of the smaller size of the rats in this group. Adrenal weight was minimal at late diestrus and this change was significant ($P<.05$) on comparison to rats at estrus. No other significant alterations in glandular weight were observed, but because of the fluctuations in uterine weight and the tendency of other weights to vary, it was considered essential to sacrifice all animals with implants which were cycling on the second day of diestrus.

Cholesterol implants. Since the cholesterol implants were used as controls for the nonspecific effects of the implantation procedure and the implants themselves, it is important to examine the results obtained with cholesterol before considering those obtained with gonadal steroids. In some cases the animals with cholesterol implants failed to cycle (Table I and III); however, the majority continued to cycle normally. Of a total of 157 rats with cholesterol implants in various loci, 115 continued to cycle. There was little or no evidence of mammary development in these rats and uterine and ovarian weights were normal, except in two of 7 groups of rats with cholesterol implanted in the ME in which uterine weight was slightly decreased ($P<.05$). Anterior hypophyseal weight was reduced in a few groups and, surprisingly, thyroidal weight was inconstantly increased. Adrenal weight increased significantly in some experiments.

Estradiol implants. These were located in various hypothalamic areas and in the anterior pituitary. Implants of estradiol in the lateral (LH) or anterior hypothalamus (AH) resulted in little alteration on comparison to implants of cholesterol (Table I). Slightly less than half of the rats continued to

TABLE I. Effects of Implants of Estrogen on Organ Weights of Female Rats.

Exp No.	No. of rats	Implant	Locus of implant	Stage of cycle	No. cycling/Total No.	Mammary development	Body wt (g)	Organ wt (mg)				
								Ovaries	Uteri	Adrenals	Thyroid	Ant. pit.
1	10	None	None	D1[9]	10/10	—[1]	214 ± 2[2]	68 ± 3.9	315 ± 19	56 ± 2.2	12 ± .8	9 ± 1
	10	"	"	D2	10/10	—	235 ± 6	60 ± 3.2	394 ± 19	52 ± 2.5	13 ± .5	12 ± .5
	10	"	"	P	10/10	—	231 ± 6	65 ± 1	434 ± 30	55 ± 2.4	13 ± .7	12 ± .6
	10	"	"	E	10/10	—	226 ± 7	67 ± 3	402 ± 18	63 ± 4	12 ± .4	12 ± .2
2	8	Chol.	L.H.[3]	D	6/8	—	215 ± 6	66 ± 5	443 ± 12	62 ± 4	17 ± .5	13 ± .7
	8	Est.[4]	L.H.	D	3/8	±	215 ± 9	63 ± 3	398 ± 18*	65 ± 3	19 ± .7	16 ± 1.2
3	7	Chol.	A.H.[5]	D	2/7	—	232 ±16	68 ± 2.6	393 ± 46	61 ± 2.5	15 ± .9	13 ± .6
	7	Est.	A.H.	D	3/7	±	222 ± 7	67 ± 1.2	432 ± 52	60 ± 3	15 ± 1.2	13 ± .8
4	8	Chol.	M.E.[6]	D	6/8	—	229 ± 5	54 ± 4.2	308 ± 32	53 ± 1.8	12 ± 1.1	9 ± .4
	14	Est.	M.E.	D	0/14	+	230 ± 6	48 ± 2	292 ± 41	53 ± 2.2	11 ± .5	13 ± .6†
	10	Chol.	M.E.	D	5/10	—		70 ± 4	459 ± 35	57 ± 2.6	15 ± 1	12 ± .9
	10	Est.	M.E.	D	0/10	+		55 ± 3 †	385 ± 21	64 ± 4.5	17 ± .6	15 ± 2 †
	11	Chol.	M.E.	D	10/11	—	253 ± 6	63 ± 3.6	371 ± 22	51 ± 1.5	14 ± .6	8 ± 1.1
	14	Est.	M.E.	D	0/14	+	266 ± 9	51 ± 2.3†	325 ± 32	57 ± 3.6	13 ± .8	13 ± 1.2†
	13	Chol.	M.E.	D	10/13	—	231 ± 5	56 ± 3.4	351 ± 8	52 ± 3.3	11 ± .6	9 ± .3
	9	Est.	M.E.	D	0/9	+	233 ± 6	50 ± 2.6	303 ± 41	53 ± 2.4	10 ± .4	15 ± 1.3†
	11	Chol.	M.E.	D	7/11	—	244 ± 4	56 ± 3.1	349 ± 20	50 ± 1.9	13 ± .8	10 ± .4
	10	Est.	M.E.	D	0/10	+	281 ± 6	52 ± 2.3	260 ± 19*	53 ± 3.2	10 ± .4	14 ± 1.7†
5	6	Chol.	M.B.[7]	D	3/6	—	257 ± 5	72 ± 5	365 ± 31	63 ± 3	12 ± 2	13 ± .9
	10	Est.	M.B.	D	0/10	+	256 ± 6	58 ± 3 *	348 ± 19	69 ± 3	13 ± 3	16 ± 1.4
6	8	Chol.	A.Pit.[8]	D	1/8	—	244 ±19	67 ± 4	363 ± 16	59 ± 3	16 ± 1	12 ± 1.4
	9	Est.	A.Pit.	D	0/9	+	226 ± 7	58 ± 3 *	307 ± 19*	64 ± 4	17 ± 1	14 ± .8
	13	Chol.	A.Pit.	D	10/13	—	230 ± 5	65 ± 3.2	385 ± 18	63 ± 2.9	16 ± .4	13 ± .4
	11	Est.	A.Pit.	D	0/11	+	253 ± 9	63 ± 3.7	376 ± 23	69 ± 3.4	17 ± .9	16 ± .8†
	13	Chol.	A.Pit.	D	13/13	—	231 ± 5	56 ± 3.4	351 ± 8	52 ± 3.3	11 ± .6	9 ± .3
	9	Est.	A.Pit.	D	0/9	+	233 ± 6	50 ± 2.6	303 ± 41*	53 ± 2.4	10 ± .4	15 ± 1.3†
	10	Chol.	A.Pit.	D	9/10	—	245 ± 7	63 ± 3.4	381 ± 28	55 ± 2.7	14 ± 1.5	10 ± .5
	9	Est.	A.Pit.	D	0/9	+	248 ± 8	49 ± 3.7*	324 ± 3.1*	48 ± 2.8	13 ± .8	13 ± .6†

[1] — = no mammary gland development; + = mammary gland development with or without secretion. [2] Mean ± SEM. [3] L.H. = lateral hypothalamus. [4] The estradiol implants were composed of equal parts of estradiol and cholesterol. [5] A.H. = anterior hypothalamus. [6] M.E. = median eminence. [7] M.B. = mammillary bodies. [8] A.P. = anterior pituitary. [9] D1 = diestrus, 1st day; D2 = diestrus, 2nd day; P = proestrus; E = estrus; D = persistent diestrus.

* $P < .05$ in comparison to cholesterol-implanted control.

† $P < .01$ in comparison to cholesterol-implanted control.

cycle normally and there was no evidence of mammary gland development. Organ weights were not altered.

Estradiol implants were located in the ME in 5 separate experiments and the rats were sacrificed 9 to 33 days after implantation. In all these experiments a pseudopregnancy-like syndrome developed as expressed by constant diestrus, mammary gland development and increase in anterior pituitary weight. All of these 57 rats developed constant diestrus whereas 38 of 54 animals with cholesterol implanted in the ME continued to cycle. Anterior pituitary weight was increased significantly by the estradiol in this locus in every group. Uterine weight was reduced in all groups and the change was significant in one of the 5. Ovarian weight was decreased significantly in 2 of 5 groups on comparison with the cholesterol-implanted controls. The decreases in ovarian and uterine weight bore no relationship to the time which had elapsed since implantation. There were no significant alterations in adrenal or thyroid weights.

In a single group of rats with implants in the mammillary bodies (MB), the findings were similar to those seen in the groups with ME implants, that is, a pseudo-pregnancy-like syndrome was induced. There was only borderline hypophyseal enlargement. Ovaries, but not uteri, were reduced in size in comparison to the cholesterol-implanted controls.

Anterior pituitary implants (AP) were also performed to determine if the steroid could directly alter adenohypophyseal function. Three separate experiments were performed. The implants were in place 13-33 days. Results were exactly similar to those obtained with placement of the steroid in the ME.

Systemic injection of estradiol. To compare the effects of implanted estrogen with that systemically administered, estradiol benzoate in oil (0.1 ml) was administered daily by subcutaneous injections in various doses for 13-14 days (Table II). There was a gradation of changes related to the dose administered. In all cases a pseudo-pregnancy-like syndrome developed. Uterine weight increased progressively as the dose was raised from 2 to 50 μg/day. Anterior pituitary weight was increased in all groups. There was clear-cut

TABLE II. Effects of Daily Subcutaneous Administration of Estrogen for 13-14 Days in the Female Rat.

Exp No.	No. of rats	Hormone	Stage of cycle	No. cycling/Total No.	Mammary development	Body wt (g)	Organ wt (mg)				
							Ovaries	Uteri	Adrenals	Thyroid	Ant. pit.
1	10	S.O.[1]	D2[4]	10/10	—[2]	236 ± 5[3]	73 ± 5	354 ± 16	67 ± 4	15 ± .5	11 ± .6
	10	Estradiol (2 μg/day)	D	0/10	+	250 ± 5	60 ± 3.4*	472 ± 14†	71 ± 3.7	14 ± .97	20 ± 1.3†
2	10	S.O.	D2	10/10	++	236 ± 6	58 ± 2.2	347 ± 15	54 ± 2.5	14 ± 1.1	10 ± .7
	10	Estradiol (20 μg/day)	D	0/10	++	243 ± 6	75 ± 3.6*	671 ± 63†	56 ± 2.2	16 ± .8	26 ± 1.6†
3	10	S.O.	D	10/10	—	243 ± 7	64 ± 2.8	366 ± 9	58 ± 3.9	14 ± .9	11 ± .8
	10	Estradiol (50 μg/day)	D	0/10	+	219 ± 4	83 ± 4.2†	843 ± 40†	46 ± 1.8†	14 ± .95	20 ± 1.3†

[1] S.O. = sesame oil treated.
[2] — = no mammary gland development; + = mammary gland development with or without secretion.
[3] Mean ± SEM.
[4] D2 = diestrus, 2nd day; D = persistent diestrus.

* $P < .05$ *vs* sesame oil-injected control.
† $P < .001$ *vs* sesame oil-injected control.

mammary development and the ovaries showed large corpora; however, there was a biphasic effect on the weight of the ovaries which declined at the 2 μg/day dose and increased progressively at the 20 and 50 μg doses. No changes in adrenal weight were detected.

Testosterone implants. The androgen was implanted in the same loci as the estradiol and the effects compared to those observed in cholesterol-implanted controls and in a composite group of normal controls sacrificed on the second day of diestrus. Small numbers of these were sacrificed throughout the experiment. Implants of testosterone in the AH had no significant effect on comparison with the cholesterol-implanted controls (Table III). LH implants were similarly without effect except that ovarian weight was significantly reduced in this group in comparison to normal controls even though the rats continued to cycle; however, it must be noted that no cholesterol-implanted controls were included with the LH group.

ME implants of testosterone evoked definite effects in that ovarian weight was significantly reduced in both experiments and uterine weight was reduced in 1 of the 2 experiments in comparison to the cholesterol-implanted controls. Most of these rats continued to cycle and showed no evidence of mammary changes. Thus, the pseudopregnancy-like syndrome which was characteristic of estrogen implants in this locus failed to develop with testosterone. No alterations in pituitary or adrenal weight were observed.

As in the case of estrogen, MB implants of testosterone also were effective, in that they reduced ovarian weight significantly. Again, the pseudo-pregnancy-like syndrome failed to develop.

When testosterone was implanted in the anterior pituitary, no changes were observed, even when two 23 gauge tubes were implanted bilaterally in an effort to obtain a more uniform distribution of the steroid to the gland.

Discussion. This study amply confirms our previous observations(2) that implants of estrogen in either the ME region or in the anterior pituitary can evoke a pseudo-preg-

TABLE III. Effects of Testosterone Implants on Organ Weights of Female Rats.

Exp No.	No. of rats	Implant	Locus of implant	Stage of cycle	No. cycling/Total No.	Mammary development	Body wt (g)	Organ wt (mg)				
								Ovaries	Uteri	Adrenals	Thyroid	Ant. pit.
	13	None	None	D2[3]	13/13	—[1]	242 ± 6[2]	72 ± 5.3	374 ± 31	57 ± 3.1	15 ± .9	11 ± .6
1	6	Chol.	A.H.	D	5/6	—	256 ±12	73 ± 4	335 ± 21	69 ± 6.2	17 ± .7	11 ± .7
	6	Test.	A.H.	D	5/6	—	271 ±14	69 ± 4	328 ± 34	66 ± 1.7	16 ± .9	11 ± .7
2	6	Test.	L.H.	D	3/6	—	259 ± 5	52 ± 3.8	333 ± 53	64 ± 4.2	16 ± .6	10 ±1.3
3	3	Chol.	M.E.	D	3/3	—	278 ± 8	74 ± 1.4	450 ± 42	75 ± 4.6	16 ± 1.2	11 ± .3
	7	Test.	M.E.	D	4/7	—	247 ±10	57 ± 2.7*	391 ± 46	80 ± 3.5	17 ± .6	10 ± .8
4	6	Chol.	M.E.	D	5/6	—	257 ±13	62 ± 3.7	360 ± 16	70 ± 4.5	14 ± .5	10 ± .2
	6	Test.	M.E.	D	3/6	—	275 ± 8	39 ± 5 *	252 ± 24*	68 ± 3.5	15 ± 1.1	10 ± .5
5	7	Chol.	M.B.	D	7/7	—	267 ± 5	72 ± 2.7	349 ± 20	67 ± 3.8	15 ± 1	10 ± .6
	7	Test.	M.B.	D	4/7	—	274 ± 7	52 ± 2.7*	323 ± 21	74 ± 5.2	17 ± .6	10 ± .8
6	8	Chol.	A.Pit.	D	8/8	—	237 ± 7	66 ± 4.4	312 ± 17	59 ± 1.3	15 ± 1.2	9 ± .3
	9	Test.	A.Pit.	D	9/9	—	245 ± 8	71 ± 6	303 ± 11	66 ± 2.6	16 ± .7	9 ± .7
	9	Chol.	A Pit.	D	10/13	—	270 ± 7	75 ± 6	309 ± 36	66 ± 5	18 ± .6	12 ± .14
	13	Test.	A.Pit.	D	10/13	—	273 ± 7	64 ± 4	368 ± 27	61 ± 3.6	16 ± .7	11 ± .4

[1] — = no mammary gland development. [2] Mean ± SEM. [3] D2 = diestrus, 2nd day; D = persistent diestrus.
* $P < .001$ in comparison to cholesterol-implanted control.

TABLE IV. Comparison of Effects of Implants of Estradiol or Testosterone into ME Region of Female Rats.

		Effect on		Change in organ wt*			
		Estrous cycle	Mammary glands†	Ovaries	Uteri	Ant. pit.	Adrenals
1	Estradiol	Diestrus	+	—	—	+	0
2	Testosterone	Cycling	—	—	—	0	0

* — = decrease in organ wt; + = increase in organ wt; 0 = no change in organ wt.
† + = mammary development, with or without secretion; — = no mammary changes.

nancy-like syndrome in the female rat (Table IV). Implants in the MB were also effective in the present study, and reductions in ovarian and uterine weight were also seen in agreement with the early results of Lisk(3) as well as our own(2).

The results with testosterone stand in contrast with those obtained with estradiol. In this case reductions in ovarian and uterine weight were seen in agreement with Lisk's observations(4), but the cycle was little affected and there was no mammary development or pituitary enlargement. In this case effects were obtained with ME and MB placements, but no effect was observed in the case of implants in the anterior pituitary.

Estradiol then appears to inhibit gonadotrophin secretion (follicle stimulating hormone and luteinizing hormone (FSH-LH)) but to augment prolactine secretion by a central action on the hypothalamo-hypophyseal unit, whereas testosterone inhibits gonadotrophine secretion without altering prolactin secretion.

The inhibition of FSH-LH secretion without augmentation of prolactin release produced by testosterone implants is of considerable interest since it appears to represent a case in which the secretion of FSH-LH is not reciprocally related to the secretion of prolactin in the rat. In most situations a reciprocal relationship holds, for example in the lactating or pseudo-pregnant rat, or after isolation of the pituitary from neural influences either by hypothalamic lesions or by grafting it to a distant site(5-10).

That the effects of the steroids were centrally mediated and not secondarily produced after systemic absorption of steroid is indicated by the experiments with injection of estradiol. Here uterine enlargement was characteristically produced, whereas the implants tended to decrease uterine weight.

It is probable that both estrogen and testosterone can act to influence secretion of gonadotrophins at a hypothalamic site, for implants of either steroid in the ME can lower the content of stored LH-releasing factor in the hypothalamus(11). No effect of testosterone was detectable when it was located in the anterior lobe; however, estradiol appeared capable of influencing anterior pituitary function by a direct action since implants in the gland also induced the pseudo-pregnancy-like condition. This is in agreement with our own previous findings(2) and those of Bogdanove(12), who found localized alterations in gonadotrophs near the site of estradiol pellets in the pituitary.

It is of interest to contrast the present results in females with those previously obtained in males(1). Gonadal steroid implants were capable of inhibiting gonadotrophin secretion in males as indicated by reductions in weights of the seminal vesicles and ventral prostate; however, in the male no evidence of mammary development was observed with estrogen implants, although they did enlarge the pituitary. This could mean that prolactin release is only stimulated in females by estrogen, or it could mean that the mammary glands of the males were unresponsive to the prolactin which was released. It is not possible to decide between these possibilities; but it is noteworthy that transient mammary secretory changes can be noted in male after ME lesions or after injection of prolactin(13).

The other noteworthy difference from the response of males was the failure of the implants to evoke adrenal enlargement in the female. An increase in adrenal size was seen regularly in males(1). This is analogous to

the behavior of females when estrogens are systemically administered (Table II), i.e., no increase in adrenal weight occurs. Similar results with systemic administration of estradiol have been reported by Kitay(14). Apparently, the normal female is already responding maximally to the influence of estrogen on ACTH output and, consequently, cannot respond with a further increase.

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Received September 12, 1966. P.S.E.B.M., 1967, v124.

Aflatoxin Toxicity in Swine. (31719)

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Feeds contaminated with aflatoxins, metabolites of *Aspergillus* flavus, have been reported to be toxic to turkeys, ducklings and pheasants(1), young pigs(2), calves(3) and monkeys(4). Liver degeneration and fibrosis were the characteristic symptoms observed. High incidences of hepatic carcinomas have been reported in rats(5) and trout(6) fed aflatoxins. Carnaghan and Crawford(7) suggest aflatoxins may produce carcinomas in swine. They cite case histories of heavy mortality in pigs fed mixtures containing cottonseed and peanut meal. The lesions described were similar to those found in pigs fed toxic peanut meal by Loosemore and Harding(2); however, the pigs that survived the acute phase were found to have 100% incidence of hepatic carcinomas.

Aflatoxins are usually mixtures of 4 closely related compounds of known structure which have been named aflatoxins B_1, B_2, G_1 and G_2. Aflatoxin B_1 is the one usually found in greatest amounts and appears to be the most toxic(8).

Reported herein are the results of 2 feeding trials in which various levels of aflatoxin B_1 were fed to young pigs.

Methods and materials. In the first trial, 6 rations containing 15% peanut meal or soybean meal were fed to pigs 12-14 weeks old. The peanut meals contained 10, 50, 340, 700 or 1550 parts per billion (ppb) aflatoxin B_1 as determined by the chemical method described by Pons and Goldblatt(9). They also contained small amounts of aflatoxins B_2 and G_1. Only naturally occurring aflatoxins were present in the 4 lots of peanut meal containing 700 ppb or less of aflatoxin B_1. Each of these 4 lots of meal was thoroughly mixed in a ribbon mixer which was equipped with paddles on the center shaft to ensure homogeneity and uniform distribution of the aflatoxin. Capacity

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