Serotonin in the Developing Mammal

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ABSTRACT Determinations have been made of the level of serotonin in various fetal and maternal tissues of goats and rabbits. In the goat, both fetal brain and blood were higher in serotonin content than comparable maternal tissues. Furthermore, in the goat fetal neocortical areas unexpectedly were found to be richer in serotonin than certain subcortical structures. In contrast to the goat, the rabbit was shown to have higher serotonin levels in maternal than in fetal blood. Moreover, when a large amount of serotonin was administered subcutaneously to pregnant rabbits, the fetuses began to die at a time when the maternal blood levels of serotonin had about doubled. This toxic action was shown to be due at least partially to the sensitivity of the umbilical vessels to the vasoconstrictor action of serotonin.

Except for a few isolated reports on endogenous 5-hydroxytryptamine (5-HT, serotonin) in fetal and early postpartum life (1–3) little is known about the occurrence and significance of this amine in the developing mammal. On the other hand, the toxic potentiality of 5-HT upon the developing fetus has been made clear. It has been reported that 5-HT administered to pregnant rats (4, 5) and mice (6, 6 *a*) can interrupt pregnancy and lead to death of the fetuses.

This paper has the double purpose of (*a*) presenting results on the content of 5-HT in several tissues of the fetuses of goats and rabbits and (*b*) of presenting data on the interruption of pregnancy in the rabbit by 5-HT and on a possible mechanism of this toxic action.

METHODS

Goat fetuses, 15 to 25 days before birth (gestation time, 145 to 150 days), were obtained by cesarean section from goats anesthetized with pentobarbital sodium. Samples of maternal and fetal blood were withdrawn simultaneously before delivery. The mother goat was sacrificed shortly after delivery by an intracardiac injection of potassium chloride, while the kid was sacrificed by light chloroform anesthesia, in both cases followed by exsanguination from the severed neck vessels. The maternal

and fetal tissues were stored in ice before extraction of 5-HT which was carried out generally 15 to 30 minutes after sacrifice of the animals.

Rabbits during the last third of pregnancy (gestation time, 27 to 31 days) were lightly anesthetized with pentobarbital sodium and cannulated for withdrawal of blood. In three experiments maternal blood pressure in the carotid artery was recorded by means of a Statham arterial pressure transducer and Gilson minipolygraph. The abdominal wall was opened along the midline and the uterus partially exposed. By a short longitudinal incision in the uterus and fetal membranes, the fetuses were exposed without interrupting their vascular supply. In the experiments in which the action of 5-HT on the umbilical vessels was studied, the fetuses were maintained alive, out of the uterus, embedded in cotton moistened with warm saline. In this way the fetoplacental circulation was not interfered with and the fetuses were kept in a viable condition for a period of 20 minutes. Special care was taken to maintain a constant temperature of 38°C around the fetuses.

In all experiments, blood from the fetuses was obtained whenever possible from the umbilical vessels by means of a 27 gauge needle. When the fetuses were too small for this procedure, blood was obtained from the severed neck vessels. Immediately after withdrawal of the blood samples the umbilical vessels were severed and certain of the fetal tissues excised and extracted for 5-HT by the method of Amin *et al.* (7). The extracts were assayed on the heart of *Mercenaria* (*Venus*) *mercenaria* within 48 hours.

Monoamine oxidase activity of the placenta was determined by the following procedure. Placental tissue (0.25 to 0.5 gm) was homogenized in 10 volumes of a modified McIlwain medium buffered at pH 7.4. Serotonin creatinine sulfate was added in amounts from 2 to 20 γ (of base) and the suspension incubated and shaken at 37°C. At the end of 1 hour the entire mixture was extracted and assayed for 5-HT as above.

5-Hydroxytryptophan decarboxylase activity was determined by the method of Gaddum and Giarman (8) with the exception that the 5-HT was assayed as above instead of on the isolated rat's uterus.

RESULTS AND DISCUSSION

1. Experiments with Goats Table I shows the levels of 5-HT found in the blood, in several parts of the brain, and in the lungs of goat fetuses, and levels of 5-HT from comparable tissues from mother goats and from a 24 hour old kid.

It appears that fetal blood in this species contains 1.5 times more 5-HT than maternal blood. Furthermore, the fetal brain is richer in 5-HT than the maternal brain, the difference being more marked in the parietal and occipital lobes than in the hippocampus and basal ganglia. This finding of 5-HT levels higher in cortical than in subcortical structures of the fetal brain was unexpected; but it may be seen that in the 24 hour old kid the relative distribution of 5-HT in the brain is already shifting toward the opposite pattern (found in

adult brains of all species thus far studied). The level in the blood of the day old kid, however, is still higher than that found in the maternal blood. 5-HT was also found in appreciable quantities in the placenta and allantoic fluid, but was almost non-detectable in amniotic fluid.

2. Experiments with Rabbits In contrast to the goat, it may be seen in the first two columns of Table II that in the rabbit the level of 5-HT in the maternal blood is almost twice as high as that found in the fetal blood. It appears that this difference is greatest in early stages of fetal development and much less at the end of pregnancy (this may be observed in Table II in which

TABLE I

5-HT DISTRIBUTION IN FETAL AND MATERNAL TISSUES OF GOAT

Tissues	Fetus			Mother			24 hr old kid	
	No.	Mean 5-HT	Range	No.	Mean 5-HT	Range	No.	Mean 5-HT
		γ/gm			γ/gm			γ/gm
Blood, γ/cc	6	3.35*	(1.83–8.00)	6	1.99*	(0.27–4.2)	1	6.2
Brain								
Parietal lobe	5	0.36‡	(0.10–0.93)	5	0.10‡	(0.07–0.12)	1	0.18
Occipital lobe	5	0.29§	(0.16–0.46)	5	0.09§	(0.08–0.11)	1	0.22
Hippocampus	4	0.25	(0.16–0.44)	3	0.15	(0.13–0.14)	1	0.10
Basal ganglia	5	0.27	(0.18–0.35)	5	0.17	(0.11–0.22)	1	0.45
Pineal body				5	3.20	(1.20–7.00)	1	0.45
Lungs	3	0.65	(0.11–1.1)	3	1.56	(0.8–2.2)	1	0.93
Amniotic fluid	3	0.02	(0.006–0.03)					
Allantoic fluid	4	0.31	(0.12–0.40)					
Placenta	3	0.26	(0.25–0.27)					

* The difference between these values is statistically significant at a level of $p < 0.01$ determined in the manner described by "Student" and Fisher.
‡ $p < 0.01$.
§ $p < 0.001$.

fetuses of light weight may be considered those of earlier development).

Table II summarizes the effect of the subcutaneous injection of 5-HT (10 mg/kg) into pregnant rabbits, on the level of 5-HT in the maternal and fetal blood, and on the viability of the fetuses.

The administration of 5-HT is obviously followed by a rise of 5-HT in the blood of both mother and fetus: the peak values were observed generally in 30 minutes in both fetal and maternal blood. It is important to note that the administration of 5-HT under these conditions caused no changes in the maternal arterial pressure recorded from the carotid artery. In all experiments, 1 hour after the administration of 5-HT all fetuses were dead and the placentas showed a marked change in color from bright to dark red. It may be

TABLE II

CHANGES OF 5-HT LEVEL IN MATERNAL AND FETAL 5-HT AFTER ADMINISTRATION OF 5-HT TO THE MOTHER RABBIT

Time, *min.*	−30		0	30		60		70	90		120	
			5-HT in maternal (M.B.) and fetal blood (F.B.)									
Fetal weight (range)	M.B.	F.B.	Dose 5-HT*	M.B.	F.B.	M.B.	F.B.	Dose 5-HT*	M.B.	F.B.	M.B.	F.B.
gm	*γ/cc*	*γ/cc*	*mg/kg*	*γ/cc*	*γ/cc*	*γ/cc*	*γ/cc*	*mg/kg*	*γ/cc*	*γ/cc*	*γ/cc*	*γ/cc*
5.9– 6.3	1.31	0.62	0	1.57	0.62	1.31	0.62	0	1.57			
13.4–16.2	2.25	1.2	0	2.25	1.2	2.25	0.9	0	2.4	1.1		
6.0–6.5	1.5	0.53	10	2.2	1.66	2.9	0.8‡	0	2.62	2.0‡		
7.0–9.7	1.2	0.6	10	3.62	1.0	2.25	0.4‡	10	4.12	0.3‡		
14.1–17.5	2.5	2.0	10	2.0	1.33	3.0	1.1‡	0	2.4	1.1	1.4	2.5
17.5–21.9	1.38	1.5	10	2.22	2.8	3.0	0.2‡	10	3.5	0.9‡	2.62	1.2
39.5–57.1	1.93	1.5	10	4.5	2.6‡	3.7	0.5‡	0				
Mean	1.72§	1.13		2.91	1.88							

* Mg/kg, subcutaneously.

‡ Dead fetuses.

§ The difference between these two means is statistically significant ($p < 0.001$) by the t test of significance described by "Student" and Fisher.

TABLE III

RABBIT: 5-HT CONTENT OF FETAL BRAIN AND FETAL INTERNAL ORGANS BEFORE AND AFTER THE INJECTION OF 5-HT (10 MG/KG, SUBCUTANEOUSLY) TO THE MOTHER

Time, *min.*	−30		0	30		60	
	5-HT level			5-HT level			
Fetal weight range	Brain	Other tissues	5-HT	Brain	Other tissues	Brain	Other tissues
	γ/gm	*γ/gm*	*mg/kg*		*γ/gm*	*γ/gm*	
5.9–6.3	0.05	0.15	Saline	0.25	0.17	0.11	0.3
13.4–16.2	0.20	0.6	Saline	—	—	—	0.63
6.0–6.5	0.09	0.13	10	0.09	0.12	0.11*	0.2
14.1–17.5	0.14	0.21	10	0.12	0.87	0.11*	2.5
39.9–57.1	0.19	—	10	0.13*	—	0.13*	—
7.0–9.7	—	0.16	10	—	0.10	—*	0.1
17.5–21.9	—	0.6	10	—	1.23	—*	2.2
Mean values	0.13	0.31‡		0.11	0.58‡	0.12	1.3

* Dead fetuses.

‡ The difference between these two values is not statistically significant as determined by Student's t test.

observed that death of the fetuses occurred when the 5-HT level in maternal blood had almost doubled. In one experiment the level had more than doubled in 30 minutes and the fetus died in this case in a shorter period of time.

The 5-HT content of the fetal blood 30 minutes after 5-HT rose proportionally as high as that of the maternal blood. This increase is, therefore, evidence that 5-HT in the maternal circulation can traverse the placental barrier. A net decrease in 5-HT was observed in the blood of the fetuses which died 60 minutes after the administration of 5-HT to the mother.

During the course of these experiments, at a time when the fetal blood was obtained for determination of 5-HT, the fetus was sacrificed and its brain and pooled internal organs (lungs, spleen, and entire gastroenteric tract) were analyzed for 5-HT content. Table III summarizes these results. It may be seen that while the administration of 5-HT to the pregnant mother led to a variable increase in the pooled internal organs of the fetus within 60 minutes, such administration failed to cause any increase in 5-HT content of fetal brain. This finding suggests that a blood-brain barrier toward 5-HT is already formed in fetal life, even in early stages of development.

Careful observation of the fetuses after the administration of 5-HT to the mother revealed a gradual and powerful vasoconstriction of the umbilical vessels. In order to investigate any inordinate sensitivity of the umbilical vessels to 5-HT, 5 γ of 5-HT in 0.5 ml of saline solution was injected directly into the small vessels forming the umbilical vein. Fig. 1A shows the fetal vessels immediately before the injection, and Fig. 1B clearly demonstrates the vasoconstriction caused by the injection of 5-HT. Fig. 1C is the control showing that the injection of 0.5 ml of isotonic saline solution did not constrict the vessels but caused only a small hemorrhage at the injection point. It was of interest to find that the injection of the same amount of 5-HT into one of the omental vessels of a pregnant rabbit caused no vasoconstriction, but did greatly increase intestinal motility.

Recently, Waugh and Pearl (5) have reported that the interruption of pregnancy in rats brought about by the administration of 5-HT is associated with renal lesions of ischemic origin analogous to those reported previously by several investigators (9–12). Histological examination of the kidneys obtained from the rabbits at the end of our experiments in which 5-HT had been administered showed extensive glomerular damage with lymphocytic infiltration.

3. 5-Hydroxytryptophan Decarboxylase and Monoamine Oxidase Activity To what extent the fetal tissues form and destroy the 5-HT present in the fetus are still unanswered questions. It has been demonstrated that fetal tissues of the rat and of the human being form large amounts of histamine (13). The kidneys of rat fetuses have also been shown to be rich in 5-hydroxytryptophan (5-HTP) decarboxylase activity (14).

In this work we have found 5-HTP decarboxylase activity in several parts of the fetal brain of the goat. In addition it was observed that the placenta of both goat and rabbit produce 5-HT from 5-HTP. Table IV summarizes these data.

Placentas of the human being, rat, rabbit, and guinea pig have been shown to possess monoamine oxidase (MAO) activity (15). In this work MAO activity was also found in the placenta of the goat.

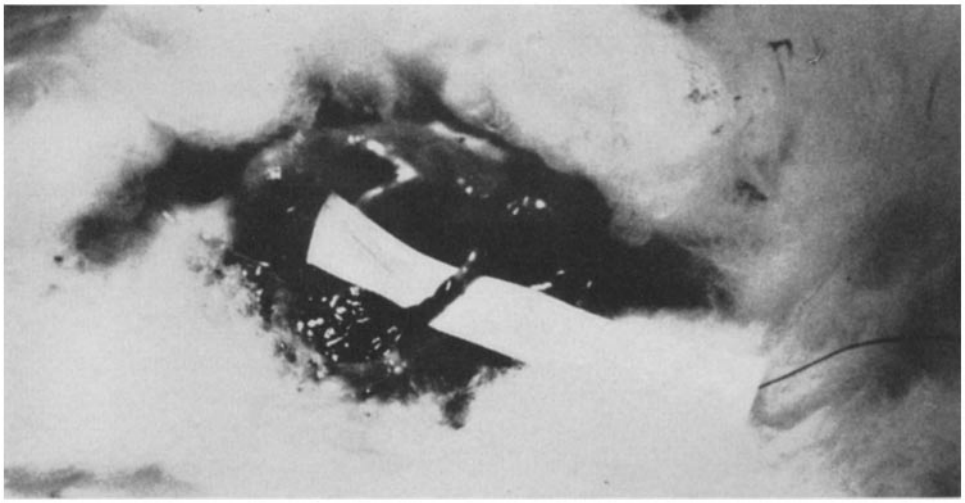

FIGURE 1A. Umbilical vein of the rabbit before injection of serotonin.

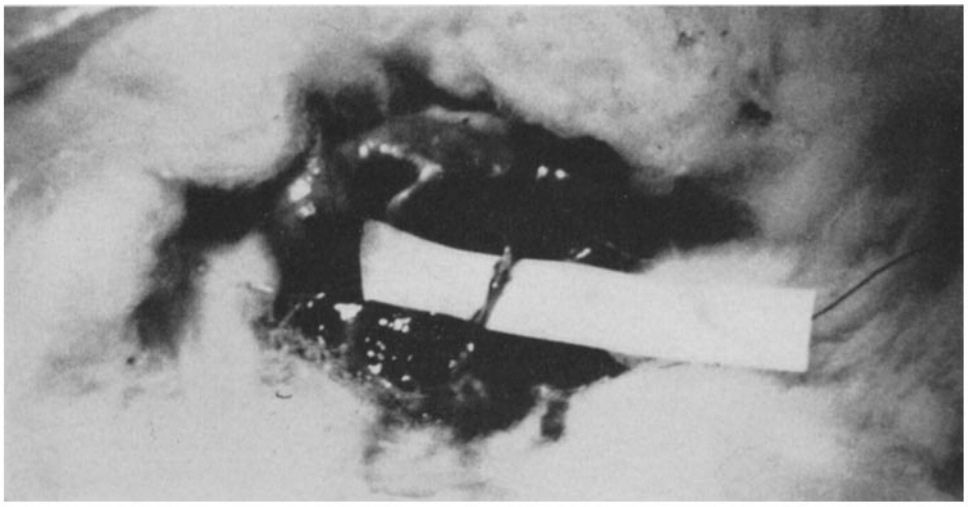

FIGURE 1B. Vasoconstriction of the umbilical vein of the rabbit immediately following injection of serotonin (5 γ in 0.5 ml saline).

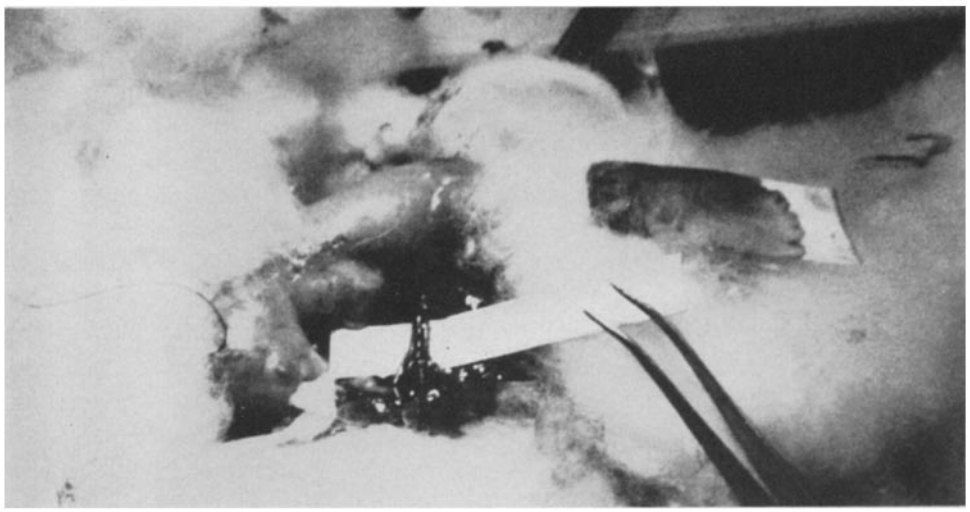

FIGURE 1C. Umbilical vein of the rabbit immediately following injection of physiological saline solution (0.5 ml).

GENERAL DISCUSSION

On the basis of experiments in the rat and guinea pig, Karki *et al.* (3) suggested a correlation between the level of brain 5-HT at birth and extent of development of the newborn. They found, in fact, that the relatively immature fetus or newborn of the rat had a relatively low level of cerebral 5-HT, while the more advanced fetus or newborn of the guinea pig had a level almost as high as in the adult. Goat fetuses at 15 to 25 days before birth are certainly well advanced, even though only few can survive if delivered out of the uterus at this stage of development. This fact, in the light of the suggestion of Karki and Brodie, might correlate with a brain level of 5-HT as high as that in the adult, but the significance of fetal levels higher than those of the adult,

TABLE IV

RELATIVE 5-HTP DECARBOXYLASE ACTIVITY IN FETAL AND ADULT TISSUES OF GOAT AND RABBIT

Species	Tissue	5-HTP decarboxylase activity* in	
		Fetus	Mother
Goat	Parietal cortex	0.7	1.0
	Diencephalon	3.8	10.0
	Placenta		6.0
Rabbit	Placenta		3.0

* Relative to activity of maternal parietal cortex as 1 (*i.e.*, conversion of 440 γ. 5-HTP to 15 γ 5-HT per gram of tissue per hour).

found in our work, is obscure. Equally difficult to explain is the presence of higher amounts of 5-HT in fetal neocortical structures than those found in the hippocampus or basal ganglia. This type of distribution of 5-HT in the fetal brain is in sharp contrast to that found in adult goats, in fetal and adult rabbits, in human adults (16), and in adult dogs (17). On the other hand, the 5-HT levels found in the brain of the rabbit fetus compared to those of the adult showed essentially the same pattern described by Karki and Brodie for the rat, and it is interesting in this context to note the rather poor development of newborn rabbits, similar to newborn rats. One consideration to be taken into account here is the role pentobarbital anesthesia may play in the levels of 5-HT found in fetal brains. The fact that barbiturates cause an elevation in brain 5-HT is amply documented (18, 19). This coupled with the finding that barbiturates are more slowly degraded in tissues of the newborn (20) and are concentrated by the fetal brain stem (21) might explain the greater level of 5-HT in fetal brains found in the goat. On the other hand, the rabbits in this study were also anesthetized with pentobarbital and fetal levels of 5-HT in this species are not higher than maternal levels.

Relatively large amounts of 5-HT were found in fetal blood and tissues of the rabbit. This fact, in addition to the findings that even larger amounts of 5-HT are present in the maternal blood and that the amine is actively formed by the placenta would seem to conflict with the observation that modest increases in circulating levels of the amine can interrupt pregnancy in the rabbit and cause death of the fetuses. Death of the fetuses in these studies occurred when the level of 5-HT in the maternal blood had just about doubled, following subcutaneous administration of the amine. To be sure, the dose of 10 mg/kg used here is a large one, but it is much lower than the subcutaneous LD_{50} in the rat which has been calculated to be 117 mg/kg (22).

Apparently, much of the toxic action upon the fetus of high circulating 5-HT in the maternal blood may be attributed to the sensitivity of umbilical vessels to the vasoconstrictor action of the amine. Such sensitivity has been demonstrated clearly in this work, as well as in the studies of Astrom and Samelius (23) on isolated human umbilical vessels, and in the more recent studies of Goerke *et al.* (24) on isolated perfused human placenta. It is somewhat surprising that death of the fetus usually supervened when the maternal circulating level of 5-HT had about doubled, in view of the fact that one can induce three- to fivefold increases in 5-HT in tissues of non-pregnant animals without any apparently adverse effects. These findings in conjunction with the extensive kidney lesions found in pregnant rabbits treated with 5-HT might prompt further investigation of a possible role of 5-HT in eclampsia.

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BIBLIOGRAPHY

1. FAUSTINI, R., *Am. J. Vet. Research*, 1955, **16**, 397.
2. DIXON, J. B., *J. Physiol.*, 1959, **147**, 144.
3. KARKI, N. T., KUNTZMAN, R., and BRODIE, B. B., *Fed. Proc.*, 1960, **19**, 282.
4. CORREL, J. T., LYTH, L. F., LONG, S., and WANDERPOEL, J. C., *Am. J. Physiol.*, 1952, **169**, 577.
5. WAUGH, D., and PEARL, M. J., *Am. J. Path.*, 1960, **36**, 431.
6. FLUCKIGER, E., and SALZMANN, R., *Experientia*, 1961, **17**, 131.

6 *a*. POULSON, E., BOTROS, M., and ROBSON, J. M., *Science*, 1960, **131**, 1101.

7. AMIN, A. H., CRAWFORD, T. B. B., and GADDUM, J. H., *J. Physiol.*, 1954, **126**, 596.
8. GADDUM, J. H., and GIARMAN, N. J., *Brit. J. Pharmacol.*, 1956, **11**, 88.
9. PAGE, E. W., and GLENDENING, M. B., *Obst. and Gynec.*, 1955, **5**, 781.
10. DELGRECO, F., MASSON, G. M. C., and CORCORAN, A. C., *Am. J. Physiol.*, 1956, **187**, 509.
11. SALGADO, E., and GREEN, D. M., *Am. J. Physiol.*, 1955, **183**, 657.
12. FIORE-DONATI, R., and ERSPAMER, V., *Am. J. Path.*, 1957, **33**, 895.
13. KAHLSON, G., ROSENGREN, E., and WHITE, T., *J. Physiol.*, 1960, **151**, 131.
14. HUANG, I., *Nature*, 1960, **186**, 117.
15. THOMPSON, R. H. S., *in* Ciba Foundation Symposium Toxemias of Pregnancy, London, J. and A. Churchill, 1950, 236.
16. COSTA, E., and APRISON, M. H., *J. Nerv. and Ment. Dis.*, 1958, **126**, 284.
17. PAASONEN, M. K., MACLEAN, P. D., and GIARMAN, N. J., *J. Neurochem.*, 1957, **1**, 326.
18. BONNYCASTLE, D. D., GIARMAN, N. J., and PAASONEN, M. K., *Brit. J. Pharmacol.*, 1957, **12**, 228.
19. ANDERSON, E., and BONNYCASTLE, B. B., *J. Pharmacol. and Exp. Therap.*, 1960, **130**, 138.
20. JONDORF, W. R., MAICKEL, R. P., and BRODIE, B. B., *Biochem. Pharmacol.*, 1958, **1**, 352.
21. PERSSON, B. H., *Acta Obst. et Gynec. Scand.*, 1960, **39**, 88.
22. FREYBURGER, W. A., GRAHAM, B. E., RAPPORT, M. M., SEAY, P. H., GOVIER, W. M., SWOAP, O. F., and VANDERBROOK, M. J., *J. Pharmacol. and Exp. Therap.*, 1952, **105**, 80.
23. ASTROM, A., and SAMELIUS, U., *Brit. J. Pharmacol. and Chemotherapy*, 1957, **12**, 410.
24. GOERKE, J. A., MCKEAN, C. A., MARGOLIS, A. J., and GLENDENING, M. B., *Am. J. Obst. and Gynec.*, 1961, **81**, 1132.