Summary. 2-Ethyl-5-methylbenzimidazole was found to be considerably more inhibitory than 2,5-dimethylbenzimidazole, or benzimidazole, for the synthesis of heme by chicken erythrocytes incubated *in vitro*. This was in the same relative order of inhibitory activities as was noted for multiplication of influenza A or B virus, and is considered to add further support to the concept that the same basic mechanism, possibly involving a fundamental role of nucleic acid in biosynthesis, underlies both processes.

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Progesterone-Like Activity in the Plasma of Ovoviviparous Snakes.*† (21139)

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Studies on the natural occurrence of progestin have been carried out primarily on mammals. This hormone has been found in a large number of mammalian species and seems to be ubiquitous in this class. The available evidence indicates that the hormone is produced in the mammal by the corpus luteum (and to a lesser extent, the pre-ovulatory follicle), the placenta, the adrenal cortex and possibly the testis. Fraps, Hooker and Forbes(1,2) reported that substances with progesterone-like activity were found in the plasma of ovulating hens, non-ovulating hens, and cocks, but not in the plasma of capons. Porto(3) reported the presence of a substance in an extract of 158 corpora lutea of two species of ovoviviparous South American snakes, *Bothrops jararaca* and *Crotalus terrificus*, that gave a 2+ endometrial reaction in the McPhail assay for progesterone(4). This is the only evidence thus far for the presence of substances with progestational activity in reptiles. It was therefore felt worthwhile to examine the blood plasma of snakes by using the sensitive bioassay method for small amounts of progesterone described by Hooker and Forbes(5). Such information might conceivably lead to a better understanding of the evolutionary significance of this hormone and its role in the lower vertebrates.

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Materials and methods. Preliminary experiments indicated the presence of substances with progesterone-like activity in the plasma of 2 pregnant water snakes, *Natrix s. sipedon.* These observations were confirmed and a quantitative study was made of the hormone content of the plasma. The animals used in these experiments were predominantly water snakes, *Natrix s. sipedon,* from Thurmont, Md., while a few observations were made on garter snakes, *Thamnophis radix,* collected in Champaign-Urbana, Ill.

Blood was obtained by cardiac puncture with a No. 18 or 20 short-beveled needle. The location of the heart was readily detected by its pulsations against the ventral body wall at scutes 25 to 30. Since the free edges of the scutes are directed caudally, the needle was inserted under the edge of one of the scutes posterior to the site of the cardiac impulse and run obliquely forward to reach the heart. The blood was treated with heparin or citrate and the plasma removed and stored at −20°C. All assays for progesterone-like activity were carried out according to the Hooker-Forbes technic(5) which has been shown to be valid for the assay of untreated serum or plasma(6). Although the identity of the substance assayed by the Hooker-Forbes test is unknown, in keeping with the previous studies, the activity of the progestin has been standardized against progesterone and will be expressed as μg equivalents of progesterone.

Experimental. Cieslak(7) states that in *Thamnophis radix* the ovarian follicles fall into 3 macroscopically distinguishable size groups. This situation has been found to obtain for *Thamnophis sirtalis* and *Natrix sipedon* and may be true for snakes in general. A new crop of follicles appears during the early summer. These undergo a long period of slow and gradual growth which culminates in their third spring with a vigorous preovulatory growth spurt. During late April and May, in the northeastern states, the ova of the largest size group increase rapidly in volume by the deposition of large quantities of yolk. Ovulation usually occurs in late May or in June. In Table I, the snakes designated as having inactive ovaries had only small follicles of the immature type. Those listed as preovulatory had ripening follicles in which appreciable amounts of yolk had already been deposited.

TABLE I. Concentration of Progesterone-Like Activity in the Plasma of Ovoviviparous Snakes.

Snake No.	Status of snake	Progesterone-like activity,† μg/ml
NM-25-52	Inactive ovaries	.3
NMT-18	Inactive ovaries with atretic follicles	1.0
NM5-52	Preovulatory	.3
NMAB-52	"	.3
NM12-52	Early ovulation	2.0
NI-1-52	Pregnant, ⅓ development*	4.0
NM26-52	" ⅓ " *	4.0
NM29R	" ⅔ " *	4.0
NMAL	" ⅔ " *	6.0
T1-11	" full term*	8.0

* Refers to the degree of development seen in the fetuses.

† Expressed in μg equivalent of progesterone.

It is extremely difficult to establish a known date of ovulation in these species. Females brought into the laboratory prior to the time the follicles are fully ripe often show some interference with ovulation. There may occur a complete failure to ovulate, a delay in ovulation or ovulation of only a portion of the maturing ova, the remainder undergoing follicular atresia. In two studies(7,8) ovulation has been found to occur in a regional population of garter snakes over a period of about three weeks. The gestation period is roughly 9 to 10 weeks. Thus an animal that ovulated early could be at full term at a time when an animal which had ovulated late was at two-thirds term. In this study, the estimation of the stage of pregnancy was made on the degree of development of the embryos and the amount of yolk remaining in each uterine ovum at the time of laparotomy or autopsy.

The plasma of snakes with preovulatory follicles or inactive ovaries contained activity equivalent to 0.3 to 1.0 μg of progesterone per ml. Following ovulation the concentration rose to 2 μg and then showed a progressive increase during pregnancy. The highest titer (8 μg per ml) was obtained at full term. This was seen in a snake which at autopsy in August contained 4 fully developed living fetuses, 3 dead but fully developed fetuses

and 7 ova in which there had been no development or in which cessation of development had occurred early in pregnancy (Table I).

The plasma of 2 male snakes with fully developed testes (June 12 and 29) showed a level equivalent to 0.3 and 1.0 μg progesterone per ml.

Discussion. The presence of a substance in the blood plasma of snakes that is capable of eliciting a reaction that is thought to be specific for progesterone or a substance very similar to progesterone raises several questions of importance to comparative endocrinology. A problem that must remain for future research is that of the chemical nature of the active agent. Yet, regardless of what it may prove to be, its physiological importance in snakes is probably quite different from that of progesterone in mammals. Bragdon(8) has shown both in the garter snake and water snake that the corpora lutea are not essential for the maintenance of pregnancy, nor does abortion occur following hypophysectomy. However, the increase in concentration of the hormone in the plasma during gestation suggests a progestational function. This in some respects is similar to the condition in the ewe (9) and rabbit(10) in which progesterone increases in the blood during pregnancy and in these animals is indispensable for a successful gestation.

Although the hormone in snake blood produces a progestional reaction in the uterus of mice, the available experimental evidence indicates that if it has a function in the snake, it is one concerned with some other physiological process than gestation(8). The fact that it is also found in the blood of male snakes tends to support this supposition and at the same time raises the question as to its probable source of origin. Beall and Reichstein (11) have isolated progesterone from the adrenal glands of cattle and progestational activity has been found in the blood of cocks (2) and in males of a variety of species ranging from amphibians to human beings(12). With regard to the ovary, it is known that during the menstrual cycle in primates, progesterone appears several days before ovulation and formation of a corpus luteum(13-15), a condition that probably is quite common among mammals(16). Thus it is evident that the presence of progesterone in the blood does not depend entirely upon the existence of a corpus luteum even in animals in which progesterone is of major importance for gestation. In fact, it is conceivable that substances having progestational activity, when tested in mammals, may be of frequent occurrence in chordates during follicular development and ovulation. Carlisle(17), by using the Hooker-Forbes technic, found progesterone-like activity in the blood and ovary of the ascidian, *Ciona,* 48 hours after experimentally induced ovulation, but not in the non-breeding animal. The chemical nature of these substances of sub-mammalian origin are, of course unknown, but should they prove to be progesterone, as suggested by the Hooker-Forbes reaction, knowledge of them may be of considerable importance for an understanding of the physiological adaptations that took place in the evolution of viviparity.

It would be of interest to know the extent to which substances with progestational activity occur throughout the chordates. The findings of Carlisle(17) support the concept that progesterone-like substances are present throughout the chordate phylum. It is indeed possible that these substances originally appeared as intermediary metabolites without a hormonal action referable to pregnancy. Such a concept is of immense significance from the evolutionary point of view and the presence of progestin in the plasma of the ovoviviparous snake is added evidence. It is also intriguing to note that the hormone is not only present but increases during gestation even though it may not have an essential hormonal role in pregnancy.

Summary and conclusion. Progesterone-like activity was determined in the plasma of the ovoviviparous snake by the Hooker-Forbes technic. Snakes with inactive ovaries or preovulatory follicles showed a concentration of 0.3 to 1.0 μg equivalent of progesterone per ml of plasma. This titre rose to 2 μg following ovulation, to 4 μg by mid-pregnancy and to 8 μg per ml of plasma at full term.

Minimal amounts of progesterone-like activity were found in the plasma of male snakes. The possible role of this substance in the reptile and its evolutionary significance are discussed.

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Bioautography of Biotin and Certain Related Compounds. (21140)

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The existence in culture filtrates of *Aspergillus niger* grown in the presence of added pimelic acid of a previously unrecognized component of natural material with biological activity equal to that of biotin in satisfying this growth factor requirement of *Neurospora crassa* has been described by Wright and Cresson(1). The factor was isolated from a large quantity of *Aspergillus niger* culture filtrate and identified as biotin L-sulfoxide(2,3). It was established that biotin L-sulfoxide originates from an *in vivo* oxidation of biotin rather than an *in vitro* (purely chemical) oxidation. Biotin L-sulfoxide has activity equal on a molar basis to biotin, biotin D-sulfoxide, biocytin, and desthiobiotin in satisfying the biotin requirement of *Neurospora crassa*. For a number of other biotin-requiring microorganisms thus far examined it is less active than biotin or the D-sulfoxide. Biotin L-sulfoxide has an ubiquitous occurrence and may be expected to have some function in metabolism.

During the investigations involved in the discovery, isolation, and characterization of biotin L-sulfoxide considerable bioautographic data with biotin, biotin L-sulfoxide, biotin D-sulfoxide, biocytin, oxybiotin, and desthiobiotin as well as mold filtrates were accumulated where butanol-water-acetic acid was used as the developing solvent and *Neurospora crassa* was the assay organism. These data are being published in detail for the interest they may have for those concerned with elucidating various aspects of the biochemistry of "naturally-occurring" forms of biotin.

Methods. Synthetic D-biotin, DL-desthiobiotin and DL-oxybiotin (O-heterobiotin) were obtained from Hoffmann-LaRoche, Inc., synthetic biotin D-sulfoxide and biotin L-sulfoxide(4) were kindly supplied by Dr. D. B. Melville, natural biotin L-sulfoxide was isolated from *Aspergillus niger* culture filtrate as described previously(2), biocytin was synthesized in the laboratories of the Chemical Division of Merck and Co., Inc.(5). The solvent mixture for the paper chromatograms was prepared by shaking together one part