tial for parthenogenetic development expressed to a greater or lesser degree in flocks of mated birds? Obviously, the answer to this question has a direct bearing upon the interpretation placed on finding cell colonies in so-called "infertile" eggs. Thus far, these usually have been considered to be instances of early embryonic mortality(8). Which of these "infertile" eggs from mated hens are developing parthenogenetically and which are cases of delayed development following fertilization? Olsen and Marsden(9) suggested that those turkey eggs from mated hens which show development only after 72 hours of incubation may be instances of parthenogenesis. Krizenecky *et al.*(10) approached the problem of morphological differences between infertile and non-fertile (from virgin hen) chicken eggs on the basis of the appearance of the germ disc. Unpublished data from this laboratory indicate that there may be similar differences in the turkey.

Another question is: Can the predisposition to parthenogenetic development serve as a deterrent to normal fertilization? It is conceivable that eggs possessing a high potential for such development would be less capable of being fertilized.

*Summary.* Evidence has been presented that: (1) Frequency of abortive parthenogenesis in the Broad Breasted Bronze turkey is as high as 80% when based on unincubated eggs studied histo-cytologically. (2) There are varietal differences which affect the frequency of occurrence of this type of development. Possibly, environmental differences also affect the frequency of abortive parthenogenesis.

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## Effect of Anesthetics and Collection Time on Corticosteroid Secretion By Rat Adrenal.* (22837)

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Measurement of corticosteroids in the adrenal vein effluent of the rat has been described by Bush(1), Singer and Stack-Dunne (2) and Rosenman *et al.*(3). However, no attempt has been made to correlate secretion rate under these circumstances with time of collection of effluent and the nature of anesthetic employed. Several lines of evidence suggest that ether anesthesia is more stressful than Nembutal (pentobarbital sodium) and evokes a more profound pituitary-adrenocortical response than the latter(4-10).

The present observations support this conclusion and demonstrate that ether, but not Nembutal, anesthesia results in a marked early elevation of corticosterone in the ad-

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renal venous effluent of the rat.

*Methods.* Adult, male Sprague-Dawley rats from our breeding colony were employed and maintained *ad libitum* on Purina laboratory chow and tap water. They weighed 300 to 500 g. Cannulation of the left renal vein was performed under ether or Nembutal anesthesia. Complete anesthesia was usually attained within 1 to 2 minutes with ether, and in approximately 15 minutes with Nembutal (50 mg/kg injected intraperitoneally). The technique of cannulation was similar to that described by Bush(1), as modified by Singer and Stack-Dunne(2), and was accomplished within 10 to 15 minutes after laparotomy. Heparin was used as an anti-coagulant. A series of timed collections was made of the left adrenal venous effluent in individual animals. The amount of blood collected was usually 2 to 4 ml for each time interval. Corticosteroids were extracted from blood samples by the method of Bush(1), as modified by Singer and Stack-Dunne(2). The extracts were then chromatographed on Whatman No. 1 filter paper employing toluene: methanol:water (4:3:1) system described by Bush(1). Known amounts of corticosterone and cortisol† were run as markers with each determination. Chromatographic development was allowed to proceed for 100 to 150 minutes at room temperature. Consistent results were obtained without temperature control. A complete change of the solvent phases within the chromatography cylinder was made only once every 3 to 4 months. Resolved steroids were located by spraying the air-dried filter paper sheets with alkaline Tetrazolium Blue‡ solution(11). The bulk of the corticosteroids in rat adrenal effluent invariably migrated in the corticosterone zone(1-3). Rarely, a trace of cortisol appeared as well(1). Elution of the stained steroids, as described by Touchston and Hsu(11), was carried out by immersing the dried chromatograms in distilled water for 3 minutes, followed by partial drying at room temperature. Sections of damp filter paper containing stained steroid were cut into small fragments and eluted twice with 5 ml of ethyl acetate:methanol (7:3). Each elution was for 10-15 minutes, with occasional shaking. Appropriate blank determinations were performed concurrently on unstained portions of the same chromatogram. These blanks usually had an absorbance of .02-.03, paralleling the average reading of .0269 reported by Touchston and Hsu(1). Photometric estimation of the dye eluted from the steroid-containing and control portions of chromatogram was made at 565 mμ in the Coleman Junior Spectrophotometer against a solvent blank set at zero density. Except for the 0 to 10 minute blood collections under Nembutal anesthesia, all density readings were equal to or greater than 0.05. The effective instrument error in this range is less than 10%. In the 0 to 10 minute Nembutal series only 1 reading was in this range. The value recorded for corticosterone secretion in this series (Table II) must therefore be considered maximal. *Quantitative chromatography* of pure corticosterone in amounts similar to those present in blood extracts yielded the results shown in Table I. The optical densities of the eluted dye followed Beer's Law (K = 0.50) over the range of 4 to 40 μg of corticosterone. The error inherent in these determinations averaged about 20% for concentrations of this steroid above 6 μg. In addition, recovery of corticosterone added to 2 ml of peripheral rat blood,

TABLE I. Photometric Analysis of Corticosterone Standards.*

| Corticosterone, μg | No. of analyses | Optical density† (565 mμ) | Error, %‡ | K§ |
|---|---|---|---|---|
| 4 | 15 | .02 ± .002 | 30 | .50 |
| 6 | 9 | .03 ± .002 | 20 | " |
| 8 | 14 | .04 ± .002 | 20 | " |
| 10 | 10 | .05 ± .003 | 18 | " |
| 16 | 15 | .08 ± .004 | 21 | " |
| 24 | 24 | .12 ± .004 | 17 | " |
| 32 | 17 | .17 ± .007 | 17 | .53 |
| 40 | 10 | .20 ± .010 | 17 | .50 |

* Corticosterone was chromatographed, stained with Tetrazolium Blue and eluted with ethyl acetate-methanol (see text).
† Mean ± stand. error.
‡ Stand. dev./mean.
§ Mean optical density/μg corticosterone.

† Samples of pure corticosterone and cortisol were generously made available by Dr. Elmer Alpert, Merck and Co., Rahway, N. J.

‡ Nutritional Biochemicals Corp., Cleveland, O.

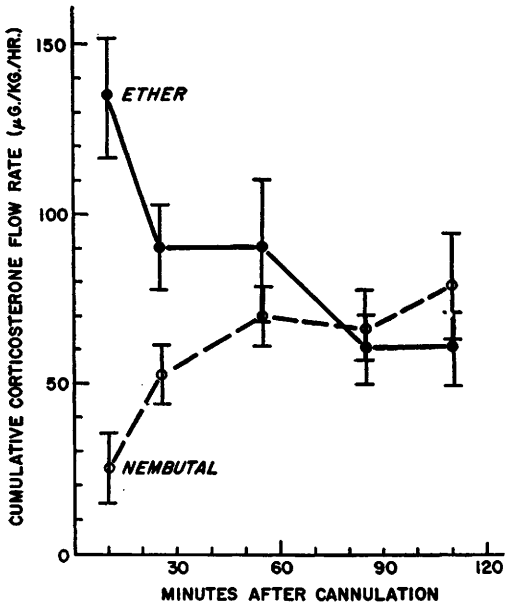


FIG. 1. Influence of anesthetic and collection time on cumulative corticosterone secretion rates of rat adrenal. Each point represents mean of 6 to 16 determinations, each on a different animal; stand. error is also shown.

and processed as described above, averaged 98% (90 to 104%) in 4 experiments with 50 μg of added steroid and 102% (88 to 144%) in 3 experiments with 10 μg of added steroid.

*Results.* The influence of time of collection and type of anesthetic on the amount of corticosterone appearing in the adrenal venous effluent of the rat following cannulation is depicted in Table II. Ether anesthesia resulted in elevated values for corticosterone concentration and corticosterone flow rate in the first 10 minutes of collection, as compared with corresponding values obtained after Nembutal anesthesia. This early difference was not evidenced in later collections (10 to 25 minutes and 25 to 85 minutes). However, cumulative values for rate of corticosterone release from the time of cannulation to the end of each collection period revealed that the differential effect of ether and Nembutal was apparent in collections made for 25 minutes or less, but not thereafter (Fig. 1).

It was not possible in the present investigations to dissociate the effects of anesthesia on corticosteroid release from those of the operative and collection procedures. It would appear likely, however, that the elevated early values observed after ether anesthesia were due, in part, to pituitary-adrenal activation associated with the administration of this anesthetic. A similar conclusion may be drawn from recent work on the effect of ether anesthesia on blood ACTH(4) and adrenal ascorbic acid levels(5) in the rat and on corticosteroid release by the dog adrenal (6). The use of ether as an anesthetic in studies on pituitary-adrenocortical function would appear to be contraindicated.

It is not certain whether the lower initial values for corticosterone release obtained after Nembutal anesthesia reflect the basal non-stressed corticosteroid output of the rat adrenal or partial inhibition of the early response to the stress of laparotomy. The latter possibility is supported by the observations of Sayers and Sayers(7), Ronzoni(8) and Cronheim and Hyder(9) on the inhibition by Nembutal of the adrenal ascorbic acid response to stress in the rat.

The similarity of corticosterone output values obtained during the later collection periods (Table II) under either Nembutal or ether anesthesia suggests that these later values represent the response of the rat adrenal cortex to the continued stresses of cannulation and blood loss. The maximal corticosterone concentrations observed in the present studies were 6.7 μg per ml of effluent with ether anesthesia and 7.0 μg per ml with Nembutal during the final collection period (25 to 85 minutes following cannulation).

The potentiating action of ether and the depressing effect of Nembutal on corticosterone secretion immediately following cannulation may be presumed to have been mediated via the hypothalamic-hypophyseal system. A central action of ether is supported by the observation of Sydnor and Sayers(4) that this anesthetic resulted in a rapid elevation of blood ACTH in the rat. However, a more direct effect of ether on the pituitary-adrenal system is not eliminated by these studies. The report by Cronheim and Hyder (9) that complete anesthesia with Nembutal blocked the response of the pituitary-adreno-

TABLE II. Effect of Anesthetic and Collection Time on Corticosterone Secretion by Rat Adrenal.

| | | Ether | | | Nembutal | | | |
|---|---|---|---|---|---|---|---|---|
| | Collection time, min.* | No. of rats | Vol of blood, ml† | Corticosterone secretion† | No. of rats | Vol of blood, ml† | Corticosterone secretion† | P value‡ |
| Corticosterone flow rate (μg/kg/hr) | 0-10 | 7 | 2.6 ± .4 | 136 ± 20 | 7 | 3.0 ± .8 | 25 ± 12 | <.001 |
| | 10-25 | 6 | 2.0 ± .3 | 62 ± 10 | 5 | 1.8 ± .2 | 44 ± 24 | .5 |
| | 25-85 | 11 | 3.8 ± .7 | 72 ± 15 | 16 | 4.4 ± .3 | 76 ± 12 | .8 |
| Corticosterone conc. (μg/ml) | 0-10 | 7 | 2.6 ± .4 | 4.1 ± 1.0 | 7 | 3.0 ± .8 | .9 ± .5 | <.001 |
| | 10-25 | 6 | 2.0 ± .3 | 3.5 ± 1.2 | 5 | 1.8 ± .2 | 2.8 ± 1.3 | .8 |
| | 25-85 | 11 | 3.8 ± .7 | 6.7 ± .8 | 16 | 4.4 ± .3 | 7.0 ± .9 | .9 |

* Mean collection time. Collection times designated as 25 min. actually varied from 20 to 30 min.; those designated as 85 min. varied from 60 to 115 min.

† Values expressed as mean ± stand. error.

‡ Significance of differences between corticosterone secretion in Nembutalized *vs* etherized animals.

cortical system in the rat to salicylic acid suggests that this anesthetic exerts its primary action on the hypothalamus. The role of the hypothalamus in the activation of the pituitary-adrenal system is well established(12).

Cumulative corticosterone flow rates in the adrenal venous effluent of the rat obtained in the present study were somewhat lower than those reported by Singer and Stack-Dunne (2) and Rosenman *et al.*(3) under similar conditions. Variations in technic and age and strain of animals may be responsible for these differences. The considerably higher values reported by Bush(1) have been attributed, in part, to the administration of exogenous ACTH to his experimental animals. However, judging from the present investigations, the short collection time (10 to 20 minutes) under ether anesthesia may also have contributed to these high values.

*Summary.* Secretion of corticosterone into the adrenal venous effluent of the rat was markedly influenced during the first 10 minutes of collection by the nature of the anesthetic employed for cannulation procedure. Ether anesthesia resulted in a significant elevation of corticosterone concentration and corticosterone flow rate during this period as compared with the values observed when Nembutal was used. This difference was eradicated during subsequent collection periods of 10 to 25 minutes and 25 to 85 minutes following cannulation. The relationship of these findings to the possible activation by ether and inhibition by Nembutal of the pituitary-adrenocortical system is discussed.

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