# Plasma Levels of Epinephrine and Norepinephrine of Chickens under Different Conditions

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# Plasma Levels of Epinephrine and Norepinephrine of Chickens under Different Conditions

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## SUMMARY

In an attemp to know plasma epinephrine (E) and norepinephrine (NE) levels of the chicken under resting conditions, blood samples of chickens under various conditions were subjected to measurement of E and NE with a conventional THI method. In blood samples obtained by venipuncture from the brachial vein, plasma E and NE levels of unanesthetized birds were 6.1 ± 1.4 ng/ml (mean ± S.E., n=8) and 11.7 ± 1.4 (n=8), respectively, and those of anesthetized birds were 4.1 ± 0.4 ng/ml (n=8) and 5.3 ± 0.3 ng/ml (n=8). The difference in the two NE levels was statistically significant. Heart puncture resulted in a marked rise of plasma E level (19.1 ± 1.5 ng/ml, n=8) with little change of plasma NE level (12.9 ± 1.8 ng/ml, n=8), but this effect was not observed under anesthesia. Two birds were anesthetized, their thoracic cavity was opened to expose the heart, and blood was drawn by a thick needle from the heart. In these samples, plasma E and NE levels were measured to be 62.3 ± 1.3 ng/ml and 73.1 ± 20.3 ng/ml, respectively. In the perfused isolated chicken rectum, E, when added to the vessel perfusate to give a concentration of 10 ng/ml or higher, stopped the spontaneous motility and reduced tone. From these results, it is safe to assume that plasma E and NE levels of the chicken during resting conditions fall within the ranges from 4 to 7 ng/ml for E and from 5 to 12 ng/ml for NE, as measured in blood samples taken by venipuncture from the brachial vein under normal conditions and by heart puncture under general anesthesia.

## INTRODUCTION

Plasma levels of E and NE have been used to measure activity of the sympathetic nervous system and responses to various stressors. Fluorimetric measurements gave 0.5 ng/ml or so for E and 1 ng/ml or so for NE in the plasma of different species of mammals[1]. However, those of chicken were contradictory, being less than 10 ng/ml for both E and NE by Lin & Sturkie[2] and Edens & Siegel[3], but 51 ng/ml for E and 16 ng/ml for NE by DeSantis *et al*[4]. The latter values seem to have received more widespread acceptance, although the plasma levels, especially of E, are far higher than in mammals.

On the other hand, the isolated chick rectum is well known for its high sensitivity to catecholamines and is used as a bioassay preparation[5]. If the chick rectum were exposed to such high concentrations of circulating catecholamines under normal conditions, it would presumably be unable to play its physiological role. This discrepancy led us to measure E and NE in blood of chickens under different conditions to determine the plasma levels during resting conditions.

## MATERIALS AND METHODS

White Leghorn cocks weighing 1.7—2.5 kg were obtained from commercial sources. Birds were housed individually in cages at least for one week before use in an experiment. Feed and water were provided *ad libitum*. Blood samples for measurement of plasma E and NE were taken by venipuncture from the brachial vein and by heart puncture. A heparinized syringe was used for taking approxi-

mately 12 ml of blood. Pentobarbital (25 mg/kg i.v.) was used for anesthesia, and blood was sampled about 5 min after anesthesia was induced. One week or more was allowed to elapse between successive blood-samplings from the same animal.

The blood was then put in 40 ml tubes, centrifuged at 3000 rpm for 20 min at 4°C and the plasma removed. Aliquots of 5 ml of the plasmas were put in 10 ml tubes containing 5 ml of 0.4 N perchloric acid, shaken in ice-cold water bath for 5 min, and centrifuged at 6000 rpm for 20 min at 4°C to remove precipitated protein. The supernatant was adjusted to pH 8.3—8.7 with concentrated sodium hydroxide solution, and then the catecholamines were adsorbed on alumina in a small polypropylene column (Seikagaku, Sepacol, mini, pp). The alumina was washed with 5 ml of distilled water, and elution of the catecholamines was performed with 0.2 N acetic acid. The eluates were stored at −20°C until assay.

The catecholamines of the eluates were determined fluorimetrically by a THI (trihydroxyindole) method described by Anton & Sayre[1] after treatments according to the protocol described by Chang[6]. Blanks were prepared by following the same procedures as for the sample except that the oxidizing agent was added after the exposure to 5 N acetic acid.

Recoveries were tested as follows: the stock solutions of these catecholamines were freshly diluted with 0.02 N acetic acid to give solutions of four different concentrations embracing the expected range, the same procedure was followed for the solutions as for the plasma samples, and their recoveries were found to be around 65 %. The values given in the results are corrected for the percentage recovery (65 %).

The isolated rectum with its vessel supply was prepared according to the method described by Komori *et al.*[7], mounted in a 200 ml organ bath filled with Tyrode solution (composition, mM : NaCl 137.0 ; KCl 2.7 ; $NaH_2PO_4$ 0.4 ; $NaHCO_3$ 12.0 ; $MgCl_2$ 1.0 ; $CaCl_2$ 1.8 and glucose 5.0) and perfused at a constant rate (5.3 to 6.0 ml/min) by means of a roller pump via the arterial cannula with Tyrode solution. The bathing and perfusing solutions were aerated and maintained at 30°C. The isometric tension developed longitudinally was picked up by a force-displacement transducer (Nihon Kohden, SB-1T), amplified by a preamplifier (Nihon Kohden, RP-3), and recorded on a potentiometric recorder (Hitachi, 056). E was chosen to test its effect on the preparation, since it has a higher potency in relaxing the rectal muscle of the chicken[8]. The drug was applied to the preparation by perfusing with Tyrode solution containing it at appropriated concentrations.

Drugs and chemicals used were *l*-epinephrine base (Merck), *l*-norepinephrine bitartrate (Merck), heparine sodium salt (153.8 units/mg) (Wako), pentobarbital sodium (Somnopentyl, Pitman-Moore), aluminumoxide (Standardisiet ; Aktivitätsstufe II-III, Merck), perchloric acid (60 %, Wako), acetic acid (Wako), EDTA-2Na (Wako) and sodium hydroxide (Wako).

Mean data are presented with their standard errors. Student's *t*-test or Cochran's test was used for comparisons between mean values. Differences were determined to be statistically significant at $P<0.05$.

## RESULTS

### 1. Blood from brachial vein

Plasma norepinephrine (NE) and epinephrine (E) levels in each animal are listed in Table 1. The birds were laid gently on their side on a table, and blood was taken by venipuncture from the brachial vein. Blood sampling was performed within a few tens of seconds, whether the animal was anesthetized or not. The obtained values tended to be more variable in unanesthetized animals. The mean E level, 6.1 ± 1.4 ng/ml, was significantly lower than NE, 11.7 ± 1.4 ng/ml ($P<0.01$), although in 2 out

Table 1. Plasma epinephrine (E) and norepinephrine (NE) levels (ng/ml) in blood samples taken by venipuncture from the brachial vein in conscious and anesthetized chickens

| Conscious | | | Anesthetized | | |
|---|---|---|---|---|---|
| Bird No. | E | NE | Bird No. | E | NE |
| 1 | 8.1 | 7.8 | 1 | 5.7 | 6.6 |
| 2 | 3.8 | 15.3 | 2 | 3.8 | 6.0 |
| 3 | 8.7 | 9.4 | 3 | 3.8 | 4.6 |
| 4 | 2.7 | 17.4 | 4 | 5.0 | 4.6 |
| 5 | 3.5 | 13.1 | 5 | 2.2 | 5.4 |
| 6 | 4.2 | 14.3 | 6 | 3.8 | 6.0 |
| 7 | 3.7 | 6.9 | 7 | 4.2 | 5.0 |
| 8 | 13.8 | 9.7 | 8 | 4.6 | 4.3 |
| Mean±S.E. | 6.1±1.4 | 11.7±1.4 | | 4.1±0.4 | 5.3±0.3 |

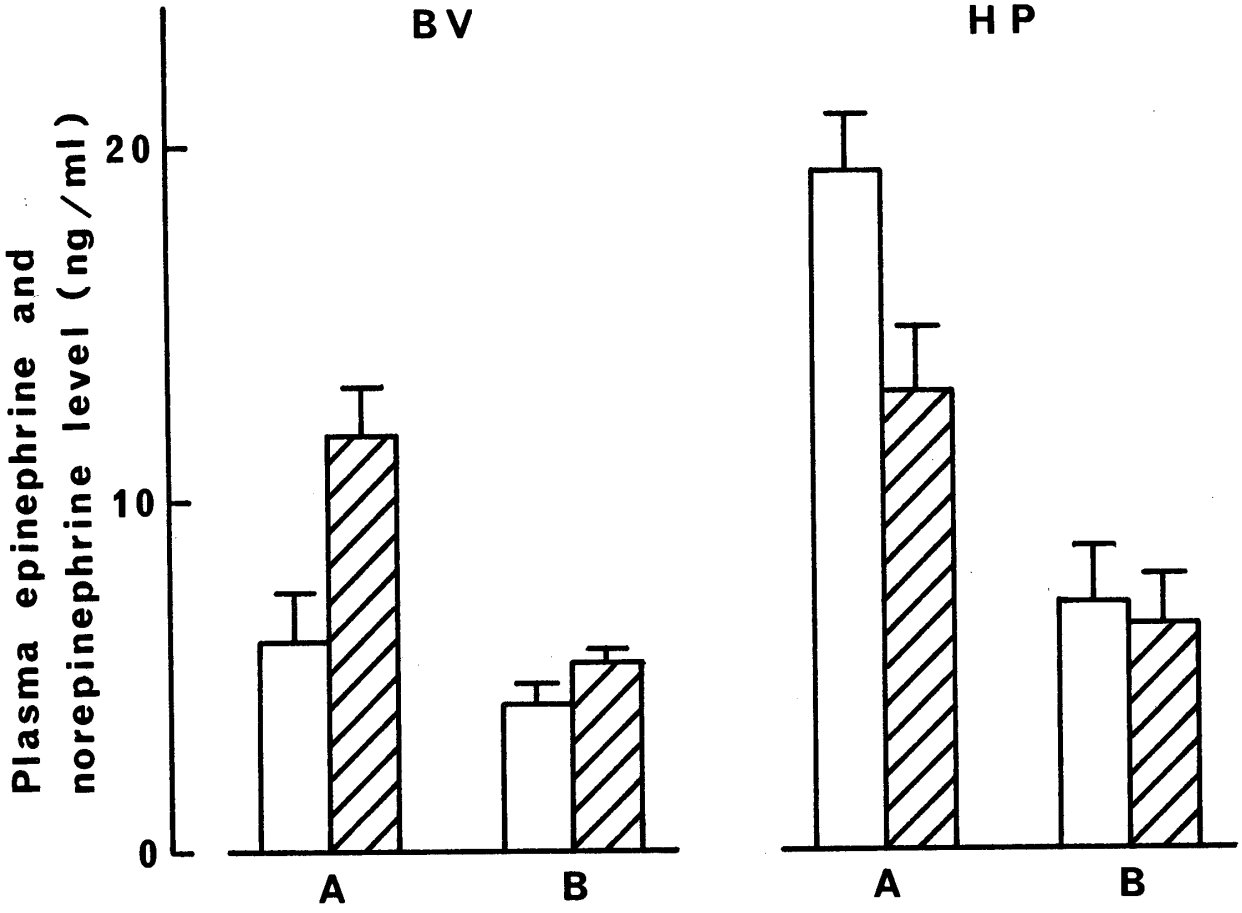


Fig. 1. Plasma epinephrine (E) (open column) and norepinephrine (NE) (hatched column) levels in blood samples taken by venipuncture from the brachial vein (BV) and by heart puncture (HP), and effect of pentobarbital anesthesia.
A : Control studies in which no anesthesia was given. B : Under anesthesia with pentobarbital (25 mg/ml, i.v.).
Heart puncture resulted in a significant rise of plasma E ($P<0.001$) which was anesthesia-sensitive. Anesthesia also caused a fall of plasma NE of BV and HP, representing about half decline. For more details see text.

of 8 animals in each group, E level was higher than NE level. As shown in Fig. 1, when the mean catecholamine levels compared between conscious and anesthetized animals, there was a significant difference in the two NE levels (11.7 ± 1.4 ng/ml and 5.3 ± 0.3 ng/ml) ($P<0.01$), whereas the difference between the E levels was small and not statistically significant.

### 2. Blood from heart puncture

Table 2 gives data from 8 conscious and 9 anesthetized animals. Birds were laid on their back on a table and immobilized by hand in a position suitable for heart puncture. Most of the birds not anesthetized struggled to escape when the needle for heart puncture pierced the thoracic wall. The time required to obtain blood varied from a few tens of seconds up to 1 min. The E level was higher than NE in 7 out of 8 conscious animals, giving the mean values of 19.1 ± 1.5 ng/ml for E and 12.9 ± 1.8 ng/ml for NE (Fig. 1). The difference between E and NE values was significant ($P<0.01$). Anesthesia resulted in a fall of both E and NE levels, and this was especially pronounced with E. As a result, the difference between the mean plasma E (6.9 ± 1.5 ng/ml) and NE (6.3 ± 1.2 ng/ml) levels became insignificant. It can be also seen in Fig. 1 that plasma E level was markedly increased by heart puncture with little change of NE, and that under anesthesia there were no significant differences between venipuncture and heart puncture in the plasma E and NE levels.

### 3. Blood removed from brachial vein under same conditions as for heart puncture

In an attempt to investigate the effect of stressors resulting from heart puncture on the plasma catecholamine levels, the birds were held under conditions similar to those for heart puncture, the

Table 2. Plasma epinephrine (E) and norepinephrine (NE) levels (ng/ml) in blood samples taken by heart puncture in conscious and anesthetized chickens

| | Conscious | | | Anesthetized | |
|---|---|---|---|---|---|
| Bird No. | E | NE | Bird No. | E | NE |
| 1 | 23.1 | 13.8 | 1 | 6.0 | 2.2 |
| 2 | 21.6 | 19.7 | 2 | 5.0 | 4.2 |
| 3 | 20.0 | 7.8 | 3 | 12.0 | 3.8 |
| 4 | 17.8 | 6.2 | 4 | 1.4 | 3.6 |
| 5 | 16.2 | 12.6 | 5 | 6.0 | 11.0 |
| 6 | 13.4 | 8.7 | 6 | 2.0 | 7.2 |
| 7 | 14.3 | 18.9 | 7 | 4.3 | 4.6 |
| 8 | 26.0 | 15.6 | 8 | 14.7 | 11.6 |
| | | | 9 | 10.0 | 8.4 |
| Mean±S.E. | 19.1±1.5 | 12.9±1.8 | | 6.9±1.5 | 6.3±1.2 |

Table 3. Plasma epinephrine (E) and norepinephrine (NE) levels (ng/ml) in blood samples taken by venipuncture from the brachial vein in the chickens under conditions similar to those for heart puncture, and then by heart puncture

| | Venipuncture | | Heart puncture | |
|---|---|---|---|---|
| Bird No. | E | NE | E | NE |
| 1 | 38.3 | 30.0 | 48.9 | 9.0 |
| 2 | 12.8 | 18.3 | 31.3 | 18.3 |
| 3 | 10.9 | 14.7 | 17.7 | 16.6 |
| 4 | 4.6 | 19.4 | 17.7 | 15.6 |
| 5 | 28.8 | 13.1 | 70.0 | 11.6 |
| 6 | 40.2 | 11.9 | 43.1 | 31.9 |
| Mean±S.E. | 22.6±6.2 | 17.9±2.7 | 38.1±8.2 | 17.2±3.3 |

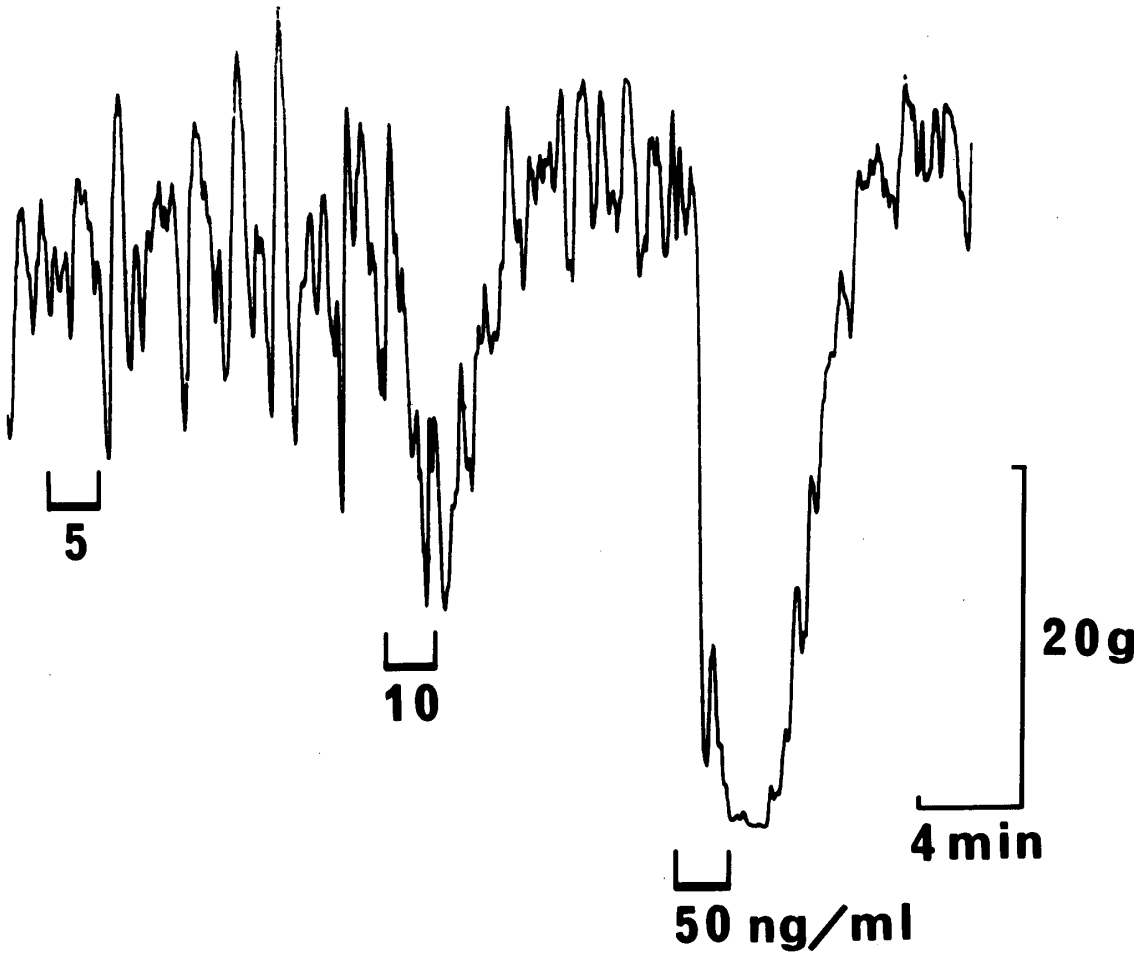


Fig. 2. Effect of epinephrine (E) on mechanical activity of the isolated perfused rectum of the chicken. E was perfused at concentrations of 5, 10 and 50 ng/ml ( ⌴ ) at 5.7 ml/min for 2 min. Tension changes were recorded isometrically.

needle for heart puncture was stuck into the thracic cavity, and then blood was taken first by venipuncture from the brachial vein and then by heart puncture. As presented in Table 3, E and NE levels in the venous blood rose to those for heart puncture. The mean E level was 22.6 ± 6.2 ng/ml and that of NE was 17.9 ± 2.7 ng/ml in the venous blood, which were much higher than those in normal controls (Table 1) and were not significantly different from the values for heart puncture of 38.1 ± 8. 2 and 17.2 ± 3.3, respectively.

4. Blood taken from heart of anesthetized birds after chest opening

As described by DeSantis *et al.*[4], two birds were anesthetized with pentobarbital (30 or 35 mg/kg, i.v.) and their chest wall was incised to expose the heart. A thick needle was inserted into the terminal region of the heart for drawing blood sample. An amount of about 12 ml blood was obtained. After a few minutes, another sample was obtained from the other region of the heart in the same way. Plasma E and NE levels in the first blood samples were 62 ± 1 ng/ml and 73 ± 20 ng/ml, respectively, which were much higher than any levels obtained above. Furthermore, E and NE levels in the second samples (299 ± 9 ng/ml and 145 ± 12 ng/ml) were about five times and two times higher than the respective values for the first samples.

5. Effect of E on isolated and perfused chicken rectum

The measured catecholamine levels in plasma varied from 3 ng/ml to 70 ng/ml according to the

conditions under which animals were placed before blood sampling. An attempt was made to determine the effect of circulating E on the rectum. E was perfused for 2 min at a constant rate at different concentrations via the blood vessel to the rectum. One of the typical results obtained from seven experiments is shown in Fig. 2. At 5 ng/ml, E had no effect on the rectum. When the concentration was increased to 10 ng/ml, tone of the rectum decreased, but spontaneous activity was still sustained. Application of E at 50 ng/ml resulted in sustained relaxation and cessation of spontaneous activity. After switching to the normal Tyrode solution, more than 6 min was required for complete recovery from the inhibitory effect.

## DISCUSSION

The changes of plasma levels of E and NE observed in this study provide evidence for fluctuations in activity of the sympathetic nervous system and adrenomedullary system during blood sampling in chicken. The activity appears to vary with conditions under which birds are placed. The plasma levels of E and NE (6.1 ± 1.4 ng/ml and 11.7 ± 1.4 ng/ml) in blood taken from the brachial vein of unanesthetized birds seem to be close to the baseline levels, since no significant influence on E level was observed during anesthesia with pentobarbital. E level is well known to be derived from adrenomedullary secretion in response to stressors[9)~12)]. The significant decrease in NE level during anesthesia suggests suppression of the activity of adrenergic neurons, since plasma NE levels result primarily from overflow from the postganglionic sympathetic neurons[9)13)14)]. Similar influence of pentobarbital anesthesia on the plasma NE level has been observed in dogs[15)]. Thus, under pentobarbital anesthesia, its inhibitory effect on sympathetic neuron activity may result in an underestimate of the baseline level of plasma NE.

The plasma levels of E and NE in blood taken by heart puncture are higher than those in blood taken by venipuncture. A simple explanation for the difference would be that immobilization by hand as well as pain during the procedure for heart puncture acts as stressful stimuli on birds which in turn lead to stimulation of the adrenomedullary system[16)]. This view is supported by the following findings: (1) There was a large and significant increase in E level which reflects an increase of the adrenomedullary secretion in response to stressors[9)~12)]. (2) Blood samples from the brachial vein of birds for which the same procedures as for heart puncture had been followed gave plasma E levels as high as for heart puncture. (3) Under anesthesia, no significant difference was found between heart and brachial venous plasma catecholamine levels. Suppression of adrenomedullary catecholamine release with barbiturates has been described in other animals[17)~19)]. In earlier work by Lin & Stukie[2)], the plasma levels of E and NE have been found to be about 6 ng/ml and 2 ng/ml, respectively, in blood obtained by heart puncture from unanesthetized birds. The origin of the difference from the present data remains uncertain. On the other hand, the plasma E and NE levels reported by Edens & Siegel[3)], who used blood samples obtained by heart puncture from anesthetized birds with pentobarbital, are in agreement with the present data.

Extraordinarily high plasma levels of E and NE in the chicken have been reported by DeSantis *et al.*[4)]. However, for the measurements they used blood samples from birds in which the thoracic cavity had been opened surgically. With two birds under similar surgical stress, marked increments of plasma catecholamines were observed. It has been described in other animals and human[11)13)] that E and NE in plasma are extremely increased during surgical stress even under general anesthesia, and this has been suggested to be related to increased release of the catecholamines in response to afferent pain stimuli from the area of trauma which are unable to be blocked with general anesthesia. Furthermore, a several-fold increase in the plasma NE and a several ten-fold increase in the plasma

E have been observed in dogs during hemorrhage or hemorrhagic shock[20]. This appears to give an explanation of the present finding that in two birds with their open chest the concentrations of E and NE in plasma were much higher in the second blood samples compared to those in the first samples (about five-fold increase in E and about two-fold increase in NE). Thus, in surgical birds, increased catecholamine levels may result from hypotensive episodes in addition to pain stimuli from the site of trauma.

The perfused isolated rectum was used as a model to determine the influence of circulating catecholamines on organs, since the high sensitivity to E is known (see **INTRODUCTION**). Infusion of E at 10 ng/ml or higher for a certain period into the preparation via the caudal mesenteric artery invariably produced inhibition of the mechanical activity. This also provides evidence that plasma E level under resting conditions is less than 10 ng/ml.

Therefore, it is safe to assume that the basal plasma levels of E and NE in the chicken under resting conditions fall within 4—7 ng/ml for E and 5—12 ng/ml for NE which obtained from blood samples taken by venipuncture with or without anesthesia and by heart puncture under anesthesia.

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# ニワトリにおける採血条件と血中カテコールアミン濃度

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## 要　　約

正常状態のニワトリの血中エピネフィリン（E）及びノルエピネフィリン（NE）量を知る目的で，色々な条件下で血液を採取し，THI法により，EとNEを測定した。翼下静脈からの採血で，無麻酔の場合，Eは6.1±1.4 ng/ml（n＝8），NEは11.7±1.4 ng/ml（n＝8）であり，麻酔下ではそれぞれ4.1±0.4 ng/ml（n＝8）と5.3±0.3 ng/ml（n＝8）であった。このNE量間の差は統計学的に有意であった。心臓穿刺による採血では，NE量（12.9±1.8 ng/ml）は殆んど変わらないのにE量（19.1±1.5 ng/ml）が著明に増大した。しかし，麻酔下ではこのようなことはみられなかった（E量，6.9±1.5 ng/ml；NE量，6.3±1.2 ng/ml）。2羽のニワトリを麻酔下で開胸し，露出した心臓から採取した血液中のEとNE量はそれぞれ62.3±1.3 ng/mlと73.2±20.3 ng/mlであった。摘出したニワトリ結直腸を血管を介して灌流しながら，Eを灌流液に添加して作用させると，10 ng/ml又はそれ以上の濃度の場合は自発運動の抑制と緊張度の低下が生じた。これらの結果から，正常状態のニワトリの血中エピネフィリン量は4から7 ng/mlの間に，又ノルエピネフィリン量は5から12 ng/mlの間にあると考えられた。