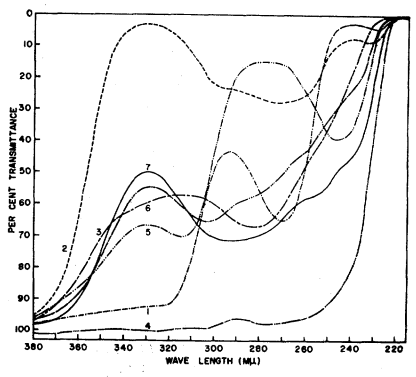

Fig. 1. Absorption peaks of tyrosine: (curve 1) 0.01 g of tyrosine per liter in 0.175*N* NaOH autoclaved 3 hours at 80 lb/in.^{2}; (curve 2) 0.01 g of tyrosine per liter in 0.175*N* NaOH; (curve 3) 3,5-diiodotyrosine in 0.125*N* NaOH autoclaved 4 hours at 80 lb/in.^{2}; (curve 4) plasma albumin in 0.125*N* NaOH; (curve 5) *p*-hydroxybenzoic acid in 0.125*N* NaOH autoclaved 4 hours at 80 lb/in.^{2}; (curve 6) plasma albumin in 0.125*N* NaOH autoclaved 3 hours at 80 lb/in.^{2}; (curve 7) tyramine in 0.125*N* NaOH autoclaved 4 hours at 80 lb/in.^{2}

same peak at 330 mμ following autoclaving with alkali (Fig. 1, curves 4 and 6); this is evidently due to the tyrosine content of the protein. The spectral evidence would indicate a structural change, perhaps to an *o*-quinoid structure, common to all the molecules mentioned above, rather than a conversion of the tyrosine to *p*-hydroxybenzoic acid (*2*).

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8 July 1957

Effects of Handling and Eating on Plasma Electrolytes

Variations in the concentrations of plasma potassium and sodium in mammalian venous blood have been principally investigated in studies of response to the administration of chemical agents or to the application of severe stressor agents. The present report (*1*) describes fluctuations in concentrations of plasma potassium and sodium in the goat which were found to occur after the handling necessary to bring the well-trained animal into the laboratory and during the eating of a greatly desired food. In addition, hematocrit value and plasma glucose concentration were measured under the same conditions, since changes in these physiological variables may help to clarify the mechanisms affecting concurrent changes in plasma potassium and sodium concentrations.

Four dioestral female and two male goats, from 2 to 2½ years of age, served as subjects. In preparing these animals for previous experiments, each animal had been handled by laboratory personnel on from 30 to 50 days. Three weeks before the experiment described in this report, the subjects were quartered by pairs in rooms in the laboratory building, where they were not disturbed except for evening feeding. Water, a legume hay, and grain (G.L.F., 18% Dairy) were continuously available ad libitum. During this time, three of the animals were subjected to five sham experimental sessions in which no blood samples were taken. The remaining three animals had recently been subjects in a study very similar to that reported here and were thus already familiar with the experimental procedure. In their living quarters the animals would neither approach an experimenter voluntarily nor allow an experimenter to approach without showing some sign of flight. However, once leashed, the animals led easily and showed no overt sign of fear of the experimenter. A few days before the experiment began, a polyethylene catheter was inserted into the external jugular of each animal and held in place with collodion for the duration of the experiment. From the first day of the experiment, the animals received restricted quantities of grain, randomly varying daily from zero to 1 qt per animal. Water and hay intake remained unrestricted.

The experimental sessions took place in the morning, and each session lasted about 3 hours. The experimenter entered the living quarters, leashed an animal, and led it down a 25-ft hallway to a experimental room. There the animal leaped onto a platform, and a strap passing through a wall ring was fastened around the abdomen. This initial stress to the animal lasted about 2 to 3 minutes and constitutes what is here referred to as "handling." The experimenter then withdrew to an adjoining room within view of the subject, approaching only to draw blood samples, painlessly, by means of the previously prepared catheter. After about 1½ hours, a pail full of grain was fastened to the platform and the subject was allowed to eat until obviously satiated—a period of from 10 to 30 minutes. All subjects showed signs of excitement and attempted to walk off the platform towards the experimenter when he entered carrying the pail, and all subjects ate as soon as the pail was sufficiently near. The experimenter then approached only to draw further samples, and the session was ended about 1½ hours after the presentation of food. Each subject was subjected to four experimental sessions, at least two of which were on successive days.

A total of 10 to 15 samples, of 5 ml each, were obtained during each experimental session (*2*). This report presents analyses of the samples obtained within 2 to 5, 15 to 25, 35 to 45, 55 to 65, and 75 to 85 minutes after the experimenter entered the animals' living quarters and of the samples obtained within the same time intervals after the presentation of grain—a total of 240 samples.

Each sample was withdrawn into a syringe containing about 0.01 mg of heparin and was immediately transferred to a 15-ml graduated tube and centrifuged for 5 minutes at 3000 rev/min in an International clinical centrifuge. The hematocrit reading was then made from the centrifuge tube, and the samples were centrifuged for an additional 10 minutes before further analyses were made. Plasma potassium and sodium concentrations were determined by the lithium internal standard method with the flame photometer (Perkin-Elmer, model 52C).

The mean data for all animals are plotted for each physiological measure in Fig. 1. The data for each of the six curves were treated independently in an analysis of the significance of linear and nonlinear regression components, by means of the method of orthogonal polynomials (*3*). Quadratic regression—that is, one point of inflexion—is significantly present beyond the 1.5-percent confidence level in five of the curves. However, only linear regression is significant for the hematocrit data obtained after eating.

It is evident from Fig. 1 that a rapid

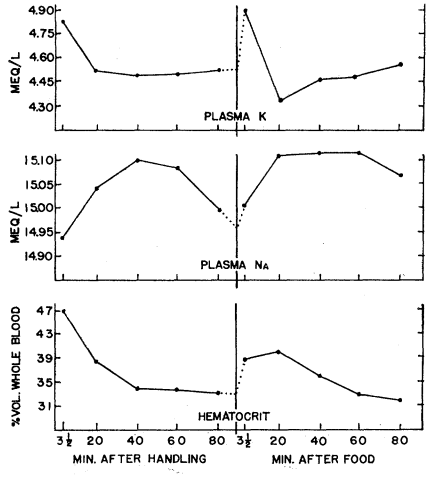

Fig. 1. Mean concentrations of plasma potassium (K) and sodium (Na) and hematocrit values for six animals as a function of time after the onset of handling, and of time after presentation of food. The central ordinate represents the time when food was presented, and the dotted lines represent linear extensions of the curves in the period between the last sample obtained before the presentation of food and the first sample obtained after the presentation of food.

rise in plasma potassium and hematocrit value occurred after the presentation of food. The significance of these rises was tested by pairing the mean value obtained for each subject within the last interval before, and the first interval after, the presentation of food. A t value of 5.32 was obtained for the differences between the paired hematocrit values, and a t of 4.34 was obtained for the potassium values. Each t is associated with 5 degrees of freedom, and both exceed the value necessary for significance at the 1 percent level.

The curve for plasma potassium obtained after handling, and its similarity to that obtained after the presentation of food, suggests that a rapid rise in plasma potassium concentration may have occurred within 2 minutes after the onset of handling. However, no samples were obtained in this interval, and direct evidence for this view is not presented here. In the case of hematocrit value, there is much evidence in the literature that a rise in hematocrit value occurs within 1 to 2 minutes after introduction of a variety of stimuli (*4*). It is therefore probable that, after handling, such a rise occurred before the fall shown in Fig. 1.

The initial rise in plasma potassium after the presentation of food is followed within 20 minutes by a marked, rapid fall below the prefood level and is then followed by a slow rise to prefood levels. The initial rise in hematocrit value is followed by a slow decline, which levels off in 60 to 80 minutes.

Plasma sodium concentration rises slowly after both handling and eating, reaches a peak within 20 to 60 minutes, and then slowly declines.

In a further study, plasma glucose concentration was followed in four of the animals in an experimental session similar to that described above. However, food was presented to the animals 30 minutes after the onset of handling, and samples were obtained at ½, 12, and 25 minutes after handling commenced and at 5, 15, and 25 minutes after the presentation of food. Plasma glucose determinations were made by the Somogyi-Shaffer-Hartmann titration method (*5*). No systematic changes in plasma glucose concentration were found to occur during the experimental sessions, and the mean of the variations which did occur was within the error of the method—that is, between 2 to 3 mg percent.

The variations in plasma potassium concentration that were found, in this study, to follow handling and eating in the well-trained goat are similar to previous results obtained after administration of adrenaline in other species (*6*). However, the lack of change in plasma glucose concentration following handling and eating suggested that effective quantities of circulating adrenaline are not present under these conditions. To obtain more evidence for this hypothesis, blood samples were taken from four of the catheterized animals at 5, 15, and 25 minutes before, and at the same times after, intracatheter injections of adrenaline chloride (*7*). It was found that, although injection of 50 μg of adrenaline produced an average peak increase in hematocrit value of 17 percent, (approximately the same increase as the average rise previously found following eating), it also produced a concurrent average peak rise of 24.4 percent in plasma glucose concentration.

Thus, although variations in concentrations of plasma potassium and sodium are found after handling and eating in the well-trained goat, these changes apparently are not mediated by the release of adrenaline. The occurrence of significant variations after handling and eating also suggests that suitable controls for these effects may be necessary in studies of blood electrolytes in vivo.

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References and Notes

1. These studies were conducted during the tenure of a research fellowship of the Social Science Research Center of Cornell University, and of a research fellowship of the U.S. Public Health Service.
2. F. Courtice [*J. Physiol.* (*London*) 102, 290 (1943)] estimates the blood-volume–weight relationship in the goat to be about 70 ml/kg. On the basis of this estimate, the blood loss per animal during an experimental session was calculated to range from 1.5 to 2.0 percent of total blood volume.
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7. Adrenaline chloride for this study was provided by Parke, Davis and Co.

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Early Sexual Maturity in the Female Short-Tailed Weasel

Delayed implantation in some of the Mustelidae has long been recognized (*1*). The age at which females first breed is not so well known. The female stoat (*Mustela erminea*) is said to first come into oestrous and ovulate spontaneously in May or June, before it is fully grown. Further ovulations take place at intervals of not less than a month before the next spring, when the animals become pregnant for the first time. Many first-year females mate at oestrous, but matings prove sterile outside of the limited breeding season (*2*). Young females of *Mustela frenata* mate when they are 3 or 4 months old, so that all females of this species may produce young in the spring, when they are about 1 year old (*3*).

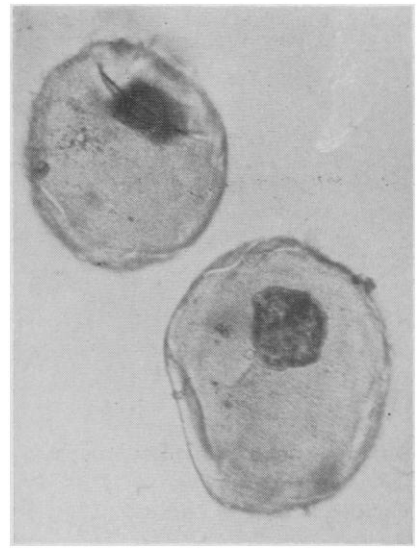

Fig. 1. Blastocysts of *Mustela erminea cicognanii*. The vesicles are partially collapsed but are as near perfect as one usually recovers in fresh animals. The inner cell mass in both embryos is slightly depressed (×52).

A female *Mustela erminea cicognanii* taken at Ithaca, New York, on 22 July 1956 was a young of the year. The skull and skeletal characters of this specimen, compared with those of young of known age, testify to its immaturity (*4*). Seven blastocysts were flushed from the uterine horns (Fig. 1). These, only slightly collapsed, measured 400 to 750 μ in diameter. The circular inner cell mass has a diameter approximately one-third that of the embryo. The hand-sectioned ovaries show well-developed corpora lutea. Although the female of this species may mate when it is 3 months old (possibly less), males do not become sexually mature until the late winter or early spring of their second year. This is the first positive evidence that the female short-tailed weasel may have a productive mating in its first summer.

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13 March 1958