Table III. Progressive disappearance of food constituents during passage through the digestive tract.

	Dry matter	Organic matter	Cellulose	Total *N*
Commelinum benghalense	100	100	100	100
Colobus A				
Saccus gastricus	90	86	82	76
Tubus gastricus	85	72	—	90
Caecum + proximal colon	80	66	56	52
Distal colon + rectum	60	55	60	41
Colobus B				
Saccus gastricus	62	59	46	67
Tubus gastricus	54	51	46	45
Caecum + proximal colon	62	51	—	45
Distal colon + rectum	74	64	—	62
Faeces	48	40	39	31

The concentration relative to lignin in the gut contents is given as a percentage of that in the food.

medium at about pH 6. The Ruminantia do this mainly by secreting large volumes of alkaline saliva[11]. The contents of both saccus and caecum-colon of our colobus monkeys were quite well buffered, 10 ml of supernatant fluid from the saccus requiring 0.7 ml N–H_2SO_4 to reduce the pH below a value of 3, and an equal volume from the caecum-colon requiring 1.5 ml N–H_2SO_4. It is not clear how the colobus maintains the buffered nature of the contents of its saccus. This compartment, like the camel's rumen[12], has a glandular epithelium which may secrete an alkaline fluid, but the colobus also has well-developed salivary glands (Table I) though nothing is known of the volume or composition of the saliva secreted.

By assuming that lignin is wholly indigestible, the apparent digestibility of the dietary constituents shown in Table II may be estimated. The amount of each component remaining undigested in successive compartments of the gut is given by its concentration per g lignin in the digesta divided by its concentration per g lignin in the food. Table III gives the values obtained, expressed as percentages of the amounts eaten. About 40–50% of dry matter, organic matter and cellulose had disappeared by the time the distal colon was reached, and some further loss appeared to take place before the faeces were voided in *Colobus* B. Both the saccus and the large intestine seemed to be important sites of digestion. Total nitrogen showed a rather greater apparent digestibility.

The absence of cellulolytic activity in colobus stomach contents reported by KUHN[6] and by OHWAKI et al.[8] was probably due to the diets of fruit and seeds being eaten by their animals. Foods rich in starch and sugar are associated with suppression of cellulolysis in cattle and sheep. Our observations indicate that microbial fermentation of food, including extensive cellulolysis, can occur in the colobus monkey receiving a leafy diet.

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Increased Plasma Creatine Kinase Activity in Rabbits: Effect of Systematically Repeated Blood Sampling[1]

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Summary. Various physical, chemical and biological factors are involved in an increased plasma creatine kinase activity. Repeated blood sampling induced in all rabbits studied a reaction of similar pattern but of different intensity, expressed by a maximum of plasma CPK activity. The physiological origins of this variation of CPK activity seems to be, as seen in control animals, the consequence of emotional stress due to handling and possibly an additional stress due to the venepunctures.

Increased plasma or serum creatine kinase activity (E.C.: 2-7-3-2; CPK) is generally used in pathology to detect cardiac (myocardial infarction) or muscular anomalies (dystrophy or hypertrophy)[2]; it would also enable the determination of the degree of stress susceptibility which partially conditions certain pathological forms of muscular hypertrophy in pigs[3–6].

Interpretation of a significant increase in this enzymatic activity may be complicated by many factors, such as ingestion of alcohol, strennuous exercise[7,8], hypothyroidism[9–11], cerebrovascular disease, burns, adaptation to cold[12], i.m. injections[13], or, as in man, emotional stress[2,14].

During experiments on several breeds of rabbits, we observed, as have many scientists, a considerable variability in the results; this led us to examine the importance of another factor which could affect the plasma CPK activity, that is systematically repeated blood sampling.

Material and methods. In a preliminary study on both anesthetized and non-anesthetized rabbits, we verified

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that the enzyme activity did not vary significantly according to the origin of the plasma studied (heart, external saphene vein, jugular vein, carotid artery, portal vein and inferior vena cava). Finally, we took the blood samples from the external saphene vein (v. tarsea recurrens) because of its accessibility, and were thus able to keep manipulations to a minimum.

The experimentation was carried out under identical conditions on 7 male New-Zealand rabbits, weighing about 3.5 kg; the blood samples (about 1.5 ml) were collected with heparinized syringes, every 3 h for 36 h, then at longer intervals from the 36th to the 81st h; all experiments were started at 09.00 h of the day. The plasma was separated in an Eppendorf 3200 centrifuge, and the CPK activity determined immediately using a Gilford Model 240 recording spectrophotometer by a method already described[15]; we verified that the heparin did not interfere with the enzymatic assay. 3 similar experimentations on sham-operated animals were then carried out. In the first control series, the rabbits were manipulated every 3 h without puncture to verify the influence of emotional stress on CPK activity. In the 2nd control series, animals were manipulated and submitted to repeated venepunctures (every 3 h) without blood sampling to verify the consequence of physical stress on the enzymatic activity. In the last control series, the rabbits were not manipulated. In the 3 control series, the blood was sampled at the initial experimental hour, at the 24th h and at the end of the 81st h of manipulation The CPK activity was then determined as previously described.

Results and discussion. Systematically repeated blood sampling induces in the rabbit a quite considerable increase in CPK activity (more than 54 times in one of the animals studied), with a maximum of activity appearing very regularly between the 21st and the 24th experimental hour.

The enzymatic activity pattern in Figure 1 can be divided into 2 successive phases: 1. Systematic CPK activity increase, reaching a maximum between the 21st and the 24th experimental h. 2. Decrease in activity until the end of the 81 h of manipulation, inspite of continued blood sampling. The CPK activity increase observed varies with the animal, which may correspond to differences in stress susceptibility, as might have been expected. Therefore, there is not conclusive evidence whether the increase of the enzyme activity is due to the blood loss itself (18 ml in the first 36 h), to the repeated venepunctures (physical stress), or to emotional stress. Control animals permitted us to compare the effects of physical or emotional stress and to test their relative importance. The results of the 3 control series (see details in material and methods) plotted in Figure 2 shows, on the one hand, that the evolutive pattern of plasma CPK activity during the 81 experimental h is similar to that previously mentioned; on the other hand that this evolutive pattern differs in intensity according to the different types of manipulations.

The activity increase described on Figure 1 is essentially due to stress during the experiments and not to the blood loss itself. If we compare the importance of the factors inducing this stress (Figure 2, a, b and c), it must be concluded that emotional stress alone, at least in rabbits, can cause a substantial increase in CPK activity; the emotional effect seems more important than the physical effect, which seems to appear (Figure 2, b and c) only in addition to emotional stress.

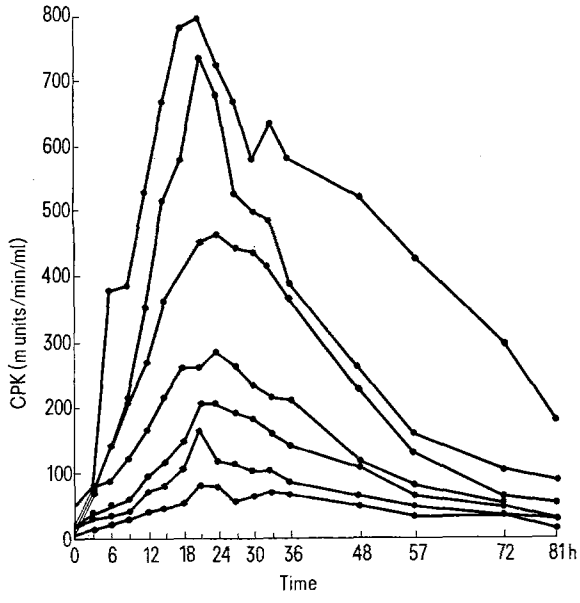

Fig. 1. Evolution of plasma CPK activity in 7 rabbits submitted to systematic blood sampling.

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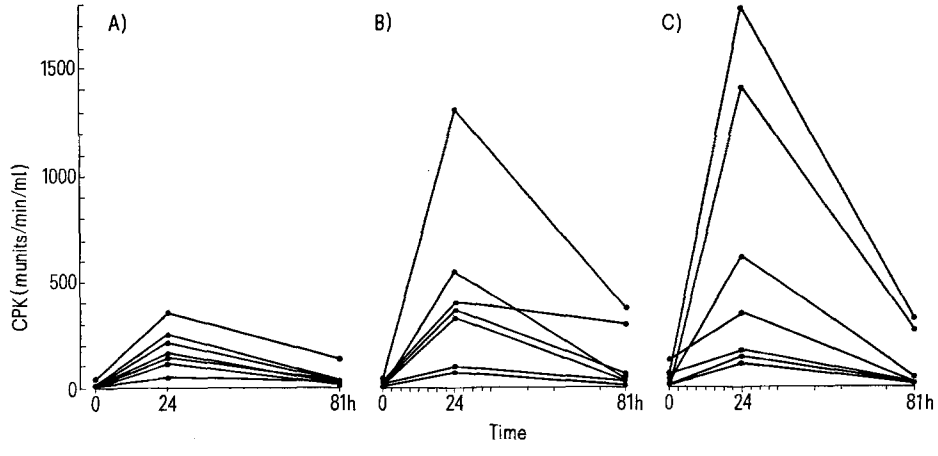

Fig. 2. Plasma CPK activity in control rabbits. A) No manipulated. B) Manipulated every 3 h without venepuncture. C) Repeated venepunctures without blood sampling.

The biochemical origins of the enzymatic activity increase itself, whether in our particular case, or in those mentioned in the introductary paragraph, remains problematic: several authors, already cited, advance the hypothesis that such increases in activity result from an increase in membrane permeability (presumably due to an influence of corticosteroids[16,17]), without however producing supporting evidence. Likewise, the light decrease in hematocrit observed during the experiments does not explain the pattern of plasma CPK activity, and particularly the existence of a peak of activity.

The appearance of a maximum between the 21st and the 24th experimental h might correspond to an infradian rhythm of stress-susceptibility[18], inducing a similar rhythm of plasma CPK activity; however, as this phenomenon did not recur during the 81 experimental h, such a hypothesis can scarcely be retained. It seems more logical that such an activity pattern represents an adaptation by the animals to experimental conditions, causing, after an increase, a decrease in the release of the enzyme, since a phenomenon as rapid cannot be explained by the modulation of the mechanism of synthesis and utilization of the CPK at organism level.

Therefore, although these results are pertinent only for rabbits, the interference of numerous factors with serum or plasma CPK activity (and particularly the stress accompanying blood sampling in rabbits) would seem to indicate that a certain care is necessary in the interpretation of results in physiology and experimental pathology[19], or of certain clinical cases[20,21] concerned with this enzyme[22,23].

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Effect of 6-Hydroxydopamine Pretreatment on Spontaneous Convulsions Induced by Barbital Withdrawal[1]

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Summary. Following withdrawal from chronic barbital administration, 6-hydroxydopamine pretreated rats show a greater number and an earlier onset of spontaneous convulsive seizures than do rats pretreated with the saline-ascorbic acid vehicle.

Abrupt withdrawal following the long term intake of barbiturates results in an abstinence syndrome characterized by the occurrence of spontaneous convulsive seizures in man (ISBELL et al.[3]) and in rats (ESSIG[4]). The biochemical mechanism underlying the appearance of these spontaneous convulsions has been only minimally investigated. Acute treatment with anesthetic dosages of of pentobarbital has been shown to decrease the turnover of noradrenaline and dopamine (CORRODI, FUXE and HÖKFELT[5], PERSSON and WALDECK[6]). Changes in the activity of noradrenaline and/or dopamine containing neurons following the long term administration of barbiturates could be related to some of the symptoms of the abstinence syndrome, i.e. spontaneous convulsions. Thus it was of interest to determine the effect that the chemical lesioning of brain noradrenaline and to a lesser extent brain dopamine nerve ending with 6-hydroxydopamine would have on the onset and incidence of spontaneous convulsions in rats withdrawn following chronic barbital treatment.

Materials and methods. A small polyethylene cannula (PE10) was implanted into each lateral ventricle of adult male Sprague-Dawley rats. The cannulae were secured in place with dental cement and 2 stainless steel screws implanted into the skull. Each cannula was kept patent by the use of a stainless steel stylet of the exact length to extend just to the tip of the cannula. 40 rats subsequently referred to as 6-hydroxydopamine pretreated were anesthetized with ether and given 100 µg of 6-hydroxydopamine hydrobromide (6-HODA) (Sigma) in 20 µl of saline (1 mg ascorbic acid per ml) in each lateral ventricle. 40 rats subsequently referred to as saline treated were also anesthetized and received only the saline vehicle. The treatment was repeated on alternate days for a total of 3 injections. After the 3rd injection, the stylets were removed, the cannulae were clipped off next to the skull and then plugged with dental cement. The animals were housed 4 per cage and exposed to a 14:10 light dark lighting regime (lights on 06.00–20.00 h). 1 week later 32 of the 6-HODA pretreated and 23 of the saline pretreated control rats were started on a dosage regimen of increasing concentrations of barbital in the drinking water. The lower sample sizes reported in the results section reflect the loss of animals during the 6 weeks of the barbital regimen. The initial concentration of barbital (not sodium salt) was 1 mg/ml which was given for 3 days. The bitter taste of barbital was disguised with saccharine (20 mg/l). The control or non-barbital treated rats were given the same concentration of saccharine in their

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