solution of sucrose containing 0.8 m*M* sodium citrate with 0.05 m*M* calcium chloride (Figure 3).

The inhibitory effect of sodium on the mechanical response suggests that sodium is not responsible for depolarization in the heart perfused with half isotonic solution of sucrose. But before the heart has adjusted to the sucrose solution, the action of sodium is stimulatory (Figure 4) and, therefore, in Ringer solution sodium is responsible for depolarization. This suggests the existence of 2 excitabilities in the frog's heart.

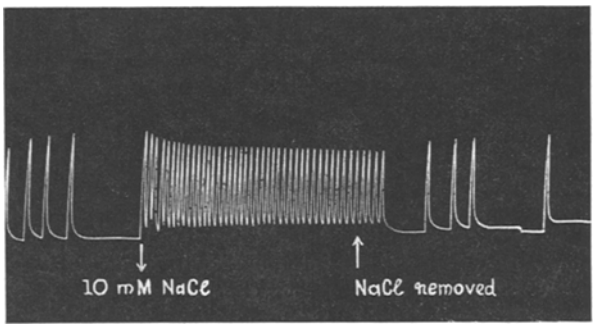

Fig. 4. The stimulatory action of sodium chloride (between arrows) on frog's heart perfused with half isotonic solution of sucrose for $^1/_2$ h.

Efflux of anions might be responsible for excitability and action potential in sucrose solution. But the effects of cation influx and anion efflux should reinforce and not antagonize each other. These experiments therefore support the suggestion that in frog's heart there are 2 independent and antagonistic mechanisms for excitability and action potential[8]. One of them is dependent upon ionic fluxes[9], and the other independent of such fluxes[5].

Résumé. Le cœur de grenouille continue à battre de lui-même et répond à une stimulation électrique durant 5–6 h s'il reçoit une injection contenant une demi solution isotonique de saccharose, de pyrophosphate de sodium et d'adénosinetriphosphate ou de citrate de sodium. Le chloride de sodium annule ces deux réactions.

I. SINGH, S. I. SINGH and N. V. RAJU

Department of Physiology, Medical College, Agra (India), 2 April 1969.

[9] A. L. HODGKIN and A. F. HUXLEY, J. Physiol., Lond. *117*, 500 (1952).

Variations of Plasma Kininogen Content due to High Sodium Intake in Rats[1]

Kininogen are plasma proteins contained in the pseudoglobulin fraction, which precipitate with ammonium sulfate between 33 and 46% saturation[2]. They are substrates to various enzymes such as trypsin[3], kallikreins[4,5] and others that liberate vasoactive peptides (Plasma kinins), when incubated in appropriate conditions.

Some physiological and pathological roles have been attributed to kinins[6–11]. Preliminary observations induced us to think of a relation between the kallikrein-kininogen-kinin system and the metabolism of sodium so far not described. The present work was done to measure the variations of the kinin substrate under conditions of sodium loading.

Materials and methods. Male albino rats (Houssay strain), weighing between 150 and 200 g were used. They were fed with standard rat food, containing about 100 mg sodium per 100 g; distilled water, 1% and 2% NaCl solutions were used as drinking fluids.

Blood (0.7 ml) was obtained by cardiopuncture (always performed between 14.00 and 15.00 h) with a heparinized syringe and immediately centrifuged at 1500 *g* at 4 °C for 30 min. Plasma was separated and kininogen samples were prepared according to FASCIOLO et al.[12]. Standard bradykinin used for bioassay was BRS640, Sandoz Lab., Basel, Switzerland.

Experimental and results. Several kinds of experiments were performed: A group of 5 rats was given distilled water ad libitum, and blood was drawn after 3 days, then water was replaced by 1% NaCl and on day 6 blood was drawn again. This procedure was repeated again, giving water for 3 days and 1% NaCl for 3 more days, and blood was drawn at the end of each period. Plasma Kininogen Content (PKC) was estimated (Figure 1). There was a significant decrease of PKC after the administration of NaCl solution as drinking fluid. The return to the water diet increased PKC in each single rat, but the values remained below the initial ones after 3 days of water intake.

Similar results were obtained in a differently designed experiment: a group of 16 rats was put in cages and given standard rat food. They drank distilled water and blood was drawn on days 0 (12 rats) and 3 (16 rats). On the third day, water was replaced by 1% NaCl and blood was drawn again on days 6 (16 rats) and 9 (13 rats, 3 died after cardiopuncture on day 6). PKC was also estimated.

[1] A preliminary report of this paper was presented at the IX Congress of the Asociación Latinoamericana de Ciencias Fisiológicas, held at Belo Horizonte, Brasil, July 7–12th, 1969.

[2] M. ROCHA E SILVA, W. T. BERALDO and G. ROSENFELD, Am. J. Physiol., *156*, 261 (1949).
[3] J. MARGOLIS, S. BRUCE, B. STARZECKI, G. H. HORNER and D. F. J. HALMAGY, Austr. J. exp. Biol. Med. Sci. *43*, 237 (1965).
[4] E. WERLE and U. BEREK, Angew Chem. *60*, A53 (1948).
[5] J. MARGOLIS and E. A. BISHOPS, Nature *194*, 749 (1962).
[6] M. E. WEBSTER, N. S. SKINNER and W. J. POWELL, Am. J. Physiol. *212*, 553 (1967).
[7] S. M. HILTON, Ann. N.Y. Acad. Sci. *104*, 275 (1963).
[8] M. ROCHA E SILVA, Ann. N.Y. Acad. Sci. *104*, 190 (1963).
[9] P. MELCHIORRI, in *Bradykinin and its Precursors* (Ed. F. SICUTERI; Centro editoriale Publictario Italiano, Rome 1963), p. 65.
[10] D. ARMSTRONG, J. B. J. JEPSON, C. A. KEELE and J. W. STEWART, J. Physiol. *135*, 350 (1957).
[11] N. S. LANDERMAN, M. E. WEBSTER, E. C. BECKER and RATCLIFFE, Jl. Alergy *33*, 330 (1962).
[12] J. C. FASCIOLO, J. ESPADA and O. A. CARRETERO, Acta physiol. latinoam. *13*, 215 (1963).

There was no significant difference between days 0 (4.1 ± 0.73 µg/ml) and 3 (4.3 ± 0.98 µg/ml; and between days 6 (2.5 ± 0.68 µg/ml) and 9 (2.6 ± 0.99 µg/ml). On the other hand, there was a highly significant difference when PKC values of rats on water intake are compared with those of the same rats on 1% NaCl intake (Figure 2). The tendency to decrease occurred in each single rat. Mean decrease was 1.6 ± 0.25, S.E.M. µg/ml ($p < 0.001$).

In the last experiment, the comparative effect on PKC due to intake of 1% and 2% NaCl was studied. 3 groups of rats were given distilled water, 1% and 2% NaCl solutions ad libitum respectively as drinking fluid for 5 days. Then PKC was estimated. The results (Figure 3) indicate that both saline solutions have a similar decreasing effect (3.06 ± 0.49 and 3.08 ± 0.27 µg/ml as compared with control rats: 4.61 ± 0.57 µg/ml, $p < 0.001$).

Blood sampling had no effect whatsoever on PKC. 11 rats were heart-punctured 3 times at intervals of 3 days, and PKC was also measured. There was no statistically significant difference between rats with no previous puncture (3.98 ± 0.82 µg/ml), and those with 1 and 2 previous punctures (4.06 ± 0.45 and 4.20 ± 0.49 µg/ml respectively).

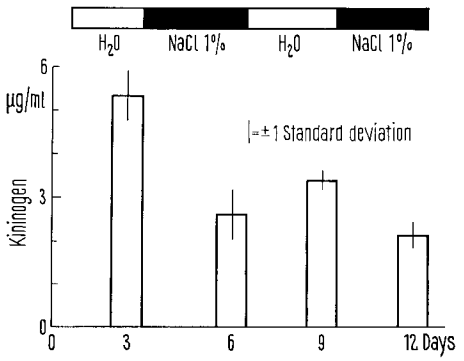

Fig. 1. Effect of 1% NaCl intake on kininogen content of plasma.

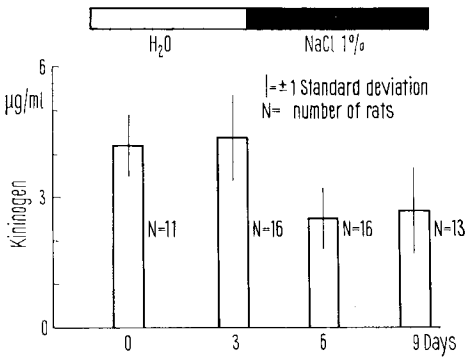

Fig. 2. Effect of 1% NaCl intake on kininogen content of plasma.

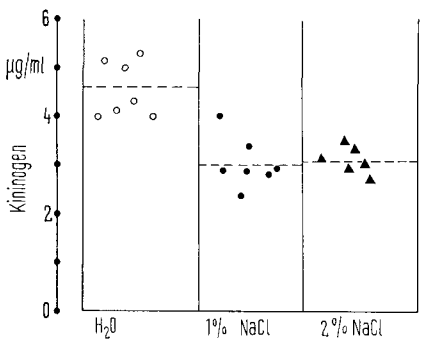

Fig. 3. Effect of 1% and 2% NaCl intake on the kininogen content of plasma.

Discussion. The close relationship between PKC and the variations of sodium intake (i.e. its decrease with higher sodium intake) suggests a correlation between the way the body regulates its sodium and water requirements and a certain function of the kininogenase-kininogen-kinin (KKK) system. This correlation might be through the ADH regulation of osmolality; or through the renin-angiotensin-aldosterone regulation of sodium reabsorption in the distal tubule; or through a separate, independent effect on sodium regulation and excretion.

These experiments do not exclude the possibility that salt loading may have produced expansion of plasma volume and thus a decrease in PKC by a dilution effect. The large decrease found in most cases, however, makes this explanation unlikely.

Since the work of JACOBSEN[15], and other workers, the existence of 2 different kininogens in plasma of several mammalians, including rats, is known. Kininogens I and II differ in molecular weight, location of the kinin moiety, affinity with ion exchange resins and susceptibility to serve as substrates to plasma and glandular kallikreins. The fact that the intensification of the high sodium diet (i.e., giving different concentrations of NaCl solutions or prolonging the experimentation time from 3–6 days) did not reduce further the kininogen content of plasma, makes us think that perhaps only one of the substrates is mainly involved in the phenomenon.

So far, the KKK system has been believed to play a role in some vasodilatations, edema forming and pain producing, in inflamation. However, there is no definite proof to attribute a physiological or a pathological role to the system, inspite of the well-known pharmacological actions of kinins. Perhaps these powerful vasoactive agents, as was the case with angiotensin, may prove to be a part of the complex mechanism of body sodium regulation[16].

Zusammenfassung. Bei männlichen Ratten nimmt bei kochsalzreicher Diät die Kininkonzentration im Plasma ab und normalisiert sich auf gemässigten Salzhaushalt.

A. NASJLETTI and M. E. AZZAM

Department of Physiology, School of Medical Sciences, Universidad Nacional de Cuyo, Mendoza (Argentina), 9 October 1969.

[13] S. JACOBSEN, Br. J. Pharmac. Chemother. *28*, 64 (1966).
[14] M. YANO, S. NAGASAWA, K. HORIUCHI and T. SUZUKI, Jl. Biochem. *62*, 504–506 (1967).
[15] M. YANO, H. KATO and T. NAGASAWA, Jl. Biochem. *62*, 386 (1967).
[16] We thank Dr. J. C. FASCIOLO for his scientific advice. Also, the technical assistance of Mr. D. GIMENEZ is acknowledged.