become inexcitable, the strength of their mechanical responses is diminished in the absence of calcium.

It is of further interest to establish whether the diminished response of a single fibre is related to electrical membrane changes. Twitch tensions, resting and action potentials were therefore measured from surface fibres of the same muscle. In Ca-free solution, action potentials steadily decreased in amplitude; the smallest recorded propagated impulses had an amplitude of approximately 30 mV. In contrast with this situation, when membrane potentials are lowered by raising the external potassium concentration (constant $[K]_0 \times [Cl]_0$ product) in the presence of calcium, the mean peak tension of excitable fibres is unaltered and the action potential continues to exhibit an overshoot. Thus calcium lack leads to a disturbance of the mechanisms governing the action potential and the active state which is not simply the result of the decreased membrane potential.

In conclusion we may say that calcium is essential for the maintenance of the membrane potential, development of the action potential and the full development of tension in the single fibre. The alteration of the action potential is closely associated with, and may be the cause of, the decrease in intensity of the active state. The decrease of mechanical response of the whole muscle in calcium free solutions is due in part to inexcitability of individual fibres and partly to a reduction in the intensity of response of excitable fibres.

Effects of zinc. The zinc content of skeletal muscle (0.8 mM/kg wet weight[10]) is similar to that of calcium, although extracellular concentrations (0.1 mM[11]) are less, and free zinc concentrations are very low. In earlier studies[12], the action of zinc as a relaxing factor in the isolated contractile system was investigated. In the present investigation another effect of zinc, an increase in the duration of the active state, has been found when zinc is added to the solution bathing an intact muscle, in confirmation of SANDOW and ISAACSON[13,14]. A distinct increase in the height and duration of twitches of the whole muscle and single surface fibres could be detected at a threshold concentration of $5 \cdot 10^{-6} M$. The tetanic tension was unaffected. Membrane potentials of surface fibres were unaffected over a period of 2 h at concentrations of zinc up to $5 \cdot 10^{-5} M$. There was an increase in the duration of the action potential simultaneously with the increase of duration of the active state. The falling phase was prolonged and the area of the action potential was increased twofold in $5 \cdot 10^{-5} M$ zinc, the rising phase and the overshoot being little affected.

It was found that zinc cannot replace calcium in the excitation-contraction process. The loss of tetanic response of the whole muscle in Ca-free solution could not be restored by adding zinc, the only effect upon the residual mechanical activity being an increase in the duration of the active state. The low membrane potentials were not affected by zinc.

It would appear that extracellular zinc is not an essential ion for contraction over a period of hours, unlike calcium, but may nevertheless be of physiological significance by modifying the duration of the active state.

Zusammenfassung. Membranpotential, Aktionspotential und Spannung der Einzelzuckung einer Einzelfaser des M. sartorius von *Rana temporaria* nehmen in der calciumfreien Badeflüssigkeit progressiv ab. Extrazelluläres Zink hingegen ist für die Kontraktion nicht notwendig, beeinflusst aber in physiologischen Konzentrationen die Dauer der Aktivität.

K. A. P. EDMAN[15] and D. W. GRIEVE[16]

Department of Physiology, University College, London (England), August 9, 1961.

[10] K. A. P. EDMAN, Acta physiol. scand. *46*, 209 (1959).
[11] B. L. VALLEE and M. D. ALTSCHULE, Physiol. Rev. *29*, 370 (1949).
[12] K. A. P. EDMAN, Acta physiol. scand. *49*, 330 (1960).
[13] A. SANDOW and A. ISAACSON, Biochem. biophys. Res. Comm. *2*, 455 (1960).
[14] A. ISAACSON and A. SANDOW, Fed. Proc. *20*, 301 (1961).
[15] Present address: Pharmacology Department University of Uppsala (Sweden).
[16] Present address: Human Biomechanics Laboratory, Medical Research Council, Hampstead, London (England).

Activity of some Enzymes in Experimental Hypertension in the Rabbit[1,2]

In 1945, PICKERING[3], demonstrated two phases of renal hypertension in rabbits by clamping one renal artery after removing the contralateral kidney. After 7 to 8 days, the clamp was removed and normal blood pressure restored, whereas hypertension still persisted if the clamp was removed after 7 to 8 weeks. This work has been repeated and confirmed by others[4,5].

It has been postulated that in the early phase, the kidney produces a renal pressor substance, probably renin, which is responsible for the maintenance of the hypertension. There seems to be agreement that in rabbits, dogs and sometimes rats, the early phase is different from the chronic phase of experimental hypertension[6]. The interpretation of the late phase of hypertension has led to many hypotheses, all of which suggest the possibility of an extra renal regulatory pressor mechanism[6]. However, there is evidence that the kidneys participate even in the pathogenesis of chronic hypertension.

The present work was designed to test the importance of the renal pressor mechanism in the various stages of hypertension in the rabbit, by observing the ability of renal tissue to produce renin and to produce and/or metabolize other pressor substances. In hypertensive rabbits, we have followed renin levels and the response to rabbit antirenin during the acute and chronic phase of hypertension. Other kidney enzymes, which might be involved in the formation and metabolism of pressor substances, have been studied, i.e., renal dopadecarboxylase (DDC) which forms dopamine from dopa and monoamine-oxidase (MAO) which forms the corresponding aldehydes by oxidation.

Material and Methods. Rabbits of both albino and black strains were used. Under nembutal anesthesia, right nephrectomy was performed through a loin incision. Two weeks later, the contralateral left renal artery was clamped with a silver clamp 10–14 mm long and 0.4–0.8 mm lumen

[1] This investigation was aided in part by grants from the Office of the Surgeon General, United States Army (no. DA-49-007-MD-429), and from the National Health Institute, United States Public Health Service (no. H-444 (C 10)).
[2] A preliminary report appears in Fed. Proc. *19*, 100 (1960).
[3] G. W. PICKERING, Cl. Sci. *5*, 229 (1945).
[4] H. A. FLEMING, J. Path. Bact. *65*, 441 (1953).
[5] P. M. DANIEL, M. M. L. PRICHARD, and J. N. WARD-MCQUAID, Cl. Sci. *13*, 247 (1954).
[6] M. A. FLOYER, Brit. Med. Bull. *13*, 29 (1957).

diameter[7]. 25 out of 120 rabbits became hypertensive in the absence of azotemia. Blood pressure was measured by the GRANT and ROTHSCHILD method[8].

Renin was assayed on heart's blood drawn in heparinized syringes. The hypertension formed on incubation was tested in cats as previously described[9]. Hypertensinase was removed by acidification to pH 3.9 for 20 min at 37°C. Antirenin was obtained by the injection of hog kidney extract into dogs. Dog plasma was used as the rabbit antirenin source. Although the activity of this plasma was not titred in dog antirenin units[10], tests of potency indicate that the antirenin effect, although definite, was small. MAO was prepared from kidney mitochondria as previously described[11]. The enzyme activity was tested by the method described by CREASEY[12]. DDC was prepared from the high speed supernatant after sedimentation of the mitochondria. The method used for assaying the activity of the DDC has been described by SCHALES and SCHALES[13].

Results. Intravenous injection of 2 cm³ of dog plasma containing antirabbit renin lowered the blood pressure by passive transfer in the three hypertensive rabbits tested. The antirenin depressor effect is most evident during the first weeks of hypertension and became less evident, but was not abolished after 5 and 8 weeks of hypertension (Figure 1). In this experiment, an identical specimen of antirenin, in equal amounts from the same source, was given to all rabbits. It was believed that the amount of antirenin administered was adequate since the blood pressure, when affected, returned to the previous normal values. Three normotensive rabbits received similar doses of antirenin without change in blood pressure. The relationship between blood pressure, renin levels (10 rabbits), MAO and DDC (12 rabbits) activity at different stages of hypertension is shown in Figure 2. During the first week of hypertension, significant amounts of renin were found in the plasma in 8 out of 10 determinations, whereas in 4 determinations between fourteen days and two months of hypertension no significant amount of renin was detected. While the plasma renin was elevated, values for MAO and DDC in the kidney remained unchanged. In the chronic phase, however, the oxygen uptake ($Q^{O_2}_{O_2}(N)$) as an expression of MAO activity decreased from 127 (range 19) to 56 (range 25). The carbon dioxide output ($Q^{N_2}_{CO_2}(N)$), as an expression of DDC activity, increased from 86 (range 20) to 148 (range 31).

Discussion. It appears from our results that the amount of plasma renin, detected by our method, decreased with prolonged hypertension. This finding corroborates with that of HAYNES and DEXTER[14] who were unable to show the presence of renin in the blood of dogs with chronic hypertension.

In a few experiments, antirenin appeared to produce some hypotensive effect even at a late stage. Antirenin has been shown often to be effective in dogs which have chronic hypertension[15].

The importance of MAO and DDC in the genesis of hypertension has been stressed by many authors[16–18]. In our experiments MAO and DDC activities in the kidney were changed only in the late phase of experimental hypertension. While, admittedly, direct relation between the activities of these enzymes in the kidney of the hypertensive animals cannot be directly related to the hypertension, the data are of interest in terms of the following hypothesis. That the kidney may be responsible for chronic hypertension in at least one species is well documented by the cure of such hypertension occasionally by unilateral nephrectomy and by the absence of hypertension in renoprival man[19].

Effect of duration of hypertension in rabbits on the depressor effect of antirenin

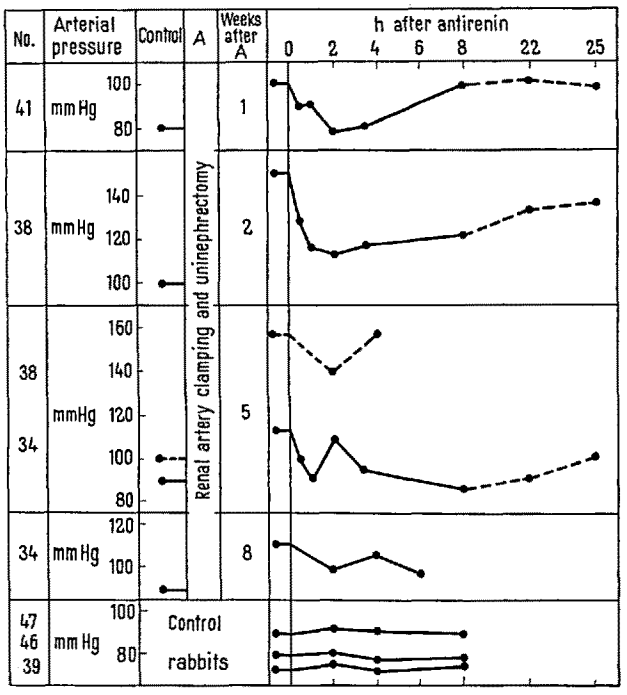

Fig. 1. Blood pressure response to anti-hog-renin in 6 rabbits.

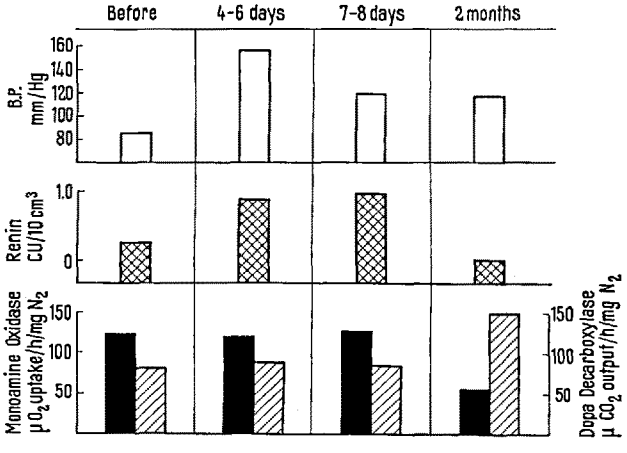

Fig. 2. Blood pressure (upper) and renin levels in cat unit (middle) are presented; (lower) MAO activity on dark bars and DDC activity on light columns.

[7] G. W. PICKERING and M. PRINZMENTAL, Cl. Sci. *3*, 357 (1938).
[8] R. T. GRANT and P. ROTHSCHILD, J. Physiol. *81*, 265 (1934).
[9] L. DEXTER, F. W. HAYNES, and W. C. BRIDGES, J. clin. Invest. *24*, 62 (1945).
[10] H. LAMFROM, E. HAAS, and H. GOLDBLATT, Amer. J. Physiol. *177*, 55 (1954).
[11] C. GIORDANO, J. BLOOM, and J. P. MERRIL, Exp. *16*, 346 (1960).
[12] N. H. CREASEY, Biochem. J. *64*, 178 (1956).
[13] O. SCHALES and S. S. SCHALES, Arch. Bioch. *24*, 83 (1949).
[14] F. W. HAYNES and L. DEXTER, Amer. J. Physiol. *150*, 190 (1946).
[15] G. E. WAKERLIN, Circulation *17*, 653 (1958).
[16] P. HOLTZ, K. CREDNER, and H. WALTER, Z. physiol. Chem. *262*, 111 (1939).
[17] R. J. BING, Amer. J. Physiol. *132*, 497 (1941).
[18] H. A. SCHROEDER, Science *95*, 306 (1942).
[19] J. P. MERRILL, C. GIORDANO, and R. D. HEETDERKS, Amer. J. Med., in press.

The absence of renin in chronic hypertension suggests another renal mechanism. Such a mechanism might be adduced from the decrease of MAO activity and the increase in DDC activity in the kidney of our hypertensive animals. At least twelve amino acids normally found in the kidney are decarboxylated, forming the corresponding pressor amines. DDC is known to act on dopa with the formation of dopamine. Thus increase in DDC activity suggests the possibility of an increased formation of the corresponding pressor amines[20]. Since all of these amines constitute substrate for MAO which oxidizes them with the formation of ammonia and the corresponding aldehyde with loss of vaso-activity, a decrease in MAO activity leads to the tentative suggestion that a decreased rate of coversion of pressor amines might play a role in continuing elevation of blood pressure. A working hypothesis of the mechanism involved in the experimental hypertension produced by constriction of the renal artery in the rabbit is represented schematically in Figure 3.

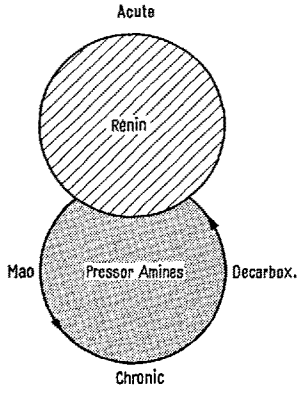

Fig. 3. Scheme of renal hypertensive mechanisms operating during acute (upper circle) and chronic (lower circle) hypertension.

This hypothesis might be in line with some interesting pharmacological work[21] performed in hypertensive patients in whom α-methyl dopa, a specific inhibitor for dopa decarboxylase, consistently lowered the blood pressure.

Zusammenfassung. Bei durch Nierenarterien-Drosselung nach kontralateraler Nephrektomie chronisch hypertonischen Kaninchen wird mit Antirenin regelmässig eine bestimmte hypotensive Wirkung erzeugt. Auffallend niedrige Reninblutwerte, stark herabgesetzte Monoaminoxydase-Aktivität und deutlich gesteigerte Dopadecarboxylase-Aktivität sind charakteristisch. Der Befund zeigt die Bedeutung der Niere in der Erzeugung der Blutdruckamine im Laufe einer chronischen Hypertonie.

C. GIORDANO[22], H. A. SAMIY, J. BLOOM, F. W. HAYNES, and J. P. MERRILL

Department of Medicine, Peter Bent Brigham Hospital and Harvard Medical School, Boston (Mass.), July 24, 1961.

[20] H. A. SCHROEDER and N. S. OLSEN, Amer. chem. Soc. Advances in Chemistry, Series No. 2, Chemical Fastors in Hypertension, May 23 (1950).
[21] J. A. OATES, JR., L. GILLESPIE, JR., R. CROUT, and A. SJOERSMA, J. clin. Invest. *39*, 1015 (1960).
[22] Present address: Renal Laboratory, Istituto di Patologia Medica, Policlinico, Napoli (Italy).

Carbon Dioxide Fixation by the Achloric Alga *Prototheca zopfii*

Whole cells of Prototheca, a genus of achloric algae morphologically related to Chlorella[1], are unable to utilize carbon dioxide[2] and the growth of the organism depends on the availability of a source of organic carbon.

Investigations in progress in this laboratory have shown that one species, *P. zopfii*, possesses enzymes of both the glycolytic and the hexose monophosphate pathways[3]. The present report deals with the fixation of radioactive carbon dioxide by cell-free extracts of this species.

Cell-free extracts of glucose-grown cells were prepared by mechanical disruption (full details will be published later) and incubated for 5 min at 37°C in glass-stoppered vials. The reaction was arrested by adding 10 *N* HCl ($^1/_{10}$ vol) and the vials aereated to remove unfixed carbon dioxide. The precipitated proteins were removed by centrifugation and aliquots of the supernatant solutions were plated onto metal planchets. Radioactivity was measured with a windowless flow counter.

The results (Table) show high carbon dioxide fixation only in the presence of P-enolpyruvate (PEP). Ribulose-1, 5-diphosphate (Ru-1, 5-P)[4] and pyruvate were not carboxylated to a comparable extent. In the case of Ru-1, 5-P, the substrate for the carboxylation is most probably still PEP that may arise from the 3-P-glycerate (3PG) present as a contaminant in the preparation of Ru-1, 5-P. Twice as much radioactivity is, indeed, fixed if only 3PG is used as a substrate. A conversion to PEP seems to be taking place also when pyruvate is used; the fixation of carbon dioxide is stimulated by ATP and ADP but not IDP nor by adding reduced di- and triphosphopyridine nucleotides, alone or with a hydrogen recycling system such as glucose-6-P and glucose-6-P dehydrogenase.

Carbon dioxide fixation by cell-free extracts of *Prototheca zopfii*

Addition	Carbon dioxide fixed (c.p.m./ml)
$MgCl_2$	93
$MnCl_2$	122
PEP + IDP	18675
PEP + $MgCl_2$	1185000
PEP + IDP + $MgCl_2$	1252000
PEP + IDP + $MnCl_2$	1142500
PEP + ADP + $MgCl_2$	1112500
Pyruvate + ATP + $MnCl_2$	1252
Pyruvate + ADP + $MnCl_2$	1322
Pyruvate + IDP + $MnCl_2$	542
Ru-1,5-P + $MgCl_2$	1572
3PG + $MgCl_2$	3227

The basal assay system contained 50 μ*M* of Tris buffer (pH 7.9); 0.1 μ*M* of Versene (pH 7.9); 0.125 μ*M* of $NaHC^{14}O_3$ (specific activity 7.8 μC/μ*M*) and 0.1 ml of enzyme preparation (0.49 mg of protein). When present, 10 μ*M* of metal activator and 1.25 μ*M* of substrate and coenzyme were added.

[1] R. CHODAT, in *Mat. Fl. Crypt. Suisse 4*, 11 (1913).
[2] P. J. CASSELTON, Nature *183*, 1404 (1959).
[3] O. CIFERRI, in preparation.
[4] The author is grateful to Dr. M. GIBBS for the generous gift of a sample of ribulose-1,5-diphosphate.