for 10 weeks. This experiment has been carried out using pooled tissue from paired animals and has been repeated 5 times.

The time course for corticosterone-stimulation of tyrosine amino transferase activity in rat liver and kidney has been measured in tissue obtained from pairs of adrenalectomised rats, killed at various times during a 6-h period following a 5 mg corticosterone injection. This experiment has been repeated.

Results and discussion. The time course for corticosterone-stimulated tyrosine amino transferase activity (Figure 1) confirms a maximum activation of the enzyme during the 4–5-h period following hormone treatment[6]. There was no hormone effect on citrate synthase activity in rat liver (Table) but a characteristic glucocorticoid-stimulation of succinate dehydrogenase and tyrosine amino transferase activity.

The apparent succinate dehydrogenase activation[2] by corticosterone is believed to reflect an increased redox state in the mitochondria, which in liver may be associated with increased fat oxidation. The failure of actinomycin D to superinduce this enzyme activity is in contrast to the superinducible tyrosine amino transferase activity. This result is taken to support the suggestion[4] that the highest affinity binding sites for corticosterone in rat liver may be responsible for all transcriptional events associated with the hormone action (Figure 2), whereas the basic corticosterone-receptors may be associated with the translational control of key gluconeogenic enzymes, acting as repressors of post transcriptional inhibitors[6]. Increased fat oxidation in the liver may have a permissive role in the gluconeogenic effect of corticosterone, giving rise to key intermediates that exert secondary control of gluconeogenic pathways.

In rat kidney there is evidence of corticosterone-activation of all 3 enzymes studied which are sensitive to actinomycin D inhibition. These enzyme activities compare with those measured in kidneys taken from animals on a low Na^+ diet. In particular the citrate synthase activity corresponds with aldosterone-stimulated levels[7]. As there was no stimulation of this enzyme in liver it is probable that this effect of high concentrations of corticosterone in kidney is mineralocorticoid.

The observed loss of a mineralocorticoid response in kidneys taken from animals on a high Na^+ diet may be understood in terms of the 'escape phenomenon'. The cause of this effect is not understood but it is of interest to note that in recent work[8] a normal hormone response has been re-established following treatment with prolactin or oxytocin.

Zusammenfassung. Die biochemischen Wirkungen von Aldosteron in der Rattenniere und Corticosteron in der Rattenleber wurden untersucht und der Mechanismus der Hormonwirkung diskutiert.

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Protein Biosynthesis and Hyperpolarization of Cells

The main idea of this report is to show that one of the most important mechanisms regulating both the membrane potential level of cell and the development of hyperpolarization is connected with protein biosynthesis. In this respect, 2 groups of facts dealt with in this report will be relevant: a) activation of protein biosynthesis leads to the development of cell membrane hyperpolarization; b) inhibitors of protein biosynthesis prevent the development of hyperpolarization.

Methods. The experiments were carried out on 120 white rats aged 8–10 months. The narcosis, urethane 0.1 g/100 g body weight, was used. The membrane potential of liver cells and muscle fibres (m. gastrocnemius, m. gracilis) was determined by method of intracellular measurement using a glass 1 μm tip diameter microelectrode[1]. The RNA content was determined by the orcinol or spectrophotometric method[2]. In order to study the RNA synthesis, the solution of C^{14}-adenine 30 μCi per 100 g body weight, or $Na_2HP^{32}O_4$ 30 μCi/100 g, were i.p. injected to animals 1 h before killing. Radioactivity was expressed in imp/min/mg RNA. Protein was estimated according to method of LOWRY et al.[3]. For electron microscopic studies, liver samples were treated by glutaraldehyde and OsO4 in cold and then embedded into epon 812. The JEM-100 electron microscope with magnification 40,000 was employed.

Results and discussion. Activation of protein synthesis in liver cells and single muscle fibres was achieved in different ways. It was shown sharp activation of protein synthesis in liver cells occurred following the bloodletting. Furthermore, it enabled the restoration of serum protein content. As indicated in Figure 1, already 12 h after bloodletting (2–3% of the body wt.) one could observe a rise in membrane potential level of liver cells as well as in RNA and protein contents. A rise was also observed in the RNA synthesis intensity. Thus, if before bloodletting, the RNA renovation rate was 928 ± 84 imp/min/mg RNA, 24 h after bloodletting it equalled 1730 ± 104 imp/min/mg RNA.

The development of cell membrane hyperpolarization and activation of protein biosynthesis coincide with obvious structural changes revealed electron-microscopically. Increased in liver cells were found the numbers of membranes of endoplasmatic net, of ribosomes as well as those of nuclei per cell (Figure 2).

Analogous findings were obtained at activation of protein biosynthesis in liver cells occurring during their regeneration. It was found that the extirpation of 2/5 of liver leads to the increase in membrane potential and RNA renovation (Figure 3).

Then, a number of hormones, as genetic inductors, can markedly effect the processes of protein biosynthesis[4,5]. As is seen in Figure 4A, rats injected by insulin (0.16

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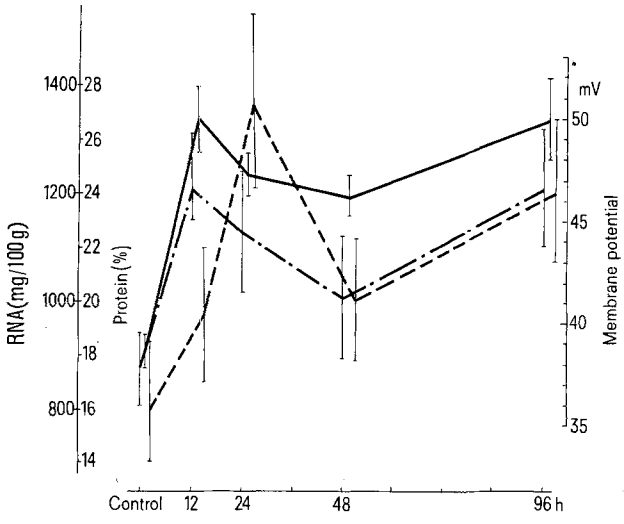

Fig. 1. Change of membrane potential level, protein content and RNA in liver cells at various terms after bloodletting. Solid line, membrane potential in mv; Broken line, protein in percent; Broken-dotted line, RNA in mg/100 g.

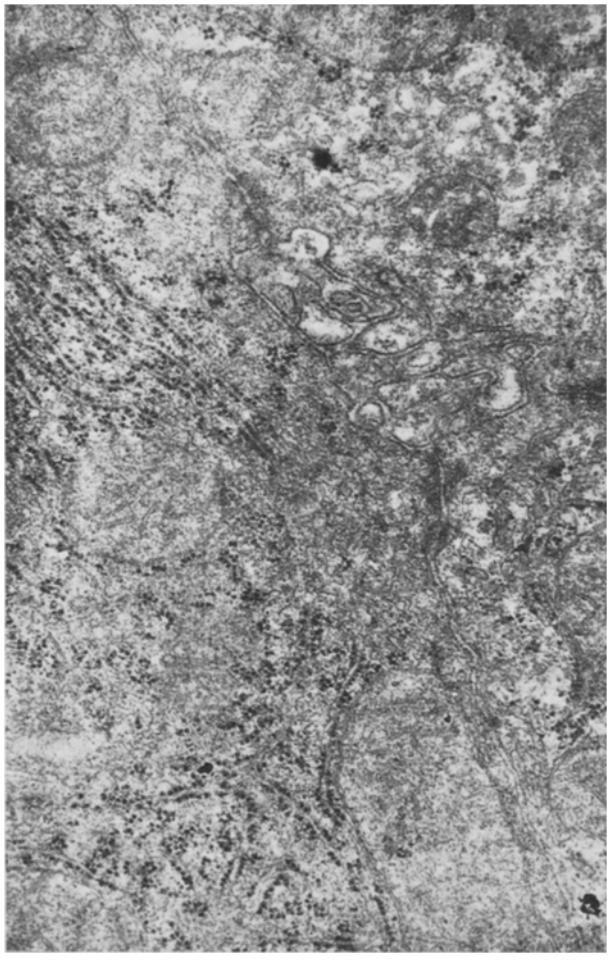

Fig. 2. Electronogram of liver 24 h after bloodletting. Plenty of ribosomes along membranes of the well-marked uneven endoplasmatic reticulum, matrix of mitochondria is somewhat thickened. × 40,000.

U/100 g), estradioldipropionate (0.5 mg/100 g) and 1-dehydromethyltestosterone (0.2 mg/100 g), the hormones greatly influencing protein biosynthesis, demonstrated an increase in the membrane potential level in muscle fibres and liver cells. Thus, activation of protein biosynthesis in different cells caused by different ways brings about one and the same results, namely, hyperpolarization.

The hypothesis put forward by us on a relationship between protein biosynthesis and the higher membrane potential level of a cell has been confirmed by findings of another series of experiments, in which inhibitors of protein biosynthesis prevent the development of hyperpolarization[6-9]. As inhibitors of protein biosynthesis, we used actinomycin D blocking the synthesis of DNA-dependent RNA; ribonuclease capable of enzymic RNA hydrolysis; and X-ray irradiation (Figure 4B).

The described influence of substances employed and ionizing irradiation is realized through shifts in protein biosynthesis. The 3 groups of facts which could be distinguished may give evidence of the following:

1. Inhibitors of energetic metabolism (NaF; monoiodacetate; 2,4-dinitrophenol) do not eliminate hyperpolarization of muscle fibres induced by hormones or denervation[8,10].

2. Hormones used in our experiments (insulin, estradioldipropionate) essentially effect the energetic process, metabolism and contents of macroergic phosphorus compounds. Inhibitors of protein biosynthesis (ribonuclease, actinomycin D), however, when used in the doses stated do not show any essential effect upon contents of adenosine triphosphoric acid and creatine phosphate.

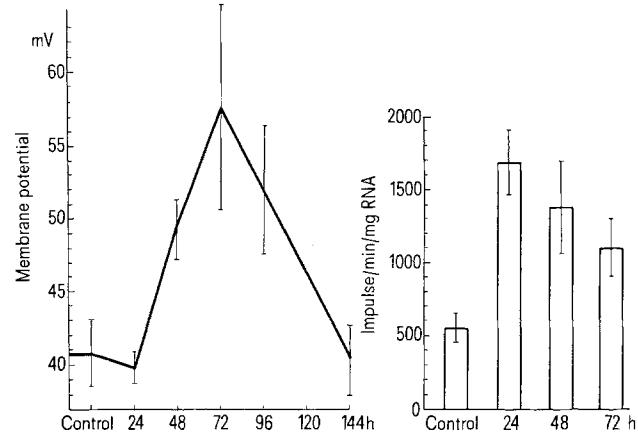

Fig. 3. Change in membrane potential of liver cells (A) and RNA renovation (B) in different periods after extirpation of 2/5 of liver tissue.

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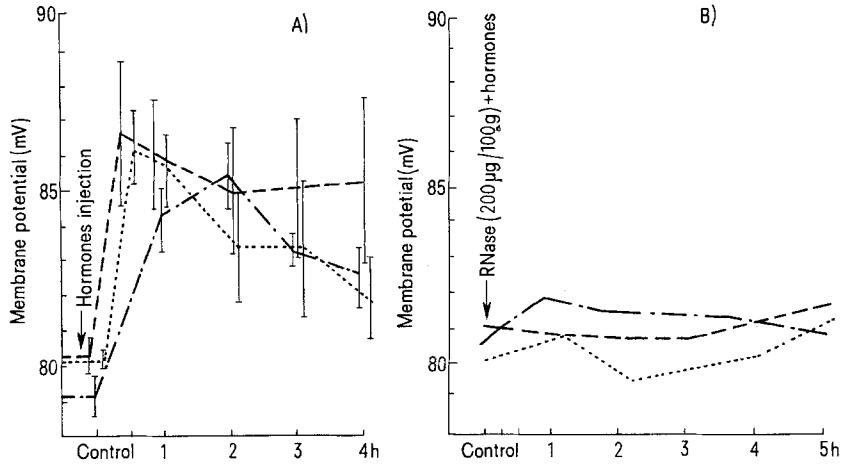

Fig. 4. Effect of ribonuclease on membrane potential level of single muscle fibres. (A) Change of membrane potential level of single muscle fibres induced by hormones. (B) Effect of ribonuclease on the change in membrane potential level caused by hormones. Broken line, insulin (0.16 units/100 g); Dotted line, estradioldipropionate (0.1 mg/100 g); Broken-dotted line, 1 dehydromethyltestosterone (0.2 mg/100 g).

3. Inhibitors of protein biosynthesis (actinomycin D, ribonuclease) prevent the activation of RNA synthesis in liver cells occurring after bloodletting and with insulin injection. Thus, 24 h after bloodletting there was an increased RNA renovation rate from 928 ± 84 imp/min/mg RNA to 1730 ± 104 imp/min/mg RNA. With a prior actinomycin D injection, the inclusion was found to increase insignificantly (1260 ± 160 imp/min/mg RNA).

Thus, with the activation of protein biosynthesis, the hyperpolarization of a cell developes. Inhibitors of protein biosynthesis prevent the development of hyperpolarization. It can be stated that a relationship between protein biosynthesis and cellular function plays an important role in the development of hyperpolarization and regulation of membrane potential level.

ВЫВОДЫ. Активация биосинтеза белка в клетке ведет к развитию ее гиперполяризации.

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29 January 1973.

Oxidative Phosphorylation in *Trypanosoma cruzi*[1]

The occurence of oxidative phosphorylation in Trypanosomatidae has been demonstrated with *Crithidia fasciculata*[2–5] and *Trypanosoma mega*[6]. In this communication we describe experiments that prove the operation of oxidative phosphorylation in *T. cruzi*, the agent of Chagas' disease. The demonstration is based on the effect of specific inhibitors on: 1. the redox state of the parasite cytochrome 'b'; 2. respiration; and 3. the intracellular concentration of high-energy phosphate and P_i.

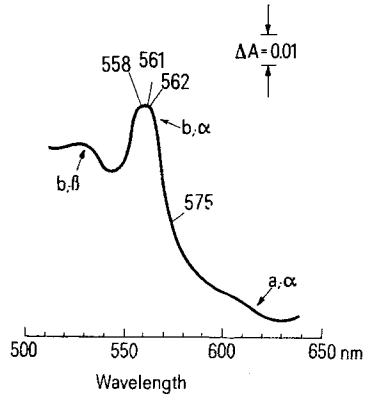

Fig. 1. Spectrum of *T. cruzi*. 27 mg epimastigotes suspended in 1.75 ml of a 30% (v/v) glycerol-standard saline medium mixture. To the measuring cuvette was added dithionite; flat oiled paper was placed in the reference cuvette[20]. 5 mm Light path. For other conditions see Methods.

Materials. *T. cruzi* epimastigotes were grown at 28 °C as described earlier[7,8]. The medium (solid phase) contained 5% (v/v) sheep blood. Unless stated otherwise the concentration of cell suspensions was measured by the weight obtained after drying washed epimastigotes at 100–104 °C for 24 h (the dry-weight was about 15% of

[1] Abbreviations. In addition to standard abbreviations, the following are used: PCP, pentachlorophenol; CCP, carbonylcyanide-*m*-chlorophenyl hydrazone; DCCD, N,N'-dicyclohexylcarbodiimide; P_i, inorganic orthophosphate; P_7, heat and acid labile phosphate; P_{as}, total acid-soluble phosphate; $P_0 = P_{as}$ less ($P_i + P_7$).

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