measured with an intracellular microelectrode, is decreased with the decrease of extracellular Ca^{++} concentration[6].

To determine whether the extracellular Ca^{++} which diffuse into the smooth muscle cell can initiate the contraction, the following experiment was performed in the K-depolarized preparation. After pretreatment of *Taenia coli* first for 30 min with a Ca^{++}-free K-Locke's solution containing 1 mM of ethylene-diamine-tetra-acetate (EDTA) and then for 10 min with a Ca^{++}-free solution without EDTA, different concentrations of Ca^{++} were added. Contractions by Ca^{++} were observed.

Figure 2 shows the relation between the initial speeds of contraction and the concentrations of Ca^{++} added. The minimal concentration of Ca^{++} required to initiate contraction varied from 10^{-6} to $10^{-5}M$ in this investigation. Initial speeds of contraction were linearly proportional to added Ca^{++} concentrations in the range from 2×10^{-5} to $1 \times 10^{-3}M$.

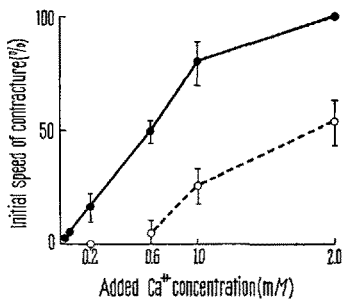

Fig. 2. The relation between initial speed of contraction produced by calcium and concentration of calcium added in the depolarized smooth muscle. This experiment was performed with isotonic measurement to observe complete relaxation of the smooth muscle. The complete relaxation was observed within 30 min of treatment with Ca-free K-Locke's solution containing 2 mM of EDTA. After an additional treatment of the preparation with Ca-free K-Locke's solution without EDTA, different concentrations of Ca were added. Initial speeds of contraction were measured and plotted against added Ca concentrations as % of the speed observed with 2 mM of Ca. Open circle, initial speeds of contraction in the presence of 1 mM manganese; closed circle, without manganese.

It is reasonable to assume that intracellular Ca^{++} in these preparations were lowered to almost identical concentrations by identical pretreatment before different concentrations of Ca^{++} were added extracellularly. If this assumption is accepted, Figure 2 shows that the initial speeds of contraction are linearly proportional to $[Ca]_0/[Ca]_i$ from 2×10^{-5} to $1 \times 10^{-3}M$. In addition, both amplitude and initial speed of contractions were strongly decreased in the presence of 1 mM of Mn^{++}, which is thought to inhibit Ca^{++} influx into the muscle cell[7].

Assuming that the action potential of the smooth muscle is 60 mV and the specific capacitance is 10 $\mu F/cm^2$, Ca influx/spike would be, roughly, more than 3×10^{-12} moles/cm^2. As it is reported that the diameter of the smooth muscle is about 4 μ and the length is about 150 μ[8], the circumferential area would be about 1.9×10^{-5} cm^2 and the volume would be about 1.9×10^{-9} cm^3. From these, it is calculated that the total Ca^{++} entering a single muscle cell is about 5.7×10^{-17} moles/spike and this Ca^{++} is also calculated to be 3×10^{-5} as a intra-cellular final concentration which may be enough to initiate the contraction of smooth muscle.

These results and calculations strongly suggest that the Ca^{++} which enter into the smooth muscle cell during the spike potential can be directly related to the contractile elements.

Further studies of the dissociation of electrical and mechanical activities should be undertaken.

Zusammenfassung. Die Ca-Wirkungen auf elektrische und mechanische Aktivität glatter Meerschweinchenmuskeln wurden untersucht. Die Resultate sprechen dafür, dass Kalziumionen, die während der Dauer des Aktionspotentials in die glatte Muskelzelle eintreten, am kontraktilen Elementarvorgang beteiligt sind.

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Metabolic Effects of Thyroxine in Infant Rats

It has been established that the thyroid hormone regulates growth, differentiation and oxidative metabolism in various animals. During the early postnatal period, thyroxine deficiency has impairing effects on the development of many organs, especially of the central nervous system, e.g. in mammals[1–4]. At about the age of 2 weeks the thyroid gland becomes important for the thermoregulation of the rat[5]. During this period, the activation of thyroid is pronounced[6,7]. Shapiro[4] found that thyroidectomy or thyroxine administration did not influence metabolic rate or body temperature in rats during the first 2 weeks of life. It was observed by Hemon[8], however, that thyroxine does not increase the oxygen consumption in rats aged 10–12 days when measured at an external temperature of 29.5 °C, but at 35 °C the increase was approximately 25%.

As the results of our pilot experiments were in conflict with the previous ones concerning the metabolic rate, the effect of thyroxine on the succinic dehydrogenase activity of liver, skeletal muscle and brain homogenates, and also

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on the oxygen consumption of the animals in various ambient temperatures, was studied.

Material and methods. Sprague-Dawley rats were used as experimental animals. Litters were reduced to 8 animals. Half of them received the experimental treatment and the other half served as controls. Thyroxine solution (dissolved in 0.1*n* NaOH and diluted with 0.9% saline) and TSH saline solution were injected s.c. once a day. The control animals received corresponding amounts of saline.

The rate of oxygen consumption was measured with Pauling's paramagnetic oxygen analyzer (Beckman E2). For continuous measurement of the body temperatures, a thermocouple was fixed on the neck skin with a piece of adhesive plaster in youngest age groups and inserted into the colon in animals aged 20 days. The oxygen consumption was measured at a falling ambient temperature of + 36 to + 15 °C. During this period, which lasted for 45–60 min, the body temperature decreased from 37 to 28 °C. The oxygen consumption was recorded at each full degree of decreasing body temperature. The results are expressed as ml/g of body weight/h.

The activity of succinic dehydrogenase complex of liver, thigh muscle and brain homogenates was measured at 37 °C with the method of KUN and ABOOD[9] as described earlier[10]. The results were calculated to give μg of reduced triphenyltetrazolium chloride (TTC) for 1 mg of tissue (fresh weight) in 10 min.

Results and discussion. The mean values of oxygen consumption of rats as a function of their body temperature are presented in Figure 1. The graphs indicate that a 3 day treatment with a thyroxine dose of 300 μg/kg day increases the metabolism significantly in 5- and 8-day-old rats ($p < 0.05$ and $p < 0.001$, respectively) and slightly but not significantly in 3-day-old ones. As the 3 day treatment was not enough to stimulate distinctly the metabolism of 20-day-old rats, the treatment was commenced 2 days earlier and thus extended to last for 5 days. The rate of oxygen consumption was then clearly increased ($p < 0.001$) as shown in Figure 1. The increased oxygen consumption of the thyroxine treated animals was also observed at lower ambient and body temperatures, although the difference in the consumption between treated and control animals was relatively slighter at lower temperatures.

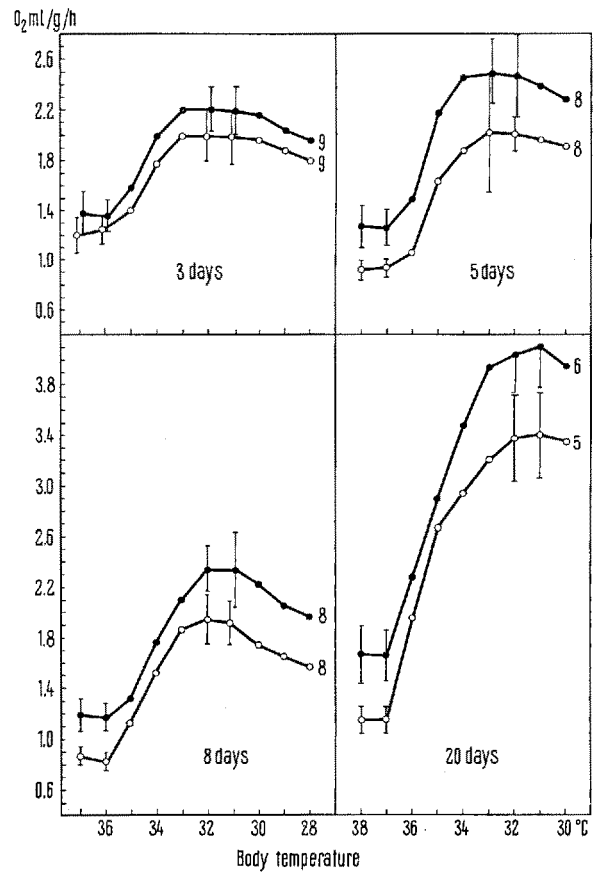

Fig. 1. Mean oxygen consumption of rats at different age levels as a function of body temperature. Open circles: controls, solid circles: thyroxine-treated animals (dose 300 μg/kg day for 3 days; for 5 days in the oldest age group). The numbers of animals and standard deviations are shown for each graph.

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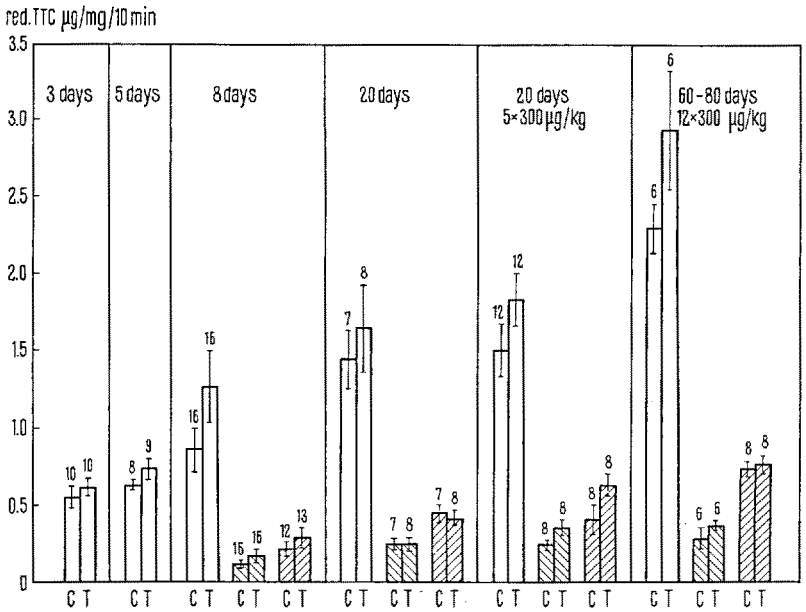

Fig. 2. Mean succinic dehydrogenase activity in liver (white columns), muscle (lining leaning left) and brain (lining leaning right) of rats at various age levels. C = controls, T = thyroxine-treated animals (dose 300 μg/kg day, for 3 days unless otherwise stated). The numbers of animals and the standard deviations are shown at the top of each column.

The negative results obtained by earlier authors could be explained to be due to the thyroxine dosage, since our experiments[11] have shown that in addition to the age of animals and the duration of treatment, also the amount of injected thyroxine strongly influences the response. Large doses of thyroxine even inhibited the metabolism.

The stimulated oxygen consumption could be explained by supposing that thyroxine activates certain enzymes of oxidative cell metabolism. Thus the effect of thyroxine treatment on the activity of succinic dehydrogenase of liver, thigh muscle and brain homogenates was studied. The results are presented in Figure 2. Three days' treatment with a dose of thyroxine 300 μg/kg day significantly increased the activity of this enzyme in the liver of rats at the age of 5 days ($p < 0.01$), although slight activation was already observed in 3-day-old rats. The effect was more pronounced in 8-day-old animals ($p < 0.001$) and was evident also in the muscle and brain homogenates. Older animals did not respond to the 3 day treatment period as strongly as the younger ones, but needed longer administration before the effect was equal. It is interesting to note that the activity in the brain tissue of the adult rats was not significantly increased, although this activation was evident in infant rats. The result corroborates previous observations which show that thyroxine administration increased the in vitro oxygen consumption of brain slices in infant rats but not in adults[12], and the succinate oxidation in liver and muscle but not in brain tissue[13].

There is much evidence that the pituitary secretion of TSH already begins during the prenatal development in various mammals[14], but in rats, for instance, TSH content in adenohypophysis and in blood is several times lower in infants than in adults[15,16]. LEVEY[15] concluded that the eventual elevation in thyroid activity in older rats results from increased production of TSH. In order to clarify this question in connection with the oxidative metabolism, the effect of TSH injections (10 IU/kg day for 3 days) on the activity of succinic dehydrogenase of liver homogenate was studied in 8-day-old rats. The results showed that the activation of this enzyme by TSH was almost the same as in thyroxine injected animals of the same age. The activity in the controls was 0.93 ± 0.04 and TSH-treated 1.23 ± 0.06 ($p < 0.001$, $N = 12 + 12$). The results are given as μg reduced TTC/mg 10 min. This indicates that the thyroid gland is functional in the control of oxidative metabolism in young rats if sufficient TSH is present[17].

Zusammenfassung. Die Behandlung 5 oder 8 Tage alter Ratten mit Thyroxin- oder TSH-Injektionen verursachte eine signifikante Stimulierung im Sauerstoffverbrauch und in der Sukzinodehydrogenase-Aktivität im Leber-, Muskel- und Gehirngewebe. Dies zeigt, dass die Inaktivität der Schilddrüse in metabolischer Kontrolle schon innerhalb von 8 Tagen nach der Geburt aufgehoben wird, wenn genügend TSH vorhanden ist.

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Disrupted Fertility of the Hidebeetle *Dermestes maculatus* (Deg.) due to Dietary Overdosage of Biotin

It has been known for some time that growth and reproduction of several insect species become inhibited upon excessive intake of certain vitamins. According to LEVINSON and BERGMANN 1959[1], fatal hypervitaminosis in pyridoxine, pantothenic, nicotinic or folic acid may be caused to housefly larvae, and AKOV and GUGGENHEIM[2] observed toxic effects due to dietary surplus in riboflavin on *Aedes aegypti*. Moreover, BENSCHOTER and PANIAGUA[3] demonstrated recently impeded reproduction of Mexican fruitflies (*Anastrepha ludens*) due to large biotin doses. Mortality in larvae of carpetbeetles was induced also by overdosage of vitamin K_1, K_2 or K_3[4]. This communication deals with a study on the influence of oversupply of B vitamins on growth and reproduction of the hidebeetle *Dermestes maculatus*. The nutritional requirements of larvae and adults are similar in this species and can be met by a semisynthetic diet[5].

Individual larvae as well as pairs of male and female beetles were maintained at 29 ± 0.5 °C and 60–70% relative humidity on diets[5] containing additions of 0.01, 0.1, 0.5 and 1.0% of single B vitamins. 15–20 larvae and adults of either sex were employed for testing each vitamin and dietary level. Rates of food consumption, development, emergence, fecundity and fertility were the criteria of these experiments.

As normal development was evident in presence of all vitamin levels, dietary excess of either nicotinic, pantothenic or folic acid, pyridoxine, biotin, thiamine, riboflavine, choline chloride or inositol has certainly no detrimental effect on growth of hidebeetle larvae. It is noteworthy, that the 5 vitamins listed first are indispensable for development of *D. maculatus*, whilst the 2 vitamins listed subsequently are needed only partly for this process[5].

However, adults were found to differ from larvae in their response to biotin overdosage. Although addition of 0.5% biotin to the control diet hardly affected the number of eggs laid, it permitted only a negligible proportion of larvae to hatch from the latter. Moreover, those larvae

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