containing TCDD, a review of chemical industries revealed a hexachlorophene producer in southwestern Missouri that accumulated distillate residues containing TCDD during TCP production in 1970 and 1971. The hexachlorophene producer contracted disposal of these residues during 1971 to a chemical distributor. The distributor subcontracted this disposal to the salvage oil company that sprayed the three affected arenas. Between February and October 1971 the salvage oil company hauled the residue, totaling approximately 68,000 liters, in six separate trips. These residues were kept in the storage tank from which the sludge for spraying the three arenas was obtained. The hexachlorophene plant discontinued operations in late 1971, leaving the tank originally used to store the distillate residue at the plant site undisturbed. The remaining residue in this tank was sampled in 1974 and analyzed by the methods described above. A portion of the oily residue was diluted with petroleum ether, washed with dilute sodium hydroxide, and then chromatographed on an alumina column. The TCDD was quantitated by methods of addition, and identification was confirmed by mass spectrometry. The waste residue contained TCDD in concentrations of 306 to 356 μg/g.

The investigation demonstrates that the improper disposal of toxic chemical wastes may have serious consequences. Human illnesses and the death of a number of valuable horses occurred when industrial wastes containing TCDD were mixed with reclaimed motor oils and lubricants. TCDD or other chlorinated dibenzodioxins or chlorinated dibenzofurans may be present in the waste products from chlorination of benzenes, phenols, and polyphenyls, since traces have been found in the commercial products (*1, 11*). Companies responsible for disposal of such wastes should be aware of the toxicity of some of these chemical waste products and of the proper methods of disposal. More extensive regulation of disposal of toxic industrial wastes may be necessary to prevent similar or more serious occurrences.

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Mechanism of Insulin-Induced Paralysis of Muscles from Potassium-Depleted Rats

Abstract. *Zinc-free insulin elicited a reduction in the potassium conductance of muscle fibers from potassium-depleted muscle, which led to depolarization, blockade of action-potential mechanism, and paralysis. These changes are proposed as the mechanism of insulin-induced paralysis in patients with hypokalemic periodic paralysis. A similar effect by concanavalin A suggests that the effect may be mediated through the insulin receptors.*

Patients with hypokalemic periodic paralysis episodically develop muscle weakness associated with a decrease in the concentration of K^+ in the serum and a depolarization of skeletal muscle cells (*1*). Although there is evidence that an elevation of serum insulin may be responsible for the triggering of these episodes (*2*), the underlying mechanism remains unclear. One hypothesis is that the paralysis results from sarcolemmal inexcitability (*3*). Gordon *et al.* (*4*) proposed that the inward rectification characteristic of resting K^+ conductances (g_K) of the sarcolemmas might contribute to the development of sustained depolarization and inexcitability when the K^+ concentration in serum is low. A second hypothesis is that excitation-contraction uncoupling may be primarily responsible for the weakness (*5*).

For more detailed studies of this problem, the K^+-depleted rat appeared to be a useful animal model because their muscles possess characteristics similar to those seen in the patients' muscles: depolarization and paralysis induced by Zn^{2+}-insulin (*6*), lower intracellular K^+ concentration and higher Na^+ concentration than normal (*7*), and morphologically altered membranous organelles (*8*). Since Zn^{2+} has a marked effect on muscle g_K (*9*), we investigated the effects of Zn^{2+}-free insulin on the electrical membrane properties of diaphragm from K^+-depleted rats. Our results indicate that in these muscles insulin alone causes a decrease in g_K, leading to depolarization, inexcitability, and weakness.

Small strips of diaphragm from rats that had been kept on a K^+-deficient diet (*6*) for 4 to 8 weeks were mounted at room temperature in low-K^+ (0.5 m*M*) Tyrode solution with 3 mg of tubucurarine chloride per liter. The serum concentration of K^+ in rats ranged from 1.5 to 2.5 m*M*. The solution was oxygenated and buffered at *p*H 7.4.

After the addition of 1 to 5 mU of Zn^{2+}-free insulin per milliliter (Lilly, lot IDG 0497193), the maximum twitch tension elicited by direct stimulation started to decrease in 30 minutes and by 1 hour had usually fallen to less than 10 percent of its original magnitude. Increasing the concentration of K^+ in the bath to 5.5 m*M* fully restored the twitch tension in 15 minutes, and insulin did not change this in either normal or K^+-depleted muscle in the solution with 5.5 m*M* K^+.

Our next step was to locate the deficit in muscle activation produced by insulin. Contractures evoked by 20 m*M* caffeine increased slightly in amplitude after insulin-induced paralysis of twitch, indicating an intact response of the contractile filaments to the release of Ca^{2+}. Contractures evoked by 120 m*M* K^+ were either unchanged or only slightly decreased, even when the twitch response was completely paralyzed by insulin. This suggests that the loss of sarcolemmal excitability rather than a deficit in excitation-contraction coupling is responsible for the insulin-induced paralysis.

To study the sarcolemmal excitability, we made intracellular recordings of the same identified single fibers of K^+-depleted muscle, both before and 1 hour after addition of insulin (5 mU/ml) in the 0.5 m*M* K^+ solution. Each surface fiber

that was recorded was identified by its position in relation to landmarks, such as nerves and blood vessels, during continuous observation under the microscope. When the muscle was paralyzed, these fibers were depolarized from (−60 to −80 mv) to (−35 to −55 mv); external stimulation generated no action potential. However, when each of these fibers was then hyperpolarized to −90 mv with injection of current for 200 msec, an action potential could often be elicited at the anodal break, accompanied always by a visible twitching of the fiber. Increasing the concentration of K^+ in the bath to 5.5 mM restored the membrane potential (E_M) and excitability. These findings indicate that the paralysis results from a loss of excitability and that the inexcitability is probably due to inactivation of the action potential mechanism by sustained depolarization. In the control muscles insulin produced a slight hyperpolarization (4 to 8 mv).

Depolarization of muscle fibers can be the result of either a decrease in g_K, an increase in the membrane Na^+ conductance (g_{Na}), a decrease in intracellular concentration of K^+, or an inhibition of the electrogenic ionic pump (*10*). The last two mechanisms can be excluded here because (i) ouabain ($10^{-3}M$) did not prevent insulin-produced paralysis, (ii) insulin enhances the Na^+ pump activity of muscles, and (iii) insulin is known not to decrease intracellular concentration of K^+ (*11*). We studied the effect of insulin on g_K by measuring the input resistance (R_i) of fibers in a solution where K^+ was the only significant permeant ion. The Na^+ was replaced by the impermeant tetramethylammonium ion (TMA) (*12*) and Cl^- by methylsulfate (*13*). The concentrations of other ions and the *p*H were the same as those in the low-K^+ Tyrode solution. Under these conditions insulin increased the R_i of identified single fibers by 38 ± 6.9 percent (± standard error) without significantly changing the E_M; thus there was a decrease in g_K.

In the attempt to investigate the effect of insulin on g_{Na}, we found that in the presence of physiological concentration of Na^+, insulin decreased the R_i of K^+-depleted muscle fibers considerably when they were markedly depolarized in low-K^+ Tyrode solution. The question is whether the decrease in R_i is the result or the cause of depolarization. To study this we measured R_i and E_M first in the absence and then in the presence of 5, 10, or 25 mM Na^+ in the solutions with 5 mU of insulin per milliliter where the concentration of K^+ was 0.5 mM. The Cl^- was replaced by methylsulfate and the remainder of monovalent cations by TMA. In the presence of insulin, increasing the concentration of Na^+ from 0 to either 5 or 10 mM did not change either R_i or E_M, and increasing it to 25 mM resulted in a slight depolarization [7.7 ± 0.9 mv (± S.E.)] without discernible change in R_i. This finding indicates that the large decrease in R_i induced by insulin in the presence of the physiological concentration of Na^+ was the result rather than the cause of a large depolarization and that insulin did not cause a large increase in g_{Na}. However, since the resting g_{Na} is very small, the possibility cannot be excluded that insulin not only decreased g_K but also caused a small increase in g_{Na} beyond detection.

We conclude that in muscles from K^+-depleted rats insulin produces a decrease in g_K and that this leads to depolarization, blockade of action potential mechanism, and paralysis. The fact that paralysis occurs only in low K^+ solution can be explained by the inward rectification property of the resting g_K. When external concentration of K^+ is low, the driving force for K^+ is outward and g_K becomes very small. Further reduction of g_K by insulin, in this condition, will have a more pronounced effect on the ratio of g_{Na} to g_K. The rectification of g_K may also explain the clinical features of human hypokalemic periodic paralysis: association of paralysis with hypokalemia, beneficial effect of K^+ administration, and abortion of attacks through exercise. Exercise results in repetitive firing of the action potentials, leading to an accumulation of K^+ in the immediate extracellular space (for example, transverse tubules) and a reversal of the driving force of K^+. It is interesting that Ba^{2+}, another g_K blocker, has been found to cause a clinical syndrome similar to hypokalemic periodic paralysis (*14*).

Although insulin has been reported to decrease both g_K (*15*) and K^+ permeability (*16*) in muscles, the insulin used contained Zn^{2+}, and in micromolar concentrations Zn^{2+} can reduce g_K (*9*). The development of Zn^{2+}-free insulin enabled us to study the effect of insulin alone.

How insulin reduces g_K is unclear. It does not do so by increasing sugar uptake because insulin paralyzed K^+-depleted muscle in the absence of external sugar. We observed that, like insulin, concanavalin A (Con A) at 200 μg per milliliter of low-K^+ solution would produce paralysis of these muscles. We could also reverse the paralysis by increasing the concentration of K^+ in the bath. It has been shown that Con A competitively binds and activates insulin receptors (*17*). It is likely that insulin reduces g_K through a receptor-mediated process. Insulin is reported to cause an increase in the intracellular concentration of guanosine 3′,5′-monophosphate (cyclic GMP) (*18*) and a decrease in the intracellular concentration of adenosine 3′,5′-monophosphate (cyclic AMP) (*19*) in some cells. In preliminary experiments, dibutyryl cyclic GMP (1 mM) did not cause paralysis of muscles from K^+-depleted rats and dibutyryl cyclic AMP (1 mM) did not prevent this effect of insulin, which is probably not mediated through such changes in the level of these cyclic nucleotides.

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