16-chromosome *M. falcata* and 32-chromosome *M. sativa*.

To summarize, it may be said that a 16-chromosome form of *M. sativa* has been found. This form is highly self-sterile and highly cross-sterile when crossed to 32-chromosome forms of *M. sativa* and *M. falcata*. It shows normal cross-fertility when crossed to 16-chromosome forms of *M. falcata*.

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Quantitative Aspects of the Action of Insulin on the Glucose and Potassium Metabolism of the Isolated Rat Diaphragm

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In a previous communication (*3*) experiments were described from which it was concluded that the utilization of glucose by the isolated rat diaphragm is associated with a shift of potassium from the medium into the tissue, and that both glucose utilization and potassium shift are increased by the addition of insulin. In order to study the quantitative aspects of these reactions, experiments were carried out with varying concentrations of insulin.

The technique was different from that used previously. Rats of 80–100-g body weight were decapitated after fasting for 24 hr; their diaphragms were removed and cut into quarters. The quarters were kept in ice-cold buffer solution (*2*) before the actual experiment started. Eight quarter-diaphragms, representing one-half of the left and right hemidiaphragms of 4 rats, were transferred to a flask containing 2 ml of buffer solution in which 200 mg % of glucose had been dissolved. After equilibration with a gas mixture containing 93% O_2 and 7% CO_2, the buffer-diaphragm system was incubated for 1 hr at 37° C, with shaking at a rate of 120/min. The remaining 8 quarter-diaphragms of the same rats were incubated in a buffer-glucose solution of the same composition that contained, in addition, insulin in the concentration to be tested.

This arrangement was chosen so as to make the conditions under which the diaphragms were incubated as similar as possible, with the only difference that insulin was present in one flask and absent from the control flask.

[1] Holding a grant from the Organon Laboratories, Inc., Oss, Netherlands.

After 1 hr the flasks were cooled and the contents centrifuged. Glucose (*5*) and potassium (*7*) concentrations in the medium were determined, and the diaphragms were weighed after blotting on filter paper. The difference (Δ glucose) between the quantities of glucose (calculated as mg/100 mg wet tissue) that have disappeared from the medium in the flask with insulin and that without insulin is the effect of the added insulin on glucose utilization.

While studying the changes in potassium content of the medium in these incubation experiments, it was found that, with the use of quarter-diaphragms, the potassium content of the medium increased as a rule, whereas it usually decreased when hemidiaphragms were used. Apparently a diffusion of potassium out of the "surviving" tissue takes place more rapidly from quarter-diaphragms than from hemidiaphragms. This is easily understandable because of the greater damage to the tissue that takes place when the diaphragm is divided into 4 parts.

In the present series of experiments the potassium content of the medium increased in both flasks, but in the vessel containing insulin the increase was invariably less than in the one without insulin. The difference (ΔK) between the increase of the potassium content (calculated as microequivalents per 100 mg wet tissue) of the medium in the flask with insulin and that without insulin is the effect of the added insulin on the potassium shift, associated with increased utilization of glucose by the tissue.

The glucose and potassium effects of increasing concentrations of insulin were plotted in a curve (Fig. 1). The

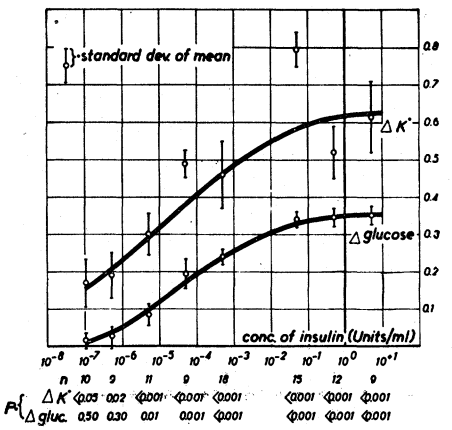

FIG. 1. Effect of varying concentrations of insulin upon glucose utilization (mg/100 mg wet tissue) and potassium shift (microequivalents/100 mg wet tissue) in experiments with isolated rat diaphragms; n = number of experiments; P = Fisher's value of probability.

graph illustrates that, with augmenting concentrations of insulin, both glucose and potassium effects increased until, at a level of about 10^{-2}–10^{1} units/ml, both effects tended to become more or less constant. In the region of lower concentrations of insulin, it was found that 10^{-7} units/ml still had a significant effect upon the potassium shift, whereas the effect upon the glucose utilization was no longer detectable. In general, the figures for the effect of insulin on potassium shift were more irregular than

those for the effect on glucose utilization; this demonstrated itself in the relatively large standard deviations of ΔK compared with those of Δ glucose. The greater irregularity of the potassium shift may be due to the varying degree of tissue damage at the excision and the ensuing leakage of potassium from the injured muscle fibers.

Extremely small concentrations of insulin can be detected by this method. Even with insulin concentrations as low as 5×10^{-3} units/ml, significant effects on the glucose and potassium metabolism of the isolated rat diaphragm were observed. Lower concentrations appeared to have a potassium effect, but the level of significance was less than 1%. Apparently this technique makes it possible to detect insulin in amounts that are much smaller than those reported by previous observers (*1*, *6*). It should be borne in mind, however, that variations in sensitivity of the diaphragms may occur.

The sample of pure insulin used in these experiments contained 28 units/mg.[2] The smallest concentration of insulin that still produced a significant increase in glucose utilization was 5×10^{-6} units/ml, which, assuming a molecular weight of insulin of 48.000, amounts to 4.5×10^{9} molecules per flask. This number of insulin molecules enabled the diaphragm to utilize about 10^{18} more molecules of glucose than diaphragms in the control flask without insulin. This extra utilization of glucose was associated with an extra shift of about half as many atoms of potassium from the medium into the tissue. With further refinements of technique, the isolated diaphragm test may offer opportunities for the determination of minute amounts of insulin.

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[2] We are indebted to J. Lens (*4*) for a gift of pure insulin.

A Method for Silver Staining of Nerve Fibers in Whole-Mount Preparations of Blood Vessels

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Humphreys (*3*, *4*) demonstrated vascular nerve fibers on cerebral blood vessels, using a modification of the Bodian (*1*) technique. He obtained better and more consistent results than with either the modified Gros-Bielschowsky technique of Penfield (*5*) or Huber's (*2*) methylene blue technique.

On cerebral blood vessels of the pia mater we have obtained more satisfactory results by use of the following modification of the Bodian technique. Blood vessels of anesthetized animals were flushed with a physiological salt solution, followed by perfusion with neutral 10% formalin. The leptomeninges containing the blood vessels were dissected from the brain and brain stem, and pinned flat on paraffin for 24 hr in a Petri dish contain-

FIG. 1. Arterial branch of vertebral artery showing the crossing and branching of large mixed fiber bundles of the adventitia. The dark stellate cells are chromatophores. Objective, 16 mm; ocular, 10 ×. Protargol.

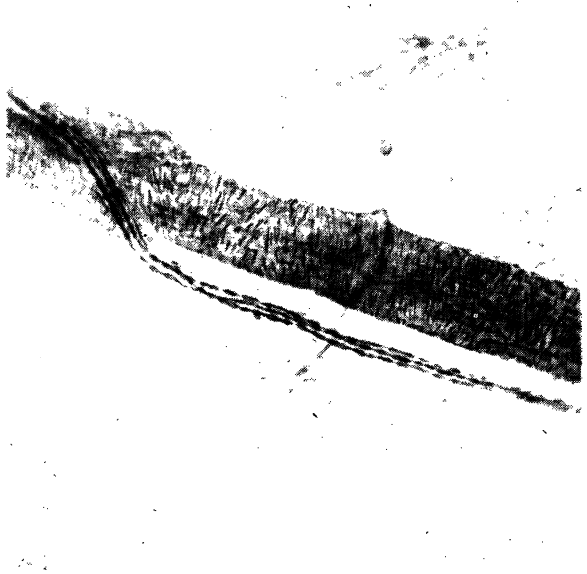

FIG. 2. Group of myelinated fibers becoming related to a branch of the basilar artery. Objective, 16 mm; ocular, 10 ×. Protargol.