

During the manipulation, the *M* linked chemically to the γ -globulin lost its virulence. If it was administered parenterally in both guinea-pigs and rabbits, antibodies were produced against γ -globulin as well as against *M*. On injecting it intracutaneously an *M* suspension into tuberculous guinea-pigs, it could be observed that γ -globulin enhanced the tuberculine sensitivity.

Parenteral administration of our vaccine did not induce protection against tuberculous infection in guinea-pigs.

Zusammenfassung. Mit Hilfe der PAULY'schen Diazo-Reaktion wird humanes γ -Globulin an humanpathogene, virulente *Mycobacterium tuberculosis*-Stämme (*M*) ge-

koppelt. Das Zustandekommen der chemischen Bindung wurde mit gefärbten Präparaten, elektronenmikroskopischen und immunoelektrophoretischen Untersuchungen der Bakterien bewiesen. Die mit γ -Globulin gekoppelten *M* haben ihre Virulenz eingebüßt. In den Versuchstieren wurden Antikörper sowohl gegen γ -Globulin als auch gegen *M* gebildet. Durch ersteres wurde das Allergisierungsvermögen von *M* gesteigert.

L. FORRÓ and Gy. KOCsis

Department of Dermatology, University Medical School, Szeged (Hungary), August 8, 1962.

Action of Isothiocyanates on Germinating Plants

Although isothiocyanates have been examined as fungicides, ascaricides, insecticides, bactericides and for their action against plant parasites, no paper dealing with their influence on the germinating process of plants has come to our knowledge.

We have determined the inhibition caused by allylisothiocyanate (AITC) on the germination and growth of pea (*Pisum sativum*), wheat (*Triticum vulgare*) and rape (*Brassica napus*, var. *oleifera*) in different concentrations ($1 \cdot 10^{-4}$ – $1 \cdot 10^{-2} M$). Germination is distinctly suppressed by AITC in concentrations of $5 \cdot 10^{-3}$ and slowed down by higher concentrations. Growth inhibition is observed by the decrease of total weight of the seedling and length of vegetative organs. The influence on water economy is not distinct. The inhibition is most efficient when the substance is applied to seeds or in the first three days of the germinating period. When the inhibitor is applied in the following days, growth of vegetative organs and weight of seedlings are also decreased, but this decrease is less distinct than in the first days. This may be explained by the fact that the degree of inhibition depends on the volume of inhibitor accepted by the plant. The increase of weight in the first three days of germination is 90% of the weight of the seed in 24 h, whereas in the next four days it is only 20%. Another possibility is that AITC blocks one or several germination processes in the first stage of germination, so that even when there is sufficient water and warmth the seeds do not start to bud.

This hypothesis was the subject of further experiments, carried out with germinating pea and rape plants, because isothiocyanates are natural substances for rape, whereas this is not proved for peas. Nitrogen metabolism was investigated by analyses of total, protein, ammonia and amid nitrogen by a modified Conway microdiffusion method¹⁻³, α -amino nitrogen was determined according to VAN SLYKE⁴ and the content of free amino acids was determined chromatographically⁵. AITC concentrations of $10^{-2} M$ slow down the metabolism of proteins in cotyledons and of nitrogen-containing substances in the vegetative organs of eight-day-old germinating pea and rape plants to the level of two-day-old plants, concentrations of $5 \cdot 10^{-3}$ and $10^{-3} M$ exert a slowing down to the level of four- and six-day-old plants. An exception is the increase of α -amino nitrogen and amino acid concentration observed, which show the decreased incorporation of amino acids into proteins.

Sugar metabolism, investigated by analysis of reducing and non-reducing sugars by SOMOGYI's method^{6,7} and of paper chromatography⁸, shows a decreased content by reducing sugars in epicotyle, roots and cotyledons of peas and an increase of non-reducing substances in the vegetative organs. The decrease in glucose, fructose and sucrose concentrations observed on chromatograms of the extracts of epicotyles and roots of plants grown in AITC solutions and after inhibitor infiltration, demonstrates an effect on saccharide decomposition. The concentration of nearly all keto-acids in germinating plants is decreased by the action of the inhibitor. AITC decreases^{9,10} the inorganic phosphorus content of the cotyledons, whereas in the epicotyles and roots it is increased. Weakly bound soluble organic phosphorus (phosphorus of adenosine di- and tri-phosphate, fructosol and 1,6-diphosphate) decreases in a marked manner in the epicotyles, similarly as strong bound soluble organic phosphorus in cotyledons and roots. The variations of the sugar content and the increased content of inorganic phosphorus in the vegetative parts of pea plants may be explained by an effect of AITC on processes connected with the formation of phosphorylated sugars.

The inhibition by isothiocyanates is not an effect on respiration, as respiration as well as anaerobic glycolysis are decreased only by 30% under conditions in which 100% inhibition of the other processes investigated was observed. Inhibition is also not caused by B avitaminosis. The effect can neither be reversed, not substantially weakened by the effect of the vitamins B₁, B₂, B₆ and B₁₂.

The distinct increase of the content of free amino acids after treating on germinating plants and the decrease of the ratio of protein nitrogen to α -amino-acid nitrogen

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suggest inhibition of protein synthesis. In order to prove this protein synthesis was investigated in slices of mouse pancreas¹¹⁻¹³. We regard the inhibition of protein synthesis by allylthiocyanate, phenylthiocyanate, cheirrolin $\text{CH}_3\text{SO}_2\text{CH}_2\text{CH}_2\text{NCS}$ and iberin $\text{CH}_3\text{SOCH}_2\text{CH}_2\text{NCS}$ as proved, because it has been simultaneously observed that phenylisothioalanine and phenylisothioleucine do not inhibit protein synthesis and that also other N-substituted derivatives of amino acids are without inhibiting effect. It is improbable that the slowing down of protein synthesis by isothiocyanates is caused by the elimination of some amino acid through bonding with isothiocyanates. When the concentration of amino acids is increased so that even the amino acid, present in the lowest concentration, is present in a concentration at least double with respect to the amount of inhibitor, the inhibiting effect of the isothiocyanates tested remains unchanged. Isothiocyanates belong to the most powerful inhibitors of protein synthesis known.

Zusammenfassung. Es wurde ein inhibitorischer Effekt des Allylthiocyanates auf die Keimung von Erbsen, Weizen und Raps beobachtet, und dabei die Veränderungen des Stickstoff- und Zuckermetabolismus verfolgt: Isothiocyanate setzen die Atmung und anaerobe Glykolyse um etwa 30% herab und legen die Proteosynthese vollständig still.

SYLVA LEBLOVÁ-SVOBODOVÁ and J. Košťál

Department of Biochemistry, Charles University, Prague (Czechoslovakia), July 26, 1962.

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Lipogenesis in Rats Adapted to Intermittent Starvation or Continuous Underfeeding

In previous papers (FÁBRY et al.¹) we have demonstrated that in rats accustomed to intermittent starvation, i.e. alternating periods of fasting and days of free access to food, a series of adaptive changes develop, most of which differ markedly from commonly known sequelae of simple continuous caloric restriction. In the present paper we are submitting the results of experiments in which we investigated changes in the carcass composition (a) of male rats submitted to intermittent starvation for 11 weeks, where the total caloric intake amounted to 46% of that in controls; (b) in rats subjected to continuous underfeeding, pair-fed the same amount of food divided into daily rations; (c) in *ad libitum* fed controls. In addition, the *in vitro* hepatic lipogenesis in female rats, intermittently starved for 3 weeks, was investigated by assessing the incorporation of 1-C^{14} -acetate into fatty acids by liver slices (for method see BARUCH and CHAIKOFF²).

Adult albino rats, Wistar strain, fed a standard laboratory diet (Larsen mixture³) were used. The intermittently starving animals were fed on alternate days during the first two weeks of the experiment, subsequently three times a week. In the pair-feeding experiment groups of animals killed by decapitation after a standard test meal were compared; hepatic lipogenesis was investigated after an unrestricted test meal over night (absorptive phase) and after a subsequent 48 h fast. For carcass analysis, a technique described by MICKELSEN⁴, was used; the extracted fat was estimated gravimetrically, protein was calculated from total nitrogen values obtained by microkjeldahlization.

All assessed parameters revealed that periodic hyperphagia on days of free access to food, by which the intermittently starving rats partly compensate for the period of starvation⁵, leads, in addition to other metabolic sequelae, to a markedly enhanced lipogenesis, which persists even after fasting. In the absorptive phase, the *in vitro* lipogenesis by liver slices (Table I) is about four times greater in intermittently starving rats than in the controls. After subsequent 48 h acute starvation, when in agreement with literary data lipogenesis is considerably suppressed in the controls, the incorporation of radio-

Tab. I. *In vitro* incorporation of 1-C^{14} -acetate into fatty acids by rat liver slices, expressed as percentage of activity added (mean \pm S.E., five animals per group)

Group	Absorptive phase	Fasting 48 h
Controls	4.22 ± 1.01	0.72 ± 0.19
Intermittent starvation (3 weeks)	15.50 ± 3.10^a	13.51 ± 1.64^b

^a Difference, as compared with the control group, is significant for $P < 0.02$.

^b for $P < 0.001$.

active acetate in intermittently fasting rats is even of a higher order than in the comparable group of controls fasting for an equal period, and is still three times greater as compared with values of controls in the absorptive phase.

The accentuated lipogenesis manifests itself also in the final carcass composition (Table II). The weight decrement of both underfed groups being equal, the carcass of intermittently starved animals contains a greater percentage of fat not only as compared with the continuously underfed group but also as compared with controls fed an unrestricted diet. The ratio of body protein, on the other hand, is lower in these animals than in the other two groups. The enhanced lipogenesis in intermittently starving animals manifests itself also by a different percentage of fat and protein increase or decrease during the period of experimental feeding (Table II).

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