acclimatized to cold ($t = 4.98$, $p < 0.001$). On the other hand, the difference between the enzyme activities in the brain homogenates from the cold acclimatized animals and from the controls is not significant ($t = 0.70$).

The present results are in agreement with the results obtained with rat liver[2], although the increase in the succinic dehydrogenase activity during the cold acclimatization in the mouse liver is greater than has been observed in the rat liver after a stay of 2 to 70 days at 1.5° to 5°C[2]. This may reflect the fact that the cold acclimatization obviously involves a greater compensatory increase in the basal heat production in mice than in rats which have a relatively smaller rate of heat loss in the cold. The acclimatization to cold did not cause any difference in the activity of the succinic dehydrogenase in the brain, a fact which indicates the relative stability of the metabolism of the central nervous system in spite of general compensatory adjustments in the other parts of the body.

Résumé. L'activité du déshydrogénase succinique dans le foie des souris mâles adultes acclimatisés pendant 7–12 jours à + 5°C est augmentée de 72%. L'activité du même enzyme dans le cerveau n'est pas altérée par cette acclimatisation au froid.

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A New Type of Antigen Induced by Chemical Linkage of *Mycobacterium tuberculosis* and γ-Globulin

The search for more effective methods to produce vaccines is very important. Hence, the authors attempted to examine whether it would not be possible to produce a new kind of chemospecific antigen which would change the antigen structure of the micro-organism and influence its ability to immunize favourably, and which could be bound by chemical linkage to a large molecule having the property to produce antibodies in considerable amounts.

Of the methods of chemical linkage which may be considered, the authors chose Pauly's[1] diazoreaction by the use of which the chemical linkage of a protein molecule to the body of the *Mycobacterium tuberculosis* (*M*) is possible[2–4].

The linkage between the *M* and γ-globulin was performed as follows: The human pathogen, virulent *M* suspension (Type: H III) was washed 5 times with distilled water and dried. Subsequently, in order to remove the lipoid layer on the surface, an amount of 5 g dry weight of the bacterium was washed with petroleum ether and then the traces of the petroleum ether were evaporated. This substance was suspended in 100 ml of 10% hydrochloric acid, and, after stirring it for 1 h in ice, 30 ml of a saturated solution of sodium nitrite was added during 4 h. 3 ml of a 10% human γ-globulin solution ('Human' Oltóanyagtermelö és Kutató Intézet, Budapest, Hungary) was dissolved in 60 ml 5*n* sodium hydroxide and added to the above mixture. After mixing for 30 min, it was centrifuged and the protein which had not been chemically bound to the bacteria was removed by washing in saline.

The chemical binding of γ-globulin to *M* was proved as follows: (1) By morphological studies of the bacterium. The *M* linked to γ-globulin loses its acid-fast character and stains well with aniline dyes. Electronmicroscopic studies show that the bacterium which, owing to the treatment with petroleum ether, absorbed electrons only poorly (Figure 1), changes again to a good absorber of electron rays through the γ-globulin coating of its surface (Figure 2).

(2) By immune electrophoresis. The *M* suspension bound to γ-globulin was incubated for the sake of comparison in a 2% γ-globulin solution for 24 h and then washed and submitted to immune electrophoresis. On the action of the current, the γ-globulin separated from the surface of the *M* incubated in it; however, the current could not separate the γ-globulin from the bacterium linked to it, but it migrated simultaneously with the *M*.

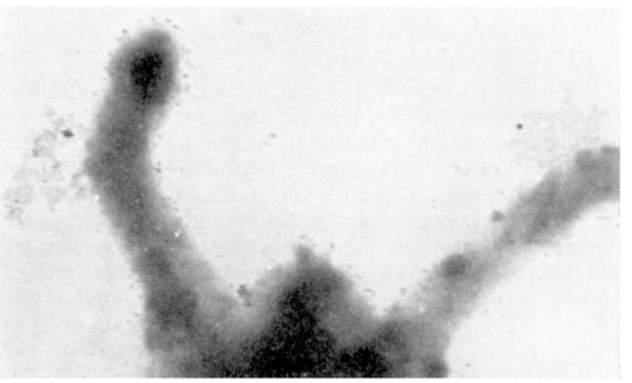

Fig. 1. Diazotized *Mycobacterium tuberculosis*. Examined without shading with a Zeiss electronmicroscope type D_2; basic magnification 12000, magnified to 20000.

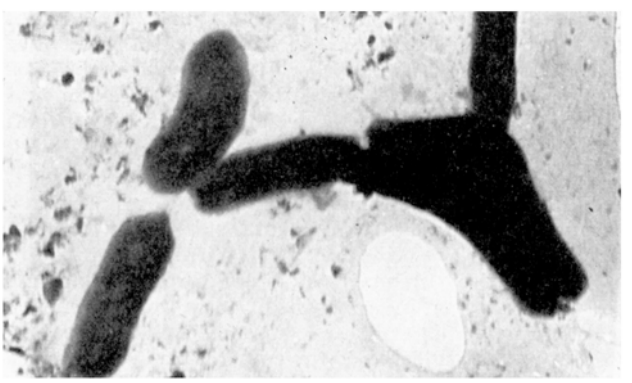

Fig. 2. *Mycobacterium tuberculosis*, linked chemically to γ-globulin. Similar data as in Figure 1.

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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*) gekoppelt. Das Zustandekommen der chemischen Bindung wurde mit gefärbten Präparaten, elektronenmikroskopischen und immunelektrophoretischen 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.

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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 (1.10^{-4}–$1.10^{-2}M$). Germination is distinctly suppressed by AITC in concentrations of 5.10^{-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 method[1–3], α-amino nitrogen was determined according to VAN SLYKE[4] and the content of free amino acids was determined chromatographically[5]. 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 5.10^{-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 chromatography[8], 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, fructoso-1 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 B_1, B_2, B_6 and B_{12}.

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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