INH % in Diät	Glutaminsäure % in Diät	Von je 5 Ratten nach 15 Tagen überlebend	Durchschnittliche Veränderung des Körpergewichtes in g	Durchschnittl. Schweregrad der periph. Nervendegeneration
0,25	–	5	6,8 (– 2 +13)	1,49
0,25	0,1	4	5,3 (– 8 +14)	1,27
0,25	0,5	5	6,8 (– 3 +16)	1,11
0,25	1,0	5	14,4 (+ 2 +20)	1,37
0,25	5,0	5	11,0 (0 +22)	1,37
0,25	10,0	5	–1,0 (– 9 + 7)	1,47
–	–	5	26,2 (+18 +33)	0

der mit Gs. behandelten Ratten ist von demjenigen der nur mit INH gefütterten Tiere nicht signifikant verschieden. Auch die als allgemein-toxischer Effekt des INH zu wertende Wachstumshemmung wurde durch Gs. nicht signifikant beeinflusst ($p > 0{,}05$).

Besprechung der Resultate. Grosse INH-Dosen führen bei Versuchstieren einerseits zu akuten, allgemein-toxischen Symptomen, wie Krämpfen und raschem Absterben, andererseits schädigen sie selektiv die Markscheiden peripherer Nerven. Während die allgemein-toxischen Symptome durch verschiedenartige Stoffe, wie Vitamin B_2, B_6, B_{12}, A, E, K, Nikotinsäureamid und Glutaminsäure, gemildert werden können, vermag nach den heutigen Kenntnissen einzig das Vitamin B_6 auch die durch grosse INH-Dosen experimentell erzeugte Schädigung der peripheren Nerven hemmend zu beeinflussen[6]. Nach den vorliegenden Ergebnissen modifiziert Gs. weder qualitativ noch quantitativ den Ablauf der experimentellen Isoniazid-«Neuritis». Dies gibt einen weiteren Hinweis, dass bei der Entstehung der Isoniazid-Schädigung peripherer Nerven bei Mensch und Tier die mit Vitamin B_6 in Beziehung stehenden Fermentsysteme betroffen sind. Substanzen, die Isoniazid über einen andern Mechanismus entgiften, besitzen keine schützende Wirkung gegenüber der neurotoxischen, auf die Markscheiden der peripheren Nerven gerichteten Wirkungskomponente des INH.

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Summary

Glutamic acid does not, either in small or in large doses, inhibit the degeneration of the medullary sheath of peripheral nerves in rats, induced within 15 days by addition of 0·25% of isoniazid to their diet. The detoxicating effect of glutamic acid, as described by Cedrangolo[1], thus only applies to the general toxic symptoms of the acute INH-intoxication, as is the case for vitamins B_2, B_{12}, A, E, K, and niacinamide.

PRO EXPERIMENTIS

Agar Diffusion Plates: The Recording of Experimental Data

The gel diffusion method of Ouchterlony[1] for the study of antigen-antibody reactions has found increasing application to immunological problems. Several descriptions of the technique have been given[2] but typically experiments are set up in Petri dishes and result in the formation of zones or fine lines of antigen-antibody precipitates in an agar gel matrix *approximately* 10 cm diameter and 3 mm deep. The line patterns observed are recorded usually by sketch or photograph but in some instances it is convenient if the whole contents of a Petri dish can be preserved. Gell[3] has demonstrated the partial desiccation of agar diffusion plates and the present communication describes an extension of this method.

Agar diffusion plates intended for preservation were cleaned by rinsing out the reagent reservoirs with water using a Pasteur pipette. Petri dishes containing the agar discs were then filled with water and the fluid changed about three times a day for three days. At the end of this time, when excess buffer and reagents had been leached from the agar, dishes and their washed contents were immersed in a large bowl of water, gently agitated, and the agar discs floated from their containers onto lantern-slide cover-glasses ($3\frac{1}{4} \times 3\frac{1}{4}$ in.). Cover-glasses and adhering discs were taken from the bowl, excess fluid removed with a Pasteur pipette and the preparations placed on a horizontal surface to dry at 18°. While drying, a large watch-glass was suspended over each disc; this allowed free access of air but prevented contamination by dust. After 3–4 days each disc dried to a tough, transparent film which adhered to its cover-glass and which differed little in diameter from an untreated disc but was *approximately* 0·5 mm in thickness. Insufficient washing resulted in opacity and the formation of salt crystals in dried preparations; hardening washed agar discs in 10% v/v formalin before transfer to cover-glasses was of no advantage in manipulation. Attempts to increase the rate of desiccation by drying either *in vacuo* or at 37° were not successful as in both cases marked distortion occurred and dried discs tended to peel off the supporting cover-glasses.

Dried preparations were best inspected against a dark background with the aid of a hand-lens. Opaque lines of precipitation were clearly seen in the transparent yellow matrix and the precipitation patterns obtained were identical with those recorded by sketch before drying (Figure). In some cases diffuse zones of precipitation were poorly preserved although the clarity of fine lines of precipitation was often enhanced, due possibly to a condensation of antigen-antibody complex on desiccation. Increased definition was obtained by staining the discs with dye before drying and such preparations could be projected as lantern-slides for demonstration purposes.

To effect staining, Petri dishes containing washed agar discs were filled with a 0·1% aqueous dye solution and allowed to stand at 18° until the dye had penetrated the discs. With protein stains this process required *approximately* 3 h at the end of which time excess dye was removed and the plates washed with water to differentiate the lines from the matrix. Since no fixative was used, differentiation was dependent upon the relative intensities of dye adsorption on the polysaccharide matrix and the protein contained in the antigen-antibody precipitates. Best results were obtained by staining the lines of precipitation with naphthol black or congo red

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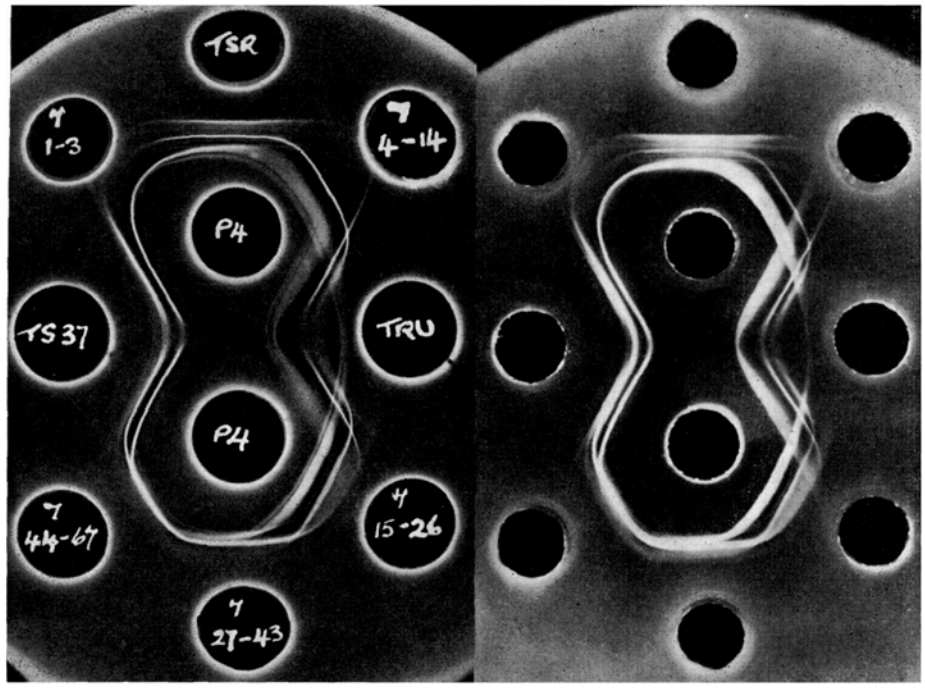

a *b*

(*a*) Agar diffusion plate stained with naphthol black and dried. The dried preparation was labelled with black waterproof ink, dyes of other inks being soluble in the mounting media solvent. (*b*) Photograph of plate *a* taken before staining and drying.

which left the agar matrix uncoloured. A photograph of such a preparation is shown in the Figure where a photograph of the same experiment taken before preservation is given also. In one case an uncoloured precipitation pattern in a deeply stained matrix was obtained by treatment with 0·1% aqueous toluidine blue followed by washing with 1% acetic acid. Attempts to use selective staining methods[4] however were not successful; basic fuchsin, mucicarmine, osmic acid and the Schiff reagents failed to give reproducible, selective staining of any of the precipitation lines produced by *Pasteurella pestis* antigens and *P. pestis* antisera[5].

Stained and dried preparations could be subjected to some degree of handling without damage. They adsorbed little or no moisture on exposure to laboratory conditions and were resistant to abrasion. On prolonged storage however, dried agar discs tended to peel away from the supporting cover-glasses and some additional protection was found desirable. Semi-permanent preparations were obtained by coating dried agar discs with Bedacryl 122 X, a colourless solution of a polymethacrylic resin in xylene. The resin dried by solvent evaporation resulting in a highly polished slide, but the surface was not very resistant to scratching and was damaged by organic solvents. More durable results were obtained by mounting each dried disc in a manner analogous to microscopic preparations. An epoxy-resin hardened with 3-diethylaminopropylamine was used for this purpose since mounting media drying by solvent evaporation could not be used due to the large enclosed surface area involved. The resin was similar in composition to 'Araldite' Type 101 (Aero Research Ltd.) but of lower viscosity. In use, hardener (1 part) was pipetted into a tube containing resin (10 parts), the solutions mixed carefully and the mixture centrifuged at 3,000 r.p.m. for 5 min to remove air bubbles. By means of a pipette with an enlarged jet 1·2–1·5 ml of the mixture was put in the centre of a dried preparation and a clean lanternslide cover-glass lowered gently on top. After keeping overnight at 37° excess hardened resin was removed from the edge of the preparation with a scalpel and the slide faces cleaned with cellosolve or acetone. To prevent the small excess of resin hardening on the slide faces and to facilitate handling while mounting it was convenient to put strips of adhesive tape along the underside edges of the dried preparations leaving 1 cm of tape free. The tape was stripped off after hardening together with most of the excess resin.

The authors wish to thank I.C.I. Ltd. (Dyestuffs Division) for a supply of Bedacryl 122 X and Mr. J. RUSSEL of C.D.E.E., Porton, for the preparation of a number of epoxy-resins.

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Microbiological Research Establishment, Ministry of Supply, Porton, England, July 30th, 1956.

Zusammenfassung

Ein Auswertungsverfahren der Antigen-Antikörper-Analyse mittels der Geléediffusionsmethode von OUCHTERLONY wird beschrieben.

Die Muster des Niederschlags im Versuch werden angefärbt und der Agar-Gelée-Nährboden mit dem Muster wird durch Austrocknung konserviert.

So gefärbte und getrocknete Präparate können, visuell geprüft, als Negative oder Diapositive verwendet werden.

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