DISPUTANDUM

Processus autoantigénique non spécifique chez le rat nourri au diméthylaminoazobenzène[1]

Nous avions antérieurement observé[2] qu'un processus autoantigénique apparaît entre la rate, le foie et le sérum d'un rat porteur de la greffe de la tumeur de Walker.

Nous avons étudié ce phénomène autoantigénique chez des rats chimiquement cancérisés dans le but de déterminer la généralité et la spécificité de ce phénomène.

Matériel et méthode. Nous avons dans cette recherche utilisé 40 rats mâles, adultes, de race Sprague Dawley, auxquels nous avons donné, comme régime alimentaire, une diète synthétique (groupe 1) additionnée soit de di*éthyl*aminoazobenzène (groupe 2), substance chimique témoin, non cancérigène, mais voisine, du point de vue de sa structure, du di*méthyl*aminoazobenzène (DAB), qu'ont reçu les animaux du 3e groupe.

Chez tous ces animaux nous avons fait, en commençant un mois après l'instauration du régime cancérigène, des analyses immunologiques du sérum et, chez deux animaux par groupe, que nous sacrifions chaque semaine, nous avons fait une étude du comportement autoantigénique de différents organes, vis-à-vis de leur propre sérum. Ces études immunologiques ont été faites à l'aide des méthodes de double diffusion en gélose[3] et d'immuno-électrophorèse[4].

Résultat. La Figure 1 illustre la relation autoantigénique qui s'est établie entre le foie, la rate et le sérum du rat nourri au DAB depuis 5 mois. Le critère immunochimique montre l'identité de la réaction entre le sérum et la rate d'une part, et le sérum et le foie d'autre part.

La réaction immunologique témoin qu'illustre la Figure 2, entre le sérum du rat nourri au diéthylaminoazobenzène et ses propres organes, soit son foie, sa rate et son rein, montre que dans ce cas aussi apparaît précocement, soit dès la 5ème semaine après l'instauration du régime qui contient le diéthylaminoazobenzène, un processus autoantigénique. Là encore, la jonction des arcs de précipitation démontre l'identité de la relation autoantigénique du foie et de la rate avec le sérum de l'animal nourri au diéthylaminoazobenzène.

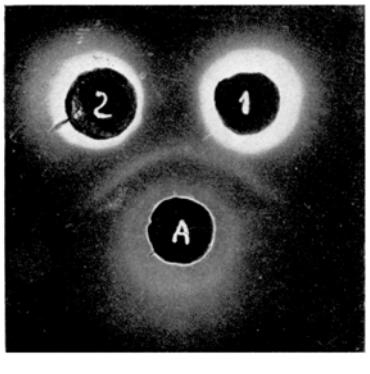

Fig. 1. Détection d'une relation autoantigénique entre le foie, la rate et le sérum du rat nourri au DAB. *Cuve 1:* Extrait de la rate du rat nourri au DAB depuis 5 mois. *Cuve 2:* Extrait du foie du rat nourri au DAB depuis 5 mois. *Cuve A:* Sérum du même rat.

Discussion. DECKERS et MAISIN[5] ont observé un phénomène comparable. Ces auteurs rapportent avoir obtenu des réactions de précipitation en double diffusion entre le sérum et des extraits d'organes normaux et de tumeurs.

En ce qui concerne ce résultat, nous sommes tentés d'attribuer l'apparition de l'autoantigénicité à l'action des colorants azoïques qui se lient aux protéines[6]. Cette association colorant azoïque-protéine pourrait en modifier légèrement la spécificité antigénique, induisant l'apparition de paraprotéines[7].

D'autre part, il est possible que ce processus autoantigénique bloque la capacité de l'organisme de répondre immunologiquement aux antigènes spécifiques qui apparaissent chez l'animal nourri au DAB[7–9].

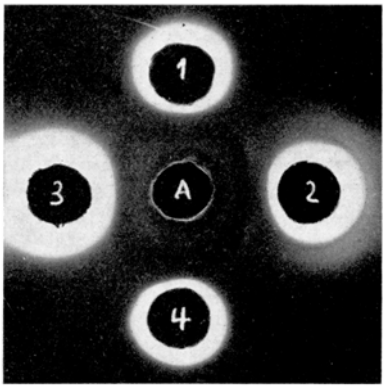

Fig. 2. Réaction autoantigénique entre le foie (cuves 1 et 3), la rate (cuve 2) et le sérum du rat nourri au 4-diéthylaminoazobenzène (A). La cuve 4 contient l'extrait du rein.

Summary. This result reports the apparition of a non-specific autoantigenic process between the liver, the spleen and the serum of rats fed on a dimethylaminoazobenzene and diethylaminoazobenzene-containing diet.

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[1] Travail subventionné conjointement par le Ministère de la Santé de la Province de Québec et l'Institut du Cancer du Canada.

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[10] Nous remercions Madame C. G.-PARÉ et Monsieur J. PROULX pour leur collaboration.

PRO EXPERIMENTIS

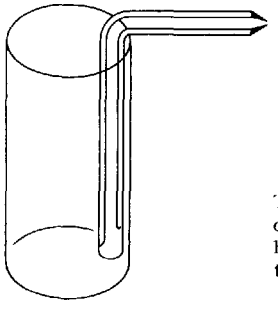
A Simple Inkwriter for Class Experiments

Writing on smoked paper is amongst the oldest and still valuable registration techniques employed in pharmacological experiments. Two of its disadvantages are the ease with which objects other than the writer may leave their traces on the smoked paper (especially in class experiments) and the need to fix the registration before it can be handled safely. These disadvantages may be overcome by using inkwriters, but many of the commercially available types are much less robust and heavier than a writer for smoked paper. However, the use of polythene tubing enabled us to develop an inkwriter (Figure) which was light enough to record the contractions of rat uterus

and ileum, and sturdy enough to be used successfully by medical students in 60 classroom experiments.

Construction. (1) Ink delivery tube: The shaft of a 20-gauge needle from which the boss has been removed but with the stylet in place is inserted into a length of closely fitting polythene tubing. The polythene is heated gently and drawn out so as to make a tight fit over the stylet. The stylet is removed, and needle and polythene tubing are bent with the fingers over the nail of the thumb to a right angle 7 mm from the blunt end of the needle: the polythene tube is cut 0.5 mm beyond the ends of the needle.

(2) Ink reservoir: This is made from polythene tubing 3–4 mm internal diameter, by heating this over a microflame and cutting it off at the appropriate length.



The finished inkwriter. The bent ink delivery tube consisting of a 20-gauge hypodermic needle encased in polythene, is glued to the wall of the polythene ink reservoir.

The ink delivery tube is glued to the side of the reservoir and left to dry for 24 h. Finally a length of aluminum wire or thin tubing is fixed to the reservoir, e.g. by inserting the toothed end of the aluminum into the gently warmed wall of the reservoir.

Our inkwriters had a weight of 90–110 mg and a capacity of 0.05 ml, which is enough to record e.g. uterine contractions for a few hours. It is essential to use a freely flowing ink, such as the ink supplied for electric recorders.

Use of this writer in class experiments provided two advantages over smoked paper writers. The work was less messy, and the students could remove their recording immediately after their experiment to take them to a discussion of the results in the lecture room. Especially the latter point was found markedly to stimulate their interest in their work.

Zusammenfassung. Unter Verwendung eines Polythänschlauches wurde ein Tintenschreiber (Gewicht 90–110 mg) zur Registrierung von Kontraktionen isolierter Organe konstruiert. Dieser Schreiber lässt sich mit Erfolg im physiologischen und pharmakologischen Pratikum verwenden.

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Department of Pharmacotherapeutics, University of Amsterdam (The Netherlands), May 8, 1963.

The Preparation of Holey Films for Electron Microscopy

Tiny holes in plastic films are useful for checking the astigmatism of an electron microscope[1], while larger holes are useful for supporting thin sections[2] and negatively stained preparations[3]. There are several methods for preparing films with holes[2,4,5], but we have found them to be unreliable or time consuming compared to the simple methods presented here.

One ordinarily makes a uniform Formvar film by allowing a solution of Formvar in ethylene dichloride to spread on a water surface. If the solution contains water droplets, the film will contain holes. Water can be introduced into the Formvar solution in a number of ways:

(1) One may spread a tiny droplet of 1% Formvar in ethylene dichloride on cold water (4 to 10°C) and immediately condense his breath on the film before the ethylene dichloride has evaporated. This procedure is simpler and quicker than the somewhat analogous method of SJÖSTRAND[2] or HARRIS[5]. However, such a Formvar film often contains bubbles in fields surrounding the zones of holes.

(2) 4–6 tiny droplets of 80% ethyl alcohol are added under shaking to 1 ml of 1% Formvar solution in ethylene dichloride. When the resulting bluish water emulsion is spread on a water surface it forms a very thin fragile film that is full of holes. Such fragile films may be picked up by allowing them to settle onto 200 or 400 mesh grids. Then before the grids have dried, they are touched to a second water surface containing a trace of a detergent so that when the grids are dry, surface tension is less likely to break the holey films.

(3) Finally, a water emulsion can be made by adding 0.001 ml of distilled water to 1 ml of a 1% Formvar solution in ethylene dichloride. The mixture is placed in a plastic tube which is inserted in the water-filled cup of a Raytheon sonic vibrator. After 30–60 sec sonic treatment at 9000 cps, the droplet of water becomes dispersed in the Formvar solution. The resulting milky blue emulsion, which is stable for about 30 min, is then mixed with 1 to 2 vol of untreated 1% Formvar solution in ethylene dichloride. When one drop of this mixture is placed on a clean water surface it spreads to form a Formvar network of veined appearance. The area between the veins contains tiny holes but here the film is again very fragile and should be picked up as previously described.

After such films have been coated with carbon they can be examined in the electron microscope (Figure 1). It is seen that many holes are suitable for correcting astigmatism. The number of holes per unit area can be reduced by decreasing the proportion of water emulsion to Formvar solution. Such mixtures also spread more uniformly on the water surface.

We also use another principle for obtaining holes of different sizes (Figure 2) by exposing the dry Formvar film to droplets of its solvent: 200 or 400 mesh grids are covered with a thin Formvar film. After drying in air, they are exposed to the very fine spray of ethylene dichloride produced by a Vaponephrin nebulizer (Vaponephrin Company, Division of Thayer Labs., Inc., 666

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