Iproniazid and implantation in mice.
Days of pregnancy were counted with day 1 as the day on which the vaginal plug was observed. Injections in saline subcutaneously

Iproniazid mg/kg	Day of treatment	Number of successful pregnancies *versus* number of ♀ with vaginal plugs
2×12	6, 7	6/6
2×25	6, 7	6/6
2×50	6, 7	3/3
2×100	6, 7	9/10
2×200	6, 7	2/2
0		8/8
100	6	4/4
100	5	3/6
100	5	3/3
0		8/10
100, 50	4, 5	3/4
100	4	2/3
0		2/3
2×100	4, 5	12/12
0		14/16

Zusammenfassung. Iproniazid-Behandlung von befruchteten Mäusen soll durch Serotoninanhäufung mit der Implantation interferieren. Unsere gezielteren Versuche ergaben bei mit Iproniazid behandelten Mäusen normale Fruchtbarkeit. Wir schliessen, dass Iproniazid nicht spezifisch mit der Implantation interferieren kann.

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Pharmakologische Laboratorien, SANDOZ AG, Basel, December 20, 1960.

Serotoninantagonismus an der Placenta

Serotonin-Injektionen lösen bei Ratte und Maus leicht Infarzierungen verschiedener Organe aus, weshalb vorgeschlagen wurde, diese Erscheinung an der Rattenniere zur Testierung von Serotoninantagonisten zu verwenden[1]; die hierfür verwendete intraperitoneale Dosis von Serotonin beträgt 100 mg/kg. Wir haben festgestellt, dass die Placenta in der zweiten Hälfte der Tragzeit bei Maus und Ratte auf bedeutend geringere Mengen subkutan verabreichtes Serotonin anspricht, ohne dass in der Niere oder einem anderen Organ makroskopisch Infarkterscheinungen beobachtet werden. Die vaskuläre Reaktion der Placenta auf Serotonin führt bei geeigneter Dosierung zum Tod der Foeten innert 3–4 h. Dieser Serotoneffekt schien uns dank seiner Eindeutigkeit und Raschheit geeignet, die Wirksamkeit von Serotoninantagonisten miteinander zu vergleichen.

Serotonin in 0,9% NaCl wurde subkutan 17–19 Tage trächtigen Mäusen 30 min nach Vorbehandlung injiziert. Die Vorbehandlung bestand in subkutaner Injektion von 0,3–0,5 cm³ 0,9% NaCl mit oder ohne Antagonist. 4 h nach der Serotonininjektion wurden die Tiere im Ätherrausch dekapitiert und geöffnet: In den eröffneten Uteri wurde die Zahl der toten und lebenden Foeten gezählt und der Zustand der Placenta notiert. Als Kriterium für den Serotoninantagonismus wurde also das Überleben von Foeten nach einer sicher tötenden Serotonindosis verwendet. Im Bereich der ED_{50} wurden pro Dosis minimal 5 Weibchen bzw. 50 Foeten zur Beurteilung der Schutzwirkung verwendet. Die ED_{50} der Serotonin-Antagonisten wurde nach der Probit-Methode graphisch ermittelt.

Folgende Stoffe wurden verglichen: 1. D-Lysergsäurediaethylamid (LSD-25, Delysid®). 2. 1-Methyl-D-Lysergsäure-propanolamid (PML-946). 3. 1-Methyl-D-Lysergsäure-butanolamid (UML-491, Deseril®).

Resultate. Nach Vorversuchen wurden als Serotonindosis 15 mg/kg und eine Wartefrist von 4 h gewählt: bei insgesamt 26 Kontrolltieren wurden so 144 von 152 Foeten getötet.

Die drei untersuchten Serotoninantagonisten erwiesen sich auch in dieser Versuchsanordnung als stark aktiv: Ihre Dosis-Wirkungskurven verlaufen im mittleren Wirkungsbereich parallel zueinander, so dass ein direkter Vergleich ihrer ED_{50} zulässig ist.

Für LSD wurde eine ED_{50} von ca. 30 µg/kg ermittelt. PML erzielte eine ED_{50} von 9 µg/kg, und für UML wurde bei 3,7 µg/kg Maus noch eine 50%ige Schutzwirkung gefunden. UML (Deseril®) erwies sich somit als rund 8mal wirksamer als LSD und ca. 2,5mal wirksamer als sein Propanolanaloges PML.

Am Serotoninödem der Rattenpfote wurde ein Aktivitätsverhältnis LSD:PML:UML von 1:2,6:4,4 gefunden[2] und am isolierten Rattenuterus ist UML 4mal stärker serotoninhemmend als LSD[3]. An der Placenta kann sich, wie die vorliegenden Versuche ergaben, der die Serotoninhemmung steigernde Effekt der 1-Methylierung der D-Lysergsäure noch stärker entfalten; dies wird besonders deutlich beim Vergleich der verwendeten Dosen von Agonist und Antagonist, die im Falle von UML (Deseril®) bei 50% Hemmwirkung an der Placenta sich wie 1:4000 verhalten.

Es dürfte deshalb von Interesse sein, den Wirkungsmechanismus, der diesem Antagonismus an der Placenta zugrunde liegt, näher zu untersuchen.

Summary. The placentae of 17 to 19 days pregnant mice respond to subcutaneous serotonin (15 mg/kg) with vascular changes which lead to the death of the fetuses in 3–4 h. Pretreatment of the females with D-lysergic acid derivatives preserves the life of the young. Thus, LSD showed an ED_{50} of only 30 µg/kg, while UML (Deseril®) gave an ED_{50} of 3.7 µg/kg. PML takes an intermediate position. At a 50% efficiency level, UML (Deseril®) antagonises the effect of a 4000 times higher dose of serotonin.

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Pharmakologische Laboratorien der SANDOZ AG., Basel, 29. Dezember 1960.

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[2] W. DOEPFNER und A. CERLETTI, Int. Arch. Allergy *12*, 89 (1958).
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PRO EXPERIMENTIS

The First Axenic Cultivation of a Rotifer Species[1]

The cultivation, in the absence of living organisms (and living cells) of other species, for long periods and with repeated subculturing—in other words, the sustained *axenic* cultivation—of members of the aschelminth Class Rotifera has not been claimed heretofore[2]. Two groups of

[1] The early part of this work was done at the Laboratory of Comparative Biology, Kaiser Foundation Research Institute, Richmond (California) and was supported, in part, by Grants G-6018 and G-13138 from the National Science Foundation.
[2] Except in a brief abstract: E. C. DOUGHERTY, B. SOLBERG, and D. JOANNE FERRAL, Anat. Rec. *138*, 344 (1960).

workers have reported brief axenic maintenance of the monogononts *Brachionus variabilis*[3,4] and *Lecane inermis*[4] and of the bdelloid *Philodina acuticornis* var. *odiosa*[4], but, for the most part, these organisms have merely survived without appreciable development. We are now able to report conditions whereunder the continuous cultivation of *L. inermis* has been realized.

During a period of almost three years numerous media, based on complex preparations, variously of vertebrate and bacterial origin, were tried, but failed to lead to axenic cultivation—first for *B. variabilis* and lately for *L. inermis* and *P. acuticornis*. The media in which, by contrast, *L. inermis* has now grown and reproduced for successive generations in serial subculture contain, as the crucial ingredient, a heated (lamb) liver extract (HLE) prepared according to a strict recipe[5] and sterilized by passage through an ultrafilter (Millipore).

The axenic *L. inermis* currently maintained by us descend from cultures originally established with rotifers that had been reared monoxenically[4] with an unidentified species of Gram-negative, rod-shaped bacteria. In the initial successful experiment, axenizing was accomplished by our selecting several dozen individuals with micropipettes, passing them through one rinse of 0.5 ml pond water previously sterilized by ultrafiltration (twice through sintered glass), and leaving them for approximately 2 h in ca. 20 ml distilled water containing, per ml, 1000 u K · penicillin G and 100 γ streptomycin · SO_4. Individuals were then transferred through one further sterile pond water rinse into 0.1% Horlick's malted milk, pending inoculation into small test tubes (10 mm outer diameter), each containing 0.2 ml medium to be tested. Each tube received a single rotifer and thereafter was tightly stoppered. Except (presumably) where injured or past reproductive age, such isolated individuals inoculated into suitable media became the progenitors of cultures consisting, at their acme, of some hundreds of active rotifers. Subsequently, subcultures have been readily initiated with single individuals from thriving axenic populations. Indefinite maintenance, in axenic culture, of *L. inermis* thus appears possible, just as has been demonstrated for several rhabditid nematodes[6], of which *Caenorhabditis briggsae*, now six years in continuous axenic cultivation in cell-free, liquid media, has been longest maintained.

HLE is not tolerated by *L. inermis* at $^1/_4$ dilution (the most concentrated tested), and only moderately well at $^1/_8$. At $^1/_{16}$ dilution growth is excellent. Initially the HLE was doubly supplemented with a 'GM' vitamin mix (at 1% the level used for *C. briggsae*[7]) plus Horlick's malted milk (at 0.075%); but current experiments demonstrate that HLE alone suffices.

Success in the axenic cultivation of *L. inermis* now opens the way to the development of chemically defined (holidic[8]) media for it, following the general pattern already set by studies with *C. briggsae*, for which a considerable advance toward such a medium has been realized[7]. These and similar organisms, grown axenically in holidic media, should in time become elegant tools with which to probe fundamental biological problems—particularly those depending for solutions upon the penetrating insights that comparative animal biochemistry and nutrition, as mature and mobilized disciplines, will inevitably provide.

Résumé. Le Rotifère monogononte *Lecane inermis*, a été mis par les auteurs en cultures axéniques capables de sous-cultivation indéfinie. C'est le premier Rotifère soumis à cette expérience.

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Laboratory for Gnotobiotic Studies, Berkeley (California), October 22, 1960.

[3] H. A. NATHAN and A. D. LADERMAN, Ann. N. Y. Acad. Sci. *77*, 96 (1959).

[4] E. C. DOUGHERTY, B. SOLBERG, and L. G. HARRIS, Anat. Rec. *137*, 350 (1960); Science *132*, 416 (1960).

[5] Originally developed by Dr. F. W. SAYRE (Laboratory of Comparative Biology) for studies on the nutrition of the nematode *Caenorhabditis briggsae* and generally prepared as follows: a liver from a freshly slaughtered lamb is allowed to age at 4°C for 24 h, then homogenized in a Waring Blendor with equal parts, w./v., distilled water, and thereafter heated in 100 ml amounts to 53°C in a waterbath and maintained at that temperature, with constant stirring, for exactly 6 min; next the product is centrifuged in the cold at 20000 G, and the supernatant (HLE) decanted and ultrafiltered immediately, or frozen pending filtration; filter d or unfiltered, it is stored at −17°C. The use of HLE in thestudy of nematode nutrition has not yet been reported in published form.

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[8] E. C. DOUGHERTY, Ann. N. Y. Acad. Sci. *77*, 51 (1959); in J. N. SASSER and W. R. JENKINS (Eds.), *Nematology, Fundamentals and Recent Advances with Emphasis on Plant Parasitic and Soil Forms* (Univ. N. Carolina Press, Chapel Hill 1960), p. 291.

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PRO EXPERIMENTIS

On the *in vitro* Bioassay for Measuring Melanophoretic Activity

This report will describe several modifications of the *in vitro* frog skin bioassay of SHIZUME et al.[1] as used in this laboratory for the estimation of melanophoretic activity. Particular attention has been directed toward the development of a 4-point assay procedure which can be easily controlled by classical mathematical analysis[2].

Materials and Methods. Pieces of skin of the legs and thighs of *Rana pipiens* frogs which have been kept in constant light for at least 2 weeks are removed, trimmed, and placed in concentric rings in a manner similar to that described previously[1]. Anodization of the aluminum parts of these rings was found to prevent completely the corrosion which occurs normally on the untreated aluminum parts when immersed in the electrolyte solutions. Anodized rings have been used here for more than 2 years without evidence of corrosion.

Variations in successive reflectance measurements on fragments of skin during control periods were encountered in early work with the assay; these were due essentially to disorientation of the skin fragment for each reading from the position in which it was first placed for the original reading. To remedy this, the bakelite outer rings[1] were designed with four equidistant V-shaped notches in the base to correspond to four ridges which we had sealed by fusion in the bottoms of 50 ml pyrex beakers. These ridges extend from the edge of the beaker about a third of the way to the center. A clear area remains in the center of the beaker through which the reflectance readings are taken. The same apparatus as employed by SHIZUME et al. (Photovolt Photoelectric Reflection Meter Model 610, Photovolt Corporation, New York) is used to indicate changes in reflectance of the skins. The use of these 'inter-

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