le procédé de stimulation utilisé n'exerce, sous ce rapport, aucun effet inhibiteur.

Du point de vue théorique, nous pensons que l'émission de beuglements de vache contribue d'une certaine façon, à replacer l'objet sexuel dans un cadre éthologique plus naturel, mais nous ne sommes pas encore en mesure de décider si l'accroissement de motivation sexuelle manifesté par le comportement du taureau, est lié à la vache elle-même, ou s'il est référé à un cadre mésologique plus large. Quelques indications recueillies au cours de la période expérimentale semblent, à première vue, être en faveur de la seconde hypothèse. Il est évident que si cette dernière était vérifiée, le champ d'application du procédé préconisé n'en serait que plus étendu. Des recherches actuellement en cours tentent de clarifier ces divers problèmes.

Tab. IV. Quantités moyennes de sperme éjaculées par les 4 taureaux utilisés au cours des deux périodes. (Valeurs en CC calculées à partir du nombre d'essais concluants, comme au Tableau III)

Animal	Période de contrôle préalable		Période expérimentale	
	Avec vache	Avec taureau	Avec vache	Avec taureau
David	–	5,16	4,25	–
Dirk	3,50	–	4,66	–
Luron	7,00	4,75	4,12	–
Ovide	4,00	4,15	–	6,00

Summary. In a preliminary series of experiments, it was noted that dairy bulls who refused to mount the cow for a long time, could be induced to mount it again when acoustically stimulated by specific calls of cows emitted by a magnetophone. In these conditions, the reaction time of the bulls was also shorter, while ejaculated quantities did not differ from those obtained before the experiments. It is suggested that this rather simple procedure could be helpful when the use of bulls as teasers raises difficulties.

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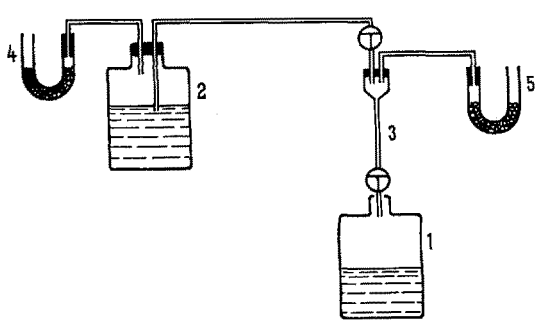
Ein Beitrag über die Art der Wirkung einiger Antituberkulotika

Die Arbeit untersucht die Beziehung zwischen Antituberkulotika und Pankreas-Lipase *in vitro*.

Apparatur. Im Versuch wurde eine Modifikation der Titrationsmethode nach WILLSTÄTTER benutzt. Die Titrationsapparatur besteht aus: (1) Gefäss mit zu titrierender Lösung, (2) Gefäss mit Titrationslösung, (3) Bürette, (4) zwei Luftfiltern mit KOH.



Technische Durchführung. Die Pankreaslipase wird durch Extraktion aus Schweinepankreas nach der Willstätter-Methode gewonnen[1,2]. Der Extrakt wird in Form seiner Glycerinlösung benutzt (10 ml Glycerin: 0,5 g des Extraktes). Das zu titrierende Gemisch besteht aus: 0,5 ml 10prozentigem Antituberkulotikum; 0,25 ml 2prozentigem $CaCl_2$; 1,0 Pufferlösung (1 Teil *n*-NH_4OH + 2 Teile *n*-NH_4Cl: pH = 8,9); 5,0 ml redestilliertem Wasser; 1,0 ml Olivenöl (zuletzt zugesetzt).

Zum Gemisch wird 0,1 ml Glycerinlösung der Pankreaslipase zugegeben. Der ganze Inhalt wird 4–5 min in einer Schüttelmaschine geschüttelt. Als Titrationsgemisch wird *n*/10 KOH in Alkohol und als Indikator 1% Thymolphthalein ebenfalls in Alkohol (2 Tropfen) benutzt.

Die Pankreaslipase wird bei 130 °C während 10 min stets unmittelbar vor jeder Titration inaktiviert. Titriert wird in folgenden Zeitabschnitten: 0, 30, 60, 120 min. Die Antituberkulotika werden in 10prozentiger Wasserlösung zu je 0,5 ml verwendet. STM-Dihydrostreptomycin (Merck Corp.); PAS-Natrium-*para*-aminosalicylicum (Spofa); INH-Nidrazid (chem. Isonicotin-hydrazidum) (Spofa); Sulfon-4,4'-di-NH_2-diphenylsulfon (Cilag); SHX-Salicylhydroxamsäure. (Das Präparat wird nicht klinisch, sondern nur vergleichsweise im Versuch verwendet.)

Ca^{++}-Ion (in Form von Calciumchlorid) hat eine wichtige Bedeutung für die Aktivierung der Pankreaslipase, weil es in der Titrationslösung die frei werdenden Fettsäuren zu unlöslichen Ca-Seifen bindet[3].

Prinzip des Experimentes beruht auf der Titration der Fettsäuren, die durch Pankreaslipase aus Olivenöl abgespaltet werden[1,2,4]. Die Titration wurde mit *n*/10 KOH in Gegenwart von Thymolphthalein durchgeführt. Die Pankreaslipaseaktivität wird durch die Anzahl der ml *n*/10 KOH, die zur Titration der Fettsäuren benötigt wurden, ausgedrückt.

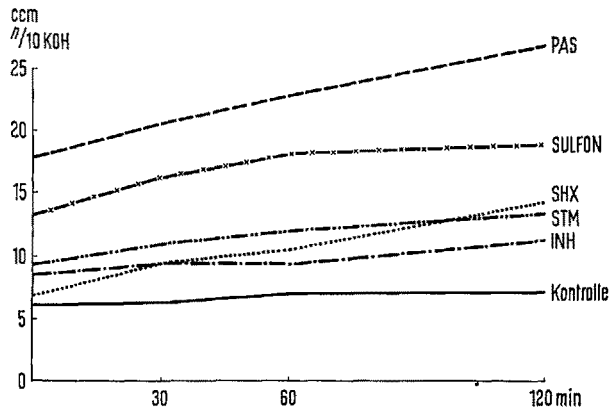
Ergebnisse. Wie die Figur zeigt, wird die Aktivität der Pankreaslipase durch PAS, INH, STM, SHX und Sulfon *in vitro* erhöht. Es handelt sich um Mittelwerte aus jeweils

[1] R. WILLSTÄTTER, E. WALDSCHMIDT-LEITZ und F. MEMMEN, Z. physiol. Chem. *125*, 93 (1923).

[2] R. WILLSTÄTTER und E. WALDSCHMIDT-LEITZ, Z. physiol. Chem. *125*, 132 (1923).

[3] P. DESNUELLE, M. NAUDET und M. J. CONSTANTIN, Biochim. biophys. Acta *5*, 561 (1950).

[4] J. B. SUMNER und G. F. SOMERS, *Chemistry and Methods of Enzymes* (Academic Press Inc., New York 1953), p. 80.



x Zeit in min. y Anzahl ccm n/10 KOH in der Alkohollösung, welche wir für die Titration der Spaltprodukte des Olivenöls (Fettsäuren) gebraucht haben.

30 Versuchen, die bei jedem Antituberkulotikum durchgeführt worden sind. Der Kontrollversuch wurde unter gleichen Bedingungen, jedoch ohne Antituberkulotikum durchgeführt.

Summary. The direct effect of any antituberculotics on pancreatic lipase was studied by the modified method of WILLSTÄTTER. The antituberculotics were found to activate the pancreatic lipase in the presence of Ca^{++}. Any cations, especially copper, decreases the lipase activity. Increasing antituberculotics concentration is accompanied by an increasing effect on the lipolytic action of lipase.

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9. Januar 1964.

Decontamination of Pleuropneumonia-Like Organism (PPLO) Infected Tissue Cultures

The problem of decontaminating cell lines infected with pleuropneumonia-like organisms (PPLO) has been approached using treatment with antibiotics[1], heat[2], or immune sera[3]. Results have been successful in the instances recorded, but the routine application of these methods has often failed. In our hands, both the thermal treatment at 41°C for 18 h, repeated several times, or the prolonged use of kanamycin (up to 600 µg/ml) and tetracycline (up to 25 µg/ml) failed to eradicate the contaminants from a line of strain L cells, originally obtained from Dr. W. R. EARLE. We decided, therefore, to test the specific antibiotic sensitivity of the PPLO isolated from this line as a guide to the selection of appropriate antibiotics. Sensitivity discs (Baltimore Biological Laboratory) were placed on plates of Bacto PPLO agar (70 parts), horse serum (20 parts), yeast extract Difco 25% (10 parts), heavily seeded with contaminated tissue culture fluids. Zones of inhibition were recorded after 7 days of incubation at 37°C. Growth of PPLO colonies was inhibited by novobiocin and chloromycetin (5 µg discs), but not by kanamycin, tetracycline, vancomycin (5 µg discs), erythromycin (2 µg discs), bacitracin (10 unit discs) and polymyxin B (50 unit discs).

The maximum non-toxic level of the two active compounds was determined and the following concentrations were eventually chosen for treatment: chloromycetin (diagnostic reagent, Chas. Pfizer & Co., Inc., Brooklyn, N.Y.), 10 µg/ml, and novobiocin, 50 µg/ml. The decontaminating procedure was carried out by establishing three cell lines: one treated with novobiocin, one with chloromycetin, and the third with both antibiotics at the concentration mentioned above.

To assess the efficacy of the decontamination procedures, transfers of the antibiotic-treated lines were carried out and each time sublines were derived to be maintained without antibiotics. When a confluent monolayer had developed, the antibiotic-free cultures were tested for PPLO by the colorimetric method based on the arginine deiminase activity[4], a test which we found to be rapid and sensitive. The PPLO-contaminated cultures were discarded. The ones which showed no evidence of PPLO were further propagated without antibiotics and tested at each transfer. When antibiotic-free sublines were consistently negative for 4 or more transfers, the treated cells were considered decontaminated from the time that the first PPLO-free subline was derived.

The results of this study showed that 8 days of treatment with novobiocin alone was sufficient to eradicate the contaminants. On the contrary, the line treated with chloromycetin was still heavily contaminated after 4 weeks. A disc sensitivity test performed at the end of this period showed that the contaminants were resistant to this antibiotic but not to novobiocin. The line treated with novobiocin and chloromycetin was decontaminated in 10 days.

In summary, the PPLO isolated in our laboratory were resistant to the most widely used antibiotics, namely kanamycin and tetracycline, at the time of the first sensitivity test and became resistant to chloromycetin, one of the two antibiotics used in the decontaminating treatment. The other, novobiocin, was effective in eradicating the contaminants in two different lines in 8 to 10 days.

As a general procedure for decontaminating PPLO-infected tissue cultures, a preliminary antibiotic sensitivity test is recommended. The use of antibiotics to which PPLO strains may easily become resistant, such as chloromycetin, kanamycin and tetracycline, should be avoided. To prevent selection of resistant mutants by the use of low concentrations of antibiotics, a test for the highest non-toxic doses should be performed in cultures different from the ones which will be eventually treated. The use of

[1] H. J. HEARN JR., J. E. OFFICER, V. ELSNER, and A. BROWN, J. Bacteriol. *78*, 575 (1959).
[2] L. HAYFLICK, Nature *185*, 783 (1960).
[3] M. E. POLLOCK and G. E. KENNY, Proc. Soc. exp. Biol. Med. *112*, 176 (1963).
[4] M. F. BARILE and R. T. SCHIMKE, Proc. Soc. exp. Biol. Med. *114*, 676 (1963).