

Diäthyl-*p*-Nitrophenylphosphat (E 600) nicht durchgeführt wurden, handelt es sich bei der von uns nachgewiesenen Esterase sowohl um die lysosomale als auch mikrosomale Fraktion dieses Enzyms. Wenn den histochemischen Methoden auch der Vorteil der Lokalisation zukommt, so ist durch ihre Anwendung allein eine exakte Aufklärung von Stoffwechselvorgängen im Gewebe nicht möglich. Ob es sich also bei der auffälligen Verminderung der Reaktionsprodukte bei behandelten Tieren um einen

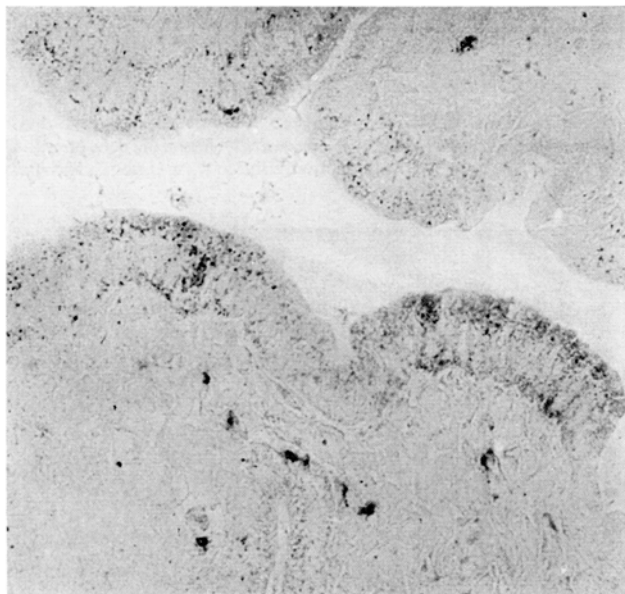


Fig. 2. Meerschweinchenuterus (2 Dragées «Anovlar» pro die, 10 Tage lang). Im Cavumepithel nur noch spärliche Reaktionsprodukte. $\times 350$.

Substanzverbrauch oder um eine tatsächliche Aktivitätsminderung handelt, lässt sich nicht sicher entscheiden und müsste quantitativ biochemisch oder zumindest elektronenmikroskopisch-zytochemisch nachgeprüft werden. Das Vorkommen lysosomaler Esterase im normalen menschlichen Endometrium während eines biphasischen Zyklus mit einem Fermentminimum in der späten Sekretionsphase wurde als ein Gelbkörperhormoneffekt angesehen (GARCIA-BUNUEL und D. BRANDES³). Dort kommt es offenbar mit der Sekretion der Uterindrüsen zur Auflösung der Lysosomen und damit zu einer Abnahme der histochemisch nachweisbaren Esteraseaktivität. Ähnlich dürften auch unsere Befunde zu deuten sein, nach denen die Gestagenkomponente der Ovulationshemmer für die Verminderung der Esteraseaktivität verantwortlich zu machen ist⁴.

Summary. Histochemical investigations were carried out in the endometrium of guinea-pigs after administration of 'Anovlar' (4 mg ethinyltestosterone-acetate and 0.05 mg ethynylestradiol). Histochemically a decrease of non-specific esterase activity was found in the endometrial as well as in the glandular epithelium after 'Anovlar' treatment. The relationship of this enzyme to synthetic steroid hormones is briefly discussed.

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³ R. GARCIA-BUNUEL und D. BRANDES, Am. J. Obstet. Gynec. 94, 1045 (1966).

⁴ Mit dankenswerter Unterstützung durch die Deutsche Forschungsgemeinschaft.

Arthritogenicity and Protective Effect Against Adjuvant Arthritis of Wax D_S Fractions (Glycolipids Without a Nitrogen-Containing Moiety) of *Mycobacterium tuberculosis* var. *hominis* and *bovis*

Waxes D from *Mycobacterium tuberculosis* var. *hominis* (as strain Canetti) are macromolecular peptido-glycolipids with important biological properties (FREUD's adjuvant effect¹; induction of adjuvant arthritis²) related to the presence of the nitrogen-containing moiety³ (amino acids and amino sugars). By ultracentrifugation in ether, JOLLÈS et al.⁴ were able to isolate a 'light' ether-soluble fraction, free from amino sugars and amino acids (wax D_S, without adjuvant effect) and 'heavy' fractions (waxes D_P), sedimentable after different time intervals, containing amino sugars and different amounts of a peptide moiety with alanine, glutamic acid and meso- α , α' -diaminopimelic acid, in approximate molar ratios 3:2:2 and possessing the adjuvant effect. Waxes D from *M. tuberculosis* var. *bovis* (as strain Marmorek) contain usually only the 'light' ether-soluble fraction (wax D_S).

Preliminary experiments by LACAPÈRE⁵ have shown that the arthritis-inducing ability of wax D_S fractions in rats is very weak when compared with that of D_P fractions. The arthritogenicity of wax D_P fractions is lost after acetylation⁶⁻⁹. We recently indicated that rats injected

with an acetylated wax D_P were protected against the arthritis-inducing ability of unaltered wax D_P not only when the acetylated wax D_P was injected 40–300 days before the unaltered wax D_P⁸, but also when the acety-

¹ J. FREUD, Fortschr. Tuberkforsch. 7, 130 (1956).

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⁶ C. M. PEARSON, F. D. WOOD and A. TANAKA, Arthritis Rheum. 7, 746 (1964).

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⁸ F. BONHOMME, C. BOUCHERON, D. MIGLIORE and P. JOLLÈS, C. r. hebdom. Séanc. Acad. Sci., Paris [D] 263, 1422 (1966).

⁹ P. JOLLÈS, D. MIGLIORE and F. BONHOMME, Immunology 14, 159 (1968).

Arthritogenicity in rats of wax D_p and D_s fractions and protective effect of wax D_s or acetylated wax D_p fractions against wax D_p arthritis

Wax D	mg	No. of rats	Arthritis in:	Severity ^a	Remaining symptoms ^b
Canetti D _{p35}	0.25	12	12/12	+++++	+++
Canetti D _{p35} acetylated	0.25	22	0/22	—	—
Canetti D _s	{ or 0.25 0.50	12	2/12	{ + +	{ + 0
Marmorek D _s	{ or 0.25 0.50	15	0/15	—	—
Canetti D _{p35} acetylated + Canetti D _{p35} (40 or 300 days later)	0.25 + 0.25	18	3/18	{ 1 + 2 ++	0 +
Canetti D _s + Canetti D _{p35} (40 days later)	0.25 + 0.25	4	4/4	+	0
Marmorek D _s + Canetti D _{p35} (40 days later)	0.25 + 0.25	15	7/15	{ 2 + 5 ++	+ +
Canetti D _{p35} acetylated + Canetti D _{p35} (the same day)	0.25 + 0.25	10	4/10	{ 1 + 2 + 1 ++	0 + ++
Marmorek D _s + Canetti D _{p35} (the same day)	0.25 + 0.25	9	9/9	++++	+++

^a Severity: +, ++, slight; +++, +++++, with osteite; +++++ malformation and ankylosis of the articulations. ^b Remaining symptoms: +, slight; ++ mean; +++ severe.

lated and the unaltered waxes D_p were injected together¹⁰. In this relationship, we want to report some results obtained with wax D_s fractions from human and bovine origins.

Adjuvant. The following formula was used: 9 mg of wax D_s from *M. tuberculosis* var. *hominis*, strain Canetti or 9 mg wax D_s from *M. tuberculosis* var. *bovis*, strain Marmorek, 4 ml vaseline oil, 1 ml Tween 80, 4 ml saline for injection. For comparisons, some assays were made with a wax D adjuvant known by previous tests to be potentially arthritogenic¹¹ (wax D_{p35}, strain Canetti). 0.25 or 0.5 ml of adjuvant is injected intradermically to each animal in the right hind foot-pad. Rats (Wistar origin) 8–10 weeks old were used. The method of scoring arthritis was previously reported^{8,10}.

Arthritogenicity of waxes D_s. Wax D_s Canetti (12 rats, 8 males and 4 females): 8 rats were tested with 0.5 ml adjuvant and 4 with 0.25 ml. 10 rats developed no arthritis; mild arthritis appeared in 2 animals, 1 tested with 0.50 ml adjuvant and the other with 0.25 ml; in 1 case the slight lesions disappeared quickly.

Wax D_s Marmorek (15 rats, 7 males and 8 females): 13 rats were tested with 0.25 ml adjuvant and 2 with 0.5 ml; none developed arthritis. The injection of wax D_s in the foot-pad provoked an edema which diminished after 48 h and disappeared completely after 15 days. A highly arthritogenic wax D_p provoked an edema which is followed by an inflammatory reaction until the apparition of the arthritis.

Protective effect of waxes D_s. Injection of the arthritogenic wax D_p 40 days after wax D_s. 4 rats injected with wax D_s Canetti in adjuvant (0.25 ml) and 15 rats injected with wax D_s Marmorek in adjuvant (0.25 ml) were re-inoculated after 40 days with the potentially arthritogenic wax D_{p35} Canetti in adjuvant (0.25 ml). Very mild arthritis appeared when wax D_s Canetti was employed: no symptoms remained after the inflammatory period.

8 rats injected with wax D_s Marmorek developed no arthritis, 2 a very slight one, and 5 a mild one.

Simultaneous injection of the arthritogenic wax D_p and wax D_s. 9 rats were tested with a mixture of waxes D_s Marmorek and D_{p35} Canetti in adjuvant (0.25 + 0.25 ml). This injection provoked in all animals the same phenomena as the injection of the arthritogenic wax alone: arthritis developed in 13 days and all the extremities and the tail were affected.

Conclusion. Our results indicate (Table) that waxes D_s and the acetylated waxes D_p^{8,9} which both develop no arthritis in rats, have a similar effect when an arthritogenic wax is injected 40 days later in these same rats⁹ but their behaviour is quite different¹⁰ when the arthritogenic wax is injected simultaneously. A detailed quantitative study of the related phenomena is actually under progress.

Résumé. Les cires D_s des Mycobactéries (souches humaines et bovines), glycolipides dépourvus de la partie azotée présente dans les cires D_p, ne provoquent pas d'arthrite chez le rat; contrairement aux cires D_p acétylées, elles ont un effet protecteur vis-à-vis d'une cire D_p arthrogène seulement si elles sont injectées 40 jours avant cette dernière.

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¹⁰ F. BONHOMME, C. BOUCHERON, D. MIGLIORE and P. JOLLÈS, C. r. hebdom. Séanc. Acad. Sci., Paris [D] 265, 2115 (1967).

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