Secondly, we compared the possibility of reactivation of intact rods of the strain *Proteus mirabilis* D 52 with that of the cells of a stable L-form of the same strain[7]; this L-form is, in fact, of a protoplast type. Rods or stable L-form cells from the stationary phase of growth were incubated 1 min at 37 °C in broth with an excess of colicin E1, before trypsin (0.25 mg/ml) was added; here the viability of elements in question was checked according to their ability to form macroscopic colonies on agar plates. For cultivation of L-colonies, 10% (v/v) of beef serum and 300 units penicillin/ml were added to meat-peptone agar.

The results are summarized in Table II. After 1 min exposure to colicin E1, 49% of rods and about 39% of L-form cells are capable of forming colonies (which corresponds to the quantitative ratio of the adsorption capacity of both kinds of elements for colicin E1[8]). After a prolonged exposure, the proportion of colony formers is no more diminished. Following the addition of trypsin, all rod cells inhibited by colicin, but no L-form cells are reactivated. So colicin E1 after 1 min exposure exerts only a bacteriostatic effect on rods, while that on stable L-form cells reaches the bactericidal phase. With regard to the fact that the stable L-form used lacks any, even chemically provable remnants of the cell wall[9], this result shows again that the cell wall considerably reduces the bactericidal effect of colicin E1.

Table I. Reactivation of rods and glycine spheroplasts of *E. coli* B1, incubated with colicin E1, by trypsin (phase-microscopic observations)

	Time of incubation with colicin E1 (min)			
	0 (controls)	1	2.5	5
Rods				
No. of elements observed	170	337	432	348
No. of viable elements	168	319	374	259
% of viable elements	98.8	94.7	86.6	74.4
Spheroplasts				
No. of elements observed	327	359	262	420
No. of viable elements	207	27	7	2
% of viable elements	63.3	7.6	2.7	0.5

Table II. Survival and reactivation of rods and stable L-form cells of *P. mirabilis* D 52, incubated with colicin E1, by trypsin (No. of colony formers)

Sample	Rods		Stable L-form cells	
	No./ml	%	No./ml	%
Original suspension	8.00×10^8	100.0	8.00×10^8	100.0
Original suspension + colicin E1 (1 min)	3.92×10^8	49.0	3.13×10^8	39.1
Original suspension + colicin E1 (1 min) + trypsin	8.03×10^8	100.4	3.12×10^8	39.0

It is thus possible to conclude that the bactericidal effect of colicin E1 on a sensitive strain proceeds the more readily, the more the wall of its cells is decomposed. In cells lacking their walls completely, the bactericidal effect is instantaneous. Thus the normal cell wall of rods does not mediate the bactericidal action of this colicin, but, on the contrary, it reduces it. This conclusion is in accordance with previous experience, concerning the action of colicin Q on *E. coli* spheroplasts[10,11] and of colicin E2 and G on stable L-form cells of *P. mirabilis*[5].

However, this conclusion may not hold for the action of all colicins in general. So it is probable that a further investigation of the effect of individual colicins on stable L-forms – and especially comparative experiments with protoplast-like stable L-forms of *P. mirabilis* and *E. coli* – may produce interesting results on the problem of the mechanism of the action of colicins in general.

Zusammenfassung. Es konnte wahrscheinlich gemacht werden, dass Zellwände von Bakterien die Wirkung bestimmter Colicine hemmen.

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[7] U. Taubeneck, Z. allg. Mikrobiol. *2*, 132 (1962).
[8] V. Obdržálek, J. Šmarda, J. Adler and O. Čech, Zentbl. Bakt. Parasitkde, Abt. 1, in press (1968).
[9] H. H. Martin, J. gen. Microbiol. *36*, 441 (1964).
[10] J. Šmarda and M. Vrba, Folia microbiol., Praha *7*, 104 (1962).
[11] J. Šmarda, Zentbl. Bakt. Parasitkde, Abt. 1, *196*, 240 (1965).
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PRO EXPERIMENTIS

The Human Chorionic Gonadotrophin Bio-Assay by Seminal Vesicles in Mice

The quantitative determination of chorionic gonadotrophin (HCG) is of importance in diagnosis and prognosis of pregnancy, as also for the diagnosis of tumours excreting chorionic gonadotrophin. From 1934, when the first method was cited for the quantitative determination, a number of biological and recently immunological methods were presented. Since the values obtained by immunological assays in second and third trimester of pregnancy are higher than those obtained by immunoassays (for lack of specificity), it was considered that immunological methods could be employed only for qualitative tests but not for quantitative determinations[1–3].

We wish to describe the biological assay method for the quantitative determination of chorionic gonadotrophin in urine based on the response in weight of seminal vesicles in immature male mice. The specificity and sensitivity of the method allow the direct injection of urine without any extraction of chorionic gonadotrophin.

Method. Male albino mice 21 days old, weighing strictly 9–10 g, were injected with native urine of total dosage levels 3, 1, 1/3, 1/9, 1/27 and 1/81 ml of 24-h urine. Doses from 1/3 to 1/81 were completed to 1 ml with borate buffer pH 9. The injections were made s.c. once daily for the 3 successive mornings. The total dose of 3 ml was injected as 3×1 ml while the other doses were in portions of 0.4, 0.3 and 0.3 ml. On the fourth morning, c. 72 h after the first injection, the animals were killed with chloroform, the seminal vesicles carefully dissected out and freshly weighed on the torsion balance 0–10 mg. 5 animals were used at each dose level. The doses were increased in geometric progression by the constant factor of 3. The calculations of the results and the tests of validity were performed by the procedure of BORTH et al.[4] using 1:2, 2:2, 2:3 or 3:3 types of calculations. As reference substance was used the Second International Standard for Human Chorionic Gonadotrophin (second IS-HCG) established 1963.

Reliability of the method. The specificity of the method was proved by the parallelism of the log-dose response curves of the second IS-HCG and many response curves of pregnancy urines (Figure 1), and by non-detection of chorionic gonadotrophin in normal urines. In order to know whether larger amounts of pituitary gonadotrophins, possibly present in nonpregnancy urines, have an influence on the results, the amounts of 2.5, 9.4, 11.0, 12.0 and 25.3 mg/24 h of the second IRP-HMG (FSH:LH = 1:1) were added to the urines of healthy individuals. After injection in mice, the increase of seminal vesicles was absent. In the third trimester of pregnancy, the concentration of the chorionic gonadotrophin in urine becomes lower but the concentration of biological active steroids increases. It had to counteract the influence of the amounts of these steroids present on increase of seminal vesicles. For this reason the mice were injected with active steroids on reproductive organs in quantities maximally present in 24-h pregnancy urines (androsterone 6 mg, testosterone 80 µg, androstenedione 40 µg, dehydroepiandrosterone 3 mg, oestrone 8 mg, oestradiol-17β 4 mg, oestriol 100 mg). Not in one case was the increase of seminal vesicles found. In order to test the accuracy, the amounts 375, 500, 800, 5000, 15,000 and 45,000 IU/24 h of the second IS-HCG were added in normal urines. Each concentration had 3 recovery experiments. The true values (the added amounts) were always within the fiducial limits $P = 0.05$. For the ascertainment of the precision the chorionic gonadotrophin was assayed in 4 urines 17–28 weeks of pregnancy in original sample and in dilutions with borate buffer 1:1 and 1:2. The individual results varied between 78 and 126% of the mean results. The sensitivity was examined by adding 250, 375, 500 and 800 IU/24 h of second IS-HCG in normal urines. At the concentration of 250 IU/24 h, not one true value was within fiducial limits, while in other amounts examined, they were. It can be considered that 375 IU/24 h is the value of sensitivity.

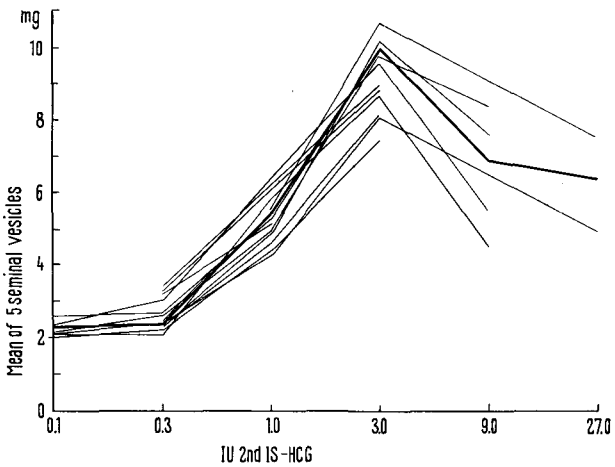

Fig. 1. The log-dose response curve of the second IS-HCG (heavy line) and response curves of the pregnancy urines.

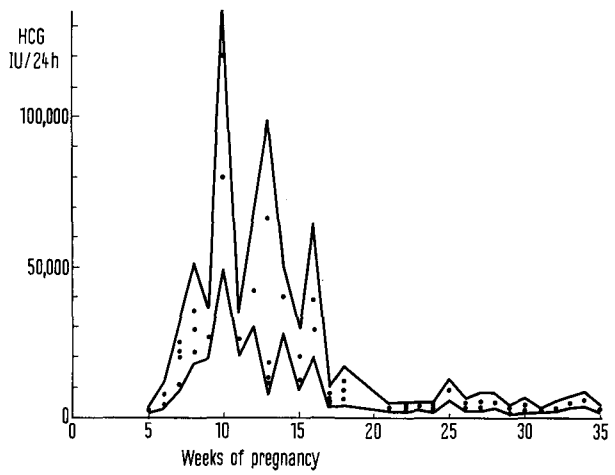

Fig. 2. The results and fiducial limits $P = 0.05$ of the HCG assays in 54 normal pregnancy urines.

In the Figure 2 the results are shown and fiducial limits $P = 0.05$ in 54 normal pregnancy urines. The values agree well with those reported by other biological methods. The index of precision of the determinations L was 5.30–14.15 ($L = 7.31 \pm 0.24$ S.E.) and the fiducial limits in order of 61–151% of the results[5].

Zusammenfassung. Biologische Methode zur Bestimmung von Choriogonadotrophin (HCG) im Harn, welche auf Gewichtszunahme von Samenblasen juveniler Mäuse basiert.

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[1] J. A. LORAINE and E. T. BELL, *Hormone Assays and Their Clinical Application* (E. & S. Livingstone Ltd., Edinburgh and London 1966), p. 90.

[2] R. BORTH, M. FERIN and A. MENZI, Acta endocr., Copenh. *50*, 335 (1965).

[3] R. HENRY, Colloque de la Société Nationale pour l'Etude de la Stérilité et de la Fécondité (Paris 1967), p. 89.

[4] R. BORTH, E. DICZFALUSY and H. D. HEINRICHS, Arch. Gynäk. *188*, 497 (1957).

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