tisch sich vermehrenden amicronucleären Ciliaten und für Mitosen.

Eine Schwierigkeit stellt sich bei Auswertung solcher Datenserien ein. Wie Figur 2 zeigt, gibt es offenbar Mischverteilungen mit unerwartet häufigen Zellen, die sich nur sehr langsam vermehren. In unserer Darstellung äussern sie sich in einer systematischen Abweichung von der Geradlinigkeit. Auch KUBITSCHEK hat auf sie hingewiesen[8]. Sie deuten sich mehr oder weniger stark an bei Verteilung 1, 3 und 4 in Figur 2, aber auch bei anderen publizierten Serien[9,10]. Diese Zellgruppe dürfte weitgehend mit der aus autoradiographischen Untersuchungen erschlossenen «non-proliferating fraction»[11,12] bei Tumoren übereinstimmen.

Der logarithmisch-normalen Verteilung der Generationszeit ist auch bei histometrischen Bestimmungen Rechnung zu tragen. Da anzunehmen ist, dass die jeweiligen Mittelwerte den gleichen Verteilungstyp besitzen, sind Häufigkeitswerte für Mitose- und Amitose-Bilder als Ausdruck mittlerer Generationszeiten bei ihren Logarithmenwerten als normalverteilt anzunehmen, wie bereits die praktische Erfahrung gezeigt hat[13,14].

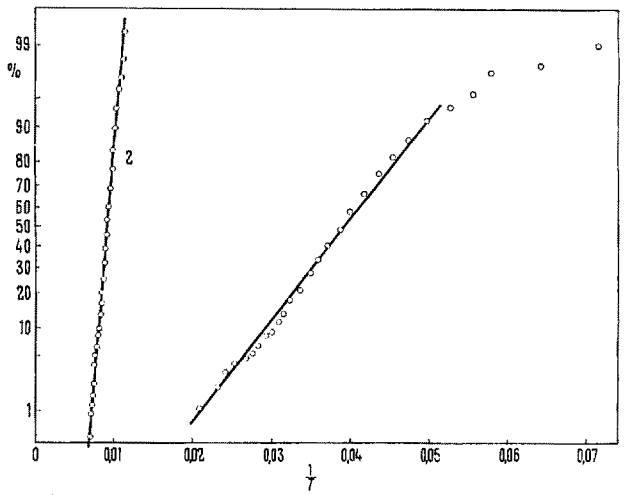

Fig. 1. Verteilung der Generationsrate $1/T$. Kurve 1: *E. coli* B/r nach[5], Kurve 2: *Tetrahymena pyriformis* HS nach[15].

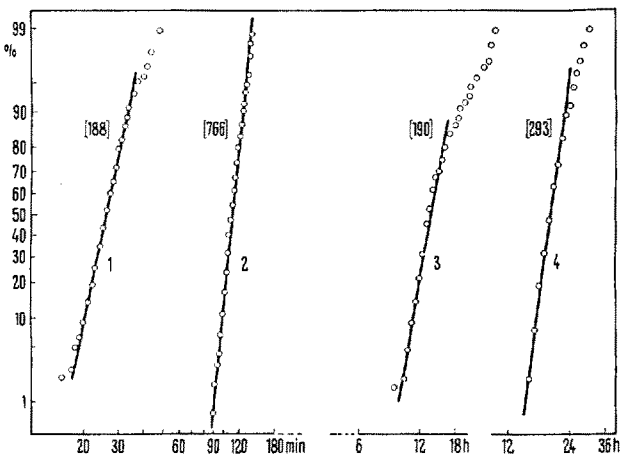

Fig. 2. Verteilung der Generationszeit T, dargestellt im Wahrscheinlichkeitsnetz mit logarithmisch geteilter Abszisse. Die Zahlen in Klammern geben die Anzahl der Messwerte an. Kurve 1, *E. coli* B/r nach[5], Kurve 2, *Tetrahymena pyriformis* HS nach[15], Kurve 3, Ratten-Sarkom S 6/1 nach[6], Kurve 4, menschliche Amnionzellen nach[16].

Summary. By application of the logarithmic-normal distribution of the generation time T, it is confirmed that, in contrast to the $1/T$-distribution, the deviation does not depend on the size of the mean.

R. SCHRÖDER

Max-Planck-Institute für Hirnforschung, Abteilung für Tumorforschung und experimentelle Pathologie, Köln-Lindenthal (Deutschland), 11. April 1967.

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Spermiogenesis in Hibernating Golden Hamsters

The investigations by WELLS[1] and FOSTER, FOSTER and MEYER[2] in *Citellus tridecemlineatus*, and of KAYSER and ARON[3] in *Cricetus cricetus* have demonstrated that gonadal activity shows a seasonal cycle which is not influenced by hibernation. After a period of rest, spermiogenesis starts again in the early months of the year even when the animal is still hibernating. A functional maximum, however, is only attained after emergence from the lethargic state.

A recent study of hibernation phenomena in the golden hamster (*Mesocricetus auratus*), using paired observations in hibernating and non-hibernating specimens, provided the opportunity to investigate more closely, and in direct comparison with the normal state, spermiogenesis during hibernation. The results to be presented in this report reveal a distinct interference of the lethargy with spermiogenesis.

Adult golden hamsters, aged 2–4 months, were grouped in pairs on the basis of equal body weights. These weights ranged from 80–130 g. One hamster was chosen at random from each pair and transferred to a cold environment (6–11 °C). The other animals were kept at room temperature, varying from 21–23 °C. They were caged separately, supplied with food and water ad libitum and exposed to 2 h of light daily. The experiment started on the 1st November.

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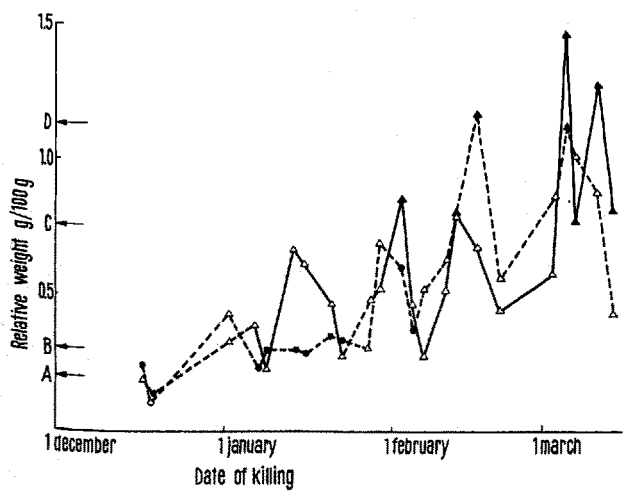
The majority of the animals kept in the cold (78%) entered hibernation in the second half of December. From this time through the middle of March, one pair of hamsters was sacrificed by decapitation on any given day. This was done only when the cold-exposed partner had hibernated at least 16 h (but less than 24 h) following a period of activity on the preceding day. The weight of the body and that of the testes were determined. The testes were fixed in Susa for 48 h and serially sectioned at 5 μ. The microscopical sections of the left testis were stained with the Pas–Ehrlich hematoxylin – light green procedure.

Left testis weight in relation to the body weight (g/100 g) and the categories of the histological pictures

Control hamsters			Hibernating hamsters		
No.	Relative weight	Classification	No.	Relative weight	Classification
57	0.198	III	39	0.234	II
55	0.137	II	52	0.098	I
13	0.317	III	5	0.441	III
46	0.400	III	36	0.235	II
6	0.221	III	38	0.299	II
21	0.674	III	28	0.303	II
9	0.618	III	48	0.292	II
49	0.473	III	1	0.357	II
70	0.271	III	31	0.339	II
34	0.475	III	30	0.302	III
42	0.531	III	7	0.697	III
26	0.862	IV	17	0.502	II
43	0.456	III	61	0.374	II
60	0.266	III	24	0.518	III
19	0.506	III	15	0.626	III
66	0.801	IV	23	0.792	III
41	0.665	III	14	1.174	IV
67	0.448	III	16	0.558	III
59	0.588	III	65	0.847	III
27	1.576	IV	54	1.132	IV
56	0.763	IV	4	1.007	III
47	1.297	IV	20	0.878	III
12	0.808	IV	50	0.422	III



Relative weight and classification of the microscopical picture of the left testis of control (——) and hibernating (– – –) golden hamsters. A: lower limit category III, control hamsters; B: lower limit category III, hibernating hamsters; C: lower limit category IV, control hamsters; D: lower limit category IV, hibernating hamsters. ○, category I; ●, category II; △, category III; ▲, category IV.

The left testis' weights are presented in the Table and the Figure. The weights are expressed as percentages of the final body weights. From the data it is evident that, under the circumstances of the experiment, the testis in both groups was in a state of regression in December, but underwent a steady growth in the following 3 months. The regression of the testis is a normal occurrence in December, but the short daily light periods may have had some additional influence (HOFFMAN and REITER[4]). However, the growth of the testis in the following months indicates firstly that short daily light periods do not prevent a reactivation of the testis, and secondly that hibernation does not interfere with testis growth in the later months. In this respect, therefore, the golden hamster does not differ from the other hibernators mentioned above.

Microscopically, the growth of the left testis was reflected by the activation of the germinal epithelium. In the completely inactive state (hamster No. 52), the germinal epithelium contained merely cells of Sertoli and spermatogonia. The lumen of the seminiferous tubules was closed and the apices of the Sertoli's cells touched each other. The revival of gonadal activity was indicated by the formation of spermatocytes and spermatids, which eventually resulted in the appearance of spermatozoa. For a detailed description of spermiogenesis in the golden hamster the reader is referred to the publication of CLERMONT[5].

In order to enable a correlation between the relative testis weight and the degree of testicular activity, the microscopical aspects of the testis were divided into 4 categories according to the diversity of the cell types in the germinal epithelium (the Sertoli cells not included): category I, spermatogonia only; category II, spermatogonia and spermatocytes; category III, spermatogonia, spermatocytes and spermatids; and finally category IV, all cell types including spermatozoa present. The results of the classification are also listed in the Table. It is apparent that category I did not occur in the controls, whereas only 1 testis met the requirements of category II. 16 out of the 23 animals fell into category III, and the presence of spermatozoa in the remainder indicated category IV. The lower limit for the relative weight of the left testis in category IV was 0.763 g/100 g, that of category III 0.198 g/100 g. The only testis representing category II weighed 0.137 g/100 g. The relative testis weights in the 3 categories did not show any overlap.

The microscopical picture in the hibernating animals, when correlated to the relative organ weight, was not equivalent to that in the controls. One testis (hamster No. 5), weighing 0.098 g/100 g, showed the histological characteristics of category I. Category II was present in 9 animals, the lower limit of relative testis weight being 0.234 g/100 g. Category III was found in 11 cases, the lowest relative testis weight being 0.302 g/100 g. Only 2 testes reached category IV, here the lower limit being 1.132 g/100 g. The weight ranges of categories II and III overlapped slightly.

From the results of the histological study, the following conclusions can be made. In the hibernating hamsters, the formation of spermatocytes, spermatids and spermatozoa is slowed down as compared with that in the control testes of the same relative weight. This is clear both from the upwards shift of the lower limits of the relative weight in the 3 defined categories, and from the much higher frequency of category II in the hibernating hamsters.

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Hibernation, therefore, interferes with spermiogenesis by a retardation of cell differentiation. Cell proliferation seems to be independent, because no difference in testis growth between the 2 groups of animals was observed. In general, an increase in the testis weight can be due either to cell proliferation or to a liquid retention, which may be attributed to a rise in the osmotic pressure in the tubules. Swelling of the latter kind, however, is only seen when the production of spermatozoa is maximal and when the seminal vesicles are enlarged. Such a state of spermiogenic activity was only found in the 2 control hamsters with the heaviest testes (hamsters Nos. 27 and 47). As was already shown, such a maximal activity cannot be reached during hibernation (WELLS[1]).

The discrepancy between cell proliferation and cell differentiation in the germinal epithelium of the testis, with regard to the influence of hibernation, possibly expresses a different endocrine balance. An analysis of hypophyseal and thyroidal microscopical states is in progress now in an attempt to reveal differences between control and hibernating hamsters at the endocrine level.

Zusammenfassung. Während einer Periode von Dezember bis März wurden von Goldhamstern im Winterschlaf und gleichzeitig von Kontrolltieren, die bei Zimmertemperatur gehalten wurden, die linken Testes untersucht. Aus der Korrelation zwischen dem histologischen Bild und dem relativen Organgewicht ging hervor, dass der Winterschlaf keinen Einfluss auf die Proliferation der Zellen des Keimepithels hat, dass sie aber die Differenzierung dieser Zellen verzögert.

J. H. SMIT-VIS and M. A. AKKERMAN-BELLAART

Anatomical-Embryological Laboratory, University of Amsterdam (The Netherlands), 17th April 1967.

Persistence of a Growth Hormone-Like Antigen in Organ Cultures of Human Placentas

The human placenta contains a substance (human placental factor, HPF[1]) that is antigenically related to human pituitary growth hormone (HGH). The active component in extracts of human placenta has been variously named 'placental lactogen'[2], 'chorionic growth hormone-prolactin'[3] and 'growth hormone-like substance'[4].

This paper reports our immunofluorescence (IF) studies on the persistence of HPF in organ cultures of 11 normal placentas (gestational ages 12–40 weeks). Normal villi were dissected under sterile conditions from 2 full-term placentas collected after spontaneous vaginal delivery and from the other placentas after caesarian section or hysterotomy. Explants (2–3 mm³) of the placental villi were maintained in a chemically-defined medium (T.C. 199) with a simple organ culture technique[5]. Representative explants from each placenta were fixed in 4% neutral buffered formaldehyde in saline after culture for 1, 3, 5, 7, 10, 12, 14 days and 3, 4, 5 and 6 weeks in vitro. The histological appearances of the trophoblast in our organ cultures, although slightly modified, indicated that the tissue is living; this conclusion was supported by the report of DNA synthesis in cytotrophoblast nuclei in similar organ cultures[6] and by evidence of glucose consumption by the explants[5].

The indirect IF staining technique has been described previously[7]. In the first stage, the sections were treated with a goat antiserum to the RABEN[8] preparation of HGH or with non-immune goat serum and 0.15 *M* NaCl as controls; fluorescein-conjugated rabbit anti-goat IgG serum was used in the second stage. The IF staining of organ-cultured explants of human placenta was reproducible as consistent results were obtained on staining sections of each block on 3 separate occasions with anti HGH serum and the controls. The specificity of this anti HGH serum in IF staining of normal uncultured human pituitary gland and placenta has been characterized previously[1,7,9]. Nevertheless it was essential to demonstrate that staining of organcultured placenta was also immunologically specific: the experiments to determine this[10] were performed on sections of 3-, 5- and 7-day explants of 2 placentas from each trimester with the methods described previously[7]. The findings were in complete concurrence with those of previous experiments in which the immunological specificity of the IF staining system had been established[7].

The sections were examined independently by both of us, without prior knowledge of the serum used, and HPF

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[10] *Firstly*, we showed that the staining capacity of the anti HGH serum diluted $^1/_{16}$ in saline (as used throughout this investigation) was removed by absorption with acetone-dried powders of human pituitary and placenta (approximately 100 mg/ml), with RABEN[8] preparation of human pituitary growth hormone (4 mg/ml) and with FRIESEN[11] extract of human placenta (4 mg/ml); absorption with acetone-dried powders of other human organs (adult liver, heart, kidney and spleen, and foetal brain, liver, spleen and skin) (approximately 100 mg/ml), purified human pituitary adrenocorticotrophic hormone (ACTH) prepared by the method of CURRIE and DAVIES[12] (4 mg/ml), purified human urinary gonadotrophins (4 mg/ml) or human IgG (4 mg/ml) did not remove the staining capacity. *Secondly*, the anti HGH serum was fractionated by chromatography on DEAE Sephadex and various fractions were used separately as the first stage in the IF staining; bright staining was obtained with the IgG fraction only. The active principle is, therefore, an immunoglobulin. *Thirdly*, it was shown in absorption experiments (50 mg absorbant/ml) that the staining capacity of the fluorescein-conjugated anti-goat IgG serum could be removed by treatment with goat IgG (prepared by DEAE Sephadex chromatography of normal goat serum) but it was not affected after treatment with other fractions of goat serum, human IgG or rabbit IgG.
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