–21 °C, mean VO_2 was 4.2 times greater and reached 58.7 ± 1.61 $ml \cdot kg^{-0.75} \cdot min^{-1}$.

With the smaller dose of epinephrine (0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$), (table 1), in dogs resting at neutral ambient temperature, there was just a slight (+8%), but significant ($p < 0.05$), increase in the glucose total disappearance rate (Rdt). This increase was not due to an increase in irreversible loss (Rdi), which remained unchanged, but it can be entirely accounted for by an increase in glucose recycling (+ 39%, $p < 0.001$). In the cold ambient temperature, Rdt remained unchanged, because of a balance between Rdi, which was significantly (–11%, $p < 0.01$) decreased, and recycling, which was significantly (+ 42%, $p < 0.001$) increased. Therefore, this smaller dose of epinephrine always increased recycling, but specially in cold it decreased the irreversible utilization.

With the larger dose of epinephrine (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$), (table 2), in dogs resting at neutral ambient temperature, there was a large increase (+ 50%, $p < 0.001$) in Rdt. This increase was mainly due to an increase in recycling (+ 115%, $p < 0.001$) which accounted for about $^2/_3$ of the increase in Rdt. Irreversible loss was less, but significantly (+ 22%, $p < 0.01$), increased by the hormonal infusion. In dogs exposed to cold, a similar situation was observed, with a significant increase (+ 40%, $p < 0.001$) in Rdt, which was mainly ($^2/_3$) due to a large (+ 128%, $p < 0.001$) increase in recycling. Therefore, this larger dose of epinephrine induced an increase in irreversible glucose loss, but this increase was smaller than that observed for recycling.

Discussion. The catecholamine secretory response to cold exposure is greatly decreased in ADMX dogs [20,21], while basal concentrations of plasma cortisol, as well as the adrenocortical response to cold stress, were not impaired. The doses of epinephrine used in the above experiment were chosen on the basis of data collected by Klepping et al.[22], who observed, by catheterization of the adrenal veins in dogs, that the output of catecholamines increased to 0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ during cold exposure.

Irreversible loss of glucose includes transformations such as oxidation to CO_2, along with conversions into metabolites which are incorporated in molecules with relatively long turnover times with regard to the duration of the experimental period. Unfortunately, in the above experiment, precise measurement of the oxidation rate is hindered by the decrease in the bicarbonate pool, which, in turn, is due to the epinephrine-induced hyperlactatemia. On the other hand, reversible loss consists of transformations which occur through utile and futile cycles[23]. The above experiment, conducted in dogs, at an energy expenditure level close to the BMR, shows that most of the extra glucose mobilized by epinephrine is recycled, rather than actually utilized. More surprinsingly, a similar situation occurred in cold-exposed dogs, even though carbohydrate needs for thermoregulation increase in cold ambient temperature[24]. These needs are probably partially covered by intramuscular glycogenolysis which is also enhanced by epinephrine.

20 G. Morin, Biol. med. *37*, 196 (1948).
21 Y. Satake, Nanzando Co Hongo, Tokyo 1955.
22 J. Klepping, M. Tanche and J. F. Cier, C. r. Soc. Biol. *151*, 1539 (1957).
23 M. S. Anwer, T. E. Chapman and R. Gronwall, Am. J. Physiol. *230*, 138 (1976).
24 Y. Minaire, A. Pernod, J. C. Vincent-Falquet and M. Mottaz, Horm. Metab. Res. *5*, 80 (1973).

Effect of antiblastokinin on rabbits pregnancy*

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Summary. On different days following coitus, i.e., during the first stages of pregnancy, adult female rabbits were treated with antiblastokinin obtained from a chicken. A high prenatal mortality was observed and a reduction of the weight in the stillborn of the rabbits treated. The antiblastokinin produces evident biological effect about the 4th day following coitus, during the period of maximum production of blastokinin by the endometrium in vivo.

Blastokinin (BKN) is a protein isolated by the uterine secretion of various mammals during the estrus or the early days of pregnancy or pseudopregnancy[1–6]. In the rabbit it is present between days 3–9 following copulation, reaching maximum about the 5th day and it appears to favour the development and implantation of fertilized ovum[3–9]. Krishnan[10] and Daniel[11] have demonstrated in the rabbit and the pig that the administration of antiblastokinin (anti-BKN) in the first stages of embryonic development, in particular during the period of preimplantation of the blastocysts, interferes with the number of foetus born[10,11], duration of gestation and appearance of successive estrus[11]. The aim of our research is to establish the biological effect of anti-BKN found by us in the chicken[12] and to establish at what time the course of administration of anti-BKN determines evident biological effect.

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1 N. Garcea, G. Porcelli, A. Caruso, S. Campo and A. Bompiani, Int. J. Fert. *19*, 73 (1974).
2 H. M. Beier, Biochim. biophys. Acta *160*, 289 (1968).
3 R. S. Krishnan and J. C. Daniel, Science *158*, 490 (1967).
4 F. A. Murray, R. W. McGaughey and M. J. Yarus, Fert. Steril. *23*, 69 (1972).
5 K. Hamana and E. S. E. Hafez, Am. J. Obstet. Gynec. *110*, 572 (1971).
6 M. A. Urzua, R. Stambaugh, G. Flickinger and L. Mastroianni, Jr, Fert. Steril. *21*, 860 (1970).
7 A. Arthur and J. C. Daniel, Fert. Steril. *23*, 115 (1972).
8 A. El-Banna and J. C. Daniel, Fert. Steril. *23*, 101 (1972).
9 A. El-Banna and J. C. Daniel, Fert. Steril. *23*, 105 (1972).
10 R. S. Krishnan, Experientia *27*, 955 (1971).
11 J. C. Daniel, Experientia *28*, 700 (1972).
12 A. Caruso, N. Garcea, S. Campo, L. Milano and A. Bompiani, Acta Eur. Fert. *5*, 295 (1974).

Materials and methods. For our experiments, we used a specific anti-BKN obtained by immunizing a chicken with purified rabbit blastokinin[12]. The chicken's γ-globulin was precipitated with ammonium sulphate[13] and the protein content was measured by the Lowry method[14]. 2 series of experiments have been carried out. In the first series, 15 adult white rabbits (New Zealand) were mated and divided into 5 groups of 3 animals each. Then 60 mg of lyophilized chicken γ-globulin containing anti-BKN, dissolved in 1 ml of physiological solution and mixed with 1 ml of complete Freund's adjuvant, were inoculated i.p.: a) to the 1st group on the 2nd day following coitus, b) to the 2nd group on the 4th day following coitus, c) to the 3rd group on the 6th day following coitus. The 4th group of animals was inoculated i.p. with 3 successive injection on the 2nd, 4th and 6th days following coitus with 20 mg of chicken γ-globulin containing anti-BKN, dissolved in 1 ml of physiological solution, incorporated in 1 ml of complete Freund's adjuvant. The 5th group (control) was treated in the same way as the 4th group with a preparation of normal chicken γ-globulin incorporated in 1 ml of physiological solution and 1 ml of complete Freund's adjuvant. All the rabbits were checked at the moment of delivery. In the 2nd series, the same rabbits of the 1st 4 groups were mated 3 months after anti-BKN treatment and checked at the moment of the successive delivery.

Effect of anti-BKN administration on rabbits prenatal mortality

	A	B	C
Number of young	58	44	18
Number of young born dead	38	3	2
%	67	7	11

A Rabbits treated with anti-BKN; *B* Same rabbits considered in A, mated 3 months after anti-BKN treatment; *C* Control rabbits.

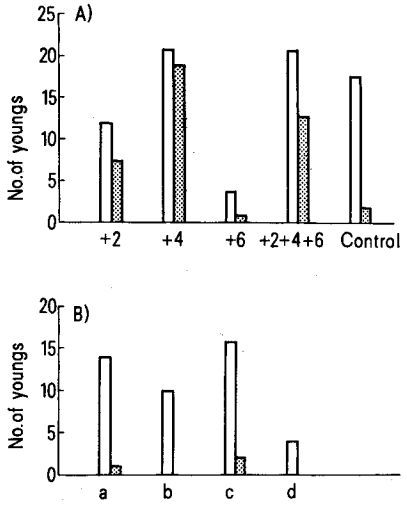

Fig. 1. Incidence of fetal mortality for each group of treatment. *A* Rabbits treated with anti-BKN; *B* Same rabbits considered in A, mated 3 months after anti-BKN treatment. Total of youngs (clear) and total of young born dead (marked) are reported.

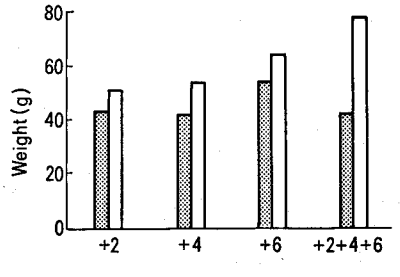

Fig. 2. Comparison between the mean of the weights of the young born dead after anti-BKN treatment (marked) and the mean of the weights of the young from the same rabbits mated 3 months after anti-BKN treatment (clear).

Results. In the table, the data are registered. Apart from the day of treatment, the data of the following groups are considered together: in A all the young of the 12 animals treated with anti-BKN; in B all the young of the same animals, mated 3 months after anti-BKN treatment; in C the youg of the control rabbits. In group A a mortality of 67% is evident, as against a spontaneous mortality of about 10% in groups B and C. The data presented in the table are showed in figure 1 analytically. The total of young born, and the total of young born dead, for each and every group of treatment are reported, both in the pregnancy successive to the anti-BKN administration (A) and in the pregnancy followed by a time span of 3 months (B).

The number of young is highly reduced in the group of rabbits treated with anti-BKN on the 2nd and 6th day following coitus. In effect, of 3 rabbits in the 1st group, one did not give birth; while of the 3 rabbits in the 3rd group, only one gave birth to 4 foetuses, of which 1 was stillborn. On the contrary, the same rabbits present the highest prolificacy at 3 months after the treatment (figure 1, a, c). The mortality is particularly high in the rabbits treated on the 4th say or on days 2, 4 and 6; and less in the other groups. Mortality of the young was nearly absent in the control and in the same rabbits 3 months later. The reduced birth in groups b and d (figure 1) is also imputable to the occasional death of a rabbit in each group. Furthermore, in group d, 1 rabbit did not give birth, probably because it was not fertilized.

The comparison between the weight of the dead young after anti-BKN treatment and that of the live young from the same rabbits after 3 months is shows in figure 2. A reduction in the weight of the stillborn is evident, especially for the young of the rabbits treated on the 4th day and on the 2nd, 4th and 6th day following coitus.

Discussion. The data demonstrate that the administration of anti-BKN in the first stages of pregnancy interferes with the normal course of the gestation in the rabbit, in accordance with the data reported by Krishnan and Daniel[10,11]. This is an indirect confirmation of the important biological role of blastokinin in the development and implantation of the blastocyst.

In fact, the prenatal mortality of the treated animals (67%) appears highly significant if confronted both with the spontaneous mortality of the control (11%) and that of the same rabbits mated 3 months after anti-BKN treatment (7%) (see the table). This excludes the possibility that the foetus mortality could be due to the administration of chicken γ-globulin or a character present in the animals used for the experiments. The number of young does not seem to be affected in a significant

13 P. Stelos, in: Handbook of Experimental Immunology, chapt. I. Ed. D. M. Weir. F. A. Davis Company, Philadelphia, 1967.
14 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Biochem. *193*, 265 (1951).

manner by the anti-BKN (see figure 1). Nevertheless, 3 rabbits treated (1 in group 1 and 2 in group 3) did not give birth. This could be caused by: absence of fertilization; defective implantation of blastocyst; or reabsorbment of embryos already nidated. These last 2 hypotheses are confirmed by the experience of Krishnan[10] and Daniel[11], respectively. In analyzing the prenatal mortality in the different groups of treatment, it is obvious that the major incidence occurs in the rabbits treated in groups 2 and 4.

The mean of the weight of the stillborn in comparison with the weight of the live young from the same rabbits after 3 months, is constantly lower (see figure 2). This phenomenon is more evident in groups 2 and 4, and it could be related to the arrest of growth resulting in uterine death of the foetus. Above all the appearance of the stillborn leads one to believe that death occurred recently before birth. Therefore the reduction in weight could be due to the under-development of the foetus. Moreover, the stillborn young under the macroscopic examination, seemed to be normal and properly formed; it therefore seems to exclude a teratogenic activity of the anti-BKN 'in vivo'. Our results did not show confirmation of defective implantation or reabsorption of the embryos produced from the anti-BKN administration; but, on the other hand, the effect on the prenatal mortality and on the weight of the young at birth was evident. It has been demonstrated that the period of greatest biological effect of anti-BKN corresponds with the period of maximum production of blastokinin by the endometrium, i.e., about the 4th–5th day following coitus[15]. This confirms the hypothesis of the importance of the production and the presence of the blastokinin in the uterus at the moment of blastocyst nidation. Nevertheless, it is not clear how the activity of the anti-BKN can produce a high prenatal mortality. We way presume that it can neutralise the blastokinin in its biological role, connecting it, in vivo, with the development of the placenta.

15 N. Garcea, A. Caruso, S. Campo, L. Milano and A. Bompiani, Fert. Steril. *27*, 442 (1976).

The effect of sleep deprivation upon variations in heart rate and respiration rate

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Summary. Variations in heart rate and respiration rate were found to be responsive to total sleep deprivation, particularly under experimental conditions more realistic to everyday life.

From recent reviews[2–4] and studies[5–7] on the effects of total sleep deprivation (TSD) in man, the major findings appear to be amongst behavioural, performance and EEG parameters rather than with physiological measures of somatic functioning. Although this suggests that human sleep may be more oriented towards the brain than to the body, there are methodological factors to consider. Firstly, because of experimental control, subjects are usually confined to a laboratory, and apart from the TSD, lead a restricted regime unlike a normal everyday existence. If human sleep aids restitution from wakefulness, then this regime may have a reduced effect than otherwise might be expected, particularly as it has been hypothesised[8,9] that a high waking visual load might potentiate TSD effects. Secondly, certain physiological measures may not be sensitive enough to TSD. Measures taken during any physiological investigation ideally need to have conceptual validity to the phenomenon under examination. Respiration is apparently[10,11] very sensitive to changes in consciousness. This factor together with: a) the apparent[12–14] interplay between cardiac regularity mechanisms, cardiac output and levels of attention, b) reports[15–17] that attention levels affect sinus arrhythmia, with respiration playing a major intermediary role, all suggest that although mean levels of heart rate (HR) and respiration rate (RR) are not sensitive to TSD[2–7], HR variation (HRv) and RR variation (RRv) may be so. The only study[18] to assess HRv found significant, but undetailed, changes. RRv has not been used in TSD studies.

Method. 6 healthy young males were paid to stay awake for 62 h, on 2 occasions. Both occasions were laboratory centred, with 1 having a high visual perceptual load and the other being a control condition containing a low load, more typical of other TSD studies. These conditions are detailed elsewhere[8]. Subjects were TSD'd in 2 groups of 3 with one group undergoing high load-low load, and the other group the reverse order. 2 weeks elapsed between conditions. 3 baseline days preceded each TSD and 2 recovery days followed. Subjects slept in a sleep laboratory on these 5 nights to ensure no additional sleep loss. Using EKG chest electrodes and nasal thermistor, HR

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2 R. T. Wilkinson, in: Physiology of Human Survival, p. 399. Ed. O. G. Edholm and A. L. Bacharach. Academic Press, London 1966.
3 P. Naitoh, in: Research in Psychophysiology, p. 153. Ed. P. H. Venables and M. J. Christie. Wiley, London 1975.
4 J. A. Horne, Physiol. Psychol., in press (1977).
5 L. C. Johnson, in: Research in Psychophysiology, p. 125. Ed. P. H. Venables and M. J. Christie. Wiley, London 1975.
6 P. Naitoh, R. O. Pasnau and E. J. Kollar, Biol. Psychiat. *3*, 309 (1971).
7 V. Fiorica, E. A. Higgins, P. F. Iampeitro, M. T. Lategola and A. W. Davis, J. appl. Physiol. *24*, 167 (1968).
8 J. A. Horne, Biol. Psychol. *4*, 107 (1976).
9 J. A. Horne and B. Walmsley, Psychophysiology *13*, 115 (1976).
10 K. Bülow, Acta physiol. scand. *59*, 1 (1963).
11 J. A. Horne and M. Whitehead, Experientia *32*, 1165 (1976).
12 M. Bonvallet, P. Dell and G. Hiebel, Electroenceph. clin. Neurophysiol. *15*, 63 (1954).
13 W. Baust, H. Niemcysk and J. Veith, Electroenceph. clin. Neuphysiol. *15*, 63 (1963).
14 J. Lacey, in: Psychological Stress, p. 14. Ed. M. H. Appley and R. Trumbull. Appleton Century Crofts, New York *1967*.
15 J. W. H. Kalsbeek, in: Measurement of Man at Work, p. 101. Ed. W. T. Singleton, J. G. Fox and D. Whitfield. Taylor Francis, London 1970.
16 B. W. Hyndman and J. R. Gregory, Ergonomics *18*, 195 (1975).
17 B. McA. Sayers, Ergonomics *16*, 17 (1973).
18 A. Ax and E. D. Luby, Archs gen. Psychiat. *4*, 55 (1961).