membrane is present in between the old and new cuticles (Fig. 2). The new cuticle increases in thickness to about 8 to 10 μ. Duration of this stage is from 2 to 3 days.

D_4 Stage: The new claws are retracted completely. The fresh cuticle increases in width to about 12 to 16 μ and becomes pale orange pigmented. The entire old endocuticle is digested by the moulting fluid and the ecdysial membrane is now attached to the inner surface of the old cuticle. This stage lasts from 6 to 9 hrs.

Stage E: This is the stage in which the animals actually shed the old cuticle starting from the cephalic region to the anal legs. This process takes place in about 70 to 100 minutes during nights.

It may be seen from the foregoing results that in centipedes there is secretion of moulting fluid, unlike in Diplopoda and Onychophora, and the old cuticle is partly digested. Therefore the feature noted in millipedes is not of general applicability to the whole of Myriapoda.

An interesting feature is that the duration of stage D is about 10 to 11 days in centipedes, compared to millipedes where this stage lasts only 3 to 4 days[1]. Even in insects where there is secretion of moulting fluid the stage D is never more than 6 days[8]. Therefore, centipedes will be ideal materials for the study of physiology of moulting in arthropods in greater detail.

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EFFECTS OF AN ACUTE DOSE OF BIOTIN ON THE REPRODUCTIVE ORGANS OF THE FEMALE RAT

THE adverse effects of biotin deficiency on the reproductive capacity and general metabolism of mammals are well documented[1,2]. On the other hand, there are no reports regarding the effects of an excess amount of biotin either in acute or chronic form on the reproductive ability and metabolism of the mammal. In certain insects, however, an excess intake of biotin causes severe degenerative effects on the ovary which leads to complete sterility[3,4]. It was of interest, therefore, to study the effects of an acute dose of biotin on the reproductive cycle, morphology of the ovary and the hepatic and uterine glycogen of the adult female rat.

Colony bred adult female rats of the Holtzman strain (approximately 3 months old) were selected after observing their normal oestrus cycle for three cycles. Eighteen rats thus selected were given biotin at a dose of 5 mg/100 g body weight dissolved in 0·2 ml of 0·1 N NaOH subcutaneously in two injections, morning and evening, at the dioestrus stage of the cycle. Groups of 6 rats each were sacrificed 7, 14 and 21 days after biotin injections. Another group of 6 rats was given an equal volume of 0·1 N NaOH at the dioestrus stage and was sacrificed 7 days after the injections. The entire experiment was performed under standardized light and dark, temperature and feeding conditions which are designed for best reproductive performances. The oestrus cycle of all the animals was studied daily on the basis of the microscopic examination of the vaginal smears. The leucocyte concentration of the vaginal smears was determined by the use of a haemocytometer after appropriate dilutions with normal saline. The histology of the ovary was studied following routine techniques and staining with haematoxylin and eosin. The liver and uterine glycogen was estimated according to the method of Montgomary[5].

The results of the present investigation showed that the NaOH-treated controls maintained normal oestrus cycles and the vaginal leucocyte numbers varied normally in relation to the stages of the oestrus cycle (Fig. 1). Since NaOH did not affect the normal oestrus cycle upto 7 days after treatment, running the controls upto 21 days was not considered necessary. The biotin-treated rats showed complete irregularity of the cycle and the vaginal leucocyte numbers progressively increased up to 14th day and sharply declined thereafter (Fig. 1). An increase in leucocyte infiltration in the vaginal lumen normally occurs during dioestrus stage in relation to the appearance of the luteal tissues in the ovary. This luteal phase of the cycle can be considered as an infertile stage of the mammalian reproductive cycle.

Therefore, biotin-induced irregularities of the oestrus cycle and massive infiltration of leucocytes in the vaginal lumen may be considered as an indication of its sterilizing effect. Biotin treatment

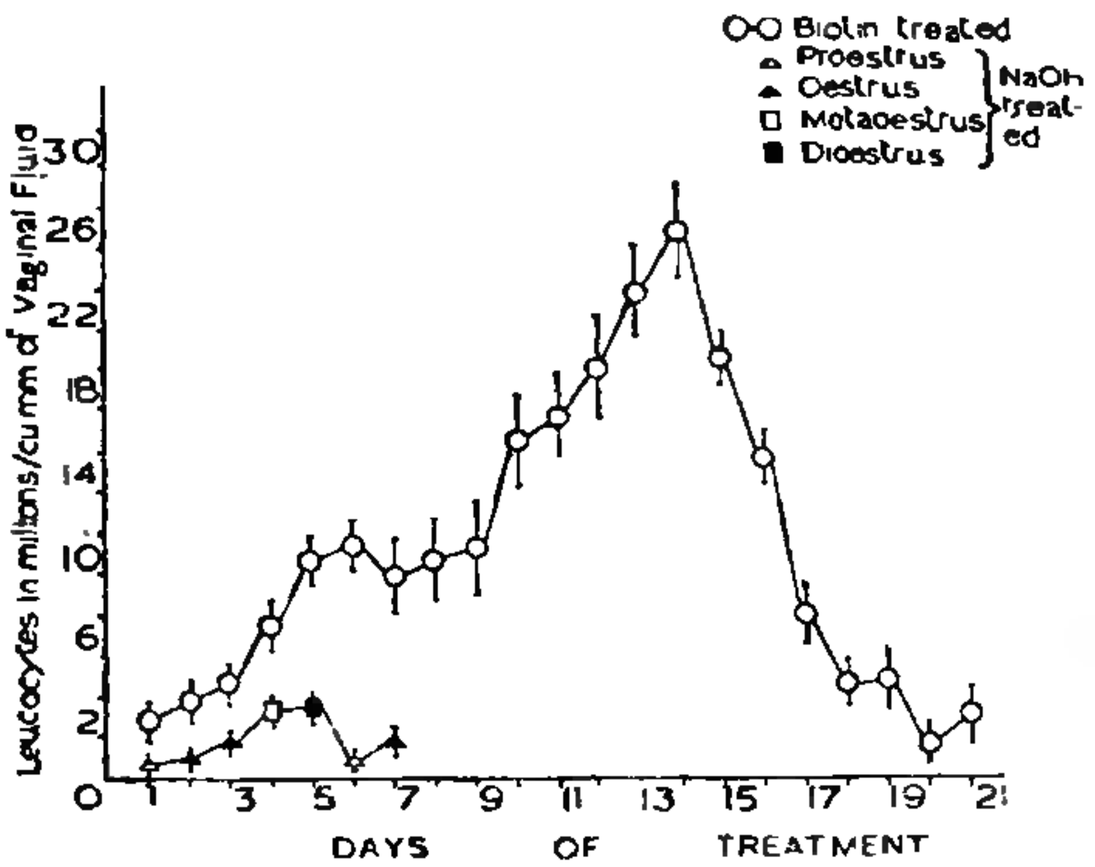

FIG. 1. Effect of biotin and NaOH on the leucocyte concentration in the vaginal fluid.

did not significantly affect the weights of the ovary, uterus, liver and normal body weight gain. The histological examination of the ovarian sections indicated that biotin treatment enhanced the process of the formation of corpora lutea, but a large number of the corpora lutea and stroma showed atrophic changes (Fig. 2).

The concentration and total liver glycogen reduced significantly 14 days after biotin treatment, but returned to the control level after 21 days (Table I). The concentration and total uterine glycogen showed a slight reduction only 21 days after biotin treatment (Table I). Terroine[1] has

TABLE I

The effect of biotin on the liver and uterine glycogen

Treatment	Liver glycogen		Uterine glycogen	
	Concentration mg/ 100 mg tissue	Total mg/liver	Concentration mg/ 100 mg tissue	Total mg/uterus
NaOH-treated control for 7 days	1·334 ±0·093	109·5 ± 1·12	0·136 ±0·112	0·469 ±0·028
7 days after biotin injections	0·567 ±0·038	43·4 ± 0·81	0·124 ±0·009	0·633 ±0·029
14 ,,	0·369 ±0·064	23·1 ± 0·58	0·144 ±0·019	0·465 ±0·025
21 ,,	1·232 ±0·095	103·8 ±1·52	0·083 ±0·010	0·305 ±0·030

There were six rats in each group.

reported that biotin deficiency in contrast to several other vitamins does not modify the glycogen content of the liver or muscle. Hence the observed reduction of hepatic and uterine glycogen is probably not a direct effect of biotin treatment. It has been suggested that progesterone inhibits glycogenic effect of oestrogen in the liver and uterus of the rat[6,7]. It is likely, therefore, that biotin is exerting its effect on hepatic and uterine glycogen through

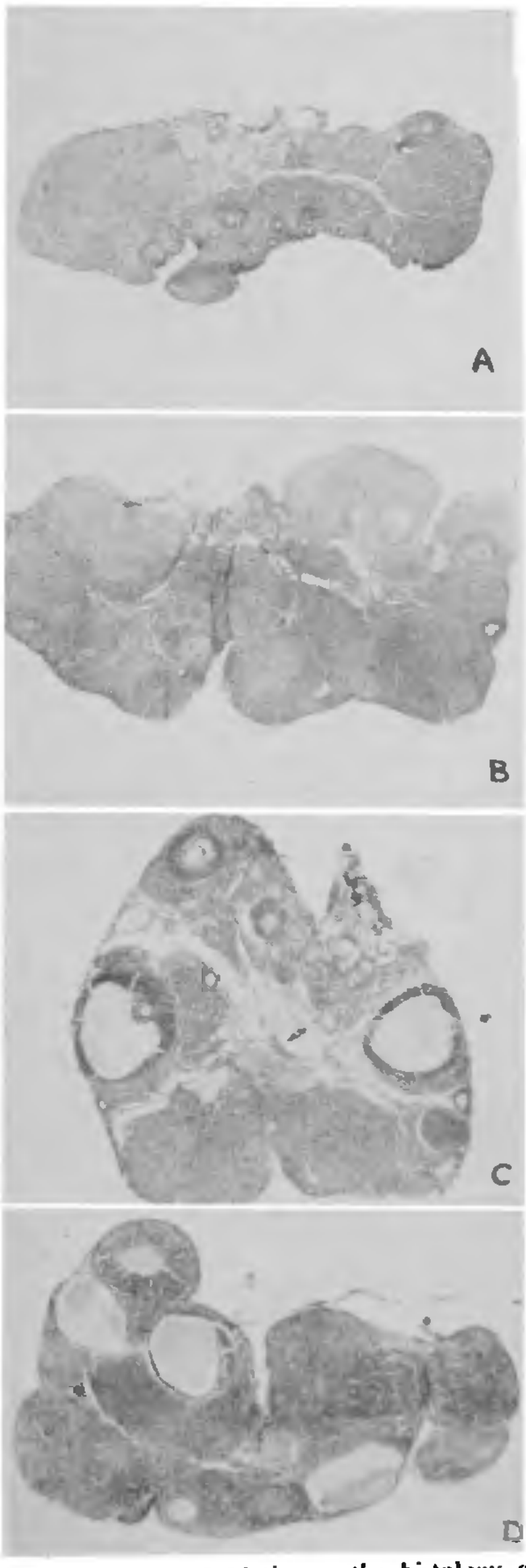

FIG. 2. Effect of biotin on the histology of the ovary. A, B, C and D—NaOH treated control (at oestrus), 7, 14 and 21 days after biotin injection respectively under low power.

an altered ratio of progesterone and oestrogen release from the ovary. The histology of the ovary of the biotin-treated rats tend to support this hypothesis.

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OXYGEN CONSUMPTION IN VIRGIN AND MATED BED BUGS

IT has been demonstrated that sperm motility in *Cimex* depends on aerobic metabolism[1]. When the O_2 supply to the sperm is cut off, the normal process of activation, as well as motility of sperm *in vivo* can be completely arrested. Also an O_2 gradient appears to be responsible for the directed migration of the sperm mass in the haemocoel. However, many problems concerning the mechanism of sperm response to molecular O_2 remain unanswered. An activity gradient in the sperm mass corresponding to an O_2 gradient is clearly indicated in the *in vitro* experiment. It would be of interest to know if a gradient in O_2 tension between the mesospermalege and the conceptacula exists in the mated bug. Prior to determining the differences in O_2 tensions, if any, between the above parts, experiments were initially performed to determine the nature of the respiratory metabolism of the virgin and inseminated bed bugs and the results are reported below.

MICRORESPIROMETER

The difference in oxygen uptake between virgin and mated females was directly determined in an all glass differential microrespirometer (Grunbaum, Siegel, Schulz and Kirk-2) with a capillary diameter 0·7 mm. In assembling the respirometer unit, 2–3 μl of kerosene were added from a 10 μl Hamilton syringe that was fitted with a needle smaller in diameter than the bore of the capillary. Microvials (3 mm × 9 mm) containing filter-paper soaked in 2% KOH were placed in the control and experimental vessels of 1 ml capacity. Respiration of a single pair of bugs was thus conveniently studied at 25° C. Except for the minor modifications mentioned above, the rest of the procedure used is as outlined by Grunbaum *et. al.*[2]. Inability to climb the glass surface prevented the insect from coming into contact with the alkali. The insects, except for occasional movements, remained quiet for long periods of time.

Unmated and mated females were placed, in the control and experimental flasks of the respirometer, which was then closed to the atmosphere. The insects used for these experiments were; (1) four to nine day old adult females fed once and (2) females of similar age but fed and mated once. Experiments were usually started 15 to 30 min. after insemination and readings were taken at intervals of 30 to 60 min. over a period of 10 to 18 hr. Differences in rate of O_2 consumption between virgins and females inseminated at 48, 64, 96, 120, 150 and 174 hr. after feeding were determined, and at each time stage, three pairs of virgin and mated bugs were tested. It was found experimentally that 48 hr. after feeding the respiratory rate of virgins reaches a more or less steady level. In the last two tests, however, the small differences in the rate of O_2 uptake were determined over a ten hr period, using a pair of virgins 140 hr and a pair 160 hr after feeding. Subsequent to these control determinations, one female of each pair was mated and the difference in rate of O_2 uptake was redetermined for each pair over a similar period. Triplicate determinations were made in these tests also.

At the end of the experiment, the inseminated females were dissected to check the extent of sperm migration and also the condition of the eggs, whether fertilized or not.

The data on difference in rate of O_2 uptake between virgin and inseminated females are summarized in Table I.

TABLE I

The increase in rate of O_2 consumption in mated over virgin females at 25° C

Age Days	Hours after feeding	No. of Pairs (Virgin and mated)	Mean increase in rate of O_2 uptake in mated over Virgin ♀ (μl/pair/hr)*
4	48	1	2·4 (±0·2)
5	64	1	2·5 (±0·1)
6	96	1	1·1 (±0·1)
7	120	1	4·6 (±0·3)
8	150	1	2·2 (±0·1)
9	174	1	2·3 (±0·1)
			$(\bar{X} = 2{\cdot}5 = \pm 1{\cdot}04)_2$

* Average for three determinations at each time stage.