

**ABSENCE OF CREATININE  
METABOLISM IN THE FUNGUS  
*ASPERGILLUS NIDULANS***

It has been shown earlier that in many fungi creatinine and creatine<sup>1,2</sup> are utilized to some extent and that the utilisation can be termed as partial. However, a number of bacteria like *Clostridium paraputrificum*<sup>3</sup> were reported to be capable of fermenting creatinine but incapable of synthesising it though creatinine induced the growth of this organism. Some soil bacteria<sup>4</sup> isolated from creatinine enriched cultures could convert creatinine to creatine by the enzyme creatino-mutase, but were incapable of independent synthesis of creatinine. It was of interest in our studies on the genetics and biochemistry of *Aspergillus nidulans*, a homothallic Ascomycete fungus, to see whether there is creatinine utilisation by the organism. The present report reveals the absence of creatinine metabolism in *A. nidulans*.

*A. nidulans* were grown in minimal medium as described by Pontecorvo *et al.*<sup>5</sup> for a period of 48 hrs on a shaker at room temperature (28–30° C). The mycelia were removed from the culture filtrate and washed with 0.05 M Tris buffer, pH 7.0. They were then homogenised in a glass Teflon tube in the same buffer at 5° C. The extract was centrifuged at 10,000 g for 10 min. at 0° C. The supernatant was assayed for the creatine phosphokinase<sup>6</sup>. For the estimation of creatine and creatinine the supernatant was treated with 10% sodium tungstate and 2/3 N sulphuric acid and the precipitate was removed. Creatine was estimated using diacetyl and  $\alpha$ -naphthol creatinine was estimated by the method of Folin and Wu<sup>8</sup> both in the extract and in the culture filtrate. Both creatine and creatinine were found to be absent.

For the induction of growth, both creatinine and creatine were substituted separately instead of sodium nitrate as the sole nitrogen source in the minimal medium. No growth was observed.

The utilisation of creatinine as the sole nitrogen source and as the energy yielding compound has been reported in the bacterium *C. paraputrificum*.<sup>9</sup> The energy liberated during the conversion of creatinine to N-methyl hydantoin was utilised for the anabolic reactions. In *A. nidulans*, neither this process nor creatine phosphate conversion by creatine phosphokinase was possible due to the absence of creatinine utilisation. This

study appears to be the first report on the total absence of creatinine metabolism in a fungus.

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**ARRANGEMENT OF THE FOETAL  
MEMBRANES AND THE OCCURRENCE  
OF A HAEMODICHORIAL PLACENTA  
IN THE VESPERTILIONID BAT,  
*PIPISTRELLUS MIMUS MIMUS***

*Pipistrellus mimus mimus* is one of the smallest mammals known and reaches sexual maturity at a body weight of 2 gm. The general topography of the foetal membranes of this bat at full term is illustrated in Fig. 1. The pregnant uterus has a diameter of 9 mm in the transverse axis and 7 mm in the cranio-caudal axis. The foetus almost completely fills up the gestation sac. The long bones are in the process of ossification. The uterine wall is highly stretched, so that the myometrium appears very thin and membranous. The chorion is in contact with the uterine wall on all the sides except on the mesometrial side of the uterus where the abembryonic wall of the chorionic sac—Trilaminar omphalopleure—hangs freely in the persistent uterine lumen. The amnion is a thin, bilaminar membrane, and is closely adhering to the body of the foetus.

The embryonic segment of the yolk-sac splanchnopleure is pushed towards the abembryonic segment of the trilaminar

to streak-like spaces and is lined by hypertrophied cuboidal endodermal cells (Fig. 2). Each endodermal cell has a centrally placed vesicular nucleus with a darkly staining nucleolus. The hypertrophied endodermal cells are highly vacuolated, and, when stained with PAS-procedure, show large amounts of PAS-positive material. The mesothelial cells have also become hypertrophied, polygonal and vacuolated.

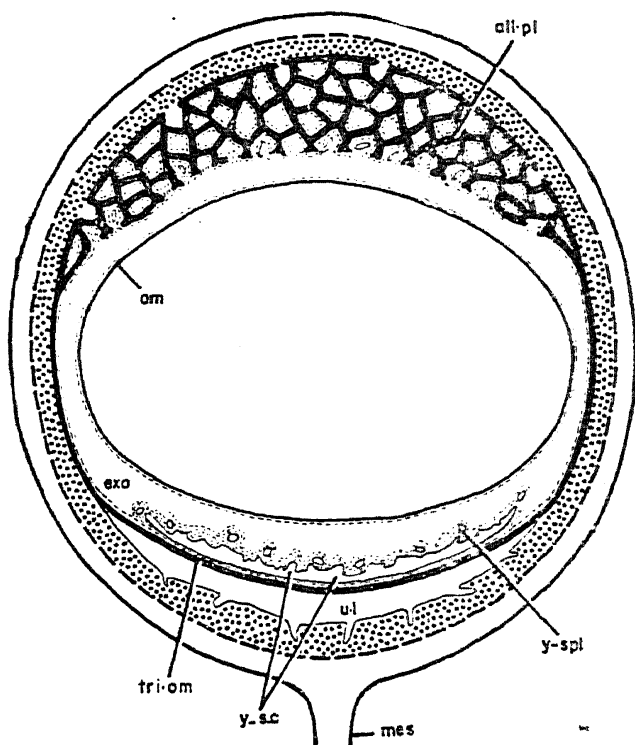
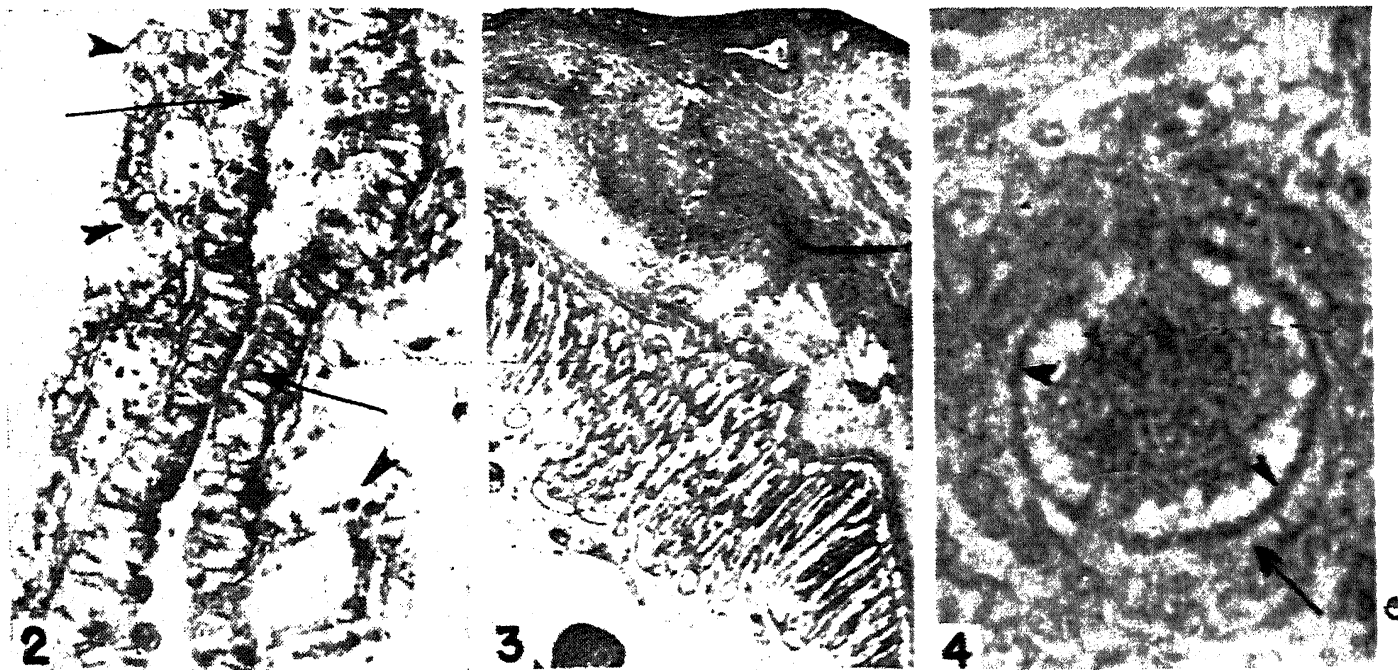


FIG. 1. Semischematic diagram to illustrate the general topography of the foetal membranes at term of *Pipistrellus mimus mimus*. *all.pl*, allantoic placenta; *am*, amnion; *exo*, exocoelom; *mes*, mesometrium; *tri-om*, trilaminar omphalopleure; *u.l*, uterine lumen; *y.s.c.*, yolk-sac cavity; *y.s.pl*, yolk-sac splanchnopleure.

The umbilical cord of *Pipistrellus mimus mimus* is relatively long and shows two or three spirals. In transverse sections the umbilical cord shows the presence of five vessels, two umbilical arteries, one umbilical vein, one vitelline artery and one vitelline vein.

The chorio-allantoic placenta is in the form of a concavo-convex disc located on the anti-mesometrial side of the uterus. The placental disc measures 7 mm in diameter and is 2 mm thick in its centre.

Histologically the chorio-allantoic placenta consists of a complex network of tubules, "placental tubules" (Gopalakrishna and Moghe, 1960), which seem to be hanging from the uterine wall, and which appear to be embedded in a matrix of allantoic mesenchyme and blood vessels (Fig. 3). From the foetal surface to the



FIGS. 2-4. Fig. 2. Photomicrograph of part of the yolk-sac splanchnopleure to show the hypertrophied endodermal (arrow) and mesodermal (arrow head) cells,  $\times 270$ . Fig. 3. Part of the allantoic placenta to show the network of placental tubules,  $\times 21$ . Fig. 4. Part of the placental tubule (PAS-staining) showing the cytotrophoblast (arrow) and the interstitial PAS-positive membrane (arrow head). The syncytiotrophoblastic lining occurs as a thin lamina on the luminal border of the tubule,  $\times 270$ .

omphalopleure on the mesometrial side of the uterus. Due to the collapse and folding of the yolk-sac wall the yolk-sac cavity is reduced

maternal blood each placental tubule (Fig. 4) consists of a layer of cellular trophoblast with lightly staining nuclei, a PAS-positive inter-

stitial membrane, which is at least partly constituted by the remnants of the basement membrane of the maternal endothelium, and a layer of enucleate eosinophilic cytoplasm, which is all that remains of the syncytiotrophoblast. Thus the placenta of *Pipistrellus mimus mimus* should be designated as "haemodichorial" according to the classification of haemochorial placentae of Enders (1965).

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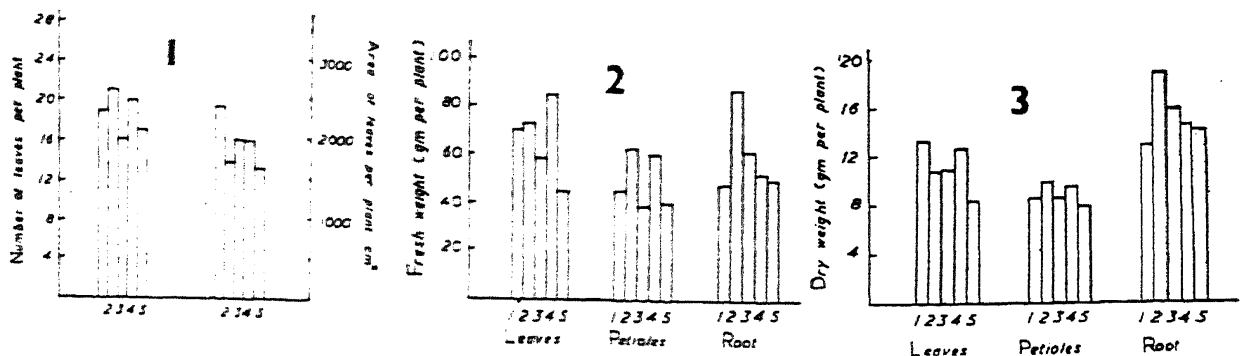
#### EFFECT OF FOLIAR APPLICATION WITH SOME MICROELEMENTS ON GROWTH AND SOME PHYSICO-CHEMICAL PROPERTIES OF SUGAR-BEET GROWN IN WINTER SEASON

FOLIAR spray with certain microelements were reported to increase cold-hardening of plants<sup>1</sup> by lowering the respiratory quotient and causing an accumulation of organic acids<sup>2</sup> as well as inducing changes in the physico-chemical properties of plant cell protoplasm biocolloids.<sup>3</sup> Therefore, the aim of this work was to investigate the effect of foliar application with Zn, Mn, Cu and B on the vegetative growth and sugar content of Beet plant as well as on the osmotic

The pots were fertilized at a rate of 0.15 g N, 0.10 g P<sub>2</sub>O<sub>5</sub> and 0.22 K per kg soil as calcium nitrate, superphosphate and potassium sulphate. After 40 days from sowing, each group of 10 pots were sprayed with 0.5 ml of one of the following solutions: 1—Distilled water as control; 2—MnSO<sub>4</sub>; 0.05%, 3—ZnSO<sub>4</sub>; 0.05%, 4—CuSO<sub>4</sub>; 0.005% and 5—H<sub>3</sub>BO<sub>3</sub>; 0.05%.

Samples of leaves, petioles and roots of both treated and control plants were collected after five months from sowing. Fresh and dry weights of the different plant parts were determined. Leaf area per plant was measured by the disc method<sup>4</sup>. In addition, number of leaves per plant were recorded. The osmotic pressure was determined for the prepared fresh leaves extract according to Loomis and Shull<sup>5</sup> as well as the electrical conductivity was determined according to Richards<sup>6</sup>. The total soluble carbohydrate content (mg glucose/gm dry weight) of different plant parts was determined using the anthrone method<sup>7</sup>.

*Vegetative growth.*—Figure 1 shows that foliar application of Mn, Zn and B to sugar-beet plants generally increased both the number and the area of leaves per plant, with varying degree according to the element applied. From Figs. 2 and 3, it could be concluded that the foliar application of either Zn or B seemed to be the most efficient for increasing the fresh and dry weights of the roots and leaves of sugar-beet plant.



FIGS. 1-3. Fig. 1. Effect of foliar application with microelements on the number and area of leaves of sugar-beet plants. (1) MnSO<sub>4</sub>, 0.05%; (2) ZnSO<sub>4</sub>, 0.05%; (3) CuSO<sub>4</sub>, 0.005%; (4) H<sub>3</sub>BO<sub>3</sub>, 0.05%; (5) control. Fig. 2. Effect of foliar application with microelements on the fresh weight of sugar-beet plant. (1-5, see Fig. 1.) Fig. 3. Effect of foliar application with microelements on the dry matter content of sugar-beet plant. (1-5, see Fig. 1.)

pressure and electrical conductivity of leaf extract during the winter season.

Fruits of *Beta vulgaris* L. (Sugar-beet) variety "A.J. Poly 2" were planted on September 9, 1970 in pots No. 30 filled with 12 kg loamy soil. After complete germination, plants were thinned and one plant per pot was left to grow.

*Total soluble carbohydrate content.*—The data in Table I show that the total soluble carbohydrate content in the root of plants sprayed with Zn was markedly increased as compared with their control, whereas other treatments were without effect. In the petioles the total soluble carbohydrate content was no