ISOLATION OF 1, 2-DIMETHYL 4, 5-DIAMINO BENZENE IN A HETEROKARYON OF *ASPERGILLUS NIDULANS*

A PAIR of pteridine derivatives namely, 6, 7-dimethyl 8-ribityl lumazine and 6-methyl 7-hydroxy 8-ribityl lumazine, which were reported to be involved in riboflavine biosynthesis[1,2] have been isolated in a heterokaryon of 2 non-allelic riboflavineless mutant of *Aspergillus nidulans* by Sadique *et al.*[3] However, it has been reported by Radha and Shanmugasundaram[4] that an isoalloxazine ring is formed by the condensation of a purine, uric acid with a benzenoid compound by the enzymes present in the mycelia of a riboflavineless mutant of *A. nidulans*. Hence, it was of interest to detect the benzenoid compound, 1, 2-dimethyl 4, 5-diamino benzene, an alleged precursor of riboflavine by Wolley.[5] The present note deals with isolation of such a compound in a heterokaryon obtained by fusing 2 non-allelic riboflavineless mutants.

An established heterokaryon of 2 non-allelic riboflavineless mutants (y ribo$_3$ and w ribo$_6$) was produced and grown in a minimal medium for 10 days at 37° C.[6] The mycelia were harvested and then homogenised with 95% ethanol containing 1% ammonia, in a cold room. The extract was centrifuged and the supernatant evaporated to a small volume at 3° to 4° C. in the cold room by perevaporation. The concentrated extract was adjusted to pH 4·0 by dilute acetic acid and chromatographed on florisil column.[7] The column is first washed with water and the filtrate concentrated by perevaporation and when tested for any fluorescent spots by chromatography gave negative results. The florisil column was then eluted with pyridine-acetic acid mixture (10 : 1), followed by washing with water. The eluent was concentrated to near dryness and then chromatographed using butanol : acetic acid : water (4 : 1 : 5). The paper was examined under ultra-violet light, when a violet fluorescent spot with a Rf value 0·68 was obtained. This was eluted with dilute HCl and rechromatographed along with authentic sample of 1, 2-dimethyl 4, 5-diamino benzene, using the same solvent. It was interesting to note that both the test as well as authentic sample of 1, 2-dimethyl 4, 5-diamino benzene had same Rf values and the ultra-violet absorption spectra obtained in a Unicam Recording Spectrophotometer (SP 700) of both had similar absorption characteristics, thus establishing the formation of 1, 2-dimethyl 4, 5-diamino benzene in the heterokaryon of *A. nidulans*.

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SIALIC ACID IN THE GENITAL ORGANS OF THE MALE RAT

THE presence of sialic acid has been reported in the testis, seminal vesicle, prostate, semen and the spermatozoa of men, the concentration being particularly high in the vesicular secretion.[1,2] The semen and spermatozoa of several other species of mammals (ram, bull, pig, dog and rabbit) have also been shown to contain this compound.[3-6] In females, sialic acid has been demonstrated in the cervical mucus, follicular fluid and zona pellucida.[4,7,8] The present communication is concerned with the distribution and concentration of sialic acid in the genital organs of the male rat.

Colony-bred young adult rats (130–150 gm.) of the Institute were used in this investigation. After removal, the tissues were blotted gently between pieces of filter-paper, weighed to the nearest 0·1 mg. in a Roller-Smith balance and then hydrolysed with 0·1 N H_2SO_4 for 1 hour at 80° C. The hydrolysate was adsorbed on to a Dowex 2 anion exchange resin (acetate form) column. Sialic acid was eluted from the resin column by acetate buffer (pH 4·6)[3] and estimated directly after development of colour by Ehrlich's reagent.[4] The readings were taken at 565 mμ in a Bausch and Lomb 'Spectronic 20' colorimeter.

It was possible to estimate sialic acid from individual testis but for the rest it was necessary to pool tissues from 5 rats for a single determination.

It will be evident from the results presented in Table I that the epididymis had a considerably higher concentration of sialic acid than the rest of the tissues ($P < 0·01$). The highest

TABLE I

The distribution of sialic acid in the genital organs of the male rat

Tissue	Sialic acid (Mg./gm.)
Testis (45)* ..	25·3† (21·2–30·7)
Caput epididymis (36) ..	61·3 (51·6–68·9)
Cauda epididymis (36) ..	80·8 (65·0–88·0)
Seminal vesicles (36) ..	25·6 (23·0–28·5)
Ventral prostate (36) ..	16·8 (12·6–22·7)

* No. of animals; † Mean with range in parenthesis.

value was recorded in the cauda epididymis ($P < 0·01$) and the lowest in the ventral prostate ($P < 0·01$). The testis and the seminal vesicles had virtually similar concentration of this compound.

The exact role of sialic acid in reproductive processes is unknown. It has been suggested that sialic acid may be involved in capacitation of spermatozoa[6] and the fertilization process.[8] The present finding on a high sialic acid concentration in the epididymis suggests a possible role of this compound in sperm maturation.

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TRANSAMINATION AND HEAT INCREMENT IN RUMINANTS

IN monogastric animals there is a high specific dynamic action of protein food[1,2] depending on the extent of oxidative deamination associated with large heat increment and of transamination with a small heat increment.[3] In ruminants, however, volatile fatty acids produced in the rumen are mainly responsible for the high heat increment,[4,5] administration of a mixture of volatile fatty acids in the rumen of a fasted sheep giving a much less heat increment than when the constituent acids were administered individually.[6,7] It was thought desirable to investigate if transamination will play any role in the genesis of heat increment of feeding in ruminants like that of the monogastric animals. An attempt was made to study the rate of transamination reaction of the rumen content and its relation with heat increment of feeding in ruminants.

Transamination reaction of the strained rumen liquor[8] and the oxygen consumption were studied with Benedict-Roth metabolism apparatus and Haldane gas analysis apparatus. Rumen fistulated sheep were maintained on 5 different feeds (low protein 6%; low protein and 5 gm urea; high protein 23% alone or along with 5 gm urea or with 30 gm casein).

TABLE I

Transaminase activity of strained rumen liquor (T) and increase in O_2 consumption percentage over the resting value (O_2)

	2 hrs. after feeding		6 hrs. after feeding	
	T	O_2	T	O_2
Low protein ..	162·8	115·50	133·9	81·08
High protein ..	231·6	19·91	188·2	18·51
Low protein + Urea ..	168·3	98·33	20·96	79·85
High protein + Urea ..	180·7	22·65	240·2	30·77
High protein + Casein ..	371·4	38·74	209·8	42·09

Table I shows that in low protein feed a low transaminase activity in the rumen was correlated with a high oxygen consumption and in high protein diet a high transaminase activity in the rumen with a low oxygen consumption. When urea was added to the low protein diet the transaminase activity was increased and oxygen consumption was decreased correspondingly. Addition of urea or casein to a high protein feed led to a higher transaminase activity and presumably with considerable diaminase activity associated with a high protein diet and thus the oxygen consumption, although lower than with low protein feed, was not proportionately so compared to the increase in transaminase activity. A lowering of heat increment with a high transaminase activity reflects the possible role of transamination in the genesis of heat increment of feeding in ruminants also.

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