

earlier described^{1,4}. The activity is expressed in units per gland and per g of glandular tissue. One unit of activity corresponds to a utilization of 1 μ l oxygen at 37°C in 30 min for succinic dehydrogenase and cytochrome oxidase, and to a change in optical density of 0.001/min at 240 m μ for fumarase.

The weight of the submaxillary gland decreased from 168 ± 2.7 (30)⁵ mg to 119 ± 3.3 (17) mg after parasympathetic denervation and to 158 ± 4.0 (8) mg after sympathetic denervation.

After parasympathetic denervation the activity of all 3 enzymes decreased when it was expressed per gland (Table). The diminished activity was especially marked for succinic dehydrogenase and fumarase. The activity of these 2 enzymes was also decreased when it was calculated per g of glandular tissue. The decrease in total activity of cytochrome oxidase corresponded to the glandular atrophy after denervation since the activity was not significantly changed when it was expressed per unit weight.

After sympathetic denervation, the activity of succinic dehydrogenase and fumarase was not significantly changed. The activity of cytochrome oxidase was decreased, however, when it was calculated per gland and per unit weight (Table).

In the Table it can be observed that the activity of all 3 enzymes differed in the 2 control groups. This was due to the fact that the experiments on parasympathetic and sympathetic denervation were carried out at different times so that the rats did not belong to the same strain and further the diet was slightly changed.

It is known that the submaxillary gland of rats atrophies after denervation. Histologically atrophic changes have been observed in all the secretory cells after parasympathetic denervation², but only in the acinar cells after sympathetic denervation³. In salivary glands of different

species, it has been shown that the activity of succinic dehydrogenase is very low in the acinar cells but marked in the duct cells⁶, and that cytochrome oxidase is more evenly distributed in the different glandular cells⁷. The localization of fumarase in salivary glands is not known. In the present experiments the denervation atrophy of the rat's submaxillary gland has been found to coincide with a reduced activity of enzymes concerned with aerobic metabolism just as previously seen in salivary glands of cats and rabbits¹. The findings indicate that the concentration of succinic dehydrogenase and fumarase decreases in the atrophic cells after parasympathetic denervation and that the concentration of cytochrome oxidase is diminished in the atrophic acinar cells after sympathetic denervation. These results suggest, furthermore, that the activity of fumarase is low in the acinar cells.

Zusammenfassung. Es wurde in der denervierten Submaxillarisdrüse der Ratte die Aktivität verschiedener respiratorischer Enzyme bestimmt. Succinodehydrase und Fumarase zeigten eine verminderte Aktivität nach parasympathischer Dezentralisierung, ebenso verhielt sich Cytochrom-Oxydase nach sympathischer Denervierung.

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⁴ P. OHLIN, Quart. J. exp. Physiol. 47, 238 (1962).

⁵ Mean \pm standard error of mean (number of observations).

⁶ H. A. PADYKULA, Am. J. Anat. 91, 107 (1952).

⁷ R. M. SCHNEIDER and P. PERSON, Exp. Cell Res. 20, 627 (1960)

Betaine Excretion by the New-Born Infant¹

In a previous communication² we reported that the 'inhibition assay' principle³ had been applied to detect myoinositol in the pooled urine of new-born infants and in the urines of a number of older children with generalized aminoaciduria. We now report that similarly betaine has been found in significant quantities in the pooled urine of healthy new-born infants and in a number of older children with generalized aminoaciduria but not in the urine of healthy older children or adults.

In this procedure biological specimens were compared by placing impregnated paper discs on the surface of an agar culture medium containing a microorganism whose growth is prevented by an appropriate inhibitor. In the process of testing the urine of new-born infants in this manner, employing *Bacillus subtilis* 6051 inhibited by β -2-thienylalanine, an activity was observed similar to that produced by phenylalanine and phenylpyruvic acid. Subsequent paper chromatography of a sample of pooled new-born infants' urine clearly demonstrated that the unknown substance was neither of these two substances. Further, if spores of *B. subtilis* were used as the inoculum instead of vegetative cells, β -2-thienylalanine inhibition was not prevented by the unknown substance. Paper

chromatography and inhibition assay of urine specimens from 100 individual new-born infants showed that this activity was uniformly present. Similar investigations of individual healthy adults failed to reveal the presence of this activity. The above data led us to undertake the isolation and identification of the activity.

Preliminary purification by paper chromatography employing three different solvent systems, followed by ion-exchange chromatography led to a purified sample which did not possess the benzenoid absorption in the ultraviolet typical of phenylalanine and its metabolites. A comparison with tables of Rf values compiled by FINK⁴, indicated that the compound had properties similar to betaine. The nuclear magnetic resonance spectrum of the

¹ This investigation was supported in part by Grant B-1960 and NB-03935 from the National Institutes of Health, U.S. Public Health Service.

² J. P. SCHIRMER JR., H. TIECKELMANN, and R. GUTHRIE, Exper. 20, 602 (1964).

³ R. GUTHRIE and H. TIECKELMANN, Proc. London Conf. Sci. Study of Mental Deficiency (May and Baker Ltd., Dagenham, England 1962), p. 672.

⁴ K. FINK, R. E. CLINE, and R. M. FINK, Anal. Chem. 35, 389 (1963).

purified material was consistent with this structure. Additional comparisons of the unknown with an authentic sample confirmed the above observations.

The role of betaine in metabolism has been discussed recently by JUKES⁵ who points out that betaine is an important intermediate in one of two mechanisms for contributing the methyl group to the biosynthesis of methionine. Further investigation is required to assess the possibility of any special role of betaine in the metabolism of the new-born infant as compared to the adult and its role in older children with generalized aminoaciduria.

Zusammenfassung. Ein Betain wurde in grösseren Mengen im aufgefangenen Urin gesunder Säuglinge und ebenfalls im Urin einiger älterer Kinder mit allgemeiner Aminoacidurie gefunden. Die Substanz besitzt eine Akti-

vität, welche die Inhibition des *Bacillus subtilis* durch β -2-Thiophenalanin verhindert. Ihre Aktivität gleicht derjenigen des Phenylalanins und der Phenylbrenztraubensäure, unterscheidet sich jedoch von diesen dadurch, dass die β -2-Thiophenalanin-Inhibition verhindert wurde, wenn Sporen des *Bacillus subtilis* als Erreger verwendet wurden.

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⁵ T. H. JUKES, Proc. 6th Internat. Congr. of Hematol. (Grune and Stratton, Boston, Mass. 1956), p. 682.

Determination of Reproductive Categories in the Life Cycle of Aphids

Some species of Aphids show warm climate populations which reproduce constantly by parthenogenesis and populations from temperate regions where amphigony also occurs. Such differences in the reproductive cycles are not to be regarded as merely phenotypic, but seem to depend upon the existence of genetic variability which is maintained even in the so-called clones by a peculiar type of meiosis (endomeiosis)^{1,2}.

Different populations of *Brevicoryne brassicae* were collected from localities having different climates: two zones each of the Appenines (Vaglio, Lama), the Po plain (Modena, Reggio), the Northern Tyrrhenian coast (Leghorn, Sestri), and the Southern Tyrrhenian coast (Naples, Salerno). Fifty parthenogenetic females of each population were isolated on cabbage leaves and kept for two months in the same thermostat which was regulated for obtaining sexuales. Table I shows the offspring of the eight populations that were kept in the thermostat for two months. Selection for parthenogenetic females was carried out and the results are shown in the Figure. The mountain populations showed no parthenogenetic females at either the 5th or 4th generation, despite selection for the parthenogenetic phenotypes. The population of Reggio showed no parthenogenetic females at the 9th generation, while in the population of Modena three parthenogenetic females, which appeared at the 3rd generation, ultimately gave rise to a parthenogenetic line where the percentage of the parthenogenetic females was increased in the next generations. The same process was obtained in previous researches, within the parthenogenetic lines of a strain³. The Mediterranean populations show on the other hand a marked tendency towards parthenogenesis: no male has ever appeared in their cultures. Amphigonic females disappeared in both populations from the Northern Tyrrhenian coast at the 9th generation. The tendency towards parthenogenesis is even more evident in the populations from the Southern Tyrrhenian coast, where conditions favourable for amphigonic reproduction exerted practically no influence at all.

The above experiments show therefore that the populations from regions with different climates are unlike in their genetic composition and form therefore different

sexual races. The environmental factors influence populations where multiple reproductive genotypes can be originated even within single parthenogenetic lines. In the same environmental conditions the individuals of different localities react differently in accordance with the reaction norms of the different sex genotypes which are present in genic pools of the populations. This explains why it is possible to obtain lines reproducing constantly by parthenogenesis from strains where amphigony has occurred.

Table I. Offspring of 8 populations of *Brevicoryne brassicae* from different regions, bred for 2 months in thermostat regulated chamber for obtaining sexuales

Vaglio				Reggio			
sex.	$\frac{\sigma\sigma}{\varphi\varphi}$	30 162	} 65%	sex.	$\frac{\sigma\sigma}{\varphi\varphi}$	20 316	} 64%
parth.		98		parth.		192	
tot.		290	35%	tot.		528	36%
Lama				Modena			
sex.	$\frac{\sigma\sigma}{\varphi\varphi}$	28 190	} 59%	sex.	$\frac{\sigma\sigma}{\varphi\varphi}$	20 273	} 50%
parth.		154		parth.		303	
tot.		372	41%	tot.		596	50%
Leghorn				Naples			
sex.	$\frac{\sigma\sigma}{\varphi\varphi}$	- 78	} 12%	sex.	$\frac{\sigma\sigma}{\varphi\varphi}$	- 38	} 6%
parth.		586		parth.		605	
tot.		664	88%	tot.		643	94%
Sestri				Salerno			
sex.	$\frac{\sigma\sigma}{\varphi\varphi}$	- 109	} 16%	sex.	$\frac{\sigma\sigma}{\varphi\varphi}$	- 19	} 3%
parth.		597		parth.		640	
tot.		716	84%	tot.		659	97%

¹ G. COGNETTI, Exper. 17, 168 (1961).
² G. COGNETTI, Boll. Zool. 29, 129 (1962).
³ G. COGNETTI and A. M. PAGLIAI, Arch. Zool. ital. 48, 329 (1963).