

slight but definite increments of $p\text{CO}_2$ observed in these experiments would also favour bicarbonate reabsorption.

CH. TOUSSAINT and P. VEREERSTRAETEN

Laboratoire de Médecine expérimentale, Université libre de Bruxelles et Fondation médicale Reine Elisabeth, Bruxelles (Belgium), March 3, 1960.

Résumé

La réabsorption tubulaire des ions sodium, chlore et bicarbonate a été étudiée au cours de perfusions de NaCl et de NaHCO_3 chez le chien normal. 61 expériences, comportant un ensemble de 295 récoltes urinaires, ont été pratiquées sur 21 animaux.

A filtration constante, la réabsorption du sodium exprimée en mM/l de filtrat glomérulaire demeure constante lorsque la natrémie est accrue par l'infusion de NaHCO_3 . Elle est, au contraire, une fonction linéaire de la natrémie lorsque celle-ci est élevée par des perfusions de NaCl.

Dans le premier cas, la réabsorption du sodium s'effectue par un double mécanisme: l'un, lié à la réabsorption du bicarbonate, est fixé par un T_m constant, l'autre, lié à la réabsorption du chlore, dépend de la chlorémie. Lorsque la natrémie est accrue par l'infusion de NaCl, le second mécanisme l'emporte nettement sur le premier. Il apparaît alors une relation évidente entre la natrémie (ou la chlorémie) et la quantité de sodium (ou de chlore) réabsorbée par les tubes rénaux.

Physiology of Intracellular Symbiotes of *Stegobium paniceum* L. with Special Reference to Amino Acid Requirements of the Host

Stegobium paniceum L. is one of the few insects where intracellular symbiosis is somewhat better understood. The yeast-like symbiotes of this beetle supply their host with certain factors of vitamin B-complex and sterol^{1,2}. The possible way by which the microbes may be supplying these growth factors has also been later suggested by GRÄBNER³. Recently it has been possible to extend such studies further with reference to amino acid requirements of larvae of this insect species.

Two batches of *Stegobium* larvae, one with and another without symbiotes, were grown on artificial diets containing various combinations of amino acids. The methods of making the basic artificial diet and eliminating the symbiotes by egg-surface sterilization have already been described earlier². The diet consisted of an amino acid mixture 15 parts, starch 85 parts, salt mixture 1 part, cholesterol 2 parts, nucleic acid 0.1 part, and vitamin solution to give a concentration of 25 $\mu\text{g/g}$ of thiamin, riboflavin, nicotinic acid, pyridoxin, pantothenic acid, and 500 $\mu\text{g/g}$ choline, 0.01 $\mu\text{g/g}$ biotin, 4 $\mu\text{g/g}$ folic acid, and 250 $\mu\text{g/g}$ inositol. 30 newly hatched larvae were put on diets deficient in one of the amino acids at a time and growth was observed. The tests were performed in small shell vials containing 2 g of diet.

The results are given in the Table, whence it can be seen that aposymbiotic condition led to an increased demand of the nutrient factors from external dietary source. For the sake of convenience, the various amino acids are so arranged in the Table that the first nine fall under the so-called 'non-essential' category while the remaining ten are under the 'essential'.

Growth of *Stegobium* larvae (with and without symbiotes) on diets lacking in one of the amino acids

Diet lacking in	With symbiotes		Without symbiotes	
	Number of adults out of 30 larvae	Developmental period in days	Number of adults out of 30 larvae	Developmental period in days
'non essential'				
DL-Alanine	29	31–36	10	72–118
DL-Aspartic acid	24	35–57	18	43–118
DL-Cystine	26	32–57	17	52–118
L-Glutamic acid	24	33–57	16	52–118
Glycine	14	64–94	0	—
L-Hydroxyproline	27	33–57	17	35–72
L-Proline	28	31–47	23	40–118
DL-Serine	28	31–50	23	43–108
L-Tyrosine	29	33–50	18	43–108
'essential'				
L-Arginine	2	51–78	0	—
L-Histidine	13	51–93	7	43–108
DL-Isoleucine	11	54–84	0	—
L-Leucine	9	54–88	0	—
L-Lysine	10	42–84	0	—
DL-Methionine	14	35–52	2	72–103
DL-Phenylalanine	18	35–86	0	—
DL-Threonine	7	57–78	0	—
L-Tryptophane	24	46–73	0	—
DL-Valine	13	42–75	0	—
No amino acids	26	38–57	—	—
Casein diet	27	27–37	29	28–40

The dietary deficiency in any one of the 'non-essential' amino acids did not adversely affect the growth of normal *Stegobium* larvae, with the single exception of glycine-deficiency which resulted in increased mortality and prolongation of larval period. Growth was, however, adversely affected when any one of the next 10 'essential' amino acids were omitted from the diet. Their deficiency resulted in marked disturbances in growth and a lower number of larvae completed metamorphosis. Without arginine only 2 larvae could become adults in 51 to 78 days and omission of threonine also proved highly detrimental. Except for tryptophane and probably phenylalanine, all the 'essential' amino acids proved comparatively more vital for larval growth than the first nine listed in the Table.

The reaction of aposymbiotic larvae was, however, entirely different. Individual amino acid deficiency became very much pronounced in the majority of cases, and was characterized by fewer larvae becoming adults and undue prolongation of larval period. In many cases, half-grown larvae survived even up to 118 days, when the experiment

¹ N. C. PANT and G. FRAENKEL, *Science* 112, 498 (1950).

² N. C. PANT and G. FRAENKEL, *Biol. Bull.* 107, 420 (1954).

³ K. E. GRÄBNER, *Z. Morph. Ökol. Tiere* 41, 471 (1954).

⁴ H. J. MÜLLER, *Z. Morph. Ökol. Tiere* 44, 459 (1956).

⁵ R. GEIGY, L. A. HALFF, and V. KOCHER, *Schweiz. med. Wschr.* 83, 928 (1953).

⁶ V. B. WIGGLESWORTH, *Tijdschr. Entomol.* 95, 63 (1952).

⁷ N. C. PANT and G. FRAENKEL, *J. zool. Soc. India* 6, 101 (1954).

⁸ L. TOTH, *Tijdschr. Entomol.* 95, 43 (1952); *Arch. Mikrobiol.* 18, 242 (1953).

was discontinued. Among 'non-essential' amino acids, glycine-deficiency resulted in total mortality of larvae, while in other cases fewer larvae became adults in longer time, as compared with normal larvae. Omission of any one 'essential' amino acid made the diet entirely unsuitable for aposymbiotic individuals since, in almost all cases, no or very few larvae became adults. It is therefore evident that the yeast-like micro-organisms supply in varying amounts almost all the ten 'essential' and most of the 'non-essential' amino acids.

The present example of the physiology of symbiotes is probably the first of its type where intracellular micro-organisms have been shown to play an important role in the amino acid requirement of an insect. Aposymbiotic condition is, however, known to cause higher mortality in *Coptosoma*⁴, *Triatoma*⁵, and *Rhodnius*⁶, or to produce additional dietary demands for vitamins of B-complex in *Lasioderma*¹ and *Oryzaephilus*⁷. Tóth⁸ has demonstrated fixation of nitrogen in the host body by microbes from atmosphere, or from nitrogenous waste products such as uric acid.

The full details of the investigation will be published elsewhere in due time.

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N. C. PANT, P. GUPTA, and J. K. NAYAR

Division of Entomology, Indian Agricultural Research Institute, New Delhi (India), July 21, 1959.

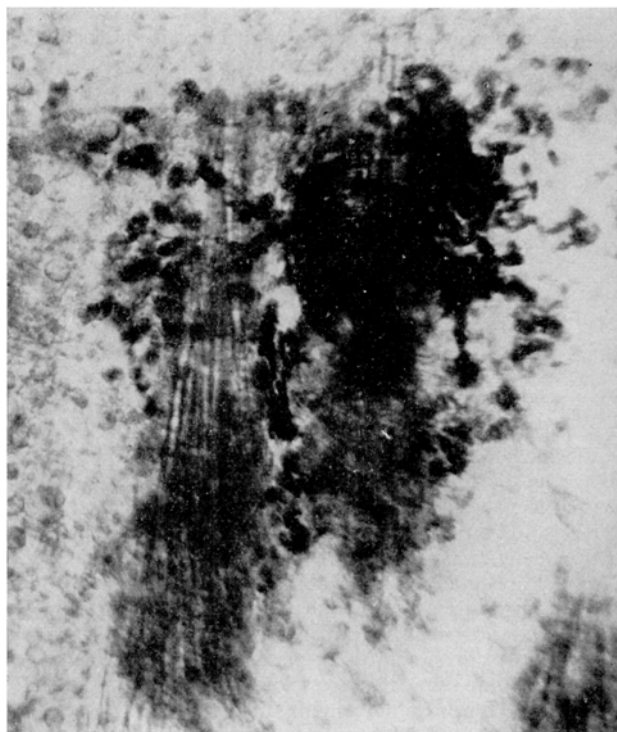
Résumé

Les auteurs ont soumis des larves de *Stegobium paniceum* L. à un régime contenant seulement des acides aminés. Ils ont aussi constaté expérimentalement que la levure, de même que les symbiotes intracellulaires fournissent des acides aminés en quantités variables selon l'hôte.

Some Etiological Factors in the Production of Malignant Melanoma

GHADIALY and BARKER¹ have found that the melanotic tumours produced in hamster skin by repeated applications of 9:10 dimethyl 1:2 benzantracene arise from a network of melanocytes surrounding some of the pilosebaceous follicles. It is well known that chemical carcinogens penetrate, accumulate, and persist in hair follicles for a considerable period of time and that an early effect of carcinogenic action is disruption of sebaceous glands². It would therefore appear that the peculiar distribution of melanocytes in the hamster skin places these cells in a most vulnerable position for attack by the carcinogen. The fact that similar networks have hitherto not been observed in mouse and rabbit skin explains why hamsters are so prone to the development of melanotic tumours while mice and rabbits are not.

Many large melanotic networks also surround the coarse hair producing giant pilosebaceous follicles situated in the costovertebral spot of the hamster. Repeated painting of the costovertebral spot, however, did not produce melanotic tumours in the spot itself, though many such tumours appeared in the surrounding skin¹. The most likely explanation of this phenomenon is that the carcinogen was rapidly flushed out by the secretion from these large sebaceous glands.



Whole mount of hamster shin showing network of dendritic melanocytes surrounding pilosebaceous follicles in the region just above and around sebaceous glands. Haematoxylin and eosin $\times 137$

Our experiments on the histogenesis of the hamster melanoma¹ suggest that a close association between melanocytes and the pilosebaceous follicles producing fine hair may be dangerous, while an association between melanocytes and large pilosebaceous follicles producing large coarse hair may not be so dangerous. In man a close association between a large collection of melanocytes and pilosebaceous apparatus, similar to that seen in the hamster, does not occur in normal skin but is sometimes seen in naevi. The hairy mole of man; a well recognised clinical entity, may be considered morphologically similar in certain respects to the costovertebral spot of the hamster for in both these sites we find large coarse hairs, large sebaceous glands, and abundant melanocytes. Since the costovertebral spot is very resistant to the action of chemical carcinogens one might suspect that the same is probably true in the case of the hairy mole of man. Clinical observation supports this idea for malignant changes very rarely occur in the hairy mole³.

It is now generally accepted that when a malignant melanoma arises in a mole or in normal human skin it is from melanocytes at the dermo-epidermal junction. In this position ultraviolet light in the form of solar radiation

¹ F. N. GHADIALY and J. F. BARKER, J. Path. Bact. 79, in press (1960).

² V. SUTZEFF, E. V. COWDRY, and A. CRONINGER, Cancer Res. 15, 637 (1955).

³ J. BELISARIO, *Cancer of the Skin* (Butterworth & Co., London 1959), p. 191.

⁴ H. O. LANCASTER, Lancet 2, 929 (1955).

⁵ H. O. LANCASTER, Med. J. Aust. 1, 1082 (1956).

⁶ E. P. CAWLEY, Arch. Derm. Syph. 65, 440 (1952).

⁷ M. GORDON, Zoologica 35, 19 (1950).

⁸ F. N. GHADIALY and M. GORDON, Cancer Res. 17, 597 (1957).

⁹ A. C. ALLEN, Arch. Derm. Syph., Chicago 69, 150 (1954).