

Summary. In over 40% of 110 newborn infants tested, in 3% of 63 healthy children, in 18% of healthy adults and in 37% of 164 pregnant women, precipitating antibodies against cow's milk could be demonstrated. All these groups form antibodies only against the whey-proteins specifically present in milk, particularly β -

lactoglobuline, and not against caseine or cow's milk proteins identical with protein present in beef serum.

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Feeding Response of Adult *Tribolium* to Carbohydrates in Relation to their Utilisation

Various species of *Tribolium* are known to flourish well on cereals and their products, in comparison with non-cereals (FRAENKEL and BLEWETT¹; SMALLMAN and AITKEN²). In the course of investigations into the factors governing establishment of *Tribolium castaneum* (Herbst.) on one and not on the other source of food, study of the role of carbohydrates in stimulating ingestion of food and their nutritional efficiency for the survival of adult insects has been undertaken.

The stimulating capacity of different carbohydrates for the adult *T. castaneum* was determined by comparing the feeding response to different test diets offered singly. For this purpose, each diet was dyed with bromophenol blue, dried and powdered. About 500 mg of the test diet was offered to 20 insects, kept in glass vials, for 30 min. At the end of this period, the insects were removed, dissected and examined under a microscope. Presence of the dye within the gut would indicate ingestion of the diet. The percentage of individuals ingesting the different carbohydrates would indicate the relative feeding preference of the insects on these sugars.

Amongst the different carbohydrates tested in pure form, feeding activity of the insect was greater on sucrose and fructose than on starch, maltose, glucose, raffinose, melibiose and agar-agar. The response to the last six carbohydrates was equally poor. Addition of sucrose to agar-agar in concentrations of 5%, 50% or 75% did not elicit any greater feeding response than the pure agar-agar. However, the feeding activity of the insect on each of the carbohydrates, listed above, was significantly lower than that on wheat flour which is the natural food of the insect.

Nutritional efficiency of the carbohydrates for survival of adult *T. castaneum* was also determined by comparing the time taken for 50% mortality of individuals kept on each carbohydrate. The insects kept on starch or maltose survived for the longest period, 50% mortality of the individuals occurring in 119 days in the case of starch and in 126 days in the case of maltose. Glucose, melibiose and lactose were less efficient and supported adult life

for a period of 76–104 days. Nutritional efficiency of the remaining carbohydrates namely, fructose, sucrose, mellezitose, raffinose and cellobiose was very poor since 50% mortality occurred in 23–30 days. Relative efficiency of the carbohydrates for the adult was thus quite different from that for the larva for which sucrose was as efficient as starch, maltose, cellobiose and glucose, but melibiose and lactose retarded its growth (BERNARD and LEMONDE³).

On the basis of all these observations, it is evident that the carbohydrates, by themselves, do not stimulate ingestion of food by adult *Tribolium* and, therefore, their presence in the cereals does not seem to play much role in the insect's preference for feeding on these foodstuffs. Nevertheless, the relative proportion of different carbohydrates in the cereals (KENT-JONES and AMOS⁴) is correlated with the capacity of the insect to digest and absorb them (KRISHNA and SAXENA⁵) and also with their efficiency to support its survival⁶.

Zusammenfassung. Die Fütterungsreaktionen beim erwachsenen *Tribolium castaneum* (Herbst.) zeigen, dass keine der verschiedenen Kohlenhydrate das Fressen besonders anregt. Der Nährwert dieser Kohlenhydrate nimmt in folgender Reihenfolge ab: Stärke und Maltose; Glukose, Melibiose und Milchzucker; Rohrzucker, Fruchtzucker, Melezitose, Raffinose und Cellobiose.

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¹ G. FRAENKEL and M. BLEWETT, *Trans. R. Ent. Soc. Lond.* **93**, 457 (1943).

² B. N. SMALLMAN and T. R. AITKEN, *Cereal Chem.* **21**, 499 (1944).

³ R. BERNARD and A. LEMONDE, *Rev. Canad. Biol.* **8**, 498 (1949).

⁴ D. W. KENT-JONES and A. J. AMOS, *Modern Cereal Chemistry* (Northern Publishing Co. Ltd., England 1957).

⁵ S. S. KRISHNA and K. N. SAXENA, *Physiol. Zool.* **35**, 66 (1962).

⁶ The authors are indebted to Prof. B. R. SESHACHAR for providing facilities for work and for making valuable suggestions in the preparation of this note.

The Suitability of Intracutaneous Infection in Guinea Pigs Induced by Virulent Tubercle Bacilli for the Use in Chemotherapeutic Trials

Intracutaneous infection of guinea pigs has been favoured by LESTER¹, RICHMOND and CUMMINGS², JENSEN et al.³ and, more recently, by BURACZEWSKA et al.⁴ for differentiation of various mycobacteria. Still more recently, this method was employed by DADDI⁵ in chemotherapeutic trials and it was reported to be suitable also for this purpose. It is the aim of this paper to present our own results with intracutaneous infection in guinea

pigs and an experience with a model chemotherapeutic trial.

¹ V. LESTER, *Acta Tuberc. Scand.* **13**, 251 (1939).

² L. RICHMOND and M. CUMMINGS, *Amer. Rev. Tuberc.* **62**, 632 (1950).

³ K. A. JENSEN, P. COLETOS, G. DADDI, E. FREERKSEN, and N. RIST, *Bull. Union int. Tuberc.* **24**, 81 (1954).

⁴ M. BURACZEWSKA, M. JANOWIEC, and B. TUSZYNSKA, *Gruzlica (Poland)* **28**, 195 (1960).

⁵ G. DADDI, *Isoxyl, a New Chemotherapeutic Agent* (Continental Pharma, Brussels 1961).

Four female guinea pigs weighing in average 390 g were infected intracutaneously into the depilated backside skin at four separate places. Each injection was accomplished by administering 1 mg of semi-dry weight of the H37Rv strain of *M. tuberculosis* suspended in 0.05 ml of saline by means of a tuberculin syringe. The mycobacterial suspension was prepared by shaking the 13-day-old culture grown on the surface of Sauton liquid medium,

after it had been partially dried between two sheets of sterile filter paper, with glass beads in sterile saline. Results of a serial dilution test performed on Löwenstein-Jensen media showed that each infection dose (1 mg of bacilli) contained 6.8×10^7 of viable mycobacterial units. After the infection the animals were put into a single cage and fed in the usual way. Once a week local changes were observed so that the longest diameter of first the infiltrate and later the ulceration, and another diameter vertical to the first one were recorded with use of a transparent millimeter scale. The values thus obtained were multiplied each with the corresponding other one and the local lesion was then expressed in square millimetres. All measurements were performed as a 'double blind' test so that two independent observers registered the respective values, each for one week, without knowing the data collected in previous weeks.

Results of this experiment are summarized in Table I, which contains mean squares of infiltrates (1st and 2nd week) or of ulcerations (3rd to 8th week) in individual animals.

It is evident from data presented in Table I that a linear correlation may be found to exist between mean squares registered in 5 guinea pigs and the time interval after the infection. This correlation is easily recognized in the Figure.

Suitability of this proceeding for chemotherapeutic purposes was checked in a model trial with isoniazid. The drug was administered to 3 guinea pigs perorally once a day in the dose of 10 mgs/kg for 6 weeks. The therapy was started 21 days after infection (appearance of ulcerations). All other conditions were exactly the same as described above. Results are presented in Table II.

Distinct regression in mean squares of local lesions in animals treated with isoniazid may be seen from data in Table II. At the end of the 6th week ulcerations in all animals finally disappeared, being replaced by cicatricous healing.

Thus, it appears that intracutaneous infection of guinea pigs represents a method well suited for chemotherapeutic trials, which finding is in agreement with the experiences of DADDI⁵, and with the general trend of evaluation as far as possible quantitative on the activity of a new chemotherapeutic compound in the course of time. Nevertheless, it must be stressed that this procedure is a very infectious one, and utmost care must be paid to protection of the staff manipulating with intracutaneously infected animals. Perhaps the spraying of lesions with some transparent acrylate dressings might prove to be advantageous in this respect.

Zusammenfassung. Versuche mit intrakutan infizierten Meerschweinchen ergaben eine lineare Abhängigkeit zwischen der Flächengrösse der entstandenen Läsionen und dem Zeitintervall nach der Infektion. Modellversuche mit Isoniazid haben die gute Brauchbarkeit dieser Methode für chemotherapeutische Zwecke bewiesen.

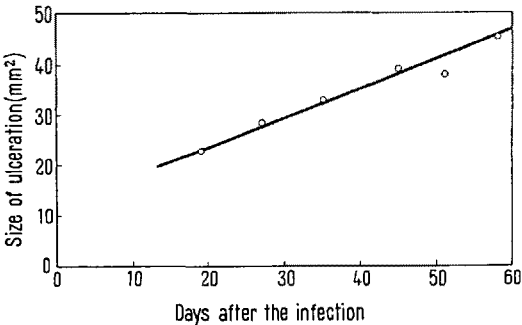
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Tab. I

Animal No.	Weeks after the infection							
	1.	2.	3.	4.	5.	6.	7.	8.
875	18.2 ^a	37.5 ^a	20.5 ^b	25.2	25.7	28.0	29.7	— ^c
873	21.6	37.8	23.0	34.6	39.0	42.7	41.5	36.0
870	28.2	57.5	30.2	29.0	36.9	42.5	41.5	52.5
872	39.7	23.2	18.7	23.2	30.5	45.5	44.7	— ^c
Mean value			23.1	28.1	33.0	39.6	39.3	44.2

^a Mean squares of infiltrates calculated from 4 lesions induced in one animal.
^b Mean squares of ulcerations calculated from 4 lesions induced in one animal.
^c This guinea pig died before the last reading could be accomplished.



Tab. II

Animal No.	Weeks after the infection					
	2.	3.	4.	5.	6.	7.
815	16.2 ^a	14.5 ^b	14.2	10.5	5.5	— ^c
802	22.0	19.6	19.3	12.6	10.6	—
817	24.7	22.7	18.5	11.5	13.5	—
Mean value	20.9	18.9	17.3	11.5	9.8	—

^a Mean squares of ulcerations calculated from 4 lesions induced in one animal.
^b This week isoniazid therapy was started in the dose of 10 mg/kg perorally.
^c At this time-interval ulcerations in all animals have been replaced by cicatricous healing.