

presence, in rabbits, of digestive group natural antibodies¹. Heat-labile positive autologous reactions were constant in all rabbits. Unheated rabbit sera were also regularly positive when tested on isologous or heterologous organs. However, unlike the results observed in rats, not all the positive isologous and heterologous reactions disappeared when sera were heated: persistence of positive staining after heating indicated the presence of a digestive group antigen-antibody reaction related to digestive-group iso- or hetero-antibodies.

Heat-labile anti-digestive antibodies were absent from the tested dog and human sera.

Discussion. These data indicate the presence in sera from healthy rats and rabbits of non-species-specific, heat-labile anti-digestive auto-, iso-, and hetero-antibodies. Such antibodies are absent in man and dog. Unlike anti-digestive antibodies induced in animals immunized with heterologous digestive mucosa extracts⁵, or digestive group antibodies¹⁻³, the present antibodies disappear after heating. They may be of the same nature as heat-labile antibodies specific for various tissue extracts which have been reported in rats⁶. Whatever may be their nature, their presence should be taken into consideration whenever digestive antigens or anti-digestive antigens antibodies are studied. This is important for studies on digestive group systems. Although digestive group antibodies may be absent from some species, such as rats, as seen from the negative results regularly observed

with heated rat sera on isologous digestive tissue sections, they appear to be present in other species such as rabbits¹, dogs^{1,2}, and man³. When heat-labile anti-digestive antibodies are present, as shown in rabbits, they must be removed to avoid attributing to digestive group antibodies positive reactions due to heat-labile antibodies. Their presence may also be a cause of error in studies on anti-digestive antigens auto-antibodies⁷.

Résumé. Les rats et les lapins normaux possèdent dans leur serum des anticorps thermolabiles qui réagissent en immunofluorescence avec des antigènes glandulaires de la muqueuse digestive autologue, isologue, et hétérologue. Ces anticorps disparaissent après chauffage à 56 °C pendant 30 min. Ces anticorps sont absents chez l'homme et le Chien.

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⁶ D. M. WEIR, R. N. PINCKARD, C. J. ELSON and D. E. SUCKLING, *Clin. exp. Immun.* 7, 433 (1966).

⁷ E. MARY COOKE, M. ISABEL FILIPE and I. M. P. DAWSON, *J. Path. Bact.* 96, 125 (1968).

Total Hemocyte Counts of Honey Bee Larvae (*Apis mellifera* L.) from Various Elevations

The hemolymph, or blood, of the honey bee, *Apis mellifera* L., is a pale yellowish fluid containing blood cells referred to variously as hemocytes, blood corpuscles, or leucocytes. The blood of larvae comprises 25–30% of the total body weight¹. Insect hemolymph is not carried in blood vessels; it fills the spaces of the body cavity and bathes the surfaces of tissues. In vertebrate blood, there is a definite correlation between the total number of erythrocytes and elevation. The present paper describes a study of the circulating hemocytes of honey bee larvae obtained from various elevations.

Combs containing larval honey bees were shipped air mail from localities at various elevations ranging from 159 feet below sea level to 7200 feet above sea level. Dates of the shipments were such that larvae were about 5 days old when they arrived. Individual 5-day-old larvae were then punctured with a sterile hypodermic needle, and the hemolymph which exuded from the wound was drawn to the 0.5 mark of a Thoma white-cell diluting pipette and diluted to the 11 mark with Toisson's fluid (1.0 g sodium chloride, 8.0 g sodium sulfate, 30 ml glycerin, 15 mg crystal violet, 160 ml distilled water). After the fluid was thoroughly mixed in the pipette for 2 min, the first three drops were discarded, and the count was made of the fourth. A Spencer bright-line hemocytometer with improved Neubauer ruling was used to count the cells in the 4 corners and the central square. Then the sum was multiplied by 40 to give the number of cells/mm³. If the cells were unevenly distributed, the sample was discarded. All average total counts reported were the results of counts from 5 samples, all taken from 5-day-old larvae. The effect of elevation on the counts was determined by regression correlation studies in which log₁₀ total hemocyte counts and log₁₀ elevation were used since the use of logarithms appeared to give the best correlation.

The average of total hemocyte counts (THC) of the 5-day-old larval honey bees are shown in Table I. Analysis showed a regression coefficient of 0.10851 which was significant at the 1% level of confidence (Table II). Therefore, a definite relationship existed between the THC and the elevation from which the insect was obtained (Figure). More circulating blood cells were present in larvae from higher elevations.

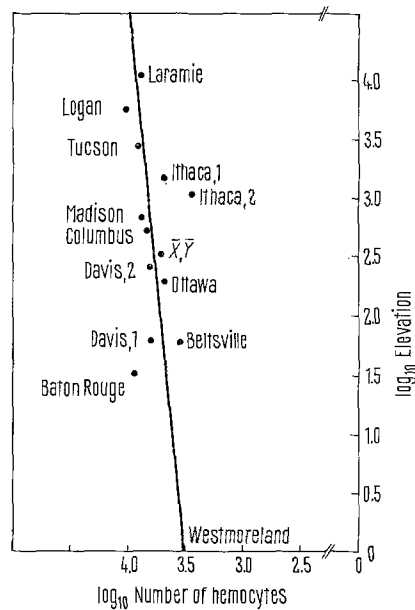
Table I. Total hemocyte counts of 5-day-old honey bee larvae from various locations

Sample number	Location from which larvae were obtained	Elevation (feet)	Avg. THC/mm ³ hemolymph
1	Westmorland, Calif.	—159	2,947
2	Baton Rouge, La.	35	7,570
3	Davis, Calif. (1)	60	5,680
4	Beltsville, Md.	61	4,176
5	Ottawa, Canada	270	6,093
6	Davis, Calif. (2)	300	5,266
7	Columbus, Ohio	760	7,140
8	Madison, Wis.	858	7,992
9	Ithaca, New York (2)	950	4,053
10	Ithaca, New York (1)	1,300	5,840
11	Tucson, Ariz.	2,543	7,667
12	Logan, Utah	4,753	9,120
13	Laramie, Wyo.	7,200	10,000

¹ G. H. BISHOP, *J. biol. Chem.* 58, 543 (1923).

Table II. Statistical data from regression correlation study of elevation versus total hemocyte counts (THC)

Formula: $\log_{10} Y = \log_{10} a + b \log X$ where X = elevation and Y = average THC (let $-159 = 1.00000$)
No. of observations = 43
Correlation coefficient between $\log Y$ and $\log X = 0.58820$
Regression coefficient = 0.10851
Standard error of regression coefficient = 0.02330
Coefficient of determination = 0.34598
Mean of $\log_{10} X = 2.54946$
Mean of $\log_{10} Y = 3.77982$



The effect of altitude on total hemocyte counts of 5-day-old honey bee larvae.

Since the regression coefficient was significant at the 1% level, increased elevation was related to a rise in the number of free hemocytes present in larval blood. The reason for this rise is not known. Perhaps factors such as pressure, blood volume, or a respiratory pigment play a role. When these studies were repeated with pooled hemolymph from adult worker bees, similar results were obtained^{2,3}.

Zusammenfassung. Ausgewertet wurde die Gesamtzahl der Hemocyten (GZH) von Proben von Honigbienenlarven, die aus verschiedenen Höhenregionen in Bereichen von 48 bis 2160 m über dem Meeresspiegel stammten. Die Untersuchung des Zusammenhanges der beiden Grössen mittels Regressionsanalyse mit logarithmischer Auftragung von $\log_{10}$ GZH gegen $\log_{10}$ Meereshöhe ergaben eine deutliche Beziehung zwischen der Zahl der Hemocyten pro mm^3 und der Meereshöhe, aus der die Insekten entnommen wurden.

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Electroimmunoprécipitation de protéines anodiques et cathodiques à pH 8,2

Il est un fait bien connu que les méthodes de précipitation en milieu gélifié sont aujourd'hui appliquées couramment dans les recherches sur de nombreuses substances antigéniques.

La réalisation même des réactions spécifiques antigène/anticorps, et notamment celles qui sont exécutées sur des plaques de gélose, avait été soumise à des modifications telles que: microméthode et macrométhode, diffusion simple et double, détermination qualitative et quantitative, augmentation de la sensibilité de la réaction de précipitation spécifique par l'utilisation de dextrans ou des immunosérums «anti-espèce fournisseuse des anticorps», lecture de résultats obtenus rendue plus sensible par la mise en jeu de l'isothiocyanate de fluorescéine ou des substances radioactives¹⁻¹².

Les résultats positifs de la précipitation de diffusion double ne se lisent pas avant 4-6 h et plus à partir du début des réactions. Ceci nécessite dans certains cas l'abrégement du temps qui s'écoule entre le début des réactions et l'apparition de résultats positifs.

En vue de surmonter cet obstacle, certains auteurs ont lié la précipitation de double diffusion sur plaques de gélose à l'électrophorèse¹³⁻¹⁶. Selon les principes de la méthode, dans la gélose coulée sur des plaques de

verre on découpe 2 rangées de réservoirs perpendiculairement au grand axe des plaques.

Dans les réservoirs situés du côté cathodique, on introduit des antigènes analysés, dans les réservoirs situés du côté anodique l'immunsérum, puis on laisse passer le courant électrique.

Sérum de cheval	Résultats positifs après (min)	
	Protéines anodiques	Protéines cathodiques
1:10	25	60
1:100	25	60
1:1000	25	60
1:2000	25	80
1:4000	60	80
1:6000	60	150
1:10,000	60	-

L'immunsérum de lapins «anti-sérum de cheval» a été préparé par nous même.