the contact inhibition phenomenon, as described in the well known studies of ABERCROMBIE and HEAYSMAN[5], although BELLAIRS and NEW[6] ascribe this property to the edge cells of the chick germ.

It would be interesting to find out how far the inhibition phenomena in this study model are specific for cancer cells. We are therefore testing, in further experiments, other cancer cells and their normal counterparts.

Zusammenfassung. Die Entwicklung vom «margin of overgrowth» junger Hühnerkeime ist verschieden nach Kontakt mit Helazellen und mit Mesonephros oder Hautgewebe; die ersteren hemmen die Entwicklung, die anderen werden vom Keim aufgenommen. Deshalb wird der «margin of overgrowth» als Modell für die Studie von Zellkontakten vorgeschlagen.

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Heat-Labile Natural Anti-Digestive Antibodies in Animals

Evidence has recently been obtained of the presence, in animals[1,2] and in man[3], of digestive group systems. Natural digestive group antibodies have been shown to be heat-resistant. Although the signification of digestive group systems is still unknown, their theoretical interest may lead to further studies. Such studies should take into consideration the presence, in healthy animals from different species, of heat-labile anti-digestive antigens antibodies.

Material and methods. The immunofluorescent indirect method was applied to sera and alcohol fixed cryostat sections of the colon, the small intestine, and the stomach from various species. Experiments were conducted on 400 healthy rats from different strains (Wistar, Long Evans, Fischer inbred rats, Fischer inbred germ-free rats), on 100 healthy rabbits, and on 200 healthy mongrel dogs. Normal human sera from 200 blood donors, and sera and colons from 40 patients without digestive disease were also tested. All sera were tested before and after heating (30 min, 56 °C). Specific fluorescent anti-γ-globulins were prepared in the laboratory[4]. Control experiments were conducted with commercial fluorescent anti-rabbit and anti-human globulins (Microbiological Associates, Bethesda).

Results. In rats, specific fluorescent staining of the goblet cells from the colon, the small intestine, and the stomach was observed with all unheated fresh sera when tested on autologous tissue sections (Figure 1). The fluorescent staining, restricted to and present in all the goblet cells, was indistinguishable from that observed with a specific anti-rat colon rabbit immune serum[5]. Same positive reactions were observed with the same unheated sera when tested on isologous and heterologous rabbit, dog, and man digestive tract tissue sections. All these reactions were negative when the sera had been previously heated (Figure 2). These results have been obtained in all the animals from the different strains of rats under experiment, including germ-free rats. This heat-labile activity was not modified when unheated sera were stored at -20 °C (up to 6 months). It disappeared after absorption with iso- or heterospecific digestive mucosa dry powder. Absorption with any one of the species-specific dry powders removed the positive staining observed on the goblet cells of all the autologous, isologous, and heterologous digestive tissue sections.

Similar results were observed in rabbits. In this species, however, some differences may be seen in relation to the

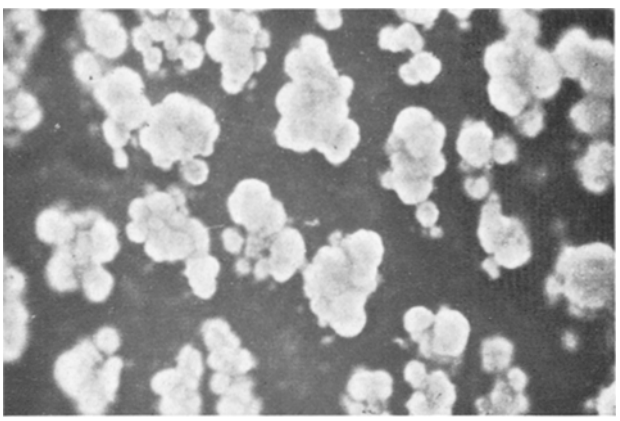

Fig. 1. Positive immunofluorescence staining observed on a rat colon with its own unheated serum.

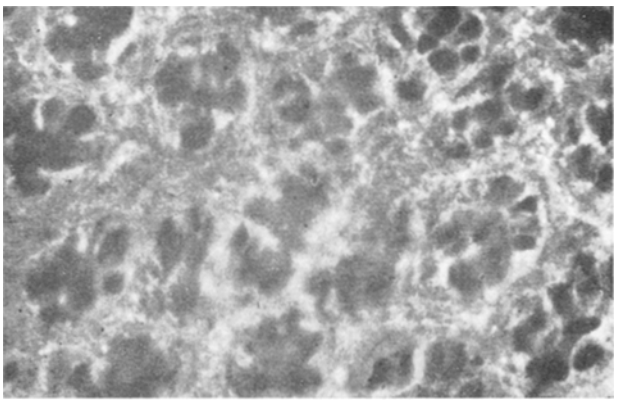

Fig. 2. Negative immunofluorescence staining observed on the same colon as in Figure 1 with the same serum heated for 30 min at 56 °C.

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presence, in rabbits, of digestive group natural antibodies[1]. 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: persistance 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 extracts[5], or digestive group antibodies[1-3], 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 rats[6]. 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 rabbits[1], dogs[1,2], and man[3]. 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-antibodies[7].

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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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 weight[1]. 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/mm^3. 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 $\log_{10}$ total hemocyte counts and $\log_{10}$ 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

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