ration de la composition normale du milieu interne» zu und verglich sie mit den Tubuli contorti der Vertebratenniere.

Die Carapaxfelder von *Argulus foliaceus* gliedern sich jederseits in einen vorderen kleinen, angenähert kreisförmigen und in einen grösseren hinteren, länglich-nierenförmigen Bereich (Figur 1). Ihre Epidermis unterscheidet sich grundlegend vom übrigen Körperepithel. Schon bei der lichtmikroskopischen Betrachtung erkennt man senkrecht zur Körperoberfläche stehende Streifungen in den zylindrischen Zellen. Das elektronenmikroskopische Bild löst diese in Mikrotubuli sowie apikale und basale Einfaltungen der Cytopalsmamembranen auf (Figuren 2–4). Die apikalen Einfaltungen, die sich zwischen Mikroleisten einsenken, sind im vorderen und im hinteren Carapaxfeld unterschiedlich: im vorderen kurz, breit und oft mit seitlichen Einbuchtungen versehen, an denen viele Vesikulationen beobachtet werden können (Figur 2), im hinteren dagegen schmaler und wesentlich tiefer mit weniger und merklich kleineren Vesikulationen (Figur 3). Wo den Mikroleisten die Kutikula aufsitzt, sind Hemidesmosomen ausgebildet (Figuren 2 und 3). Zwischen den Mikroleisten ist der Interzellularraum mit flockigem, elektronenoptisch wenig dichtem Material gefüllt, welches dem Inhalt der Vesikel gleicht. Die Basis der Epithelzellen (Figur 4) wird von einer starken Basalmembran, in die Kollagenfibrillen eingelagert sind, begrenzt. Die Basen der Zellen sind vielfach miteinander verzahnt. Dadurch entstehen interzellulare Räume, in die hinein sich die Basalmembran erstrecken kann (Figur 4, *). Die mit den Verzahnungen erzielte Oberflächenvergrösserung wird durch schlauchförmige Einfaltungen der basalen Cytoplasmamembranen verstärkt. Meist lösen sich ganze Abschnitte der Einfaltungen in Reihen von Bläschen auf. Stellenweise kann man die Einfaltungen bzw. die Bläschenreihen von der Basis der Zellen bis zur Basis der apikalen Mikroleisten verfolgen (Figur 2, Pfeile). Eine morphologisch erkennbare Polarität der Epithelzellen kommt auch in der Orientierung anderer Zellstrukturen zum Ausdruck. Zahlreiche Bündel von Mikrotubuli (Figur 2, Pfeilspitzen), die sich verzweigen können, sind über desmosomenartige Bildungen sowohl mit der basalen wie auch mit der apikalen Cytoplasmamembran verbunden. Dadurch könnte grössere Festigkeit, aber auch eine Kompartimentierung und Strömungsorientierung des Cytoplasma erzielt werden.

Der Kern liegt im zentralen Zellbereich; er ist schwach gelappt und meist seitlich depress, das Kernmaterial diffus verteilt. Ein oder mehrere Nucleoli sind deutlich. Sehr zahlreich sind vor allem freie Ribosomen (Figuren 3 und 4). Golgi-Komplexe findet man dagegen nur vereinzelt. Die Mitochondrien sind auffällig gross und häufig (Figuren 2 und 3).

Die Zellen ähneln den Chloridzellen der Trichopteren-Larven[4]. Vergleichbare Strukturen fanden sich auch in den Salzdrüsen der Silbermöve[5] und in den Salz absorbierenden Kiemenzellen von *Callinectes sapidus*[6]. Diese Analogien sowie der histochemische Nachweis z. B. von Chlorionen vor allem in den Mikroleisten der hinteren Carapaxfelder machen es wahrscheinlich, dass die Schalenfelder von *Argulus foliaceus* Bereiche der Körperoberfläche darstellen, die dem aktiven Ionentransport dienen. In Anlehnung an frühere Untersuchungen[4] sollen sie Chloridzellen-Organe genannt werden. Wenn man annimmt, dass der Gasaustausch durch das Integument der gesamten Körperoberflöche erfolgt, dann sollte den Carapaxfeldern zusätzlich eine ionenregulatorische Funktion zuerkannt werden.

Ausführlichere Untersuchungen der Ultrastruktur und der Funktion der ventralen Carapaxfelder werden demnächst an anderer Stelle veröffentlicht.

Summary. The specialized areas of the carapace of *Argulus foliaceus* hitherto known as respiratory areas, are most probably identified as iontransporting epithelia by electron microscope and histochemical investigations.

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Affinity of ^{14}C-Nicotine to Some Cells of the Lymfomyeloid System Demonstrated by Autoradiography

Blood dyscrasias associated with exposure to drugs is one of the serious problems encountered in therapy today. However, very little is known about the mechanisms by which drugs cause blood disorders. In some cases, an altered immunity or an allergic mechanism has been held responsible[1, 2, 15]. In other cases, a direct toxic effect of the drugs on the circulating blood cells or on the bone marrow has been demonstrated[2–4]. In the latter instance, a specific accumulation of the drug to toxic levels in the bone marrow might lead to hemopoietic damage, and therefore it would be of great interest to demonstrate whether any particular drug has the ability to accumulate in the hemopoietic tissues.

Work in this department on the distribution of nicotine in the body by means of autoradiography showed a significant accumulation in some hemopoietic tissues, such as the spleen and the bone marrow[5, 6]. In the present investigation, an attempt was made to perform a more detailed study of the nicotine distribution in the bone marrow and other tissues of the lymphomyeloid system of the mouse, using autoradiographic and histochemical techniques.

Four 3-month-old C_3H/TifBom male mice were each injected i.v. with 5 µCi (N-methyl-^{14}C) nicotine bitartrate, spec. activity 24 mCi/mmol (Radiochemical Center, Amersham, England). The dose corresponded to 1.4 mg/kg (calculated as a base). At 5 min, 30 min, 4 and 8 h after injection the mice were killed by immersion in a mixture of hexane and solid CO_2 (−78 °C) and embedded in carboxy-

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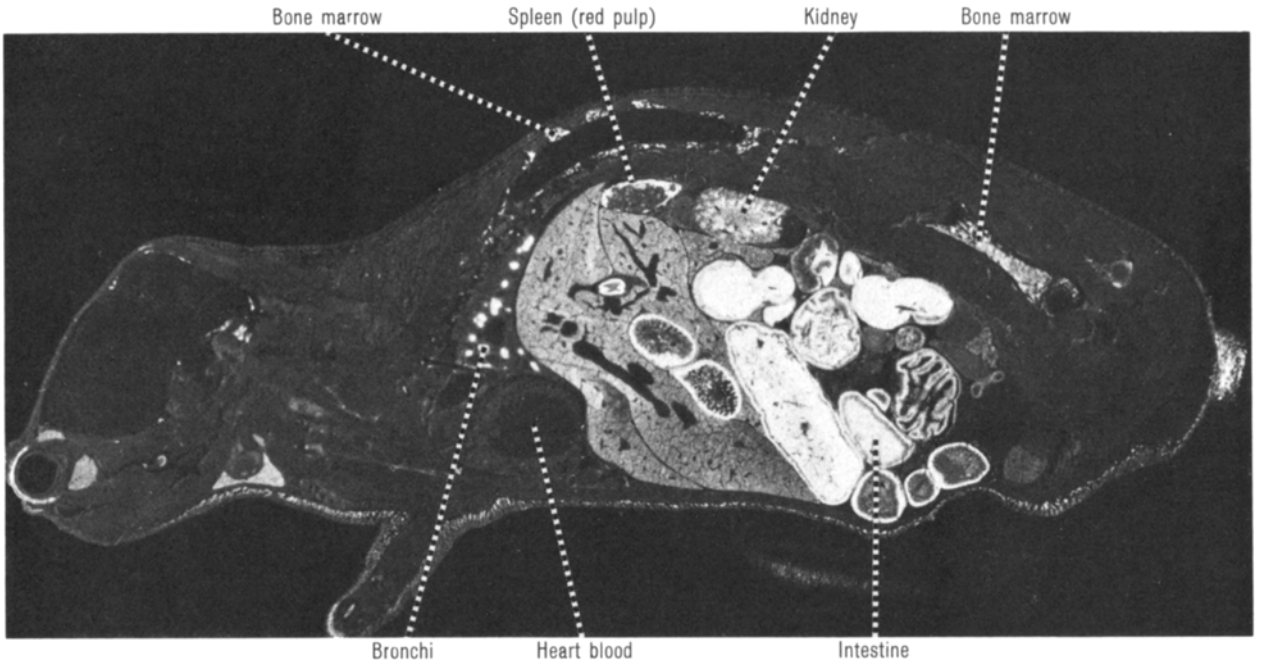

Fig. 1. Whole-body autoradiogram of a mouse 8 h after i.v. injection of ^{14}C-nicotine. Note the accumulation of radioactivity (light areas) in the bone marrow and the red pulp of the spleen. A high concentration of radioactivity is also present in the kidney, gastrointestinal tract and the bronchi.

methylcellulose mixed with water. Sagittal sections (20 or 30 μm thick) of the whole frozen animals were cut and processed according to ULLBERG's[7,8] whole-body autoratiographic technique. For semiquantitative evaluation of whole-body autoradiograms, the radioactivity in different organs was compared with the autoradiograms of simultaneously exposed ^{14}C-isotope standard[9].

In order to establish which cells of the bone marrow accumulate the label after injection of the radioactive nicotine, the whole-body autoradiographic technique was applied to lethally irradiated mice which were grafted with syngeneic bone marrow cells and injected with ^{14}C-nicotine. In such irradiated mice, the grafted stem cells from the bone marrow give rise to macroscopic colonies in the spleen which are composed of differentiated hemic cells[10]. Thus a localization of the labelled nicotine to these colonies might serve as an indication of an affinity of the drug to certain blood cell precursors.

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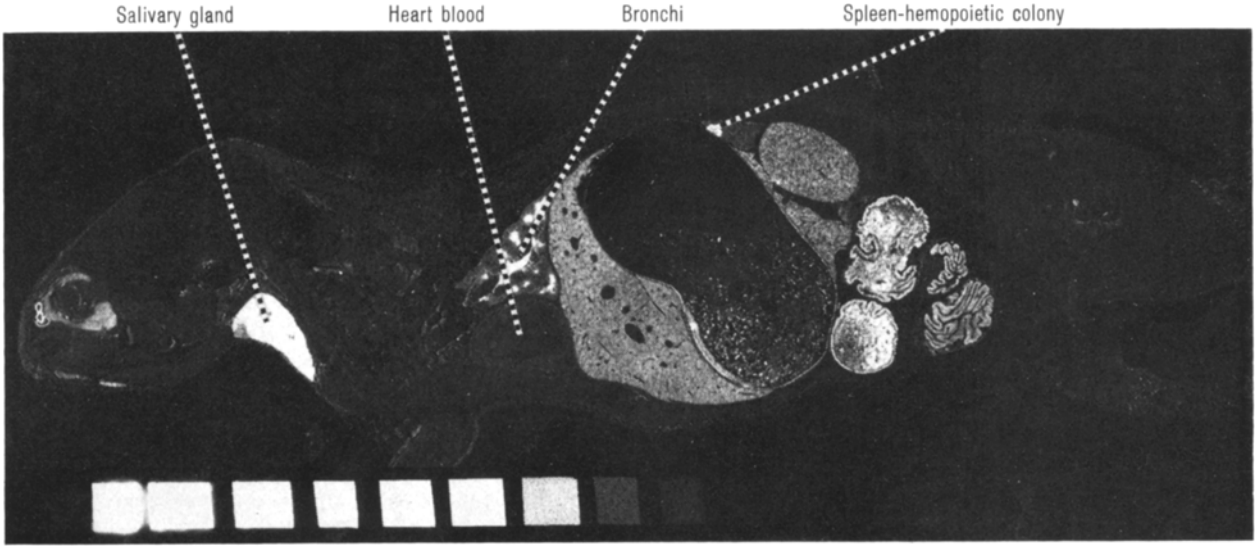

Fig. 2. Whole-body autoradiogram of an irradiated mouse with implanted bone marrow cells 4 h after i.v. injection of ^{14}C-nicotine. A high uptake of radioactivity (light areas) can be seen in the hemopoietic colony in the spleen. An accumulation of radioactivity is also present in the salivary glands and the bronchi.

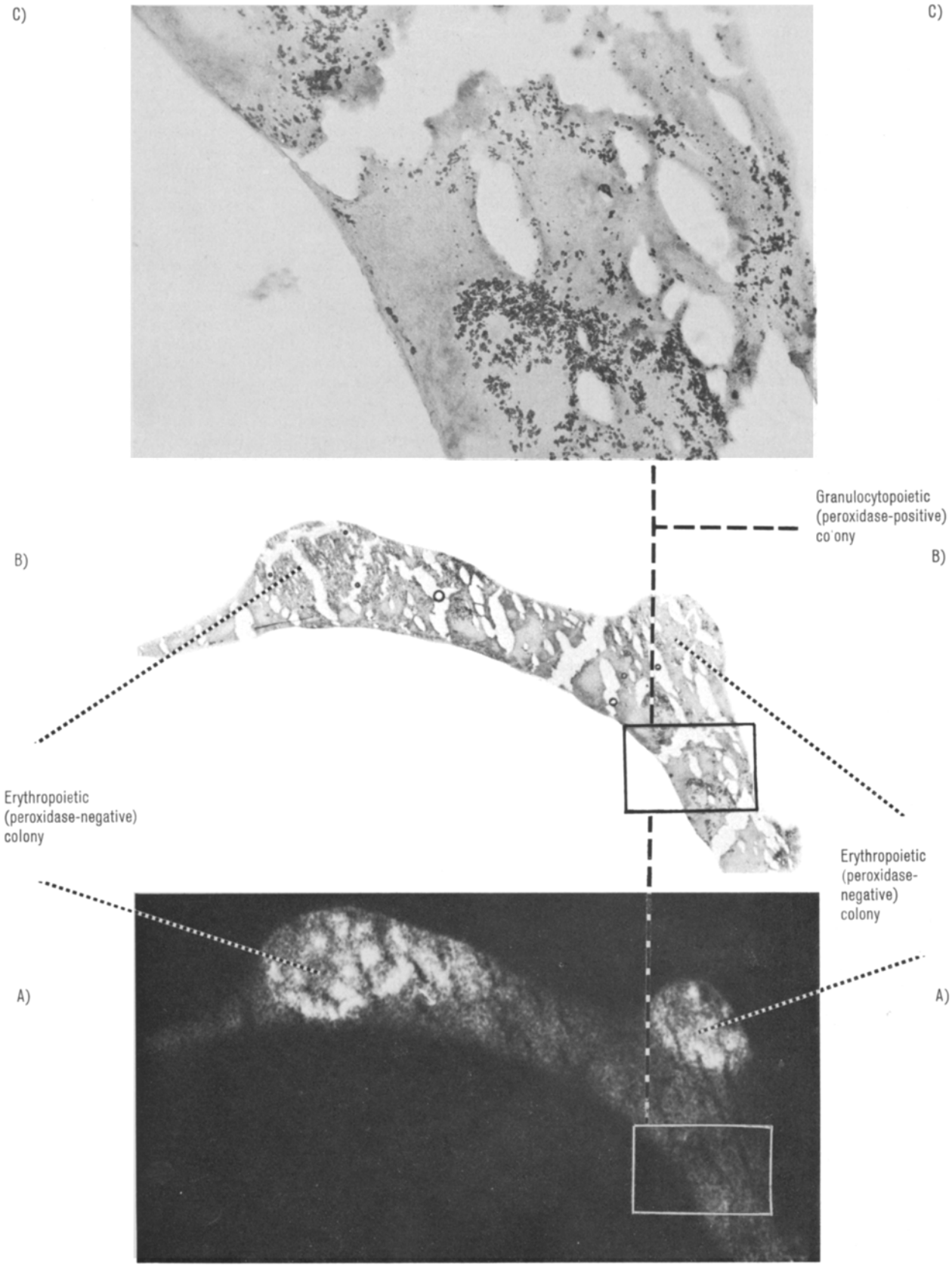

Fig. 3. A) Autoradiogram of a spleen from an irradiated mouse with implanted bone marrow cells 4 h after i.v. injection of ^{14}C-nicotine. B) Corresponding section treated cytochemically for a demonstration of peroxidase in the granulocytopoetic cells. C) Magnification of a splenic area from Figure B) showing a granulocytopoetic/peroxidase-positive/colony. As can be seen from the Figure, the radioactivity is mainly localized in the peroxidase-negative (erythropoietic) colonies. The peroxidase-positive/granulocytopoietic/colonies show a very low level of radioactive labelling.

Totally 12 male C_3H mice (b.wt. 25 g) were irradiated (Co^{60}-850R) and each was injected i.v. with 5×10^4 bone marrow cells. 6 of the animals were then injected i.v. with 4 μCi ^{14}C-nicotine on the 7th day after irradiation and another 6 mice received ^{14}C-nicotine on the 10th day after irradiation. The survival times were 30 min and 4 h (3 animals at each time interval) for both groups. After the killing, either the whole mouse or the removed spleen were embedded in carboxymethylcellulose, sectioned and subjected to whole-body autoradiography as described above. Several irradiated mice which were injected with ^{14}C-nicotine (but not with bone marrow cells) were run as controls to ensure that the irradiation itself did not result in changes in the distribution pattern of ^{14}C-nicotine.

To differentiate between the erythropoietic and granulopoietic colonies, serial sections (10 μm thick) of the spleens were made from mice with bone marrow transplanted and subsequently injected with ^{14}C-nicotine. One section was always exposed on film (autoradiographed) and stained afterwards with hematoxylin-eosine. The adjacent section was then subjected to a cytochemical method for demonstration of peroxidase which is specific for neutrophilic and eosinophilic granulocytes and its precursors[11,12]. In this way it was possible to compare directly the uptake of label in the peroxidase positive- (granulocytic) and the peroxidase-negative (erythropoetic) colonies.

The whole-body autoradiograms of normal mice injected with ^{14}C-nicotine (Figure 1) showed a marked accumulation of radioactivity in some organs of the lymfomyeloid system, such as the bone marrow and the spleen (mainly red pulp). The accumulation of radioactivity was highest 5 min after injection, exceeding that of the blood by about 4 times. After 30 min the labelling intensity dropped to about half the original level, but remained unchanged up to 8 h following the injection of ^{14}C-nicotine. At that time no or very little radioactivity could be seen in the blood and most of the tissues (Figure 1). At shorter time intervals, the uptake of radioactivity could also be observed in the thymus (cortex) and lymph nodes but it soon disappeared. After 4 h no radioactivity was detectable in these tissues. The irradiation of the mice did not seem to result in any change of the nicotine distribution picture excepting the hemopoietic tissues (in which the lack of radioactivity coincides with the depletion of highly radiosensitive hemopoietic cells).

On the autoradiograms of irradiated mice grafted with the bone marrow cells, a high and prolonged accumulation of radioactivity could be seen in the splenic colonies at all time intervals after the injection of ^{14}C-nicotine (Figures 2 and 3). The intensity of labelling in the colonies was about 4 times higher than in the rest of the splenic tissue and remained on that level during the entire period of the investigation (Figure 3). Actually the uptake of label was of the same order of magnitude as the labelling in the other organs of the body showing a pronounced accumulation of nicotine – such as the bronchi, the liver and the excretory pathways (Figure 2).

Among the colonies, the larger ones, detectable mainly on the 10th day after irradiation, seemed to exert the highest uptake. Since this sort of colony is mostly of the erythrocytic type[13], the result would indicate an affinity of nicotine and/or its metabolities for the erythropoietic cell precursors. This was further confirmed when the uptake of radiactivity was compared with the peroxidase activity (specific for the granulocytopoetic cells – see above) in the colonies. In the small colonies that exerted a positive reaction on peroxidase, a very slight uptake of radioactivity was seen as compared with the large peroxidase-negative ones (see Figure 3). Thus the precursor cells able to take up and accumulate radioactivity after the injection of ^{14}C-nicotine seem to be of the erythropoietic type.

No attempts were made in the present investigation to determine whether the radioactivity in the bone marrow and/or in the splenic colonies represents the nicotine or its metabolites. However, the experiments with various tissues in vitro have shown the liver to be the only organ with a significant capacity to metabolise nicotine[14]. This fact, together with the early localization of nicotine in the bone marrow observed in this investigation, would suggest that at least part of the radioactivity accumulated in the hemopoietic tissue represents the unchanged drug[15].

The present results do not allow any conclusions on the functional significance of nicotine in the hemopoietic tissues. However, the specific and prolonged accumulation of the drug in the erythropoietic cells could indicate some kind of nicotine interference with the proliferative capacity and/or maturation of the erythrocyte precursors – as well as an interference with the functional activity of the mature erythrocytes. Further experimental studies are needed to elucidate this point, and also to explain the precise functional significance of nicotine in the hematopoietic tissues.

Zusammenfassung. Nach intravenöser Injektion von ^{14}C-Nikotin wurde bei der normalen Maus mit Hilfe der Ganzkörperautoradiographie im Knochenmark und in der roten Pulpa der Milz eine kräftige und langanhaltende Ansammlung der Radioaktivität festgestellt. Kombinierte autoradiographische und histochemische Untersuchungen der Milz bestrahlter, mit Knochenmark transplantierter Mäuse haben weiter gezeigt, dass das radioaktive Nikotin und/oder dessen Metabolite innerhalb des hämatopoietischen Gewebes hauptsächlich in den erythropoietischen Zellen lokalisiert ist.

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Genetic Influence on Development of Reticulum Cell Sarcomas in SJL/J Mice

Initially, it was reported that SJL/J mice exhibit a distinct elevation in the plasma levels of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) after infection with the LDH virus[1]. It was then shown that this unique response is a recessive trait under the control of an autosomal gene[2]. This paper presents initial evidence for