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HOST METABOLIC ALTERATIONS DURING VENEZUELAN EQUINE ENCEPHALITI--ETC(U)
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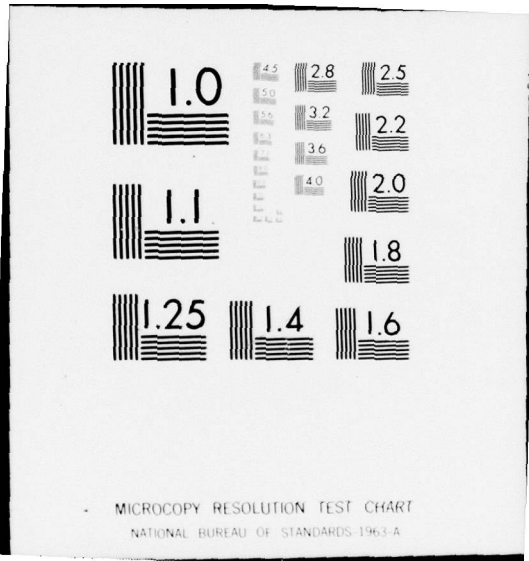
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Although an effective vaccine exists to protect against Venezuelan equine encephalitis (VEE), not all people who may be exposed to this disease are likely to be vaccinated. The disease most often presents as a short febrile illness but the convalescence period may be protracted, and death due to encephalitis does occur in a small percentage of those infected. Knowledge of the metabolic alterations which occur during VEE may materially aid in its treatment. Use of the V-198 strain of VEE in the rat produces a uniform model in which to study		

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metabolic alterations. Changes that occur early in the disease include viremia, neutrophilia, a decrease in plasma zinc and transferrin and increased amino acid uptake into liver. Plasma zinc depression persists into the later stage of the disease, but to a lesser degree. An increase in plasma copper and seromucoid occurs late in the disease concurrent with the development of pronounced encephalitis. Hypoalbuminemia and decreased ketonemia occur during both the early and late stages of the disease. Taken together, these metabolic alterations chronicle the development of VEE in the rat and thus may be useful as prognostic indicators, in formulating supportive therapy, and as monitors of potential antiviral therapy.

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Host metabolic alterations during Venezuelan equine encephalitis in the rat

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Running Title: Metabolic alterations during VEE in the rat

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In conducting the research described in this report, the investigators
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Abstract

Although an effective vaccine exists to protect against Venezuelan equine encephalitis (VEE), not all people who may be exposed to this disease are likely to be vaccinated. The disease most often presents as a short febrile illness but the convalescence period may be protracted, and death due to encephalitis does occur in a small percentage of those infected. Knowledge of the metabolic alterations which occur during VEE may materially aid in its treatment. Use of the V-198 strain of VEE in the rat produces a uniform model in which to study metabolic alterations. Changes that occur early in the disease include viremia, neutrophilia, a decrease in plasma zinc and transferrin and increased amino acid uptake into liver. Plasma zinc depression persists into the later stage of the disease, but to a lesser degree. An increase in plasma copper and seromucoid occurs late in the disease concurrent with the development of pronounced encephalitis. Hypoalbuminemia and decreased ketonemia occur during both the early and late stages of the disease. Taken together, these metabolic alterations chronicle the development of VEE in the rat and thus may be useful as prognostic indicators, in formulating supportive therapy, and as monitors of potential antiviral therapy.

Venezuelan equine encephalitis (VEE) virus causes considerable morbidity in man, about 5 to 10% encephalitis and about 0.5 to 1% mortality, with death generally due to encephalitis. Although VEE generally presents as a short febrile illness, convalescence may take as long as 3 weeks.¹ Although an extremely effective vaccine has been developed,² it is not feasible to vaccinate all people who may be exposed to the disease. For those who became ill only symptomatic therapy is presently available. Effective supportive, in contrast to symptomatic, therapy depends on knowing the effects of the disease on the physiology and metabolism of the host. Concern with host metabolism during VEE also reflects the fact that potential antiviral agents, such as stabilized poly(ICLC), induce metabolic alterations themselves which may have to be taken in consideration when such drugs are used.^{3,4} Also, it has been suggested that certain metabolic alterations which occur during the acute phase of an illness, notably those induced by leukocyte derived factors, may be a part of the overall host defense system.⁵ If this is so, then a knowledge of the metabolic sequelae of VEE may aid in formulating appropriate supplemental, supportive therapy, such as parenteral alimentation solutions, to hasten convalescence and/or treat specific aspects of the disease. For example, knowledge of the alterations in amino acid metabolism which occur in patients with chronic cirrhosis has allowed development of alimentation solutions which provide adequate nutrition without resulting in hepatic encephalopathy.⁶

The present animal model was thus devised to allow us to correlate the observed pathology with virologic manifestations and host metabolic alterations. The rat was chosen as the host, in part because it is one

of the best studied animals as regards its metabolism and because previous studies indicated that metabolic alterations could in fact be related to a specific aspect of a disease⁷ and be used as prognostic indicators⁸ during bacterial illness. The V-198 strain, of those strains of VEE tested, was found to produce the most uniform pattern of disease culminating in encephalitis and death (Jahrling et al. manuscript in preparation). This paper reports on certain of the host metabolic alterations which occur in rats following exposure to the V-198 strain of VEE virus. In-depth presentations of the virologic and pathologic manifestations are in preparation.

Materials and methods

Male, Fisher-Dunning rats, weighing approximately 240 to 270 Gm. were purchased from Microbiological Associates (Walkersville, MD). The rats were maintained in light and temperature controlled rooms [12 hours light (0600-1800 hours), 12 hours dark, 25° C.] for one week before use. They were fed Purina pellets ad lib. until initiation of the experiment. One day prior to the initiation of the infection the rats were injected subcutaneously (s.c.) with 2 μ Ci. of [14 C]aminoisobutyric acid ([14 C]AIB) per 100 Gm. body weight (New England Nuclear, Boston, Mass.). On the day of initiation of infection the rats were assigned to one of three groups: a) fasted rats receiving $10^{4.3}$ plaque forming units (p.f.u.) of the V-198 strain of VEE virus s.c.; b) fasted rats receiving the same dose of heat-killed V-198 strain virus, and c) fed rats. Eight rats each from groups a and b and 4 rats from group c were studied on 1, 2, 3, 4, and 5 days after the initiation of infection in group a. Eight fed rats were also studied on day 0. Rectal temperatures were recorded (Yellow Springs telethermometer) and then the rats were exsanguinated under halothane anesthesia by transecting the vena cava and collecting the blood from the pleural cavity. Heparin (100 units per 10 ml.) was used in collecting the blood. A blood sample was taken to determine white blood cell and differential counts and viremia. The rest of the blood was then centrifuged to obtain plasma which was exposed to ultraviolet light to inactivate the virus. The plasma was analyzed for zinc and copper,⁹ seromucoid,¹⁰ albumin and transferrin,¹¹ free fatty acids¹² and ketone bodies.¹³ A 1-Gm. portion of the liver was also removed (after perfusion with normal saline) for assessment of hepatic [14 C]AIB uptake.¹⁴

Strain V-198 VEE virus, third mouse brain passage, was used. The inoculum, $4.3 \log_{10}$ p.f.u., was contained in 0.2 ml. of 1 per cent bovine serum albumin in Hank's solution, pH 7.6. Inactivated virus was obtained by heating the virus inoculum suspension at 80° C. for 60 minutes. Viremia was determined by titrating 0.2-ml. blood samples on duck embryo cell culture monolayers grown in 10-cm² wells of plastic plates. The duck cells were maintained under media containing 1 per cent agarose in a 5% CO₂ atmosphere; p.f.u. were counted and compared with those produced by stock suspension containing known amounts of virus.¹⁵

Statistical significance was determined by one way analysis of variance. The fed rat control data did not display any significant daily variation and so were summed together and shown as a stippled horizontal bar in the accompanying figures.

Results

The s.c. inoculation of $4.3 \log_{10}$ p.f.u. of the V-198 strain of VEE virus eventually results in death to all rats which were inoculated (Table I). Food was provided ad libitum after day 6 to obviate death due merely to starvation. Providing food to infected rats from the time of inoculation of the virus does not alter the time to death. Histologic analysis reveals lymphoid necrosis on days 1, 2, and 3. By day 3 there is evidence of encephalitis which increases in severity thereafter (De Paoli et al. manuscript in preparation).

The concentration of virus present in the blood at various times after the initiation of the infection is depicted in Fig. 1. Peak viremia occurred on or before day 1; by day 3 only 25 per cent of the rats were viremic and by day 4 no virus was detectable in the blood. Virus was found in thymus and brain during the later stages of the disease (Jahrling *et al.* manuscript in preparation). A significant increase in rectal temperature was evident as early as day 1; the temperature became further elevated on days 3 and 4. By day 5 the body temperature in both fasted-infected and fasted-control rats began to decline (Fig. 2 top left). Pronounced neutrophilia was evident on day 1, but the percentage of neutrophils quickly returned to normal levels (Fig. 2 bottom left).

Plasma zinc was significantly decreased in infected rats by day 1 and remained low throughout the experiment (Fig. 2 top right). Fasting by itself eventually also resulted in a decline in plasma zinc values. Plasma copper values in the infected rats were somewhat below those of the fasted rats during the first 3 days of illness, but increased significantly over fasting values on days 4 and 5 (Fig. 2 bottom right).

[¹⁴C]AIB uptake by liver is presented as per cent of fasted control values because the tissue content of [¹⁴C]AIB decreases with time in both fasted and fed control rats (Fig. 3 top left). Peak response occurs on day 2 and tapers off thereafter. Plasma seromucoid concentration appears to peak on day 4 and then decreases (Fig. 3 top right). Albumin in contrast begins to decline by day 2 and remains below fasting values throughout the study. Fasting itself has little effect on albumin concentration (Fig. 3 bottom left). Transferrin concentration decreases with fasting; infection produces a further decline on days 2 and 3 and an increase on day 4 (Fig. 3 bottom right).

Plasma ketone body concentration was significantly decreased within one day of exposure as compared to fasted-control rats, was further depressed on day 2, rebounded toward fasting values on day 3, but was depressed again on day 4 and 5 (Fig. 4top). Plasma free fatty acids are decreased within one day of fasting. Infection causes a slight but insignificant additional decrement (Fig. 4 bottom).

Discussion

The s.c. inoculation of $4.3 \log_{10}$ p.f.u. of the V-198 strain of VEE virus in rats results in lymphoid necrosis followed by encephalitis and death. The metabolic alterations in this study then reflect changes in animals for which death is imminent; thus the values will not be distorted or skewed by the presence of survivors. Since viremia and neutrophilia quickly disappeared and fever was always present, more selective indices of the stage of illness were sought. Taken in concert the metabolic alterations described here appear to chronicle the sequence of pathophysiologic events that lead to death.

Pekarek and coworkers⁹ noted small but significant decreases in plasma zinc concomitant with fever in volunteers who received live, attenuated VEE virus vaccine and who subsequently became ill. An increase in plasma copper was also noted which began when fever was subsiding. In rats, plasma zinc was significantly decreased within one day after exposure to the virulent virus and plasma copper was significantly elevated on days 4 and 5. In those infections in which it has been studied, it appears that the zinc which is removed from the plasma is taken up by the liver.⁷ The uptake of zinc by the liver may be related to the increase in plasma acute phase globulins and the role zinc appears to play in nucleic acid, protein and amino acid metabolism.^{5,7} The rise in plasma copper is usually associated with an increase in ceruloplasmin¹⁶ indicative of acute-phase globulin release or increased synthesis.

An increase in amino acid uptake by liver has been noted during a number of infections^{7,14,17} and appears to reflect a redistribution of amino acids from muscle to liver,^{17,18} perhaps to supply substrate for increased gluconeogenesis¹⁹ and/or increased acute-phase globulin synthesis.⁵ It is not certain whether the transient aspect of this metabolic response is a true reflection of the events taking place in the animal. Conceivably the depletion of the amino acid analogue, which is not metabolized but is excreted,²⁰ precludes detection of increased amino acid uptake late in the disease.

Consonant with the idea of increased acute-phase globulin synthesis and/or release in the later stages of the disease is the increase in seromucoid. A 2- to 4-fold increase in seromucoid is associated with most infections.²¹ In man the seromucoid fraction is comprised of

α_1 -acid glycoprotein, haptoglobin, α_1 -antitrypsin and a number of other carbohydrate-rich proteins which are normally found in small amounts in plasma.²² The composition of the seromuroid fraction may vary from species to species, yet it appears to be a good indication of infection and/or inflammation.^{7,8,10} Lust²³ observed an increase in hepatic microsomal protein synthesis in the late stages of the disease in mice given the Trinidad strain of VEE. These findings could represent increased plasma protein synthesis, since plasma proteins are formed on the bound ribosomes²⁴ which would be precipitated along with microsomes.

Decreased plasma albumin is a concomitant of most infections.²¹ The present data clearly indicate that this decrease is not a function of fasting. Whether the decrease in albumin represents increased degradation, decreased synthesis and/or altered distribution²⁵ has not yet been satisfactorily determined. It has been suggested that albumin functions, as a storage form of amino acids.^{26,27} If so the decrease in albumin may make amino acids available for the synthesis of more critically needed proteins and/or gluconeogenesis. Transferrin which along with albumin has been considered an indicator of nutritional status²⁸ and which is often decreased in infections²¹ was decreased in part by fasting and somewhat more by infection early in the disease, but there was a slight indication of an increase in this protein late in infection. The significance of these changes remain to be ascertained.

As in pneumococcal sepsis, murine typhoid and tularemia,²⁹ there was a decrease in plasma β -hydroxybutyrate and total ketone bodies compared to fasting levels despite the fact that the infected rats were also fasted. This inhibition of ketosis is not simply a function of reduced availability of fatty acids as substrate since there was no

difference between fasted-infected rats and fasted-control rats in regard to the plasma free fatty acid level. The mechanism of this inhibition of ketosis may be related to the seemingly inappropriate increase in plasma insulin, which often occurs during severe infections,³⁰⁻³² or to some reorientation of hepatic metabolism.^{32,33} Of more interest is the role of this phenomenon in the host response to infection. It has been postulated that the inhibition of ketosis would allow the muscle to be catabolized^{34,35} to provide amino acids for the increased gluconeogenesis which seems to occur during infection and for increased acute phase globulin synthesis.⁵ It may thus play a permissive but vital role in the host response to infection.

Based on preliminary pathology findings one could conceivably divide the illness into at least two phases, an early phase (days 1, 2 and 3) in which lymphoid necrosis is quite evident and a second phase (days 3 to 5) in which encephalitis is present and developing. Conceivably there could be a third phase when deaths begin to occur but we do not have such data as yet. If one accepts this designation, one can observe that there are qualitative and quantitative differences in regard to metabolism between these two phases (Table II). For example, amino acid uptake by liver is only significantly increased and transferrin significantly decreased in phase I. The plasma copper, seromuroid and transferrin increases are only evident in phase II. The plasma zinc depression, though present in both phases, is diminished in phase II, as compared to the fasted controls. Inhibition of ketosis is prominent in both phases, perhaps indicating that it is a fundamental aspect of the host response to infection. It thus appears that a progression of metabolic events does occur which could be used as an

indicator to monitor the efficacy of potential antiviral agents and alternative therapies. If the self-same progression occurs in humans with this or other encephalitic diseases, then these metabolic sequelae could also be used as prognostic indicators if the disease is not uniformly fatal.

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Table I. Incidence of mortality in rats exposed to VEE

<u>Day Postexposure</u>	<u>Number Dead/20</u>
5	0
6	6
7	11
8	11
9	11
10	11
11	12
12	12
13	14
14	16
15	17
16	20

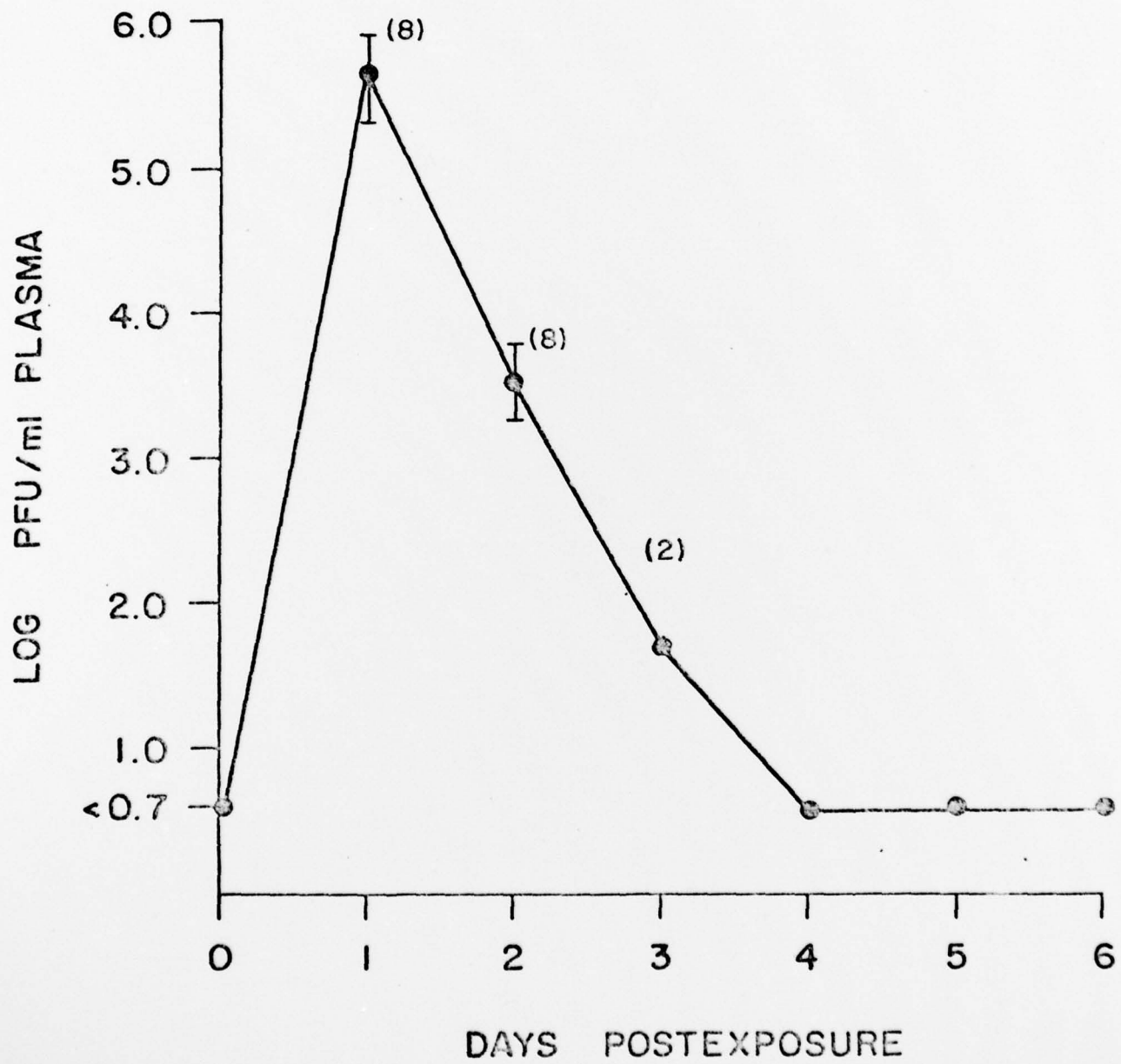
Table II. Summary of metabolic alterations during VEE in the rat

<u>Response</u>	<u>Occurrence</u>	
	<u>Phase I</u> (<u>days 1-3</u>)	<u>Phase II</u> (<u>days 3-5</u>)
Plasma zinc depression	++	+
Plasma copper increase	-	++
Hepatic amino acid uptake	++	-
Plasma seromucoid increase	-	++
Plasma albumin decrease	+	++
Plasma transferrin decrease	++	-
Plasma transferrin increase	-	+
Ketone body inhibition	++	++

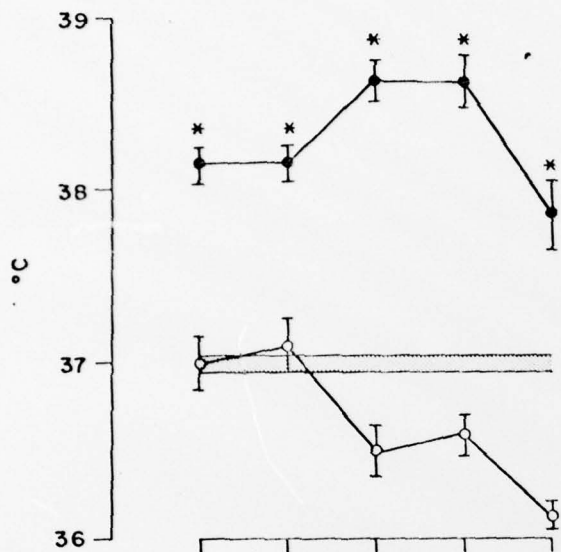
- = No change; + = moderate change; ++ = Marked change.

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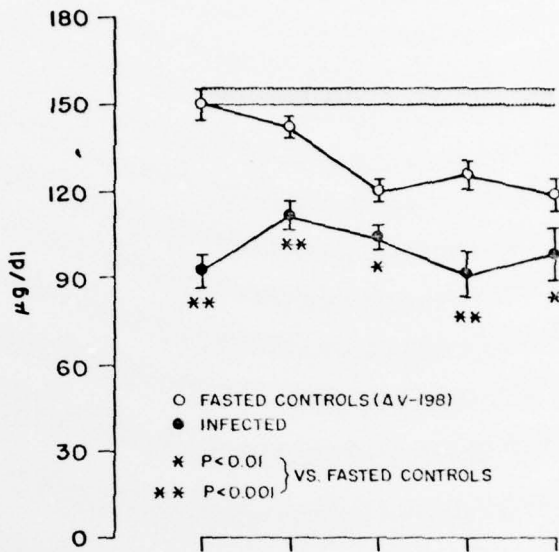
- Fig. 1. Viremia in adult white rats inoculated s.c. with $10^{4.3}$ pfu V-198 VEE virus. Eight rats were sampled at each time period. The number in paranthesis indicates the number with detectable viremia.
- Fig. 2. Rectal temperature (top left), percent neutrophils (bottom left), plasma zinc concentration (top right), plasma copper concentration (bottom right). Data from the fed controls (28 animals) are represented as a stipled horizontal bar. There were eight infected and eight fasted control rats per point per day.
- Fig. 3. [14 C]-aminoisobutyrate uptake by liver (top left), plasma seromucoid concentration (top right), plasma albumin concentration (bottom left), plasma transferrin concentration (bottom right). Data from the fed controls (28 animals) are represented as a stipled horizontal bar. There were eight infected and eight fasted control rats per point per day.
- Fig. 4. Plasma ketone body concentration (top). Plasma free fatty acids (bottom). Data from the fed controls (28 animals) are represented as a stipled horizontal bar. There were eight infected and eight fasted control rats per point per day.



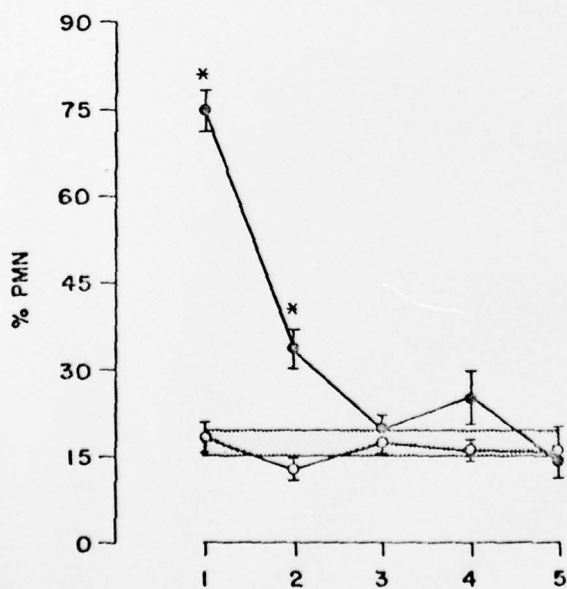
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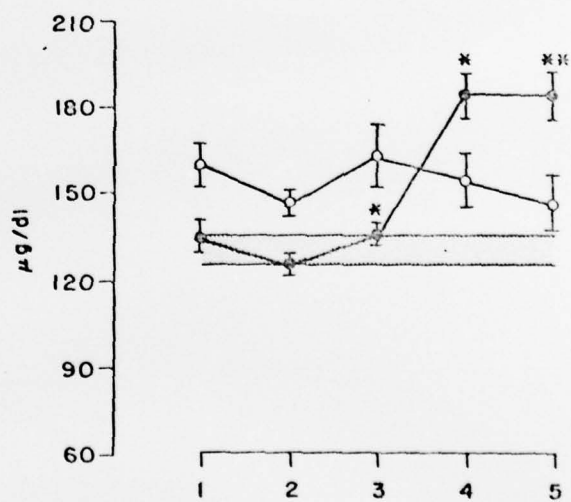
PLASMA ZINC



NEUTROPHILS



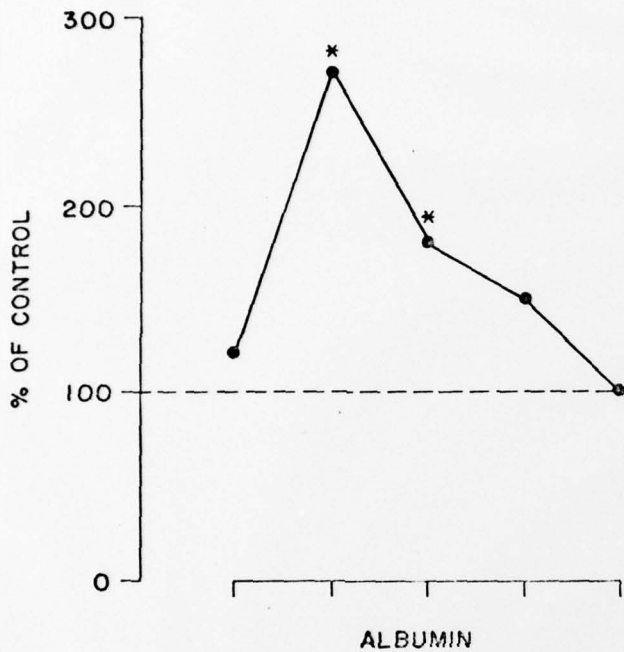
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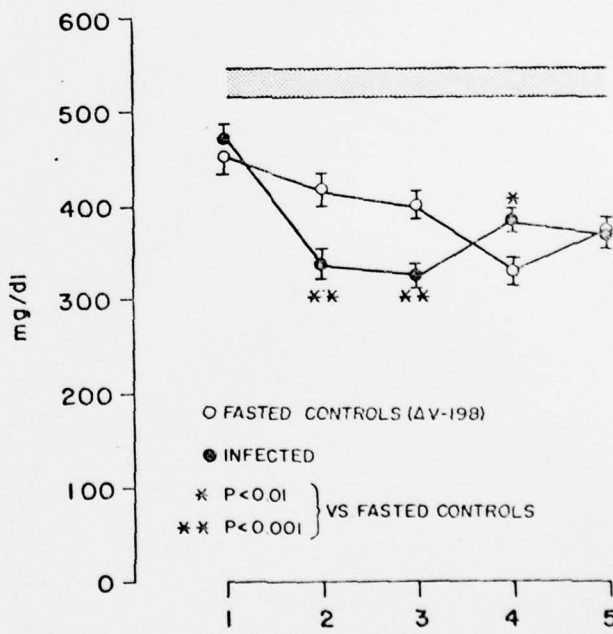
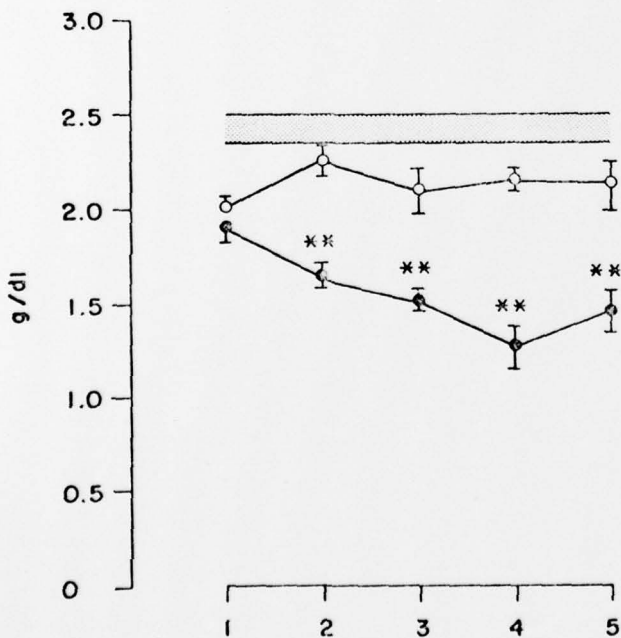
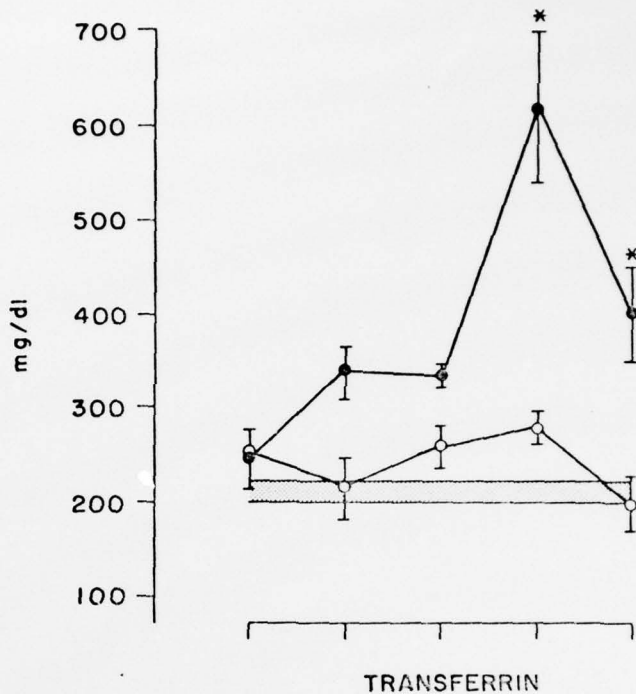
○ FASTED CONTROLS (ΔV-198)
 ● INFECTED
 * P < 0.01
 ** P < 0.001 } VS. FASTED CONTROLS

DAYS POSTEXPOSURE

[¹⁴C] AIB UPTAKE BY LIVER



SEROMUCOID



DAYS POSTEXPOSURE

○ FASTED CONTROLS (ΔV-198)
 ● INFECTED
 * P < 0.01
 ** P < 0.001 } VS FASTED CONTROLS

