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THE EFFECT OF HYPERTHERMIA
ON PROTEIN TURNOVER IN INFECTION

IRVING GRAY, COLONEL, MSC
and PAUL V. HILDEBRANDT, CAPTAIN, VC
U. S. ARMY MEDICAL UNIT, FORT DETRICK, MARYLAND

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The general interrelationship between infection and nutrition has been well established and recent reviews have summarized our present knowledge (1-4). The effect of infection on nitrogen metabolism has been investigated (5,6) and has been shown (7) to cause a shift toward negative nitrogen balance in virus infections (8). However, despite the fact that fever normally accompanies infections, little has been done to determine the relative contribution of the hyperthermia and the microorganism to the altered protein metabolism. The hypermetabolic state of individuals during febrile periods has long been recognized (9). It has been shown that for each °F rise in body temperature, the metabolic rate increases about 7% above basal (9). In addition, there are marked changes in electrolyte and carbohydrate metabolism. Protein metabolism is likewise affected primarily toward an increased breakdown of body protein (10,11). In our laboratories preliminary experiments have shown that rabbits infected with Pasteurella tularensis have an increased uptake of methionine-S-³⁵ during the early course of this febrile disease (12). Furthermore, we have observed and reported a marked increase in protein synthesis in a cell-free system derived from the brains of mice infected with the virus of Venezuelan equine encephalomyelitis (VEE) (13). In the studies to be reported here, we have tried to delineate the contribution of the hyperthermia and of the microorganism to the observed changes. To this end, we have studied protein turnover during controlled fever alone as well as during virus infection plus controlled fever.

MATERIALS AND METHODS

Three young chimpanzees, weighing approximately 17 kilograms, were used. Food was withheld for 24 hours prior to anesthesia. Each chimpanzee was placed under light surgical anesthesia with pentobarbital sodium. A superficial vein of an arm or leg was catheterized with polyethylene tubing for the administration of

fluids, anesthetic agents, or the taking of blood specimens. A catheter was inserted to collect urine samples and measure urine output.

When the animal had been prepared, it was placed between plastic mats through which thermostatically controlled water circulated. A rectal thermister attached to the water-regulating apparatus (Gorman-Rupp Co. K-Thermia) automatically controlled the temperature. The temperature of the chimpanzee was held at 98-99°F as control; 105-106°F as hyperthermic. The experimental period lasted 24 hours from the time the temperature of the animal reached and stabilized at the selected temperature. Normal physiological saline in 5% glucose was administered as a slow drip via the intravenous catheter. Approximately 800 ml were administered during periods of euthermia and 1,000 ml during periods of hyperthermia.

Following zero time blood and urine samples, 14 mg of L-methionine- S^{35} (Me- S^{35}), containing 500 μ c, dissolved in 2 ml isotonic NaCl were injected intravenously into the femoral vein opposite the catheterized limb. Blood samples (5 ml) were collected in vials containing potassium oxalate at 1, 2, 4, 8, 16 and 24 hours after the administration of the methionine. Each time a blood sample was drawn, the urine volume excreted subsequent to the previous sample was noted and a portion saved for radioactivity assay and routine analysis.

The plasma was removed from the blood and 1 ml was fractionated into globulin and albumin fractions by the method of Pillimer-Hutchison (14). The soluble albumin was precipitated with trichloroacetic acid (TCA). Each fraction was washed with 5% TCA and dissolved in 1 ml of 0.5N NaOH. 100 μ l of each fraction were analyzed for nitrogen content by the Biuret method (15).

All planchets were counted in a gas flow counter to an error of less than 3%. The appropriate standard of the injected Me- S^{35} was counted and data reported as a fraction of the injected dose.

Protein turnover was followed under 3 different conditions in each animal. The first was at 98-99°F (control). The second was identical to the first except that the rectal temperature was raised to 106°F (fever). The third was identical to the second except the chimpanzee had been inoculated with 10^8 MIPLD₅₀'s (mouse intraperitoneal lethal dose 50) of VEE virus 24 hours prior to protein turnover studies. In chimpanzees, the virus of VEE produced its initial febrile response approximately 12 to 24 hours post inoculation. An additional 1 ml of blood was drawn at each sampling time for viremia determinations.

RESULTS

In the infected animals, the concentration of virus in the blood was $10^{3.75}$ MIPLD₅₀'s per ml when protein turnover studies were initiated. Baseline serology indicated that none of the chimpanzees had had previous experience with this virus, and in the infected animals, serology following the experiment indicated hemagglutination inhibition (HI) and complement fixation (CF) titers as summarized in Table I.

CHIMP NO.	HI	CF
1	5,120	128
2	≤10,240	512
3	≤10,210	256

TABLE I. Hemagglutination Inhibition (HI) and Complement Fixation (CF) Titers in the Serum of Chimpanzees Challenged with VEE.

In Figures 1a, b, c we see the marked effect on protein turnover resulting from fever. The decrease in the uptake of the labeled methionine into the plasma globulin is quite definite in two of the chimpanzees (Sam, Denise) but still apparent in the third (Ralph). No data are reported concerning the albumin fraction inasmuch as there was no observable change under the conditions of the experiment. However, when the same degree of hyperthermia is maintained in the presence of the virus, there is an obvious increase in protein turnover. In all 3 experimental situations, control, hyperthermic, hyperthermic with virus, the significant uptake of the Me-S³⁵ has occurred in the first 4 hours following the administration of the tracer. If we plot the ratio of each of the experimental curves to its own control from the 4-hour point on, we obtain the curves in Figure 2. It appears that in each of these experimental situations, the rate of loss of the S³⁵, the measure of the rate of protein breakdown, is the same but increased over the control rate. The increased rate of breakdown in the presence of a virus infection or fever could, if continued, contribute toward an increased nitrogen excretion. That virus infection does cause increased nitrogen excretion has recently been clearly demonstrated (7,8). This effect, related specifically to amino acids in bacterial infections, has been demonstrated in these laboratories (16). Furthermore, fever itself has been shown to

have a similar effect on the breakdown of protein and nitrogen excretion (10,11). Thus, it is not unreasonable to conclude that a principal cause of increased nitrogen loss during acute infectious disease results from the fever rather than the microorganism.

Finally, if we consider that the breakdown rate of the globulin during fever alone is the same as fever with virus, then the increased uptake of the Me-S³⁵ in the presence of the virus must be due to an increased synthetic or uptake rate resulting from the direct effect of the microorganism. Our recent report (13) demonstrating the stimulation of protein synthesis by microsomes obtained from animals infected with VEE lends further support to this latter conclusion.

SUMMARY

Protein metabolism has been followed in control, hyperthermia and hyperthermia plus virus.

Breakdown is increased in both experimental groups over that in the control group. It is concluded that this increase, caused primarily by the fever, contributed to the previously observed increased nitrogen excretion.

Evidence is presented to support the concept that protein synthesis is stimulated by the presence of virus at a time simultaneous with that of the observed protein breakdown.

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GRAY and HILDEBRANDT

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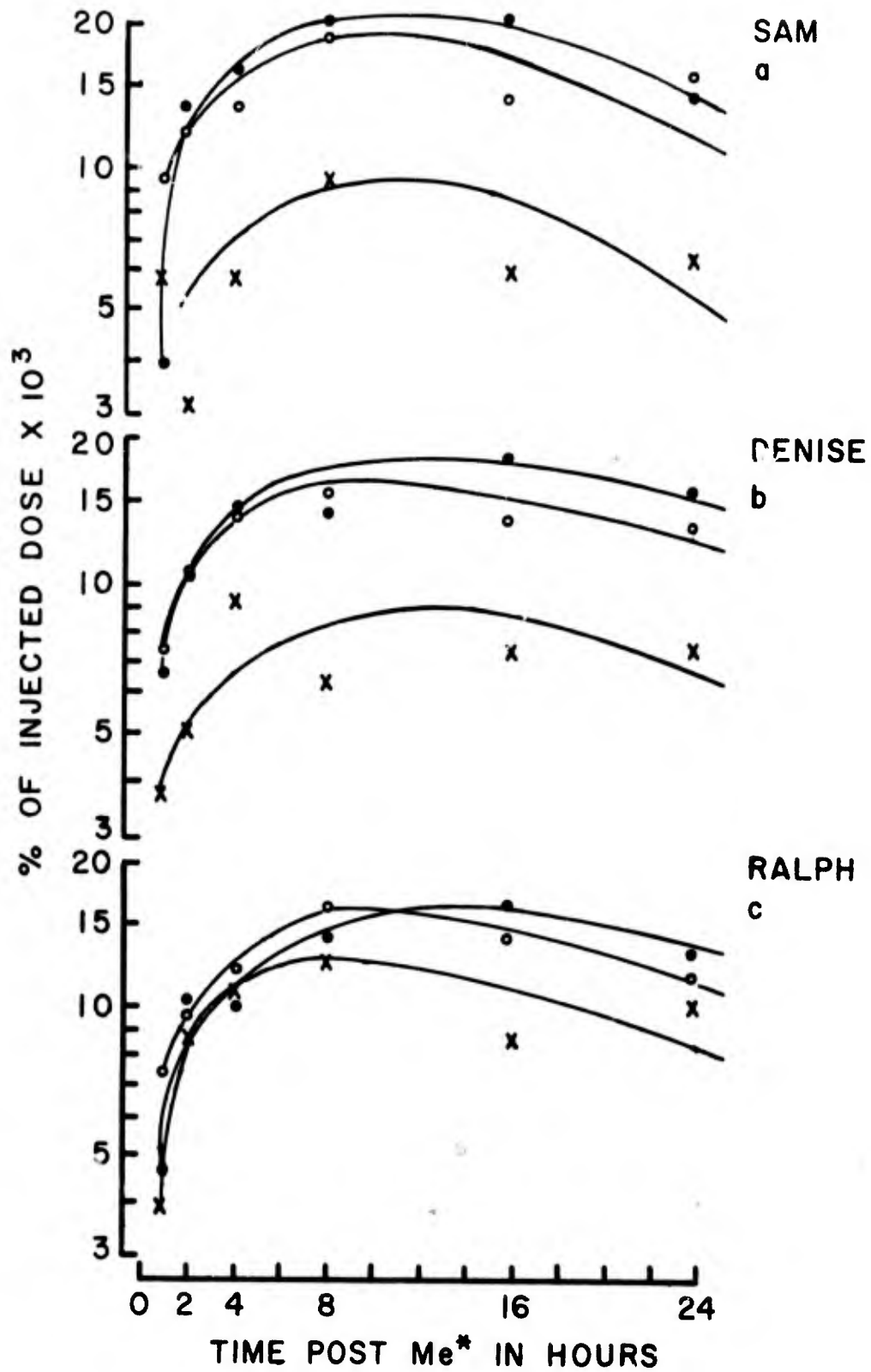


Figure 1: Plasma Globulin Turnover in Hyperthermic and Infected Chimpanzees.
 ● - Control, X = Hyperthermia, ○ = Hyperthermia + Virus

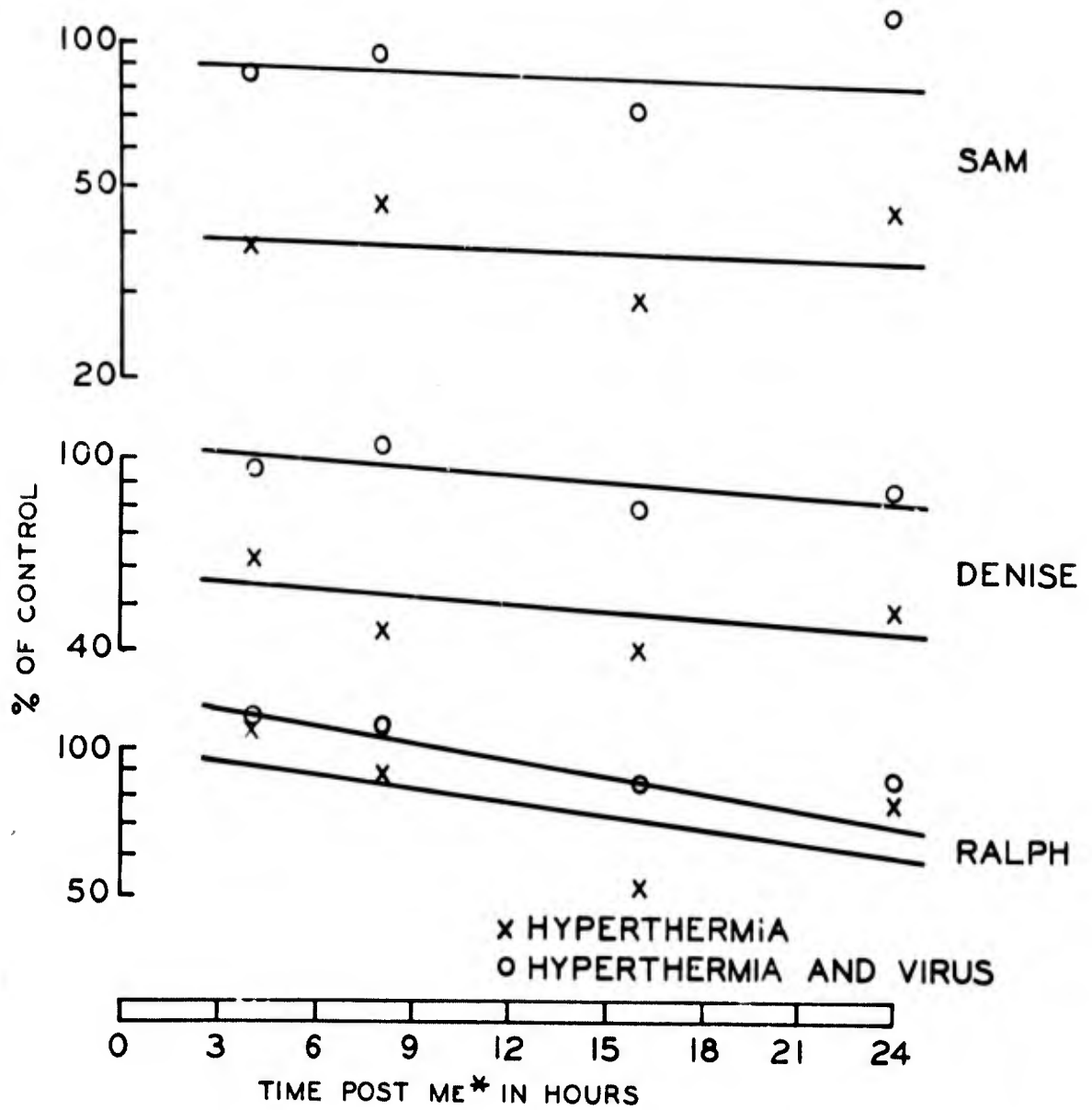


Figure 2: Plasma Globulin Breakdown in Hyperthermic and Infected Chimpanzees.