TABLE III. Mortality Data for Non-splenectomized Bartonella-Free Rats (8/Group) Injected with Whole Blood or Fractions from X-Irradiated Bartonella Carrier Rats.

Exp. III

Material inj. (.2 cc)	Previous treatment	No. deaths on days post inj. 1	2	3	4	5	6	7	8	9
Whole blood	X-irrad.	1				1				
Red cells	"							1		
Plasma	"		2			1				
Whole blood	None									
Red cells	"	No deaths in any group								
Plasma	"									

protective effect of an intact spleen. The results of the previous experiments, that X-irradiation activated the Bartonella organism to give a more lethal infection, were confirmed.

Discussion. The existence of *Bartonella muris* infections in rodents is well established (3). It has also been established(1,2) that X-irradiation activates this infection. Treatment with antibiotics and hematological responses have been reported(4) but actual transmission of the disease by inoculation is presented here. The results indicate the protective role of the spleen in controlling the disease, since activated plasma was not rapidly lethal in non-splenectomized animals as was observed in the splenectomized group. The results also suggest that the Bartonella infective response may be obtained with equal efficiency whether plasma or washed-cells from irradiated infected animals are used as the transmision medium. Since the spleen appears to be essential in controlling the effects of this infection, it is possible to suggest a dual role for this organ as a radiosensitive tissue: first, as a hematopoietic organ; secondly, as an organ exerting a controlling effect on latent Bartonella infection.

Summary. The experimental results confirm previous reports that X-irradiation activates latent bartonellosis in rats to produce a fulminating infection. It was also found that whole blood, red blood cells or plasma from infected irradiated rats were equally lethal when injected into *Bartonella muris*-free rats. The lethality was much less when the recipient rats had not been splenectomized.

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Effect of Caffeine on Spinal Reflexes.* (27176)

G. SANT'AMBROGIO,† D. T. FRAZIER AND L. L. BOYARSKY
(Introduced by L. D. Carlson)
Department of Physiology, University of Kentucky Medical Center, Lexington

There have been few studies of the effect of caffeine on the mammalian spinal cord. Wood(1) found an increase in the patellar reflex in man following caffeine ingestion, which lasted for several hours. Gualtierotti *et al.*(2) observed an increase in the patellar response and a decrease in latency in man. Danilov(3) reported a decrease of tendon jerks in spinal and intact dogs and an increase in flexor responses. The present study is an attempt to examine the effect of caffeine on monosynaptic and polysynaptic reflexes of decapitate cats using electrophysiological methods. The amplitude of the responses, the input-output relation of the polysynap-

* Assisted by grant from Nat. Inst. Health.

† Present address: Istituto di Fisiol. Umana, Univ. of Milano, Italy.

tic reflex and inhibition of the reflexes were investigated.

Methods. Thirty-nine cats with spinal cord transected at C_1 were used as subjects. The S_1 ventral root was isolated and cut peripherally. The spinal cord was covered with a pool of warm mineral oil. Oil-pool and rectal temperatures were maintained between 37° and 38° C throughout the experiment with an infra-red lamp. The monosynaptic reflex was obtained by stimulating the biceps-semitendinosus nerves with a stimulus maximal for group I fibres. The pure polysynaptic reflex was obtained by stimulating the sural nerve with a stimulus maximal for group II fibres. Reflex responses were recorded from the S_1 ventral root. In the case of the polysynaptic reflex, the afferent volley was also monitored with electrodes on the sural nerve.

The input and output volleys of the polysynaptic reflex were photographed simultaneously on a Tektronix 502 double-beam oscilloscope. Photographs of the output were traced from a projection and the cutouts of the tracings were weighed. These areas were taken as measures of output of the reflex response. The height of the action potential of the afferent volley was used as the measure of input. The monosynaptic reflex was inhibited by stimulating the nerve to the ipsilateral quadriceps muscle at appropriate intervals before delivering a shock to the semitendinosus nerve. The polysynaptic reflex was inhibited by stimulating the contralateral sural nerve at intervals of 0-200 msec. before stimulating the ipsilateral sural nerve.

Caffeine (U.S.P., alkaloid) was administered intravenously in isotonic saline. Stimuli were square waves 0.1 msec. in duration delivered at 0.3/sec. from a Grass S-4 stimulator and isolation unit. Responses were amplified with a Grass P-4 preamplifier coupled to a Tektronix 502 oscilloscope. A Grass camera was used to photograph the responses.

Results. Monosynaptic reflex. The monosynaptic reflex was studied in 14 animals. Caffeine has only a slight effect on the amplitude of the monosynaptic response. Fig. 1 (A_1 and A_2) shows the monosynaptic reflex

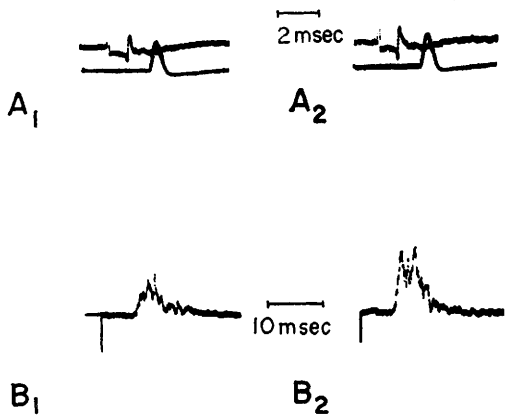

FIG. 1. Effect of caffeine on monosynaptic and polysynaptic reflexes. Upper traces in A_1 and A_2 are afferent action potentials. Lower figures are monosynaptic response. A_1, before caffeine; A_2, 15 min. following inj. of 20 mg/kg caffeine; B_1, polysynaptic reflex before caffeine; B_2, polysynaptic response 15 min. after inj. of 20 mg/kg caffeine. Excitatory volley for monosynaptic reflex was to biceps-semitendinosus and for polysynaptic reflex was to sural nerve.

response to a dose of 20 mg/kg caffeine. No changes in amplitude, reflex latency, conduction velocity or duration of response were observed. Up to doses of 20 mg/kg there was no perceptible effect on the amplitude of the monosynaptic reflex. At higher doses, an increase in response usually occurred but the effect was highly variable. Fig. 2 (filled circles) shows the responses to a variety of doses of caffeine 15 minutes after administration.

Caffeine had no effect on the time course of direct inhibition. Fig. 3 shows that caffeine at levels up to 64 mg/kg did not affect the direct inhibition of the monosynaptic reflex. As a control on whether the system was capable of being inhibited, strychnine (0.16 mg/kg) was injected following the caffeine trials. A prompt disappearance of inhibition took place.

Polysynaptic reflexes. Fourteen cats were used in the study of the polysynaptic reflexes. Following doses of caffeine at relatively low levels (5-10 mg/kg), the amplitude of the polysynaptic reflex increased. Fig. 1 (B_1 and B_2) shows the effect of caffeine at a level of 20 mg/kg on the pure polysynaptic reflex. An increase in amplitude of the response, with no change in its latency or duration has occurred. Fig. 2 (open circles) summarizes data obtained in this group of ani-

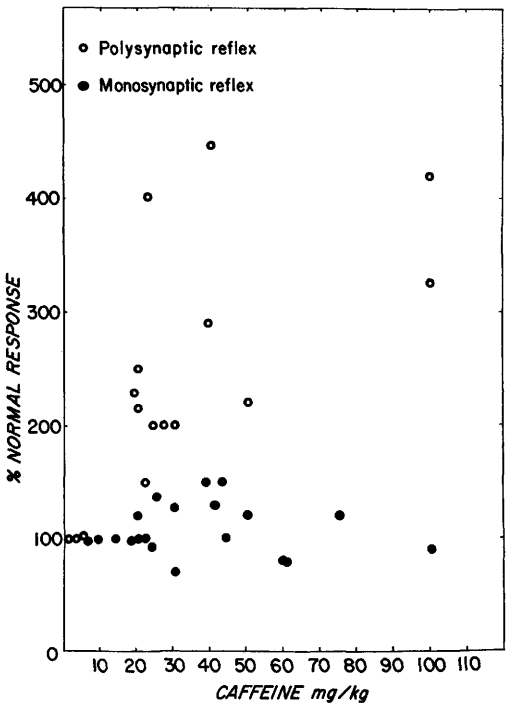

FIG. 2. Dose-response relation of monosynaptic (●) and polysynaptic (○) reflexes. Responses obtained 15 min. after caffeine inj.

mals. At doses of 20 mg/kg there is a 2-3-fold increase in amplitude of response: at 40 mg/kg the response has increased 3-4-fold. The effect of caffeine persisted for 3-6 hours.

In 3 cats the input-output relationship of the polysynaptic reflex was studied as a function of dose of caffeine. Fig. 4 shows the typical result. The linear relation between input and output is characteristic of the polysynaptic reflex(4). In these experiments, the threshold response was reached at intensities which were 20% of maximum input. A slight change in minimum threshold of the input occurred at the highest dose used (40.5 mg/kg). Below this dose level there is no apparent change in threshold but an increase in amplitude of response at a given threshold. In 3 other cats inhibition of the polysynaptic reflex was studied as a function of caffeine level. Caffeine up to doses of 60 mg/kg had no effect on the course of indirect inhibition. Strychnine in these animals, likewise, had no effect on the course of indirect inhibition (at a dose of 0.2 mg/kg).

Discussion. The principal finding of these experiments is that caffeine increases the amplitude of the polysynaptic reflex but has little or no effect on the monosynaptic reflex. Similarly, strychnine has no effect on monosynaptic reflex amplitude but increases the polysynaptic responses in the spinal cord (5). Caffeine is also reported to have no effect on monosynaptic reflexes of the spinal cord following close intraarterial injection (6). The slight increase in monosynaptic response reported here may be a consequence of the peripheral vasodilitation reported to occur following high doses of caffeine(7).

The explanation of the increased polysynaptic response to caffeine differs from that proposed to explain the action of strychnine in the spinal cord. Strychnine abolishes direct inhibition so that its effects on the polysynaptic responses have been ascribed to a depression of inhibition rather than an increased facilitation(5). Our experiments show that the effects of caffeine cannot be explained by depression of inhibition leading to increased activity since inhibition is unaffected by caffeine even at very high doses.

The effects of caffeine may therefore be explained as a result of increased responsiveness of neurones. From the effect of caffeine on the input-output curves, it is clear that neurones are sensitive at all levels of input and that the increased response of the polysynaptic reflex does not result from a synchronization of neurones so that neurones re-

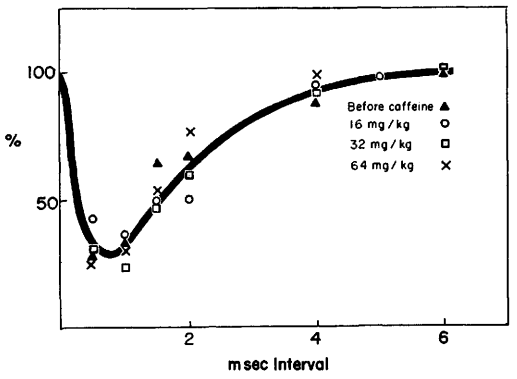

FIG. 3. Effect of caffeine on curve of direct inhibition. Ordinate: monosynaptic response as % of uninhibited response. Abscissa: interval between inhibiting and test shock. Inhibiting volley from quadriceps nerve; excitatory volley was from biceps-semitendinosus.

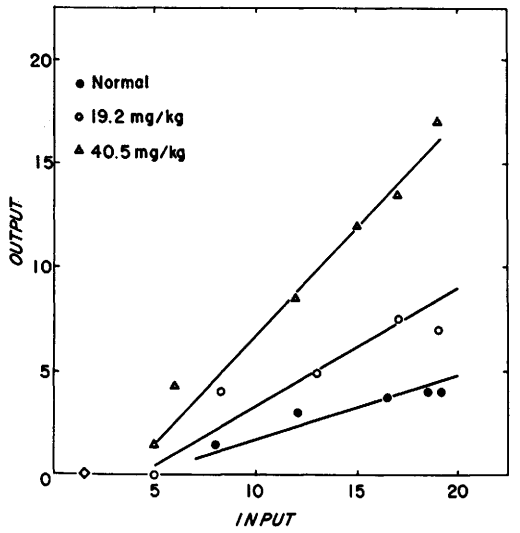

FIG. 4. Input-output relation of polysynaptic reflex as a function of dose of caffeine. Input is height of action potential of sural volley. Output is area of polysynaptic response from ventral root S_1. Initial outputs of all curves begin between the diamond symbol and input level 5.

spond only to low levels of input. Whether the increased responsiveness is a result of pre- or post-synaptic events cannot be ascertained from our experiments. That presynaptic fibres are probably not involved is implied by experiments demonstrating that dorsal root potential is unaffected by caffeine at doses up to 40 mg/kg. Above this dose the dorsal root potential is diminished rather than increased (unpublished results). Since the dorsal root potential is probably a sign of the excitability of pre-synaptic terminals, its diminution indicates a depression of presynaptic activity by caffeine(8).

An increase in the patellar reflex of the human after caffeine was obtained at doses of 2-5 mg/kg(1,2). In the spinal animal no effects can be obtained with such doses. The effects on the patellar reflex in humans, therefore, are to be attributed to alterations in cortical or other higher influences playing upon the interneuronal reflex pool of the spinal cord rather than to a direct effect upon the spinal cord.

Summary. The effect of caffeine on spinal reflexes has been studied by electrophysiological methods. The monosynaptic reflex evoked by stimulating the biceps-semitendinosus nerves does not show consistent change with doses of caffeine up to 100 mg/kg. The time course of direct inhibition of the monosynaptic reflex is not affected by caffeine. The polysynaptic reflex evoked by stimulation of the sural nerve shows a marked increase. The minimum effective dose is approximately 5 mg/kg. The input-output relationship of the polysynaptic reflex after caffeine suggests that the effect of caffeine is to recruit neurones at all levels of input rather than to shift all neurones toward the same threshold.

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