experiment. Temporary ischemia of the spinal cord, for example, might have led to loss of interneurons, decrease in net inhibition, and resultant sustained discharge of motoneurons including gamma units(8). However, in our chronic animals, ischemic rigidity was avoided, since arteries entering along ventral roots were undisturbed, those along dorsal roots were spared and, in some preparations, compensatory adjustments in circulation were favored by making the transection and deafferentation at different times. Lesser effects due to relative ischemia of long duration, or to sensitization of denervated neurons are excluded in animals prepared acutely.

The flurry of injury discharge which follows procedure of isolating a ventral root filament may dwindle to a slow discharge that must be distinguished from spontaneous activity, particularly since myelinated axons of small size are more prone to demonstrate injury potentials(9). That units were not accepted as truly spontaneous unless firing was sustained for over 5 minutes, and that some were followed for periods to an hour is reassuring. Moreover, injury potentials in ventral roots due to handling were eliminated as a factor in experiments in which afferent units were monitored. Triggering of cord activity from hyperexcitable tissue lying adjacent to cord transection is a further possibility.

The volume of discharge heard in ventral rootlets of these preparations is not as great as the similarly pure fusimotor activity in roots leading from deafferented levels of the cord in decerebrate cats. This and the observation that silent units of probable fusimotor nature can be aroused to activity with stimulation of the isolated cord segment suggests that only a minor portion of available gamma units is spontaneously active.

Summary. Presence of spiking discharge in fibers of ventral roots leading from lumbosacral cord completely deafferented and transected at the L_1 level is described. This activity was noted in adult cats either chronically or acutely prepared. On the bases of the small relative height of these spikes and the demonstration in afferent discharge from muscle spindles of tonic fusimotor influence, it is concluded that ventral root activity arises in gamma efferent fibers. Probably only a minor portion of gamma fibers present is participating.

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Received November 16, 1959. P.S.E.B.M., 1960, v103.

Experimental Shigella Infections. III. Sensitivity of Normal, Starved and Carbon Tetrachloride Treated Guinea Pigs to Endotoxin. (25541)

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Hartley strain guinea pigs may be rendered susceptible to a fatal enteric infection with a strain of *Shigella flexneri* 2a if they are either deprived of food for 4 days prior to challenge or injected subcutaneously with carbon tetrachloride 24 to 48 hours before oral administration of viable bacteria(1,2). The mechanism of this increased susceptibility is not clear.

Certainly some alteration of the animal must occur to allow the dysentery bacilli to multiply. In addition, it is conceivable that death results because pretreatment also might render the animal more susceptible to toxic products elaborated by the multiplying dysentery bacilli.

One toxic product of the dysentery bacillus is its endotoxin or somatic antigen. This communication presents results of experiments comparing susceptibility of normal, starved and carbon tetrachloride treated guinea pigs to intravenously injected endotoxin, and attempts to relate differences in susceptibility to *in vivo* distribution of endotoxin.

Material and methods. Hartley or Walter Reed strain guinea pigs weighing 300 to 400 g were used in all experiments. Those which were starved were deprived of food for 4 days before intravenous administration of endotoxin. Carbon tetrachloride treatment consisted of injecting 0.15 ml of this material subcutaneously usually 24 hours before endotoxin was injected intravenously. Animals were observed for 96 hours, and the LD_{50} calculated by the method of Miller and Tainter (3).

Endotoxin of the Boivin type was prepared from *S. flexneri* 2a strain 2457 which was used in our previous work(1,2). The toxin was extracted from acetone treated cells with trichloroacetic acid and further purified by ethyl alcohol-salt (NaCl) fractionation(4). Extracted material was dissolved in physiological saline to a final concentration of 2.64 mg/ml. This solution contained 0.05 mg N_2/ml. In some experiments Cr^{51} labeled endotoxin was employed(5) and distribution of Cr^{51} in organs of animals determined by procedures previously described(6).

Results. Pooled results of experiments comparing susceptibility of normal, starved and carbon tetrachloride treated guinea pigs to intravenously administered *S. flexneri* 2a endotoxin are summarized in Table I. Normal guinea pigs were quite resistant, LD_{50} being in excess of 1320 μg of endotoxin. In contrast the sensitivity of animals was greatly increased following either starvation or pretreatment with CCl_4. Normal and starved animals succumbing to endotoxin usually did so 24 to 48 hours following administration, whereas carbon tetrachloride treated guinea pigs succumbing to endotoxin usually expired 6 hours to 12 hours following injection.

TABLE I. Mortality Following Intravenous Administration of Bacterial Endotoxin to Normal, Starved and Carbon Tetrachloride Treated Hartley Strain Guinea Pigs.*

Endotoxin dose (μg)	Treatment Normal†	CCl_4	Starved
1320	10/25		5/ 5
264	3/25	8/ 8	6/10
52.8	0/25	34/39	3/10
10.6		13/23	2/10
2.1		13/30	
.4		0/15	
LD_{50}	2500 μg	4.0 μg	52.8 μg
S.E.	1766 μg	3.5 μg	26.4 μg

* Starved animals were deprived of food 4 days prior to administration of the endotoxin; CCl_4 treated animals received 0.15 ml of this drug subcut. 30 hr prior to administration of endotoxin.
† Deaths/Total.

An experiment was next conducted to determine the effect of time of administration of carbon tetrachloride on susceptibility of guinea pigs. Animals of the Walter Reed strain were employed, and at the time this experiment was carried out, carbon tetrachloride treated animals of this strain were more sensitive to endotoxin than the Hartley strain animal. Thus a dose of approximately 10 μg sufficed to kill virtually all treated animals. Results of this study show that pretreatment with carbon tetrachloride was effective in decreasing resistance to endotoxin when administered 24, 48 or 72 hours before the toxin (100, 100 and 70% respectively of the animals in these groups died), but had little effect when given at the same time or 1 hour or 4 hours before endotoxin challenge (0, 10 and 10% respectively of the animals in these groups died).

Since carbon tetrachloride treated guinea pigs are more susceptible to endotoxin than are normal animals, experiments were conducted to determine if differences in distribution of endotoxin exist in treated as compared to normal animals. Preliminary experiments in normal animals indicated that most Cr^{51} bound to endotoxin was in the liver and remained there for long periods while free Cr^{51}

Cl_3 was more generally distributed and appeared early in the urine in considerable amounts. A dose of 52.8 μg of Cr^{51} labeled endotoxin was employed because normal animals survive this level while a large number of pretreated guinea pigs do not. Carbon tetrachloride treated and normal animals were paired prior to injection with endotoxin. When carbon tetrachloride treated animals expired their counterparts were sacrificed and distribution of the chromium label in their organs was determined. The results show that no apparent differences in distribution of the chromium label in the liver, spleen or lung were observed in the normal group which survived or treated animals which succumbed. The mean endotoxin content of each of these organs was 6.4, 24.3 and 3.0 μg respectively. Radioactivity of brain, cecum, large intestine, small intestine, kidneys and blood was very small and well within counting error. Additional experiments indicated that no differences in distribution of labeled endotoxin could be detected one hour after its intravenous administration to normal or carbon tetrachloride treated guinea pigs. In addition rate of clearance of the endotoxin from the blood of the 2 types of animals did not differ. The mean endotoxin content found in blood 1, 2, 3 and 5 hours following the intravenous inoculation of 200 μg was 6.2, 5.6, 4.2 and 3.8 μg respectively.

Discussion. Our results show that carbon tetrachloride treated or starved guinea pigs are more susceptible to intravenously injected endotoxin than are normal animals. Liver damage is the major change caused by these treatments. Starvation brings about a fatty metamorphosis while injection of carbon tetrachloride causes a central lobular necrosis in addition to fatty metamorphosis(1,2).

The short period of time required for the carbon tetrachloride treated animals to succumb is reminiscent of the results of McLean and Weil(7) who found that dogs might die in 2 hours following intravenous injection of endotoxin if the liver was bypassed by an Eck's fistula. The possibility must be considered that the carbon tetrachloride sufficiently damaged the liver to render it ineffective thus producing, as far as its function with respect to endotoxin is concerned, what amounts to an Eck's fistula. That the damaged liver trapped as much Cr^{51} as the normal liver might be used as a legitimate argument against this explanation. However, if we assume that the Cr^{51} label represents endotoxin—and we agree that this can be only an asumption (Braude *et al.*(5))—we could postulate that while the damaged liver could remove the endotoxin from the blood stream, it could no longer render it pharmacologically inactive.

It is not known what role this increased susceptibility to endotoxin has in rendering starved or carbon tetrachloride treated guinea pigs susceptible to a fatal enteric infection with *Shigella flexneri.* We have not been able to demonstrate this material in the blood stream or organs of animals experimentally infected with this organism, but it may be that the available technics are not sufficiently sensitive to detect it. Thus at present we may only speculate that decrease in resistance to this toxic product may be a factor which brings about death of the infected guinea pig.

Summary. Pretreatment of Hartley strain guinea pigs either by subcutaneous injection with carbon tetrachloride or by 4 day period of starvation renders them more susceptible to intravenously administered endotoxin. The LD_{50} for normal animals is in excess of 1300 μg; for starved animals 52.8 μg and for carbon tetrachloride treated animals 4.2 μg. Distribution of Cr^{51} label of Cr^{51} tagged endotoxin in organs of normal animals which survive intravenous dose of 52 μg of endotoxin is the same as that in carbon tetrachloride treated animals which succumb following this challenge.

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Received November 20, 1959. P.S.E.B.M., 1960, v103.

Radiofluoride Distribution in Tissues of Normal and Nephrectomized Rats.* (25542)

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The beneficial effect of the fluoride ion upon incidence of dental caries has stimulated interest in the metabolism of fluoride both in calcified and in non-calcified tissues. The kidney has been of particular interest because it is the major route of excretion of absorbed fluoride. The amount of fluoride in non-calcified tissues has been difficult to evaluate analytically because it is found in minute quantities (micromoles/kg). However, development of the crystal scintillation counter has allowed application of radiotracer technics to certain facets of soft tissue fluoride metabolism. The most useful radioisotope of fluoride is F^{18} which is a positron emitter with a half-life of 109.7 minutes(1).

Procedure. Each of 4 male rats (A-D) was given a 1 ml intraperitoneal injection of radiofluoride in a saline solution containing 0.2 ppm stable fluoride. Two other animals (E,F) were each given a 2 ml injection of the solution. Three of the animals (A,C,E), were heminephrectomized 10 days before the experiment, and the remaining kidney was removed on the morning of the experiment. Each animal was sacrificed 80 minutes after injection. The soft tissues, digested to solution in 5 N NaOH on a steam bath, were diluted to a 25 ml volume. The humeri were dissolved by shaking them in dilute HCl. Radiofluoride contents of the tissues were determined by using a well-type crystal scintillation counter to measure radioactivity in a 10 ml aliquot of the dissolved tissue. These measurements were made over 8-minute periods and were corrected for background count, volume and radioactive decay to one point in time. Chloride contents were determined by the wet ash procedure described by Caster, MacDonald and Armstrong(2). Water contents of the fresh tissues were evaluated by loss in weight on drying at 100°C.

Results. Table I presents in 3 ways the mean radiofluoride concentrations found in the soft tissues of normal and nephrectomized animals. These data (Columns II and III) show an elevation of the absolute radiofluoride concentration in the tissues of the animals deprived of kidney function. However, no marked change in the pattern of soft tissue distribution of fluoride occurred as a result of nephrectomy. This fact is best seen from Table II in which radiofluoride concentration in the water of the soft tissues is compared with that of the plasma water. This ratio of fluoride concentration does not appear to differ in normal and nephrectomized animals. The tissues can be divided (Table II) into 4 groups: 1) Brain tissue had lowest relative radiofluoride concentration. 2) Tail tendon was the only soft tissue which had a radiofluoride concentration greater than plasma water. 3) Heart muscle and skeletal muscle had a radiofluoride concentration close to one-half the concentration present in plasma water. 4) Skin, liver and testicular tissue showed a range of concentrations, when compared to plasma water concentrations, with a high of 0.90 for skin tissue water and a low of 0.68 for liver tissue water.

Table I also gives tissue concentrations of radiofluoride in counts per microequivalent

* This study supported by grant from U.S.P.H.S. The authors thank the Linac Staff of Physics Dept. for help in preparation of the radiofluoride.

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