performance of the stock colony during the year before use of chlortetracycline and the year following. The number of females indicated includes all animals that were in the colony during the year regardless of the period of time. The number in period I was greater because of higher mortality and hence a larger number of replacements. During period I, about 13% of the animals were affected with cervical lymphadenitis‡ or lumpy jaw, but there was only 1 case after the use of chlortetracycline. Since that time there have been no cases in the colony. The percentage of abortions was markedly lower in period II. The litter size, birth weight and weaning percentage were slightly, but perhaps not significantly, higher during period II. At the present time a large proportion of our experimental animals are among the fourth generation since chlortetracycline has been routinely used in the colony diet.

In view of the observations of Roine *et al.* (1), one might suspect that the successful use of dietary chlortetracycline may be due to the absence of certain types of bacteria in the intestinal flora of our animals. Listeria was thought to be the causative agent in the Finland colony. In view of widespread occurrence of *Listeria monocytogenes,* it seems unlikely that our colony is free of this organism. In fact, in at least one case it has been cultured from an animal during a routine pathological check.

Summary. Chlortetracycline hydrochloride has been included in various diets of a guinea pig colony for 3 years without evidence of toxicity. It had no effect on growth rate and caused no mortality when added to a purified diet at levels from 25 to 200 mg/kg. At the routine level of 25 mg/kg, the antibiotic decreased abortions and adult mortality and eliminated cervical lymphadenitis from the breeding colony. Although chlortetracycline may be toxic to guinea pigs under some conditions, in our laboratory it has been beneficial in the maintenance of a breeding colony and has been used routinely in purified experimental diets for growth studies.

‡ The causative organism, which belonged to the genus Streptococcus, was isolated and identified by Dr. L. D. Kintner, Veterinary Pathology Department.

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Accumulation of Calcium^{45} by Salivary Glands. (23538)

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Many investigators have compared Ca^{45} exchange in various tissues(1,2,3), but none appear to have included the salivary glands in their studies. Chemical determinations of calcium concentration in the salivary glands of cats has revealed that the concentration was approximately twice that of plasma(4). In dogs, no difference was apparent(4). Histochemical determination of calcium in mouse tissues did not reveal a greater concentration in salivary glands than in other tissues(5). The present report compares the changes in specific activity with time after administration of calcium^{45} in the submaxillary gland, kidney, and blood of female rats. Total calcium in submaxillary glands, kidneys, and blood of rats is also determined.

Methods. Calcium^{45} chloride* in a dose of 10 μc was given to ether-anesthetized 6-month-old virgin female Long-Evans rats by injection into a foot vein. At intervals from

* Calcium^{45} was purchased from Union Carbide Nuclear Co., Oak Ridge, Tenn. under license number 4-918-1. The specific activity was 92 Mc/g.

1 minute to 48 hours animals were etherized and organs removed, blotted, and weighed in tared 10 x 75 mm boro-silicate glass tubes. Blood was also collected and weighed in the same way. Samples were wet-ashed at temperatures below 120°C with fuming nitric acid, perchloric acid, and 30% hydrogen peroxide. Total calcium was analyzed by flame photometry using a Beckman DU, oxy-acetylene burner, and a specially built high-sensitivity, stable, photomultiplier recording attachment.† Emission interference was estimated by the addition of a known amount of calcium to the unknown and repeating the analysis(6). Exhaust gases from the flame were passed by suction through a double thickness of Fiberglas No. PF105 to remove Ca^{45}.‡ Radioactivity of the calcium present in each sample was determined by oxalate precipitation of an aliquot after addition of sufficient carrier calcium to assure complete recovery. The precipitate was spread on copper planchets and counted with an end-window GM tube. Samples were counted in the infinitely thin region (less than 1 mg/cm^2) as shown by recoveries of added Ca^{45}. Initially, samples were taken of liver, kidney, submaxillary gland, parotid gland, pancreas, ovary, and blood, but for comparison, only blood, kidney, and submaxillary gland will be discussed. Specific activities in liver, pancreas, and ovary were similar to kidney while parotid gland was similar to submaxillary gland.

Results. 1) Calcium concentration in rat organs: In 22 normal female Long-Evans rats, the mean and standard errors of stable calcium concentrations were as follows: Blood 3.66 ± .14 meq/kg; kidney 4.0 ± 0.1 meq/kg; submaxillary gland 21.6 ± 1.0 meq/kg.

2) Exchange of Ca^{45} in rat organs: Table I details the changes in specific activity in kidney, blood, and submaxillary gland at various times after intravenous administration of 10 μc $Ca^{45}Cl_2$. Differences in exchange by kidney and submaxillary glands are obvious at 5 minutes and at 8 hours. The rapid fall in blood specific activity represents exchange of calcium^{45} with bone calcium.

TABLE I. Specific Activity* in Blood, Kidney, and Submaxillary Gland at Various Times after Intravenous Administration of 10 μc Ca^{45} i.v. (3 Rats at Each Time Interval, Each Line Represents 1 Animal).

Time	Blood	Kidney	Submaxillary gland
5 min.	249	160	51
	249	175	42
	235	215	77
1 hr	45	44	29
	50	43	30
	43	67	19
4	33	32	49
	29	24	43
	35	32	50
8	17	22	130
	28	39	70
	45	48	76
12	15	12	27
	17	17	32
	13	10	32
18	41	35	39
	22	22	33
	24	39	45
24	7.5	9.5	12
	4.4	7.2	9.3
	4.5	7.7	8.8
48	2.7	2.3	4.0
	1.9	4.3	4.0
	7.2	6.2	12.0

* Counts/sec./meq Ca.

Because the specific activity level in blood of different animals was variable, the ratios of specific activity in various organs in the same animal was used as a better means of comparison. Thus, retention of Ca^{45} in one organ as compared to blood or to another organ would give a ratio greater than one. On the other hand, delayed exchange in an organ would give a ratio less than one. Table II presents the ratios of specific activity of Ca in the submaxillary gland as compared to the kidney and blood, and the ratio of specific activity in kidney as compared to blood.

The ratios of specific activity of Ca^{45} in submaxillary gland as compared to kidney or blood shows a rise from 5 minutes to 8 hours. These ratios then fall and at 18 hours the difference between blood, kidney, and the submaxillary gland is no longer significant. The probability of chance occurrence of these dif-

† Description available on request.

‡ Filter designed by Mr. Charles Lapple, Stanford Research Institute, Menlo Park, Calif. Fiberglas supplied by Fiberglas Engineering Supply Division, Owens-Corning Glass, San Francisco, Calif.

TABLE II. Ratios* of Specific Activities of Submaxillary Gland as Compared to Blood or Kidney, and of Kidney Compared to Blood at Various Times after Intravenous Injection of 10 μc Ca^{45} (6 Rats at Each Time Interval).

	Submaxillary gland		Kidney
	Kidney	Blood	Blood
1 min.			.96 ± .06
5	.20 ± .05	.18 ± .04	.90 ± .09
4 hr	1.40 ± .10	1.30 ± .20	1.00 ± .10
8	2.5 ± .7	2.9 ± 1.0	1.1 ± .1
12	2.06 ± .13	2.2 ± .25	.97 ± .06
18	1.2 ± .1	1.2 ± .2	1.3 ± .3

* Mean ± stand. error.

ferences was less than .03 at 5 minutes, 8 hours, and 12 hours. The differences at the other time intervals were not less than .05. The data indicate a significant delay in the exchange of Ca in the submaxillary gland. The equilibration time appears to be greater than 4 hours, although exact determination is difficult since the blood specific activity is falling rapidly.

In another experiment, the rapidity of exchange in the kidney was determined by killing the rats 1 minute after the injection of Ca^{45}. The mean ratio of kidney specific activity to blood specific activity in 6 rats was 0.96 with a standard error of ± .06, indicating that exchange was complete even at this short time interval.

Discussion. Various mechanisms can be postulated to explain the slow exchange of radio-calcium in the submaxillary gland. First, the slow exchange may represent a metabolically dependent, rate-limiting process for exchanging calcium across the cell membrane or the membrane of an intracellular particle. The fact that total calcium in the submaxillary gland is approximately 5 times as high as in the kidney indicates fundamental differences in the way the two tissues handle calcium.

Secondly, the slow exchange may represent a slow rate of dissociation of a tightly bound Ca complex either inside the cell or the cell surface. The specific activity ratio at 5 minutes between actively exchanging calcium in the submaxillary gland and total kidney calcium was recalculated on the assumption that the actively exchanging calcium in the gland was equal to total kidney calcium. This recalculated ratio was .80 ± .06 indicating that the exchange was still slower than kidney.

Thirdly, the slower exchange of Ca^{45} in the submaxillary gland could also be explained if the blood flow per unit weight in the gland was extremely small as compared to the kidney. Ca^{45} exchange within the kidney is complete in one minute, and presuming a renal blood flow (RBF) of 2.5 ml/g/minute this would indicate that a blood flow of 2.5 ml was sufficient to equilibrate 4.02 μeq of Ca (kidney Ca conc. $= K_c$). Since the submaxillary gland contains 21.6 μeq Ca/g (submaxillary gland Ca conc. $= S_c$), a flow of 13.5 ml of blood should be sufficient for equilibration (S_c/K_c x RBF). If an equilibration time (ET) of 4 hours is accepted, then blood flow would need to be as low as 3.3 ml/g/hour $\left(\frac{S_c/K_c \times RBF}{ET}\right)$ to be a limiting factor. Burgen(7) found the venous outflow rate from the unstimulated submaxillary gland in dogs was 13 ml/g/hour. This presumably is low since it was taken after surgical isolation of the blood vessels. Thus, if the submaxillary circulatory volume of rats is at all comparable to that in dogs, the rate of circulation is apparently not the only factor controlling the slow exchange of Ca^{45} in the submaxillary gland.

Studies are being made to elucidate the mechanisms controlling the high salivary gland Ca, the slow exchange with blood Ca, and of the part this Ca plays in the secretion of saliva calcium.

Conclusion. 1. Ca^{45} accumulation and loss in the submaxillary gland is much slower than in the kidney. Equilibrium between the gland and blood is reached approximately 4 hours after administration, while at 8 hours the specific activity of calcium in blood and kidneys has fallen to less than one-half that in the submaxillary gland. Equilibrium is again reached at 18 hours. 2. In female Long-Evans rats, total calcium in the submaxillary gland was 21.6 ± 1.0 meq/kg, in the kidney 4.0 ± 0.1 meq/kg and in blood 3.66 ± .14 meq/kg.

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Host Resistance to Hemorrhagic Shock X. Induction of Resistance by Shock Plasma and by Endotoxins.* (23539)

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The blood and tissues of an animal (dog, rabbit) in prolonged hemorrhagic shock contains a toxin which is the product of bacterial activity(1). It accumulates in consequence of injury to the antibacterial defense mechanisms and produces irreversibility to transfusion and death. If the integrity of the antibacterial mechanisms could be sustained, so that these toxins would be neutralized, or their production suppressed, the shock, even if severe and prolonged, should remain reversible to transfusion.

Accordingly, experiments were designed to explore the possibility of inducing tolerance to hemorrhagic shock, *i.e.* preventing development of irreversibility to transfusion, by prior exposure to known bacterial endotoxins. Should tolerance to shock be so induced, the same result might also be obtained by prior exposure to plasma from irreversibly shocked animals, if the toxic factor in this plasma which produces irreversibility is in fact a bacterial endotoxin. The data in this communication demonstrate that protection against irreversibility to transfusion and death from severe and prolonged hemorrhagic shock can be secured by prior treatment with plasma from severely shocked animals, as well as by prior treatment with known endotoxins.

Method. Rabbits were selected as the most suitable species in which to produce tolerance to endotoxin, and subsequently to gauge the effect of such tolerance on the course of hemorrhagic shock. The MLD/100 of Difco *E. coli* Endotoxin (Batch No. 0127-B8) was found to be 2 mg/kg body weight in normal rabbits. The MSD/100 (maximal surviving dose, *i.e.* the highest dose that can be given with a zero mortality rate) was 0.03 mg/kg. Seven groups of adult albino rabbits (average weight 2-3 kg) were trained for 3 days so that spontaneous fever would not occur in response to the required manipulations(2). Each rabbit then received a daily dose (1 MSD/100 or less—see Table I) of endotoxin intravenously for 7 successive days. In every instance observations were made on the fever response, and on the total and differential leucocyte count during the subsequent 6 hours. The fever response was characteristic for endotoxin and showed the anticipated daily decline, so that by the seventh day the curve was characteristic of induced tolerance to the endotoxin (Fig. 1). On the eighth day hemorrhagic shock was produced by a standard method described elsewhere(3), and tolerance to shock observed, especially with regard to rate of "take-up" (*i.e.* spontaneous return of the shed blood to the animal from the elevated reservoir), pressor response to transfusion, survival time and survival rate. One of the 7

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