toxin *per se* in the meninges or cerebral ventricles.

To elucidate further the importance of the granulocyte, endotoxin was injected into the basilar cistern of animals previously given nitrogen mustard. Although all such animals were debilitated, their responses to endogenous pyrogen depressed, and hypothermia observed in those given only intra-cisternal saline, appreciable febrile responses occurred following basal cisternal endotoxin instillation. Indeed, several rabbits with few or no circulating granulocytes remained capable of responding with high temperatures. The production of fever by the intra-cisternal injection of endotoxin must therefore represent either a direct central nervous system action of endotoxin as proposed by Bennett and co-workers(2-4), or is mediated by cell types other than the granulocyte.

Conclusion. Endotoxin injected into previously untraumatized basal cisterns of curarized rabbits evoked higher and more sustained febrile responses than comparable amounts given intravenously. Endotoxin injected into the basal cistern also evoked appreciable febrile responses in nitrogen mustard treated rabbits with few or no circulating granulocytes; the highest responses were observed in the absence of circulating granulocytes. Release of granulocytic pyrogen does not appear to constitute a necessary intermediate step for the cerebral pyrogenic activity of endotoxin.

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Turnover Rate and Distribution Pattern of Radiostrontium in the Skeleton of the Laying Hen.*† (31557)

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Approximately 75% of the Ca required for egg shell formation by the laying hen is dietary in origin, and 25% is obtained from skeletal Ca(1). The exact site of endogenous Ca depletion for egg shell formation is not known. It is feasible, however, to assume that the skeletal structures of the body participate in egg shell formation at different rates. Radiostrontium is a bone seeking isotope and its turnover rate would be expected to be influenced by the mineral requirement of egg shell formation. However, the elimination and turnover rate for Sr^{89} may vary with

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bone structure. The purpose of this study was to determine which bones accumulate Sr^{89} and which are the first to release Sr^{89} for egg shell formation since the egg shell is one of the major excretory routes for radiostrontium in the laying hen.

Procedure. Single-comb White Leghorn females 7 months of age with an average body weight of 1600 g, were used to study the turnover rate of radiostrontium in various bones of laying hens. Hens were kept in individual cages and fed a standard laying diet, *ad libitum,* containing accepted calcium and phosphorus levels (N. R. C. 1960 Publication #327). Fourteen laying hens were each administered 0.5 ml solution containing 10 μc of Sr^{89} (pH = 5.9) intramuscularly (pectoral muscles) immediately after oviposition. Two birds were killed at each of the following time intervals: 12 hours, 1, 2, 4, 8, 16, and 24 days following administration of Sr^{89}. All the birds were autoclaved at 120°C and 15 psi for 10 minutes. The following bones were removed, cleaned, defatted, dried, ashed, and analyzed for Sr^{89} activity by the method of Creger *et al*(2): (1) skull, (2) mandible, (3) cervical vertebrae, scapula and first 4 thoracic vertebrae, (4) furculum and coracoid, (5) sternum and xiphoid process, (6) ribs and uncinate processes, (7) synsacrum, ischium and pubis, (8) humerus, ulna, radius, ulnare and radiale, (9) femur, (10) tibiotarsus, (11) tarsometatarsus, and (12) toe digits.

Results and discussion. Fig. 1 and 2 show the retention of Sr^{89} in the bones following a single intramuscular injection in the laying hen. Sr^{89} activity in all the bones was at a maximum at 12 hours post injection and decreased exponentially by the following function:

$$R(t) = c_1 e^{-\lambda_1 t} + c_2 e^{-\lambda_2 t} + c_3 \quad (1)$$

where: c_1, c_2, c_3 are constants, and λ_1 and λ_2 are elimination constants equal to 0.121 and 0.043, respectively, calculated by the method of Colvin *et al*(3).

It was observed that all the bony tissues participate in Sr^{89} exchange and all have a reasonably consistent common pattern. The final constant term in the equation (1) must be presumed to represent material which is being excreted from the bone at a very slow rate, as the terminal part of the cuve is almost parallel to the time axis on a semilog plot.

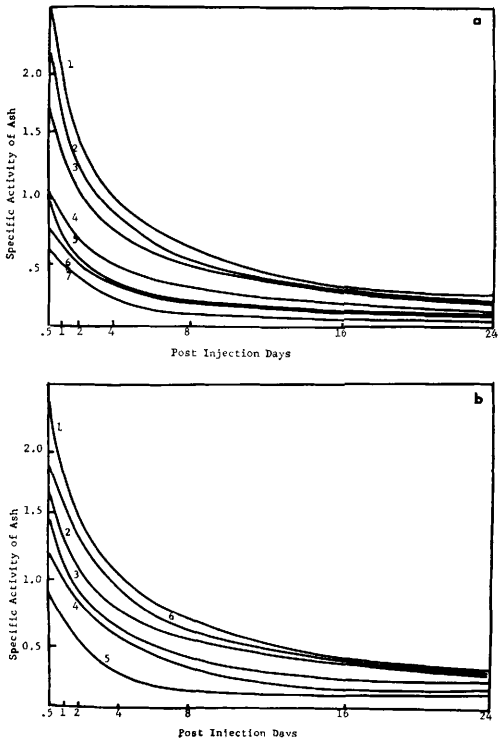

FIG. 1a. Elimination rate of deposited Sr^{89} in different bones after an intramuscular injection of 10 μc $Sr^{89}Cl_2$. (% of the injected dose per g of the bone ash—specific activity—vs time.) 1. Ribs and uncinate processes. 2. Femur. 3. Humerus, ulna, radius, ulnare and radiale. 4. Mandible. 5. Tarsometatarsus. 6. Skull. 7. Phalanges.

Fig. 1b. Elimination rate of deposited Sr^{89} in different bones after an intramuscular injection of 10 μc $Sr^{89}Cl_2$. (% of injected dose per g of the bone ash—specific activity—vs time). 1. Sternum and xiphoid process. 2. Synsacrum, ischium and pubis. 3. Tibiotarsus. 4. Furculum and coracoid. 5. Claws. 6. Cervical and thoracic vertebrae, and scapula.

Great variations were found in the extent to which the different bones retained Sr^{89}. The skull, tarsometatarsus and toe digits lost only small amounts of strontium, whereas the ribs (and uncinate processes); sternum (including xiphoid process); synsacrum, ischium and pubis; and cervical vertebrae, scapula, and thoracic vertebrae lost Sr^{89} at a rapid rate, as can be seen from the turnover values in Table I.

TABLE I. Turnover Rate of Sr^{89} in Different Bones of the Laying Hen.

Bone	Avg sp. act. in 12 hr post inj of 2 birds*	Avg turnover in 24 days of 2 birds†
Ribs & uncinate process	2.60	2.40
Femur	2.40	2.30
Sternum & xiphoid process	2.40	2.20
Cervical & thoracic vertebrae & scapula	1.85	1.60
Synsacrum, ischium & pubis	1.70	1.50
Humerus, ulna, radius, ulnare & radiale	1.75	1.50
Tibiotarsus	1.55	1.45
Furculum & coracoid	1.20	1.10
Mandible	1.05	.98
Tarsometatarsus	1.00	.95
Claws	.92	.82
Phalanges	.70	.60
Skull	.75	.50

* $\frac{\mu c/g \text{ of ash} \times 100}{\mu c \text{ in the dose}}$.

† Specific activity after 12 hr post injection minus specific activity after 24 days post injection.

Although the immediate source of calcium for shell formation is the blood, it is clear that the reserve source must be located elsewhere. Since there are no large stores of calcium in the soft tissue, the most probable sources of shell calcium are the food and the skeletal tissues.

In the bird, an entirely new system of medullary bone, produced chiefly as an outgrowth from the endosteal lining of the shafts of the long bones, such as femur and tibiotarsus, is produced at the onset of the laying cycle and serves to accumulate calcium to be used for egg shell formation(4). In the pigeon, the process of accumulation and destruction of medullary bone is extremely rapid, and is accomplished in about 48 hours(5,6). Since metabolic studies have indicated that strontium behaves qualitatively like calcium(7,8), it may be assumed that if strontium were available, it would be incorporated into the newly formed bone crystals of the medullary bone just as would be calcium. Therefore, a bird in a high state of production would be expected to have a high turnover of minerals in the bones involved in the medullary system. From Table I, it can be seen that the femur had a very rapid turnover and the tibiotarsus a less rapid turnover of strontium.

The bones of the pectoral girdle had turnovers intermediate between femur and tibiotarsus, except for the furculum and coracoids which had turnovers less than the tibiotarsus.

Bones which contain primarily more spongy than compact bone tissue, *e.g.*, ribs and sternum, were found to have higher turnovers of strontium than did more compact bones. However, data are not available which would indicate that minerals are drawn from the spongy bone tissue for use in egg shell formation. Rather these data would indicate simply that strontium is more quickly eliminated from spongy bone tissue than from bones of a more compact construction, presumably due to the generous blood supply of the former.

On the basis of these results it is concluded that (1) all the bony tissues participate in the Sr^{89} exchange, (2) all bones show similar retention patterns, (3) turnover of Sr^{89} in different bones is in the following decreasing order: ribs (with uncinate processes); sternum (including xiphoid process); femur; synsacum, ischium and pubis; cervical and thoracic vertebrae, and scapula, furculum and coracoid; humerus, ulna, radius, ulnare and radiale; tibiotarsus; mandible; skull; tarsometatarsus; claws and phalanges.

Summary. Fourteen laying hens were administered 10 μc Sr^{89} each (intramuscularly), and 2 birds were killed at 12 hours, 1, 2, 4, 8, 16, and 24 days, respectively. The soft tissues were cleared from the skeleton and all bones were analyzed for Sr^{89} content by liquid scintillation. It was observed that all bony tissues participate in Sr^{89} exchange, and that the ribs had the highest turnover rate, whereas, the skull had the lowest turnover rate. In general, it was found that bones composed primarily of spongy bone tissue turned over Sr faster than those of more compact construction.

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Prevention and Interruption of SV40 Induced Transplantation Immunity with Tumor Cell Extracts.* (31558)

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Adult hamsters immunized with papovavirus SV40 resist transplantation of cells transformed by the homologous virus(1-4). These rejection studies have suggested the presence of virus specific transplantation antigens which are presumably located at the surface of transformed cells. Recently, the presence of new surface antigens in cells transformed by SV40 has been demonstrated using immunofluorescence(5,6) and cytotoxic tests(7,6).

Animals inoculated with antigens in neonatal life often fail to respond immunologically in adult life to the same antigens(8). This report describes successful attempts to induce a state of immunological unresponsiveness in hamsters against SV40 transplantation antigens by inoculation of cell surface material from SV40-transformed cells. It also describes experiments which revealed that the protection induced by SV40 against cells transformed by the homologous virus could be reduced by treatment of the animals with cell extracts from the virus-transformed cells.

Materials and methods. Viruses. Simian virus 40 was the Baylor reference strain described previously(9). The virus was grown in African green monkey kidney (GMK) cultures maintained in lactalbumin hydrolysate medium (M-H) without serum.

Cell lines. The H-50 cells were derived from a hamster tumor induced by SV40(10). The cells synthesize the intranuclear SV40 tumor (T) antigen(11) and produce tumors in unvaccinated weanling hamsters but not in hamsters vaccinated with SV40(1). The cells also contain a specific surface (S) antigen which can be detected with sera from SV40 vaccinated hamsters resisting transplantation of cells transformed by SV40(5). Specific surface antigens have also been demonstrated by cytotoxic tests using heterologous rabbit serum(7). The cells are free of infectious SV40(12).

The cells were grown in Eagle's medium supplemented with 10% heat-inactivated calf serum, 100 units of penicillin, and 100 μg of streptomycin per ml.

Preparation of cell ghosts. Cell ghosts were prepared according to the method of Haughton(13). Briefly, 2.5×10^8 cells were dispersed with 0.2% trypsin and washed with tris buffered saline (TBS), pH 7.4. The cells were extracted successively with 100 ml of 3, 6, and 15% sodium chloride in the cold. Final wash was carried out in distilled water. No viable cells remained when tested by dye exclusion test(7). The ghost cell preparations were suspended in 4 ml of TBS.

Preparation of ruptured cells. H-50 cells were removed from 16-oz bottles with the help of a rubber policeman. The cells were washed with TBS, sedimented by low speed centrifugation and resuspended in 1 ml of TBS per 16-oz bottle. The cells were frozen and thawed 2 times and treated for 30 seconds in a Raytheon sonic oscillator at 12 kc per second.

Animal experiments. Two sets of experiments were carried out:

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