Table 1. Incorporation of $I^{131}$-iododeoxyuridine into DNA after irradiation. The figures represent percentages relative to incorporation into unirradiated controls at 20 hours after injection of the iododeoxyuridine (three to five mice per tabulated number).

| Exposure (r) | Incorporation at intervals between x-radiation and injection (%) | | | |
|---|---|---|---|---|
| | 2 hr | 6 hr | 24 hr | 48 hr |
| 10 | 110 | 68 | | |
| 25 | 52 | 39 | | |
| 52 | 37 | 29 | 102 | |
| 104 | 24 | 23 | 55 | |
| 208 | 20 | 23 | 43 | |
| 312 | 18 | | 39 | |
| 416 | 12 | | 26 | |
| 520 | 13 | | 22 | |
| 624 | 12 | | 20 | 62 |

tissue. This precipitate contains the DNA together with most of the tissue proteins. Analysis of various organs revealed that the amount of acid-insoluble radioactivity reached a maximum within an hour after injection of labeled iododeoxyuridine. The acid-soluble radioactivity after this period was primarily due to the presence of iodide, resulting from degradation of the labeled compound. By 20 hours, almost all the body radioactivity was insoluble in trichloracetic acid. Of the radioactivity lost from the body, over 95 percent was excreted in the urine, largely as iodide.

The unirradiated animal, 21 hours after the injection of labeled iododeoxyuridine, retained an average of 8.3 percent of the injected dose. The retained activity was proportioned 41 percent to the gastrointestinal tract (none of this was in its contents), 21 percent to the skin (about half of this represented contaminating iodide), and 15 percent to the bones (including bone marrow). Only 2.7, 2.3, 1.7, and 1.1 percent were present in the skeletal muscles, liver, thymus, and spleen, respectively. While the rates at which radioactivity

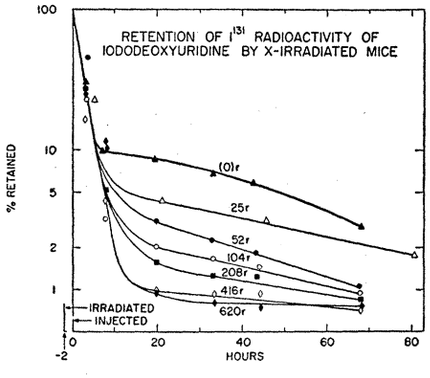


Fig. 1. Disappearance of total radioactivity in mice after injection of $I^{131}$ iododeoxyuridine. Tracer was given 2 hours after x-radiation. "O" dose curve shows unirradiated controls. Three to five mice per point.

disappeared from the various organs were quite different, the preponderance of radioactivity in the gut and the steepness of the decline from this compartment after 40 hours largely account for the shape of the curve for whole-body retention.

Since the extent of incorporation of labeled iododeoxyuridine in the intact mouse could not be determined until after the degradation products had been eliminated, the amount of radioactivity present at 20 hours was chosen as a measure of incorporation. Table 1 shows that the incorporation was a function of the irradiation dose and of the time between irradiation and injection. Maximum inhibition of iododeoxyuridine retention for any given x-ray dose occurred approximately 6 hours after irradiation and was already close to maximum 2 hours after irradiation. Recovery, in that iododeoxyuridine retention returned to normal, began within 24 hours after administration of 52 r, but was not yet complete even 48 hours after administration of 624 r.

The inhibition produced by the larger doses is even greater than the graph indicates, for an iodide retention curve would approximate that for labeled iododeoxyuridine after administration of 624 r, and in fact, over 80 percent of the radioactivity "incorporated" when the labeled compound was given 2 to 24 hours after 624 r of radiation was found in the skin, largely as iodide (*4*). The greatest inhibition of incorporation was suffered by the gastrointestinal tract, and the greatest recovery, 48 hours after irradiation with 624 r, also occurred in this organ, little or no incorporation then being observed in the spleen, lymph nodes, or thymus. These results parallel those recently reported by Nygaard and Potter (*5*) for the effect of x-rays on the incorporation of thymidine-2-$C^{14}$ into the DNA of rat intestine, thymus, and spleen.

Irradiation given 2 hours *after* the injection of $I^{131}$-iododeoxyuridine produced a small depression in the curve for retained radioactivity, and irradiation given 24 hours after injection produced barely detectable changes in the shape of the retention curve, indicating that early death of labeled cells can account for only a fraction of the inhibition observed. The maximum inhibition observed 6 hours postirradiation (Table 1) may result from inhibition of mitosis and the resultant depletion of the proliferating pool, since this interval is close to the generation time for the rapidly proliferating cells of the gut. However, inhibitions observed at earlier times (Fig. 1) obviously cannot be so explained and must therefore represent either a slowing of DNA synthesis within cells or a less efficient utilization of label because of altered cell permeability or increased pool size of DNA precursors.

In any event, the sensitivity of iododeoxyuridine incorporation in mice to as little as 10 r of precedent x-radiation suggests the possibility of developing this technique as an index of radiation injury in man after either accidental or therapeutic exposure. Of course, the possible mutagenic effects of labeled iododeoxyuridine, either from the compound itself or from its radiation, must be considered in any such study (*6*).

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## Fallout Radioactivity in Cattle and Its Effects

*Abstract.* The levels of strontium-90 and cesium-137 in cattle grazed on the Nevada Test Site and elsewhere in Nevada are similar to those in cattle from other parts of the country. Gastrointestinal absorption of the relatively large amounts of radioactive cerium-praseodymium, ruthenium-rhodium, and zirconium-niobium present in the rumina is very small. Zinc-65 made its first appearance in samples of muscle and liver in November 1958 and has persisted in later samplings. There has been no evidence of biological damage to date, either histologically or grossly.

In 1957, the U.S. Atomic Energy Commission authorized a project (*1*) to determine what effects, if any, the radioactivity produced in atomic bomb tests at the Nevada Test Site was having on cattle grazing in areas heavily contaminated by fallout as contrasted

Table 1. Strontium-90 in grazing animals and in people.

| No. of animals | Location * | Sample date | $Sr^{90}/Ca$ ($\mu\mu c/g$) | | | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | | Rumen | Feces | Rib | Femur | Milk | Milk/bone | |
| | | | *Cattle* | | | | | | |
| 5 | NTS | Fall 1957 | 436 | 602 | 13.9 | 15.6 | | | |
| 6 | DV | Fall 1957 | 117 | 103 | 13.4 | 15.5 | | | |
| 4 | NTS | Spring 1958 | | 190 | 8.6 | 16.1 | | | |
| 3 | DV | Spring 1958 | 56 | 62 | 17.6 | 15.5 | 3.1 | 0.19 | |
| 3 | KC | Spring 1958 | 172 | 80 | 12.3 | 22.3 | 5.1 | 0.29 | |
| 4 | NTS | Fall 1958 | 159 | 193 | 7.4 | 27.1 | | | |
| 4 | DV | Fall 1958 | 57 | 55 | 9.6 | 14.3 | 1.8 | 0.15 | |
| 4 | KC | Fall 1958 | 231 | 329 | 19.1 | 21.0 | 4.7 | 0.23 | |
| | | | *Sheep* | | | | | | |
| | Br. Col., Can. | May 1956 | | | | 32 | | | (6) |
| | Chile | May 1956 | | | | 7 | | | (6) |
| | England | July 1957 | | | | 200 | | | (7) |
| | | | *Caribou* | | | | | | |
| | Alaska | May 1956 | | | | 50–112 | | | (6) |
| | | | *Cattle* | | | | | | |
| | Utah | Jan.–Apr. 1958 | | | | | 4.0 | | (3) |
| | Arizona | Jan.–Apr. 1958 | | | | | 1.0 | | (3) |
| | U.S. (av.) | 1958 | | | | | 6.7–10 | | (4) |
| | | | *Human beings (7–12 months)* | | | | | | |
| | North America | 1957–58 | | | | 1.8 | | | (8) |

* NTS, Nevada Test Site; DV, Delamar Valley, Nev.; KC, Knoll Creek, Nev.

with cattle, at a distance, grazing on more normal pastures. Accordingly, 50 head of Hereford cattle, born and raised in the Delamar Valley just east-northeast of the Nevada Test Site, were purchased in November 1957; these animals were then grazed within the test site itself and comprised the maximum radiation exposure herd. A cooperative program was inaugurated with the University of Nevada to maintain two additional herds located at distances of 75 miles east (Delamar Valley, Caliente, Nevada) and 300 miles north (Knoll Creek, Contact, Nevada) of the test site. Twice yearly (May and November) five animals from each herd were killed. Samples of bone, liver, muscle, thyroid, milk, rumen content, and feces were collected and radioassayed; a detailed autopsy was performed on each animal, and tissue sections were examined for histopathology (*2*). Film badges in small leather pouches on the neck chain of each animal were replaced bimonthly. Results of radioactivity analyses are summarized in Tables 1 and 2.

Considering the $Sr^{90}$ data first, it is clear that there are only small differences in the ratio of $Sr^{90}/Ca$ among the three herds and between these cattle and grazing animals from other parts of the Northern Hemisphere. In fact, the Nevada values are among the lower ones. The Southern Hemisphere value (Chile) is the lowest, which is consistent with world-wide fallout patterns. The values for human bone are less than those in any of the grazing animals, because of discrimination against strontium in moving up the food chain to man.

Milk samples also have $Sr^{90}/Ca$ ratios close to the values for nearby Western states. The Nevada, Utah, and Arizona values are all similar and are less than the 1958 national average of 8.2 $\mu\mu c/g$ (*4*), which is what one would expect if most of the $Sr^{90}$ fallout comes from the stratospheric reservoir and is influenced on the local scale by rainfall patterns. The $Sr^{90}/Ca$ ratio in milk is consistently lower than the ratio for bone in the Nevada cattle, the milk-to-bone ratio averaging about 0.2. Probably the milk-producing organs are better at rejecting $Sr^{90}$ than the bone; Comar (*5*) reports that in cows the ratio of $Sr^{90}/Ca$ is 0.11 in milk and 0.18 in the skeleton.

The $Sr^{90}/Ca$ ratio in the rumen and fecal samples, reflecting the $Sr^{90}/Ca$ ratio in the feed shortly before slaughter, is higher than the bone values by something like an order of magnitude. In addition, these ratios are affected markedly by local fallout patterns and hence show larger fluctuations. Thus, the large $Sr^{90}/Ca$ ratios in the 1957 samples is probably due to the Plumbbob test series (spring and summer of 1957 at the Nevada Test Site); there was a reduction in the values in the spring of 1958 (a period of no testing).

The most striking feature of the results on $\gamma$-emitters is that the tissues contain so little radioactivity, in spite of the fact that the rumina contain relatively large amounts of the intermediate-lived fission products commonly found in dust and dirt. In Table 2, only $Zr^{95}/Nb^{95}$ values are listed; $Ce^{144}/Pr^{144}$ values are similar, while $Ru^{106}/Rh^{106}$ are generally lower by a factor of 10. These are nonphysiological elements, and absorption through the gut wall is very small. Cesium-$^{137}$, while certainly present, is swamped and not observed in the rumen contents, but is the main fission product in the samples of muscle and liver. It is significant that the $Cs^{137}/K$ ratios are quite similar to those for commercial beef from widely separated locations. This means that the $Cs^{137}$ is coming mainly from the stratosphere, as was the case with $Sr^{90}$, and not from local fallout.

Fisson product levels in the rumen contents and fecal samples from the Nevada Test Site herd showed a marked correlation with Nevada atomic bomb tests. The test series occurred during the spring and summer of 1957 (Plumbbob) and in the fall of 1958

Table 2. Gamma-ray emitters in cattle. (N.D., not detected.)

| Sample | Location * | Date | $K^{40}$ (g K/kg) | $Cs^{137}$ ($\mu\mu c$/g K) | $Zn^{65}$ ($\mu\mu c$/kg) | $Zr^{95}$ ($\mu\mu c$/kg) | $Nb^{95}$ ($\mu\mu c$/kg) |
|---|---|---|---|---|---|---|---|
| Muscle | NTS | Dec. 1957 | 3.9 | 100 | N.D. | N.D. | N.D. |
| | DV | Dec. 1957 | 3.8 | 93 | N.D. | N.D. | N.D. |
| | Pooled | May 1958 | 3.9 | 120 | N.D. | N.D. | N.D. |
| | Pooled | Nov. 1958 | 4.7 | 160 | 140 | N.D. | N.D. |
| Liver | Pooled | Nov. 1958 | 5.2 | 70 | 320 | Trace | Trace |
| Bone | Pooled | Nov. 1958 | Trace | Trace | Trace | N.D. | N.D. |
| Muscle | | | | | | | |
| (2-yr steer) | NTS | May 1959 | 2.8 | 122 | 107 | N.D. | N.D. |
| (1-yr steer) | NTS | May 1959 | 3.0 | 76 | 77 | N.D. | N.D. |
| (1-mo calf) | NTS | May 1959 | 4.5 | 112 | 153 | N.D. | N.D. |
| Liver | | | | | | | |
| (2-yr steer) | NTS | May 1959 | 4.6 | 61 | 195 | N.D. | N.D. |
| (1-yr steer) | NTS | May 1959 | 5.0 | 28 | 154 | N.D. | N.D. |
| (1-mo calf) | NTS | May 1959 | 3.1 | 58 | 97 | N.D. | N.D. |
| Hamburger | Local † | 1959 | 2.3 | 87 | 30 | N.D. | N.D. |
| Beef liver | Local † | 1959 | 2.8 | 64 | 50 | 30 | 60 |
| Rumen | | | | | | | |
| contents (av.) | NTS | Dec. 1957 | N.D. | N.D. | N.D. | 210,000 | 420,000 |
| | DV | Dec. 1957 | N.D. | N.D. | N.D. | 35,000 | 70,000 |
| | NTS | May 1958 | N.D. | N.D. | N.D. | 20,000 | 37,000 |
| | DV | May 1958 | N.D. | N.D. | N.D. | 1,600 | 3,000 |
| | KC | May 1958 | N.D. | N.D. | N.D. | 20,000 | 37,000 |
| | NTS | Nov. 1958 | N.D. | N.D. | N.D. | 215,000 | 430,000 |
| | DV | Nov. 1958 | N.D. | N.D. | N.D. | 58,000 | 116,000 |
| | KC | Nov. 1958 | N.D. | N.D. | N.D. | 23,000 | 47,000 |

* NTS, Nevada Test Site; DV, Delamar Valley, Nev.; KC, Knoll Creek, Nev. † Local purchase.

(Hardtack II), and the fission product levels in the Nevada Test Site herd were much higher in December 1957 and November 1958 than between tests (May 1958). The other two herds showed little change. This shows the effect of the relatively intense local fallout on the test site itself from smaller nuclear devices; this would be much more important for short-lived and intermediate-lived fission products than for the long-lived ones (such as $Cs^{137}$ and $Sr^{90}$, the concentrations of which are not elevated in any of the three Nevada herds).

It is interesting that a new radioactivity, $Zn^{65}$, makes its first appearance in the November 1958 soft-tissue samples and remains present in the May 1959 samples. Zinc-65 is not a fission product, but is formed by neutron interaction on stable zinc. It has been detected in the general food supply (*9*), it is well absorbed from the gastrointestinal tract, and body retention is high (*10*). The amounts observed in the samples of liver and muscle were comparable to the $Cs^{137}$ content.

External $\gamma$-ray dose and bone plutonium levels are trivial; the same was true of thyroid $I^{131}$ levels with the exception of the Nevada Test Site herd soon after Hardtack II, when levels were of the order of the human maximum permissible level.

All reports from the Armed Forces Institute of Pathology indicate nothing significant from microscopic pathological examination. To date, no gross effects have been observed. The reproduction rate is normal, the animals are in a satisfactory state of nutrition, and there has been no increased incidence of any diseased condition.

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1. This work was performed under the auspices of the U.S. Atomic Energy Commission.
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## One-Trial Interhemispheric Transfer of a Learning Engram

*Abstract.* By using spreading depression to decorticate temporarily one hemisphere in rats, a learning trace was established in the opposite functional hemisphere. Spontaneous transfer of the engram from the *trained* hemisphere to the untrained hemisphere does not occur when both hemispheres are functional. If, however, the animal was allowed to make one reinforced response, complete transfer of the engram to the untrained hemisphere occurred.

We have been investigating (*1*) the use of spreading depression to decorticate temporarily one hemisphere and thereby to localize a conditioned performance to the other hemisphere as first described by Bureš (*2*). This approach derives from the work of Sperry and his co-workers (*3*), who showed a similar localization of learning engrams in the split-brain preparation. In our technique, cups were implanted chronically around small holes drilled through the skull of male albino rats, one over each hemisphere. The dura was left intact. Plungers fitted into the cups prevented dehydration of the brain. A small pledglet of cotton soaked with 25 percent potassium chloride was placed in the cup on one side to produce repetitive cycles of spreading depression restricted to that particular hemisphere. These spreading depressions inactivate the cortex for 3 to 4 hours (*2*).

The rats were given 1-hour daily sessions of operant training with one cortical hemisphere depressed. Under these conditions the functional cortex was exposed to bar-press conditioning under a continuous reinforcement schedule for food (*4*). In Fig. 1 the depressed cortical hemisphere is shown schematically, for each day, by cross-hatching.

On the first two days (Fig. 1), operant level scores were obtained, that is, measures of the animal's basal predisposition to respond without any training. On the 3rd and 4th days, the animal was reinforced for every response, and the increased rates indicate the animal had learned. The engram was shown to have been established in the cortex that remained functional during training, when this trained cortex was depressed by spreading depression. This is shown in Fig. 1, for spreading depression was produced in the *trained* cortex on day 5, and the animal's response rate declined to a basal level. It should be noted that the responses on day 5 were not reinforced, and thus they were obtained under extinction conditions. On day 6 the trained cortex was again functional, spreading depression was initiated on the untrained side, and the return of a higher rate of responding coinciding with the functional return of the trained cortex indicates that the engram was not impaired by the presence of spreading depression on the previous day.

The procedure of trying the trained side and the untrained side under conditions of reinforcement and extinction was carried out on the same animal over a number of days (Fig. 1). On days 5 and 9 the amount of responding of the animal under extinction with the trained cortex depressed is shown. As has been noted, this performance does not differ from the operant level performance prior to conditioning. These extinction scores are in contrast with the score obtained on day 11, when the trained cortex was functional during extinction. The presence of an engram is shown in that hemisphere by the retention of learning in extinction.

This use of extinction tests as a retention measure of learning shows more rigorously the presence of a unilateral engram than does Bureš's use of speed of learning scores, that is, the number of trials required to reach a criterion of conditioned performance. This operant technique is also flexible in terms

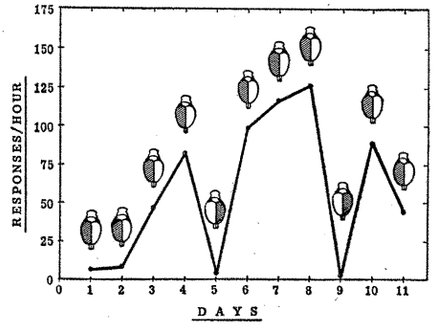


Fig. 1. Unilateral learning obtained by use of spreading depression as a technique of temporary decortication. The shading indicates the hemisphere depressed. The first two days give the operant level of responding before conditioning. Subsequent days show the increase in responding during training. On days 5, 9, and 11, responses are made during extinction. On days 5 and 9 the response level when the trained cortex is depressed is shown to be low as compared to day 11 when the trained cortex is functional.