TABLE 1

ESTROGENIC ACTIVITY IN AN EXTRACT OF A COMMERCIAL FOOD RATION (B) FOR LABORATORY ANIMALS

| No. of mice | Av. body wt., g | Treatment | Mean uterine wt., mg |
|---|---|---|---|
| 8 | 28.6 | 16 days castrated | 11.2 ± 1.4 |
| 8 | 31.2 | 23 days castrated | 13.3 ± 1.3 |
| 10 | 30.5 | Estradiol, 0.002 μg/day | 23.1 ± 2.7 |
| 10 | 29.2 | Estradiol, 0.005 μg/day | 24.2 ± 1.9 |
| 10 | 31.5 | Estradiol, 0.01 μg/day | 40.0 ± 1.4 |
| 10 | 29.2 | Estradiol, 0.1 μg/day | 97.3 ± 8.7 |
| 10 | 29.1 | Food extract, 0.05 ml/day | 24.6 ± 2.1 |
| 9 | 28.7 | Food extract, 0.1 ml/day | 53.6 ± 2.0 |

The animals were rested for 15 days postcastration and then injected daily for 7 days. The mice were killed 24 hr after the last injection, the uteri dissected out, split longitudinally to remove luminal water, and weighed immediately on a torsion balance.

It may be seen from the data in Table 1 that castration atrophy was complete by the 16th day. The mean uterine weight of mice castrated for 16 days was found to be 11.2 mg and that from mice castrated for 23 days was 13.3 mg. Since the body weights averaged approximately the same for each group of mice, it was not deemed necessary to convert the uterine weights to a per cent body weight figure. A standard dose-response curve was established with estradiol over a dosage range of 0.002 μg to 0.1 μg/day; these concentrations gave an adequate curve for assay purposes. The extract was injected at two dose levels, and in all instances a constant volume of 0.1 ml was injected. The equivalent of 0.05 ml of the extract increased the mean uterine weight to 24.6 mg, whereas 0.1 ml resulted in a mean uterine weight of 53.6 mg. Analysis of the data revealed that 1 kg of food contained an estrogenic potency equivalent to 3.75 μg of estradiol.

Although no direct evidence is available for the nature of the estrogen, it would not appear to be estradiol since the substance showed high oral activity. If one speculates on the source of the estrogen as due to implanted pellets used in the poultry industry (*1, 2*) and as having gotten into the food as waste scraps of meat, then one could conclude that the substance is a synthetic estrogen since these are the substances used by poultry breeders. It is of interest to note that several years ago the feeding of neck scraps from implanted birds caused sterility in mink (*3*).

There is also a possibility that the estrogen came from a plant source. Investigators have shown the presence of estrogenic activity in glycyrrhiza (*4*), willow (*5*), wheat germ oil (*6*), date palm tree (*7*), clover (*8, 9*), and alfalfa (*9*). Recently an isoflavone derivative has been isolated from soybean oil meal with estrogenic activity (*10*). Thus it is highly possible that the source of the hormone could have been from added plant material.

Regardless of the source of the estrogen, an appreciable amount of the hormone was found in a commercial food ration. The concentration was sufficiently high to prevent castration atrophy as noted by changes in the uterus and the vagina. This could easily invalidate many experiments and would definitely interfere with any assay for estrogen or with the Hooker-Forbes test for progesterone (*11, 12*). It would thus appear essential that proper controls be maintained at all times for possible food contamination with an estrogen.

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## Comparative Studies in the Uptake of Phosphorus by Tissues under Different Doses of Injected Radioactive Phosphorus $P^{32}$ [1]


K. L. Bhattacharya,[2] K. P. Chakraborty,[2] A. Bose,[2] and N. N. Das Gupta

*Chittaranjan Cancer Hospital and Institute of Nuclear Physics, Calcutta, India*


Radioactive phosphorus ($P^{32}$) is one of the few radioisotopes that have been used in therapy (*1, 2*). When a radioisotope in soluble form is injected or administered by mouth, it is deposited with greater or less selectivity in different organs. A knowledge of this differential uptake is essential for a correct estimate of the radiation dosage and for successful radioisotope therapy (*3*). The results of early investigations on the relative concentration of $P^{32}$ in different organs of experimental animals and of the time variation of the concentration have been summarized by Hevesy (*4*) and Chaikoff and Zilversmit (*5*).

In this work we have attempted to find out how the relative concentration of phosphorus by tissues is affected by different doses of the injected radioisotope. This seems to be of fundamental importance, not only for the correct estimation of the therapeutic dose of radioactive material, but also for showing the disturbances in metabolism produced by different doses of beta irradiation from $P^{32}$.


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TABLE 1

PERCENTAGE OF ADMINISTERED $P^{32}$ RECOVERED 72 HR AFTER INJECTION

| Organ | Dose, 0.3 μc/g | | Dose, 0.5 μc/g | | Dose, 1 μc/g | | Dose, 4.5 μc/g | |
|---|---|---|---|---|---|---|---|---|
| | 1 g tissue | Whole organ | 1 g tissue | Whole organ | 1 g tissue | Whole organ | 1 g tissue | Whole organ |
| Bone | 2.08 | 18.6 | 2.24 | 20.0 | 2.28 | 20.4 | 1.8 | 16.0 |
| Liver | 1.26 | 8.9 | 1.16 | 8.2 | 1.37 | 9.6 | 1.47 | 10.3 |
| Spleen | .92 | .32 | .95 | .33 | 1.1 | .38 | 1.1 | .38 |
| Small intestine | .74 | 1.9 | .78 | 2.0 | 1.1 | 2.9 | .9 | 2.3 |
| Stomach | .66 | .59 | .79 | .70 | .7 | .62 | .87 | .77 |
| Kidney | .69 | .86 | .47 | .58 | .57 | .71 | 1.0 | 1.2 |
| Muscle | .6 | 30.0 | .53 | 27.0 | .57 | 29.0 | .6 | 30.0 |
| Lung | .49 | .41 | .49 | .41 | .56 | .46 | .63 | .53 |
| Heart | .44 | .25 | .32 | .19 | .47 | .27 | .63 | .36 |
| Skin and hair | .35 | 6.3 | .36 | 6.5 | .27 | 4.9 | .35 | 6.3 |
| Brain | .14 | .24 | .14 | .24 | .21 | .37 | .23 | .41 |
| Blood | .13 | 1.10 | .13 | 1.10 | .16 | 1.32 | .15 | 1.23 |
| Total | | 69.47 | | 67.25 | | 70.93 | | 69.78 |

$P^{32}$ was injected into rats in amounts of 0.3, 0.5, 1.0, and 4.5 μc/g of body weight. The maximum dose of 4.5 μc/g of body weight was lethal (*6, 7*). All rats receiving that dose of radioisotope died within 12 days after the injection.

In all, 48 albino rats divided into 4 groups were used. Each animal was injected intraperitoneally with a single dose of carrier-free radioactive phosphorus in the form of isotonic $Na_2HPO_4$.[3] At the end of 72 hr the animals were anesthetized and tissues and blood samples obtained. The tissues were weighed wet and a portion thereof hydrolyzed or fused and assayed for radioactivity.

All uptake measurements were carried out with a hundred scaler circuit, shielded manual sample changer, and end-window type of Geiger-Müller counters with a window thickness of about 4 mg/cm$^2$. In order to allow for decay, during the period between the measurement of uptake and the injection of radiophosphorus, the beta activity in the tissue sample was always compared with that of a standard aliquot of the administered dose measured under identical conditions. The activity in the total organ was obtained by multiplying the mean concentration per gram of tissue by the average weight of the whole organ.

Table 1 indicates the activity in different tissues, both per gram of fresh tissue and in the whole organ, 72 hr after injection of $P^{32}$, expressed as percentage of the administered dose.

For the 4 doses of $P^{32}$ used in these experiments, the average of the total activity recovered from all organs listed was about 69%. The activity in the excreta measured during the same period accounted for 21% and the remaining 10% of the activity was concentrated in organs not listed in the table.

For each dose, bone had the highest concentration of $P^{32}$/g of tissue; next in order of concentration were liver and spleen. Among the other organs, the activity per gram of tissue decreased in the order in which they are listed. Although the dose of radioisotope varied from 0.3 μc/g to 4.5 μc/g of body weight, the percentage of uptake was found to be practically independent of the dose. The slight differences noted in the table are not statistically significant, even for the lethal dose of 4.5 μc/g of $P^{32}$. Assuming homogeneous distribution of $P^{32}$ throughout the tissues, with the doses of 0.3, 0.5, 1.0, and 4.5 μc/g of body weight, the animals were subjected to cumulative whole body beta irradiation throughout the first 3 days at the average rates of 10, 17, 34, and 153 r/day (*6*). Although the calculated doses are approximate, the present experiment shows that with these rates of beta irradiation, no appreciable difference is noticeable in phosphorus metabolism within the first 3 days.

If the relative concentration of $P^{32}$ in different tissues reaches approximate equilibrium within the first 3 days of its administration (*7, 8*), and thereafter it is eliminated with an effective half-life dependent only on physical decay and excretion, then the dose of beta irradiation delivered to different organs is roughly proportional to the concentration of $P^{32}$/g of fresh tissue. A comparison of the measured uptakes (Table 1) shows that with this method of administration, the bones receive 3–4 times as much radiation as the muscles, which again receive about 4 times as much radiation as blood.

[3] Obtained from A.E.R.E., Harwell.

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