A Tracer Study of Distribution of Iodoacetate in Rat Tissues.* (18868)

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In the course of a study of the mechanism by which iodoacetate inhibits dental caries, it was found desirable to determine the distribution in rat tissues of iodoacetate labelled with I^{131}. Since there is a possibility of slight decomposition of iodoacetic acid with liberation of iodine, it was necessary to make a comparative determination of the distribution of I^{131} as iodide in order to be sure that the observed results were due to iodoacetate rather than to iodide in the tissues. Previous studies of the distribution of I^{131} in rabbits(1,2) and rats (3) have indicated that, except in the thyroid gland, there is no selective uptake of radioactive iodine by body tissues.

Experimental. Synthesis of labelled iodoacetic acid. The method was based on that of Guggenheim(4). An aqueous solution of 200 mg of potassium iodide and 200 mg of monochloroacetic acid to which had been added 1.75 millicuries of carrier-free I^{131} was heated one hour at 50-60°. Sodium bisulfite was then added to remove any free iodine, and the solution was twice extracted with ether. The water layer was treated with 50 mg of iodoacetic acid and once more extracted with ether. The ether extracts were evaporated to dryness, and the residue taken up in distilled water and diluted to the desired strength. Yields averaged 48%. The freshly prepared solutions gave no color with starch, and were used immediately. When the solutions were allowed to stand for a week, enough iodine was liberated to give a faint starch test.

Distribution studies. Mann, Bale, Hodge, and Warren(2) showed that the presence of I^{127} reduces the uptake of I^{131} by the tissues. In order to compensate for the possibility of I^{127} which might have accompanied the iodoacetate through the procedure for its isolation, the iodide solutions to be used were prepared as follows: A solution of 400 mg of KI in 100 cc of water was extracted 3 times with ether. The ether was evaporated and the residue taken up in water to which the carrier-free I^{131} was added. Any I^{127} which was carried through the process should then be present in both the iodide and iodoacetate solutions in comparable amounts.

Toxic effects were noted in rats when iodoacetate was injected in concentrations slightly under 50 mg per kg. In preliminary studies to determine the time required for maximum uptake of iodoacetate by the teeth, 10 rats weighing approximately 100 g were injected intraperitoneally with about 4 mg of iodoacetate having an activity of 15-20 microcuries. Two rats were sacrificed at 1, 3, 6.5, 24, and 50 hours each. The tissues to be studied were removed, weighed fresh and dried at 56° for 48 hours. After determining the dry weight, they were ground to a fine powder and placed under a Geiger-Muller tube for counting in porcelain planchets having an area of 14 cm^2. Each sample was counted for 30 minutes. This allowed for a 5% error in counting statistics as all samples were from 4 to 18 times above background count. An aliquot of the dose given to the animals was run concurrently with the samples. This obviated any calculation that would otherwise be necessary in correcting for the decay of the I^{131} in the iodoacetate

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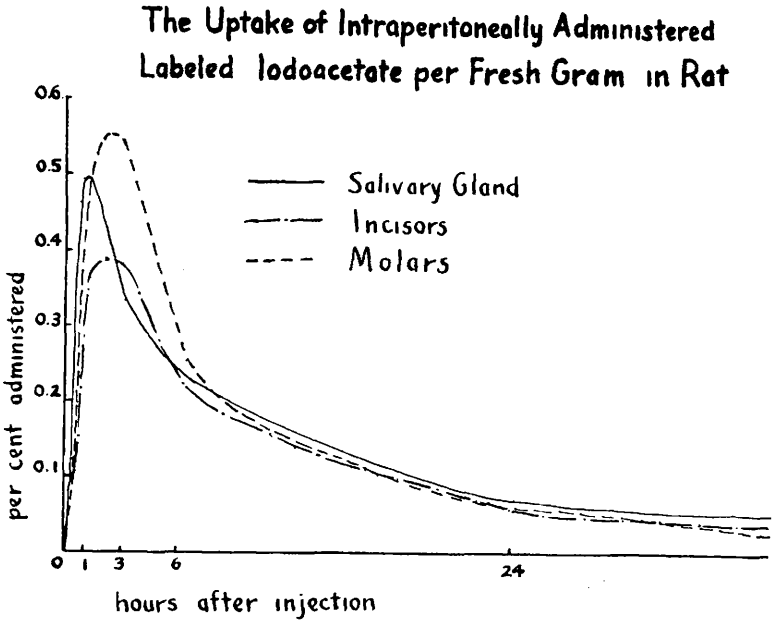

FIG. 1.

molecule. It was necessary to apply a mass absorption correction factor, as varying amounts of dried tissue were employed in the samples. In order to determine the degree of absorption of the radiation from I^{131} by varying amounts of mass, an aliquot of the dose was prepared and measured for activity with no mass present. Small amounts of water were added and after each addition a determination of the activity was made. From this a curve was derived giving the relationship between the mass of the sample and the percent of activity absorbed.

The same procedure was followed in the distribution studies. In this case, a total of 6 rats each were used for the iodoacetate and iodine studies, and the animals were sacrificed three hours after the intraperitoneal injections. The rats in the iodoacetate study received the same dosage as in the pilot study; the rats in the iodine study received a dosage with an activity of about 15 μc of I^{131}.

Results and discussion. The results of the preliminary study, shown in Fig. 1, indicate that the maximum uptake of labelled iodoacetate by the oral tissues occurs about 3 hours after injection. All further studies were therefore made at this time interval. Direct comparison of uptake of labelled iodoacetate and iodine by rat tissues after injection under comparable conditions is given in Table I. The range of individual variations in the rats was not great enough to alter the general findings.

The most striking effect shown in this table is the uptake of the two substances by the thyroid gland, as compared with the uptake by all the other tissues. Iodide is absorbed selectively, as expected, and the other tissues show only a small content of I^{131}. Uptake of iodoacetate, while higher in the thyroid than in the other tissues, is taken up to a much smaller degree than is iodide. In the other tissues, however, the content of iodoacetate is nearly twice that of iodide. In view of the fact that on standing, iodoacetate liberates some iodine, it is very possible that at all times a little iodine is present. If this is so, this free iodine will be taken from the blood by the thyroid, and may very possibly account for the higher values obtained in the thyroid with iodoacetate. The results observed in the other organs would then be due entirely to iodoacetate. It is noteworthy that in all organs except the thyroid, the uptake of iodoacetate is from 1.5 to 2 times as high as the

TABLE I. % of Administered Dose of Labelled Substance per g of Fresh Wt of Rat Tissues ± σm (Stand. Deviation of the Mean).

Tissue	Iodoacetate	I^{131}
Thyroid	8.304 ± .777	152.448 ± 10.542
Blood	1.201 ± .162	.698 ± .112
Saliva	.948 ± .265	——
Adrenals	.648 ± .159	.226 ± .057
Molars	.505 ± .109	.211 ± .031
Liver	.467 ± .078	.255 ± .033
Kidney	.402 ± .131	.266 ± .034
Incisors	.397 ± .138	.184 ± .017
Salivary glands	.358 ± .078	.250 ± .013
Skeleton	.285 ± .085	.187 ± .015
Striated muscle	.159 ± .065	.111 ± .031

uptake of I^{131}. This is probably due to the higher concentration of iodoacetate in the blood, almost double that of blood iodide, which is rapidly lowered by the thyroid. This fact, together with the rapid decrease in concentration with time seen in the oral tissues (Fig. 1) indicates that there is probably no selective uptake of iodoacetate. A diffusion into and out of the organs is indicated, a behavior characteristic also of iodine, where the thyroid is not involved.

Summary. (1) The distribution of iodoacetate and of potassium iodide, both labelled with I^{131}, has been determined in rats three hours after intraperitoneal injection. (2) The thyroid gland does not show a high selective uptake of iodoacetate. (3) Uptake of iodoacetate by tissues other than the thyroid is about twice that of iodide. There is no evidence of selective absorption by any tissue studied.

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Response of Castrated Male and Female Hyperthyroid Rats to Vitamin B_{12}.* (18869)

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Recent studies in this laboratory(1) indicated that the addition of vit. B_{12} to a purified ration containing toxic levels of iodinated casein effected a greater growth response in male than in female rats. Carrick(2) observed a sex difference in the response of chicks to fish solubles added to a ration presumably deficient in vit. B_{12}. An assay method for the determination of vit. B_{12} employing the hyperthyroid rat was developed by Frost *et al.*(3). These workers reported no sex difference in growth response to supplementation. Male and female offspring of vit. B_{12}-depleted rats showed a decided difference in growth response in studies reported by McCollum and Chow(4). Male rats grew more rapidly in the absence of vit. B_{12} supplementation than did their litter-mate females, suggesting a lower need for or more storage of this nutrient in the male rat. These results seemed to warrant further investigation of the influence of sex on the growth-promoting effect of vit. B_{12} in the hyperthyroid rat. Studies involving normal and castrate rats of both sexes were therefore initiated.

Materials and methods. Weanling rats of the Sprague-Dawley strain were used in this study. Thirty male and 18 female rats were

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