Effect of Bioflavones upon Metabolism of Iodine and Iodinated Compounds in the Rat.* (19920)

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During studies on the effect of various "Vitamin P" compounds upon the development of transmissible tumors in rats, it was observed that one of these substances, hesperidine methyl chalcone, was capable of changing the normal distribution and excretion of iodide and diiodotyrosine, using I^{131} as a tag. This report is a summary of our studies of the effect of this compound and other bioflavones upon the fate of I^{131} and I^{131}-tagged tyrosine in rats.

Methods. Adult rats of both sexes, weighing from 190 to 250 g, were used as experimental animals; they were of the Long-Evans, Curtis-Dunning, or Slonaker strains. The I^{131} was obtained from the Oak Ridge National Laboratory. The diiodoI^{131}tyrosine employed was tagged with radioiodine, using the method previously reported by one of us(1). Both the radioiodine and diiodoI^{131}tyrosine were administered to the rats via the external jugular. After the administration of one or the other of these substances, animals other than controls were given varying amounts of hesperidine methyl chalcone or other bioflavones. They were administered as a saline solution by the intragastric or subcutaneous routes. Radioactive assays of the I^{131} were made with a scintillation counter composed of a thallium-activated sodium iodide crystal and an R.C.A. 5819 photomultiplier tube. The sensitivity of this counter was such that 8.5% of all of the disintegrations occurring in the material taken as a sample were observed as counts. Approximately one μc of I^{131} was administered to each animal, this amount being in the tracer dose level. The data are expressed as the per cent of the tagged iodine administered present in wet weight tissue or excreta. In the case of the diiodoI^{131}tyrosine, 2 μg were administered along with the I^{131} tag to each animal studied with this compound. Whatman No. 1 filter paper was used in the paper chromatograms.

Results. In experiments in which the inorganic iodide pool of the rats was labeled using I^{131}, it was found that the intragastric administration of H.M.C. in doses from 40 to 1,000 mg per kilo was effective in increasing the uptake of I^{131} by the thyroid, skin, and stomach plus its contents, so that the values observed were significantly greater than those

TABLE I. Fate of I^{131} in the Rat 15 Hr after Its Intravenous Administration. Animals other than controls received hesperidine methyl chalcone by the intragastric route at the same time. Data are expressed as % of I^{131} given per g of blood, liver, kidney, and skin, wet wt, and % per organ for thyroid and stomach plus contents. Five rats in each group.

	Control	mg H.M.C. administered		
		10	50	250
Blood	.11 ± .03*	.17 ± .04	.23 ± .08	.25 ± .03
Liver	.11 ± .02	.12 ± .02	.16 ± .04	.18 ± .02
Kidney	.08 ± .01	.12 ± .02	.22 ± .08	.18 ± .03
Stomach	1.25 ± .27	3.6 ± .11	3.5 ± .45	3.5 ± .8
Skin	.19 ± .01	.34 ± .09	.31 ± .08	.29 ± .04
Thyroid	16 ±1.2	25 ±2.1	20 ±3.5	21 ±1.9
Remains	.01	.01	.04	.02
Urine	67	53	44	48

* All values are means ± stand. error = $\sqrt{\frac{\Sigma\, dov^2}{(N)\ (N-1)}}$.

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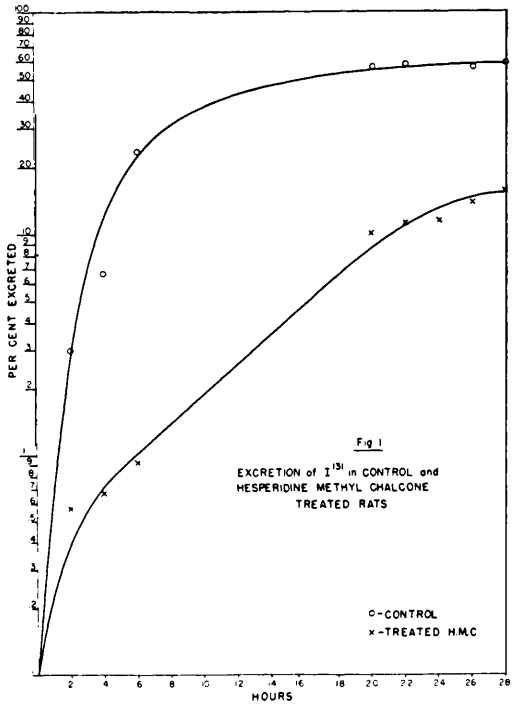
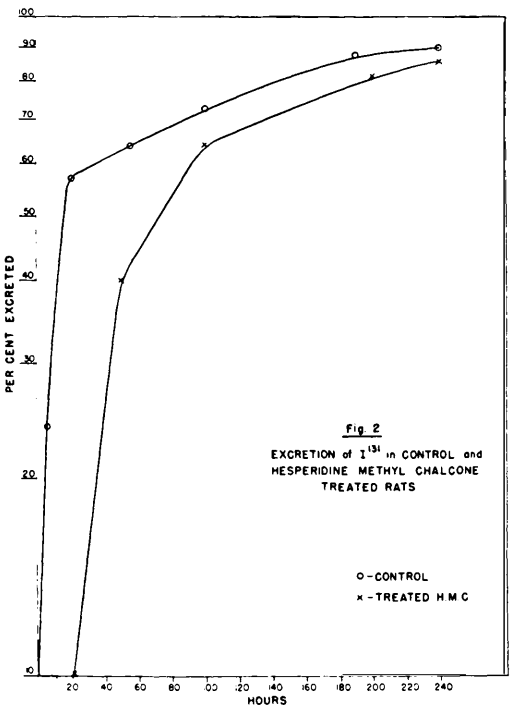




observed in the controls (Table I). A depression of the excretion of the I^{131} was observed in the H.M.C.-treated animals, as can be seen from Table I. Additional data for other tissues are also shown in Table I. When H.M.C. was given as a subcutaneous injection at the same time that I^{131} was administered intravenously, an even greater depression of the urinary excretion of I^{131} was observed. Urine samples taken at various time periods up to 240 hours after the administration of the drug and the radioactive tag showed that the urinary excretion of I^{131} was markedly diminished during the first 24 hours, as shown in Fig. 1. Following this time period, the treated animals exhibited I^{131} excretion rates which were similar to those of the controls. The relative excretion rates of I^{131} by the control and H.M.C. animals throughout the entire time period studied are shown in Fig. 2. The excretion curves presented suggest that the H.M.C.-treated animals did not catch up with the controls at time periods as long as 240 hours after the initial tagging of the iodide pool with I^{131}. The lag in excretion of the H.M.C.-treated animals resulted in an increase of the I^{131} content of thyroid, skin, and balance which was 185, 375, and 200% greater than normal respectively, 240 hours after the administration of I^{131}.

When 5 mg of sodium iodide was administered to rats as a carrier along with the I^{131} which was essentially carrier free, the depression of iodine excretion as observed with I^{131} was still obtained. For example, at 2, 5, 8, 24, 32, and 48 hours after the administration of I^{131} plus carrier, the controls excreted an accumulated total of 11, 32, 42, 87, 91, and 95% of the dose, respectively. When rats were given 80 mg of the H.M.C. subcutaneously, the excretion was found to be 1.4, 5, 5.4, 83 and 96% respectively for the time periods stated above.

The repeated administration of H.M.C. at 12- or 24-hour intervals did not depress the excretion of I^{131} indefinitely. These data suggest that hesperidine methyl chalcone can effect only a transitory depression of iodide excretion.

When diiodoI^{131}tyrosine was administered to rats which were given H.M.C. subcutaneously at dosage levels shown in Table II, a depression of urinary excretion of the I^{131} tag was observed which was similar to that

TABLE II. Fate of DiiodoI^{131}tyrosine in Rats 15 Hr after i.v. Administration. Rats other than controls received hesperidine methyl chalcone subcut. at the same time. Values are given as % of dose per g wet wt of all tissues except thyroid and stomach; these are reported as % per organ.

	Control	mg of H.M.C. per rat				
		10	20	50	80	240
No. of rats in group	30	5	5	5	5	5
Blood	.08 ± .01	.36 ± .09	.44 ± .05	.42 ± .21	.25 ± .04	.21 ± .03
Liver	.08 ± .01	.28 ± .05	.34 ± .04	.37 ± .04	.32 ± .02	.33 ± .09
Kidney	.17 ± .02	.39 ± .03	.54 ± .06	.67 ± .03	.55 ± .03	.48 ± .05
Stomach + cont.	1.7 ± .71	3.1 ± .48	7.3 ±1.1	6.5 ±1.3	12 ± .25	29 ±3.9
Skin	.17 ± .02	.59 ± .03	.72 ± .06	.91 ± .20	.81 ± .11	.55 ± .05
Thyroid	12 ± .61	25 ±1.4	14 ±1.7	13 ±2.2	8.8 ±1	4.9 ± .05
Remains*	.012± .0003	.04	.04	.04	.05	.04
Urine	69.6 ± .45	10.5	9.5	5	7.8	3.5

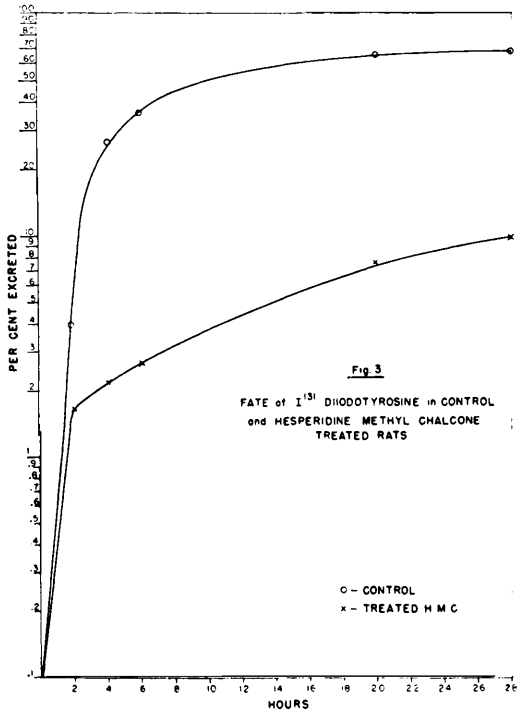
* In groups receiving H.M.C., remains and urine were measured for the group and not separately. Hence, statistical evaluation was not possible.

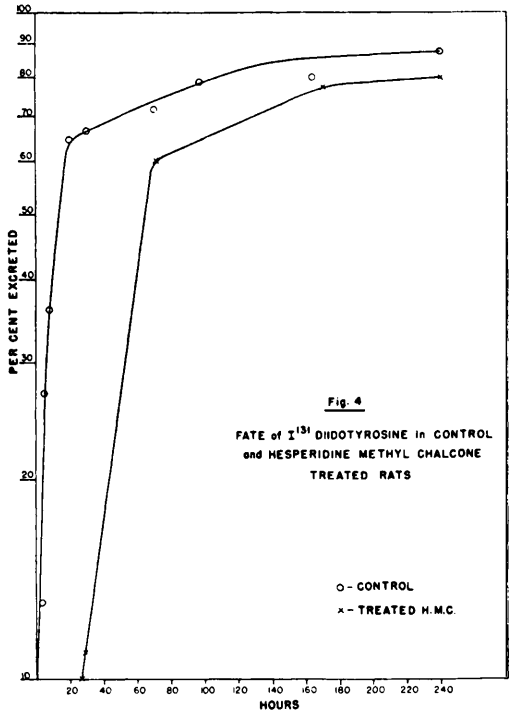
seen with inorganic iodide. The thyroid uptake of the diiodoI^{131}tyrosine was almost twice as great as normal in the group receiving 10 mg of H.M.C., whereas the higher dose levels showed only slightly less than normal uptake and finally, at the highest dose level, the thyroid uptake was depressed. A maximum uptake for skin was observed at the 50 mg dose level, although the H.M.C.-treated animals showed an uptake which was greater than normal at all dose levels. Hesperidine methyl chalcone caused an increase in the I^{131} tag of diiodotyrosine which was found in the stomach and its contents. The increase observed above normal appeared to be directly related to the amount of the drug given. The diiodoI^{131}tyrosine levels of other tissues were significantly increased when H.M.C. was administered, as shown in Table II.

Rats which were given 80 mg of H.M.C. in addition to diiodoI^{131}tyrosine and sacrificed 240 hours later showed a preliminary depression of excretion during the early time periods (Fig. 3 and 4). The tissue of these animals when sacrificed contained more of the original tag than the controls. This effect was reflected primarily by higher uptakes which were observed in thyroid and skin. The thyroids of the controls contained 2 ± .82% of the dose, whereas the thyroids of the H.M.C.-treated rats contained 3.4 ± .61% of the dose. The difference between these 2 groups appears to be significant. A comparison of the means of the 2 groups gave a p value of less than .01.

In an effort to gain additional information concerning the mechanism whereby H.M.C. reduces the excretion of iodide, studies were made upon the relative concentrations of I^{131} present in the red cells of normal and H.M.C.-treated rats; this result was compared to the I^{131} plasma concentration. As shown by one of us(2), this test may be employed to determine the degree of binding of iodide into a molecule which may be too large to penetrate the red cell membrane. Thus the ratio of the iodide space in the red cell of the rat compared to that of plasma when all of the I^{131} is present as iodide, was found to be 0.76 ± 7% in the control animals used. Similar measurements upon blood samples drawn from rats previously treated with 80 mg of H.M.C. gave ratios of 0.74 ± 7%. These results indicated that the H.M.C. did not bind the inorganic iodine into a complex which was too large to penetrate the red cell membrane. The greatly reduced urinary excretion which was observed can not be explained by virtue of the fact that the iodide is bound in a molecule which is too large to escape filtration through the glomerular membrane.

One dimension water chromatograms of varying amounts of iodide and hesperidine methyl chalcone revealed that these 2 substances appear to combine in water solutions. Using I^{131} as a tag, the movement on paper for iodide differed from that of hesperidine methyl chalcone plus iodide. An examination of the chromatogram with ultraviolet light revealed that iodide plus H.M.C. formed





fluorescent compounds which had R.F. values which differed from that seen for H.M.C.

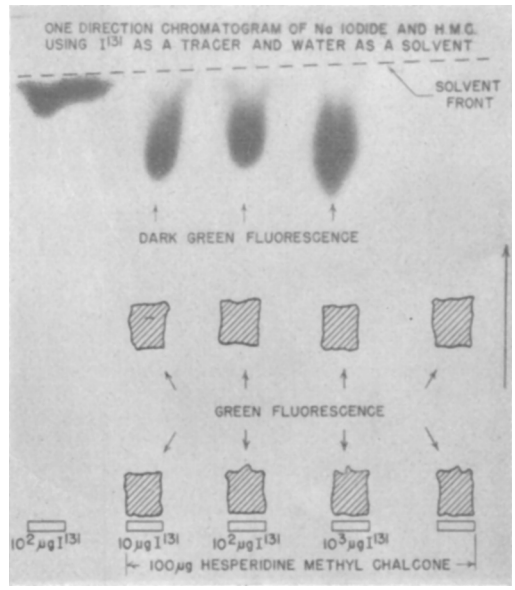


FIG. 5.

alone. The chromatogram is shown in Fig. 5.

Studies with other bioflavones and related compounds, such as sodium hesperidine chalcone, eriodictin, and sodium rutin revealed that these substances were able to influence the metabolic pattern of a tagged dose of I^{131} in a manner similar to that observed with H.M.C. These data are summarized in Table III.

It has been postulated by Parrot *et al.*(3), and Wilson *et al.*(4) that the bioflavones exert a sparing action upon epinephrine in the body. In these studies it was found that the administration of ephedrine, which blocks the destruction of epinephrine by amine oxidase(5), affected the fate of diiodoI^{131}tyrosine in a manner similar to H.M.C. For example, rats given 50 mg of ephedrine in 6 ml of saline solution subcutaneously, along with an intravenous dose of diiodoI^{131}tyrosine, and sacrificed 15 hours later showed a urinary excretion of the I^{131} tag which was only 12.2% of normal. Values for skin, thyroid, and stomach plus contents were observed to be 300, 75, and 1500% of normal respectively. When epinephrine itself was administered to rats, a reduction of the excretion of the I^{131} tag of diiodoI^{131}tyrosine was observed and was 48% of normal. Values for skin, thyroid, and stomach plus contents were observed to be

TABLE III. Distribution of I^{131} 15 Hr after Intrav. Administration in Rats Treated with Various Flavones. Values given are presented as % of uptake observed in normals. 80 mg of the flavone listed were given to each rat in 6 ml of solution. Five animals in each group.

	Rutin	Hesperidine chalcone	Eriodictin
Skin	415	415	505
Thyroid	91	74	74
Stomach	425	920	670
Urine	28.5	17	21

230, 44, and 830% of normal respectively.

Discussion. These studies show that the bioflavone derivative, hesperidine methyl chalcone, is able to depress the excretion of iodide by the rat. The administration of a dose of carrier sodium iodide, which was approximately 2,500 times greater than the normal total thyroid iodide content(6), with the I^{131} gave similar results. Hesperidine methyl chalcone was not able to block the excretion of iodide indefinitely, since repeated doses showed no greater effect upon iodide excretion than one single dose. Presumably, because of the depression of excretion of iodide, an increased uptake of I^{131} by the thyroid and skin was observed when H.M.C. was given orally. No essential difference was observed between doses of 10 mg or 250 mg. These data suggest that only small amounts of H.M.C. are absorbed by the intestinal tract. Paper chromatograms of iodide and H.M.C. suggest that bioflavones alter the metabolic pattern of iodide in the body by acting as an iodine "trap".

Summary. Studies with bioflavones such as hesperidine methyl chalcone showed that these compounds are capable of altering the thyroidal accumulation of I^{131} and of diiodoI^{131}-tyrosine. The intragastric administration of various amounts of H.M.C. caused an increased uptake of I^{131} or I^{131}-tagged material by the thyroid and skin. A reduction in the I^{131} excreted was observed. When H.M.C. was administered subcutaneously, the excretion of I^{131} was even more markedly reduced. Studies with other bioflavones such as sodium hesperidine chalcone, eriodictin, and sodium rutin showed these compounds to effect iodine metabolism in the same manner as H.M.C. These studies suggest that the effects observed with H.M.C. upon iodine metabolism may be related to its epinephrine sparing action when present in small amounts. The administration of large amounts of H.M.C. depresses thyroidal accumulation of I^{131}. Paper chromatograms indicate that larger amounts of H.M.C. in the body act as an iodide "trap" and thereby prevent the accumulation of I^{131} by the thyroid.

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