seasonal influences.

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Effect of Butazolidin[R]* upon the Fate of I^{131} in the Rat. (20049)

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During the course of the evaluation of other drugs upon the thyroidal accumulation of iodine in patients, it was observed that a patient being given butazolidin[R] had a very low I^{131} thyroid uptake. In this study rats were used in order to measure more completely the effect of this drug upon iodine metabolism.

Methods. Young albino rats were given butazolidine[R] by subcutaneous injection at dosage levels ranging from 8 to 200 mg/kg body weight. In some groups of animals this was followed immediately by a tracer dose of 5 μc of I^{131} intraperitoneally. No additional iodide was added in these studies; thus the administration of the I^{131} served only to label the iodide pool of the body. The total amount of iodide present in 5 μc of I^{131} is calculated to be less than 1×10^{-3} μg. As will be noted in Table I, the uptake of I^{131} by control animals varied as much as 100%. Although the experimental and control animals for each group are of the same lot and origin, both Sprague Dawley and Slonaker strains of rats were employed in the several experiments combined in Table I. In our hands, Sprague Dawley rats recently received from Wisconsin have much higher thyroid uptake of I^{131} than that observed in rats bred locally. We believe that this merely represents a smaller iodide pool in rats imported from the Midwest since this higher uptake is reduced to that seen in local rats after a period of several weeks in their new environment. These animals were sacrificed 20 hours following I^{131} administration. Other groups of rats were given the drug daily for periods of 10 to 16 days in which the last injection of the drug was followed by 5 μc of I^{131}. After sacrifice selected organs of all of the rats were measured for I^{131} with a scintillation counter in which the error in measurement was less than $\pm 4\%$. The geometry of the scintillation counter was such that 8% of all of the γ disintegrations occurring in the sample was observed as counts. The beta particles associated with I^{131} decay were completely absorbed with a 200 mg cm^{-2} aluminum filter and were not seen by the counter. Because of this, the wet tissues were counted directly for I^{131} by placing them in metal dishes 5 cm from the crystal of the counter. Corrections for mass absorption or small differences in geometry were not necessary when the samples were counted in this manner. The tissues of animals which were given butazolidine[R] for longer periods of time were also examined for pathological changes and compared to the controls.

Results. The data as summarized in Table I show that the administration of butazolidine[R] once to rats will reduce the amount of a test dose of I^{131} accumulated by the thyroid. The degree of reduction is directly related to the quantity of the drug given. More of the I^{131} given was excreted in the urine of the animals receiving the drug than by the controls. This amounted to 167, 171, 191, 400% of normal for the 1, 4, 10 and 20 mg dose levels respectively. Relatively less of the I^{131} tag was observed in plasma, kidney, GI tract plus contents, liver and skin in the group receiving 20 mg of the drug and it was observed to be about 50% of normal as shown in Table II.

* Butazolidin[R] is 3,5-dioxo-1,2-diphenyl-4-n butyl-pyrazolidin. Butazolidin[R] was synthesized and supplied by Geigy Co., New York.

TABLE I. % of I^{131} Taken Up by Thyroid in Control Rats and in Rats Receiving Amounts of Butazolidin^R Shown Below. 5 rats in each group.

	mg to each rat				
	1	4	10	20	40
% in thyroid	10 ± 2.6*	5.4 ± 1.6	1.5 ± .1	1.8 ± .4	.33 ± .02
Avg body wt	119	126	119	207	190
	Control rats for each group				
% in thyroid	42 ± 1.7	42 ± 1.7	42 ± 1.7	44 ± 3.2	21.4 ± .22
Avg body wt	120	120	120	201	188
Origin of rat	S.D.†	S.D.	S.D.	S.D.	Slonaker

* Stand. mean error $= \pm \sqrt{\frac{\Sigma \text{ dev}^2}{n(n-1)}}$.

† Sprague Dawley.

TABLE II. Comparison of Relative I^{131} Concentration in Tissues of Rats Receiving One Dose Butazolidine^R and 10 Doses of the Drug over a 10 Day Period. Animals sacrificed 24 hr after I.P. administration of I^{131}. 5 rats in each group.

	% of normal tissue uptake	
Tissue	20 mg butazolidin^R once, %	2 mg butazolidin^R daily, 10 days, %
Plasma	46	140
Kidney	52	133
G.I. tract	41	160
Liver	50	129
Skin	60	134

The balance of the rat which was composed of muscle primarily contained as much of the I^{131} tag as the controls.

When rats were given 2 mg of the drug daily for a period of 10 days and in another case 5 mg daily for 16 days, the thyroid uptake of I^{131} returned to almost normal limits. For example, in the former group the controls took up 22.2 ± 2.2% and the treated rats 19.4 ± 3.1%. In the latter group receiving 5 mg of the drug daily, the controls took up 23 ± 1.7% as compared to an uptake of 23 ± 2.6% for the treated animals.

The administration of butazolidin^R for a period of 10 days resulted in an increase of the I^{131} tag in the tissues of the rats which was observed to be 30 to 60% greater than the controls and in contrast to the lower tissue concentration of I^{131} observed when the drug was administered once (Table II).

An examination of the thyroid, bone marrow, liver, lungs, heart, spleen, muscle, ureter, adrenals, retroperitoneal fat, spinal cord, thymus, and pancreas for histological changes revealed no changes which could be attributed specifically to the butazolidin administered.

A one dimensional water chromatogram using distilled water with increasing amounts of iodide plus I^{131} suggests that butazolidin^R is capable of combining directly with iodine as iodide (Fig. 1). The mode of action of the drug in preventing iodide accumulation by the thyroid appears to be of the nature of a competition for iodide in the body.

Discussion. These data suggest that the drug butazolidin^R can alter the manner in which iodide is normally handled in the body. *In vitro* studies with chromatograms suggest that this presumably is due to the ability of the drug to combine with iodide in the body. By

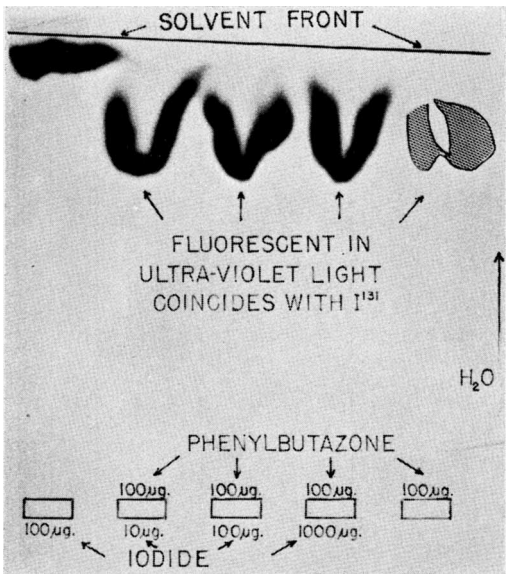

FIG. 1. A chromatogram of iodide and butazolidin^R plus iodide using I^{131} as a tracer and water as a solvent.

virtue of this capture, thyroidal accumulation of iodide and tissue content of iodide is reduced to a level much lower than normal. When the drug is administered daily, "a steady state" of drug concentration is achieved in the body. The presence of the drug in the tissues of the body increases their iodine concentration to values which are greater than normal. When the iodine affinity of the drug remaining in the body is satisfied, then thyroidal accumulation of iodine returns to normal levels.

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Diffusibility of Dyes in Buffered and Unbuffered Solutions.* (20050)

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In aqueous solutions the rate of diffusion of a substance depends primarily on its concentration gradient. In biological fluids the presence of buffers and other cell constituents may have a significant influence on the rate of diffusion. The purpose of this investigation was to compare the diffusion of basic dyes in aqueous medium, and in acid buffer. The acetate buffer was chosen because most dyes are relatively soluble in this buffer.

Method. The two half-cells were made from centrifuge tubes. Dimensions: 6.0 cm high and 2.5 cm in diameter with a side arm 1.0 cm in diameter and 1.0 cm long at a distance of 1.0 cm from the bottom of the main tube. The cellophane membrane (Eimer and Amend 8-667) was clamped between 2 rubber stoppers of 1.0 cm thickness with a bore of 0.9 cm. The side arms of the half-cells were then inserted into the rubber stoppers. Each half-cell contained either 15 ml of dye (1×10^{-4} M) in acetate buffer (pH 5.0) or water. The intensity of the diffused dyes was measured at appropriate intervals in a Beckman Spectrophotometer (Model DU). For each dye there was determined a standard concentration curve in order to transform the observed optical densities into micrograms. The *cell constant* was determined by allowing a 0.1 N HCl solution to diffuse into water. The diffused acid was measured with a Beckman pH-meter, or titrated with 0.01 N NaOH using phenolphthalein as an indicator. Ohölm(1) found that the diffusion coefficient "D" for 0.1 N HCl at 5° = 1.85 cm^2/day. The cell constant $K = D \times t/Q$. Q equals the number of ml of diffused acid expressed in units of the original concentration (the latter having a unit value of 1), and t equals time. It was found that 0.238 ml of 0.1 N HCl diffused during 30 minutes. Therefore, $K = 1.85 \times 0.0208/0.238 = 0.162$. K represents the ratio h/A, h is the effective distance through which diffusion takes place. A is the effective area of the surface available for diffusion. The diffusion coefficient $D = K \times Q/t$. The values for D are given in Tables I and II, t = 1 day. Q = micromoles (1×10^{-6} M) of diffused dye in 24 hours. In the first group of experiments the dyes were dissolved in water and allowed to diffuse into water. In the second group, the dyes were dissolved in acetate buffer (pH 5.0) and allowed to diffuse into the same buffer. In the third group there were four sections. Table III: a) The buffer in the half-cell (A) which contained the dye was diluted while that in half-cell (B) remained constant; b) the buffer in both half-cells was diluted simultaneously; c) the buffer in half-cell (A) was diluted with 10% water; and d) the concentration of the dye was varied in both water and buffer. The results are reproducible within 2-5% depending on the tinctorial power of the dye.

Results. In Table I we find the dyes arranged in decreasing order of their diffusibility "D" in water. "Stomach/blood" is the ratio of the concentration of the dye found in the

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