changes color only in the presence of the metal. In neither type does the histochemical demonstration of the metal require reprecipitation; thus, it may be possible to stain the metal without removing it from its naturally bound site.

Meurexide is a colored chelating agent that has been used as an indicator in the analysis of calcium in aqueous solution (*3*). Both the free compound and the calcium chelate have similar solubilities in water, and when calcium is added to a saturated solution of meurexide, no precipitation occurs. If used as a calcium stain, the meurexide reaction is of type 1; that is, it is adsorbed and bound only in the presence of the metal, staining the calcium an orange-red and leaving the background a nearly neutral color. No diffusion is apparent with periodic visual examination for at least 1 hr.

Procedure. (i) Remove paraffin from section with xylol; (ii) hydrate by bathing in two solutions of absolute alcohol, then 95-percent alcohol, and then 80-percent alcohol; (iii) stain 5 min in a solution that is both 0.1*N* in NaOH and 0.1*N* in KCN and is saturated with meurexide (*4*); (iv) wash, briefly either in distilled water or in 50-percent alcohol, so as not to decolorize excessively; (v) dehydrate and mount in Harleco Synthetic Resin (*5*).

Specificity. The stability constants of most of the meurexide-metal chelates are unknown (*1, 3*). Comparisons of serial sections stained for iron and calcium by various methods indicate that iron is complexed by the cyanide in the staining solution and does not itself stain. It seems likely that copper might be similarly bound, since it is known that present analytic determinations of calcium in water and serum using meurexide and a cyanide solution are not interfered with by the afore-mentioned metal in appreciable amounts.

Comparison with other methods. Areas stained by the Von Kossa method (silver stain for phosphate) stain with meurexide (except in areas where the phosphate is known to be present as iron phosphate). Areas that did not appear colored in the meurexide or silver-treated sections were stained by the alizarin precipitate method (*6*), but the precise role of greater specificity as opposed to less sensitivity has not been investigated. Conclusions as to the sensitivity of the meurexide stain based on the use of meurexide as an indicator in solution were not thought justified. Quantitative data on the color change of the indicator in solution may not be related to the adsorption of the dye on the metal in tissue section and may be misleading.

In addition to the afore-mentioned type-1 stain, preliminary work indicates that a substance such as Eirochrome Schwarz T may be usable as a type-2 histologic stain (*7*). Although areas of calcification appeared a midnight blue and the background remained a bright green with Eirochrome Schwarz T, this particular agent was less satisfactory than the meurexide for histologic work since the cellular detail was obliterated by the depth of the background stain. Work is in progress, however, for testing it as a magnesium stain for the magnesium ribonucleate complex of gram-positive organisms, and thus an alternate for the gram stain.

The use of water-soluble chelating agents in histochemical staining may eliminate some of the theoretical disadvantages of methods employing reprecipitation and may permit the use in histochemistry and pathology of a whole group of relatively unexplored compounds, of which meurexide is only one. Perhaps similar agents may make the localization of many different metals possible and aid the study of their metabolism and distribution.

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References and Notes

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2. We wish to thank Arthur T. Hertig whose interest and encouragement made this work possible.
3. G. Schwartzenbach, W. Biederman, and F. Bangerter, *Helv. Chim. Acta* **29**, 811 (1946); G. Schwartzenbach and H. Gysling, *ibid.* **32**, 1108 (1944); G. Schwartzenbach and H. Gysling, *ibid.* **37**, 1314 (1949).
4. Original meurexide was obtained from C. E. Bricker, Department of Chemistry, Princeton University. Subsequent meurexide stain, Calcium Indicator Capsules, was obtained from Hagan Corp., Pittsburgh, Pa.
5. Harleco Synthetic Resin, a mounting medium made by Hartman Leddon Co., Philadelphia, Pa.
6. L. K. Dahl, *Proc. Soc. Exptl. Biol. Med.* **80**, 474 (1952).
7. Eirochrome Schwarz T and information on its use were obtained from Bersworth Chemical Co., Framingham, Mass.

30 July 1954.

Aureomycin and the Thyroid Gland

A recent report (*1*) suggested that the growth-stimulating properties of antibiotics (*2*) were attributable to an antithyroid effect. The present study constituted a repetition and extension of this report (*1*) and failed to confirm any antithyroid effect of the antibiotic Aureomycin (*3*) chlortetracycline. After our manuscript had been submitted, findings similar to ours by Libby and Meites appeared (*4*).

In a preliminary trial (Table 1), Aureomycin, 1 mg/kg of the diet, and propylthiouracil, 2 percent of the diet, were used with two different diets to repeat

Table 1. Effect of Aureomycin on thyroid weight.

Diet	Addition per kg of diet	Thyroid weight (mg/100 g body weight)	"p" values
1	None	7.5* ± 0.3	
1	1 mg Aureomycin	6.6 ± .5	0.3
1	20 mg Aureomycin	9.2 ± .3	.05
2	None	9.8 ± .4	
2	1 mg Aureomycin	11.1 ± .2	.1
2	20 mg Aureomycin	9.4 ± .1	.6

* Mean ± S.E.

Table 2. Effects of Aureomycin and propylthiouracil on thyroid weight and on uptake of I^{131} in rats.

Diet	Addition per kg of diet	Body weight (means g)	Thyroid weight (means g)	Mean thyroid weight (mg/100 g body weight)	"p" values	24 hr	"p" values	48 hr	"p" values
1	None	224	15.9	7.1* ± 0.4		130* ± 7		82* ± 10	
1	1 mg Aureomycin	222	13.7	6.2 ± .4	0.3	141 ± 15	0.05	83 ± 9	0.8
1	20 mg Aureomycin	235	14.5	6.2 ± .5	.3	191 ± 27	.001	97 ± 13	.02
1	Propylthiouracil	86	33.5	38.7 ± 1.3	.001	54 ± 9	.001	46 ± 8	.001
2	None	185	16.8	8.6 ± 0.5		221 ± 21		215 ± 17	
2	1 mg Aureomycin	208	14.9	7.2 ± .8	.2	266 ± 14	.001	208 ± 20	.3
2	20 mg Aureomycin	207	16.3	8.1 ± .4	.6	259 ± 17	.002	199 ± 12	.02
2	Propylthiouracil	152	63.7	41.6 ± 9.0	.001	8.2 ± 0.6	.001	1.8 ± 0.9	.005

* Mean ± S.E.

the earlier study (*1*). Diet 1 contained ground yellow corn, soybean meal, corn gluten meal, fish meal, alfalfa meal, distillers' solubles, vitamin concentrates, and minerals; diet 2 was Pratt's Nurishmix. Both diets contained adequate iodine and permitted rapid growth and development. Eight weanling rats were used in each group and were maintained for 42 days. In no case did Aureomycin produce significant changes in the weight of the thyroid gland after 42 days of feeding. The rats fed propylthiouracil at 2 percent of the diet, as used by the Hahnemann group (*1*), lived only a week or two and are thus not included.

A second trial was made with the same diets and supplements using 10 rats per group. Propylthiouracil was added at levels of 0.2 and 0.02 percent of the diet, and the animals survived the entire period. After 42 days of feeding, 5 μc I^{131} was injected intraperitoneally. Half of the animals were sacrificed 24 hr later, and the radioactivity of the excised thyroid glands was measured by a scintillation detector; 48 hr after the injection, the remaining animals were similarly treated. The results are shown in Table 2. The addition of Aureomycin did not cause a change in the thyroid weights of any groups. The striking increase in the weight of the thyroid glands produced by propylthiouracil confirms much earlier work.

The results of this study differ from those of the other report (*1*), not only in degree, but in direction. This group reported a threefold thyroid weight increase and a fourfold I^{131} uptake decrease following 1 mg/kg of the Aureomycin diet. In our hands, 20 times that level of Aureomycin failed to produce a significant change in gland size and actually caused a small but definite increase in the uptake of I^{131} by the thyroid gland. The effect of antibiotics on the thyroid gland is being studied further.

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References and Notes

1. B. Calesnick, W. D. Harris, and R. S. Jones, *Science* **119**, 128 (1954).
2. T. H. Jukes and W. L. Williams, *Pharmacol. Revs.* **5**, 381 (1953).
3. Aureomycin is the trademark of the American Cyanamid Company for the antibiotic chlortetracycline.
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1 July 1954.

Association Affairs

Pullman Meeting of the Pacific Division

The Pacific Division of the American Association for the Advancement of Science held its 35th annual meeting, 21–26 June 1954, on the campus of the State College of Washington at Pullman. Seventeen societies participated in a program of 315 scientific papers. Arrangements for the meeting were ably handled by a local committee and various subcommittees, under the general chairmanship of Adolph Hecht.

This was the second Pullman meeting of the Pacific Division, the first having been held in 1932. Persons who had the pleasure of attending both of these meetings, a little more than two decades apart, were greatly impressed with the growth of the State College of Washington, the expansion of its physical plant, and the continuous improvement of its facilities for both teaching and research.

Registration headquarters were in the Wilson Compton Union Building, an impressive and commodious student union building that was recently completed, with a cafeteria, dining rooms of assorted sizes for large and small groups, residence quarters where a number of delegates were housed, and ballrooms that can be used for social gatherings in the evening and can be divided during the day by movable partitions to serve as meeting places for smaller gatherings.