thiouracil it may very well be that the oxidation of At^0 to AtO^- is prevented permitting the continued accumulation and retention of astatine in some type of loose organic binding.

Summary. A study has been made of the accumulation of I^{131} and At^{211} in normal and propyl thiouracil-treated rats. A very marked enhancement of the accumulation of At^{211} in the thyriod gland has been observed following administration of propyl thiouracil. This is in contrast to the diminution of the uptake of I^{131} by the thyroid glands of the rats receiving propyl thiouracil.

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1. Taurog, A., Chaikoff, I. L., and Feller, D. D., *J. Biol. Chem.,* 1947, v171, 189.

2. Astwood, E. B., *Ann. N. Y. Acad. Sci.,* 1949, v50, No. 5, 419.

3. Lahey, F. H., and Bartels, E. C., *Ann. Surg,* 1947, v125, 572.

4. McCullagh, E. P., *Ohio State Med. J.,* 1950, v46, 127.

5. Johnson, G. L., Leininger, R. F., and Segré, E., *J. Chem. Phys.,* 1949, v17, 1.

6. Hollander, J. M., Perlman, I., and Seaborg, G. T., *Rev. Mod. Phys.,* 1953, v25, 469.

7. Hamilton, J. G., and Soley, M. H., *Proc. Nat. Acad. Sci.,* 1940, v26, 483.

8. Hamilton, J. G., Asling, C. W., Garrison, W. M., and Scott, K. G., *Univ. Calif. Pub. Pharmacol.,* 1953, v2, No. 21, 283.

9. Hamilton, J. G., Durbin, P. W., and Parrott, M. W., submitted for publication.

10. Shellabarger, C. J., and Godwin, J. T., 1954, Am. Goiter Assn., submitted for publication.

11. Durbin, P. W., Hamilton, J. G., and Parrott, M. W., submitted for publication.

12. Fisher, R. A., *Statistical Methods for Research Workers,* 1950, Oliver and Boyd, Edinburgh.

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Localization of Antimony in Blood. (21102)

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This study is an outgrowth of an observation by Andrews and Hamilton of this laboratory. Rats to which they gave a single intravenous injection of fuadin labeled with Sb 122, 124 showed a relatively high concentration of the antimony in the erythrocytes. The concentration tended to be maximal in approximately 8 days. Earlier workers had noted high concentration of antimony in red cells following administration of tartar emetic and other antimonial drugs(1,2). Several alternative hypotheses, which might be offered to account for these observations, have been explored. The results obtained are consistent with the hypothesis that the antimony in the red cells is attached to the protein portion of the hemoglobin.

Methods. The labeled antimony catechol disulfonate complex (fuadin) for this work was prepared as follows: A quartz ampoule containing 75 mg of Sb_2O_3 was irradiated in the Oak Ridge pile 1 week or more. The ampoule was transferred to a holder behind a lead screen, the neck was broken off, and 0.5 ml of concentrated HCl was introduced by means of a pipette. The mixture was stirred 10 minutes by a slow stream of air bubbles from a capillary tube. A solution of 0.63 g of sodium catechol disulfonate† in a little water was added while stirring was continued. These quantities provided an excess of the complexing agent. The solution was washed into a graduated test tube, neutralized with 10% NaOH to pH 7, and was

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diluted to 25 ml with water.

Results. The apparent increase of the relative concentration of antimony in the red cell fraction with time up to 8 days suggested that the antimony found in the red cells may have entered during their development. The following experiments, however, show that this is not the only way in which antimony can enter the red cell fraction. Two ml of a dilute solution of labeled fuadin in 0.9% NaCl, 10 ml of 0.9% NaCl, and 1 ml of whole, fresh, unheparinized rat blood were shaken 3 minutes. After standing 45 minutes at room temperature, the mixture was centrifuged 30 minutes. The counting of an aliquot portion of the clear supernatant liquid showed that over 10% of the antimony had been removed beyond the effect of mere dilution. It seemed possible that reaction of the antimony with the plasma protein might have formed an insoluble precipitate, which was thrown down by centrifuging. However, a 4.7 ml portion of the plasma was mixed with 5.4 ml of 0.9% NaCl and 2.0 ml of the dilute fuadin solution, allowed to stand 30 minutes, centrifuged and filtered. An aliquot portion of the clear filtrate showed only 2% reduction in counts per minute, which was well within the limit of error of the counting equipment.

The next hypothesis examined was that the antimony is removed from the solution by becoming adsorbed on the surface of the red cells. Various quantities of a suspension of repeatedly washed rat red blood cells were mixed with 2.0 ml portions of the fuadin solution, diluted to 11 ml with 0.9% NaCl, shaken 15 minutes, and centrifuged 20 minutes. The results shown in Table I indicate that, although there may have been some removal of antimony by the cells, it was not so rapid as would be expected for surface adsorption.

Another experiment demonstrated that removal of antimony by the red cells is marked when more time is allowed. In each of 2 centrifuge tubes, 3.0 ml of defibrinated rat blood was mixed with 10 ml of 0.9% NaCl containing a trace of fuadin and was allowed to stand at room temperature with occasional gentle agitation. In the tube that was centrifuged at the end of 2 hours the ratio of the counts per minute per ml of cells to the counts per minute per ml of supernatant liquid was 4.9. At the end of 6.7 hours the ratio was 6.2. These results were taken as an indication that the antimony penetrates the cells and becomes attached at a moderate rate of speed.

TABLE I. Removal of Antimony from Fuadin Solution by Short Contact with Red Cells of the Rat.

Quantity of cell suspension, ml	c/min.
.0	3531
.1	3520
.65	3305
1.6	3356
6.0	3343

A 386 g white, male Wistar rat, which 10 days previously had received 1.2 ml of fuadin solution, was sacrificed. A sample of the blood was defibrinated and centrifuged. The red cells were washed repeatedly with 0.9% NaCl, then suspended in 0.9% NaCl solution, and laked by shaking with a little ether. Cell stroma were removed from one portion of the mixture by centrifugation and from another portion by mixing with aluminum hydroxide and filtering. The hemoglobin was then isolated. The activities of samples, counted after dilution to 10 ml, are shown in Table II. Wintrobe(3) gives 13.0 ± 1.2 as the % of hemoglobin in rat blood. Even if the lower figure, 11.8%, is assumed, the activity of the hemoglobin fraction was sufficient to account for all the activity in the defibrinated blood.

One further step was to check the possibility that antimony might have taken the place of iron in the hemin portion of the hemoglobin molecules. The method of Fischer(4) was used to isolate hemin from the pooled blood of 10 rats, which had received intravenous injections of fuadin 8 days before sacrifice. The activity of the hemin was found to be only 299 counts per minute per gram when counted dry, whereas the acetic acid mother liquor retained 2335 counts per minute per ml when an aliquot portion was counted by the usual liquid counting method. If all the antimony were held in the hemoglobin just as iron is held, the activity of the hemin fraction should be many times as great as that of the mother liquor. The antimony might

TABLE II. Antimony Content of Samples from Blood of Rat.

Sample	c/min./g
Defibrinated blood	8600
Plasma	65
Hemoglobin (aluminum hydroxide method)	73800
Hemoglobin (centrifugation only)	74100

have been loosely attached to the hemin, but it seems more likely that it was attached to the protein portion of the hemoglobin. An added reason for believing this is the fact that the red cells of the dog do not seem to take up the antimony nearly so avidly as those of the rat. Presumably there is no difference between the hemin of the rat and of the dog, but the protein portions of the molecules are different. Support by analogy is found in the fact that arsenic, a member of the same family of elements as antimony, when administered as sodium arsenite, has been shown to be taken up by the red cells of the rat and to go with the globin and acid-acetone soluble heme fractions(5).

Summary. Labeled fuadin prepared from neutron irradiated antimony trioxide has been used in a study of the localization of antimony in the red cell fraction of rat blood. The results are consistent with the hypothesis that the antimony is attached to the globin portion of hemoglobin.

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1. Ness, A. T., Brady, F. J., Cowie, D. B., and Lawton, A. H., *J. Pharmacol. and Exp. Therap.*, 1947, v90, 174.

2. Otto, G. F., and Maren, T. H., *Am. J. Hyg.*, 1950, v51, 353; *ibid.*, 1950, v51, 370.

3. Wintrobe, M. M., *Clinical Hematology*, Lea & Febiger, Philadelphia, 1949, 819.

4. Fischer, H., John Wiley & Sons, New York, *Organic Synthesis*, 1941, v21, 53.

5. Lowry, O. H., Hunter, F. T., Kip, A. F., and Irvine, J. W., Jr., *J. Pharmacol. and Exp. Therap.*, 1942, v76, 221.

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The Polysaccharides of *Candida albicans.** (21103)

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The presence of serologically active polysaccharides in organisms belonging to the genus Candida has held the attention of many investigators interested in devising serologic tests for the diagnosis of Moniliasis, and demonstrating cross reactions between Candida and other closely related yeast-like organisms (1-3). The present report studies the homogeneity of a *Candida albicans* polysaccharide preparation by quantitative immunologic technics.

Materials and methods. A strain of *Candida albicans*‡ giving typical chlamydospore formation on corn meal agar, sugar fermentation reactions consistent with the species, and requisite pathogenicity in the rabbit was selected for use in this work. The organism was cultured on Sabouraud's agar in Roux flasks at 30°C for 3 days. Growth was washed off gently with acetate buffer, pH 6.03, and adjusted to approximately 50% concentration by volume. The suspension was autoclaved at 15 lb pressure for 10 minutes and then centrifuged to recover the supernatant. Addition of one volume of 95% alcohol precipitated the polysaccharide and protein constituents, which after solution in distilled

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