reticulocytes, might contain protoporphyrin. In this connection it is of interest that the amount of non-respiratory iron porphyrin compounds was increased in immature red cells.[27] Furthermore, during liver-induced remission in patients with pernicious anemia, there has been observed a precipitous fall in serum iron values[28] which preceded, but was almost reciprocal with, the rise in reticulocytes.[29]

[27] Burmester, B. R., *Folia Haemat.*, 1936-37, **56**, 372.

[28] Heilmeyer, L., and Plötner, K., *Serumeisen und die Eisenmangelkrankheit*, G. Fischer, Jena, 1937.

Summary. The occurrence of protoporphyrin was studied in 133 sternal bone marrow specimens. Protoporphyrin was most regularly present in marrow samples containing predominately normoblastic young red blood cells.

Ninety-six sternal bone marrow specimens from 12 patients with Addisonian pernicious anemia before and after liver extract injection were obtained by serial sternal punctures at 24-hr intervals. The appearance of protoporphyrin was coincidental with the increase of immature red cells of normoblastic type in the marrow.

[29] Moore, C. V., Doan, C. A., and Arrowsmith, W. R., *J. Clin. Invest.*, 1937, **16**, 627.

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Blood Iodine in Dogs Receiving Thyroxin or Phlorhizin.

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Since an increase in the basal metabolic rate may be due to various causes, supplementary tests have long been sought which would aid in differentiating thyroid disease from other conditions in which a rise in metabolism occurs. The intimate connection between iodine metabolism and the thyroid suggests that blood iodine determinations might be of particular value for this purpose. Extensive use of microdeterminations of iodine in balance studies and blood analyses has enabled several investigators to extend our knowledge of iodine metabolism and of the course of thyroid disease.[1,2] The same studies also indicate some of the limitations to be expected of blood iodine determinations. Known cases of hyperthyroidism, following a period of mobilization and loss of thyroid iodine, may show normal blood iodine levels but retain their high metabolic rate. Thus there appears to be no immediate relationship between the concentration of iodine in the blood and the basal metabolic rate at a given time. Occurrence of high values for blood iodine in non-thyroid conditions has also been mentioned.[2]

It therefore appeared of interest to study the relationship between basal metabolic rate and blood iodine in dogs receiving injections of thyroxin or phlorhizin. The calorigenic response to thyroxin is presumably due to the injected hormone itself. Phlorhizin, on the other hand, although it produces its principal effect in both normal and thyroidectomized dogs, sharply increases the metabolic rate only if the thyroid is present.[3,4] Can involvement of the thyroid in this experimental condition be detected by determining blood iodine?

In the following experiments normal adult

[1] Curtis, G. M., and Puppel, I. D., *Ann. Surg.*, 1938, **108**, 574.

[2] Perkin, H. J., and Cattell, R. B., *Surg., Gynecol., Obstet.*, 1939, **68**, 744; *N. Y. State J. Med.*, 1936, **36**, 1033.

[3] Lusk, G., *The Elements of the Science of Nutrition*, 4th Ed., Philadelphia and London, 1931, W. B. Saunders Co.

[4] Gaebler, O. H., and Zimmerman, W. J., *Am. J. Physiol.*, 1939, **128**, 111.

female dogs, thoroughly accustomed to living in metabolism cages were used. The first group (Table I) received a stock diet of cracker meal, casein, corn oil, yeast, bone ash, and Karr's salt mixture[5] without added

TABLE I.
Blood Iodine of Dogs After Intravenous Injections of 10 mg of Thyroxin.

Hr after thyroxin	Blood iodine mcg/100 cc			
	dog 11 16.0 kg	dog 11	dog 32 12.8 kg	dog 35 13.9 kg
Control	1.0	3.1	2.1	2.1
"	2.1	2.1	2.1	1.1
0.5	159.		93.	200.
1	136.		88.	169.
2	134.	76.	73.	164.
4	99.	70.	56.	108.
8	59.		39.	86.
12	50.	40.	29.	64.
24	29.	29.6	19.1	40.
48		9.5	5.3	15.8
72	2.1	6.3	7.4	7.4
96		7.4	3.1	5.3
120		5.3	2.1	5.3
144		4.2		2.1
168		4.2		
192		3.1		

iodine. Crystalline thyroxin (Squibb) was carefully weighed in a 4 cc test tube, dissolved therein by adding a drop of 10% sodium hydroxide and water, transferred with rinsings to a 10 cc syringe, and injected into a leg vein. Blood samples were drawn from the jugular veins. Determinations of iodine were made by a method similar to that of Trevorrow and Fashena,[6] but after the initial oxidation the digest was transferred to a one-piece all glass outfit for distillation without aeration. The final reoxidation to iodate and titration of this were performed as described elsewhere.[7]

The rapidity with which amino acids disappear from the circulation when protein digests are injected intravenously is well known. In keeping with this the blood iodine during the first 3 min after injections of thyroxin never exceeded 302 mcg per 100 cc. Had the 10 mg of injected thyroxin been distributed uniformly through the circulation and remained there, the value should have exceeded 600 mcg per 100 cc of blood in these animals. It is however equally worthy of note that while half of the thyroxin was never detected and 90% of the remainder disappeared from the circulation in 24 hr the presence of an increased amount of iodine was detectable in the blood for 3 to 6 days.

In 2 highly trained animals the calorigenic action of 10 mg doses of thyroxin was determined, by using a mask[8] and the Tissot method. We are indebted to Miss Annette Moore for the numerous gas analyses. In 7 experiments the time curve of the calorigenic effect was essentially that previously reported by others.[9] It may therefore be stated that during the first 24 hr following intravenous injection of 10 mg of thyroxin the metabolic rate is rising rapidly while blood iodine is falling rapidly; during the second and third 24-hr periods the metabolic rate remains high, while blood iodine continues a rapid return to normal. There is thus no parallelism between blood iodine and the metabolic rate in such experiments.

TABLE II.
Blood Iodine of Dogs Before and During Phlorhizin Injections.

	Blood iodine, mcg per 100 cc		
	Dog 14	Dog 11	Dog 35
Before phlorhizin			
5 days			3.2
4 "	1.5		4.2
3 "	(4.2)		4.2
2 "	1.5	1.1	5.3
1 "	2.1	2.1	3.5
During phlorhizin			
1 day	2.1	1.1	1.1
2 days	1.5	1.1	5.3
3 "	2.1	0.9	1.1
4 "	2.1	1.5	3.2

Results obtained in phlorhizinized dogs are shown in Table II. These animals received 750 g of horse meat and 40 g of bone ash daily, so that the diet would be comparable with the meat diets used in earlier studies

[5] Karr, W. G., *J. Biol. Chem.*, 1920, **44**, 255.

[6] Trevorrow, V., and Fashena, G. J., *J. Biol. Chem.*, 1935, **110**, 29; 1936, **114**, 351.

[7] Gaebler, O. H., and Baty, M., *Ind. and Eng. Chem., Anal. Ed.*, 1941, **13**, 442.

[8] Gaebler, O. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 500.

[9] Wilhelmj, C. M., and Boothby, W. M., *Am. J. Physiol.*, 1930, **92**, 568.

on the effects of phlorhizin on the respiratory metabolism of dogs. Only 10 cc samples of blood were used, and since the blood iodine values are all at the normal level the titrations were too small for a high degree of accuracy. However, additions to blood of thyroxin equivalent to 10 mcg of iodine per 100 cc were recovered with an accuracy of over 90%. It is evident that no definite rise in blood iodine occurred in the phlorhizinized animals. This is especially significant if one considers the fact that the basal metabolic rate of intact dogs receiving this amount of phlorhizin increases 70%.[3,4] In cases of thyroid disease presenting such an elevation of metabolism the blood iodine may be increased 20 times or more.

Conclusion. Neither in normal dogs receiving 10 mg doses of thyroxin intravenously, nor in intact dogs receiving phlorhizin, is there any parallelism between the blood iodine level and the basal metabolic rate. The former result conforms with expectations, since the blood is not the site of action of thyroxin. The latter finding is more unexpected, since the increase in metabolism which phlorhizin produces in dogs is very large and is abolished by thyroidectomy.

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Effect of Direct Applications of Tyrothricin and Allantoin to Cells *in vitro*.*

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Recent interest in the direct application of sulfonamide crystals to wound surfaces and to body cavities has stimulated research on the effect of such treatment upon cells cultivated *in vitro*.[1,2] In spite of the higher resistance of embryonic cells in tissue cultures to the action of inhibitory agents, workers in this field hold that *in vitro* experiments offer stringent and easily controlled conditions for the analysis of drug toxicity.

In addition to the sulfonamides, a new class of compounds characterized by high bactericidal action is being derived from microörganisms. Of these, tyrothricin and its derivatives, are described as unsafe at present for general use, especially when brought in direct contact with the blood stream.[3,4] Tyrothricin, however, has been found not to interfere with the healing of wounds[3] and therefore may become useful in this direction.

Somewhat better known, allantoin, the chemical associated with the benefits of maggot therapy, has already proved useful at 2% concentrations in stimulating the granulation of chronic suppurative wounds.[5,6] Its action does not appear to be bactericidal and therefore it has been used in association with some disinfectant. Veal[7] has found that allantoin combined with sulfanilamide in a greaseless base was extremely valuable

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1 Jacoby, F., Medawar, P. B., and Willmer, E. N., *Brit. M. J.*, 1941, **2**, 149.

2 Pomerat, C. M., Capouya, M., and Greenleaf, F. I., to be published.

3 Herrell, W. E., and Brown, A. E., *Minnesota Med.*, 1941, **24**, 1059.

4 Robinson, H. J., and Molitor, H., *J. Pharm. and Exp. Therap.*, 1942, **74**, 75.

5 Robinson, W., *J. Bone and Joint Surg.*, 1935, **17**, 267.

6 Greenbaum, F. R., *Am. J. Surg.*, 1936, **34**, 259.

7 Veal, Ross, *Med. Ann. of Dist. of Col.*, 1941, **10**, 2.