by any one of 16 amino acids at some concentration. The rabbit albumin could not be replaced by a mixture of amino acids simulating the amino acid composition of the albumin determined by microbiological assay. Thiamine was found to be the only vitamin essential for the growth of *L. canicola*. *L. canicola, L. pomona, L. ballum, L. sejroe,* and *L. icterohaemorrhagiæ* could be subcultured on a simple medium containing only salts, thiamine, asparagine, and rabbit serum albumin.

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Received December 10, 1952. P.S.E.B.M., 1953, v82.

Physiologic Responses Attending Administration of Antimony, Alone or with Simultaneous Injections of Thyroxin.* (20022)

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Brady, Cowie, and also Maren and Otto have reported the high localization of antimony in the thyroid gland of the dog(1-3). Apparently this localization is not species specific since similar results were obtained by Smith(4) in the hamster, and by Kramer(5) in the rabbit. In this laboratory a strong affinity for antimony was demonstrated by the thyroids of rats exposed to the inhalation of finely divided antimony trioxide, and by rats fed a low level of antimony trioxide in their diet(6). The growth curve for the latter group of animals showed a slightly greater increase in body weight than the controls. The increase in body weight and the sluggish behavior of these animals, accompanied by the marked accumulation of antimony in the thyroid, were interpreted as a tendency toward hypothyroidism. If antimony did have antithyroidal activity when administered alone, one would expect that when given with simultaneous injections of thyroxin, their antagonistic actions should result in a nearly normal metabolic state.

In view of the above facts the following experiments were designed to determine the effect on thyroid function of antimony administered alone or in combination with thyroxin. Three experiments were conducted, 2 using albino rats and one using albino rabbits. The body weight and oxygen consumption were employed as measures of thyroid activity. The concentration of antimony in the tissues was determined in order to ascertain whether or not the presence of thyroxin influenced the pattern of distribution of antimony in the body.

Methods. Oxygen consumption was deter-

* Presented as a partial fulfillment of the degree of Doctor of Philosophy, University of Pittsburgh.

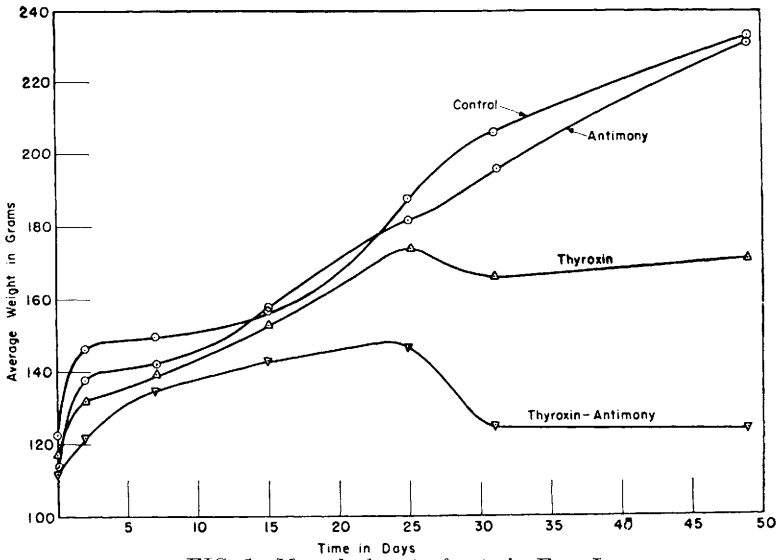

FIG. 1. Mean body wt of rats in Exp. I.

mined by using the apparatus devised by D'Amour and Blood(7). The animals were deprived of food for 12 hours prior to performing the test. Inasmuch as metabolism studies may be influenced by the time of day, the temperature, the amount of noise and activity in the animal room, an effort was made to keep these conditions relatively constant in the experiments. Maren's method for the microdetermination of antimony in the presence of organic material was used(8). In those cases where excessive amounts of iron interfered with the procedure (*e.g.*, liver and spleen) it was necessary to follow Freedman's modification(9) of Maren's method.

Procedure. The male albino rats of the Sprague-Dawley strain used in Exp. I and II were approximately 5 weeks of age at the beginning of the experiment. Each rat received 15 g of synthetic casein diet daily, previously determined as adequate for the normal growth and development of the control animals. *Exp. I.* Twenty rats were divided into 4 groups of 5, as follows: A. Control group; B. Thyroxin-treated group; C. Thyroxin-treated antimony trioxide group; D. Antimony-trioxide group. The animals in Groups B and C received subcutaneous injections of thyroxin daily, the dosage being 0.1 mg/100 g body weight. The antimony dosage in Groups C and D was 2% antimony trioxide added to the casein diet. The rats were weighed periodically. The oxygen consumption was determined after one, 2, 3, 4, and 6 weeks of treatment. At the end of 49 days the rats were anesthetized with ether and bled by cardiac puncture. Blocks of organs were removed for histologic study and preserved in 10% formalin. The concentration of antimony was determined in specimens from Groups C and D. *Exp. II.* Twenty-four rats were divided into 4 groups of 6, as in Exp. I. This study was the same as the preceding one with regard to the concentration of thyroxin and the level of antimony, but the oxygen consumption was not determined until the 54th day. The experiment was terminated after 56 days following the same procedure used in Exp. I. *Exp. III.* Eight full-grown male albino rabbits of approximately the same age and body weight were paired into 4 groups on the basis of treatment as in the previous experiments. The rabbits were fed *ad libitum* on Purina stock diet. The antimony trioxide was force fed in gelatin capsules to insure a daily dose of 13 mg/kg of body weight. The thyroxin was injected subcutaneously, the daily dose being 0.33 mg/kg body weight. The oxygen uptake was measured on the 20th day. The animals were sacrificed by air embolism on the 21st day. Specimens from Groups C and D were analyzed for antimony.

TABLE I. Changes in Oxygen Consumption (cc O_2/100 g Body Wt/Min.) over a 6-Wk Period.†

Time on test, wk	O_2 consumption: Control (Mean)	S.D.)	Thyroxin (Mean	S.D.)	Thyroxin-Antimony (Mean	S.D.)	Antimony (Mean	S.D.)
1	2.74	.34	3.21	.37	3.13	.35	3.24	.12
2	2.46	.16	2.95	.55	2.90	.37	2.53	.21
3	1.75	.17	3.14*	.49	3.26*	.33	1.72	.18
4	2.41	.33	4.61*	.51	6.12*	.56	2.62	.29
6	2.15	.16	4.38*	.22	5.37*	.42	2.54	.37

* Denotes statistically significant difference when compared with the control value ("p" = .01).

† Five rats in each group.

TABLE II. Mean Body Weights and Oxygen Consumption Values (on the 54th Day) of Rats in Exp. II.†

Group	Mean body wt (g) Initial	Final	Oxygen consumption, cc O_2/100 g body wt/min. (Mean	S.D.)
Control	123.6	185.6	2.72	.27
Thyroxin	121	127	3.71*	.17
Thyroxin-antimony	118.8	112.8	4.89*	.31
Antimony	122.6	192.1	1.98*	.31

* Denotes statistically significant difference when compared with the control value ("p" = .01).

† Six rats in each group.

TABLE III. Initial Weight of Rabbits, Weight of Animals on Autopsy and Oxygen Consumption Values Obtained after 20 Days of Treatment.

Group	Rabbit No.	Body wt (kg) Initial	Final	Oxygen consumption (cc O_2/kg body wt/min.)
Control	1	2.1	2.3	5.95
	2	2.3	2.3	5.62
Thyroxin	1	3.2	2.4	10.25
	2	3.1	2.5	6.51
Thyroxin-antimony	1	3.3	2.4	17.52
	2	3.2	2.4	16.84
Antimony	1	2.8	3	4.59
	2	2.7	2.7	5.75

Results. The results of the growth studies of Exp. I are presented in Fig. 1. The average weight of the antimony group parallels that of the control group. The thyroxin-antimony group showed only a slight gain in weight during the first 3 weeks and suffered a drastic loss of weight in the fourth week. The oxygen consumption studies (Table I) indicate that the oxygen uptake of the antimony group did not vary significantly from that of the control group during the 6-week period. In the third week thyroxin exerted comparable effects on the oxygen consumption of the thyroxin and thyroxin-antimony groups, but a greater increase in oxygen consumption was observed in the thyroxin-antimony group in the latter half of the experiment.

The results of Exp. II are set forth in Table II. The average body weights of these animals were of the same order as those of Exp. I. The oxygen consumption values are similar to those of the preceding experiment. That is, in the order of increasing oxygen uptake the groups are: antimony, control, thyroxin, and thyroxin-antimony.

The results of Exp. III (Table III) indicate that the rabbit was similarly affected by the treatments. The metabolism of the antimony group, as reflected in the body weight and oxygen uptake, did not differ from the control group. The hypermetabolic condition of the thyroxin-antimony group was more pronounced than that of the thyroxin group.

In view of the metabolic changes brought about by the combined effects of thyroxin and antimony, the relative distribution of antimony in the tissues of the thyroxin-antimony group was compared with that of the antimony group (Table IV). The mean antimony concentration for each organ of the thyroxin-antimony group, except the adrenal, exceeds that of the antimony group. The results are not statistically valid, however, because of the wide individual variations within the sample.

The findings of the chemical analyses of the rabbit tissues yielded a distribution pattern of antimony in the tissues very similar to that determined in the rat, although the concentrations were lower because of fewer doses.

TABLE IV. Distribution of Antimony in Rat Tissues. 8 rats.

	Organ, concentration of antimony (γ/g)						
	Liver	Kidney	Heart	Spleen	Lung	Adrenal	Thyroid
				Thyroxin-antimony			
	10.6	6.4	12.1	52.9	—	—	117.6
	7.8	4.1	8.6	8.9	—	51.1	111.6
	6.3	8.9	13.2	11.1	—	56	313.7
	13.2	5.6	5.9	15.9	9.8	52.3	35.5
	14.4	9.6	8.1	17.7	14.3	14.5	17.2
	23.7	16.7	17.5	39.9	26.4	52.4	57.7
	14.4	11	13	12.4	12.8	30	—
	21.4	19.7	16.2	15.4	24.9	90	33.4
Avg	14	10.3	11.8	21.8	17.6	49.5	98.1
				Antimony			
	2.1	4.7	4.9	4.6	—	—	30
	3	3.5	4.8	7.6	—	87.5	10.7
	3.6	3.9	10.2	27.6	—	44.3	28.7
	16.4	10.2	10.9	26.8	18.6	96.6	280
	16.5	7.7	1.2	24.3	10	52.7	118.4
	7.8	5.9	4.2	20.4	12.5	—	106.6
	23.2	10	12.7	—	28.4	50	154.5
	1.9	6.9	5.8	20.7	10.1	76	47.3
	5.7	6.3	12.5	—	4.7	—	23.5
Avg	8.9	6.7	7.6	18.9	14	67.8	88.9

The histologic differences found in the thyroids of the thyroxin and the thyroxin-antimony groups did not vary significantly from those observed in the glands of the control groups during phases of the normal secretory cycle. The thyroids of the antimony group displayed some hyperplasia.

Discussion. Tissue respiration has been shown to be inhibited by antimony in the kidney(10) and in the liver(11). Chen *et al.* (12), and Barron and Kalnitsky(13) have further demonstrated that antimony interferes with cellular metabolism by combining with the sulfhydryl group of the respiratory enzymes. On the basis of these observations one would expect a general depression of metabolism following antimony administration. Kramer(5), however, recently reported that antimony did not affect the metabolic rate as reflected by the oxygen uptake and serum cholesterol level. The results of Exp. I and III confirm Kramer's observation that antimony does not influence oxygen consumption. The diminished oxygen uptake of the antimony group in Exp. II appears to be atypical in that further studies in progress substantiated the results obtained in Exp. I and III.

The results obtained in the thyroxin-antimony groups do not support the original hypothesis, namely, that thyroxin and antimony have antagonistic actions. It appears that antimony enhances the action of thyroxin resulting in a hypermetabolic condition which is more severe than that produced by thyroxin alone. The apparent absence of histologic change in the thyroid gland of animals given thyroxin and antimony suggests that the mode of action of the test materials in this group may be extrathyroidal, whereas the action of antimony alone appears to be within the gland in the nature of a thyroid block.

The results of the chemical analyses indicate that antimony is widely distributed throughout the tissues. With the exception of the thyroid concentration the results are not completely in agreement with those reported previously(1,2,4,5,14-16). The route of administration, the level of dosage and the number of doses are factors which may have influenced the relative distribution in the tissues. In addition to the thyroid, the adrenal showed a strong affinity for antimony as also did the lung and spleen. The accumulation of antimony in the latter 2 organs may be explained by the hypothesis that the reticulo-endothelial cells are involved in the removal of antimony from the system.

Summary. A study was made on the meta-

bolic effects and the tissue distribution of antimony when administered alone or with simultaneous injections of thyroxin. It was found that: 1) there appeared to be little effect on the body weight or oxygen uptake following ingestion of antimony trioxide in the amounts employed in these studies; 2) thyroxin activity was intensified by antimony trioxide resulting in a greater degree of hypermetabolism than was observed with thyroxin alone; 3) the relative distribution of antimony in the tissues was not significantly altered by injections of thyroxin; 4) the glandular hyperplasia observed in the thyroids of the antimony group was not present in the thyroxin-antimony group which suggests a difference in the mechanism of action.

Grateful acknowledgement is made to Dr. H. H. Schrenk for his suggestions, guidance, and interest throughout the preparation of this manuscript and to Dr. J. H. U. Brown for originally suggesting the problem. Dr. Paul Gross kindly reviewed the histologic sections.

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Received December 11, 1952. P.S.E.B.M., 1953, v82.

Influence of Insulin on Uptake of Monosaccharides by the Isolated Rat Diaphragm. (20023)

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The mechanism whereby insulin produces an increase in the phosphorylation of glucose is unknown. The effect of insulin may be due to some direct action on the hexokinase system or to some action on the permeability of the cell which makes more substrate available for phosphorylation. The latter hypothesis was developed by Levine *et al.*(1) who demonstrated that the volume of body fluid throughout which d-galactose is distributed in the eviscerated, nephrectomized dog is increased after the administration of insulin. Since galactose is neither utilized nor excreted by the eviscerated dog, Levine postulated that insulin exerts its effect by facilitating the rate at which the hexose enters the cell. Subsequently Levine and his colleagues(2) reported that insulin exerts a similar effect on other sugars having the same configuration as d-glucose in the first 3 C atoms.

In order to test further the hypothesis that insulin exerts an action on the transfer of sugars across the cell membrane, the effect of insulin on the *in vitro* uptake of several hexoses and pentoses by the isolated rat diaphragm was studied. The sugars used were highly purified preparations of d-glucose,