centrations which have been shown to be effective against gram-positive organisms,[5] it would seem that this substance should prove useful in the treatment of certain infections of the biliary system.

Conclusions. The administration of penicillin in man results in the excretion of the substance by the liver in somewhat higher concentrations than are present in the blood stream. No penicillin could be demonstrated in the gastric juice.

Marjorie Jewell, Thelma Maxon and Grace Helm assisted in this work.

[5] Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, to be published.

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Production of Pulmonary Edema by Thiourea in the Rat, and Its Relation to Age.*

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In earlier communications[1,2,3] we reported that the oral administration of certain sulfonamides, and of thiourea and its derivatives produces hyperplastic enlargement of the thyroid gland, concomitant with a marked drop in the B.M.R. in weanling and adult rats. Thyroidectomy cells were also found in the anterior pituitary. All of these changes were abolished by the parenteral administration of thyroxin. Iodine was of no avail in the prevention of this "thyroid-metabolic" effect. The hypothesis was advanced that the syndrome is due to an inhibition of thyroxin formation. A consequence of this inhibition is increased thyrotropic hormone formation by the pituitary with the resultant cellular stimulation of the thyroid. Astwood *et al.*[4] have published similar observations, and furthermore have met with success in the treatment of hyperthyroidism with thiourea and thiouracil.[5] Kennedy[6] reported that allyl thiourea will cause hyperplasia of the rat thyroid, together with changes in the cells of the anterior pituitary, an observation in agreement with our findings with respect to both thiourea and allyl thiourea.

Richter and Clisby[7] reported that 1-2 mg doses of phenyl thiourea usually proved to be lethal for their rats. Adult animals weighing between 164 and 350 g when given from 1-10 mg of this substance by various routes, showed signs of acute respiratory distress, accompanied by a marked drop in body temperature in less than 30 minutes. Their survival period varied from 2-18 hours. The only detectable pathologic signs were marked edema of the lungs and pleural effusion. These workers suggested that the depression in body temperature might be an indirect effect of the lowered body metabolism, resulting from the pleural effusion and respiratory distress. If the phenyl thiourea was administered in *sub-lethal doses* for a few days, and then gradually increased, the adult animals developed a tolerance for this substance. Adult rats, treated for several months in this

* Supported by a grant from the Rockefeller Foundation.

[1] Mackenzie, J. B., Mackenzie, C. G., and McCollum, E. V., *Science*, 1941, **94**, 518.

[2] Mackenzie, J. B., and Mackenzie, C. G., *Federation Proc.*, 1942, **1**, 122.

[3] Mackenzie, C. G., and Mackenzie, J. B., *Endocrinology*, 1943, **32**, 185.

[4] Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R., *Endocrinology*, 1943, **32**, 210.

[5] Astwood, E. B., *J. A. M. A.*, 1943, **122**, 74.

[6] Kennedy, T. H., *Nature*, 1942, **150**, 233.

[7] Richter, C. P., and Clisby, K. H., *Arch. Path.*, 1942, **33**, 46.

manner did not show any *thoracic changes* at autopsy; however, the thyroid glands were hyperplastic.

Binet[8] found that the subcutaneous injection of 600 mg of thiourea into a young 120 g rat resulted in a depression of body temperature, a slowing of the heart beat and a gradual collapse of the animal. Autopsy of this animal, which succumbed the following day, disclosed serous effusion in the pleura, unaccompanied by pulmonary congestion.

Hartzell[9] reported that the intravenous injection of 75 mg of thiourea into a rat (age and size not stated) caused the animal to die approximately 2 days later—the autopsy findings included passive congestion of the lungs, spleen and kidneys.

Our studies on thiourea at dietary levels of 0.05 to 2% were, with the exception of 2 adult animals, confined to weanling rats.[2,3] The 2 adult rats received the stock diet containing 1% thiourea for 2 months. After 2 weeks a definite depression in the B.M.R. was observed. Astwood[10] found that the daily oral administration of 2 g of thiourea to adult rats was not lethal over a period of 10 days.

In the course of subsequent experiments dealing with the effects of prolonged administration of sulfaguanidine on the thyroid gland of the rat, it was observed that these animals died with very striking symptoms when given for one night a diet containing 1% thiourea. Three out of 4 adult animals, previously fed the stock diet containing 2% sulfaguanidine for a period of 7½ months, when transferred to a diet containing 1% thiourea were found dead the following morning, while the fourth was moribund. The surviving animal showed signs of acute respiratory distress. The body temperature was below 92°F. Autopsy of this animal revealed the presence of 7 cc of clear, straw-colored serous fluid in the pleural cavity and marked areas of hemorrhage in the lungs. The fluid contained many cells and clotted rapidly on exposure to the air. The free drug level of sulfaguanidine in this animal was 8.4 mg %. The same condition was found in the 3 dead animals (Group A, Table I). Histologic examination of the lungs of our animals showed changes typical of those seen in pulmonary edema. The response of these adult rats to the oral ingestion of thiourea was identical to that observed by Richter and Clisby[7] in adult rats given phenyl thiourea.

In view of these findings, it seemed likely that the administration of thiourea to rats was incompatible with the administration of sulfaguanidine, possibly due to the formation of phenyl thiourea. This hypothesis was tested by feeding 2% sulfaguanidine and 0.5% thiourea simultaneously to young adult rats (5 months of age) and to immature animals (1¼-1½ months of age). As can be seen from Table I, in the former instance, 14 out of 16 animals died over night, while in the latter case only 2 out of 8 rats succumbed (Groups B and C). However, similar results were obtained with thiourea alone, indicating not only that it is the offending substance, but also that young adult animals (5 months of age) are very susceptible to its *toxic action*, as compared with immature rats (Groups D and F). Young adult rats taken from our stock colony,† and allowed access to the stock diet containing 1% thiourea were found dead the following morning. The pleural cavity contained fluid in amounts varying from 7-15 cc (Group D). Food consumption records revealed that as little as 5 mg of thiourea was sufficient to cause death in many young adult animals. In this group of animals, 2 females who consumed 3 and 4 g respectively of the diet (30 and 40 mg of thiourea) did not succumb. Autopsy of one of these animals revealed hemorrhagic sites in the lungs, but no pleural fluid. The other animal has been allowed to stay on the 1% thiourea diet and has survived.

Three 2-months-old female rats (Group E) consuming 10-40 mg of thiourea did not die overnight, but were found to have between 2.75 and 4 cc of serous fluid in the pleural

[8] Binet, P., *Rev. Med. Suisse Romande*, 1893, **13**, 628.

[9] Hartzell, A., *Contrib. Boyce Thompson Inst.*, 1940, **11**, 249.

[10] Astwood, E. B., *J. Pharm. and Exp. Therap.*, 1943, **78**, 79.

† *Mus Norvegicus.*

TABLE I.
Effect of Parenteral and Oral Administration of Thiourea on the Rat and Its Relation to Age.

Group	No. of animals	Age, approximate, mo	Range of body wt, g	Addition to stock diet	Dose of thiourea, mg	Time to death, hr	% dying	Amt of fluid in pleural cavity, cc
A	3M 1F	8½	167-265	1% thiourea		<16	100	4.5-7
B	6M	5	320-393	2% sulfaguanidine + 0.5% thiourea		<16	100	5-8
	6M 4F	''	260-405	''		<16	80	5-13
C	6M 2F	1¼-1½	75-92	''		<16	25	2-3
D	14M	5	300-390	1% thiourea		<16	100	7-15
	6F	''	248-280	''	5-30	<16	66	9-14
E	3F	2	120-144	''	10-40	>24	0	2.75-4
F	4M 4F	<1	40-50	''	20-70	<16	0	—
G	4M	2½	220-230		27 mg intraperit.	<3	100	5.5-8
	4M	''	210-239		18 mg orally	Died within 2-3 hrs	100	3-5
	5M	''	215-220		18 mg intraperit.	''	100	4.5-6
H	5M	5	350-380		18 mg orally	Died bet. 2¾-3¾ hrs	100	6-9
	3M 3F	''	255-317		18 mg intraperit.	Died within 3-5 hrs	83	6-15.5
I	4F	5	212-316		50 mg per kilo body wt intraperit.	<16	50	12-15
	4F	''	267-300		100 mg per kilo body wt intraperit.	<4	100	6-9
J	2M 4F	1	51-59		18 mg orally		0	—
	6M 2F	''	51-57		72 mg intraperit.		0	—
	4M 4F	''	48-52		90 mg intraperit.		0	—
	4M 3F	''	45-57		180 mg intraperit.		0	—
	6M	''	50-55		180 mg intraperit. (twice in 4 hrs)		0	—
	5M 5F	1¼-1½	86-92		90 mg intraperit.		0	—

cavity when sacrificed the following morning. The higher tolerance of the weanling rat to thiourea was shown in Group F where no deaths took place, even though as great or greater amounts of this substance were consumed than in the case of older animals.

When thiourea‡ was injected intraperitoneally, a single dose of 18 mg killed some young adult animals within a 2-hour period. Death was usually preceded by a convulsion. The oral route was found to be as effective as the intraperitoneal route (Groups G and

‡ C.P. product obtained from Pfanstiehl Chemical Company, Waukegan, Illinois. Melting point, 179.5°C (uncor.). Melting point after 3 recrystallizations, 179-180°C (uncor.). Nitrogen—% recovered: 98.8, 99.6 and 99.7. We are indebted to Harry Eisenberg for this analysis.

H). When 50 mg per kilo of body weight of thiourea was given intraperitoneally to 4 young adult females, 2 animals died in 16 hours and 2 survived and were killed at the end of 24 hours. Fluid was found in the pleural cavity in every case. Increasing the level of thiourea to 100 mg per kilo of body weight caused death preceded by convulsions in the 4 young adult animals tested (Group I).

The results with 35 weanling rats (1 month of age) and 10 rats (1¼-1½ months of age) were in striking contrast to those reported above. Weanling rats injected intraperitoneally with 72, 90 and 180 mg of thiourea survived. Following the intraperitoneal injection of 90 mg of thiourea (1 cc solution) or 180 mg (2 cc solution) the animals appeared to be acutely ill and comatose for several hours. However, despite the 180 mg dose which on the basis of body weight is roughly 50 times that required to kill a 250 g adult rat, the weanling animals survived and were bright and alert the following day. Six weanling rats given 360 mg in 2 divided doses in a 4-hour interval, survived this treatment; 2 of these sacrificed at the end of 6 hours still had some of the injected fluid in the peritoneal cavity. The lungs of these animals appeared to be somewhat redder than usual, but no fluid was found in the pleural cavity (Group J).

The results with weanling and immature rats confirm our earlier findings with respect to thiourea, for we not only fed thiourea in the diet up to a 2% level, but administered 110 mg daily parenterally to weanling rats.[3] In the case of the 2 adult rats fed thiourea at a 1% level in the diet and reported in a previous publication, we can attribute their survival to a high tolerance to thiourea. It is pertinent to point out here that a search of our autopsy records disclosed that both animals had areas of hemorrhage in their lungs.

Since thiourea causes pulmonary edema in the rat, and also causes hyperplasia of the thyroid gland, and a lowering of the B.M.R. the possibility existed that the thyroid was in some way involved in the acute pulmonary effect. Two adult thyroidectomized rats (3 months after operation and with B.M.R.'s indicating complete thyroidectomy) weighing 211 and 230 g were injected with 18 mg of thiourea intraperitoneally. Both animals showed acute respiratory distress within 30 minutes after injection, and died 24 hours later. The toxic response was similar in all respects to that seen in non-thyroidectomized animals, except for a longer period of survival. At autopsy no thyroid tissue was found in either animal. This eliminates the thyroid gland as being essential for the pulmonary reaction. Furthermore unpublished data have shown that thyroid enlargement and a decrease in the B.M.R. occurs in the absence of pulmonary edema or a marked depression of body temperature. Studies on the effect of thiourea on body temperature of young animals are in progress. However, there remains the possibility that the resistance of the immature rat to pulmonary edema is associated with the greater increase in size of the thyroid produced by thiourea in these animals. In view of Astwood's findings that adult animals can tolerate 2 g of thiourea orally per day, it is evident that either the *strain of rat* he employed possessed a high tolerance for this substance, or that the *basal diet* used exerted a protective action with respect to pulmonary edema.

Conclusions. Thiourea, in addition to effecting thyroid enlargement and a decline in the B.M.R., produces in appropriate doses, rapid and fatal pulmonary edema in adult rats. No necessary relation between these two reactions has been demonstrated. The immature rat is much less susceptible to the pulmonary edema and more susceptible to the thyroid enlargement produced by this compound. On a weight basis it tolerates 50 times the lethal dose for adult rats.