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Increased Susceptibility to Chloroform Poisoning Produced in the Albino Rat by Injection of Crystalline Thyroxin.

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There is a considerable volume of literature dealing with the deleterious effects upon the liver of abnormal amounts of thyroid hormone. These changes in the liver have been found in laboratory animals following ingestion of thyroid preparations,[1, 2] and have been reported from clinical and pathological studies on human beings.[3-9] Briefly, they consist in different types of degenerative processes in the liver, detected by histological examination of post-mortem material, and in diminished liver function as shown by various tests.

There is much difference of opinion as to the importance of liver changes occurring in hyperthyroidism. Many believe that they may be of considerable significance in the clinical picture, particularly in connection with the thyroid crises and postoperative "storms." Others hold that they are not of basic significance: Means,[10] for example, believes that they are not due to direct injury by the thyroid hormone. Whatever may be the correct interpretation of the observed phenomena, however, it is probably well established that degenerative changes in the liver, and diminished liver function, are often associated with hyperthyroidism; and that jaundice not infrequently occurs in connection with a thyroid crisis.

The following experiments were undertaken primarily to discover whether the picture produced by liver injury in the presence of an increased amount of thyroid hormone in the circulation is different

1 Farrant, R., *Brit. M. J.*, 1913, 1363.

2 Hashimoto, H., *Endocrinology*, 1921, **5**, 579.

3 Cameron, G. R., and Karunaratne, W. A. E., *J. Path. and Bact.*, 1935, **41**, 267.

4 Weller, C. V., *Ann. Int. Med.*, 1933, **7**, 543.

5 Goodpasture, E. W., *J. Am. Med. Assn.*, 1921, **76**, 1545.

6 Boyce, F. H., and McFetridge, E. M., *Arch. Surg.*, 1938, **37**, 401.

7 Bartels, E. C., and Perkin, H. J., *New England J. Med.*, 1937, **216**, 1051.

8 Maddock, W. G., Pedersen, S., and Coller, F. A., *J. Am. Med. Assn.*, 1937, **109**, 2130.

9 Beaver, D. C., and Pemberton, J. deJ., *Ann. Int. Med.*, 1933, **7**, 687.

10 Means, J. H., *The Thyroid and Its Diseases*, pub. Phila., J. B. Lippincott, 1937, p. 292.

from that produced by liver injury unassociated with hyperthyroidism.

Method. The experimental animal was the albino rat; liver injury was produced by injection of chloroform. This animal and drug were selected because of the extensive data that have been accumulated on the effects of chloroform on rats. The rats were in healthy, active condition. They were weighed every 3 days, and also within 24 hours before the injection of chloroform. In most instances the body temperature was taken by rectum once or more daily. The animals were kept on a standard diet (Purina Dog Chow).

Crystalline thyroxin (Squibb's) was administered daily, by subcutaneous injection, over a period varying from 7 to 20 days,* in doses varying from .1 to .2 mg, the crystals being dissolved in an alkaline solution and the total volume for each dose made up to ½ cc with sterile distilled water.

At the end of the period of thyroxin administration, the animals were injected subcutaneously with varying doses of chloroform (Squibb's) in mineral oil. The doses of chloroform were based on the findings of Moise and Smith,[11] that the majority of their animals on standard diet receiving 1.5 cc of chloroform per kg of rat survived. Opie and Alford[12] have reported the survival of animals on standard diet with as high a concentration of chloroform as 2.0 cc per kg of rat. In the present experiments, the chloroform varied in amount from 1.5 to .4 cc per kg of rat, and was diluted with sterile mineral oil so that the total volume per dose was .5 cc.

All the animals that died were autopsied as soon as possible. Certain animals were sacrificed for histological study, as indicated in Table I.

Results. The animals, during the period in which they were receiving thyroxin injections, showed few if any clinical symptoms—although they were perhaps more active and lively than the control animals. In general, their weight was well maintained or, in a few instances, increased, and their hair remained in good condition.

The results of the chloroform injections are shown in Tables I and II. Table I includes rats receiving from 1.5 to .8 cc of chloroform per kg of body weight. The survival period of these animals varied from 12 to 36½ hours, with the exception of one rat who made a complete recovery. The survival period for the control animals who

* A few animals received desiccated thyroid tablets, but they were too few in number to include. The results, however, would seem to be comparable with the crystalline thyroxin.

11 Moise, T. S., and Smith, A. H., *J. Exp. Med.*, 1924, **40**, 13.

12 Opie, E. L., and Alford, L. B., *J. Am. Med. Assn.*, 1914, **62**, 895.

TABLE I.

Tables I and II, Survival Period of Rats Following the Injection of Chloroform, with and without Previous Administration of Thyroxin.

Rat No.	Initial wt, g	Administration of crystalline thyroxin: Total amt mg	Administration of crystalline thyroxin: Duration days	Wt at time chloroform injected, g	Dose of chloroform per kilo of body wt, cc	Survival period	Remarks
4	215	1.3	10	190	1.5	12 hr	
17	210	2.0	16	214	.8	16½ ''	
7	209	.7	7	167	1.5	18½ ''	
30	204	2.4	16	233	.8	18½ ''	
31	228	2.4	16	247	1.5	23 ''	
22	183	2.0	13	228	.8	24 ''	
28	201	3.2	20	233	1.5	35 ''	Rec'd .2 mg cryst. thyrox. in 1 dose, after chloroform.
21	185	2.0	13	232	.8	36½ ''	
27	214	2.4	16	233	.8	Recovered	
			Control				
48	205			203	1.5	4 days	
56	166			185	1.5	5 ''	
46	173			191	.8	5 ''	
52	177			191	.8	5 ''	
32	138			158	.8	5 ''	
42	157			191	1.5	6 ''	
50	188			196	1.5	Recovered	
47	179			191	.8	''	
43	138			147	.8	''	
39	166			204	.8	''	
37	170			193	.8	''	
33	119			137	.8	''	
8	182			156	1.5	Sacrificed at 48 hr	No symptoms of chloroform poisoning.
11	218			210	1.5	Sacrificed at 48 hr	'' '' '' '' ''

TABLE II.

Rat No.	Initial wt, g	Administration of crystalline thyroxin: Total amt mg	Administration of crystalline thyroxin: Duration days	Wt at time chloroform injected g	Dose of chloroform per kilo of body wt cc	Survival period	Remarks
24	192	2.0	15	222	.4	12½ hr	
26	162	2.4	16	179	.4	13½ "	
14	216	2.0	16	213	.4	17½ "	Temp. 104.6° on day of death.
29	153	2.4	16	147	.4	Recovered	Rec'd .6 mg cryst. thyrox. in 3 doses, after chloroform.
23	197	2.0	13	210	.4	"	
12	210	.7	8	215	.4	"	
		Control					
35	110			120	.4	"	
34	128			141	.4	"	
44	138			171	.4	"	
54	152			190	.4	"	
55	155			181	.4	"	
57	178			190	.4	"	

received corresponding doses of chloroform without the preceding administration of thyroxin, varied in 6 instances from 4 to 6 days, while 6 rats recovered. In 2 other instances the rats were sacrificed at the end of 48 hours for histological study; they showed no symptoms of chloroform poisoning. Table II shows a group of 6 animals who, following administration of crystalline thyroxin, received .4 cc of chloroform per kg of body weight. Three of these animals survived for 12½, 13½ and 21 hours, respectively; the other 3 recovered. Of the 6 controls who received .4 cc of chloroform per kg of body weight, all recovered.

Histological Studies. On autopsy, the animals which had received thyroxin injections followed by chloroform showed extensive liver damage, the lesions consisting of varying degrees of central necrosis, usually extreme. The kidney lesions were not impressive, the changes consisting merely in cloudy swelling. In the majority of the cases, examination of the lungs showed marked engorgement of the vessels of the alveolar walls, and evidence of edema. In a few instances there was actual hemorrhage into the alveolae.

Control animals Nos. 48, 56, 46, 52, 32, and 42, died from 4 to 6 days after administration of chloroform. In some instances it was difficult to find evidence of damage to the liver; in other instances there was evidence of reparatory processes. It seemed unlikely that the liver pathology had been responsible for the death of any of these animals. The kidneys showed interesting lesions, the most outstanding being extensive damage to the convoluted tubules, with areas of calcification, the picture resembling that seen in bichloride of mercury poisoning.

Discussion. The above results would seem to indicate that rats on a standard diet who have been injected with crystalline thyroxin over a period of time show increased susceptibility to chloroform poisoning.† As has long been known,[12] the action of chloroform is profoundly influenced by diet—starvation, or diets high in fat, rendering the animals particularly susceptible to chloroform poisoning, while high carbohydrate and high protein diets afford considerable

† The fact that administration of thyroid preparations may influence the action of a drug was first demonstrated by the well-known experiments of Hunt[13] in which protection against the toxic effects of acetonitril was afforded to mice by the administration of the thyroid hormone. The only experiments which I have found on chloroform poisoning in the presence of hyperthyroidism were carried out by Davis and Whipple[14]: following the influence of Hunt's work they fed thyroid hormone to dogs prior to the administration of chloroform, but found that it was without effect.

13 Hunt, R., *J. Biol. Chem.*, 1905, **1**, 33.

14 Davis, N. C., and Whipple, G. H., *Arch. Int. Med.*, 1919, **23**, 612.

protection.[11, 15, 16] Since the administration of thyroid preparations affects the glycogen content of the liver, as shown by Coggeshall and Greene,[17] it might be supposed that this was a prime factor in the increased susceptibility to chloroform poisoning in hyperthyroidism. However, it is by no means certain that the explanation is so simple: and in a few preliminary experiments of the present study we were not able to protect the animals who were receiving thyroxin, from the effects of chloroform, by supplementing the diet with intraperitoneal injections of glucose.

The mechanism whereby hyperthyroidism in rats increases their susceptibility to chloroform poisoning would seem to be worth further investigation, in the hope that it might throw some light on the relation between thyroid and liver, particularly as manifested in the thyroid crisis. It is interesting also to speculate as to the possible application of the present findings to the choice of anesthetic for operations upon patients with hyperthyroidism. Although few anesthetics have as deleterious an effect upon the liver as does chloroform, a number of them are to a certain degree harmful to this organ; and it may prove that their ill effects as well as those of chloroform are intensified in the hyperthyroid patient. This may be of particular significance in regard to tribromethanol (avertin) because of the chemical relationship of this drug to chloroform.

Conclusions. The susceptibility of the albino rat to chloroform poisoning is increased by the administration of crystalline thyroxin.

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15 Goldschmidt, S., Vars, H. A., and Ravdin, I. S., *J. Clin. Inv.*, 1939, **18**, 277.

16 Miller, L. L., and Whipple, G. H., *Am. J. M. Sc.*, 1940, **199**, 204.

17 Coggeshall, H. C., and Greene, J. A., *Am. J. Physiol.*, 1933, **105**, 103.