fall into the range of those of the heavy metals and therefore are not likely to form triple acetate salts. One must admit, however, that other factors, such as the solubility of an individual acetate or the solubility of a double salt, together with the coordination number of the ion, may limit the possibility of the formation of a triple salt. It is interesting to note, therefore, that although a triple acetate has been reported for beryllium (*1*), the findings are open to question according to the work of another investigator (*4*). To date, no triple acetate has been reported for calcium, although very early work (*6*) reported two varieties of double salt, one of which might have been a triple salt. No triple salts of strontium or barium are known. Hence, it appears that the size of the ionic radius of a divalent ion not only affects the solubility of the triple acetate salt within the group listed in Table 1 but also provides a means of predicting whether or not any divalent cation is likely to form a triple acetate salt.

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The Mechanism of the Therapeutic Effect of Iodine on the Thyroid Gland

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It is now a well-established fact that in cases of toxic goiters the iodine produces an effect which, clinically, shows relief of the symptoms and, biochemically, decrease of the circulating thyroid hormone and an increase in the gland of total iodine, both free and organically bound. Histologically, this effect is manifested by the deposit of the colloid inside the follicles. These facts are generally interpreted as a blockage of the release of the secretion by iodine, but its mechanism is still not very well understood.

The theory of a mechanical blockage, supported by several authors (*5, 6*), can be hardly maintained in view of the modern concepts of enzymatic chemistry and histophysiology of the thyroid gland.

Salter and Lerman (*7*), as the result of a study of enzymatic synthesis carried out with proteases as catalysts, suggested that the therapeutic effect of iodine is due to the mass-law phenomenon, which acts by "forcing" the reaction in the direction of a synthesis and, in this way, inducing the colloid formation and storage.

In 1941 one of us (*1*) demonstrated that the colloid of rat thyroids, extracted from a single follicle, has a definite proteolytic activity, and established a correlation between this activity and the function of the thyroid gland. From these results, later confirmed by Dziemian (*3*), the conclusion was drawn that in the reabsorption of the colloid an enzymatic mechanism is involved which is responsible for the proteolysis of thyreoglobulin. It also was found that iodine, after a certain time, inhibits this proteolytic activity.

TABLE 1

PROTEOLYTIC ACTIVITY OF TOXIC GOITERS BEFORE AND AFTER TREATMENT *in Vitro* WITH IODINE

Blank Mg. of tyrosine and tryptophane set free	Iodinized extract Mg. of tyrosine and tryptophane set free	Per cent inhibition
0.116	0.031	73.3
0.164	0.035	77.1
0.094	0.042	55.3
0.225	0.105	53.4
0.092	0.011	88.5

Recently we found (*2*) in human thyreotoxicosis that the proteolytic activity of the total gland, as measured by the amount of tryptophane and tyrosine set free, is probably also decreased through the action of iodine administered in therapeutic doses. These results and those of Henrriott (*4*) on the inhibition of pepsin activity by iodization *in vitro,* led us to suppose that in the case of iodine treatment the clinical effect is due to an inhibition of the proteolytic enzyme system.

In order to test this assumption, glycerol extracts of human thyroid gland (toxic goiters) were iodinized *in vitro* with a final concentration (0.05 M) of iodine, and the proteolytic activity was determined by the amount of tyrosine and tryptophane set free after a 4-hour incubation at 37° C. with edestin as substrate. The details of this method were described in our previous paper (*2*). Here we wish only to add that the glycerol extracts, after iodinization, were dyalized for 24–48 hours at 3° C. Also, the blank (*i.e.* the same extract, but without iodine) was treated in the same way.

Certain of the results of this experiment are given in Table 1, from which the conclusion may be drawn that, under these conditions, there is 53.4 to 88.5 per cent inhibition of the proteolytic activity of the thyroid gland. It is interesting to point out that this

inhibition is due to free iodine, as control experiments with potassium iodide (which serves as a solvent medium for the iodine in our iodinization experiments) show no inhibiting effect whatever. The same may be said about the thiourea, which obviously acts through a different mechanism.

From our experiments we conclude that the therapeutic action of iodine on the thyroid gland is due to the inhibition of the proteolytic enzyme system, probably responsible for the release of the follicular colloid.

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Brain Involvement as a Possible Cause of Relapse After Treatment in Spirochetal Relapsing Fever

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Schuhardt and O'Bryan (*1*) have reported that doses of 40,000+ units (per kilogram body weight) of penicillin sodium, when injected intraperitoneally over a period of 72 hours beginning at onset of the first attack, will cure blood-stream involvement and prevent most brain involvements in *Ornithodorus turicata*-transmitted relapsing fever of the white rat. Equivalent intraperitoneal doses given at later stages of the infection failed to cure brain involvement. Subsequently, these workers (*2, 3*) reported cure of brain involvement by the intracranial injection of 1,000 units of penicillin. These observations provide a means of testing the theory that relapse after treatment in spirochetal relapsing fever can be a consequence of the persistence of spirochetes in the central nervous system of the treated animals.

This tendency to relapse after treatment is a fundamental problem, not only in the case of spirochetal relapsing fever but also in that of spirochetal diseases generally. Also, the problem of therapy in neurosyphilis conceivably is related to the problems here involved. Furthermore, we believe that much of the confusion in the literature relative to the use of relapsing-fever-spirochete infected animals in testing spirocheticidal agents stems from the irregular tendency of these animals to relapse after treatment.

In our experimental work 53 rats were infected by the intraperitoneal injection into each of 0.01± ml. of onset positive heart blood from tick (*O. turicata*)-infected rats. The infection status of these rats, both before and after treatment, was followed by uniform (0.01 ml. of a 1: 20 dil.), daily, dark-field examination of tail blood. Penicillin therapy was begun in the test animals on the second or third day of dark-field positivity which, in each case, was the fourth or fifth day of the infection.

Twenty-five of the rats were anesthetized with 3–4 mg. of nembutal per 100 grams body weight, and each received 1,000 units of penicillin in a single intracranial injection (0.05 ml. of a 20,000 units/ml. solution in phosphate buffer pH 7.0). Although many of these rats showed severe convulsive reactions, only 2 died as a result of this injection. The 23 surviving rats received 1 to 14 intraperitoneal injections of penicillin at three-hour intervals, resulting in doses ranging from 4,400 to 50,900 units/kg. body weight.

Nineteen rats received no intracranial penicillin, but received 1 to 16 intraperitoneal injections at intervals of three hours, resulting in penicillin doses of from 10,100 to 52,500 units/kg. body weight. These rats served as controls for comparing the effect of combined (intracranial and intraperitoneal) therapy with intraperitoneal therapy alone. Nine additional rats served as untreated controls. Eight of these relapsed one or more times during the experiment, and all 9 were brain-blood-passage positive at the end of 31 days.

Too few rats were included in the group receiving 30,000 to 40,000 units/kg. body weight intraperitoneally to draw final conclusions relative to the mini-

TABLE 1

No. rats	Penicillin therapy			No. showing microscopic relapse	Brain-blood passage results	
	Intracranial	Intraperitoneal				
	Amount	No. inj.	Units/kg.		No. passed	No. pos.
13	1,000 units	1 to 10	4,400–22,800	12	1	1
14	None	1 to 10	10,100–35,900	12	2	2
6	1,000 units	4	41,000–50,500	Not examined	6	3
4	1,000 units	10 to 14	42,400–50,900	0	4	0
5	None	10 to 16	47,600–52,500	3	2	2
9	None	None		8	9	9