Summary. Post-extrasystolic potentiation (PEP) of cardiac contractility was studied in rabbit auricular muscle. It is shown that potentiation is related to the interval between basal beat and extrasystolic beat. An increase in driving rate up to about 3 per second increased the extent of potentiation and decreased the interval between basal beat and the extrasystole necessary for the production of maximal potentiation. Quinidine increased the effective refractory period and the interval for producing PEP and decreased the degree of potentiation produced by the extrasystole.

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Received March 9, 1956. P.S.E.B.M., 1956, v91.

Metabolic Actions of Acetic Acid Analogs of Thyroxine and Triiodothyronine.* (22361)

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In the field of thyroxine analogs and possible derivatives which may have special metabolic characteristics, the most striking recent observations were those of Gross and Pitt-Rivers(1) and of Roche, Lissitzky and Michel(2) that 3,5,3′-triiodothyronine (Trit) was several times more active than thyroxine. Many workers have since confirmed this on a variety of test animals(3). The time of delay before the metabolic effects of Trit are seen is now recognized as shorter than with thyroxine(4-7), but still present. The duration of Trit action is also more brief, effects which are more striking in the myxedematous human than in experimental animals(8,9).

Thibault and Pitt-Rivers(10,11) reported that 3,5,3′,5′-tetraiodothyroacetic acid (Tetrac) and 3,5,3′-triiodothyroacetic acid (Triac) produced an immediate *in vitro* stimulation of kidney and liver slices appearing at concentrations about 0.01 μg Triac per ml or 0.05 μg Tetrac per ml and reaching a maximum at respectively, 1.0 and 5.0 μg per ml. In addition, Tetrac was stated to cause a considerable rise in metabolic rate of thyroidectomized rats in 2 hours followed by a return to the starting level after 4 hours. Pitt-Rivers had previously reported on the activity of Tetrac and Triac(12), noting no unusual results except for unexpectedly high toxicities. The present report concerns possible qualitatively distinct properties of Tetrac and Triac.†

Methods. All rats used were from the Sprague-Dawley strain, thyroidectomized at least 6 weeks previously. Most tissues were prepared as previously discussed(13). Ehrlich mouse ascites tumor cells, cultured *in vivo* in Carworth white mice,‡ were washed sev-

* This work was supported by grants from the Council on Pharmacy and Chemistry of American Medical Assn., the Smith, Kline, and French Foundation, and Committee on Growth of American Cancer Society.

† Samples of Tetrac and Triac were obtained through the generosity of Dr. Pitt-Rivers and of Dr. John A. King, Warner-Chilcott Laboratories.

‡ These cells from a Southern Research Institute culture were obtained through the courtesy of Dr. Warner W. Carlson and Mr. J. T. Huggins.

TABLE I. Effect of Triac or Tetrac Added *In Vitro* to Tissue Slices Prepared from Thyroidectomized Rats.

Compound added, μg/ml	No. of animals	% of control Q_{O_2} Kidney 0–15	Kidney 0–60	Liver 0–15	Liver 0–60	Heart 0–15	Heart 0–60
Triac: .01	8	99.3	100.0	102.0	103.5	115.0	111.4
.10	8	97.2	100.0	101.7	102.3	107.5	111.1
1.0	8	94.1	97.3	97.1	93.7	104.9	110.4
5.0	2	97.9	99.0				
10.0	8	95.2	96.1	93.7	89.4	85.7	84.7
Tetrac: .05	8	87.8	92.7	93.8	88.3		
.50	8	96.9	97.1	101.4	98.7	96.4	100.8
1.0	2	103.6	100.9				
5.0	8	95.7	97.8	105.5	102.7	86.7	98.3
10.0	2	98.2	95.3			77.4	91.7
50.0	8	73.4	65.1	101.0	101.8	52.7	55.7

eral times by suspension in Krebs' Ringer solution followed by mild centrifugation. A 10% v/v suspension was made from the final packed tumor cells using a solution appropriate to the determination of oxygen consumption (Krebs' Ringer-phosphate) or glycolysis (Krebs' Ringer-bicarbonate). Any thyroxine analog being studied was present in 0.005 N NaOH final concentration. Control vessels always contained the same amount of alkali and 0.9% NaCl.

Results. Findings are divided into 3 groups: (1) *In vitro* effects on rat tissues, (2) *In vivo* injection into thyroidectomized rats, (3) *In vitro* effects on tumor cells.

1. *In vitro Triac and Tetrac effects on tissue metabolism.* Table I shows oxygen consumption values calculated as percentages of control values for 0-15 and 0-60 minutes of observation. Liver, kidney and heart were not stimulated significantly over these time intervals. Kidney was clearly depressed by 50 μg Tetrac/ml solution and heart by both 10 μg Triac/ml and 50 μg Tetrac/ml. Heart also appeared to have been inhibited the first 15 minutes by 5 and 10 μg Tetrac/ml, but escaped by 1 hour. Liver metabolism was not altered markedly by either material, although it was slightly depressed by the highest level of Triac. Readings actually were continued for a second 60 minutes, with no significant changes beyond those already discussed. Rates of shaking of the apparatus were also varied over the range of 80 to 120 per minute. The data published by Thibault and Pitt-Rivers(10) for kidney yield the following averages: with 5 μg Tetrac/ml solution, 73% increase for 15 minutes and 30% for 60 minutes; with 1 μg Triac/ml, 106% and 30% for 15 and 60 minutes, respectively. In no instance did the results obtained in this laboratory approach these increases. The later report by these workers(11) indicated a more evanescent effect of Triac on liver slices. Concentrations from 0.01 to 1.0 μg/ml all gave increases averaging 28% for 15 minutes and 7% over 60 minutes.

2. *In vivo effects of injected Triac and Tetrac.* The reported immediate increase of BMR in thyroidectomized animals produced by Tetrac injection(10) might be due to undesirable non-specific effects. We have studied the metabolism of tissues removed from thyroidectomized rats 1.5 hours after injection of 2 mg Tetrac/kg or 1 mg Triac/kg. The tissues were immediately chilled to 5°C and kept at this temperature throughout the period of preparation, about 0.5 hour. The effective time after injection is considered to include only the 15 minute equilibration time at 37° in addition to the 1.5 hours after injection.

The results in Table II fail to show any rapid response following injection of the acetic acid analogs into thyroidectomized animals. Heart was included because it has been found to respond with such high sensitivity to injected thyroxine and skeletal muscle because it makes up so much of the metabolizing body mass. Preliminary experiments with liver and kidney have shown the same absence of immediate energy metabol-

TABLE II. Oxygen Consumption of Tissues Removed from Thyroidectomized Rats Injected with Tetrac (2 mg/kg) or Triac (1 mg/kg).

Hr after inj.*	% of control Q_{O_2} Tetrac-injected			Triac-injected		
	Heart	Diaphr.	Skel. M.	Heart	Diaphr.	Skel. M.
1¾–2	94.9	94.4	109.2	102.8	97.8	94.7
2 –2¼	106.9	96.1	105.3	97.9	93.4	93.4
2¼–2½	96.3	90.0	104.6	98.1	102.9	90.8
2½–2¾	92.3	91.9	113.6	100.3	97.3	95.5
2¾–3¼	111.1	94.9	105.0	92.2	96.6	88.3
3¼–3¾	88.0	92.5	100.0	89.5	98.1	83.6

* Not including approximately ½ hr required for preparation of tissues, during which time tissues were kept at 0°C. Four animals used in each category.

TABLE III. Metabolism of Tissues Excised from Thyroidectomized Rats Injected with Thyroxine, Tetrac or Triac.

Tissue	Thyroidectomized Q_{O_2}	% increase above control Q_{O_2} L-thyroxine*	Tetrac*	Triac*
Liver	.98	59.2	53.1	52.0
Diaphragm	.72	34.7	23.6	33.3
Heart	.44	170.6	147.7	277.3
Skel. M.	.68	27.9	17.6	23.5
Pancreas	.83	39.8	20.5	25.3
Saliv. gl.	1.86	31.7	22.0	20.4
Kidney	3.27	35.5	21.7	45.9

* Compound specified in each case was inj. at a dose of 2 mg/kg body wt/day for 4 days and animal sacrificed on fifth day. Three animals in each group.

ism change.

Thyroidectomized rats were also injected with Triac and Tetrac at several dose levels once per day for 4 days, and sacrificed on 5th day. The most consistent responses were obtained at a level equimolar to 2 mg thyroxine/kg/day. These are shown in Table III, together with responses to L-thyroxine.§ The cumulative effect from these materials, as with thyroxine, suggests that metabolism is stimulated for longer than 2 to 4 hours. It may be estimated that Tetrac is about three-quarters as active as L-thyroxine. Since Triac stimulated tissues to approximately the same extent as thyroxine, it is about one-fourth as active as L-triiodothyronine. The apparently greater effect of Triac on the heart requires further study.

3. *In vitro responses of Ehrlich's mouse ascites tumor cells.* This tumor cell appears to be a useful test object with Triac and Tetrac(14). Table IV gives the Q_{O_2}, $Q^{O_2}_{CO_2}$ and $Q^{N_2}_{CO_2}$ values obtained in terms of the wet weight of tumor tissue. Since the dry weight averaged 11.0% of the wet weight, multiplication by a factor of 9.1 yields dry weight Q's. The endogenous metabolic rate was the same as that with pyruvate, but glucose caused a 40% decrease. The nature of the foodstuff used in the absence of added substrate is not known, but the repeated washings with saline should have removed such substances as pyruvate or lactate. As is typical of tumor cells, addition of glucose caused a tremendous increase in glycolysis over the non-nutrient, about 12-fold for aerobic and 32-fold for anaerobic.

The tumor cell Q_{O_2} was not affected by Triac from 0.01 to 10 μg/ml, or Tetrac 0.05 to 5, except for 8-10% increases at levels of 0.5 to 1.0 μg/ml. Triac at 50 and 100 μg/ml and Tetrac at 50 showed increasing inhibition. In contrast, as shown in Fig. 1 and 2, both aerobic and anaerobic glycolyses were enhanced by 50 μg Triac/ml, the former by 52% and the latter 22%. Somewhat lesser effects were shown by 60 μg Tetrac/ml. However, the thyroxine isomer, B-5522, N-(3,5-diiodo-4-hydroxybenzoyl) -3,5-diiodotyrosine, devoid of thyroxine-like, metabolism-stimulating properties when injected into thyroidectomized animals, also gave increases in both aerobic and anaerobic glycolyses. In addition, thyroxine itself accelerated aerobic glycolysis and Trit anaerobic.

In general, these results confirm the observations of Heimberg *et al.*(14) on the *in*

§ Sodium L-thyroxine was kindly provided by Dr. H. L. Fevold, of the Travenol division of Baxter Laboratories.

TABLE IV. Oxygen Consumption, Aerobic Glycolysis and Anaerobic Glycolysis of Ehrlich Mouse Ascites Tumor Cells.

Determ.	Substrate	Control	Triac, μg/ml (as % of control) .01	.10	1.0	10	50	100	Tetrac, μg/ml (% of control) .05	.5	5.0	50
Q_{O_2}	None	.90				90.0	33.3	10.0				
	Glucose	.56	97.0	98.0	110.1	98.2	41.1	16.1	102.0	108.1	99.0	45.5
	Pyruvate	.98				93.9	55.1	22.4				
$Q^{O_2}_{CO_2}$	None	.14										
	Glucose	1.75				104.6	152.0	87.4				
$Q^{N_2}_{O_2}$	None	.08										
	Glucose	2.59	102.5	99.5	95.8	113.5	121.6	70.2	100.0	99.3	101.5	

vitro stimulation of glycolytic activity by Tetrac and Triac. However, our findings that thyroxine and Trit have similar effects throws considerable doubt on interpreting these changes as being specifically related to an action of the iodothyroacetate derivatives. Furthermore, the comparable activity exhibited by a physiologically inactive thyroxine isomer, B-5522, suggests that the entire phenomenon is related more generally to some structural configuration of these compounds (such as the 3,5-diiodo-4-hydroxyphenyl group) than is the complete oxidative pathway. It would be quite premature to insist that one function or the other was the more "genuine" action of the thyroid hormone.

The present studies seem to put the acetic acid analogs of thyroxine and Trit into a rather less dramatic relationship to the "physiologically active" thyroid hormone than was indicated by Thibault and Pitt-Rivers. Both Triac and Tetrac are thyroactive compounds, but show no clearly qualitative differences from thyroxine or, more appropriately, Trit. Other studies being carried out at present indicate that Triac, Tetrac and Trit may all produce a more rapid response on the sensitive heart than thyroxine when injected into thyroidectomized animals. Whether the acetic acid analogs have, also like Trit, a more rapid turnover, remains to be determined.

If Trit is, as has been suggested, the "active" form of the thyroid hormone produced from thyroxine, it would seem unlikely that it is transformed into Triac prior to exerting its final actions. An alternate interpretation of these interconversions might be that any compound with the proper configuration is potentially active in its own right and that metabolism of thyroxine may yield a series of compounds, including Trit, Tetrac, Triac, and other derivatives.

Summary. Tetraiodothyroacetic acid (Tetrac) and triiodothyroacetic acid (Triac), in-

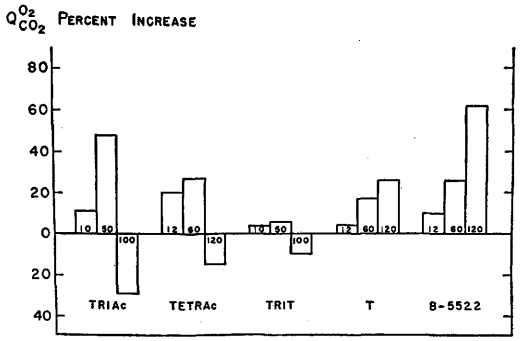

FIG. 1. Aerobic glycolysis of Ehrlich mouse ascites tumor cells as affected by thyroxine, Trit and related materials at various concentrations, shown as μg/ml.

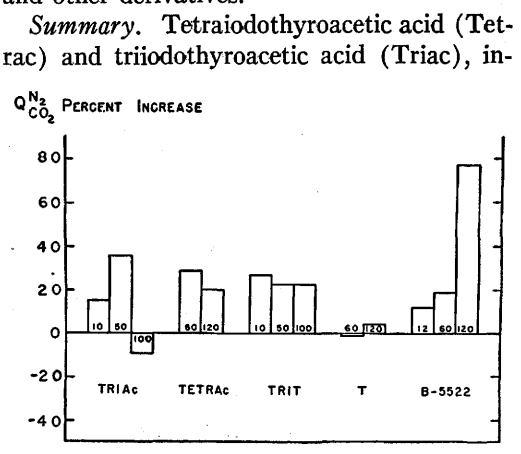

FIG. 2. Anaerobic glycolysis of Ehrlich mouse ascites tumor cells as affected by thyroxine, Trit and related materials at various concentrations, shown as μg/ml.

jected into thyroidectomized rats over a period of several days, have the same order of activity as an equimolar dose of L-thyroxine, as judged from the oxidative metabolism of excised tissues. Triac and Tetrac do not appear to produce an immediate metabolism-stimulating effect, either injected into animals or incubated *in vitro* with tissue slices. At levels which depress oxygen consumption of Ehrlich mouse ascites tumor cells, Triac and Tetrac enhance aerobic and anaerobic glycolyses. However, this effect is not considered specific to the acetate analogs, since thyroxine, triiodothyronine, and a physiologically inactive thyroxine isomer all are capable of producing similar changes.

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Received March 9, 1956. P.S.E.B.M., 1956, v91.

Radiation-Induced Alteration of Fibrinogen Clotting Rate and Clot Lability.* (22362)

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Studies of the effect of radiation on the biological activity of proteins have been confined almost exclusively to enzymes. In the case of the latter, an important body of information leading to a better understanding of radiation effects has been obtained(1). Investigations of proteins exposed to ionizing radiation have yielded a wealth of physicochemical data which must, however, be supplemented by examination of the radiation-induced modifications of their biological functions. In the light of the extensive work that has already been done on purified fibrinogen, it was decided to investigate the effects of x-radiation of fibrinogen on its subsequent clotting by thrombin. Fibrinogen has already been studied physicochemically after irradiation in the dry state(2) and in concentrated solution(3). In these studies, the doses of radiation employed were of such high magnitude as to have little practical significance from the point of view of mammalian radiation damage. The present study is concerned with the influence of low doses of radiation.

Materials and methods. Purified bovine fibrinogen (lot No. 55199) was generously supplied by Dr. E. C. Loomis of Parke, Davis and Co., and was shown to contain 90-93% clottable protein. Solutions of this fibrinogen were prepared in 0.15 M NaCl buffered with

* This work was performed under U. S. Atomic Energy Commission contract AT(30-1) 1554, administered by L. V. Heilbrunn.