Our findings are also important in relation to treponeme identification and their future taxonomy based on antigenic analysis. This investigation has shown that treponemes may be differentiated rather easily by fluorescent antibody (FA) methods and suggests an expanded study.

Statements relative to the inhibition or blocking technic have been mostly of a reference nature. Where absorptions have been indicated in the present work the blocking antibody technic has been shown to demonstrate equal results. Because of its simplicity, this technic would be preferred in future FTA test modifications.

Summary. 1. The antigens of intact T.p., the R.t., T.m. and the free-living mud organism, T.z., are compared. 2. An antigen appearing to be identical to the Reiter Protein antigen was found to be a common or shared component in all of the treponemes studied. 3. By means of absorption or blocking procedures and employing FA methods, all treponemes could be identified. 4. It is suggested that FA methods can play an important part in future taxonomic studies of treponemes and that the FTA test can be modified so as to increase both sensitivity and specificity.

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Acetylcholine and Iodine Metabolism in Thyroid Slices.* (27516)

GEORGE S. SERIF†

Department of Biochemistry, School of Medicine, State University of South Dakota, Vermillion

For many years the thyrotrophic hormone (TSH) has been known to control the metabolism of iodine in the thyroid gland *in vivo*. More recently *in vitro* TSH effects have been observed in the iodine metabolism of thyroid slices(1,2,3). In addition to *in vitro* iodine effects, Field and his colleagues (4,5) have also demonstrated that TSH stimulates oxidation of glucose $-C^{14}$ to $C^{14}O_2$, and production of TPN from DPN in thyroid slices as well. Further work by the Field group(6) has suggested that the neurohormone acetylcholine has a similar specific function in the thyroid gland in the control of glucose oxidation. Some differences exist, however, between the actions of TSH and acetylcholine in glucose metabolism. In particular, TSH preferentially increases the oxidation of glucose *via* the hexose monophosphate pathway (*i.e.*, C_1 carbon oxidation) whereas acetylcholine stimulates both C_1 and C_6 carbon oxidation equally. In view of these similarities and differences in control of carbohydrate oxidation, it is of interest to establish whether acetylcholine, like TSH, has a controlling influence on iodine metabolism in thyroid slices, and if so whether the effects observed are similar to those observed with TSH. This paper reports the experiments designed to answer these questions.

Experimental. Thyroid glands obtained from nembutal-anesthetized dogs were kept at ice-water temperature, trimmed of all ex-

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† Present address: Dept. of Agricultural Biochemistry, Ohio State Univ., Columbus.

traneous tissue, and sliced with a Stadie-Riggs microtome to a thickness of approximately 0.3 mm. The slices were sectioned to an average size of 3 × 3 mm and pooled, to eliminate biochemical differences which might exist in the various areas of a given gland.

In iodine concentration studies 100-130 mg of tissue (wet weight) were incubated in stoppered flasks containing air as the gas phase and 3.0 ml of Krebs-Ringer phosphate buffer solution (minus calcium ion) at a pH of 7.4. This medium contained in addition 1×10^{-6} M KI, 0.01 M glucose, and I^{131} at a level of 4 μc/100 ml of medium. Where applicable this medium also contained acetylcholine chloride (2.8×10^{-4} M), eserine sulphate (3.6×10^{-4} M), and thiourea (1×10^{-3} M). Flasks were incubated with shaking at a temperature of 37°C for periods ranging up to 2.5 hours. After incubation slices were blotted and reweighed since a loss in weight occurs during incubation. Thyroid concentration of iodine was evaluated by a comparison of the I^{131} present in the thyroid slices and the incubation medium. This was accomplished by counting the thyroid slices and one ml of incubation medium in the well of a crystal scintillation detector. From these counts the concentration of I^{131} could be expressed in terms of the ratio of the counts of I^{131}/g of tissue (T) to the counts of I^{131}/ml of fluid (M)(7).

In studies of the iodinated amino acid distribution in thyroid slices the incubation procedures were the same as those described above with the exceptions that I^{131} was added to the flasks at higher levels (5 μc/flask) to facilitate subsequent operations, and tissue weights in the range 70-77 mg were used. After a 2.5-hour incubation these slices were removed, blotted, and homogenized in 1 ml of Krebs-Ringers phosphate buffer (pH 7.4) containing 5 mg of thiourea to prevent iodine exchange in subsequent handling. The homogenates were hydrolyzed with pancreatin, identical aliquots chromatographed, and the chromatograms radioautographed using procedures already described(8,9). An estimation of the radioactivity present in the various components was achieved by cutting out the respective areas of the chromatograms, folding, and counting them in a well-type crystal-scintillation detector.

TABLE I. Influence of Acetylcholine and Thiourea on *in vitro* I^{131} Concentration in Dog Thyroid Slices.

Addition	T/M* Control	T/M* Acetylcholine	Acetylcholine / Control × 100
Thiourea	31.1	26.8	86
"	19.4	18.5	95
"	16.6	17.8	107
"	25.8	34.9	135
"	16.4	13.0	79
			Avg = 100
None	15.2	29.4	190
"	29.6	48.9	165
"	25.2	34.9	138
"	11.2	25.3	226
"	34.6	40.7	118
"	13.7	25.3	185
"	15.1	24.6	163
			Avg = 170

A probability level $p < 0.01$, derived from $t = \frac{\bar{d}}{s_{\bar{d}}}$, was established from the paired control and acetylcholine slices in the absence of thiourea. The probability level in the presence of thiourea indicated no significant difference. Comparison of acetylcholine stimulations in thiourea treated slices *vs* slices not treated with thiourea yields a value $p < 0.01$, derived from $t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$.

* 2-hr incubation.

Results. The influence of acetylcholine-eserine on the ability of dog thyroid slices to concentrate iodide I^{131} from the incubation medium in presence and absence of thiourea is shown in Table I. It is seen that acetylcholine-eserine induces a significant increase in I^{131} concentration in the absence of thiourea ($p < 0.01$). The presence of thiourea, however, inhibits the stimulation of I^{131} concentration since there is no significant difference between acetylcholine and control slices in the presence of this drug.

The radioautographs of Fig. 1 and the data of Table II reaffirm the observations made in Table I since more I^{131} is found in the aliquots from acetylcholine-eserine treated slices than in the controls. The radioautographs and the counts of the chromatogram components show that this increased concentration of I^{131} is present as organically bound

iodine since the iodide ion concentration (counts per minute) remained essentially the same in both test and control experiments. The radioautographs of the hydrolyzed slices reveal this increased organically bound iodine to be accounted for mainly as monoiodotyrosine and diiodotyrosine. Further, the increase in formation of monoiodotyrosine and diiodotyrosine in the presence of acetylcholine-eserine is shown to be highly significant ($p < 0.001$ Table II).

Chromatographic analysis of hydrolyzed thiourea treated slices revealed only iodide ion indicating that no organic binding of iodine occurs in the presence of this compound under the conditions of this experiment.

Virtually no I^{131} labeled thyroxine was observed in these studies. The ratio of monoiodotyrosine to diiodotyrosine was observed to change only slightly in the direction of increased diiodotyrosine (*i.e.*, 1.7:1 with acetylcholine-eserine treatment and 2.0:1 for control slices).

Since there is an increased quantity of iodide ion in acetylcholine treated slices as shown in Table II then by difference there is a decreased quantity of I^{131} left in the incubation medium of acetylcholine treated slices as compared to control slices. This conclusion was verified by chromatographing equal aliquots from the unhydrolyzed incubation medium of a control and a test flask. The data obtained are shown in Table III. Enzymic hydrolysis of the organic I^{131} found in the incubation medium released only monoiodotyrosine and diiodotyrosine.

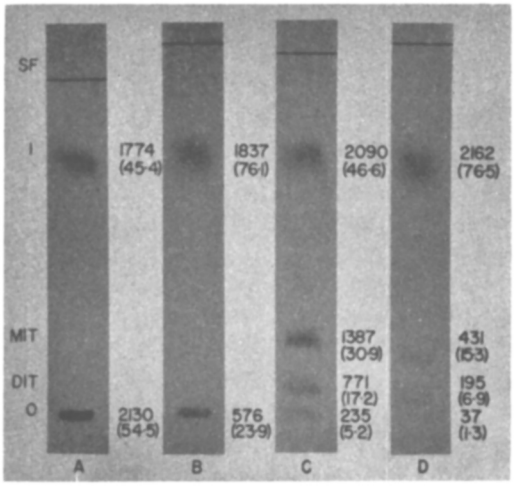

FIG. 1. Typical radioautographs of chromatograms of I^{131} distribution in incubated thyroid slices (4 exp).

A. Thyroid slices treated with acetylcholine and eserine.
B. Thyroid slices, untreated.
C. Thyroid slices treated with acetylcholine and eserine and hydrolyzed with pancreatin.
D. Thyroid slices, untreated, and hydrolyzed with pancreatin.

Figures to the right of the respective radioautographs indicate counts per minute and percentage contribution of each component to total activity on the chromatogram; O refers to origin; DIT refers to diiodotyrosine; MIT refers to monoiodotyrosine; I refers to iodide ion; S. F. refers to solvent front. The solvent used was collidine: water (125 : 44) with ammonia in the gas phase (8).

Discussion. The data of this report dem-

TABLE II. I^{131} Distribution in Enzyme Hydrolyzed Dog Thyroid Slices Incubated with I^{131} Iodide Ion in Presence and Absence of Acetylcholine.

Addition	Origin (cpm)	DIT (cpm)	MIT (cpm)	I^- (cpm)	% organic I^{131}
Acetylcholine & eserine	235	771	1387	2090	53.4
"	170	758	1270	2229	49.7
"	224	863	1467	2361	52.0
"	226	882	1467	2312	52.4
				Avg	52.1*
None	37	195	431	2162	23.5
"	76	386	729	2531	32.0
"	54	237	455	2277	24.7
"	91	317	667	2258	32.3
				Avg	28.1*

* A comparison of acetylcholine treated and control slices with Student's test, $t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$, yields a value $p < .001$.

TABLE III. I^{131} Content of Unhydrolyzed Incubation Medium of Acetylcholine Treated and Control Thyroid Slices Post Incubation.

Addition	Origin (cpm)	Iodide (cpm)
Acetylcholine	368	1202
None	212	2476

onstrate conclusively that acetylcholine can effect a stimulatory action on the organic binding of I^{131} in thyroid slices. The mechanism of this effect, however, appears obscure. Pastan *et al.*(6) have shown that glucose penetration and utilization in thyroid cells are increased in the presence of acetylcholine. It is possible that the increased incorporation of I^{131} into organically bound form may be a secondary response to an increased glucose metabolism.

It is of interest that the effects observed here with acetylcholine are similar to those observed by other workers using TSH as a stimulant. Thus in thyroid slices(3) TSH has been demonstrated to increase I^{131} uptake and conversion of I^{131} iodide to organically bound forms. Whether this *in vitro* similarity is of physiological importance remains to be established.

Summary. The action of acetylcholine on I^{131} metabolism in dog thyroid slices was investigated. It was established that acetylcholine, like the thyrotrophic hormone, increased the ability of thyroid slices to concentrate iodine. This augmented iodine uptake was found mainly in the form of increased amounts of protein bound monoiodotyrosine and diiodotyrosine.

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Electrophoretic Studies of Bovine Serum. V. Complement-Fixing Antibodies in Serum Fractions in Anaplasmosis.* (27517)

THOMAS E. ROGERS AND GEORGE T. DIMOPOULLOS

Dept. of Veterinary Science, Agric. Exp. Station, Louisiana State University, Baton Rouge, La.

A previous report from this laboratory described changes in the serum proteins of calves infected with *Anaplasma marginale*(1). It was suggested that the complement-fixing (CF) antibody activity might be associated with the *alpha-* and *beta-*globulins during the acute phase of anaplasmosis whereas this activity would appear in the *gamma-*globulin fraction during convalescence.

The present study was undertaken to determine the location of *Anaplasma* CF antibodies in serum protein fractions of calves in the acute and convalescent stages of anaplasmosis. The relationship between concentrations of globulins and CF antibody titers was also studied.

Materials and methods. The methods for infecting and maintaining the calves and for observations during the disease have been described(1-4). Blood samples were obtained from 30 infected calves at various times during the acute and convalescent stages of the disease and from 18 normal animals. Sera collected during the acute phase of the disease were selected from calves exhibiting a severe anemia and a high *Ana-*

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