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)

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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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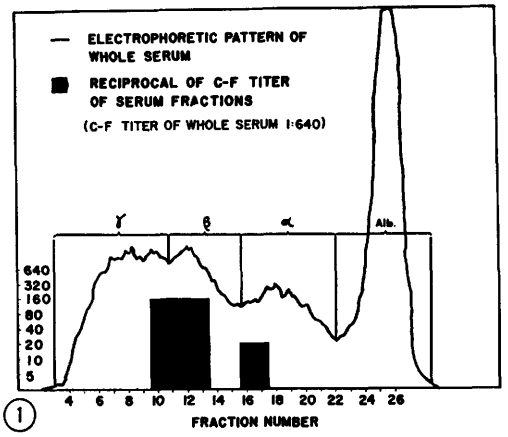
plasma body (AB)† count. Convalescent sera were obtained from animals showing an increase in number of erythrocytes with an accompanying decrease in AB count. Sera were tested for CF activity according to the methods outlined for diagnosis of anaplasmosis(4). The CF antigen‡ was prepared from hemolyzed, *Anaplasma*-infected erythrocytes(5). Sera were analyzed for total protein content by the biuret method as modified by Weichselbaum(6) and for serum protein composition by paper strip electrophoresis using veronal buffer at pH 8.6, 0.075 ionic strength as described earlier(1-3). All large volume serum fractionations were made using a continuous paper electrophoresis apparatus (Model CP, Spinco). In each case involving the fractionation of normal, acute, and convalescent sera all samples were diluted 1:2 with veronal buffer, pH 8.6, 0.025 ionic strength and 110 ml of this mixture were allowed to flow on the paper curtain (S & S 470) in similar buffer. The electrophoretic cell was maintained at approximately 1°C during the fractionations. A current of 93 ma was applied for 96 hr. An average total of 22 fractions that contained protein were collected in tubes from drip points along the paper curtain using a fraction collector (Fraction Collector, Spinco). All fluids originating from the same drip-point were pooled at the end of fractionation and kept at 4°C until they were dialyzed and concentrated. The paper curtains were dried at 125°C for 30 min. and stained with bromphenol blue to locate the protein bands and their respective drip-points. Fractions were dialyzed against distilled water to remove buffer salts and water was removed by further dialysis against 15% polyvinylpyrrolidone and freeze-drying. The dried fractions were reconstituted with sufficient 0.85% NaCl solution at the rate of approximately 2 ml per fraction to give the same total volume of serum that had flowed on the curtain from the sample reservoir. Total protein determinations were conducted on all fractions using the biuret method to calculate per cent protein recovered. Each fraction was also analyzed for homogeneity by paper strip electrophoresis. Aliquots from each serum fraction were assayed for *Anaplasma* CF antibody activity. To eliminate the anticomplementary activity usually encountered in concentrated serum fractions(7-9), the samples were initially diluted 1:5 with normal bovine serum rather than with the veronal-sodium chloride buffer solution which is ordinarily employed in the CF test. The samples were then inactivated at 58°C for 35 min. and further dilutions were made with veronal-sodium chloride buffer solution to determine CF antibody titers. Appropriate controls of positive and negative sera, serum fractions, antigen, complement and sensitized sheep cells were included in all tests.

Results. Fractionation by continuous electrophoresis of sera from normal calves and calves in the acute and convalescent stages of anaplasmosis resulted in separation of albumin, *alpha-*, *beta-*, and *gamma*-globulins. Average total recovery based on protein analyses after fractionation was 72%. The relative CF antibody activity in fractions of sera from calves with acute anaplasmosis is shown in Fig. 1. In all instances sera contained CF antibodies which were associated with the fractions of *alpha-* and *beta*-globulins of lower mobilities and the *gamma*-globulins of higher mobility. Most of the antibody activity was present in fractions which were identified as *beta*-globulins. Fig. 2 indicates the location of CF antibody activity in fractions of sera obtained from calves during the convalescent phases of anaplasmosis. Antibody activity in low concentration was found in the *beta*-globulin fractions of lower mobility. The *gamma*-globulins of high and intermediate mobilities possessed antibodies in high concentration. These convalescent sera did not contain CF antibodies in fractions migrating with the *alpha*-globulins, which was in contrast to those detected in acute phase sera. The means of the absolute concentrations of serum protein fractions of the acute, convalescent, and normal sera as determined by paper strip electrophoresis are given in Table I. Sera obtained during the acute phase of infection, as demonstrated by a large

† *Anaplasma* body (AB) count denotes per cent of erythrocytes containing marginal bodies.

‡ Supplied through the courtesy of ADED, U. S. Dept. of Agric.



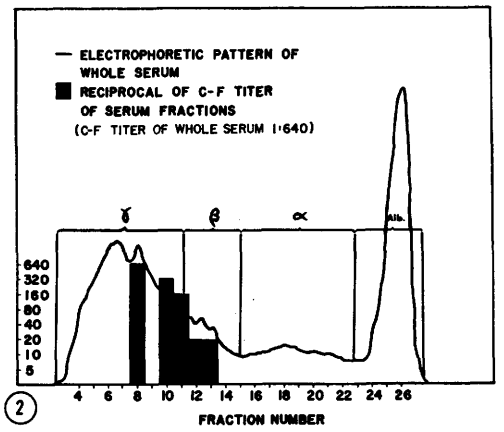


FIG. 1. Distribution and titers of complement-fixing antibodies in serum fractions of acute phase anti-*Anaplasma marginale* serum.

FIG. 2. Distribution and titers of complement-fixing antibodies in serum fractions of convalescent phase anti-*Anaplasma marginale* serum.

number of erythrocytes containing AB, exhibited decreases in absolute values of total serum protein, albumin, *gamma*-globulin, and total globulins. The concentration of *alpha*-globulin increased whereas a significant change in concentration of *beta*-globulin was not observed. During the convalescent stages, when the number of erythrocytes that contained AB decreased in number and approached the 1% level, an increase in concentration of *gamma*-globulin occurred and levels of *alpha*- and *beta*-globulins decreased below normal values. Studies of normal bovine sera did not reveal CF activity in the fractions that were separated, neither did the whole serum show antibody activity.

Discussion. The results described here confirm earlier observations(1) on changes which occur in the serum proteins of calves infected with *A. marginale*. The present studies also give support to a previous suggestion(1) concerning a shift in mobility of the CF antibodies which are resident in the serum globulins. This shift is coincident with simultaneous changes in concentrations of serum globulins as animals progress from the acute to the convalescent phase of the disease. Similar changes in the electrophoretic mobility of other antibodies have been reported(10-14). Even though the present findings cannot be strictly compared with the others the similarity of changes in antibody location within the serum proteins cannot be overlooked. In the present studies there was a disappearance of CF activity in the *alpha*-globulins during the convalescent phase as compared to the activity present in this fraction in the acute phase. There was no appreciable change in distribution of CF activity in the *beta*-globulins, but lower CF titers were observed in this fraction during the convalescent stages. The greatest changes were found in the *gamma*-globulin fractions. In the acute phases only the fraction of highest mobility showed CF activity, whereas during the convalescent stages the CF activity of the *gamma*-globulin became associated with the fractions of higher and intermediate mobilities. The apparent shift of CF activity toward globulins of lower mobilities may be due to inherent structural differences of the CF antibodies which develop during the period between the acute and convalescent phases. These changes could account for a

TABLE I. Means* of Absolute Concentrations of Serum Protein Fractions in Acute and Convalescent Stages of Anaplasmosis.

Fraction	Protein concentration in g/100 ml		
	Acute serum	Convalescent serum	Normal serum
γ-globulin	.92 ±.08	2.02 ±.31	1.54 ±.19
β- "	1.43 ±.11	1.07 ±.07	1.49 ±.14
α- "	1.62 ±.27	1.02 ±.14	1.34 ±.08
Albumin	2.09 ±.13	3.12 ±.24	3.10 ±.12
T.S.P.†	6.06 ±.19	7.23 ±.24	7.47 ±.33

* Mean values for 16 calves in acute stages, 14 calves in convalescent stages, and 18 normal calves.
† T.S.P.—Total serum protein.

difference in net charge as measured by these electrophoretic methods and would be evidenced by changes in the location of CF activity due to the different electrophoretic mobilities.

Summary. Fractionation of acute phase anti-*Anaplasma* sera and subsequent study of the fractions by CF showed that serologic activity was associated with the *alpha-* and *beta*-globulins of lower mobilities, and the *gamma*-globulins of highest mobility. Similar studies with convalescent sera showed a complete absence of CF activity in the *alpha*-globulins. CF activity in the *beta*-globulin fractions was distributed essentially similar to that found in acute phase sera although the titers of the individual fractions were diminished. The CF activity in the *gamma*-globulins during the convalescent stages was associated with fractions of high and intermediate mobilities. It was confirmed that a relationship existed between the concentrations of globulin components and CF antibody titers.

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Identification of the Hormones Secreted by an Autonomous Mammotropic Pituitary Tumor in Rats. (27518)

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A very useful laboratory tool for studies on pituitary hormones has been provided by Furth who has developed several strains of hormone-secreting pituitary tumors in rats and mice(1,2,3). The purpose of this study is to identify the hormones elaborated by one of Furth's autonomous mammotropic tumors, MtT-F4. On the basis of organ examination and hormone assay, Furth *et al.*(2,4,5) had concluded that the tumor elaborated a single pituitary hormone, mammotropin, which was responsible for body growth, growth and secretion of mammary glands and hypertrophy of the adrenals.

Methods. The primary tumor, MtT-F4, had been induced by Furth by implantation

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