Anal. Calcd. for $C_{12}H_{11}S_2Cl_3$: Cl, 32.70. Found: Cl, 32.26.

Discussion of Results

1,1,1-Trichloro-2,2-bis-(thienyl)-ethane was previously reported melting at 76.0°. Using the same procedure in preparation of this compound, we obtained a compound, when pure, melting at 78.4°.[8] However, analysis of this product proves it to be the desired product.

Attempts to determine the structure of 1,1,1-trichloro-2,2-bis-(chlorothienyl)-ethane were carried out by the method of Cristol and Haller.[9]

This method failed to give the product anticipated. Treatment of 1,1-dichloro-2,2-bis-(chlorothienyl)-ethylene with chromic oxide in boiling glacial acetic acid gave a yellow oil which failed to crystallize. Since the bis-chlorothienyl ketone was expected to be a solid, it was concluded the susceptibility of the thiophene nucleus to oxidation led to decomposition.

When the olefin was refluxed with alkaline and neutral potassium permanganate, the original product was recovered. Hydrolysis of the olefin with barium hydroxide[10] in ethylene glycol gave a neutral oil and a trace of acidic material. The yield of neutral product was insufficient for characterization, but a qualitative test showed the presence of sulfur and a trace of halogen. A halogen analysis indicated approximately 1.0% chlorine. Evidently the chlorine in the thiophene nucleus was removed by barium hydroxide as well as that attached to the ethylenic chain. No definite structure has been assigned to these compounds, but on the basis of analysis and the known high reactivity of the 2,5-positions of thiophene, it is suspected that the thiophene nucleus is joined at the 5-position.

Laboratory tests of the insecticidal properties indicate that 1,1,1-trichloro-2,2-bis-(chlorothienyl)-ethane is the most effective compound against cockroaches; however, the derivatives of 2-bromothiophene and 2-iodothiophene show some activity. The derivative of 2-methylthiophene shows no insecticidal activities. 1-Trichloro-2,2-bis-(chlorothienyl)-ethane seems to be as active as DDT against cockroaches.

(8) Peter, ref. 4, p. 1345.

(9) Cristol and Haller, ref. 5, p. 140.

(10) Cristol, Soloway and Haller, THIS JOURNAL, **69**, 510 (1947).

Summary

A series of thiophene analogs of DDT have been prepared. The ones not previously reported are: 1-trichloro-2,2-bis-(chlorothienyl)-ethane, 1-dichloro-2,2-bis-(chlorothienyl)-ethylene, 1-trichloro-2,2-bis-(bromothienyl)-ethane, 1-trichloro-2,2-bis-(iodothienyl)-ethane, 1-trichloro-2,2-bis-(methylthienyl)-ethane.

AMES, IOWA RECEIVED AUGUST 7, 1947

[CONTRIBUTION FROM THE LABORATORIES OF PHYSICAL AND PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

Biophysical Studies of Blood Plasma Proteins. VII. Separation of γ-Globulin from the Sera of Various Animals[1]

BY J. CHARLES NICHOL AND H. F. DEUTSCH

The separation of the components of biological tissues and fluids by ethanol fractionation as carried out by Cohn, *et al.*,[2] is directed to the isolation and recovery of all recognizable entities of the system in question. Often, however, in the interests of expediting the recovery or of increasing the yield of a given component it may be desirable to separate such an entity with immediate (but not necessarily eventual) disregard for other constituents. The antibodies of various animal species immunized to different antigens are known to possess the gross physical-chemical characteristics of the γ-globulins and to separate from solution with them. The scientific and technical importance of these antibody-rich fractions is the incentive which has led us to the development of a simple and effective procedure for removal of the normal γ-globulins from the sera of human beings and of the goat, dog, rabbit, rat, chicken and guinea pig.

It is found that in the individual species the chemical treatment may vary somewhat, but in all cases there is an initial and important step in which the antibody-rich γ-globulins are precipitated from a diluted serum which may be followed by a purification treatment to remove certain small amounts of contaminant β-globulins. In this way the γ-globulins are obtained in relatively pure form. The methods used involve variations in ethanol and salt concentrations and *p*H such as were used previously in studies on human γ-globulin.[3,4,5]

The general scheme, based in part upon our previous work,[3,4] consists in diluting one volume of serum with three volumes of water, adjusting the

(1) This work was supported in part by grants from Eli Lilly and Company, the Wisconsin Alumni Research Foundation, and the U. S. Public Health Service.

(2) (a) E. J. Cohn, J. A. Luetscher, Jr., J. L. Oncley, S. H. Armstrong, Jr., and B. D. Davis, THIS JOURNAL, **62**, 3396 (1940); (b) E. J. Cohn, L. E. Strong, W. L. Hughes, D. J. Mulford, J. N. Ashworth, M. Melin and H. L. Taylor, *ibid.*, **68**, 459 (1946).

(3) H. F. Deutsch, L. J. Gosting, R. A. Alberty and J. W. Williams, *J. Biol. Chem.*, **164**, 109 (1946).

(4) H. F. Deutsch, R. A. Alberty and L. J. Gosting, *ibid.*, **165**, 21 (1946).

(5) J. L. Oncley, M. Melin, D. A. Richert, J. W. Cameron and P. M. Gross, Jr., in press.

*p*H to 7.6–7.7, and adding 50% ethanol to give a final ethanol concentration of 20%. Temperatures are maintained to within one degree of the freezing point at all times. The precipitate which forms (ppt. A) contains most of the serum γ-globulins in admixture with some β-globulins. After removal by centrifugation, this precipitate is suspended in cold distilled water at a concentration of 0.5–1.0% and the suspension is adjusted to a *p*H ranging from 5.0 to 5.2. Various concentrations of ethanol and salt are used at this point, depending upon the species from which the original serum was obtained, to effect removal of the β-globulin (ppt. B), while maintaining the major portion of the γ-globulins in solution. The ppt. A from all the sera which were studied contains two families of γ-globulins, γ_1- and γ_2-globulins in our nomenclature.[4]

To effect the separation of the globulin of higher electrophoretic mobility (γ_1) when it was not removed entirely in ppt. B, the supernatant in this precipitation is brought to *p*H 5.6–6.0, the ethanol concentration to 10% and the ionic strength to 0.01, giving ppt. C-1. This precipitate consists predominantly of γ_1-globulin, along with some γ_2-globulin. The proteins remaining in solution at this point are essentially pure γ_2-globulins. Supernatants to ppt. B or ppt. C-1 are adjusted to *p*H 7.2–7.4 and the ethanol concentration is brought to 25% to effect precipitation. The γ_2-globulins, ppt. C-2, obtained in this manner are removed by centrifugation, suspended in distilled water, frozen and dried *in vacuo*.

A general scheme for the fractionation of an animal serum for γ_2-globulin is shown in Fig. 1. The conditions found suitable for that step of the fractionation process involving the separation of the beta from the gamma globulins for the various sera are shown in Table I. In the case of dog and rat sera it was necessary to introduce an additional step at *p*H 5.6–6.0 to remove γ_1-globulin (ppt. C-1) prior to the precipitation of the γ_2-globulin (ppt. C-2). An alternative procedure designed to remove γ_1-globulins with ppt. B is to raise the alcohol concentration above the values shown in Table I for the rat and dog systems.

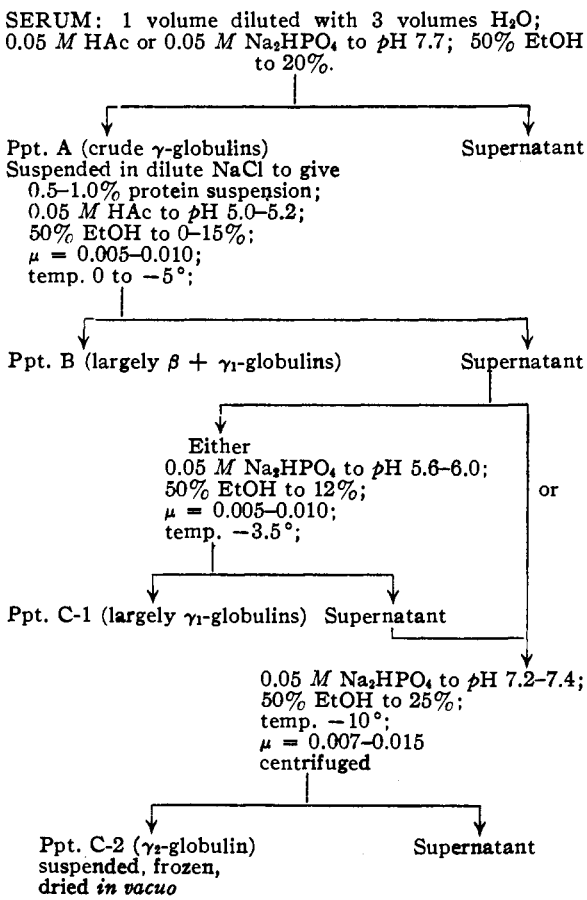

Fig. 1.—Fractionation scheme for isolation of γ_2-globulin from animal sera.

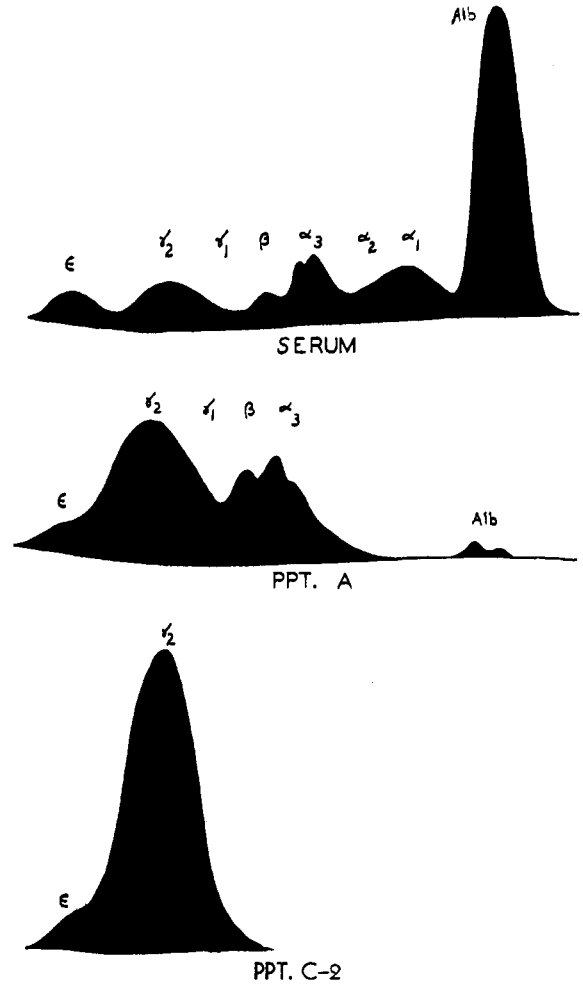

Fig. 2.—Descending electrophoretic patterns showing the course of fractionation of goat serum. Electrophoretic experiments were performed using barbiturate–citrate buffer, μ = 0.088, *p*H 8.6; duration of experiments 7800 sec. at potential gradient of 8.5 volts per cm.

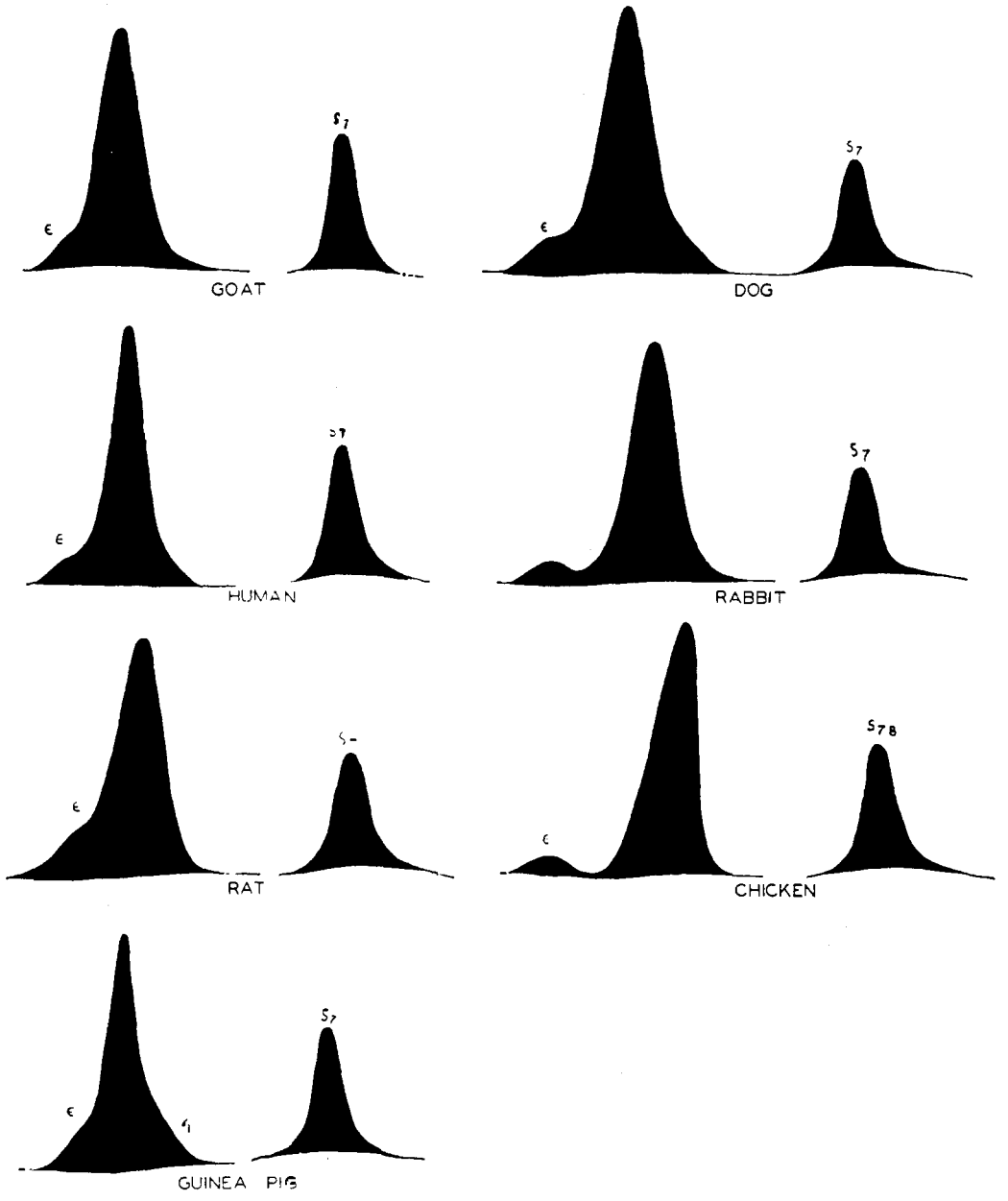

Fig. 3.—Descending electrophoretic patterns and sedimentation pictures of the γ_2-globulin of various animals (electrophoretic diagrams on left). Electrophoretic experiments were performed using barbiturate buffer, $\mu = 0.1$, pH 8.6 at a potential gradient of 6.5 volts per cm.; duration of experiments in seconds, goat 7800; human 8400; dog 11,000; rabbit 12,000; rat 7200; chicken 7800; and guinea pig 7200. Sedimentation pictures were taken after sixty minutes in the ultracentrifuge at 50,400 r. p. m.

However, by carrying out the precipitation step C-1 (Fig. 1) one can also obtain concentrates of γ_1-globulins whereas by the above procedure they are carried down into a precipitate consisting largely of β-globulins.

The electrophoretic patterns following the course of the fractionation of goat serum as outlined in Fig. 1 are shown in Fig. 2.

The fractionations were evaluated in terms of yield (recovery) and electrophoretic "purity" of product. The latter analyses were carried out in a pH 8.6 diethylbarbiturate buffer of ionic strength 0.1 at a constant potential gradient of approximately 6.5 volts per cm. The duration of experiments was from 7200 to 12000 seconds. Mobilities were determined by using the center of the initial boundary as the reference point.

Sedimentation analyses were carried out with γ-globulin solution in the high-velocity oil-turbine ultracentrifuge at 50,400 r.p.m., using a

TABLE I

CONDITIONS USED FOR THE SECOND PRECIPITATION STEP (PPT. B)

Serum	pH	Ethanol concentration, vol.%	Ionic strength, μ
Goat	5.2	0	0.01
Human	5.1	15	.01
Dog[a]	5.2	6	.005
Rabbit	5.2	10	.01
Rat[a]	5.0	10	.01
Chicken	5.0	10	.01
Guinea pig	5.1	15	.01

[a] Supernatants contain γ_1-globulins which were removed by step C.

schlieren method to record the position of the boundaries as a function of time.

Diffusion studies performed in this Laboratory by Polson[6] with solutions of our rabbit γ_2-globulin gave normal scale line displacement-distance curves to give $D_{20w} = 4.1 \times 10^{-7}$ sq. cm./sec. The method of moments was used in the computation. From this value and our sedimentation constant $s_{20w} = 7.05\ S$ a molecular weight of 160,000 is calculated. It is of interest that this value is in agreement with data of Kabat[7] for immune rabbit globulin.

The γ_2-globulins of the various animals are quite similar as regards their electrophoretic and sedimentation behavior (Table II, Fig. 3). However, it is apparent that chicken γ-globulin deviates somewhat in its properties, because it can be seen from Fig. 3 that two closely related electrophoretic components are present. The average mobility of this fraction is considerably higher than that of the γ_2-globulins of the other species studied. The sedimentation experiments show a single, somewhat broadened, peak but the sedimentation constant is somewhat higher than that of the other γ_2-globulins and heterogeneity is indicated. This fraction is free of lipid and was designated as a γ_2-globulin since it represented the component of lowest electrophoretic mobility in chicken serum. We have found this protein fraction to contain antibody.

Except as just noted, all γ_2-globulins sedimented as relatively homogeneous proteins with $s_{20w} = 7S$. Ppt. B of dog serum, consisting largely of γ_1-globulin, contained a component of high molecular weight ($s_{20w} = 18S$). γ_1-Globulins from other species were not separated from the main γ-globulin fraction for study, although electrophoretic analyses of the various fractions indicated their presence. From the point of view of their physical properties, the several animal γ_2-globulins appear to be quite similar to those found in human plasma. In addition, the γ_1-globulin of the dog shows similar molecular and electrokinetic behavior as compared to that of the corresponding fraction in human plasma.

(6) A. Polson, personal communication.

(7) E. A. Kabat, *J. Exptl. Med.*, **69**, 103 (1939).

TABLE II

PHYSICAL CONSTANTS AND YIELDS OF γ_2-GLOBULIN

Serum	$-U \times 10^5$ cm.^{2} volt^{-1} sec.$^{-1}$[a]	% Component of sedimentation constant $s_{20w} = 7S$	γ_2-Globulin recovered per 100 ml. serum, g.	γ_2-Globulin in product, % (electrophoresis)	% Yield of γ_2-globulin[b]
Goat	1.5	99	0.65	98	60–65
Human	1.3	93	.5	98	60–70[c]
Dog	1.2	95	.25	98	55–60
Rabbit	1.7	95	1.0	98	70–75
Rat	1.8	95	0.45	98	50–55
Chicken	2.6	92[d]	.6	95	(30–35)[e]
Guinea pig	1.1	98	.3	80	75–70

[a] Mobilities measured from center of the initial boundary. [b] Based on per cent. of γ_2-globulin in original serum as determined by electrophoresis. [c] Source material was old reconstituted dried plasma. [d] $s_{20w} = 7.8$ for the chicken γ_2-globulin. [e] Electrophoretic pattern suggests the presence of two closely related proteins. Lipoproteins having the same electrophoretic mobility as those of the gamma globulins in chicken sera make it impossible to give an exact estimate of the γ-globulin yield.

After being shell-frozen and dried, the final products (γ_2-globulins) of the fractionation are recovered as white powders which are readily soluble in 0.15 *M* sodium chloride to give clear and stable solutions. Cruder products which are contaminated with γ_1- and β-globulins may give solutions showing considerable amounts of suspended material and turbidity.

Acknowledgments.—The authors wish to acknowledge the many helpful suggestions of Dr. J. W. Williams during the course of these investigations. The valuable technical assistance of Mr. E. M. Hanson and Mrs. Alice McGilvery is gratefully recognized.

Summary

A method for the separation of γ_2-globulins from normal animal sera has been developed. It has been applied successfully to human, goat, dog, rabbit, guinea pig, rat and chicken sera.

MADISON, WISCONSIN RECEIVED NOVEMBER 12, 1946