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THE RELATION OF PROTEIN, LIPOIDS AND SALTS TO THE WASSERMANN REACTION.[1]

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In the present work on the Wassermann reaction in syphilis and general paralysis several points have been especially considered, namely: (1) the quantitative estimation of the active substances in syphilitic blood sera and paralytic cerebro-spinal fluids; (2) the effects of physical and chemical agents upon the active substances; (3) the quantitative estimation of the globulin content of syphilitic blood sera and paralytic spinal fluids; (4) the alcohol-extractibility of the active substances of syphilitic sera and paralytic spinal fluids; and (5) the antigenic lipoids and salts entering into Wassermann's reaction.

THE QUANTITATIVE ESTIMATION OF THE ACTIVE SUBSTANCES OF SYPHILITIC BLOOD SERA AND PARALYTIC CEREBRO-SPINAL FLUIDS.

The blood sera from fifty-five syphilitic patients have been studied with the view of determining the minimal quantity of serum just sufficing to produce complete fixation[2] of complement, the amount of which was contained uniformly throughout the entire work in 0.1 c.c. of fresh serum of the guinea-pig. Of eight cases of primary syphilis, five gave positive reactions, the titers ranging from 0.1 to a little over 0.2 c.c. The majority of the titers were 0.2 c.c. Of forty-two cases of secondary syphilis, six have been untreated and gave positive reactions in titers of 0.0006, 0.002, 0.003, 0.004, 0.01 and 0.04 c.c. respectively. Seventeen cases had

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[2] In each tube 3 hæmolytic units of anti-sheep amboceptor (rabbit serum) were employed, the quantity being 0.003 c.c. The alcoholic extracts of the blood and liver of man or rabbits were used as "antigens" in quantities of 0.1 to 0.2 c.c. of a 0.1 per cent. or 0.2 per cent. suspension. Purified preparations of lecithin were also employed as "antigens."

been slightly treated and gave positive reactions in titers of 0.01 to 0.2 c.c., or more frequently of 0.02 to 0.06 c.c. This group and the group of untreated cases of secondary syphilis gave the highest titers of all the cases examined. Seven cases were moderately treated and gave positive reactions in titers of 0.2 or a little more than 0.2 c.c. Twelve cases were in the late secondary stage under steady and prolonged treatment, of which only two gave positive reactions in a titer weaker than 0.2 c.c. The remaining ten cases gave negative results. Five cases of tertiary syphilis, which had been under steady and prolonged treatment, gave negative reactions excepting one in which the test was positive and the patient had lesions of the nervous and osseous systems.

Thirty-four control sera were examined; 11 were from normal persons and were uniformly negative; 23 were from persons suffering with other diseases than syphilis; of these five reacted positively in titers from 0.05 to 0.2 c.c. I could not exclude absolutely a syphilitic infection in the latter.

The cerebro-spinal fluid from cases of general paralysis gave in the majority of instances titer of 0.1 to 0.2 c.c. It was rarely that a sample with a titer of 0.02 c.c. was met with. I have not met an instance in which 0.01 c.c. produced complete inhibition of hæmolysis.

Hence the highest titer of any syphilitic serum was 0.0006 c.c., and of any cerebro-spinal fluid, 0.02 c.c.

THE EFFECTS OF PHYSICAL AND CHEMICAL AGENTS UPON THE ACTIVE SUBSTANCES.

The active substances of unfractionated syphilitic serum or of the globulin fractions obtained by half-saturation with ammonium sulphate, or through dialysis, are inactivated at 70° to 76° C. in twenty minutes. I may add that the activity of the euglobulin fraction obtained through dialysis disappears more easily when heated in distilled water than when heated in 0.9 per cent. saline solution. It was almost completely destroyed by a temperature of 65° C.

Drying syphilitic serum at 24° C. does not affect its activity in regard to the Wassermann reaction, and heating in a dry state to

temperatures below 100° C. for twenty minutes leaves its activity unimpaired. The temperatures of 120° and 135° C. produce partial insolubility of the dry serum and render it almost inactive.

The cerebro-spinal fluids from cases of general paralysis were, in my experience, which agrees with the findings of Marie and Levaditi,[3] rendered inactive in ten to twenty minutes by temperatures of 70° to 80° C.

Concerning the deterioration of the active substances with age, I may state that a sample of serum which gave a titer of 0.004 c.c. after the first twenty-four hours was reduced to a titer of 0.01 c.c. after fifty-six days in the refrigerator at 2° C. Some spinal fluids obtained from cases of general paralysis and almost a year old gave a titer of 0.1 to 0.2 c.c., no quantitative observations having been made when they were first collected. Bruck and Stern[4] state that the active principle in syphilitic sera will retain its strength with little deterioration when kept in the refrigerator, no length of time being assigned.

The activity of cerebro-spinal fluids from patients suffering from general paralysis is destroyed by acids or alkalies. When 0.2 c.c. of a fairly active sample of spinal fluid of a paralytic is mixed with 1.6 c.c. of a 0.9 per cent salt solution with an acidity or an alkalinity corresponding to $N/40$ concentration, the fluid becomes so modified that after removal of acid (HCl) or alkali (NaOH) by neutralization, at the end of one hour at 37° C., it no longer gives the Wassermann reaction. A concentration above $N/40$ of hydrochloric acid or sodium hydroxide in the same amount causes marked reduction of hæmolysis. This antihæmolytic phenomenon may possibly be due simply to increase in the salt tonicity after the neutralization.

Trypsin (Grübler's Trypsin sicc.) in neutral or a weakly alkaline (0.5 per cent. Na_2CO_3) medium caused complete inactivation of the syphilitic sera or cerebrospinal fluids. The ferment was allowed to act at 37° C. for three hours before the tests were made. It was customary to subject 0.2 c.c. of the serum or spinal fluid to the digestion and then to test the entire mixture, after neutralization in case of alkaline reaction, for the Wassermann reaction.

[3] Marie and Levaditi, *Annales de l'Inst. Pasteur,* 1907, xxi, 138.

[4] Bruck and Stern, *Berl. klin. Woch.,* 1908, xlv, 301.

Pepsin (Grübler's Pepsin puriss.) was found to be without effect when added to the serum or spinal fluids of syphilitics and paralytics in neutral medium. In the presence of a 0.2 per cent. hydrochloric acid it destroyed the activity of these fluids very promptly and completely. The acid alone had been found to be injurious when used in that strength, although a more complete disappearance of the active substances in the presence of pepsin conclusively demonstrated the effect of this ferment. It seems to be of some interest to recall here that, in spite of the pronounced antienzymotic properties of blood serum, the active substances of the serum and spinal fluid of syphilitics and paralytics are easily destroyed by tryptic and peptic digestion.

The activity of the syphilitic sera and paralytic cerebro-spinal fluids disappears when they are exposed to the combined effects of a photodynamic substance and direct sunlight. Eosin "rein" in dilution of 0.03 per cent. destroys the substances within three hours in the presence of direct sunlight. No visible precipitation occurs in the exposed eosinized mixtures. A three hours' exposure of non-eosinized controls to direct sunlight produces a perceptible diminution of the activity. On the other hand, no deteriorating effect is exerted by the dye in the dark. Whether the destructive action of eosin in direct sunlight is, in part or *in toto,* due to the acid formation in the mixture has not been determined.

THE QUANTITATIVE ESTIMATION OF THE GLOBULIN CONTENT OF SYPHILITIC SERA AND PARALYTIC CEREBRO-SPINAL FLUIDS.

Klausner[5] found that by adding adequate quantities of distilled water to syphilitic sera a precipitate would form, and Sachs and Altmann[6] observed a similar precipitate when a weak alcohol is employed instead of distilled water. The differences between syphilitic and non-syphilitic sera in these two tests are quantitative and become indistinguishable upon prolonged standing. Klausner thought his reaction due to an increase in globulins in syphilitic sera. Porges and Meier[7, 8] discovered that the sera of syphilitic patients

[5] Klausner, *Wien. klin. Woch.*, 1908, xxi, 214, 363.
[6] Sachs and Altmann, *Berl. klin. Woch.*, 1908, xlv, 522.
[7] Wassermann, *Berl. klin. Woch.*, 1907, xliv, 1599, 1634.
[8] Porges, *Wien. klin. Woch.*, 1908, xxi, 206.

can be differentiated from normal sera by the appearance of a quicker and more abundant precipitation of lecithin emulsion upon addition of these sera.

The diagnostic value of the Wassermann reaction and the Porges-Meier reaction for syphilis has been found to be, to a great extent, similar. Considering the rôle which lecithin plays in both reactions, it would seem not improbable that the active substances entering into these reactions are identical. Many chemicals to which antigenic properties had been assigned are also found to produce the precipitation phenomenon with syphilitic fluids. Thus sodium oleate and sodium glycocholate were shown by Nobl and Arzt[9] and Fritz and Kren[10] to give a similar reaction to lecithin. According to Elias, Neubauer, Porges and Salomon[11] the active substances belong to globulins and are present both in normal and syphilitic sera, differing only in the quantities which are greater in the latter than in the former.

No quantitative estimation of the serum globulin content of the syphilitic sera has yet been systematically made.

The presence in the spinal fluid of general paralytics and tabetics of the active substances has repeatedly been shown by previous workers.[12, 13, 14] Long before the Wassermann reaction had been applied to these cases an increase in proteins in the cerebro-spinal fluid had been known to be characteristic to these diseases.[15]

In my present investigations thirty-three cases of syphilis and twenty cases of other diseases than syphilis, including five normal individuals, have been subjected to simultaneous tests for the globulin content and for the Wassermann reaction. The method of estimating the quantity of serum globulins was a relative one and consisted of weighing the precipitate obtained by half-saturation of the sera with ammonium sulphate (or 1 part of serum + 4 parts of a

[9] Nobl and Arzt, *Wien. klin. Woch.*, 1908, xxi, 287.
[10] Fritz and Kren, *Wien. klin. Woch.*, 1903, xxi, 386.
[11] Elias, Neubauer, Porges and Salomon, *Wien. klin. Woch.*, 1908, xxi, 748, 831.
[12] Wassermann and Plaut, *Deutsch. med. Woch.*, 1906, xxxii, 1769.
[13] Marie and Levaditi, *Annales de l'Inst. Pasteur*, 1907, xxi, 138.
[14] Morgenroth and Sterz, *Virchow's Archiv*, 1907, clxxxviii, 166.
[15] The serum must not contain hæmoglobin.

half-saturated solution of $(NH_4)_2SO_4$). The precipitate was first centrifugalized at a rate of 5,000 revolutions per minute, during twenty minutes, decanted, the clear supernatant fluid poured off, the residual moisture absorbed with soft filter paper, and the solid parts then weighed in the centrifuge tube, whose own weight had been previously known. Beside this direct estimation, I found the following indirect method to be of some use in avoiding tedious weighing of the globulin deposit. This indirect estimation is based upon the precipitation of serum globulins by weak solution of acids. Take 1 c.c. of serum and mix it with 4 c.c. of half-saturated solution of ammonium sulphate, centrifugalize, pour the supernatant fluid away, then dissolve the deposit (globulins) in 10 c.c. of 0.9 per cent. salt solution. The clear globulin solution so prepared is then mixed with an equal volume of 10–15 per cent. butyric acid[16] solution containing 0.9 per cent. sodium chloride, shaken well and left at room temperature. The test is observed from time to time. With the globulin solution from normal serum there is, as a rule, no, or at most, slight opalescence even at the end of two hours from the time of acidification. With the globulin solution from syphilitic serum, which gives positive Wassermann test, a dense cloudiness arises promptly and within thirty minutes or later it becomes flocculent, and finally a deposit in the bottom of the test tube occurs. In the following table some of my experiments on this point are given. The ciphers given under the column "Weight of serum-globulins" denote the weight of the moist deposit of the globulins precipitated from 1 c.c. of serum and approximately correspond with eight to nine times the real weight as obtained by proper processes (coagulation with alcohol, hot water, removal of salts, extraction of fatty substances, and drying to constant weight).

The foregoing experiments show that in the blood sera of untreated or slightly treated cases of primary and secondary syphilis there is a definite increase in the globulin content. The results obtained by means of the direct and indirect methods of globulin estimation indicate that an increase in the globulin content is paralleled by the positive results of the Wasserman test, the only difference be-

[16] Various acids other than butyric have been found to be less satisfactory for this purpose.

tween the two being the slightly higher percentage of positive results with the indirect globulin method. There was no quantitative parallelism between the globulin content and the titers of the active substances of syphilitic sera. A syphilitic serum can have a low titer or be active for the Wassermann reaction without showing a correspondingly high globulin content. Among the controls the sera from the normal individuals and gonorrheal patients gave in-

TABLE I.

Syphilitic Sera.

	Stage.	Treatment.	Direct Estimation. Weight of the Globulins Weighed as Moist Deposit.	Indirect Estimation. Flocculent Precipitation of Globulin Solution with 10 per cent. Butyric Acid.	Wassermann Reaction.
1	I	o	0.348 gram	++	+ > 0.2 c.c.
2	"	o	0.321	++	+ 0.2
3	"	o	0.355	++	+ 0.2
4	"	o	0.280	+	—
5	"	×	0.200	—	—
6	II	o	0.553 (!)	++++	+ 0.04
7	"	×	0.395	+++	+ 0.02
8	"	×	0.260	+	+ 0.2
9	"	×	0.250	+	+ > 0.2
10	"	×××	0.257	+	—
11	"	×	0.411	++++	+ 0.02
12	"	×	0.402	+++	+ 0.2
13	"	×	0.353	++	+ 0.05
14	"	×	0.279	+	+ > 0.2
15	"	×	0.300	++	+ > 0.2
16	"	××	0.425	+++	+ 0.2
17	"	××	0.298	++	+ 0.1
18	"	××	0.365	+++	+ 0.2
19	"	××	0.393	++	+ > 0.2
20	"	×××	0.251	—	—
21	"	×××	0.343	+++	+ > 0.2
22	"	×××	0.161	—	—
23	"	×××	0.149	—	—
24	"	×××	0.200	—	—
25	"	×××	0.141	—	—
26	"	×××	0.210	—	—
27	"	×××	0.140	—	—
28	"	×××	0.253	+	—
29	"	×××	0.230	+	—
30	"	×××	0.252	+	—
31	III	×	0.450	++++	+ 0.1
32	"	××××	0.110	—	—
33	"	××××	0.105 (!)	—	—

Under column "Treatment" o = no treatment, × = slight treatment, ×× = moderate treatment, ××× = much treatment, ×××× = treatment extending over two years.

TABLE II.

Non-syphilitic[17] *and Normal Sera.*

Nature of Disease.	Direct Estimation of Globulins.	Indirect Estimation of Globulins.	Wassermann Reaction with the Serum.
Gonorrhœa.	0.205	—	—
"	0.135	—	—
"	0.175	—	—
"	0.130	—	—
"	0.110	—	—
"	0.140	—	—
Rheumatic fever.	0.183	—	—
Gastric ulcer.	0.170	—	—
Cirrhosis of liver.	0.350	+++	+ 0.05 c.c.
Chronic valvular disease, etc.	0.305	++	+ 0.2
Tuberculosis.	0.283	+	+ 0.2
Pneumonia lobar.	0.166	—	—
Chronic interstitial nephritis.	0.251	—	—
Malaria.	0.312	++	—
Tuberculosis.	0.321	++	+ 0.05
Normal.	0.165	—	—
"	0.163	—	—
"	0.184	—	—
"	0.205	—	—
"	0.204	—	—

variably negative results and no increase in or abnormally large globulin content could be found by direct estimation. Of the rest of the controls four sera showed an increase of globulin and reacted positively to the Wassermann test.

My experiments on cerebro-spinal fluids from forty-three cases of general paralysis[18] showed that there is a closer relation between the increase of leucocytes and that of globulins than between the increase of globulins and the positive reaction to the Wassermann test. In other words, the results obtained by cytological diagnosis and globulin estimation are in good harmony, while the Wassermann reaction was in some instances absent although the globulin was increased. In forty-three cases, thirty-seven positive results were obtained. In two of forty control cases, where the globulin tests were all negative, the Wassermann reaction was faintly positive.

[17] The possibility of a previous syphilitic infection could not be excluded in some of the sera giving the positive reaction for the Wassermann test.

[18] A fuller account of this series and of still other cases will be published elsewhere in conjunction with Dr. J. W. Moore.

The method which I employed for detecting an increase of the protein in the cerebro-spinal fluids may be mentioned here, as it affords a much sharper and more enduring means to differentiate normal from paralytic spinal fluid than the Nonne method of half-saturation with ammonium sulphate.[19] It is very simple and can be made with small quantities of material in less than half an hour. Its diagnostic value has been found to be equivalent, if not superior, to that of cytological examination. The technic is as follows:

To 0.1 c.c. of spinal fluid[20] add 0.5 c.c. of the 10 per cent. butyric acid,[21] boil briefly over a flame, then add quickly 0.1 c.c. of normal solution of sodium hydroxide and reheat briefly. Allow the test tube to stand in a frame and observe the reaction. Read the reaction for diagnosis within three hours; longer standing, extending to twenty-four hours, offers no advantage, and the characteristic appearance of the reaction becomes less obvious after the lapse of this time. In the spinal fluid from cases of general paralysis *a coarse granular or flocculent precipitate* appears, which gradually settles down to the bottom of the test-tube leaving the fluid above clear. In the majority of cases of general paralysis, tabes dorsalis and cerebro-spinal lues, the precipitate becomes granular within ten to twenty minutes and settles in a short time afterwards. In the spinal fluid from cases of alcoholic psychosis, dementia præcox, and epilepsy as well as from normal subjects, there occurs *a slight and uniform opalescence* only, and no coarse precipitate forms even after several hours. In case of an ambiguous reaction a test with 0.2 c.c. of the spinal fluid must be made which usually decides the point.

In determining the quantities of proteins, especially those precipitable by half-saturation of ammonium sulphate in cerebro-spinal

[19] This method consists of mixing 2 c.c. of spinal fluid with an equal volume of saturated solution of ammonium sulphate (strictly neutral) and reading the result within three minutes. The differentiating value gradually diminishes as time elapses beyond three minutes.

[20] Specimens which had been preserved in the refrigerator for about one year, or those from which all cellular elements have previously been removed by centrifugalization, are equally good for the test. It is entirely to one's individual taste whether one or a multiple of the quantities indicated above will have to be employed.

[21] A number of inorganic and organic acids have been tried, but were found to be less satisfactory.

fluid, I found that the ones which are peculiarly rich in the paralytic spinal fluid, are chiefly globulins or globulin-like substances which are the main substances causing the characteristic coarse or flocculent precipitate in the above test as well as the ones entering into the Wassermann reaction. The average quantities of these proteins contained in 1 c.c. of fluid ranged from 0.01 to 0.02 gram[22] weighed as a moist deposit of constant density. A similar estimation with non-paralytic[23] and normal spinal fluids, showed that the amount of the protein never exceeded one-tenth of that found in the specimens from paralytics.

CAN THE ACTIVE SUBSTANCES OF SYPHILITIC SERA AND PARALYTIC CEREBRO-SPINAL FLUIDS BE EXTRACTED?

Levaditi and Yamanouchi[24] claim that the reacting substances of syphilitic sera and paralytic and tabetic spinal fluids can be extracted with alcohol. By alcoholic extraction they obtained a certain amount of lipoids and of salts. Both sets of substances were inherently antihæmolytic when used in large enough quantity. Mixed in smaller quantity with liver extract or other "syphilis antigens" they gave increased inhibition of hæmolysis. Apparently Levaditi and Yamanouchi consider the Wasserman reactions as a summation of the anticomplementary effect of two sets of lipoids and salts. They give no quantitative details.

My own experiments in this respect failed to convince me that the Wassermann reaction is caused by alcohol-soluble constituents of serum and spinal fluid.

By means of dialysis of the serum in a celloidin sac the englobulin fraction of the serum was obtained free from dialysable salts. The euglobulin fractions were redissolved in 0.9 per cent. salt solution and tested for the Wasserman reaction. Three samples of syphilitic and two samples of normal sera examined showed that the euglobulin obtained from syphilitic sera possesses the property of giving the Wassermann reaction while that from normal sera did not. The titers of the globulin fractions were the same as those of the corresponding sera.

[22] To obtain an approximate absolute weight the ciphers must be divided by 8 to 9.

[23] Cases of tabes dorsalis are not included.

[24] Levaditi and Yamanouchi, *Compt. rend. de la Soc. de Biol.*, 1908, lxiv, 27.

The above experiment shows that the active substances binding complement with lipoids remain in the celloidin sac on dialysis. The removal of dialysable salts from the serum does not affect the activity of the specific substances which are precipitated with the euglobulin fraction. A qualitative difference is shown to exist between normal and syphilitic serum-globulins.

The lipoidal bodies of syphilitic sera and paralytic spinal fluids are alleged by Levaditi and Yamanouchi[25] to represent the specific active syphilitic substance of these fluids. In studying this question I have undertaken numerous quantitative estimations of alcohol-extractable constituents of blood sera, blood coagula and spinal fluids of normal, syphilitic and paralytic subjects, in order to ascertain if there exist qualitative or quantitative difference between the lipoids derived from normal and from syphilitic materials. Two syphilitic sera and one normal serum were extracted with ten volumes of 95 per cent. alcohol for five days at 37° C. The weights of the alcohol soluble matters were, after drying, 0.205, 0.210 and 0.202 gram, respectively, per 11.5 c.c. of the serum (ca. 1.79 per cent. average). The sample which yielded 0.205 gram of alcohol-extractable substances had a titer of 0.004 c.c., that which yielded 0.210 gram had a titer of 0.01 c.c., and that which yielded 0.202 gram was normal and had no effect on complement in Wassermann's test. The alcoholic extracts were dissolved in 0.9 per cent. saline solution (11.5 c.c., the original volume of serum) respectively, and tested for the property of "syphilitic antibody." When used in quantities over 0.3 c.c. these solutions were slightly antihæmolytic, but there was no regular complement fixation even when used in combination with antigenic substances (lecithin, alcoholic extract of liver, etc.). There was no difference between the solutions obtained from normal and from syphilitic sera, both being entirely devoid of the "antibody" property which characterizes the whole syphilitic sera.

Three samples of blood coagula of syphilitic subjects (manifest secondary stage) and three of normal persons were extracted with ten volumes of 95 per cent. alcohol at 37° C. for five days. The quantities of the coagula and of the lipoids and salts extracted with alcohol may be stated as follows:

[25] *Loc. cit.*

Syphilitic coagula I	20 grams.	Extract 0.230 gram.
II	17 grams.	Extract 0.219 gram.
III	15 grams.	Extract 0.190 gram.
Normal coagula I	32 grams.	Extract 0.330 gram.
II	20 grams.	Extract 0.210 gram.
III	28 grams.	Extract 0.299 gram.

The average content of syphilitic and normal blood coagula in alcohol-extractable matters is approximately 1.1 per cent., of which salts make up a large percentage.

The experiments in regard to the existence of specific active syphilitic substance in these extracts have been negative. No positive reaction for the Wassermann test was obtained with any of them.

Similar extraction experiments were made with three samples of general paralytic spinal fluids and one of a non-paralytic patient. None of the extracts gave a positive Wassermann test conclusive enough for establishing a "syphilitic antibody" nature. In a similar way to Levaditi and Yamanouchi, I observed in one of the paralytic extracts more or less marked inhibition of hæmolysis in combination with lecithin, but I am not convinced that this reaction is to be regarded as the regular Wassermann reaction.

ANTIGENIC LIPOIDS AND SALTS FOR WASSERMANN'S REACTION.

Landsteiner, Müller and Plötzl[26] discovered the alcohol solubility of the antigenic principles of syphilitic and normal tissues. Porges and Meier[27, 28] found lecithin to act as antigen, while Levaditi and Yamanouchi[29] place sodium glycocholate, sodium taurocholate, protagon and cholin among the syphilitic "antigens," and found lecithin to be less active than sodium glycocholate. Sachs and Altmann[30] state that sodium oleate has a similar action. Cholesterin and vaselin were effective as antigens in the hands of Fleisch-

[26] Landsteiner, Müller and Plötzl, *Wien. klin. Woch.*, 1907, xx, 1421, 1565.
[27] Wassermann, *Berl. klin. Woch.*, 1907, xliv, 1599, 1634.
[28] Porges, *Wien. klin. Woch.*, 1908, xxi, 206.
[29] Levaditi and Yamanouchi, *Compt. rend. de la Soc. de Biol.*, 1907, lxiii, 740; 1908, lxiv, 349.
[30] Sachs and Altman, *Berl. klin. Woch.*, 1908, xlv, 494.

mann[31] and cholesterin was found inactive by Levaditi and Yamanouchi.

My study of this topic was directed to the ascertainment whether qualitative or quantitative differences existed between extracts of normal and syphilitic blood and liver. The extraction was made with eight volumes of 95 per cent. alcohol at 42° C. for six days. The liver was made into a pasty mass by grinding and straining before extraction. The sera and coagula from syphilitic and normal bloods were extracted with ten volumes of 95 per cent. alcohol at 37 C. for five days. Before testing "the antigenic value" of the extract of each sample I removed salts by taking up the lipoids with ether. The ether soluble substances were then dried and fractionated with acetone.[32]

The following table gives the results:

			Acetone Insoluble.	Acetone Soluble.
Liver of a congenital syphilitic child.		15 grams	0.105 gram[33]	0.195 gram
Liver of a non-syphilitic child.		35 grams	0.665	0.250
Liver of a normal rabbit.		20 grams	0.540	0.140
Syphilitic coagulum.	I	20 grams	0.054	0.070
	II	17 grams	0·035	0.050
	III	15 grams	0.015	0.050
Normal coagulum.	I	32 grams	0.059	0.070
	II	20 grams	0.026	0.042
	III	28 grams	0.035	0.058
Syphilitic serum.	I	11.5 c.c.	Alcoholic extract without fractionation 0.205 gram.	
	II	11.5 c.c.	Alcoholic extract without fractionation 0.210 gram.	
Normal serum.	I	11.5 c.c.	Alcoholic extract without fractionation 0.202 gram.	

The lipoidal content of different samples of blood coagula varied rather irregularly, but this may be due to variable consistence of the coagula. In general it appears to be somewhat lower than with the sera, and many times less than with the livers.

[31] Fleischmann, *Berl. klin. Woch.*, 1908, xlv, 490.

[32] For redistilled acetone employed in the experiment I am obliged to Dr. P. A. Levene.

[33] The ratio between the acetone-insoluble and acetone-soluble portion in this sample of syphilitic liver is very different from the normal specimens, but I found it impossible, at the time, to secure a second syphilitic liver to confirm this result.

The ratio between the acetone-insoluble and acetone-soluble portion is quite the reverse in the liver and the blood coagula. In the liver the acetone-insoluble lipoids exceed the acetone-soluble lipoids, while the contrary is the case with the coagula.

Two cubic centimeters of the cerebro-spinal fluids derived from three cases of general paralysis and one non-paralytic patient yielded upon extraction 0.003 to 0.004 gram of lipoids each.

In determining the antigenic value of these extracts solutions of uniform concentration were made with the acetone-insoluble and acetone-soluble fraction of each coagulum or liver: thus 0.1 gram of the substance in question was dissolved in a few cubic centimeters of methyl alcohol and then emulsified in 100 c.c. of 0.9 per cent. salt solution. For the standard of antigenic value lecithin from two different sources (Lecithin "Agfa" Merck and one of my own preparations from beef blood corpuscles), were used. A syphilitic serum with the titer of 0.004 c.c. was employed as "antibody" in the amount of 0.1 c.c.

It was found that the antigenic strength of the acetone-soluble fraction of the extract of blood coagula, irrespective of their sources, was somewhat stronger than the acetone-insoluble fraction. With the extracts of livers the acetone-insoluble fraction was slightly more effective than the acetone-soluble fraction.

It was also found that *the lipoids extracted from syphilitic sera or blood coagula with alcohol can act as "antigens" for the "antibody" found in corresponding native sera.* There was no evidence that any qualitative or quantitative difference exists between the lipoids extracted from syphilitic and those extracted from normal liver, blood sera and blood coagula.

Just which lipoids are responsible for the powerful antigenic properties of the acetone-soluble portion has not been ascertained, but I can exclude cholesterin from the possible agents; for I tested negatively cholesterin preparations from sheep's brain, and gall stones and a sample prepared by Merck. Protagon, cholin and neurin did not exhibit in my hands convincing antigenic properties. I can confirm Levaditi in the finding that sodium glycocholate and sodium taurocholate can act as "antigens" but I found the

quantities required very large compared with lecithin and other antigenic lipoids. Sodium cholate acted in a similar way to the other two bile salts.

SUMMARY.

1. The high value in respect to complement-binding exhibited by blood sera from syphilitics and spinal fluids from general paralytics is associated with an excessively high content of globulin, but there does not exist a direct quantitative relation between the two. Cases of secondary syphilis which have been under prolonged and proper medication do not exhibit the globulin increase and usually fail to give the Wassermann reaction. The active substances entering into the Wassermann reaction are precipitable with the globulin and chiefly with the euglobulin fraction of the fluids.

2. Temperatures of 70° to 76° C. destroy the active substances. Exposed to sunlight the active substances deteriorate slowly. A photodynamic substance such as eosin, under the direct influence of the sun, brings about their complete and rapid destruction. This effect does not occur in the dark. The active substances are subject to tryptic and peptic digestion and are destroyed by weak acids and alkalies.

3. The active substances in the blood sera and spinal fluids cannot be separated from them or from the globulin precipitate by alcohol.

4. There are contained in the alcoholic extracts of normal and syphilitic blood and organs certain acetone-soluble lipoids which possess high antigenic values for the Wasserman reaction. Cholesterin is inactive and the bile salts less active than the lipoidal bodies.

5. Sodium cholate is about as active as sodium taurocholate and glycocholate, but neurin and cholin are inactive.

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