	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Indol.
Streptococcus pyogenes.....	+	+	+	+	*c*	—	—
Staphylococcus pyogenes.....	+	+	+	+	*c/p*	+	—
Mic. tetragenus.....	+	+	+	+	*c*	—	—
Mic. melitensis.....	+	+	+	+	+	—	—
Mic. zymogenes.....	+	+	+	+	*c/p*	+	—

XXX. The Acid Fast Group.

This group is a very extensive one, possessing, in common, a waxy envelope which comprises from 15 to 30% of the total dried weight of the organisms, and which confers on them their remarkable staining properties. Dynamically there is the widest range in their activities from the exquisitely pathogenic *B. tuberculosis* and *B. leprae* to the very ubiquitous grass bacilli.

The members of the group considered here comprise three strains of Müller's grass bacillus, *B. leprae* "Hardy" from the Hygienic Laboratory in Washington, *B. leprae* "Duval" from New Orleans and the organism known as the "Nasal Secretion" bacillus of Karlinski. The latter clearly belongs to the grass bacillus type.

A prominent chemical characteristic of the grass bacilli is the "negative ammonia phase" in glucose broth. This is also exhibited by the "Nasal Secretion" culture.

Leprosy culture "Hardy" produced practically no measurable change in either plain or glucose broth, although it grew with moderate luxuriance. The group as a whole is culturally inactive.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY, NO. 216.]

THE WEIGHT OF A FALLING DROP AND THE LAWS OF TATE. XI. THE DROP WEIGHT AND SURFACE TENSION OF BLOOD SERUM.[1]

BY J. LIVINGSTON R. MORGAN AND HAROLD E. WOODWARD.

Received July 17, 1913.

Object of the Investigation.

In a series of papers from this laboratory[2] it has been shown by one of us, with co-workers, that the weight of a drop of liquid falling from a properly constructed tip, as determined by aid of a special apparatus (which

[1] We are deeply indebted in this work to Drs. Bailey, Hopkins and Smith of St. Luke's Hospital, to Drs. Butterfield and Bronfenbrenner of the Rockefeller Institute, and to Dr. Warren of Roosevelt Hospital, and take this occasion to express our thanks to them.

[2] THIS JOURNAL, 30, 360–76, 1055–68; 33, 349–62, 643–57, 657–72, 672–84, 1041–60, 1060–71, 1275–90, and 1713–27.

reduces the possible error, due to excessive speed of formation, loss by evaporation, etc., to a minimum value) is strictly proportional to the surface tension of that liquid. In short, it has been found that at any one temperature the relationship existing is

$$\text{surface tension} = \text{drop weight} \times \text{constant},$$

where the value of the "constant" depends only upon the diameter of the tip employed. One great advantage of this method over that based upon the measurement of the capillary rise, for example, lies in the fact that here the surface tension is found *directly* from the experimental result, *without any knowledge of the density of the liquid at that temperature*, a factor so vitally necessary when the capillary rise is employed.[1]

The object of this investigation was two-fold:

First. The study of the surface tension of normal and pathological blood serum from different mammals.

Second. The testing by the aid of the very accurate Morgan drop-weight apparatus, of the so-called "Meiostagmin" reaction of Ascoli and Izar,[2] *viz., that the immunizing reaction in pathological blood serum is always accompanied by a lowering of the surface tension of the serum;* as shown by their work on syphilis, typhoid, tuberculosis and cancer, where the results were positive in 90% of the cases observed.

These topics will be considered in the above order, from the point of view of one interested in the possibility of getting results which are concordant and satisfactory, rather than in the physiological meaning of the results themselves.

The Standardization of the Tips Used.

Three tips were used in the course of this investigation, one of the original ones having cracked. As in past work, to avoid loss of time while cleaning a tip thoroughly between observations, two pieces of apparatus were employed, as it has been found that the results when calculated in terms of surface tension were perfectly interchangeable, the difference never amounting to more than a few hundredths of 1%.

The tip designated as Tip I was calibrated at 30° by determining the weight of 30 drops of benzene falling very slowly from it. Subtracting from this weight the weight of 5 drops, determined as a blank, allowing the fifth drop to hang from the tip until the total time elapsed for the 5, is the same as that for the 30, gives then the weight of 25 drops of benzene, free from any effect of evaporation; for the same *total* weight must be

[1] The experimental work of this paper was carried out during the winter of 1911–1912, and the above conclusion was drawn from the previous study of some 60 liquids. Since that time about 80 other pure liquids (as well as 60 solutions) have been studied, with the result that this conclusion is now even more evident.

A report on this further work will appear in the near future.

[2] See Juhnke, *Interstate Med. J.*, **18**, 233 (Feb. 1911).

lost by evaporation from the 5 as from the 30.[1] One twenty-fifth of this weight then is the weight of one drop, w, and the surface tension in dynes per centimeter, γ, of benzene at 30° can then be found by aid of the equation

$$\gamma = 2.1148/Kw(= \text{constant} \times w)$$

where

$$K = w(78/d)^{2/3}/(288.5 - 30 - 6),$$

d being the density of benzene at 30°[2]. When the value of the "constant," *viz.*, $2.1148/K$, is once found in this way for benzene, it is then to be used for any other liquid, after *its* value of w is found, no matter what the temperature may be. The surface tension γ, however, is naturally always for the same temperature as that at which the drop-weight w was determined.

Tip I gave an average of 11.7113 grams for vessel and 30 drops of benzene at 30°, the average of the blank of 5, at the same temperature, being 10.9695, which gives for one drop the weight of 0.029672 gram, or 29.672 milligrams. Since the density of benzene at 30° is 0.8679, we find by aid of the above formula that $K = 2.3572$ or

$$\gamma = 0.8972\,w \text{ for Tip I.}$$

Tips II and III were standardized by the use of water, employing the value of δ surface tension of water found by Morgan and McAfee[3] from their drop weights. According to them the surface tension of water varies with the temperature as shown by the equation

$$\gamma_t = 75.872 - 0.1547t - 0.000222t^2,$$

which at 37° would give the value 69.844 dynes per centimeter.

Tip II gave 13.2203 grams for 30 drops of water at 37°, and 11.3082 grams for the blank of 5, which gives 0.07648 gram, or 76.48 milligrams as the weight of one drop. Surface tensions and drop weights then must always be related in this way on this tip according to the proportion

$$w : \gamma :: 76.48 : 69.844,$$

from which we find

$$\gamma = 0.9122w \text{ for Tip II.}$$

K for this tip then (from $\gamma = 2.1148/Kw$) is equal to 2.3158.

Tip III gave a weight in average of 30 drops of water at 37° equal to 12.9084, the blank of 5 weighing 11.0108, which gives one drop a weight of 75.904 milligrams, from which we find

[1] This method of procedure is so satisfactory that it is possible to make observations within a few degrees of the boiling point, where the blank must consist of 10 drops in place of 5, owing to the large total evaporation.

[2] For details of the standardization of a tip, see, for example, THIS JOURNAL, 33, 360–61, 1713–16.

[3] THIS JOURNAL, 33, 1275.

$$\gamma = 0.92016w \text{ for Tip III.}$$

K for this tip is 2.2983.[1]

From the K values above it is now possible to calculate the effective diameter of the tips.[2] Such a calculation shows the diameters to be, respectively, 5.580, 5.483 and 5.441 millimeters for Tips I, II and III.

Since the sera to be studied in this investigation were to be very viscous, and very viscous liquids, unless special care be taken, usually lead to results of drop weight which rather tend to be high (owing to the peculiar way in which the drop separates from the remaining mass, apparently) it was thought wise first to make a preliminary investigation with this type of liquid to see that the results were not too high, and further to ascertain whether or not constant, consistent results in general were obtainable. For this purpose we selected glycol and solutions of cane sugar in water.

Glycol, with its high boiling point, 200°, has such a small vapor pressure at the temperatures worked at that it was found possible to do without the blank determination, consequently series of 10 drops were weighed at these temperatures, with the results given in the following table. All the weights are given here to show just what the accuracy is with such a viscous liquid.

GLYCOL, $(CH_2OH)_2$.

Temp.	Tip.	10 drops.	Av.	1 drop mgs.	γ.
		0.5265			
		0.5266			
0°	I	0.5273	0.5268	52.68	47.26
		0.5269			
		0.5270			
		0.5268			
		0.5051			
		0.5053			
30°	I	0.5047	0.5050	50.50	45.31
		0.5051			
		0.5050			
		0.4728			
55°	III	0.4733	0.4731	47.31	43.53
		0.4734			
		0.4729			

From the results of surface tension γ, we find by the aid of least squares its relationship to the temperature to be

$$\gamma_t = 47.277 - 0.0675t.$$

[1] This standardization of the tip is a most important thing and much time is consequently spent upon it. The results above are simply the average of a number of determinations which agree for the 30's, for example, to within a few tenths of a milligram. The results are given as above simply to save space.

[2] Morgan and Cann, THIS JOURNAL, 33, 1060.

Walden[1] gives the formula, from results of capillary rise in the literature, as

$$\gamma_t = 48.48 - 0.0994t.$$

Comparing the results from his formula with ours as above at the three temperatures we find

t.	M. & W.	Walden's formula.
0	47.26	48.48
30	45.31	45.50
55	43.53	43.01

It must be remembered here that the capillary rise method also finds difficulty with a viscous liquid, especially if it have a low density, for if it be drawn too high in the tube its weight is not sufficient to draw it away from the walls, and if it is not drawn high enough, its viscosity may prevent it rising further. At any rate the above comparison shows that the results by drop weight are not too high, if the results of capillary rise are correct. Somewhere between 30° and 55° the results by the two methods agree. The results by drop weight are certainly not too high, even when there might be danger of their being so, *viz.*, at 0°, where the liquid is most viscous. As to constancy it will be seen that the drop weight results leave nothing to be desired.

The results for 0.5, 1, 1.5, and 2 molar sugar solutions at 37° were also satisfactory as to constancy, so that if there be any special technique necessary to handle viscous liquids it may be assumed that it was obtained in these preliminary determinations. The change of the surface tension of sugar solutions with the concentration, at 37°, it was found could be accurately expressed according to these results by the formula

$$\gamma = 69.878 + 0.456C + 0.5202C^2,$$

where C represents the number of moles of sugar per liter of solution.

Surface Tensions of Human and Animal Blood Sera.

Dog blood serum was studied first, the dogs being kept in special cages and fed the regular daily diet (15 grams meat, 4 grams cracker meal, 3 grams lard and 35 cc. water per kilogram of body weight) until they were in normal condition.[2] It was found that serum obtained by clotting remained clear longer than that obtained by centrifuging, so the former method was employed throughout the work. Experiments seemed to show the same surface tension, for blood itself as for the serum, but as blood decomposes more readily, it was decided better to use the serum throughout.

After a large number of results had been obtained under various conditions it was found that satisfactory enough agreements could be obtained by weighing one single drop of serum after it had fallen very slowly from

[1] *Z. physik. Chem.*, 63, 143.

[2] These dogs were placed at our disposal by Professor W. J. Gies, and we take this occasion to thank him for his kindness.

the tip. At 37° the weight of vapor from the first hanging drop before it fell, which saturated the air space of the weighing vessel, was found to be small, and in average equal to 0.5 milligram—so this weight as well as that of the empty vessel was subtracted from the weight of vessel and drop. A great advantage of this procedure was that several determinations could be made in a comparatively short time, with the probability that the serum itself had not changed during the series.

The blood serum of horse, rabbit, guinea pig and sheep were obtained either from the Rockefeller Institute or St. Luke's Hospital. The results here do not agree so well among themselves as was the case with dog serum, due to the fact that the condition of these animals was not governed as carefully as with the dogs.

The results on human serum also vary among themselves more than those on dog serum, for the amount and kind of food eaten seems to have a considerable effect on the surface tension of the serum, and the conditions governing the human subjects were not especially regulated Most of this serum was obtained from St. Luke's or Sloan Hospitals, and some of it was given by fellow-workers in the laboratory.

DOG SERUM.

No.	Tip.	Wt. 1 drop. Mgs.	Av.	γ.	Remarks.
1	II	49.7 49.4	49.6	45.7	Normal dog
2	III	49.8 49.5 49.8	49.7	45.7	Normal dog
3	III	48.9 48.8	48.9	45.0	Normal dog
4	III	49.7 49.7	49.7	45.7	Dog; centrifuged
		49.4 49.2	49.3	45.4	Dog; clotted
5		49.5 49.3	49.4	45.5	Normal dog
6		49.3 49.3	49.3	45.4	Same dog, next day

HORSE SERUM.

7	III	51.1 50.9 50.9	51.0	46.9	
8		48.3 48.3 48.3	48.3	44.5	

RABBIT SERUM.

No.	Tip.	Wt. 1 drop. Mgs.	Av.	γ.	Remarks.
9	III	49.1 49.0	49.1	45.2	
10	III	50.1 50.0	50.1	46.1	
11	III	51.6 51.6 51.6	51.6	47.5	

GUINEA PIG SERUM.

12	III	49.8 49.8	49.8	45.3	

SHEEP SERUM.

13	III	51.7 51.8	51.7	47.6	
14	III	48.7 48.9	48.8	44.9	

HUMAN SERUM.

15	I	49.5 49.4 49.5	49.5	44.5	
16	I	51.5 51.5	51.5	46.2	Placental
17	II	50.2	50.2	45.9	Placental
18	II	50.6 50.9 50.7	50.8	46.4	Placental
19	II	49.7 49.8	49.8	45.5	Placental
20	II	51.5 51.6 51.5	51.6	47.1	Mixed
21	II	52.5 52.7	52.6	48.0	Kidney trouble
22	III	55.8 56.0	55.9	51.4	Chronic nephritis
23	III	51.2 51.4	51.3	47.1	Same case; later sample
24	I	49.4 49.6	49.5	45.1	Same case; later sample
25	III	53.0 53.0	53.0	48.8	Locomotor ataxia
26	III	48.7	48.7	44.8	Apoplexy
	II	49.0	49.0	44.7	

HUMAN SERUM (*continued*).

No.	Tip.	Wt. 1 drop. Mgs.	Av.	γ.	Remarks.
27	III	48.4 48.6 48.7	48.6	44.7	J. S. B.
28	III	48.3 48.0 48.1	48.1	44.3	J. S. B.
29	III	49.4 49.3	49.4	45.4	F. R. E.
30	III	50.1 50.1 50.2	50.1	46.1	H. E. W.
31	III	49.2 49.0 49.1	49.1	45.2	H. E. W.

COMPARISON OF SURFACE TENSIONS.

Human serum	44.3–46.4	Av. 45.4
Dog serum	45.0–45.7	45.4
Horse serum	44.5–46.9	45.7
Rabbit serum	45.2–47.5	46.3
Guinea pig serum	45.3	45.3
Sheep serum	44.9–47.6	46.2

Experiments 1–6 inclusive show conclusively that, when perfectly normal, the blood serum of dogs is practically a constant value at 37°, *viz.*, 45.4 dynes per centimeter. With the other animals and the human subjects such a regulation to certain definit conditions was not possible, as has already been mentioned. The sera in experiments 15–19 inclusive and 27–31 are probably perfectly normal and give an average value of 45.4 dynes, but most of them vary considerably from this because the condition was not specially controlled. The placental sera (Nos. 16–19) are rather high (average 46.2), probably because the patients had not been eating as much as the others (average 45.4). The blood in Nos. 27 and 28 was obtained in the afternoon while it was taking up the digested food, which is the reason for its low value. No. 29 was obtained about an hour after eating, before the blood could absorb any food. No. 30 was taken at noon—about eighteen hours after eating, since nothing was eaten that morning—while a few days later No. 31 was taken about four hours after eating, thus showing the great possible variation in one individual at different hours of the day.

Several of the pathological sera show a higher surface tension than would be expected even from a low diet. In the case of kidney trouble and nephritis this is probably due to retention of salt and in the latter it also accompanies a high blood pressure.

The surface tension of blood serum, it will be observed, is much below

that of water (45.4 as compared to 69.84 at 37°), but this is not surprising when the number of substances in solution is considered. The list includes neucleo protein, serum globulin, serum albumin, glucose, fat, enzymes, lecethin, cholesterol and esters, gases, coloring matter and inorganic salts such as chlorides, phosphates, carbonates, and sulfates of sodium, potassium and magnesium. The only things on this list that would raise the surface tension of water are the inorganic salts, while the proteins, being colloids, would lower it somewhat and emulsions of fat, lecethin and cholesterol would lower it still more.

Iscovesco[1] has determined the surface tension of blood serum using the "stalagmometer" of Traube. By this method the number of drops of serum in a certain volume is counted and compared with the number of drops of water in the same volume of water, and the surface tension found by aid of the relation

$$\frac{\text{No. drops of water} \times \text{density of serum}}{\text{No. of drops of serum}} \times 75 = \text{surface tension.}$$

Since this investigator found it necessary to determin the water "constant" several times a day, the method can hardly be considered accurate. The use of 75 for the surface tension of water shows the results to be found at approximately 5°, though no temperature is mentioned in the work. The solutions of serum in water that he worked with gave values lying between 69 and 74 (almost equal to pure water). We made a few determinations of such solutions by the more accurate method and found a value of about 65 dynes at 0°, but solutions of serum decompose more easily than the pure serum, so that it seemed better to make all our measurements on the latter.

Probably the cause of the difficulty which necessitated the redetermination of the water constant in the above work was the insufficient cleanness of the tip. In fact in this work, wherever aqueous solutions are employed, the greatest care must be taken to keep the tip free from all foreign material. In fact, no satisfactory agreements can be obtained with such liquids unless the tip is cleaned with alkalin permanganate solution followed by chromic acid in diluted sulfuric acid, and then washed with distilled water and dried by suction—and this after each determination.

Fano and Mayer[2] have used the capillary rise method on blood serum, but as their work was at nearly every temperature except that of the blood, no comparison with ours is possible. Their work would seem to indicate that the surface tension of the serum varies according to the species and the individual, which is quite contrary to our conclusions above that

[1] *Comp. rend. soc. biol.*, **69**, 353; 70, 1166.

[2] *Arch. fisiol.*, **4**, 165.

the variation in any one individual may be as great as between individuals, and that the value for any individual lies between 45 and 46 dynes.

The Meiostagmin Reaction.

Ascoli and Izar, of the University of Pavia, have shown by aid of the stalagmometer of Traube, *that when a specific antigen is added to a specific serum, the surface tension of the liquid is lowered after the antibody, present in the pathological serum, has bound the antigen by incubation for two hours at 37°.* This reaction was tested by them on typhoid, syphilis, tuberculosis and cancer serum, being positive in about 90% of the cases which were positive according to standard methods, and nearly always negative in negative cases.

This reaction we have tried with the Morgan drop weight apparatus in a series of syphilis cases—about 25 being positive by the standard Wassermann test, and 5 negative. These sera came from St. Luke's and Roosevelt Hospitals, the results of the Wassermann test made in the hospital being also given us, with the serum, together with a clinical history of the case.

We decided here to work at 0° rather than a higher temperature, so that no reaction might take place in the serum during the measurement, and water was used for diluting since there were no corpuscles present —otherwise the directions of Ascoli and Izar were followed. The reaction does not seem to be as rapid as many such reactions. When red blood corpuscles, for example, are treated at 0° with an antibody (from the serum of an animal which has been immunized against these corpuscles), and the corpuscles are immediately centrifuged away from the solution, it is found that they have already combined with all the antibody in the few seconds they were together. In the Meiostagmin reaction, however, it takes about two hours for complete union of the antibody and antigen at 37° and there seems to be almost no reaction (union) at all at 0°.

Izar used an alcoholic ethereal splenic extract from a syphilitic fetus for an antigen, but in our work the antigens were from beef or guinea pig heart. The following antigens were used in our work:

Antigen I.—The acetone insoluble part of an alcoholic and ethereal extract of beef heart. Titrated strength is 0.04 cc. to 0.1 cc. serum.

Antigen II.—An alcoholic extract of guinea pig heart. Strength is 0.06 to 0.1 cc. serum.

Antigen III.—Same as *I.* Strength is 0.01 cc. to 0.1 cc. serum.

The solutions were made up usually so that the amount of serum was 1 in 25 parts by volume.

Antigen I.....	0.2 cc.	II	0.3 cc.	III	0.05 cc.
Water.....	11.8		11.7		11.95
Serum.....	0.5		0.5		0.50
Total.....	12.5		12.5		12.5

Half of the 12.5 cc. is then put in an incubating water bath at 37°, while the other half is placed in the drop-weight apparatus, its temperature reduced to 0° and its drop weight found. The weight of five drops was found here and the determination repeated until satisfactory checks within 1 milligram were obtained—no blank was needed here as the evaporation at 0° was utterly negligible. An error of 1 milligram on the five drops, which would be very large, would be about 0.25%, which is not of such great moment since all real change would be considerably over 0.5%. After the other half had incubated for the two-hour period it was placed in the drop-weight apparatus, reduced to 0° and treated as the other had been.

It seemed to make no difference whether the serum was inactivated (by heating to 55° for 30 minutes) or not, but most of the samples we used were inactivated, as they had already been used for the Wassermann test in which the serum must contain no complement. In one case the Meiostagmin reaction was tried both before and after inactivation of the serum. Inactivation destroys the complement (which is present in all serum), but leaves the immune body, and it is only the latter that is necessary in the Meiostagmin reaction. The table below shows this conclusively:

FRESH SERUM.

Tip III. Antigen III.

Before incubation.			After incubation.		
Wt. 5 drops.	w.	γ.	Wt. 5 drops.	w.	γ.
0.3976 0.3981	79.57	73.22	0.3955 0.3965	79.20	72.88
Change = 0.46% of original value.					
SAME SERUM AFTER 30 MINUTES AT 55°.					
0.4072 0.4070	81.42	74.35	0.4057 0.4047	81.04	74.01
Change = 0.46% of original value.					

The change being the same in both cases shows that the heating has not destroyed the portion important for the Meiostagmin reaction; and that the reaction will work whether the serum is fresh and contains complement, or has lost its complement by keeping it three or four days, or by heating it.

Sera from five non-syphilitic subjects was next studied. Four of these had shown negative results by the Wassermann test, while the fifth (No. 36) was from the same source as Nos. 30 and 31, being used simply as a control. The results on these five samples are given in the following table.

The negatives do not show more than a 0.2% decrease, and that is about the limit of accuracy with these solutions, using 5 drops, as it means a check of 0.8 mg. on this weight before and after incubation. No. 33 where

NON-SYPHILITIC SERA.

Tip III.

		Before incubation.			After incubation.			
No.	Antigen.	Wt. 5 drops.	w.	γ.	Wt. 5 drops.	w.	γ.	% change.
32	I	0.3753 0.3749	75.02	68.51	0.3757 0.3742	74.99	68.49	0
33	II	0.3577 0.3586	71.62	65.36	0.3593 0.3600	71.93	65.66	+0.5
34	III	0.4056 0.4051	81.07	74.60	0.4048 0.4043	80.90	74.45	—0.2
35	III	0.4006 0.4010	80.16	73.76	0.4008	80.16	73.76	0
36	III	0.3997 0.4007	80.04	73.65	0.3999 0.4005	80.04	73.65	0

antigen II is used increases slightly in weight probably owing to the large amount of alcohol in that antigen and the possibility of its loss during incubation at 37° by evaporation—which would cause an increase in the drop weight and surface tension.

In the table below are given the results of the Meiostagmin reaction in sera showing positive clinical evidence of syphilis, and also in most cases by the Wassermann test.

SYPHILITIC SERA.

Tip III.

		Before incubation.			After incubation.			
No.	Antigen.	Wt. 5 drops.	w.	γ.	Wt. 5 drops.	w.	γ	% change.
37	I	0.3758 0.3749	75.03	69.04	0.3706 0.3708	74.14	67.30	—2.5
38[1]	I	0.3337 0.3343	66.80	61.00	0.3261 0.3261	65.25	59.54	—2.4
39	I	0.3635 0.3632	72.67	66.87	0.3565 0.3571	71.36	65.67	—1.8
40[1]	I	0.3963 0.3966	79.30	72.42	0.3969 0.3959	79.28	72.40	0
41	II	0.3537 0.3547	70.84	65.15	0.3343 0.3350	66.92	61.60	—5.5
42	II	0.3591 0.3591	71.82	66.07	0.3523	70.46	64.87	—1.8
43	II	0.3575 0.3567	71.42	65.72	0.3550 0.3539	70.89	65.23	—0.8
44	III	0.3783 0.3797	75.80	69.75	0.3710 0.3716	74.26	68.33	—2.0
45	III	0.3963 0.3974	79.37	73.04	0.3885 0.3881	77.67	71.55	—2.0
46	III	0.3881 0.3872	77.53	71.34	0.3818 0.3810	76.28	70.19	—1.6

[1] Nos. 38 and 40 were from Tip II.

SYPHILITIC SERA (*continued*).

		Before incubation.			After incubation.			
No.	Antigen.	Wt. 5 drops.	w.	γ.	Wt. 5 drops.	w.	γ.	% change.
47	III	0.3955 0.3951	79.06	72.75	0.3914 0.3915	78.29	72.04	—1.0
48	III	0.3976 0.3981	79.57	73.22	0.3955 0.3965	79.20	72.88	—0.5
49	III	0.4070 0.4064	81.34	74.85	0.4053 0.4048	81.01	74.55	—0.4
50	III	0.4033 0.4031	80.64	74.20	0.4025 0.4017	80.42	74.00	—0.3
51	III	0.3965 0.3961	79.26	72.94	0.3951 0.3955	79.06	72.75	—0.26
52	III	0.4046 0.4050	80.96	74.50	0.4039 0.4045	80.84	74.39	—0.15
53	III	0.4027 0.4033	80.60	74.17	0.4027 0.4027	80.54	74.11	—0.1
54	III	0.3909 0.3919	78.28	72.03	0.3721 0.3731	74.52	68.57	—4.8
55	III	0.3949 0.3954	79.03	72.72	0.3873 0.3883	77.56	71.37	—1.9
56	III	0.3985 0.3979	79.64	73.28	0.3918 0.3910	78.28	72.03	—1.7
57	III	0.3992 0.3992	79.84	73.47	0.3944 0.3933	78.27	72.49	—1.3
58	III	0.4024 0.4030	80.54	74.10	0.3999 0.4005	80.04	73.65	—0.6
59	III	0.3997 0.3990	79.97	73.59	0.3974 0.3984	79.58	73.23	—0.3

In Nos. 54–59 the solutions were made up so that the serum was 1 volume in 10 instead of 1 in 25, with apparently no difference in the result. It is to be noted that the actual surface tension means nothing here, it is only the *change* in surface tension caused by the incubation. Results of 4 other positive cases are omitted here as the results did not check very closely: but they were decidedly positive.

In all but three of the above tests there is a decided decrease in surface tension after incubations, but in Nos. 40, 52 and 53 the change when present is no greater than might be found in a negative case so that these cannot be designated as positive by the Meiostagmin reaction. The history of these three cases, however, offers a possible explanation. No. 40 was a case where the primary occurred twenty-five years ago, when it was cured by the regular treatment The Wassermann test was a very weak positive, as titration showed only two units of antibody while an active case contains eight or ten times as much. No. 53 was a case of

tertiary syphilis in which the primary was ten years ago. It gave a positive Wassermann test with one antigen and negative with another. The Wassermann test was not tried with the antigen we employed in the Meiostagmin reaction. No. 52 was a case of early tabes probably on syphilic basis. No. 51 which gives a weak positive Meiostagmin, gave only a doubtful positive Wassermann test; this case had been treated for about eight months with mercury and potassium iodide.

The Meiostagmin reaction is evidently not quite as delicate as the Wassermann test, but it is a simpler test to make, for it only requires serum and antigen, while the Wassermann requires inactivated serum, antigen, guinea pig serum for complement, sheep blood corpuscles, and rabbit serum which has been injected with sheep corpuscles, all of which must be tested to see if they can be used together.

Summary.

The results of this investigation may be summarized very briefly as follows:

1. The surface tension of blood serum in an individual may change during the day, depending upon the food that is being absorbed by the blood.

2. The surface tension of the blood serum of different individuals and different species is approximately the same, if account be taken of the possibility of a daily change in any one individual.

3. The surface tension of blood serum seems to be abnormally high in certain diseases, especially those in which the kidneys are affected.

4. The Meiostagmin reaction was found to be positive in more than 80% of the cases of clinically positive syphilis investigated.

5. The clinically positive cases of syphilis in which the Meiostagmin reaction was not positive were those in which the Wassermann test was but weak or rather doubtful.

LABORATORY OF PHYSICAL CHEMISTRY.

[PAPER NO 24 FROM THE BIO-CHEMICAL LABORATORY OF THE STATION FOR EXPERIMENTAL EVOLUTION, THE CARNEGIE INSTITUTION OF WASHINGTON.]

STUDIES ON MELANIN V. A COMPARISON OF CERTAIN NITROGEN RATIOS IN BLACK AND IN WHITE WOOL FROM THE SAME ANIMAL.

BY ROSS AIKEN GORTNER.

Received July 17, 1913.

Introduction.

Historical.—In earlier publications upon the chemical nature of melanin and the chemistry of melanin formation (Gortner, 1910 (*a*), (*c*), 1911 (*a*), (*c*), (*d*), 1912 (*a*), (*b*)) I have shown the following: