The Use of Acetamide in the Meiostagmin Reaction

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The original meiostagmin reaction (MR) was based on the reduction of the surface tension of cancer serum after incubation with tumor extracts (1–4). The numerous variations and modifications of this method have been reviewed by Stern and Willheim (21). The MR shares the lack of specificity with other lability tests; but, apart from pregnancy, only very few noncancerous conditions are known to interfere, and these are readily diagnosed by clinical methods and auxiliary technics of the MR (14, 23). Keller and Künzel (11) used Lecomte du Noüy's dynamic principle (13) to observe surface tension variations in serum-sodium oleate systems. Significant differences between cancer and normal sera were noted. The surface tension buffering power of sera against strong surface-active reagents has been investigated in connection with the MR, but buffering in the presence of reagents expected to raise the surface tension of cancer sera has not been investigated so far. The present work describes experiments of the latter kind. Keller and Künzel explained their results in terms of a combination of soap and serum proteins. More recent work (5) supports such views and indicates that similar combinations may take place between amides and serum proteins. For reasons of simplicity the effects of acetamide, urea, and diacetamide (15) were tested in our experiments. The first two are denaturing agents, the former being weaker than the latter (20). The denaturing effect of diacetamide is the same as that of acetamide, judging from the titration of various proteins in isotonic aqueous saline with absolute alcohol, using Keller's method (10) with nephelometric standards.

METHODS

Cancer and normal sera were taken at random at the Royal Hobart Hospital, Launceston General Hospital, and Sydney Hospital in 5–10-ml. lots under sterile conditions. Oxalated and citrated sera were found unsuitable. The samples were defibrinated at the sampling stations. Different methods of defibrination did not affect the results, nor did the use of slightly hemolysed samples, contrary to observations with an early form of the MR (19).

The effects of delays in testing are shown in Table 3. Centrifuging immediately after sampling gave better quantitative results. Sodium cyanide helps to counteract the effects of a delay in testing, but it was not used in the reported experiments. Sodium fluoride has no effect and copper sulfate an adverse effect.

The sera were diluted with 5 per cent aqueous solutions of the amides. With lower concentrations the results became inaccurate, and higher concentrations were avoided to minimize denaturing effects. The amide solutions were added to the sera up to 512 dilutions. The solutions were homogenized by gently tilting the stoppered test tubes 10 times, pouring into standard watch glasses of 4-ml. capacity with a surface: volume ratio of 4.0, and allowing the solutions to come to equilibrium. The latter process required a few minutes and 3–4 preliminary measurements. The surface tensions (γ) of the original and diluted sera were measured at 18° C. in a thermostatically controlled room with a Cambridge Du Noüy Tensiometer. The average of 3–6 measurements was taken as the correct value as soon as the measurements became constant within $\pm$ 0.1 dyne/cm. Slightly fermented sera have low surface tensions in the undiluted state (γ = 49–52 dyne/cm) and were discarded.

RESULTS AND DISCUSSION

With urea (γ = 73.0 dyne/cm in 5 per cent solution), no significant effect could be observed. Since weaker denaturing agents such as acetamide and diacetamide gave more pronounced effects, these must be due mainly to causes other than denaturation. No consistent results could be obtained with diacetamide (γ = 67.8 dyne/cm in 5 per cent solution) other than a sharp initial drop of the surface tension at low dilutions (7).

With acetamide purified by distillation alone, curves of different shapes could be obtained for cancer and normal sera, but the findings could not be duplicated with accuracy. The effect is ascribed to the odorous impurity of acetamide which is absent when acetamide is repeatedly recrystal-

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lized from benzene and ethyl acetate and washed with ether. Acetamide solutions keep unchanged for 4 hours, but inaccurate results are obtained with solutions 24–30 hours old.

With fresh 5 per cent solutions of acetamide (γ= 70.1 dyne/cm), the surface tension versus dilution curves rise to a maximum at 16–50 dilutions. At further dilutions transient cloudiness appears, and the surface tension values fall to a minimum at 100–200 dilutions. This minimum value ($\gamma_{min.}$) is lower than the surface tension of the undiluted serum (γ_0) with cancer, pregnancy, and some other sera. Since the rise to higher surface tension values past the minimum may be slow or fast, it was attempted to characterize the general behavior of the curves by a conventional measure termed "critical area" (CA). A positive CA is defined as the area inclosed by the curve and a straight line drawn through γ_0 parallel with the dilution axis. If this line is a tangent of the curve at its minimum, the CA is reported as 0. Otherwise, the CA is reported as negative (−). In sera exhibiting powerful buffering effects around the value of $\gamma_{min.}$ it may happen that 512 dilutions are not sufficient to circumscribe the positive CA. In a few cases of this kind the area was completed by a line drawn perpendicularly through the point corresponding to 512 dilutions on the abscissa. A positive CA may be evaluated by counting squares or by planimetry. Figures reported in Tables 1, 2, 3 and 4 have been rounded off to the nearest ten with an average error of about 10 units. Examples of typical cancer (C41S) and noncancer (N11) curves are shown in Figure 1.

TABLE 1

RESULTS OBTAINED ON CANCER SERA

Number	Sex	Age	Diagnosis	Treatment	γ_0	γ_{min}	G	CA
C 1	F	60	C., cervix of uterus		58.0	56.0	2.0	320
C 2	M	54	C., stomach		58.0	54.2	3.8	950
C 3	F	44	C., cervix of uterus		57.0	55.0	2.0	370
C 4	F	62	C., uterus		55.0	53.8	1.2	80
C 5	F	35	C., ovaries	morphia	57.2	53.4	3.8	910
C 6	F	75	C., cervix of uterus		57.3	53.8	3.5	210
C 7	M	61	C., lower bowel	O(5 d.)	59.0	57.9	1.1	90
C 8	F	58	C., lower bowel	O(2 w.)	58.5	57.8	0.7	50
C 9	M	66	C., head of pancreas	O	58.2	55.9	2.3	520
C 10	F	62	C., breast, metastases	O(1 w.)	57.0	55.3	1.7	370
C 11	M	57	C., stomach, "	morphia	57.0	55.6	1.4	320
C 12	M	62	C., stomach	O, penicillin	57.5	57.1	0.4	30
C 13S	F	68	C., breast	O	56.0	55.2	0.8	60
C 14S	F	40	C., cervix of uterus		58.9	57.3	1.6	210
C 15S	M	63	Squam. carc. ani		55.3	54.3	1.0	290
C 16S	M	51	Carc. piriform fossa		56.9	54.6	2.3	380
C 17S	M	59	Squam. carc., hypopharynx		56.2	55.7	0.5	30
C 18L	F	49	Carc., cheek	X, O(7 m.)	57.4	54.8	2.6	650
C 19L	M	45	Astrocytoma pariet.	X, PB	57.8	56.7	1.1	70
C 20L	M	49	Carc., larynx	X	58.9	57.9	1.0	270
C 21L	F	63	Carc., breast	X, PB	58.0	55.3	2.7	490
C 22L	F	74	Carc., tongue	X	58.0	57.1	0.9	100
C 23L	F	47	Astrocytoma pariet.	X, PB	57.7	56.8	0.9	130
C 24	F	59	Endocervic. carc., II		59.5	57.8	1.7	190
C 25S	M	36	Carc., bladder	O(11 m.)	57.0	56.7	0.3	10
C 26S	M	62	Squam. carc., lip		58.4	57.4	1.0	80
C 27L	M	72	Carc., jaw, 7 years	X	57.8	56.7	1.1	210
C 28L	F	38	Pituitary tumor	X	57.4	56.6	0.8	110
C 29L	F	76	Squam. carc., temple	X	56.5	54.8	1.7	280
C 30L	F	55	Adenocarc., uterus		56.7	56.0	0.7	80
C 31S	F	68	Carc., cervix, II		57.8	57.2	0.6	50
C 32S	F	39	Carc., cervix, II		58.8	57.7	1.1	150
C 33S	F	54	Scirrh. carc., breast	O(4 m.)	56.0	56.0	0.0	0
C 34L	F	71	Carc., breast, 22 years		57.0	55.8	1.2	100
C 35L	F	38	Epithelioma, nose		58.0	56.7	1.3	230
C 36L	F	66	Carc., breast		57.1	55.9	1.2	260
C 37L	M	52	Carc., neck and chin	X	56.4	57.0	−0.6	
C 38S	F	35	Carc., cervix, II	radiotherapy	57.0	56.0	1.0	190
C 39S	M	60	Obstruction of esophagus, anaplast. carc.		56.3	55.8	0.5	40
C 40S	M	52	Malignant angioma	radiotherapy	56.8	56.0	0.8	70
C 41S	M	60	Carc., lip		57.2	55.3	1.9	230
C 42	F	62	C., breast	O, B(4 d.)	56.8	55.0	1.8	230
C 43	M	60	C., pancreas	O(1 w.), B(1 d.)	54.9	55.5	−0.6	
C 44	M	48	C., esophagus	B(2 d.)	53.0	56.2	−3.2	

L: samples from Launceston; S: samples from Sydney; unmarked samples from Hobart; O: operated; B: transfusion of 2 pints of blood; d: days; w: weeks; m: months of operation or transfusion before test; X: X-ray treatment; PB: phenobarbitol.

The use of CA values has the statistical disadvantage of unsymmetrical representations, but CA values are correlated with the symmetrical expression $G = \gamma_0 - \gamma_{min}$. Results on cancer sera are shown in Table 1 and on other sera in Table 2. An analysis of CA values by anatomical sites indicates that great variations are not to be expected (Table 4).

Cases C42, C43, and C44 show the effects of blood transfusion. The CA values in the latter two cases are not comparable with the rest of the data. In the normal series no clinical data were available on Cases N4 and N15. Radiologically treated cases appear to be correlated with flat, elongated curves. It is reasonable to assume that the low CA of C37L is a consequence of successful radiological treatment, but the same could not be said about C12—a case of carcinoma of the stomach treated with penicillin.

Excluding cases C43 and C44 in the cancer series and those of pregnancy, one obtains average CA values (rounded off to the nearest ten) of 220 for 41 cancer cases and of 40 for 11 noncancer cases with positive or zero CA values. Better percentages could be reported by omitting postoperative or radiologically treated cases with low CA values. The average values of G (rounded off to the first decimal) are 2.5 for 4 pregnancy cases, 1.4 for 42 cancer, and −0.8 for 27 noncancer cases. Only one noncancer and nonpregnancy case (4 per cent) exceeds the average G value for cancer, and all cancer cases have G values above the noncancer and nonpregnancy average. A histogram of the G values, shown in Figure 2, illustrates the difference between the results obtained in the cancer and

TABLE 2

RESULTS OBTAINED ON OTHER SERA

Number	Sex	Age	Condition	γ_0	γ_{min}	G	CA
N 1L	M	65	Gastr. hyperacidity	57.2	57.1	−0.1	0
N 2	M	25		55.0	56.3	−1.3	
N 3	F	25		55.2	55.5	−0.3	
N 4	F	20	?	57.8	56.8	1.0	80
N 5	M	24		58.0	57.5	0.5	30
N 6	M	26		58.0	58.9	−0.9	
N 7	M	21		58.6	59.0	−0.4	
N 8	M	39		52.0	54.7	−2.7	
N 9	M	18		57.0	56.5	0.5	20
N 10	M	23		56.5	57.1	−0.6	
N 11	M	24		56.1	58.0	−1.9	
N 12	M	42	Fract. tibia	55.0	56.7	−1.7	
N 13	F	31		56.0	59.2	−3.2	
N 14	M	30	Traum. hydrocele	57.8	57.8	0.0	0
N 15	F	62	Arthritis (?)	57.8	56.8	1.0	70
N 16	F	45	Disloc. ankle	57.0	57.0	0.0	0
N 17	M	35		58.0	58.0	0.0	0
N 18	M	27	Acute lumbago	56.8	58.2	−1.4	
N 19	F	61		56.0	57.5	−1.5	
D 20	F	69	10 years	57.3	57.4	−0.1	0
D 21	M	70		57.0	55.4	1.6	150
D 22	F	76	Cardiac failure	58.4	58.2	0.2	20
D 23L	F	66	3 months	60.2	61.4	−1.2	
D 24L	M	67	3 years	59.0	60.4	−1.4	
D 25L	F	56	2 years	56.8	60.1	−3.3	
D 26L	F	68	11 years	56.4	59.4	−3.0	
D 27L	F	47	14 years	54.0	?	?	
P 28	F	37	37 weeks	56.3	54.1	2.2	380
P 29	F	21	38 weeks	57.0	54.4	2.6	350
P 30	F	22	35 weeks	56.8	54.6	2.2	190
P 31	F	27	24 weeks, twins	57.8	55.0	2.8	500

N: normal; D: diabetes; P: pregnancy; L: samples from Launceston; unmarked samples from Hobart. Treatment: 1L X-ray; 20, 22 insulin; 13 pentothal; 18 sodium salicylate; 20 phenobarbitol.

TABLE 3

EFFECT OF DELAY ON TESTING SERA

Origin	Delay	Average CA	Average G
Hobart	0	320	2.0
Launceston	24 hours	210	1.1
Sydney	48 hours	130	0.9

TABLE 4

ANALYSIS OF CA VALUES BY ANATOMICAL SITES

	Cases	Average CA	Average G
Stomach, esophagus	4	330	1.5
Uterus, ovaries	11	250	1.7
Breast	7	220	1.3
Neck, throat, mouth	9	220	1.0
Head	4	180	1.3
Lower intestine	3	180	0.9

noncancer series (the latter excluding cases of pregnancy). The difference between the corrected standard deviations for G in the cancer and noncancer series is 0.38 ± 0.19. A better indication of the significance of the MR is obtained from the

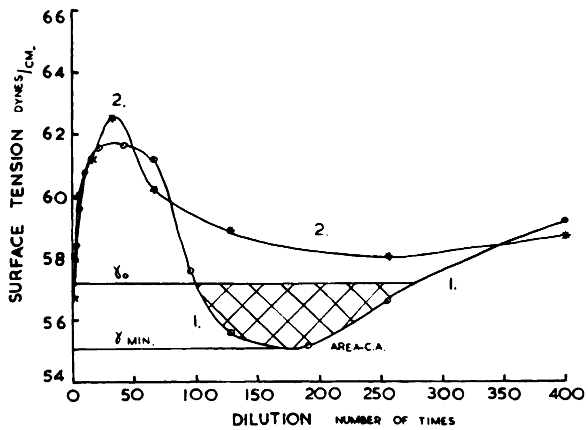

FIG. 1.—Surface tension of cancer (1) and normal (2) serum on dilution with 5 per cent acetamide.

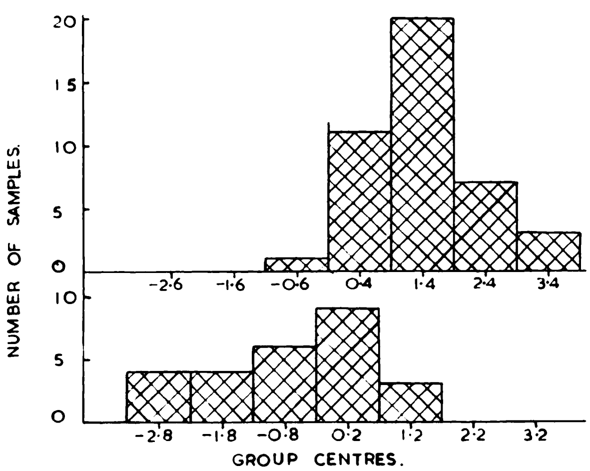

FIG. 2.—Histogram of G values for cancer (upper figure) and noncancer (lower figure) cases. The latter exclude cases of pregnancy.

t-test. For the calculated 8.0 for t and 66 degrees of freedom, a probability percentage point of well below 0.1 per cent is obtained. A variance test gives $F(25, 41) = 2.0$, corresponding to a probability percentage point of about 3. The significance of the CA values in the cancer and noncancer series can be estimated by the χ^2-test. Dividing the samples into cancer and noncancer classes and classes with positive and negative (including zero) CA values, χ^2 is obtained as 39.5, which, for 1 degree of freedom, corresponds to a probability percentage point of well below 0.1 per cent. Almost the same result is obtained if the χ^2-test is applied to the G values with classes comprising positive and negative (the latter including zero) G values.

The effect of dilution with buffer solutions has been studied in the case of cancer, diabetes, and normal sera. Figures 3 and 4 show the results of a

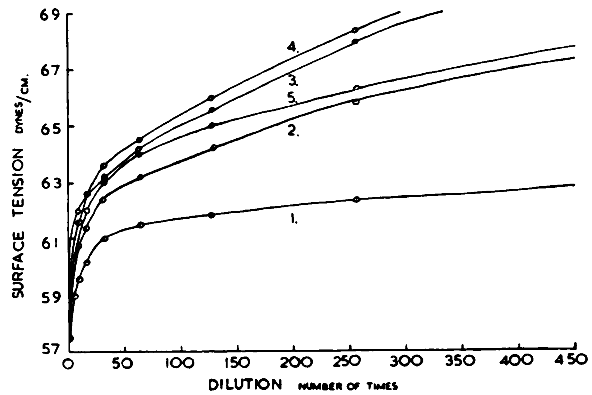

FIG. 3.—Surface tension of serum on dilution with buffer solutions. 1: pH 6.00; 2: pH 6.72; 3: pH 7.30; 4: pH 7.72; 5: pH 8.59.

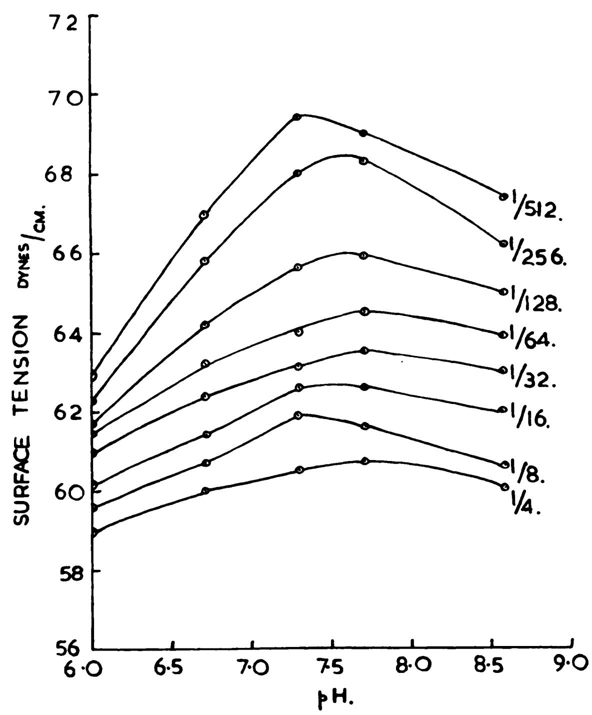

FIG. 4.—Surface tension of serum as a function of pH at various dilutions.

typical set of experiments on sample C12 which may be regarded as intermediate between typical cancer and noncancer sera. With borate and succinate buffers (6) the surface tension versus dilution curves are different from those obtained with acetamide solutions and approximate the curves obtained by diluting sera with water (Fig. 3). The variation of the surface tension with pH exhibits maxima between 7.3 and 7.8 (12).

The surface tension versus dilution curves present a close analogy with the curves obtained for the system diluted serum-sodium cholate (22), which show a sharp surface tension minimum at 20–200 dilutions, approximately as in our experiments. This effect has been explained in terms of the combination of serum proteins with sodium cholate, which tends to displace lipids from the lipoproteins. The effect is more pronounced with sera from which the lipids have been removed. This, however, is difficult to apply to the MR, since γ_0 values of cancer sera are not higher than those of normal sera, in spite of the raised lipid content of the former; and the surface tension maximum observed with lipid-free sera by Tayeau and Blanquet and in our experiments is not explained. Conversely, Elkes and Finean in their work on the hemoglobin-sodium hexadecyl sulfate system noted the formation of soluble and insoluble complexes at significantly different, limited ratios of detergent to protein (8). Elkes and Finean suggested different mechanisms of combination on the acid and alkaline sides of the isoelectric point. In the case of the amphoteric acetamide an approximately symmetrical behavior is expected. The behavior of the chemically simpler system acetamide-diacetamide (7) confirms the suggestion that the characteristic lowering of the surface tension in our MR is due to a combination of acetamide with serum proteins, resulting in the attraction of protein molecules into the surface. According to modern views on the nature of acetamide (17, 18), the combination is assumed to take place through hydrogen bonding. The finer mechanism of the process—in particular, whether film penetration occurs (9)—cannot be decided from the existing evidence. The maximum of the curves may represent the opposite process, which is reversed when a certain stage of denaturation has been achieved. At dilutions beyond this point a cloudiness occurs. This is not due to the precipitation of globulins owing to dilution, since globulin fractions of cancer sera dissolved in saline to the protein concentration of the original serum give a MR with acetamide which is almost identical with the MR of the original serum. Finally, it should be noted that the MR with acetamide is closely related to modern technics of tensiometric titrations (16, 24).

SUMMARY

1. The surface tensions of cancer and noncancer sera were determined in progressive dilutions with 5 per cent acetamide.
2. The difference between the initial and minimum surface tension values was found to be positive in pregnancy and most of the cancer samples, whereas it was negative in most of the other samples.
3. A conventional unit, the critical area, was defined and found to be positive in pregnant and most of the cancerous samples, whereas it was negative or slightly positive only in other samples.
4. The modified MR is discussed from the point of view of the combination theory.

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REFERENCES

1. ASCOLI, M. Die spezifische Meiostagminreaktion. München. med. Wchnschr., **57**:62–63, 1910.
2. ASCOLI, M., and IZAR, G. Die Meiostagminreaktion bei bösartigen Geschwülsten. München. med. Wchnschr., **57**: 403–5, 1910.
3. ———. Die spezifische Meiostagminreaktion II. *Ibid.*, pp. 954–56, 1910.
4. ———. Zur Technik der Meiostagminreaktion bei bösartigen Geschwülsten. *Ibid.*, pp. 2129–31, 1910.
5. BOYER, P. D.; LUM, F. G.; BALLOU, G. A.; LUCK, J. M.; and RICE, R. G. The Combination of Fatty Acids and Related Compounds with Serum Albumin. I. Stabilization against Heat Denaturation. J. Biol. Chem., **162**:181–98, 1946.
6. BRITTON, H. T. S. Hydrogen Ions (Tables 42 and 50), pp. 219 and 222. London: Chapman & Hall, 1932.
7. DUNN, P., and POLYA, J. B. Amides. VI. Studies on Acetamide and Diacetamide. Rec. trav. chim. (in press).
8. ELKES, J., and FINEAN, J. B. Observations on the Association of Hemoglobin and Sodium Hexadecyl Sulphate in Bulk Solution. Surface Chemistry, pp. 281–87. London: Butterworth Scientific Publications, 1949.
9. HARKINS, W. D.; MITTELMANN, R.; and CORRIN, M. L. Types of Solubilization in Solutions of Long-chain Colloidal Electrolytes. J. Phys. Coll. Chem., **53**:1350–61, 1949.
10. KELLER, C. J. Nephelometric Studies in Biological Protein Solutions. Ztschr. f. d. ges. exper. Med., **103**:427–45, 1938. "Abstr.," Chem. Abstr., **32**:7942, 1938.
11. KELLER, C. J., and KÜNZEL, O. Untersuchungen über die Oberflächenspannung im normalen und pathologischen Serum. II. Ztschr. f. klin. Med., **134**:287–99, 1938.
12. ———. Influence of Hydrogen-ion Concentration on the Surface Tension of Serum. Ztschr. f. klin. Med., **136**:507–16, 1939. "Abstr.," Chem. Abstr., **34**:4881, 1940.
13. LECOMTE DU NOÜY, P. Studies in Biophysics. The Critical Temperature of Serum. New York: Reinhold Publishing Corp., 1945.
14. MICHELI, F., and CATTORETTI, F. Die Meiostagminreaktion bei bösartigen Geschwülsten. München. med. Wchnschr., **57**:1122–23, 1910.

15. POLYA, J. B., and TARDREW, P. L. The Preparation of Diacetamide. J. Chem. Soc., 1081–83, 1948.
16. PRESTON, J. M. Tensiometric Analysis of Surface Active Electrolytes. J. Soc. Dyers Colour., **61**: 165–66, 1945.
17. RICHARDS, R. E. The Force Constants of Some OH and NH Linkages. Trans. Faraday Soc., **44**: 40–44, 1948.
18. RICHARDS, R. E., and THOMPSON, H. W. Spectroscopic Studies of the Amide Linkage. J. Chem. Soc., 1248–60, 1947.
19. ROSENBERG, M. Zur Frage der serologischen Karzinomdiagnostik. II. Die Meiostagminreaktion. Deutsche med. Wchnschr., **39**: 926–28, 1913.
20. STEINHARDT, J. Properties of Hemoglobin and Pepsin in Solutions of Urea and Other Amides. J. Biol. Chem., **123**: 543–75, 1938.
21. STERN, K., and WILLHEIM, R. The Biochemistry of Malignant Tumors, pp. 255–65, 863–68. New York: Reference Press, 1943.
22. TAYEAU, F., and BLANQUET, P. Tension superficielle des mélanges sérum-sel biliaire. Surface Chemistry, pp. 329–34. London: Butterworth Scientific Publications, 1949.
23. VERCESI, F., and GUERCIO, F. Biochemische Untersuchungen über das Verhalten des Serums bei malignen Tumoren, Schwangerschaft und gynäkologischen Erkrankungen. Biochem. Ztschr., **279**: 229–32, 1935.
24. WIJGA, P. W. O. Bepaling van oppervlakte-actieven stoffen in verdunde oplossingen. Chem. Weekbl., **45**: 477–80, 1949.